**TH-FC001**

**De Novo Acute Kidney Injury (AKI) and Acute-on-Chronic AKI Share Similarly Poor Long-Term Survival** Michael Heung,[1] Vahakn B. Shahinian,[1] Elizabeth Hedgeman,[1] Chi-Yuan Hsu,[2] Neil R. Powe,[2] Paul W. Eggers,[3] Desmond Williams,[4] Rajiv Saran.[1] *[1]University of Michigan, Ann Arbor, MI; [2]University of California San Francisco, San Francisco, CA; [3]National Institutes of Health, Bethesda, MD; [4]Centers for Disease Control and Prevention, Atlanta, GA.*

Chronic kidney disease (CKD) is a known risk factor for AKI, but comparative outcomes of acute-on-chronic AKI vs. de novo AKI are not well characterized. We sought to describe the risk for AKI in patients with and without pre-existing CKD, and to compare their long-term mortality.

A 5% sample of the national U.S. Veterans Affairs database for 2005 was analyzed for hospitalizations of ≥2 days. Patients with AKI (AKIN criteria) were categorized into those with prior CKD (60mL/min>eGFR>10mL/min) or no prior CKD. Survival was assessed up to 3 years post-admission with time-dependent Cox regression.

Incidence of AKI was 7.9% among the 16343 hospitalizations and was higher among those with prior CKD than those without (22.1% vs. 6.1%, p<0.0001). Mean age of patients with AKI was 68 years, 97.5% were male and 31.7% had pre-existing CKD; mean length of stay was 14.6 days. The overall 3-year mortality of this AKI cohort was 38.3% vs. 13.9% in those without AKI. While crude 3-year mortality was significantly higher in AKI patients with prior CKD versus those without CKD (44.3% vs. 35.6%, p<0.01), adjusted Cox regression suggested increasing survival risk over time for those with prior CKD (Table 1).

While past studies have focused on short-term or intensive care outcomes, we provide insight into long-term outcomes following AKI. Trends were toward higher short-term mortality with de novo AKI but higher long-term mortality in the acute-on-chronic group. Overall, de novo AKI and acute-on-chronic AKI share higher long-term mortality, emphasizing the need for improved AKI prevention, especially in those with CKD.

Table 1: Time-Dependent Mortality in AKI Patients by Prior CKD Status[a]

| Time Period (from hospital admission) | Hazard Ratio (Prior CKD vs. No-CKD) | 95% CI | P-value |
|---|---|---|---|
| 0-1096 days (proportionality test)[b] | - | - | 0.04 |
| 0-30 days | 0.72 | 0.49, 1.06 | 0.10 |
| 30-90 days | 0.88 | 0.59, 1.32 | 0.54 |
| >90 days | 1.18 | 0.95, 1.46 | 0.13 |

[a]Adjusted for age, sex, race/ethnicity, diabetes and hypertension status.
[b]Due to violation of proportional hazards assumption for prior CKD we included time-dependent covariates to model the varying hazard ratios over time.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC002**

**Long-Term Influence of Prior Chronic Kidney Disease on Post-Operative Patients with Acute Kidney Injury in the Surgical Intensive Care Unit: The 5 Year NSARF Experience** Hung-Bin Tsai,[1,2] Vincent Wu.[3] *[1]Department of Internal Medicine, Buddhist Dalin Tzu Chi General Hospital, Chiayi, Taiwan; [2]School of Medicine, Tzu Chi University, Hualian, Taiwan; [3]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan.*

**Background.** Prior chronic kidney disease (CKD) is among the most potent predictors of acute kidney injury (AKI) following major surgery. The long term influence after acute on CKD (ACKD) in the critical setting is poorly studied. This study was to examine whether critical patients with ACKD experience different disease courses and outcomes than merely AKI or patients with chronic dialysis. **Method.** This non-concurrent prospective, multi-center, observational study enrolled 10,903 patients admitted after major operations since 2003 to 2009 and a total of 9524 patients survived to hospital discharge. CKD was defined according to estimated glomerular filtration rate (eGFR) ≤ 45 mL/ min/ 1.73m^2 .AKI were stratified according to the maximal RIFLE classification. The baseline sCr was the data obtained at hospital discharge from the previous admission, or the nadir creatinine value during the hospital course. **Result.** During the index admission, 1379 patients expired, 4393 (46.6%) of them survived to hospital discharge had an episode of AKI: 2434 (25.8%) RIFLE-R, 979 (10.4%) RIFLE-I, 745 (7.9%) RIFLE-F and 235 (2.5%) AKI patients had prior CKD. The Cox proportional hazard model showed ACKD patients had worse long term survival (HR, 4.00; 95% CI, 3.20-5.15) than patients without prior CKD. Hazard ratio (HR) for death increased with the severity of AKI: RIFLE-f (HR, 2.96), RIFLE-I (HR, 2.42) and RIFLE-R, (HR, 1.61) then the counter part. After a median follow–up of 3.89 years, the incidence of long-term dialysis were 0.17 versus 22.4 per 100 person–years among patients without CKD and with CKD, corresponding to an adjusted HR of 19.4 (95% CI, 8.2-45.8) and 56.2 (95% CI, 26.7-118.5). **Conclusion.** The patients with prior CKD were at higher risk for long term mortality and dialysis dependence after major surgery. ACKD patients with partial renal recovery during hospitalization were associated with an increased risk of death and long term dialysis.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC003**

**Acute Kidney Injury (AKI) Episodes as a Time-Dependent Predictor of Progression of Chronic Kidney Disease (CKD) in Diabetes Mellitus (DM)** Charuhas V. Thakar,[1,2] Annette Christianson,[2] Jonathan Himmelfarb,[3] Anthony Leonard.[2] *[1]University of Cincinnati, Cincinnati, OH; [2]Cincinnati VA, Cincinnati, OH; [3]University of Washington, Seattle, WA.*

Studies of long-term outcomes in AKI have examined the impact of a single event of AKI. We assessed the effects of AKI episodes during multiple hospitalizations as predictors of advanced CKD in a cohort of diabetics.

4,082 patients with DM, seeking longitudinal care within a VA healthcare system between 1999 - 04 were followed until 07. Primary outcome was Stage IV CKD [outpatient glomerular filtration rate (GFR) of < 30 ml/min/1.73m2]. Excluded were patients with < 3 outpatient creatinine (Cr) values (n, 278), and with baseline GFR of < 30 (n, 125), leaving 3,679 for analysis. Baseline demographics (age, gender, race), and co-morbidity information (initial diagnosis of hypertension, obesity, ischemic heart disease, congestive heart failure, peripheral vascular disease, and proteinuria) was extracted. AKI during hospitalization was defined as 0.3 mg/dl or 1.5 times increase in Cr relative to baseline. Cox-survival models examined the effect of first inpatient AKI episode vs no AKI, and up to 3 AKI episodes as time-dependent covariates, on Stage IV CKD. Covariates included demographics, baseline Cr, and time-dependent co-morbid diagnoses.

The sample was 97% male, median age was 62 yrs, and mean baseline GFR was 81 ml/min/1.73m2. Median follow-up was 62 months. 1,822 (49%) patients required >/= 1 hospitalization. 530/1,822 (29%) patients experienced at least one AKI episode during the study;30% of those experienced >/= 2 AKI episodes. Frequency of Stage IV CKD was 13% in those never hospitalized, 10% in those hospitalized but no AKI, and 23% in hospitalized with AKI (p < 0.0001). In multivariable cox-proportional hazards models, first inpatient AKI vs. no AKI was a time-dependent risk factor for Stage IV CKD [hazard ratio (hr), 3.6, 95% CI, 2.8, 4.6]; each AKI episode doubled that risk (hr, 2.0; 95% CI, 1.8, 2.3).

AKI increases risk of advanced CKD in diabetics, independent of co-morbidities in a time-dependent analysis; there is a dose-response effect associated with the number of AKI episodes.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC004**

**Predicting Which Patients Who Survive an Episode of AKI Will Progress to CKD: Validation of a Risk Score** Lakhmir S. Chawla,[1] Richard Amdur,[2] Carlos E. Palant,[2] Paul L. Kimmel.[1] *[1]Division of Renal Diseases, George Washington University, Washington, DC; [2]Research and Medical Service, Veterans Affairs Medical Center, Washington, DC.*

Intro: Patients who survive an episode of AKI are at high risk for progressing to advanced stages of CKD. Up to 20% of patients with an inpatient diagnosis of ATN progress to CKD4 or higher within 24 months. We hypothesized that if patients who survive AKI that are at highest risk for progression to CKD can be identified, opportunities to intervene might be realized.

Methods: We assessed all patients in the VA healthcare system who were admitted with a primary diagnosis indicating AKI (ARF or ATN ICD9 codes 584.xx) from 10-1-99 to 12-31-05. In the exploratory phase, we used logistic regression to test three multivariate prediction models for progression to CKD4. In the confirmatory study phase, we validated the models in all VA patients admitted for MI or pneumonia during the same time frame (CON).

Results: There were 5358 patients in the AKI population. 13.6% entered CKD4 post-admission. Full model with stepwise entry: This model was significant (p<.0001) and good predictive accuracy (ROC curve = 0.82). Simple model: Predictors tested included age, mean-albumin-during, mean-SC-during, and time at risk. This model was significant (p<.0001) and good predictive accuracy (ROC curve = 0.81) User-friendly model: Predictors entered included age, time at risk, pre-eGFR, mean-albumin, and RIFLE score. This model was significant (p<.0001), and acceptable predictive accuracy (ROC curve = 0.77). User friendly model validation- When the AKI-derived user-friendly model was tested in CON subjects, it was significant (p<.0001), and had moderate effect size (R2 = .12) and good predictive accuracy ( ROC = 0.82). Using a cut-point of -2.0, the model had sensitivity 0.76, specificity 0.63, and OR = 5.21 for those with positive compared to negative risk.

Conclusions: We developed 3 models to identify AKI survivors at high risk for progression to advanced CKD. Such tools can allow for identification of patients who should receive additional follow-up and candidates for interventional clinical trials.

Disclosure of Financial Relationships: Consultancy: Astute Medical, Nxstage Medical; Ownership: MAKO OrthopedicsResearch Funding: Eli Lilly; Honoraria: Sanofi-Aventis, Angiodynamics; Scientific Advisor: Nxstage medical scientific advisory board, Covidien scientific advisory board.

**TH-FC005**

**CKD, ESRD and Death after Reversible Acute Kidney Injury in Patients with Normal Baseline Renal Function** Ion D. Bucaloiu,[1] H. Lester Kirchner,[2] Evan Norfolk,[1] James E. Hartle,[1] Robert M. Perkins.[1] *[1]Nephrology, Geisinger Medical Center, Danville, PA; [2]Biostatistics and Data Core, Center for Health Research, Danville, PA.*

**Background:** Acute kidney injury (AKI) accelerates established CKD. The long term risks of de-novo CKD, ESRD, and death after reversible AKI in patients with normal baseline kidney function are unknown. **Methods:** All adult discharges with normal baseline

renal function from a tertiary medical center between January 2004 and December 2007 were identified and followed for outcomes through December 2009. The exposure of interest was reversible (to baseline eGFR) hospital-associated AKI. Patients with and without AKI were compared for the outcomes of CKD stages 3, 4, 5 and death, after propensity-score matching, using a marginal Cox proportional hazards model. **Results:** Of 21,373 eligible patients, 2,633 sustained a reversible AKI episode. Of these, 2,571 were successfully matched with up to 3 controls (n = 6,403). Median (IQR) follow up was 39.3 (28.3-50.6) months. The incident rate ratios (95%CI) of stage 3, 4, and 5 CKD, as well as death, were significantly higher in the AKI vs. non-AKI group [2.86 (2.66-3.07), 4.85 (4.08-5.76), 7.67 (5.14-11.46), 2.26 (2.05-2.49), respectively].

Adjusted long term risk of de novo stages 3, 4, and 5 CKD and death after AKI and subsequent renal recovery, among patients with normal pre-hospitalization renal function*

| Outcome | HR (95% CI) |
|---|---|
| Stage 3 CKD | 2.70 (2.52, 2.91)† |
| Stage 4 CKD | 4.80 (4.04, 5.70)† |
| Stage 5 CKD | 7.82 (5.19, 11.78)† |
| Death | 2.14 (1.94, 2.36)† |

*Model adjusted for baseline eGFR, Charlson Co-Morbidity Index, coronary artery disease, and procedure code for echocardiogram. † p < 0.0001.

**Conclusion:** A single, reversible AKI event in patients with normal baseline kidney function independently confers mortality risk, and associates with the development of CKD and ESRD.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC006

**Comparison of Urinary L-FABP with NGAL and IL-18 in an Adult Heterogeneous ICU Population** Kent Doi,[1] Kousuke Negishi,[1] Tomoko Ishii,[1] Toshiro Fujita,[1] Takehiro Matsubara,[1] Naoki Yahagi,[1] Takeshi Sugaya,[2] Eisei Noiri.[1] *[1]University of Tokyo; [2]CMIC Co., Ltd.*

Acute kidney injury (AKI) significantly worsens the outcomes of critically ill patients. Biomarkers that detect AKI early and reflect the severity will be useful to improve the outcomes. Although several new AKI biomarkers have been reported, evaluation in heterogeneous disease-oriented populations is necessary to confirm their reliability before transition to clinical use. Three hundred thirty nine adult critically ill patients who admitted to a mixed intensive care unit (ICU) were prospectively studied. Five urinary biomarkers including L-type fatty acid-binding protein (L-FABP), neutrophil gelatinase-associated lipocalin (NGAL), IL-18, N-acetyl-β-D-glucosaminidase (NAG), and albumin were measured at ICU admission. One hundred thirty one patients (38.6%) were diagnosed as AKI by the RIFLE criteria. Urinary L-FABP could detect AKI better than the other biomarkers [the area under the receiver operating characteristic curves, AUC-ROC, for L-FABP 0.748 (95%CI 0.691–0.798), NGAL 0.695 (0.634–0.750), IL-18 0.686 (0.623–0.743), NAG 0.621 (0.558–0.680), albumin 0.687 (0.626–0.741)]. Urinary L-FABP was also able to predict later onset AKI after ICU admission. Moreover, L-FABP, NGAL, and IL-18 were able to predict 14-day mortality better than serum creatinine (sCr) [AUC-ROC for L-FABP 0.896 (0.835–0.937), NGAL 0.827 (0.688–0.912), IL-18 0.826 (0.679–0.914), sCr 0.733 (0.614–0.826)]. Multiple logistic regression analysis revealed only urinary L-FABP was associated with 14-day mortality, although urinary NGAL also showed virtually the same tendency without reaching statistical significance ($p = 0.0507$) . Combination of urinary L-FABP and NGAL achieved the highest sensitivity and specificity for mortality prediction [AUC-ROC 0.903 (0.847–0.940)]. This is the first study to compare new urinary biomarkers of L-FABP, NGAL, and IL-18 directly for AKI detection and prediction with a cohort of heterogeneous patients in a mixed ICU. Our data indicate urinary L-FABP can contribute to development of new AKI diagnostic tools in critical care.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC007

**Comparative Analysis of Urinary Biomarkers in the Diagnosis of Acute Kidney Injury and the Prediction of Renal Replacement and Mortality at the Time of Admission to the Hospital. A Multicenter-International Study** Thomas L. Nickolas,[1] Kai M. Schmidt-Ott,[2,4,5] Pietro A. Canetta,[1] Omar H. Maarouf,[3] Meghan E. Sise,[1] Catherine Forster,[1] David Sola-Del Valle,[1] Eugenia Singer,[4,5] Saban Elitok,[5] Antje Elger,[4] Ralph Kettritz,[4,5] Friedrich C. Luft,[4,5] Jonathan M. Barasch.[1] *[1]Columbia University; [2]Max Delbruck Center for Molecular Medicine; [3]Staten Island University Hospital; [4]Charite'-Universitatmedizin; [5]Helios Clinics Berlin.*

NGAL, Kim-1, IL-18 and L-FABP are released by renal tubules in response to acute kidney injury (AKI) and along with Cystatin C have been shown in small studies to respond to stimuli (ischemia, sepsis, nephrotoxicity) which generally induce AKI. We have conducted the largest, prospective, multicenter study to date to discern the prognostic significance of urinary biomarkers. The study comprised 1677 patients entering Emergency Departments in the United States and in Germany, including 53% female, 9.5% black and 18% Caribbean Hispanics. Diagnostic adjudication, performed by a panel of nephrologists was certain or likely in 1212 patients (72.3%). AKI was found in 94 patients (7.7%), Prerenal Azotemia in 252 patients (20.8%), Stable Chronic Kidney Disease in 150 patients (12.4%), and Normal kidney function in 716 patients (59.1%). AUC-ROC for the prediction of AKI was highest for NGAL (0.81; CI 0.76-0.86) followed by Kim-1 (0.71; CI 0.65-0.76)**, LFABP (0.71; CI 0.65-0.76) ***, IL-18 (0.63; CI 0.57-0.70)***, and Cystatin C (0.65; 0.58 - 0.72)***, (** p 0.001; *** p 0.0001 vs. NGAL). Additionally, the median values of NGAL were nearly 8 fold higher in AKI than in Prerenal Azotemia, but the other biomarkers were elevated 2 or less. NGAL, Kim-1, and LFABP independently predicted a poor clinical outcome in the subsequent week in the hospital prognosticating dialysis initiation and/or mortality, when corrected for age, gender, ethnicity, comorbidities, and serum creatinine. These data show that upon presentation to hospital, urinary biomarkers have both individual and combined value in both the prediction of AKI and a poor outcome in hospital, NGAL being the most highly dynamic and discriminatory for AKI.

Disclosure of Financial Relationships: Consultancy: Abbott DiagnosticsResearch Funding: Abbott Diagnostics: A study of NGAL in the Emergency Room; Patent: Columbia University has licensed NGAL to Abbott and Biosite.

## TH-FC008

**Cystatin C: A Superior Filtration Marker for Pre-Operative Acute Kidney Injury Risk Stratification – The TRIBE-AKI Consortium** Michael Shlipak,[1] Amit X. Garg,[2] Steven G. Coca,[3,4] Jay L. Koyner,[5] Zhu Wang,[3,4] Heather Thiessen Philbrook,[2] Uptal D. Patel,[6] Prasad Devarajan,[7] Chirag R. Parikh.[3,4] *[1]SFVA; UCSF, San Francisco, CA; [2]University of Western Ontario; [3]Yale University, New Haven, CT; [4]VA CT HealthCare System, New Haven, CT; [5]University of Chicago, Chicago, IL; [6]Duke University, Durham, NC; [7]University of Cincinnati, Cincinnati, OH.*

Acute kidney injury (AKI) following cardiac surgery is associated with poor outcomes, but is challenging to predict from clinical information. The TRIBE-AKI Consortium prospectively enrolled a cohort of adults undergoing cardiac surgery at 6 academic centers during 2007-2009. All participants were at high risk for AKI, defined by one or more of the following: emergency surgery, baseline creatinine (Cr)>2 mg/dL, left ventricular dysfunction, age>70, diabetes, concomitant bypass and valve surgery, or repeat revascularization surgery. Pre-operative serum Cr was measured by each hospital's clinical lab, and eGFR was estimated by the CKD-EPI equation. Cystatin C (CysC) was measured from frozen sera by nephelometry (Siemens). Each kidney predictor was categorized as Low (quintiles 1-2), Medium (quintiles 3-4), and High (quintile 5). The outcome was AKI by AKIN Stage 1 or higher (≥0.3mg/dL or 50% rise in Cr). Analyses were adjusted for characteristics used for pre-operative risk stratification by the Society of Thoracic Surgeons. The 1,147 participants with pre-operative Cr and CysC levels had average age of 71±10, Cr 1.1±0.3, eGFR 74±9, and CysC 0.93±0.32. 407 (36%) participants developed AKI during hospitalization. CysC categories had the strongest and most linear associations with AKI. Adjusted odds ratios (95% CI) were 1.9 (1.4-2.5) for Medium and 4.4 (3.0-6.4) for High CysC, compared with 1.2 (0.9-1.6) and 2.0 (1.4-2.8) for Cr categories, and 1.0 (0.7-1.3) and 1.7 (1.2-2.4) for eGFR categories, respectively. After adjustment for clinical predictors, the c-statistic to predict AKI was 0.62 without kidney markers, 0.64 with Cr, 0.63 with eGFR, and 0.68 with CysC. In conclusion, CysC appears to offer substantial improvement over Cr and eGFR as a pre-operative marker to forecast the risk of AKI after cardiac surgery.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC009

**Mobilization of Vascular Progenitors and Recovery of Renal Function in Humans with Acute Kidney Injury** Yuan Chung, Yuhua Li, Rhian Touyz, Lionel G. Filion, Lauralyn Mcintyre, David Allan, Kevin D. Burns. *Medicine, The Ottawa Hospital, OHRI, University of Ottawa, Ottawa, ON, Canada.*

**Background:** Endothelial-like vascular progenitor cells (VPCs) are blood-derived angiogenic precursors that may facilitate vascular repair following ischemic injury. We determined if acute kidney injury (AKI) in humans is associated with mobilization of peripheral blood VPCs, and if VPC levels correlate with renal recovery. **Methods:** After obtaining informed consent, blood samples were drawn from 30 patients admitted to the intensive care unit, at times 0, 1, 3, 7 and 14 days after diagnosis of AKI using the RIFLE criteria. VPC levels were determined by cell cluster-forming assay and cell populations enriched for angiogenic precursors were enumerated by flow cytometry. **Results:** 30 patients were enrolled (16 males, mean age 62.4 yrs) with the following RIFLE categories of AKI at time 0: Risk (n=5), Injury (n=11) and Failure (n=14). Most admissions to intensive care were due to sepsis (n=19), and 20 patients had pneumonia or others causes of respiratory failure. A significant time-dependent increase in VPC clusters was observed over 14 days following AKI in the overall cohort. Greater mobilization of VPCs was observed at 14 days in patients with more severe AKI (I and F categories at enrollment) compared with patients presenting with Risk (p=0.043) as demonstrated by increases in VPC clusters and proportion of circulating CD34+,CD133+,VEGFR2+ angiogenic precursors. In patients with improvement in renal function, there was a significantly greater mobilization of VPC clusters, compared to patients who experienced stable or worsening RIFLE scores (p<0.03). Greater VPC mobilization was also observed in patients with improvement in Sequential Organ Failure Assessment (SOFA) scores during the study period (p=0.046). **Conclusion:** Time-dependent increases in circulating VPCs occur in patients with AKI. Greater mobilization of VPCs is associated with global improvement and recovery of renal function, suggesting a potential biological role for VPCs in repair of kidney injury in humans.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC010**

**Duration of Injury and Baseline Renal Function Define Biomarker Performance in Acute Kidney Injury** Zoltan H. Endre,[1] John W. Pickering,[1] Robert J. Walker,[2] Prasad Devarajan,[3] Charles L. Edelstein,[4] Joseph V. Bonventre.[5] *[1]Department of Medicine, University of Otago Christchurch, Christchurch, New Zealand; [2]Department of Medicine and Surgery, University of Otago, Dunedin, New Zealand; [3]Department of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Centre, Cincinnati, OH; [4]Division of Nephrology, University of Colorado, Denver, CO; [5]Renal Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA.*

Background

Biomarker performance in acute kidney injury (AKI) is usually based on populations homogeneous with respect to time and type of renal insult. We investigated urinary biomarkers in a high-risk heterogeneous population.

Methods

In a prospective observational study six urinary biomarkers in 529 patients admitted to two general intensive care units, we compared diagnostic and predictive performance of urinary γ-glutamyltranspeptidase (GGT), alkaline phosphatase (AP), neutrophil-gelatinase-associated-lipocalin (NGAL), Cystatin C (CysC), Kidney Injury Molecule-1 (KIM-1) and Interleukin-18 (IL-18). Biomarker concentrations were compared using the area under the receiver operator characteristic curve (AUC) for diagnosis or prediction of AKI, dialysis or death. Performance was reassessed after stratification according to pre-admission (baseline) renal function (eGFR) and time after renal insult.

Results

On entry, all biomarkers except KIM-1 predicted death at 7 days (0.61<AUC<0.69). Without stratification, no biomarker had an AUC above 0.7 in diagnosis or prediction of AKI; NGAL, CysC and IL18 predicted dialysis with AUC>0.7. AKI was detected in patients with eGFR<60 ml/min by GGT, CysC, NGAL and IL18 with an AUC≥0.85, but only between 12 and 36 hrs after insult; with eGFR≥60ml/min, GGT detected AKI with AUC ≥0.73, IL18, with AUC≥0.63, and AP with AUC≥0.62, for up to 12 hours following insult. NGAL, with AUC≥0.68, CysC, with AUC≥0.65, and KIM1, with AUC≥0.62, detected AKI for up to 36 hours.

Conclusions

All biomarkers predicted death and dialysis. For detection of AKI in a heterogeneous population, urinary biomarker performance is critically dependent on both timing and baseline function before renal insult.

Disclosure of Financial Relationships: Honoraria: Travel & accommodation support to speak at meetings from Inverness (AACB, Brisbane, Australia 2009; APCN, Seoul 2010) and Abbott (Architect user meeting; Rotorua 2010).

**TH-FC011**

**Paneth Cell Activation after Acute Kidney Injury Causes Liver and Intestine Injury and Systemic Inflammation in Mice** Sang Won Park,[1] Mihwa Kim,[1] Kevin M. Brown,[1] Vivette D. D'Agati,[2] H. Thomas Lee.[1] *[1]Anesthesiology, Columbia University, New York, NY; [2]Pathology, Columbia University, New York, NY.*

Patients with acute kidney injury (AKI) frequently suffer from extra-renal organ dysfunction and systemic inflammation. These extra-renal complications from AKI are the leading causes of mortality in the intensive care unit. We aimed to determine the mechanisms of ischemic and non-ischemic AKI induced systemic complications. C57BL/6 mice were subjected to sham surgery, 20 min renal ischemia or unilateral nephrectomy. Both ischemic (ALT=201±22 U/l, N=6, p<0.01) and non-ischemic AKI (ALT=154±17 U/l, N=6, p<0.01) caused significant hepatic injury compared to sham-operated mice (ALT=53±6 U/l, N=6) in 5 hrs. The rises in plasma ALT correlated with focused peri-portal hepatocyte necrosis, vacuolization, neutrophil infiltration and pro-inflammatory mRNA upregulation. Plasma levels of TNF-α and IL-17A were also significantly elevated 5 hrs after ischemic (TNF-α=21.8±1.5 pg/ml, N=4, P<0.01; IL-17A=98.8±10.0 pg/ml, N=4, p<0.01) or non-ischemic AKI (TNF-α=20.5±2.9 pg/ml, N=4, p<0.01; IL-17A=103.7±12.0 pg/ml, N=4, P<0.01) compared to sham-operated mice (TNF-α=3.4±1.5 pg/ml, N=4; IL-17A=0.0±0.0 pg/ml, N=4). Neutralization or genetic deletion of TNF-α or IL-17A prevented AKI induced hepatic and intestine injury. Small intestine histology after AKI showed massive epithelial necrosis and apoptosis as well as profound endothelial apoptosis. Remarkable hyperplasia and degranulation of Paneth cells were observed. Laser capture microdissection demonstrated increased mRNAs for TNF-α and IL-17A in Paneth cells of mice subjected to AKI. Finally, wild type mice treated with dithizone to deplete Paneth cells or Paneth cell deficient (*Sox9$^{flox/flox}$;VilCre*) mice were protected against hepatic and intestine injury after ischemic or non-ischemic AKI. Taken together, we propose that Paneth cell activation/degranulation after AKI produces hepatic damage and systemic inflammation via cytokine induction and intestinal barrier dysfunction. Modulating Paneth cell dysregulation may have important therapeutic implications in reducing systemic complications arising from AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC012**

**Proximal Tubular Epithelial Cell Specific Deletion of Netrin-1 Receptor UNC5B in Mice Exacerbate Ischemia Reperfusion Injury of the Kidney** Ganesan Ramesh,[1] Sutip Navankasattusas,[2] Dean Y. Li,[2] Weiwei Wang.[1] *[1]Medicine, Penn State University College of Medicine, Hershey, PA; [2]Medicine, University of Utah, Salt Lake City, UT.*

The axon guidance molecules netrin-1 was shown to regulate inflammation and apoptosis during ischemia reperfusion injury of the kidney. However, the receptor through which netrin-1 suppresses tubular epithelial cell apoptosis was unknown. Kidney proximal tubular epithelial cells are known to express specific netrin-1 receptor called UNC5B in the apical surface of the tubules. To determine whether netrin-1 mediates its protective effect against ischemia reperfusion injury of the kidney through UNC5B, we have generated a proximal tubular epithelial cell specific UNC5B knockout in mice using cre-lox technology. To enhance cre pentrance for floxed-UNC5B gene deletion, UNC5B-flox mice were mated with UNC5B heterozygous knockout mice to create UNC5B–/Flox mice. Deletion of UNC5B was confirmed by genomic PCR and immunohistochemical staining for UNC5B in kidney cortex. Tissue specific knockout mice and their littermate control mice without cre were subjected to 26 minutes or 22 minutes of ischemia followed by 72 h of reperfusion. Kidney function was monitored by measuring BUN and creatinine. Both heterozygous UNC5B knockout mice and tissue specific homozygous knockout mice are highly susceptible to kidney injury and did not survive beyond 24 h after reperfusion as compared to WT control mice. However, when these mice are subjected to mild 22 minutes of ischemia, heterozygous knockout (UNC5B–/Flox) mice without cre did not show any increase in serum creatinine where as proximal tubule specific knockout mice (UNC5B–/flox/GGT-cre) showed a dramatic increase in kidney injury as shown by increase in serum creatinine ( 0.81±0.2 vs. 0.15±0.02, p<0.001, 24h). These results suggest that netrin-1 receptor UNC5B plays critical role in tubular epithelial cell survival and deletion of UNC5B gene in mice exacerbate kidney injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC013**

**Deletion of VHL in the Thick Ascending Limb Protects from Ischemic Acute Kidney Injury** Gunnar Schley,[1] Bernd Klanke,[1] Kai-Uwe Eckardt,[1] Patrick Maxwell,[2] Carsten Willam.[1] *[1]Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany; [2]Division of Medicine, University College London, London, United Kingdom.*

Ischemia is a central pathophysiological factor in the development of acute kidney injury (AKI). In murine models of AKI, like in ischemia/reperfusion experiments, tubular ischemic necrosis and activation of the hypoxia-inducible factors (HIF) occur in particular in the outer medulla of the kidney involving the proximal tubule and the thick ascending limb of Henle's loop (TAL). In this study we investigated the effects of genetic HIF induction in the TAL in ischemia/reperfusion experiments.

To target the TAL the Tamm-Horsfall protein (THP) promoter was used to drive Cre recombinase expression in transgenic mice which were then crossed with mice carrying floxed von Hippel-Lindau (VHL) gene. Ischemic AKI was induced by clamping both renal pedicles for 25 min, with 3 days of reperfusion afterwards.

TAL specific knockout of the VHL gene led to stable expression of HIF1alpha. No further HIF induction was seen in other organs and no erythrocytosis was noted. HIF1 accumulation in the TAL was associated with cellular target gene induction (e. g. Glucose transporter-1). Renal morphology and functional parameters were not different at control conditions. Clamping of the renal pedicles led to profound ischemic injury in the outer medulla. Compared to wildtype mice, VHL deletion in the TAL functionally (creatinine: sham 0,08±0,01 vs. wt 0,78±0,12 vs. ko 0,32±,03 mg/dl, p=0.003; urea: sham 48,10±3,69 vs. wt 470,86±66,53 vs. ko 185,30±21,68 mg/dl, p=0,001) and morphologically protected mice from ischemic AKI. Although VHL has several biological functions, the protective effect of VHL knockout is presumably HIF-dependent and might be mediated by HIF target genes. Our findings underscore the role of the TAL in the pathophysiology of AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC014**

**Cystathionine-γ-lyase Is an Endogenous Modulator of Oxidative Stress** Eelke M. Bos,[1,2,3] Henrike Jekel,[1] Pauline M. Snijder,[1,2] Guangdong Yang,[3] Henri G. D. Leuvenink,[2] Harry Van Goor,[1] Rui Wang.[3] *[1]Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Netherlands; [2]Department of Surgery, University Medical Center Groningen, University of Groningen, Netherlands; [3]Cardiovascular Research Lab, Lakehead University, Thunder Bay, ON, Canada.*

Cystathionine-γ-lyase (CSE) is the main $H_2S$-producing enzyme in the mammalian cardiovascular system. Exogenous $H_2S$ treatment is highly beneficial in renal ischemia/reperfusion injury (IRI). Here, we investigated whether endogenous $H_2S$-production is protective against IRI, and if overexpression of CSE reduces mitochondrial ROS production.

Male C57BL/6 wildtype (WT) or $CSE^{-/-}$ were subjected to 30 min of bilateral renal ischemia. After 24h kidneys and plasma were collected. Renal $H_2S$-production rate in untreated WT and $CSE^{-/-}$ mice was measured using the Zn-trap method. HEK293 cells were transfected with pIRES2-EGFP-CSE plasmid to overexpress CSE. Mitochondrial superoxide production or or cytoplasmatic ROS level were measured using MitoSOX probe or dihydroethidine (DHE), respectively.

Renal IRI caused considerable mortality in $CSE^{-/-}$ mice, while WT mice showed no deaths post reperfusion (64% vs. 100% survival). Tubular necrotic area in the renal cortex of $CSE^{-/-}$ animals was 60% higher than that of WT ($p<0.01$). This coincides with a 91% reduced renal production of $H_2S$ in $CSE^{-/-}$ mice ($p<0.01$). In-vitro results showed that NaHS (a donor of $H_2S$) concentration-dependently reduced Antymicin A-induced mitochondrial superoxide production with a significant reduction at 10 μM (61%, $p<0.01$), max reduction at 1 mM (83%, $p<0.01$). Cytoplasmatic ROS levels were significantly reduced by NaHS at 10 μM (44%, $p<0.05$) with a max reduction at 1 mM (95%, $p<0.001$)). CSE overexpression in HEK293 cells significantly reduced the increase in MitoSOX (75%) and DHE (49%) fluorescence after Antimycin treatment when compared with mock-transfected cells ($p<0.01$).

It is concluded that endogenous $H_2S$-production by CSE plays a beneficial role in the response to renal IRI. This role is mediated in part by the antioxidant effect of $H_2S$, involving the reduction of ROS levels in both cytosol and mitochondria.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC015**

**Absence or Blockade of SR-BI/II and CD36 Receptors Improves Sepsis Survival and Acute Kidney Injury in Mice** Asada Leelahavanichkul, Alexander V. Bocharov, Roger Kurlander, Irina Baranova, Tatyana G. Vishnyakova, Xuzhen Hu, Kent Doi, Gyorgy Csako, Robert A. Star, Thomas L. Eggerman, Peter S. Yuen. *NIDDK, NIH, Bethesda, MD.*

Class B scavenger receptors bind/internalize oxidized LDL, apoptotic cells, and bacteria, which initiates pro-inflammatory cell signaling. A synthetic SR-B antagonist, L-37pA, reduced bacterial uptake and bacteria-induced cytokine secretion. Therefore, we tested L-37pA in a cecal ligation puncture (CLP) mouse sepsis model. L-37pA reduced organ damage, including serum LDH, and improved kidney and liver function (lower creatinine/BUN/ tubule damage, AST/ALT, respectively). Serum IL-6, TNF-α and IL-10 levels were also diminished by L-37pA, indicating reduced systemic inflammation. In contrast to typical anti-inflammatory agents, L-37pA increased peritoneal granulocyte counts and modestly reduced peritoneal bacterial load. These complementary actions improved the survival rate from 6% to 27%. Mice deficient in either SR-BI/II or CD36, were further protected from CLP-induced sepsis. In both knockout strains, 2- to 3- fold less systemic inflammation and organ damage were accompanied by an 8-fold increase of peritoneal granulocyte accumulation and a ~50-fold reduction of peritoneal bacterial counts, relative to CLP in wild-type mice. CD36-deficient mice survival was 58% vs 17% in control mice. SR-BI/II- deficient mice, which showed mineralocorticoid and glucocorticoid deficiency due to defective HDL uptake, demonstrated almost 50% survival vs 5% in steroid-compensated experiments. Cultured granulocytes, deficient in either SR-BI/II or CD36, had reduced cytokine response to exogenous bacteria and lived longer (more resistant to bacteria induced cell death). Our data indicate that L-37pA promotes local peritoneal granulocyte accumulation, bacterial killing, and inhibits systemic inflammation. These results demonstrate that targeting class B scavenger receptors are anti-inflammatory and indirectly anti-bacterial, without being immunosuppressive.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC016**

**Dendritic Cell (DC) Deficiency of Sphingosine 1-Phosphate Receptor 3 ($S1P_3R$) Attenuates Natural Killer T (NKT) Cell Activation and Kidney Ischemia-Reperfusion Injury (IRI)** Amandeep Bajwa, Liping Huang, Li Li, Mark D. Okusa. *Medicine and the Center for Immunity, Inflammation and Regenerative Medicine, University of Virginia, Charlottesville, VA.*

Sphingosine 1-phosphate (S1P), a sphingolipid that is the natural ligand for a family of five G-protein coupled receptors ($S1P_{1\text{-}5}Rs$), regulates cell survival, differentiation, cytoskeletal rearrangements, angiogenesis, calcium regulation, and lymphocyte circulation. $S1P_3Rs$ on vascular endothelial and epithelial cells have been linked to enhanced vascular and epithelial permeability, respectively, and dendritic cell (DC) $S1P_3R$ deficiency protects mice from lethal sepsis. Previous work has demonstrated that kidney IRI is dependent on the DC activation of NKT-mediated production of interferon gamma (IFN-γ). We hypothesized that DC $S1P_3Rs$ are involved in mediating DC-NKT interaction in kidney IRI. Both kidney pedicles were clamped in C57BL/6 mice for 26 min and then released for 24 hrs. FACS, ELISA, H&E staining and plasma creatinine (mg/dL) measurement were performed. WT mice injected with alpha-galactosylceramide (αGalCer; 10μg/mouse) had significantly higher levels of plasma IFN-γ (81.1%, $p<0.05$) at 6 and (95%, $p<0.05$) at 24h compared to $S1P_3KO$ mice. We next transferred WT or $S1P_3KO$ DCs loaded with aGalCer (DC-αGalCer) into WT mice and performed kidney IRI. Mice reconstituted with WT DC-αGalCer had more kidney IRI with higher plasma creatinine (57.1%, $p<0.01$), neutrophil infiltration (84.5%, $p<0.001$), NKT infiltration (61.9%, $p<0.001$) compared to mice reconstituted with $S1P_3KO$ DC-αGalCer. Additionally, mice reconstituted with WT DC-αGalCer prior to kidney IR had had significantly higher mRNA levels of proinflammatory cytokines (TNFα, IL-12p40, IL-1β, IL-6) and chemokines (CXCL1,2,5) compared to $S1P_3KO$ DC-αGalCer reconstituted mice. We conclude that absence of DC $S1P_3Rs$ attenuates NKT cell activation, neutrophil infiltration and proinflammatory cytokine production in response to kidney IRI. Thus the development of $S1P_3$ antagonists may lead to novel therapeutic approach for the prevention and treatment of kidney IRI.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC017**

**CD4 T Cells and CXCL1 in Cisplatin-Induced Acute Kidney Injury** Ali Akcay, Kultigin Turkmen, Quocan Nguyen, Zhibin He, Sarah Faubel, Alkesh Jani, Charles L. Edelstein. *Univ of Colorado Denver.*

We have previously demonstrated that neither neutrophil depletion nor macrophage depletion is protective in cisplatin-induced acute kidney injury (Cis-AKI). Thus we further investigated CD4 T cells in Cis-AKI. The aim of the study was to determine the role of CD4 T cells and CD4 T cell cytokines like CXCL1 (also known as IL-8 or KC) in Cis-AKI. Mice were injected with Cis 25 mg/kg and sacrificed on days 1, 2 and 3. BUN and serum creatinine (SCr) were elevated on Day 3. Flow cytometry for CD4 T cells was performed on single cell extracts of kidney. CD45+ (common leukocyte antigen) gated events were analyzed for expression of CD3 and CD4. The increase in CD4+ cells in Cis-AKI was not seen in CD4 -/- mice. Both CD4 T cell depletion with GK 1.5 antibody (10 mg/kg IP on day 7, 3 and 0 before Cis) and CD4 T cell -/- mice were protective against Cis-AKI. SCr was 1.5 in wild type (WT) mice+Cis and 0.9 in CD4 -/- mice+Cis ($P<0.05$, n=20). SCr (mg/dL) was 2.1 in Cis+vehicle and 0.9 in Cis+GK1.5 ($P<0.05$, n=6). CD4 T cells are known to produce CXCL1. CXCL1 (pg/mg) in whole kidney was 1.4 in vehicle and 15 on day 1, 61 on day 2 and 72 on day 3 (all $P<0.01$ *vs.* vehicle, n=4). The increase in CXCL1 in Cis-AKI was decreased in CD4 T cell -/- mice demonstrating that CD4 T cells result in an increase of CXCL1 in Cis-AKI. CXCL1 (pg/mg) in whole kidney was 98 in WT mice+Cis and 21 in CD4 -/- mice+Cis ($P<0.001$, n=5). To determine whether CXCL1 is a mediator of Cis-AKI, CXCR2 -/- that lack the CXCL1 receptor were studied. CXCR1 -/- mice were protected against C-AKI. SCr was 2.6 in WT mice+Cis and 0.8 in CXCR1 -/- mice+Cis ($P<0.01$, n=10). In summary, in Cis-AKI: 1) CD4 T cell depletion is protective, 2) CXCL1 is increased in kidney, 3) CXCL1 is decreased in CD4 -/- mice, 4) inhibition of the action of CXCL1 in CXCR1 -/- mice is protective. In conclusion, the injurious effect of IL-8 from CD4 T cells merits further study in Cis-AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC018**

**NGAL Reporter Mouse Illuminates Tubular Cell Stressors In Vivo** Neal A. Paragas,[1] Andong Qiu,[1] Jonathan M. Barasch.[1] *[1]Medicine, Columbia University, New York, NY; [2]; [3].*

We generated a difusion reporter knockin mouse, Ngal-Luc2/mC, under the control of the NGAL promoter. The NGAL-Luc2/mC knockin mouse was constructed by fusing luciferase (Luc2) and mCherry (mC) genes by overlap PCR and this product was inserted immediately 3' of the NGAL promoter using Bac recombineering. Unilateral IR (30 minutes) was performed on the right or left kidney of the NGAL-Luc2/mC reporter mouse while the contralateral kidney was left undisturbed as an internal control. NGAL expression was measured by both NGAL-Luc2 and NGAL-mC activities. NGAL expression was specific to the damaged kidney and was found to be dependent on the dose of ischemia, namely, NGAL-Luc2 activity rose as much as 40 fold after a 30min dose of ischemia, but only 10 fold after 15min of ischemia, whereas the contralateral kidney did not express the reporter. NGAL-Luc2 expression appeared within 3 hours of insult and conversely extinguished by 24 hours. NGAL-Luc2 activity correlated in timing and intensity with the appearance and concentration of urinary NGAL. Similar data were obtained in a bilateral IR model, sepsis model, and cisplatin nephrotoxic model. Using in situ hybridization as well as mCherry fluorescence, we show NGAL expression was expressed in the TALH and α Intercalated Cells of the Collecting Ducts. Cells extracted from NGAL Reporter Kidneys responded to nephrotoxins (cisplatin, cobalt and LPS) and bacterial products, and were modulated by novel Nf-κβ inhibitors. In contrast to these studies, simple volume depletion sufficient to increase serum Na and reduce body weight did not induce NGAL-Luc2 expression. In sum, in a living animal, Ngal-Luc2/mC revealed rapid expression, dose dependence, reversibility and kidney specificity during stressed conditions. Furthermore, we show that kidney NGAL directly correlates with uNGAL confirming reports that kidney epithelia secrete uNGAL. Additionally, primary cultures of adult kidney cells reveal that NGAL expression is mediated by Nf-κβ. Ngal-Luc2/mC mice and primary cell lines will permit the study of signaling pathways that trigger massive NGAL expression resulting in its utility as a biomarker of kidney epithelial stress.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC019**

**siRNA Against P53 Minimizes Recurrent AKI and Prevents Development and Exacerbation of CKD** Swetlana Boldin-Adamsky,[1] Silvia B. Campos-Bilderback,[2] Ruben M. Sandoval,[2] Elena Feinstein,[1] Bruce A. Molitoris.[2] *[1]Quark Pharmaceutical, Ness Ziona, Israel; [2]Dept. of Medicine/Division of Nephrology, Indiana University, Indianapolis, IN.*

Recent evidence from experimental and clinical investigations indicates repeated AKI episodes trigger CKD and accelerate its progression. Thus, prevention of AKI may minimize CKD development and reduce its progression to ESRD. As we have previously shown that siRNA against P53 (siP53) minimized ischemia/reperfusion AKI, we assessed whether its use during repeated AKI would limit CKD progression. First, we tested whether siP53 was capable of preventing CKD development caused by recurrent AKI. Male SD rats underwent 5 recurrent (at monthly interval) 45 min bilateral renal pedicle clamp followed by i.v. injection of siP53. As expected, each consecutive AKI cycle resulted in higher SCr levels in untreated rats but not in siP53 treated rats. Ten days after the fifth AKI, untreated AKI rats displayed lower 24 hr GFR ($p<0.01$) and more advanced proteinuria and histological signs of CKD than siP53-treated ($p< 0.01$). We next investigated the efficacy of siP53 in minimizing AKI exacerbation of CKD with pre-existing CKD. CKD was induced in SD rats

by unilateral nephrectomy, followed by 3-4 bimonthly cycles of AKI with rats on high salt diet. When CKD was confirmed (high SCr, proteinuria and low GFR), rats were subjected to AKI (40 min clamp) followed by treatment with either control siRNA or siP53. Dynamics of post-AKI changes of SCr indicated treatment with siP53 markedly attenuated AKI and lead to a rapid return of increased SCr levels to baseline. In contrast, SCr levels in control siRNA treated CKD rats reached 2-fold higher levels of SCr as a result of AKI and SCr remained elevated for at least one week. Histopathological evaluation showed less signs of both AKI and CKD in siP53-treated rats($p< 0.01$). Finally, we observed by two-photon in vivo imaging that Cy-3 siRNA uptake by proximal tubule cells was not impaired in CKD rats, but the cellular siRNA half-life was prolonged with CKD progression.

Thus, siP53 is efficacious with recurrent use in AKI, and prevents the development of CKD and progression of CKD in a proteinuric CKD model.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC020**

**Sestrin2 Is Up-Regulated and Causes Autophagy of Renal Tubular Cells in the Acute Kidney Injury In Vitro and In Vivo** Masayuki Ishihara. *Endocrinology, Metabolism and Nephrology, Kochi Medical School, Japan.*

Autophagy is one of the systems which protects life from stresses. We reported autophagy occurred in mice AKI model before. Recently it was reported sestrin2(sesn2) induce osteosarcoma cells to autophagy. Autophagy is thought to play an important role for preventing AKI from stresses. However, little is known about the role of sesn2 in AKI.

The purpose of this study is to understand the roles of sesn2 in autophagy in renal tubular cells, and the regulation of sesn2 in AKI. To clarify the significance of sesn2 in AKI, we used a rat I/R AKI model in vivo and cultured renal tubular cells as an in vitro model. We observed sesn2 expression in the time course of a rat AKI model by Western blot analysis. We also evaluated sesn2 expression by immunostaining in vivo. To elucidate the regulation of sesn2, we evaluated the expression of sesn2 in MCT cells under H2O2 and hypoxia conditions by RT-PCR and Western blot analyses. Furthermore, to examine sesn2 regulates autophagy or not, we established MCT cells which stably transfected with LC3-GFP as a marker of autophagy. Using this cell line, we detect autophagy as a GFP-positive-autophagosome.

In Western blot analysis, sesn2 expressed significantly in AKI model in 3-12 hours after I/R. The peak time of sesn2 expression was 6 hours. In immunohistological examination, sesn2 expressed in the proximal tubule cells. Sesn2 gene and protein expressed strongly in the stress condition of MCT cells. Overexpression of sesn2 induces autophagy in MCT cells. In this study, we showed sesn2 expression increased in the stresses in vitro and in the AKI model in vivo. Increments of sesn2 expression caused autophagy in renal tubular cells. These results indicate the up-regulated sesn2 induces damaged tubular cells to autophagy, and play a role in the pathophysiology in AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC021**

**Genome-Wide RNAi Screen Identifies a Role for the Proteasome and the MAPK Pathway in Metazoan Phosphate Sensing** Clemens Bergwitz,[1] Sumi Sinha,[1] Charles Derobertis,[1] Hway Helen Chen,[1] Meghana M. Kulkarni,[2] Michael Schnall-Levin,[3] Harald Jueppner,[1] Stephanie Mohr,[2] Bonnie Berger,[3] Norbert Perrimon.[2] *[1]Endocrine Unit and Dept. of Pediatrics, MGH, Boston, MA; [2]Dept. of Genetics, HMS/HMI, Boston, MA; [3]CSAIL, MIT, Cambridge, MA.*

Traditional sequence homology-based database searches to find metazoan orthologs of the bacterial and yeast phosphate sensors have been unsuccessful. We performed a genome-wide RNAi screen using the Drosophila hemocyte-like cell line S2R+ with phosphate-induced activation of MAPK as a readout. Members of the canonical MAPK pathway were among a set of 124 genes identified in the screen and subsequently validated with independent RNAi reagents. Using inhibitors of the MAPK and Akt pathways in S2R+ and murine C2C12 cells, we confirmed that activation of the canonical MAPK pathway in response to phosphate is evolutionarily conserved. However, our results also indicate that the MAPK pathway may not be specific for phosphate; rather, its activation by phosphate may reflect cross-talk between the insulin- and phosphate-sensing pathways. We, therefore, re-screened all 124 genes with insulin as a stimulus and identified 69 genes for which insulin-induced MAPK signaling was unaffected. Six of these phosphate-specific genes encode for regulatory subunits of the 26S proteasome. This finding was validated using the proteasomal inhibitor MG132, which blocks phosphate-induced activation of MAPK in S2R+ cells, as well as in the murine bone marrow ST2 stromal cells and rat osteosarcoma UMR106 cells. MG132, furthermore, reduces phosphate-induced expression of osteopontin in ST2 and UMR106 cells, a gene well-known to be regulated by phosphate. Proteasomal activity has been demonstrated to regulate gene expression via degradation of cytoplasmic inhibitors resulting in the activation of transcription factors, or via the select degradation of various transcription factors. Identification of the phosphate-related target(s) of the proteasome will therefore be important to understanding how phosphate activates MAPK and regulates osteopontin gene expression.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC022**

**Characterization of Phosphate Metabolism in NaPi-IIb Conditional Knock-Out Mice** Yasuhiro Ichida,[1] Hiroko Segawa,[2] Otoya Ueda,[1] Mami Kakefuda,[1] Naoshi Horiba,[1] Etsuyo Hanabusa,[2] Naoko A. Wada,[1] Takanori Tachibe,[1] Yosuke Kawase,[1] Kumiko Koguchi,[1] Ichiro Kaneko,[2] Shoji Kuwahara,[2] Sawako Tatsumi,[2] Hirotake Takai,[1] Shuichi Ohtomo,[1] Kou-Ichi Jishage,[1] Naoshi Fukushima,[1] Ken-Ichi Miyamoto.[2] *[1]Chugai Pharmaceutical Co., Ltd., Gotemba, Shizuoka, Japan; [2]Tokushima University, Tokushima, Japan.*

Elevated plasma phosphate (Pi) is a major risk factor associated with the increased cardiovascular morbidity and mortality of dialysis patients. Plasma Pi is regulated by dietary Pi intake in small intestine and secretion from kidney. Dietary Pi is efficiently absorbed (60-70%) through the small intestine by both active and passive mechanisms. Although active transport of Pi is mediated primarily via the type IIb Na/Pi cotransporter (Slc34a2/NaPi-IIb), the contribution ratio of active transport is not clear. To analyse the role of NaPi-IIb *in vivo*, we have generated *NaPi-IIb* gene knockout mice. At the last ASN meeting we reported that intestinal Pi absorption was decreased in *NaPi-IIb*$^{+/-}$ mice. This time, we generated *NaPi-IIb* conditional KO mice with a tamoxifen-inducible Cre-loxP system. After induction of *NaPi-IIb* gene disruption, *NaPi-IIb*$^{flox/-}$ mice were indistinguishable from wild-type mice littermates with respect to size, body weight, and behavior. However not only urinary Pi excretion but also plasma Pi concentration was significantly decreased in *NaPi-IIb*$^{flox/-}$ mice compared with wild-type mice. *NaPi-IIb*$^{flox/-}$ mice also exhibited significant low plasma FGF23 levels and high plasma $1,25(OH)_2D_3$ levels. Intestinal $Na^+$-dependent Pi uptake was significantly decreased in *NaPi-IIb*$^{flox/-}$ mice compared with wild-type mice. The present study indicates that the active Pi transport (NaPi-IIb) system of the small intestine plays an important role to regulate Pi absorption, and NaPi-IIb should be a potential drug target of hyperphosphateima.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC023**

**Conditional Knock-Out of the Calcium-Sensing Receptor in Mouse Kidney** Hakan R. Toka,[1] Martin R. Pollak.[2] *[1]Nephrology, Brigham and Women's Hospital, Boston, MA; [2]Nephrology, Beth Israel Deaconess Medical Center, Boston, MA.*

High urinary calcium excretion is a major risk factor for the development of kidney stones. Rare gain-of-function mutations in the Calcium-sensing receptor (CaSR) can lead to high urinary calcium excretion. Furthermore, population studies suggest a role for CaSR variants in explaining human variation in renal calcium excretion. The CaSR was reported to be expressed along the entire nephron including proximal tubule, thick ascending limb and distal nephron. However the exact localization and function of CaSR in these various parts of the nephron, and its role in regulating calcium excretion are poorly understood.

A conditional CaSR KO mouse for targeted inactivation of exon 3 was generated. Germline knock-out mice showed increased embryonic lethality. Viable animals displayed failure-to-thrive with hypercalcemia and severe osteomalacia recapitulating the phenotype of neonatal severe hyperparathyroidism (NSHPT) in humans. These results confirm that deletion of exon 3 leads to complete loss of CaSR function.

A nephron-specific knock-out of CaSR was studied by crossing conditional exon3-less CaSR mouse with animals expressing Cre under the Six2 promoter. Homozygous exon3-floxed Six2Cre mice were viable without apparent phenotype. Six2Cre is expressed in the metanephric mesenchyme during kidney development and therefore targets renal tubular epithelial cells in the entire nephron except the collecting duct. RT-PCR and Western blotting showed absent CaSR protein in whole kidney of floxed Six2Cre mice. Baseline serum and urine Calcium levels as well as PTH data showed no difference between experimental and control mice. There was also no difference in serum Calcium levels when kidney-specific CaSR KO mice were challenged with increased dietary Calcium intake. These results suggest that renal expression of CaSR is not required for maintaining calcium homeostasis under basal conditions. Future experiments will address the importance of renal CaSR under conditions of dietary calcium load, PTH infusion, vitamin D loading, and phosphate depletion.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC024**

**Tissue Transglutaminase Inhibits TRPV5-Dependent Calcium Transport in a N-Glycosylation-Dependent Manner** Sandor Boros, Henrik Dimke, Rene J. Bindels, Joost G. Hoenderop. *Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Tissue transglutaminase (tTG) is a multifunctional $Ca^{2+}$-dependent enzyme, catalyzing the covalent crosslinking of proteins. Recent studies have shown that the transient receptor potential vanilloid (TRPV) family of cation channels can contribute to the activity of tTG in keratinocytes and hence skin barrier formation. In the kidney, where active transcellular $Ca^{2+}$ transport via TRPV5 predominates, the potential effect of tTG remains untested. TRPV5 is regulated by a multitude of factors, many (such as klotho or tissue kallikrein) secreted into the urine, acting from the extracellular side. tTG was detected in mouse urine and in the apical medium of polarized cultures of rabbit connecting tubule and cortical collecting duct (CNT/CCD) cells. Importantly, patch clamp experiments revealed that extracellular application of tTG significantly reduced TRPV5 activity in human embryonic kidney (HEK 293) cells transiently expressing the channel. Similarly, a strong inhibition of transepithelial $Ca^{2+}$ transport was observed after apical application of purified tTG to polarized rabbit CNT/CCD cells. tTG promoted the aggregation of the plasma membrane-associated fraction of

TRPV5. Using (whole cell) patch clamp, we observed a clear reduction in the pore size after tTG treatment, indicating distinct structural changes in TRPV5 upon crosslinking by tTG. As N-linked glycosylation of TRPV5 is a key step in the extracellular regulation of channel function, we determined the effect of tTG in the N-glycosylation-deficient TRPV5 mutant. In the absence of N-linked glycosylation, the TRPV5 channel was insensitive to tTG treatment. Taken together, these observations suggest that tTG is a novel extracellular enzyme inhibiting the activity of TRPV5 via catalyzing covalent heterotetramer formation. The inhibition of TRPV5 occurs in an N-glycosylation-dependent manner, signifying a common final pathway by which different extracellular factors regulate the activity of the channel.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC025

**Adaptations in Renal and Intestinal Phosphate Handling in the Rodent Roux-en-Y Gastric Bypass Model** Joanne Marks,[1] Marco Bueter,[2] Havovi Chichger,[1] Carel W. Le Roux,[2] Edward S. Debnam,[1] Robert J. Unwin.[1] *[1]Neuroscience, Physiology and Pharmology, University College London, London, United Kingdom; [2]Investigative Medicine, Imperial College London, London, United Kingdom.*

Recent studies have shown that phosphate (Pi) absorption by the small intestine (SI) rapidly induces changes in renal Pi reabsorption. Acute administration of a high Pi diet to rats previously maintained on a low Pi diet increases duodenal, but not jejunal, brush boarder membrane (BBM) Pi uptake and NaPi-IIb protein levels; this adaptation is associated with transient postprandial hyperphosphatemia and reduced renal BBM Pi uptake and NaPi-IIa protein expression (1). Additionally, infusion of a duodenal mucosal homogenate evokes a rapid increase in renal Pi excretion (2). The current study used the Roux-en-Y gastric bypass (RYGB) model, in which the duodenum is 'bypassed', to investigate further the gut-renal axis controlling Pi homeostasis.

RYGB or sham surgery was performed on male Wister rats according to the protocol of Bueter *et al* (3). After 16 weeks, the kidneys and segments corresponding to the duodenum (biliopancreatic limb), jejunum (alimentary limb) and ileum (common limb) were harvested for determination of Pi transporter mRNA and protein levels. RYGB resulted in a significant increase in NaPi-IIb mRNA expression in the alimentary limb (sham: 0.88±0.54 vs. RYGB: 6.78±2.17 ^^Ct, P<0.05). Interestingly, PiT1 and PiT2 mRNA expression was increased in the common limb (PiT1: sham: 2.1±0.8 vs. RYGB: 4.8±3.5 and PiT2: sham: 4.5±1.5 vs. RYGB: 21.6±9.5 ^^Ct, P<0.05). In keeping with previous findings, up-regulation of NaPi-IIb in the alimentary limb had no effect on renal NaPi-IIa or NaPi-IIc mRNA or protein levels, and it did not affect serum Pi concentration.

These findings suggest that the RYGB model may be a useful tool in defining the functional role and adaptation of different segments of the small intestine in response to dietary Pi changes, and more specifically, in exploring the mechanisms of cross-talk between the SI and kidney in controlling Pi homeostasis.

1) Giral H et al AJP 297: F1466-75, 2009
2) Berndt T et al PNAS 104: 11085-90, 2007
3) Bueter M et al Gastroenterology 138: 1845-53, 2010

Disclosure of Financial Relationships: nothing to disclose

### TH-FC026

**Mechanisms of Apical Membrane Trafficking of Sodium Phosphate Cotransporters** Eleanor D. Lederer,[1,2] Syed J. Khundmiri,[2] Francesca Pribble,[2] Silvia Mercedes Uriarte,[2] Kenneth R. McLeish.[1,2] *[1]Medical Service, Robley Rex VA Medical Center, Louisville, KY; [2]Medicine, University of Louisville, Louisville, KY.*

Regulation of phosphate homeostasis is a function of the brush border membrane (BBM) expression of type IIa sodium phosphate cotransporters (Npt2a) in proximal renal tubule cells; however, the mechanisms for BBM trafficking and insertion of Npt2a are unknown. We recently found that NHERF1 deficient proximal tubule cells exhibit impaired BBM trafficking in association with a decrease in Munc-18 expression. Based on these findings, we hypothesize that Npt2a exocytosis occurs through a vesicle trafficking pathway involving SNARE (Soluble NSF Attachment Protein Receptors) protein interactions. We stimulated Npt2a forward trafficking by treating opossum kidney (OK) cells with low phosphate medium after Npt2a depletion by pretreatment with either PTH (100 nM, 6h) or high phosphate medium (24h). We examined the effect of actin depolymerization agents (Colchicine and Cytochalasin D), a dynein inhibitor (EHNA), and a SNAP23 TAT fusion peptide which inhibits SNARE protein interactions on low phosphate-stimulated Npt2a BBM expression by Western blot, confocal microscopy, and radiolabeled phosphate uptake. Pretreatment with PTH or high phosphate decreased the expression of Npt2a by 40% while subsequent treatment with low phosphate medium for 24 hours restored the BBM expression of Npt2a to control levels. The addition of actin depolymerization agents, EHNA, or the SNAP23 TAT fusion peptide in the presence of low phosphate medium blunted the increase in BBM expression of Npt2a stimulated by low phosphate medium treatment by 60%, 50%, and 60% respectively as determined by Western blot. Confocal imaging and phosphate transport confirmed the inhibition of low phosphate-stimulated Npt2a exocytosis by all three classes of inhibitors. Western blot identified the presence of SNARE proteins syntaxin 2 and 4, VAMP2, and SNAP 25 in OK cells. We conclude that exocytosis of Npt2a stimulated by low phosphate occurs through trafficking of Npt2a-containing vesicles through a pathway involving actin polymerization, dynein motor, and SNARE protein interactions.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC027

**TRPV6 Is Downregulated by OCRL, a Protein Associated with Oculocerebrorenal Syndrome of Lowe and Dent Disease** Guojin Wu, Wei Zhang, Tao Na, Haiyan Jing, Ji-Bin Peng. *Nephrology Research and Training Center, Division of Nephrology, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.*

Oculocerebrorenal syndrome of Lowe (OCRL) gene product is a phosphatidyl inositol 4,5-bisphosphate [PI(4,5)$P_2$] 5-phosphatase, and mutations of OCRL cause Lowe syndrome and Dent disease, both of which are frequently associated with hypercalciuria. Transient Receptor Potential, Vanilloid subfamily, subtype 6 (TRPV6), is an intestinal epithelial calcium (Ca) channel mediating active Ca absorption. Hyperabsorption of Ca was found in patients of Dent disease with increased Ca excretion. In this study, we tested whether TRPV6 is regulated by OCRL and if so, to what extent it is altered by Dent-causing OCRL mutations using *Xenopus* oocyte expression system. Co-expression of OCRL decreased TRPV6-mediated Ca uptake in a dose-dependent manner. The decrease in Ca uptake was associated with lowered TRPV6 protein abundance. The PI(4,5)$P_2$ 5-phosphatase domain of OCRL alone was sufficient to downregulate TRPV6, whereas other domains alone had little effect. Deletion of RhoGAP domain in OCRL further increased the inhibitory effect of OCRL on TRPV6, and mutations that disrupt interactions between OCRL and Rab proteins, including S564P and G664D, attenuated it. Thus, the PI(4,5)$P_2$ 5-phosphatase domain is essential to the inhibitory effect of OCRL on TRPV6, and the RhoGAP and Rab binding domains are also involved in this regulation. To assess whether the regulation of TRPV6 by OCRL is impaired under Dent disease condition, the effects of 7 OCRL Dent-causing mutants on TRPV6 were evaluated. All the Dent-causing mutants of OCRL tested, including F243S, I274T, R318C, Y479C, R493W and D523N in the phosphatase domain and E737D in the RhoGAP domain, exhibited decreased ability (ranging from 0 to 80% compared to wild-type) to inhibit TRPV6. In conclusion, OCRL downregulates TRPV6 and the disruption of this regulation by Dent-causing mutations in OCRL may lead to increased intestinal Ca absorption and in turn, hypercalciuria.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC028

**Regulation of the Epithelial Calcium Channel TRPV5 by Sex Hormones** Yu-Juei Hsu,[1,2] Shih-Che Hsu,[2] Joost G. Hoenderop,[2] Rene J. Bindels.[2] *[1]Division of Nephrology, Tri-Service General Hospital, Taipei, Taiwan; [2]Physiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.*

Sex hormones play a key role in sex differentiation and development, as well as in the regulation of skeletal growth and maturation. Long-term female sex hormone estrogen and male sex hormone androgen deficiency resulted in a negative $Ca^{2+}$ balance and osteoporosis among elderly women and men, respectively. Studies have reported gender differences in urinary $Ca^{2+}$ excretion, showing a greater urinary $Ca^{2+}$ loss in males than in females. However, the molecular mechanisms underlying different renal $Ca^{2+}$ handling by sex hormones remain unexplored. To determine whether sex hormones affect active $Ca^{2+}$ reabsorption by regulating the $Ca^{2+}$ transport proteins Transient Receptor Potential Vanilloid-subtype 5 (TRPV5) and calbindin-D28K, the castrated mice model was used to investigate the regulation of TRPV5 and calbindin-D28K by sex hormones. Male mice, compared to females, had a higher urinary $Ca^{2+}$ excretion accompanied by reduced renal $Ca^{2+}$ transporter expression. Androgen deficient bilaterally orchidectomized (ORX) mice excreted less $Ca^{2+}$ in their urine than sham-operated controls. ORX-induced hypocalciuria was normalized after testosterone replacement. Consistently, androgen deficiency resulted in augmentation of both renal mRNA and protein abundance of TRPV5 and calbindin-D28K, which in turn was suppressed by testosterone treatment. Moreover, there is no significant difference in serum 1,25$(OH)_2D_3$ levels between controls, ORX mice, and testosterone-supplemented ORX mice. In contrast, supplementation with 17β-estradiol to ovariectomized (OVX) rats normalized renal mRNA levels of TRPV5 and calbindin-D28K and increased the protein abundance of TRPV5. This was confirmed in 25-hydroxyvitamin $D_3$ 1-1α-hydroxylase-knockout mice where 17β-estradiol replacement therapy enhanced renal expression of TRPV5, leading to the normalization of serum $Ca^{2+}$ levels. In conclusion, we demonstrated that sex differences in renal $Ca^{2+}$ reabsorption, which are mediated by the inhibitory effects of androgen and the stimulatory effects of estrogen on TRPV5-mediated renal $Ca^{2+}$ transport in a 1,25$(OH)_2D_3$-independent manner.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC029

**Regulation of Intestinal and Renal NaPi Transporters by Liver X Receptor** Yupanqui A. Caldas,[1,2] Michael A. Cortázar,[1,3] Hector Giral-Arnal,[1] Victor Sorribas,[2] Judith Blaine,[1] Kayo Okamura,[1] Moshe Levi.[1] *[1]University of Colorado Denver, Aurora, CO; [2]Universidad de Zaragoza, Zaragoza, Spain; [3]Universidad del Valle, Cali, Colombia.*

The Liver X Receptor (LXR) agonists have shown to modulate the lipid composition of different tissues including the vascular tissue, the intestine, the liver and the kidney through modulation of the ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1), which mediate reverse cholesterol transport. The effects of LXR agonists on phosphate transporters however are not known. Phosphate homeostasis is regulated by the intestinal and renal sodium-phosphate (NaPi) transporters that are expressed in the brush border membrane (BBM) of these organs. Recent studies indicate that hyperphosphatemia presumably mediated through upregulation of the NaPi transporters play an important role in cardiovascular disease. We used both an in vitro and in vivo system to study the effect of the LXR agonists on intestinal and renal NaPi transporters. C57BL/6 mice fed

chronically with either of the LXR agonists TO901317 and DMHCA show marked and significant reductions in the abundance of renal BBM NaPi transport proteins: 57% for NaPi-2a, 40% for NaPi-2c, and 30% for PiT-2, as well as intestinal BBM NaPi transporter: 66% for NaPi-2b. The decrease of the expression of these NaPi cotransporter proteins was correlated with significant decreases in the activity of 32Pi uptake, 63% in the ileum and 20% in the kidney BBM. Even more significant, serum Pi levels were decreased by 40% while Pi urine excretion was increased by 30%. After incubation for 24 hours with TO901317 and DMHCA the same effect was also observed in opossum kidney cells (OK: a cell culture model of the renal proximal tubule) where NaPi4 protein abundance (the main type II NaPi transporter in OK cells) was reduced by 55% and Pi transport by 49%. In this cell line we have demonstrated increased nuclear expression of the endogenous LXR receptor, and several of the target genes, following treatment with the LXR agonists. Our studies therefore document a novel role for the LXR agonists in modulation of intestinal and renal NaPi transporters and serum Pi levels.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC030

**Phosphate Homeostasis in Osteocyte-Ablated Mice** Sawako Tatsumi, Seiichi Yamaguchi, Tatsuya Kamatani, Yuji Shiozaki, Kengo Nomura, Yukiko Saito, Shinsuke Kido, Hiroko Segawa, Ken-Ichi Miyamoto. *Molecular Nutrition, The University of Tokushima Graduate School, Tokushima, Japan.*

Recent studies have shown that alterations in osteocytes metabolism occur in very early stages of chronic renal disease (CKD) and likely mediate altered bone and mineral metabolism in patients with even very mild degree of renal dysfunction. The fibroblast growth factor 23 (FGF23) and dentin matrix protein 1 (DMP1) genetic mutations cause phosphorous (Pi) metabolic disorders. FGF23 and DMP1 are made primarily in osteocytes. These are suggesting that the osteocyte plays the total systemic Pi regulation. In a previous study, we have established a transgenic mouse model, based on the diphtheria toxin (DT) receptor-mediated cell knockout (TRECK) system, in which inducible and specific ablation of osteocytes is achieved in vivo (Tatsumi S et al. Cell Metab 2007). Within 48 hours of DT administration, more than 70% the osteocytes were killed. "Osteocyte-ablated" mice exhibited excessive bone resorption, impaired mineralization and adipose tissue proliferation in marrow space, all of which are hallmarks of the ageing skeleton. To analysis the role of osteocyte in Pi homeostasis, we investigated renal Pi handling in the osteocyte-ablate mice. Plasma Pi and calcium concentration were not changed in the ablated mice. Plasma FGF23 levels were significantly decreased and plasma PTH levels were not changed in the ablated mice. Urinary Pi excretion was markedly increased and renal sodium dependent Pi cotransporter NaPi-IIa and NaPi-IIc protein levels were significantly decreased in the ablated mice. Thus, the osteocyte-ablated mice show increased renal Pi excretion. We will discuss the mechanisms of hyperphosphaturia in the osteocyte-ablated mice.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC031

**Hemodialysis in Pregnant Women with ESRD (PW-ESRD) a Tertiary Care Hospital Experience** Miguel Angel González Alfaro,[1] Enrique Rojas-Campos,[2] Benjamin Gomez-Navarro.[1] *[1]Nephrology, Hospital de Especialidades, CMNO, IMSS, Guadalajara, Jalisco, Mexico; [2]Medical Research Unit in Renal Diseases, Hospital de Especialidades, CMNO, IMSS, Guadalajara, Jalisco, Mexico.*

INTRODUCTION. There is low information regarding maternal and perinatal outcomes in Pregnant women with ESRD. We report the experience of PW-ESRD in a tertiary care hospital in the west of Mexico. METHODS. 45 pregnant women who were on HD from Jan-2006 to Dec-2009. They receive a 20-Hrs standard weekly HD (5 sessions), all conceptions were before ESRD diagnosis. RESULTS. Age 26±7 yrs; gestational age at start HD 13±6wks. All neonatal deaths require Neonatal ICU (N-ICU). Main maternal-perinatal results are shown in table.

Maternal and Neonatal Outcomes

| Variable | N=45 |
|---|---|
| Hypertension during pregnancy N (%) | 32 (71) |
| Uncontrolled hypertension | 80 % |
| Pre-eclamsia/Eclamsia N (%) | 10 (23)/4 (9) |
| Antihypertensive treatment N (%) | 35 (78) |
| Antihypertensive drugs N (min-max) | 3±1.5 (1-7) |
| Neurological complications N (%) | 5 (11) |
| Polyhidramnios N (%) | 13 (29) |
| Newborn variables | |
| Gestational age (Wks) | 28±8 |
| Weight at birth (Grams) | 1,750±634 |
| Live/neonatal death (ND)/abortion N (%) | 33(73) / 4(9) / 8(18) |
| Apgar 1/5 minutes | |
| Live | 8±1 / 9±1 |
| ND | 5±1 / 8±1 |
| Weight at birth Live/ND (grams) | 1930 / 1128* |
| Neonatal-ICU N (%) | 32 (71) |
| N-ICU Live / ND | 85%/100% |
| Diagnosis to N-ICU Live/ ND N (%) | |
| Respiratory distress | 12 (43)/4 (100)* |
| Ictericia | 16 (57) / 0 |

*p<0.05

CONCLUSION: This is one of the largest reports regarding outcome in pregnant with ESRD. The majority of patients develop and had uncontrolled hypertension, they receive 3 antihypertensive drugs. Living newborns had higher weight, higher apgar and require less frequently N-ICU.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC032

**Greater Dialysate Endotoxin Level Asociated with Increased Mortality of Hemodialysis Patients in Japan** Takeshi Hasegawa,[1, 2] Shigeru Nakai,[2] Ikuto Masakane,[2] Kunitoshi Iseki,[2] Yoshiharu Tsubakihara.[2] *[1]Division of Nephrology, Showa University Fujigaoka Hospital, Yokohama, Japan; [2]Comittee of Renal Data Registry, The Japanese Society for Dialysis Therapy, Tokyo, Japan.*

**INTRODUCTION AND AIMS:** The Japanese Society for Dialysis Therapy (JSDT) has advocated the strictest standards for dialysate. The aim of this study was to evaluate the impact of water quality by dialysate endotoxin (ET) level on mortality of hemodialysis (HD) patients in Japan.

**METHODS:** We analyzed 135,814 patients on HD (at the end of 2006: on HD > one year, three times HD per week, during 2007: no changing facilities and modalities) from a nationwide annual survey of the Japan Renal Data Registry (JRDR) by the JSDT. Main exposure to be tested was ET level (EU/ml) reported by 2,942 facilities at the end of 2006 (categorized into 5groups: <0.001, 0.001 to 0.01, 0.01 to 0.05, 0.05 to 0.1, and 0.1<). Logistic regression analysis was employed to estimate the relative risk (RR) of all cause death during 2007, adjusted for age, gender, time on end-stage renal disease (ESRD), diabetes mellitus, Kt/V, normal protein catabolic rate (nPCR) , treatment time, serum albumin, and hemoglobin. Other outcome of interest was odds ratio (OR) of ET level higher than the limit level (0.05EU/ml) of the JSDT standards by attach rates of ET filter.

**RESULTS:** 92.2% of HD patients followed the limit of ET level in Japan (<0.05EU/ml). There were no significant differences in patient characteristics among groups by ET level. Patients who were exposed to greater ET level (0.1EU/ml<) displayed a 26% increased risk of all-cause death compared to those who with lower ET level (<0.01 EU/ml). Wide variation was seen across facilities in attach rate of ET filter (0 to 97.9%). Higher attach rates of ET filter associated with lower odds of exceeding the limit of ET level in Japan (<0.05EU/ml) .

**CONCLUSIONS:** These results suggested that greater ET level was related to increased risk of all-cause death in Japanese HD patients and that higher attach rates of ET filter decreased the likelihood of ET level greater than the limit advocated by the JSDT. Correcting this modifiable water management practice may improve outcomes of HD patients in Japan.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC033

**Accelerated Coronary Artery Calcification and Hyperparathyroidism in Hemodialysis Patients Receiving Low Calcium Dialysate** Soo Jin Kim, Youngki Lee, Ji Eun Oh, Young Rim Song, Sung Gyun Kim, Ja-Ryong Koo, Jung-Woo Noh. *Department of Internal Medicine, Hallym Kidney Research Institute, College of Medicine, Hallym University, Seoul, Korea.*

Background: Coronary artery calcification (CAC) has been shown to be a significant predictor of cardiovascular mortality and morbidity in hemodialysis (HD) patients. Some concern regarding the calcium loading is raised as an inducer of CAC. We hypothesized that lowering of dialysate calcium levels would result in decreased the progression rate of CAC with compared to that of standard calcium dialysate.

Methods: Seventy-six HD patients were randomized to receive low calcium dialysate (LCD; 1.25 mmol/L, n=36) or continue on standard calcium dialysate (SCD; 1.5 mmol/L, n=40) for 12 months. The 64-slice multidetector computed tomography was performed at entry into the study and again at 12 months to calculate coronary artery calcium scores (CACS). Biochemical data were evaluated every 3 months.

Results: Baseline demographic or clinical characteristics were not different between two groups. Serum calcium, phosphorus and calcium x phosphorus product at baseline, 3, 6, 9, 12 month were similar in both group. However, intact-PTH levels of LCD group showed an increase at 3 month and maintained higher thereafter. At 12 month, the CACS significantly increased in LCD group and not at all in SCD group (LCD: median 64.1 at baseline *vs* 328.9 at 12 months, *P*=0.001, SCD: median 40.1 *vs* 199.7, *P*=0.9, between group *P*=0.03). There was no difference in doses of a calcium-based phosphate binder and the frequency of intradialytic hypotension between two treatments.

Conclusions: Use of LCD appears to be associated with more progression of CAC than use of SCD. While the exact mechanisms accounting for this result cannot be ascertained, increased serum PTH levels in LCD group might have influenced on the progression of CAC. However, a larger study should be undertaken to confirm these results.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC034**

**Tolerance to Hemodialysis in Insulin-Requiring Diabetic Patients: A Prospective Randomized, Cross-Over Multicenter Study between Bicarbonate Dialysis (BD) and Blood Volume Controlled Acetate-Free Biofiltration (BVC-AFB) (THIRD Study)** Antonio Santoro,[1] Ezio Movilli,[2] Elena Mancini,[1] Giovanni Cancarini.[2] *[1]O.U. Nephrology, Dialysis, Hypertension, Policlinico S.Orsola-Malpighi, Bologna, Italy; [2]O.U. of Nephrology, A.O. Spedali Civili & University, Brescia, Italy.*

To investigate a possible better tolerance to dialysis for the fragile diabetic (D) patient, eliminating acetate in the dialysate bath (AFB) and using an automatic blood volume control (BVC), we evaluated cardiovascular stability and frequency of intradialysis symptoms in a prospective,randomized,cross-over study,comparing conventional BD to BVC-AFB.

55 insulin-requiring D patients took part in the study (72% male, 28% female;68±8 years, dialysis vintage 36±15 months): 68% of them completed it (30% dropout). Each patient was treated for 3 months with BD, then for 3 months with BVC-AFB. Primary end point was the frequency of dialysis complicated by hypotensive events (systolic blood pressure value(SBP)<90mmHg, or drop of SBP>25mmHg from the predialysis value and requiring therapy). Frequency of sessions requiring nurse call/intervenction were accounted. Sessions with hypotensive events and nurse calls were analyzed by crosstabs and Chi$^2$, while the ΔSBP by ANOVA. Dialysis treatments were comparable as time lenght, weight loss, electrolyte content of the bath.BVC-AFB treatments have an 2,3±0,3 (L/h) infusion flow rate. Not all the hypotensive events required therapies or nurses inteventions. A significant reduction of ΔBP was observed.

Table1

| | BD | BVC-AFB | p |
|---|---|---|---|
| Symptomatic dialysis | 11,0% | 5,1% | p<0,01 |
| Dialysis with acute hypotension | 19,5% | 14,1% | p<0,01 |
| ΔSBP lying (mmHg) | -7,5 ± 0,6 | -0,8 ± 0,6 | p<0,01 |
| ΔHR lying (bpm) | +2,3 ± 0,3 | +0,6 ± 0,3 | p<0,01 |
| Dialysis sessions with nursing call/intervention | 17,5% | 5,4% | p<0,01 |

Main results as frequency, or M ±SEM

The BVC-AFB association seems to improve the cardiovascular response to dialysis treatment in D patients.We observed a reduction in hypotensions frequency, in ΔBP and an increase in call-free sessions. Besides, the BVC-AFB combination seems to increase the nurse time saving.

Disclosure of Financial Relationships: nothing to disclose

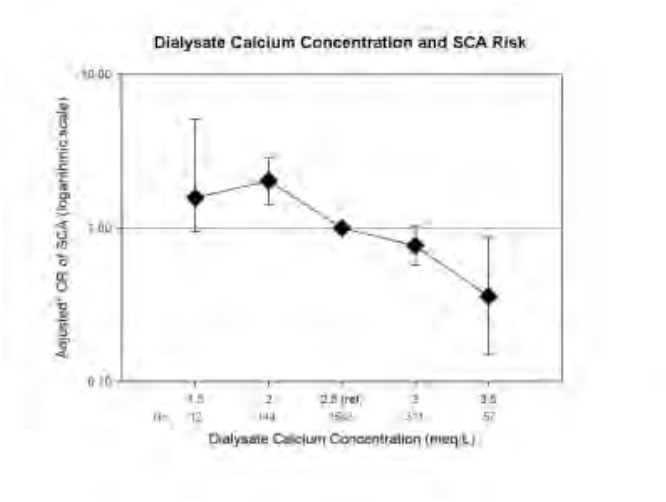
**TH-FC035**

**Sudden Cardiac Arrest Risk Associated with Low Calcium Dialysate in Hemodialysis Patients** Patrick H. Pun, Ruediger W. Lehrich, John Paul Middleton. *Nephrology, Duke University, Durham, NC.*

The optimal dialysate calcium (Ca) concentration to maintain normal mineralization and reduce risk of cardiovascular events is in debate. KDOQI guidelines suggest that the dialysate Ca concentration should be lowered to 2.5 mEq/L among patients receiving oral Ca to avoid potential risks of vascular and soft tissue calcification. However, arrhythmias may be more likely to occur with lower dialysate Ca secondary to worsening of QT prolongation. We sought to examine the influence of low dialysate Ca on the risk of witnessed sudden cardiac arrest (SCA) within dialysis clinics.

We previously designed a case control study from among 43,200 US Davita hemodialysis patients between 2002-2005. 502 patients who experienced a witnessed SCA were compared with 1632 randomly selected age and dialysis-vintage-matched controls. We examined baseline clinical and dialysis characteristics including last prescribed dialysate Ca concentration and concurrent prescription of medications associated with QT prolongation. Adjusted risk of SCA was modeled using logistic regression techniques.

82% (N=1699) of patients received dialysate Ca <=2.5 meq/L at the time of event (cases) or index date (controls). After adjusting for differences in demographics, comorbidity, serum Ca levels and other baseline differences, use of dialysate Ca<=2.5 meq/L was independently associated with risk of SCA (adjusted OR 1.5, CI 1.1-2.1). Concurrent exposure to QT prolonging medications (OR 2.23, 95% CI 1.42-3.49) and low potassium dialysate (OR 2.9, 95% CI 1.9-4.3) conferred additional risk.




Lowering of dialysate Ca concentration is associated with an increased risk of SCA. Our study suggests that inherent or acquired cardiac conduction disturbances and coincident medication exposures should be considered in addition to Ca absorption and bone turnover in determining the optimal dialysate Ca prescription.

Disclosure of Financial Relationships: Research Funding: Satellite Health Care, Davita Clinical Research; Scientific Advisor: Genzyme.

**TH-FC036**

**Dialysate Na Prescription in Patients Undergoing Conventional HD: A Stealthy Foe?** Jair Munoz Mendoza,[1,2] Sumi Sun,[2] Sheila Doss,[2] Glenn M. Chertow,[1] John E. Moran,[1,2] Brigitte Schiller.[1,2] *[1]Department of Nephrology, Stanford University, Palo Alto, CA; [2]Department of Research, Satellite Healthcare, Mountain View, CA.*

Fluid control is critical in patients with end stage renal disease (ESRD). While higher dietary sodium (Na) intake is associated with higher interdialytic weight gain (IDWG), the role of dialysate Na concentration on IDWG is less well understood. We conducted a cross-sectional study of 1084 stable, anuric hemodialysis (HD) patients to assess the relations among pre-dialysis plasma Na, dialysate Na prescription, IDWG, blood pressure (BP), and thirst. The Na gradient was calculated by subtracting plasma Na drawn prior to the first HD of the week from the dialysate Na prescription. IDWG and pre-HD BP were averaged over the previous six treatments. Self-reported degree of thirst was studied in a subset of 432 patients.

The dialysate Na prescription varied with a median dialysate Na of 140 mEq/L (range 133 to 149). 52% of patients were dialyzed at Na=140, 36% with Na >140, only 12% at Na <140 mEq/L. The mean Na gradient was 4.6, (range -7 to 24). Only one-third of patients were dialyzed with a gradient ≤ 2 mEq/L.

IDWG was higher in the dialysate Na >140 mEq/L group compared to Na=140 mEq/L (2.99 vs. 2.64 Kg) ($p<0.05$). However, there was no difference in pre-HD MAP between these two groups ($p>0.05$). Patients with a Na gradient >6 mEq/L gained on average 700 mL more per session than patients with a Na gradient ≤ 2 mEq/L ($p<0.0001$).

IDWG was estimated as 70 mL higher per each mEq/L increase in Na gradient ($p<0.0001$). Self-reported thirst was directly, albeit weakly correlated with Na gradient ($r=0.11$, $p=0.02$). Intradialytic hypotension was not significantly related to the dialysate Na concentrations ($p=0.54$) or Na gradient ($p=0.91$).

There are statistically significant and clinically meaningful associations among the dialysate Na prescription, Na gradient, and IDWG in stable HD patients. Insufficient attention to Na dialysate prescription may be an unrecognized contributor to increased IDWG in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC037**

**Heparin-Grafted Dialysis Membrane Permits Minimal Systemic Anticoagulation and a Lower Anti Xa Level at Treatment End** Michele Kessler,[1] Thanh Cao Huu,[1] Alberto Gutierrez,[2] Marie-Jeanne Coudert-Krier,[3] Jan T. Kielstein,[4] Concetta Gangemi,[5] Roula Galland,[6] Françoise Moussion-Schott.[7] *[1]CHU, Nancy, France; [2]Karolinska Hospital, Stockholm, Sweden; [3]ALTIR, Nancy, France; [4]Medical School, Hanover, Germany; [5]OCM Borgo Trento, Verona, Italy; [6]Clinique St Exupery, Toulouse, France.*

**INTRODUCTION AND AIMS:** A new Hepran (H) dialysis membrane that has unfractionated heparin (UFH) grafted to its surface was evaluated for the possibility to reduce the systemic anticoagulation during hemodialysis (HD) and the resulting change in anti Xa activity at treatment end.

**METHODS:** In a prospective multicenter clinical study in stable HD patients (n=41), the H membrane was first used with the previous heparin (hep) dose followed by a stepwise weekly reduction of hep dose. Heparin reduction was stopped when two or more treatments in a week showed very early signs of clotting (circuit pressures, signs of clot formation, quality of rinse-back using a visual scale).

**RESULTS:** Regardless of the type of hep used, with the H membrane, the hep dose was reduced from 68±17 to 38±14 IU/kg for 29 patients lowering antiXa at treatment end from 0.45±0.26 to 0.22±0.11 IU/ml (p<0.001) (51% reduction).The reduction of the usual hep dose (64±14 to 35±12 IU/kg for LMWH and 81±18 to 45±13 IU/kg for UFH) resulted in an antiXa level at treatment end of 0.25 ± 0.11 IU/ml for LMWH and 0.14 ± 0.07 IU/ml for UFH-treated patients. Failure to further decrease hep dose was related to signs of clotting in blood lines (56%), in dialyzer (10%), or both (33% of treatments). For 12 patients (1 LMWH ,11 UFH patients) for whom hep dose reduction was not possible (usual hep dose 76±21 IU/kg), usual AntiXa level at treatment end was 0.23 ± 0.08 IU/ml. **CONCLUSIONS:** When using the H membrane significant reduction of pre-study hep dose and post-HD anti Xa level was possible in regular HD patients. Different responses in LMWH and UFH groups may relate to different ways of hep administration and patient profiles. Our data indicate that a heparin-grafted membrane can be beneficial for HD patients who require low anti Xa activity at the end of treatment to reduce bleeding risk and possibly other heparin side effects

Disclosure of Financial Relationships: nothing to disclose

## TH-FC038

**Increasing Dialysate Flow Rate in Dialyzers with Enhanced Dialysate Flow Distribution Does Not Increase the Delivered Dose of Dialysis in Terms of $Kt/V_{urea}$** Richard A. Ward,[1] John W. Idoux,[2] Rosemary Ouseph,[1] Thomas A. Depner,[3] Thomas A. Golper.[2] *[1]Medicine, University of Louisville, Louisville, KY; [2]Medicine, Vanderbilt University, Nashville, TN; [3]Medicine, University of California Davis, Sacramento, CA.*

Previous in vitro and clinical studies showed that the urea mass transfer - area coefficient ($K_oA$) increased with increasing dialysate flow rate. This observation led to increased dialysate flow rates in an attempt to maximize the delivered dose of dialysis ($Kt/V_{urea}$) during a given treatment. Recently, we showed that urea $K_oA$ was independent of dialysate flow rate in the range 500 - 800 mL/min for dialyzers incorporating features to enhance dialysate flow distribution. This finding suggested the hypothesis that increasing the dialysate flow rate with such dialyzers will not significantly increase delivered $Kt/V_{urea}$. To test this hypothesis, we performed a multi-center randomized clinical trial of dialysate flow rates, 600 mL/min (A) and 800 mL/min (B), in 28 patients using an ABAB or BABA sequence. All other aspects of the dialysis prescription, including treatment time (223 ± 33 min), blood flow rate (435 ± 23 mL/min), and dialyzer (Revaclear or Revaclear MAX), were kept constant for a given patient. Delivered $Kt/V_{urea}$ was determined as single-pool (Kt/Vsp) and equilibrated (eKt/V) $Kt/V_{urea}$ from pre- and post-dialysis plasma urea concentrations according to Daugirdas and as ionic Kt/V from serial measurements of ionic dialysance made throughout each treatment. The three measures of $Kt/V_{urea}$ are shown in the table for the two dialysate flow rates.

| Dialysate Flow Rate | Kt/Vsp | eKt/V | ionic Kt/V |
|---|---|---|---|
| 600 mL/min | 1.67 ± 0.04 | 1.39 ± 0.03 | 1.44 ± 0.04 |
| 800 mL/min | 1.65 ± 0.04 | 1.38 ± 0.03 | 1.48 ± 0.04 |

Data presented as mean ± SE for n = 28

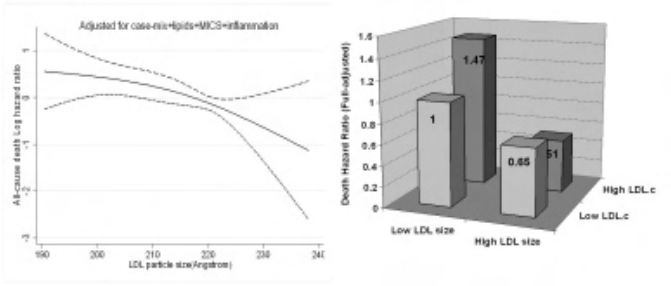
By analysis of variance, there was no significant difference between the two dialysate flow rates in any of the three measures of $Kt/V_{urea}$ ($p > 0.284$). These data suggest that increasing the dialysate flow rate beyond 600 mL/min for these dialyzers offers no benefit in terms of delivered $Kt/V_{urea}$.

Disclosure of Financial Relationships: Research Funding: Gambro Medical Products, Rockwell Medical Technologies; Honoraria: Fresenius Medical Services, DCI, Renal Advantage, Renal Research Institute, Gambro.

## TH-FC039

**Novel LDL Particle Diameter Measurement by Ion Mobility Predicts Mortality in Maintenance Hemodialysis Patients** Nazanin Noori,[1] Csaba P. Kovesdy,[2] Michael Caulfield,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]Nephrology, Salem VA, Salem, VA; [3]Quest Diagnostics, Irvine, CA.*

**Background:** It is not clear whether different aspects of LDL-cholesterol other than its conventionally measured concentration are associated with mortality in maintenance hemodialysis (MHD) patients. We hypothesized that the LDL particle diameter, measured by novel ion mobility method, may be a better outcome predictor. **Methods:** We examined the survival predictability LDL particle diameters using Cox proportional hazard regression in 235 MHD patients who were followed for up to 6 years (2001-07) and performed incremental levels of multivariate adjustment for case-mix (age, gender, race, diabetes, dialysis vintage , Charlson comorbidity score and Kt/V), malnutrition-inflammation complex syndrome (albumin, creatinine, phosphorus, calcium, ferritin, hemoglobin, nPNA [nPCR]; and body mass index).and additional inflammatory markers (CRP, IL-6, and TNFα).**Results:** The highest quartile of LDL particle diameter (compared to the lowest quartile) was associated with a 54% greater survival (see spline figure): Death hazard ratio (95% confidence interval) was 0.46 (0.21-0.98). Lower mortality persisted with higher LDL diameter (above median) when combined with either high or low total LDL concentration (see bar diagram).




**Conclusions:** Larger LDL particles appear associated with greater survival in MHD patients independent of LDL concentration or other confounders. Use of LDL subfraction morphology in risk-stratification of dialysis pts warrants additional studies.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC040

**Sleep-Disordered Breathing Is a Novel Risk Factor for Cardiovascular Events and Mortality in Hemodialysis Patients** Takahiro Masuda,[1,2] Mitsunobu Murata,[3] Sumiko Honma,[1] Yoshitaka Iwazu,[2] Nobuhiro Sasaki,[1,2] Manabu Ogura,[2] Akira Onishi,[2] Yasuhiro Ando,[2] Shigeaki Muto,[2] Kazuomi Kario,[3] Eiji Kusano,[2] Yasushi Asano.[1] *[1]Department of Nephrology, Koga Red Cross Hospital, Koga, Ibaraki, Japan; [2]Division of Nephrology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan; [3]Division of Cardiovascular Medicine, Deparment of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan.*

Sleep-disordered breathing (SDB), characterized by repetitive apnea and hypopnea during sleep, is a risk factor for cardiovascular events in the general population. However, the links between SDB and cardiovascular events in hemodialysis (HD) patients remains unclear. We followed the clinical outcome of 94 HD patients, who underwent overnight pulse oximetry on dialysis day. The SDB group was defined as 3% oxygen desaturation index (3%ODI) over 5 events per hour, and the others were the normal group. Forty-four patients (46.8%) were classified into the SDB group. Body mass index (BMI), diabetes mellitus, 3%ODI and Epworth Sleepness Scale, a questionnaire about daytime sleepiness were significantly higher; and duration of dialysis, Kt/V, normalized protein catabolism rate (nPCR) and hemoglobin were lower in the SDB group than in the normal group. During a median 55 months follow-up, Kaplan-Meier analysis revealed that the SDB group had significantly higher cardiovascular events and all-cause mortality than the normal group. Age, cardiothoracic ratio, serum albumin and 3%ODI were predictors of cardiovascular events and all-cause mortality at univariate Cox regression analysis. After adjustment for age, gender, BMI, duration of dialysis, diabetes mellitus, Kt/V, nPCR, cardiothorac ratio, systolic blood pressure, hemoglobin, serum albumin and total cholesterol, SDB is an independent predictor of increased cardiovascular events (hazard ratio 2.93; 95% confidence interval [CI], 1.13-7.61; *P*=0.027) and all-cause mortality (hazard ratio 3.73; 95% CI, 1.03-13.54; *P*=0.045).These results indicate that SDB is a novel risk factor for cardiovascular events and mortality in HD patients. Effective and earlier treatment for these patients is needed to improve clinical outcome.

Disclosure of Financial Relationships: nothing to disclose

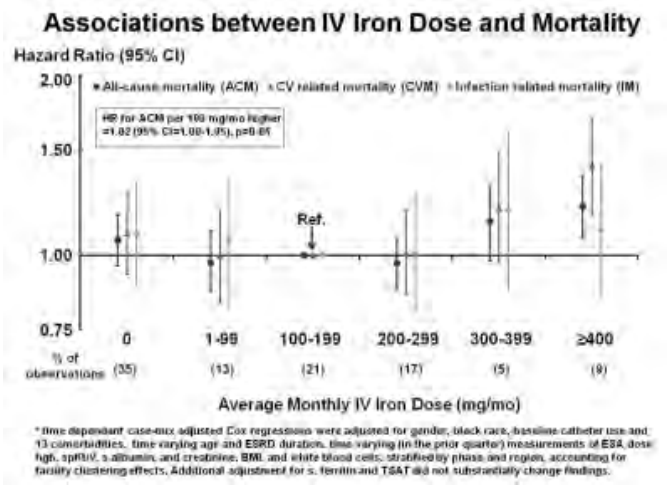
## TH-FC041

**Association of Intravenous Iron (ivFe) Dosing with Mortality: Findings from the DOPPS** George R. Bailie,[1] Lin Tong,[2] Yun Li,[3] Nancy A. Mason,[3] Ronald L. Pisoni,[2] David A. Goodkin,[2] Francesco Locatelli,[4] Mark R. Marshall,[5] Masaaki Inaba,[6] Bruce M. Robinson.[2,3] *[1]Albany College of Pharm. & Health Sci.; [2]Arbor Research; [3]Univ. of Michigan; [4]Ospedale A. Manzoni; [5]Middlemore Hosp.; [6]Osaka City Univ. Grad. Sch. of Med.*

Previous studies found equivocal associations between ivFe and clinical outcomes but did not compare countries. We examined associations between ivFe dosing and mortality using international Dialysis Outcomes and Practice Patterns Study (DOPPS) data.

IvFe data were collected from 2002 to 2008 for 20,445 HD pts in 584 facilities in US, Jap, Australia, Can, NZ and 7 European countries. Using time dependent, case-mix adjusted Cox regression, we examined associations of longitudinal ivFe use with all-cause (ACM), cardiovascular (CVM), and infection-related (IM) mortality for 4-month ivFe dose categories (expressed as average dose/mo). Instrumental variable analysis (using facility as the instrument) was also performed due to potential for confounding of pt-level ivFe dosing by indication.

In the 1st 4-mo after study enrollment, % receiving ivFe ≥400 mg/mo varied from 0.7% (Jap) to 21% (Swed). Compared to pts receiving ivFe 100-199 mg/mo, pts with average dose ivFe ≥400 mg/mo had 21% higher risk of ACM, 41% higher risk of CVM, and no elevation of IM risk (Figure). Higher total ivFe dose over 1 year was associated with increased risk of ACM (HR per 100 mg/mo higher =1.05 [95% CI=1.01-1.09], p=0.02). Instrumental variable analysis yielded consistent findings (elevated mortality risk at average monthly ivFe ≥400 mg, p=0.02 for ACM and 0.007 for CVM).




IvFe dosing varies across HD facilities and countries. Higher ivFe doses (above 300 or 400 mg/mo) are associated with higher ACM and CVM. Differences by region, ivFe agent, and use of regular maintenance doses compared with intermittent repletion, require evaluation.

Disclosure of Financial Relationships: Consultancy: Fresenius Medical Care, American Regent, Vifor Pharma, Genzyme; Honoraria: Fresenius Medical Care, American Regent, Vifor Pharma.

### TH-FC042

**The Beneficial Effect of Dual Renin-Angiotensin System (RAS) Blockage Against Peritoneal Membrane Dysfunction in CAPD Patients: A Randomized Controlled Study** Talerngsak Kanjanabuch,[1,2] Wassawon Wontanatawatot,[1] Piyatida Chuengsaman,[3] Pisut Katavetin,[1] Somchai Eiam-Ong.[1] *[1]Chulalongkorn University, Thailand; [2]Kidney&Metabolic Disorders Research Center, Thailand; [3]Banphaeo Hospital, Thailand.*

To investigated the protective effect of RAS blockades over peritoneal membrane dysfunction in CAPD patients.

**Patients&Methods:** A stratified randomized controlled single-blinded study was conducted in 93 hypertensive (HT) & naïve CAPD patients. There were 3 groups of patients: 1) ACEI (enalapril 40 mg/day,N=31), ACEI+ARB (40mg-daily enalapril+50mg-daily losartan,N=31), and Placebo (N=31). The dosage of anti-HT agents in all groups were titrated to achieve the target BP≤130/80 mmHg. Peritoneal equilibration test with 3.86%G solution, 24-hr dialysate protein, 8-hr dialysate CA125, residual renal function (RRF), and dialysis adequacy were examined at the beginning and 6-month periods.

**Results:** 69 patients had completed study. Half of patients in all groups had diabetes. No differences were observed in baseline demographics, including peritoneal membrane parameters. The mean arterial BP level after treatment was not different among groups and throughout the study period. There were significant improving of these parameters in the ACEI and ACEI+ARB groups when comparing with the Placebo: D/D0 glucose (0.45±0.05 *vs.* 0.36±0.05 *vs.* 0.31±0.04, $p$=0.03), 24-hr net UF (1,114.6±497.7 *vs.* 855.7±357.0 *vs.* 690.0±289.4 ml., $p$=0.01), 24-hr dialysate protein loss (3.5±0.4 *vs.* 4.5±0.4 *vs.* 5.3±0.4 gm, $p$<0.01), serum albumin (3.7±0.4 *vs.* 3.8±0.4 *vs.* 3.2±0.5 gm/dl, $p$<0.01), dialysate CA125 appearance rate (63.5±54.5 *vs.* 76.7±64.9 *vs.* 37.1±19.2 U/min, $p$<0.01). Adding ARB to ACEI showed further increments of serum albumin and dialysate CA125 appearance rate compared to single RAS blockage. Although adequacy and $D/P_{Cr}$ were no significant differences at 6 mo among 3 groups, there were trend to improvement in both treatment groups. RAS blockades did not yield in serious adverse effects including hyperkalemia.

**Conclusions:** Blocking of RAS significantly increased UF and preserve peritoneal membrane function CAPD patients. RAAS Blockage should be selected as a first-line anti-HT agent in CAPD patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC043

**Has the Probability of Death on Peritoneal Dialysis Been Overestimated? The Importance of Competing Risks in Survival Analysis** Jean-Baptiste Beuscart,[1,2] Dominique C. Pagniez,[2] Celia Lessore,[2] Luc Frimat,[3] Alain Duhamel.[1] *[1]Biostatistics, EA2694, UDSL, Lille, France; [2]Nephrology, CHU Lille, France; [3]Nephrology, EA 4003, CHU Nancy, France.*

Background: The Kaplan-Meier method may be suboptimal in survival analysis on peritoneal dialysis (PD). This method considers one event only, usually death. Transfer to haemodialysis (HD) and renal transplantation are not taken into account, although these events alter the probability of death and hinder its observation in PD. It has been shown in other fields of medicine that the competing risks method was more appropriate in such situations.

Methods: All consecutive incident patients starting PD in our center between 1992 and 2008 were enrolled in this study. Cumulative incidence was estimated for death during PD, transfer to HD, and renal transplantation, using both the Kaplan-Meier and the competing risks methods.

Results: At five years, of 383 patients included, 107 (28%) had died during PD treatment, 109 (28%) had been transferred to HD, and 91 (24%) had underwent renal transplantation. The Kaplan Meier method systematically overestimated the probabilities of these events: the estimated risks at five years were 50% for death, 59% for transfer to HD, and 39% for renal transplantation.

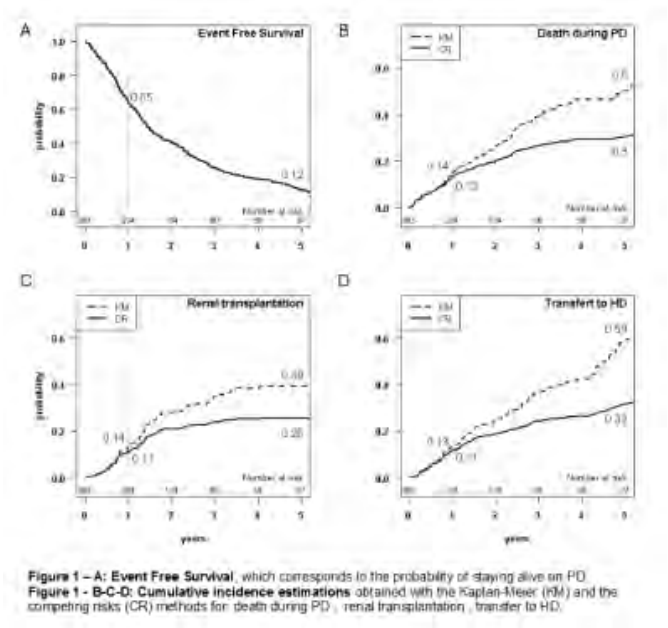



**Figure 1 – A: Event Free Survival**, which corresponds to the probability of staying alive on PD.
**Figure 1 - B-C-D: Cumulative incidence estimations** obtained with the Kaplan-Meier (KM) and the competing risks (CR) methods for: death during PD , renal transplantation , transfer to HD.

The sum of these three probabilities exceeded 100% at five years, which means that patients are expected to experience more than one event, which is impossible. With the competing risks method, the estimated probabilities for death during PD, transfer to HD and renal transplantation were 30%, 32% and 26% at five years, respectively. The sum of probabilities estimated with this method never exceeded 100% at any times.

Conclusion: The competing risks method appears to be a more appropriate and reliable way than the Kaplan Meier method to estimate the probability of events in PD.

Disclosure of Financial Relationships: Research Funding: Baxter Healthcare Corp. (Deerfield, IL).

### TH-FC044

**Higher eGFR at Dialysis Initiation Is Associated with Increased Mortality** William F. Clark,[1] Yingbo Na,[2] Steven J. Rosansky,[3] Jessica M. Sontrop,[1] Jennifer J. Macnab,[1] Richard J. Glassock,[4] Paul W. Eggers,[5] Kirby L. Jackson,[3] Louise M. Moist.[1] *[1]University of Western Ontario; [2]Canadian Institute of Health Information; [3]University of South Carolina; [4]UCLA; [5]National Institute of Diabetes and Digestive and Kidney Diseases.*

Background: Recent studies report a trend towards earlier dialysis initiation [at higher levels of glomerular filtration rate (GFR)], and an association between early initiation and increased mortality. Here, we examine trends in hemodialysis initiation within Canada, and compare the mortality risk between early and late dialysis initiators.

Methods: The analytic cohort included 25,901 incident hemodialysis patients >18 years from the Canadian Organ Replacement Registry (2001-7). Early dialysis initiation was defined as estimated GFR >10.5 mL/min/1.73m$^2$. Time-dependant proportional hazards Cox models were fitted to compare the risk for mortality between early and late dialysis initiators.

Results: From 2001-7, mean eGFR at dialysis initiation increased from 9.3 (+/-5.2) to 10.2 (+/-7.1) (p<0.001) and the proportion of early starts rose from 28% [95% confidence interval (CI): 27%-30%] to 36% (95% CI: 34%-37%). Mean eGFR among early vs. late starts was 15.5 (+/-7.7) and 7.1 (+/-2.0), respectively. The unadjusted hazard ratio (HR) for mortality in early vs. late starts was 1.48 (95% CI: 1.43-1.54), which decreased to 1.18 (95% CI: 1.13-1.23) after adjusting for demographics, comorbidities, serum albumin, vascular access and transplant status. The mortality differential between early and late initiators per 1000 patient-years narrowed after one year of follow-up, but never crossed and began widening after 24-months of follow-up; differences were significant at 6, 12, 30 and 36 months.

Conclusion: In Canada, patients are initiating hemodialysis at increasingly higher levels of eGFR. A higher eGFR at dialysis initiation is associated with an increased risk for mortality that is not fully explained by differences in baseline characteristics.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC045**

**Weekend Admissions Predict Higher Mortality in Patients with End Stage Renal Disease** Ankit Sakhuja, Nilay Kumar, Rahul S. Nanchal, Aaron T. Dall, Gagan Kumar. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

**INTRODUCTION:**
End stage renal disease (ESRD) is common medical condition affecting an increasing number Americans. Patients are commonly admitted with acute care issues like infection or electrolyte disturbances. There are limitations to hemodialysis services and manpower on weekends. Poor outcomes on weekends for acute medical conditions have been described in acute renal failure. We hypothesize that weekend admissions are associated with higher mortality and fewer hemodialysis procedures compared to weekdays.

**METHODS:**
Retrospective case control design was utilized to analyze the Nationwide Inpatient Sample from year 2007. Adult patients (age ≥ 18 years) with ESRD admitted on weekdays formed the controls and patients admitted on weekends formed the cases. Primary outcomes measured were all cause in-hospital mortality and use of hemodialysis on weekends. Pearson correlation and Chi square were used to compare the variables for unadjusted analysis and logistic regression was used to obtain adjusted odds ratios. α was set at 0.05.

**RESULTS:**
Of the 836,550 estimated admissions with ESRD, 19.7% were admitted on a weekend. Patients admitted on a weekend had significantly higher mortality (7.6% vs. 6.6%) than those admitted on a weekday. After adjusting for age, sex, race, patient's co-morbidities and hospital characteristics, the mortality was found to be significantly higher on the weekend (OR 1.17, 95%CI 1.11-1.24). The time to dialysis was 0.29 days longer (95%CI 0.21-0.36) on weekends.

**CONCLUSIONS:**This nationwide inpatient observational study holds up the inference that weekend admissions of ESRD patients have significantly increased mortality over weekday admissions. Other factors that affect this 'weekend phenomena' warrant further studies.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC046**

**The Effect of Hemodiafiltration on Quality of Life in a Randomized Controlled Trial (CONTRAST Study)** Albert H. Mazairac,[1] G. Ardine De Wit,[2] E. Lars Penne,[1,3] Neelke C. Van Der Weerd,[1,3] Claire H. Den Hoedt,[1,4] Muriel Grooteman,[3] Marinus A. Van Den Dorpel,[4] Pieter M. Ter Wee,[3] Michiel Bots,[2] Peter J. Blankestijn.[1] *[1]UMCU, Utrecht, Netherlands; [2]JULIUS, Utrecht, Netherlands; [3]VUmc, Amsterdam, Netherlands; [4]Maasstad Hospital, Rotterdam, Netherlands.*

**Background:** It is unclear if hemodiafiltration (HDF) leads to a preferable health status as compared to hemodialysis (HD). The overall results of small randomized trials or observational studies are indecisive.

**Aim:** To assess the effect of HDF on quality of life (QOL) as compared to HD in a cohort of chronic dialysis patients.

**Methods:** We performed a 24 month analysis in 312 patients from the CONvective TRAnsport Study (CONTRAST; NCT00205556), an ongoing randomized controlled trial on the effect of online HDF versus continuation of low-flux HD on all-cause mortality. QOL was assessed with the KDQOL-SF. This questionnaire provides data for a physical (PCS) and mental (MCS) composite score, and describes kidney disease-specific QOL in 12 domains.

**Results:** Over 24 months time, the PCS and MCS declined in HD patients (ΔPCS: -3.0 points, 95% CI -5.0 to -1.0, p=0.001; ΔMCS: -2.3 points, 95% CI -4.5 to -0.1, p=0.043), but not in HDF (ΔPCS: -0.9 points, 95% CI -2.8 to 1.0, p=0.827; ΔMCS: -0.4 points, 95% CI -2.2 to 1.4, p=1.00). As both domains were somewhat better at baseline in HD patients, this did not lead to statistical significant differences in generic QOL between HD and HDF (PCS: p=0.07; MCS: p=0.09). Kidney disease-specific QOL domains did not vary between both dialysis modalities over 24 months. There was a strong decline in patient satisfaction in both HD (-11.1, 95% CI -16.1 to -6.1, p<0.001) and HDF patients (-10, 95% CI -16.8 to -3.3, p=0.001).

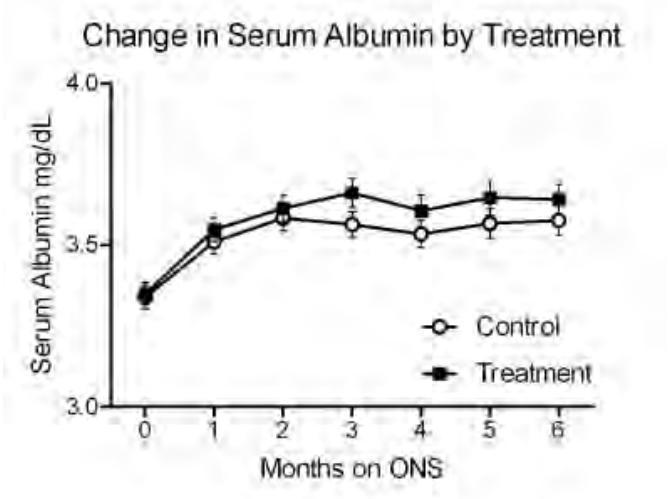
**Conclusion:** Whereas generic QOL declined in HD patients over time, it remained stable in HDF. After 24 months of follow-up, these changes did however not reach a statistical significant difference between HD and HDF. The remarkable decline in patient satisfaction over time needs further evaluation.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC047**

**Oral Nutritional Supplement (ONS) Provided during Dialysis Is Not Superior to Dietary Counseling Provided by Registered Dietitians (RDs) in Improving Serum Albumin** Deborah A. Benner, Mary B. Burgess, Marcia Davis, Steven M. Wilson, Tracy Jack Mayne, Allen R. Nissenson. *DaVita Inc., Denver, CO.*

**Background:** In hemodialysis (HD) patients, albumin is decreased by nutritional factors (protein, calorie, vitamin, and fluid intake) as well as non-nutritional factors (infection, inflammation, catabolism, and surgery). The extent of nutritional vs. non-nutritional factors affect albumin levels in HD patients is not well understood. **Objective:** Determine the effect of a 3 times-a-week ONS provided during dialysis vs. nutritional counseling alone on serum albumin levels in malnourished (serum albumin <3.5 g/dl) prevalent maintenance HD patients. **Methods:** Phase 4 trial with HD patients randomized to receive 1 serving of ONS during each dialysis treatment along with nutritional counseling (n=63) vs. nutritional counseling alone (n=72) for 6 months. Nutritional counseling was standardized for both groups and provided by RDs. Additional ONS use was prohibited. The primary endpoint was change in serum albumin from baseline to months 3 and 6. Primary analysis was conducted using mixed linear models. **Results:** There was a main effect of time between months 0 and months 3 & 6, but no significant effect of treatment group or treatment by time iteration on serum albumin. Pre-albumin levels did not differ by treatment at baseline (treatment 22.9 ± 1.1 mg/dL vs. control 23.6 ± 1.1 mg/dL) or month 6 (treatment 26.0 ± 1.4 mg/dL vs. control 23.1 ± 1.4 mg/dL).




**Conclusion:** Both groups showed improved serum albumin. The addition of an ONS (425 kcal /19 g protein), provided 3x/wk during dialysis, however, was not superior to nutritional counseling alone, when provided by RDs. It is not known whether more frequent ONS would have a greater effect on albumin. These results reinforce the importance of the dietitian's role in modifying patient behavior to improve biomarkers.

Disclosure of Financial Relationships: Employer: DaVita Inc.

**TH-FC048**

**Chronic Inflammation and Interleukin 1 beta (IL-1b) Inhibition in Chronic Hemodialysis (CHD) Patients** Adriana Hung,[1,2] Charles D. Ellis,[1] Ayumi Shintani,[1] Cindy Booker,[1] T. Alp Ikizler.[1,2] *[1]Vanderbilt University, TN; [2]Nashville VA, Nashville, TN.*

Chronic inflammation is highly prevalent in CHD patients and has been consistently associated with poor outcomes. IL-1b is a major pro-inflammatory cytokine which is elevated in CHD patients. IL-1 family gene polymorphism has also been associated with development of ESRD. A balance between IL-1b and its naturally occurring receptor antagonist IL-1ra may play a pivotal role in controlling the inflammatory response and its consequences in this population.

We conducted a pilot and feasibility double blinded RCT to evaluate the safety and the efficacy of the administration of recombinant human IL-1ra (Anakinra) on biomarkers of inflammation and nutrition. Inclusion criteria included hsCRP > 5 mg/dL for 3 consecutive months. Exclusion criteria included active infection and use of HD catheters.

Seventeen CHD patients (mean age 49±13 yrs, 71% African American, 71% males, 21% diabetics, mean BMI 31.8 ± 8, mean KTV 1.60±0.33, median vintage 35 months) were randomly assigned to placebo or Anakinra (100 mg at each HD session for 4 weeks) in a 1:1 ratio. The median CRP and IL-6 at baseline were 10.8 mg/dL (IQR 7.85, 17.4) and 4.76 pg/mL (IQR 2.89, 10.6), respectively. Patients in the intervention arm had a mean % drop in serum CRP of 53% vs. 1% in the placebo arm (p=0.008) and a mean % drop in IL-6 of 40% vs. a mean increase of 20% in the placebo arm (p=0.03). Serum pre-albumin increased 23% in the intervention arm and 6% in the placebo arm (p=0.1). Serum albumin increased by 3% in the intervention arm and there was no change in the placebo arm. There were no serious adverse events but there were two injection site reactions and a mild soft tissue infection at the access site in the intervention arm--these 3 subjects were withdrawn from the study, but 1 had outcomes recorded. A subject had asthma requiring steroid in the placebo arm was also withdrawn.

Administration of IL-1ra is safe and effective in lowering biomarkers of inflammation in CHD patients. These data provide strong rationale for future studies evaluating the impact of this anti-cytokine treatment on other intermediate and long-term (hospitalization and death) outcomes.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC049**

**Rosuvastatin and LDL-Cholesterol in Diabetic Patients Receiving Hemodialysis – A Post Hoc Analysis of the AURORA Trial** Hallvard Holdaas,[1] Bengt C. Fellstrom,[2] Ingar Holme,[3] Roland E. Schmieder,[4] Faiez Zannad,[5] Alan G. Jardine.[6] *[1]Oslo University Hospital, Rikshospitalet, Oslo, Norway; [2]Uppsala University, Uppsala, Sweden; [3]Ulleval Hospital, Oslo, Norway; [4]University Hospital Erlangen, Erlangen, Germany; [5]CHU & University Henri Poincare, Nancy, France; [6]University of Glasgow, Glasgow, United Kingdom.*

**Background:** Patients with chronic kidney disease and renal transplanted recipients are at high risk for cardiovascular events. Use of statins reduces cardiovascular risk in these populations. Two randomized controlled trials in hemodialysis populations (4D, AURORA) showed no benefit of statin treatment.

**Methods:** We performed a post hoc-analysis of the pre-specified subgroup of diabetes patients in the AURORA trial. Of the 2776 patients recruited to the AURORA trial, 731 patients had a diagnosis of diabetes mellitus at inclusion. We examined whether the effect of rosuvastatin treatment on clinical outcome was dependent on LDL cholesterol at baseline.

**Results:** Patients randomized to rosuvastatin in the highest quartile of LDL cholesterol (greater than 3.04 mmol/L) had a 37% reduced risk of the primary combined endpoint; death from cardiovascular causes, or nonfatal myocardial infarction, or nonfatal stroke (hazard ratio [HR] 0.63, confidence interval [CI] 0.40 – 0.99, p = 0.045).There was also a 46% risk reduction in the secondary cardiac event; cardiac death or nonfatal myocardial infarction (HR 0.54, CI 0.31 – 0.93, p = 0.026). No such decreases were observed in any of the other quartiles of LDL cholesterol at baseline.

The clinical events for total mortality and stroke did not differ significantly in any quartile of LDL cholesterol.

**Conclusion**: Use of rosuvastatin reduced the risk of death from cardiovascular causes, nonfatal myocardial infarction and nonfatal stroke in diabetic hemodialysis patients with LDL cholesterol > 3,04 mmol/L. Rosuvastatin also reduced the risk of the secondary cardiac endpoint. Diabetic patients undergoing hemodialysis with elevated LDL cholesterol may be considered for lipid lowering therapy.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC050**

**Long QTc, Alteration of Calcium Phosphate Product, Prevalence of Ventricular Arrhytmias and Sudden Death in Peritoneal Dialysis Patients: An Holter Study** Pierluigi Di Loreto,[1] Francesca K. Martino,[1] Claudio Ronco,[1] Giorgio Vescovo.[2] *[1]Nephrology, S Bortolo Hospital; [2]Internal Medicine, S Bortolo Hospital.*

Patients in peritoneal dialysis very often show elongation of the QTc interval. This can carry an arrhythmogenic risk that can be worsened by ischaemia or by electrolitic abnormalities.

We have measured the duration of the QTc interval and recorded a 24 h ECG Holter in 79 patients in peritoneal dialysis and we correlated the duration of QTc and the arrthymias with plasma levels of Ca++, PO4--, PTH, K+, Na+ and Mg++. Qtc was calculated on 12 leads surface ECG with the Bazzetts formula. Statistical analysis performed in SPSS version 10 (SPSS Inc., Chicago, IL, USA).

Mean QTc duration in dialyzed patients was 0.445±0.04 sec. 55 patients showed a long QTc (>0.45 sec).PTH was 344±25 pg/ml, Ca++ 9.27±0.11 mg/dl, PO4-- 5.5±1,5 mg/dl, Na+ 139,6± 3,4 mMol, K+ 4.04±0.64 mMol/L,. Mg 2.52±043 mg/dl. We found at 24 h ECG Holter supraventricular arrhytmias in 38 patients, complex premature ventricular contractions in 44 patients, monomorphic premature ventricular contractions in 16 patients, non sustained ventricular tachycardia (NSVT) in 10 patients. We also found the following statistically significant positive correlations: QTc / P r= 0.045 p<0.05,QTc / PTH r= 0.077 p<0.02, QTc / Ca r= 0.076 p<0.02. Five patients had sudden death and their QTc was 465± 0,03 msec, 11 patients belonged to Lown class 4a or 4b and their QTc was 465±0,02 msec, 10 patients showed NSVT and their QTc was 464±0,03 msec. The 5 sudden death patients showed a NSTV and belonged to Lown class 4.

QTc is prolonged in the majority of patients with peritoneal dialysis. The QTc value is correlated with the plasma levels of PTH, PO4—and Ca++. To our knowledge this is the first study to examine the relationship between phosphorus and arrthymias in peritoneal dialysis patients. Long Qtc seems to be correlated to increased prevalence of ventricular arrhytmias that may be in turn the cause of sudden cardiac death. The role of hyperparathyroidism and elevated levels of calcium and phosphates in the prolongation of QTc and in the alterations of ventricular repolarization has to be established with perspective studies.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC051**

**Coagulation Activation Induces Increased NADPH-Dependent Reactive Oxygen Species (ROS) Generation in Haemodialysis (HD)** S. Simone, M. Cariello, S. Pietanza, Francesco Paolo Schena, G. Grandaliano, G. Pertosa. *Nephrology, Dialysis and Transplantation Unit, Univ. of Bari, Italy.*

Increased oxidative stress (OS) is a non-traditional cardiovascular risk factor in HD patients (pts). We demonstrated that EVAL, a low coagulation-activating membrane, reduces inflammation in HD. The aims of the study were to investigate: i) the role of NADPH oxidase in ROS generation in HD pts ii) the link between coagulation system activation and ROS production in these pts. Intracellular ROS generation (2',7'-dichlorodihydrofluorescein) and NADPH-dependent superoxide generation (chemiluminescence) were evaluated in peripheral blood mononuclear cell (PBMC) isolated from HD pts dialyzed for at least 6 months with synthetic membranes (S group) (polysulphon/polyamide, n=15) or EVAL (E group, n=15) and in healthy subjects (C, n=15). Protein expression of NADPH oxidase subunits, NOX2, p22phoX was analyzed by western-blot analysis. The activation of coagulation factor X (FX) was evaluated measuring plasma levels of prothrombin fragment F1+2 by ELISA. Coagulation FX activation, intracellular ROS and superoxide generation, NOX2 and p22 protein expression were markedly induced in the S group and significantly reduced by EVAL treatment (table, mean±SEM).

| | C | S group | E group | p value |
|---|---|---|---|---|
| F1+2 plasma levels (pmol/ml) | 184.9±7.3 | 388.5±64.3 | 225.1±60.2 | .03 S vs E .01 C vs S |
| ROS generation (AU) | .10±.05 | .33±.28 | .19±.15 | .03 S vs C |
| NADPH-dependent superoxide generation (RLU/mg) | 15.4±.8 | 36.7±5.9 | 20.1±2.6 | .01 S vs C .05 S vs E |
| NOX2 protein expression (AU) | 1.0±1.0 | 3.4±1.4 | 2.8±1.9 | .0005 S vs C .02 S vs E |
| P22phox protein expression (AU) | .8±.4 | 3.3±1.9 | 2.8±.7 | .0008 S vs C <.0001 S vs E |

An increase of NADPH-dependent superoxide generation was observed only in S Group after HD treatment (2.5±.13; p=.01). Incubation of PBMC with activated FX induced a significant increase of superoxide generation (p=.04) and NOX2 protein expression (p=.03). In conclusion, the use of EVAL, by reducing coagulation FX activation, inhibits NADPH-related ROS production in HD pts, contributing to improve their cardiovascular risk profile.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC052**

**Effects of Liposome Encapsulated Clodronate on Chlorhexidine Gluconate-Induced Peritoneal Fibrosis in Rats** Taketoshi Kushiyama,[1] Takashi Oda,[1] Keishi Higashi,[1] Kojiro Yamamoto,[1] Takahiro Uchida,[1] Naoki Oshima,[1] Yutaka Sakurai,[2] Soichiro Miura,[1] Hiroo Kumagai.[1] *[1]Nephrology, National Defense Medical College, Tokorozawa, Saitama, Japan; [2]Preventive Medicine and Public Health, National Defense Medical College, Tokorozawa, Saitama, Japan.*

**Backgound.** Long-term peritoneal dialysis (PD) causes morphologic and functional changes in the peritoneum, which hampers the continuation of PD therapy. Macrophage plays important roles in the development of the peritoneal fibrosis. We examined the effect of liposome encapsulated clodronate (LC), which induce apoptosis of macrophage, on chlorhexidine gluconate (CG)-induced peritoneal fibrosis in rats. **Methods.** Fifty Sprague-Dawley rats were intraperitoneally injected either with saline (n=10) or with CG (1.5ml/100g, n=40) three times a week. Forty CG injected rats were randomly assigned into 4 groups (n=10, each): without intravenous injection (CG group), 3 groups with intravenous injection twice a week (10mg of LC, 20mg of LC, and 10mg of non-encapsulated clodronate). After 21 days, the rats were sacrificed and the parietal peritoneum was harvested. **Results.** CG induced massive infiltration of ED-1 positive macrophage with prominent peritoneal thickening. LC administration significantly reduced the number of peritoneal macrophages (27.2±2.8 cells/field in LC20 vs 92.0±4.6 cells/field in CG). This decrease in macrophage number paralleled well with decreases in mRNA expression for TGF-β1, collagen type I and III, and in peritoneal thickening (59.6±5.4 μm in LC20, 147±9.7 μm in CG). Double staining for cytokeratin and α-smooth muscle actin (α-SMA) revealed that α-SMA-positive-mesothelial-cells were prominently increased by CG injection and significantly decreased by LC treatment. **Conclusions.** These data suggest the critical role of macrophages in the development of peritoneal fibrosis. LC may be a new therapeutic strategy for the treatment of peritoneal fibrosis in PD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC053**

**Association of Ankle Brachial Pressure Index and C-Reactive Protein with Cardiovascular and All-Cause Mortality in Hemodialysis Patients** Tetsuya Yamada,[#1] Takanobu Toriyama,[#2] Seiichi Matsuo,[#3] Hirohisa Kawahara.[#2] *[1]Seto Kyoritsu Clinic, Seto, Aichi, Japan; [2]Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Aichi, Japan; [3]Nephrology, Nagoya University Hospital, Nagoya, Aichi, Japan.*

**Background:** Ankle Brachial Pressure Index (ABPI) has been established as an indicator of not only peripheral artery disease but also systemic atherosclerosis. C-reactive protein (CRP) also has been reported to reflect vascular wall inflammation and can predict future cardiovascular (CV) events. We investigated the association of ABPI, CRP and their joint role with prediction of CV and all-cause mortality in patients on hemodialysis (HD).

**Methods:** ABPI and serum CRP levels were measured consecutively in 442 HD patients. The patients were divided into tertiles according to ABPI levels; tertile 1 (T1): <1.02, T2: 1.02-1.20 and T3: > 1.20, and also to serum CRP levels; T1: <0.9 mg/l, T2: 0.9-3.7mg/l and T3: >3.7mg/l, respectively. All patients were prospectively followed up to 8 years.

**Results:** Serum CRP levels was 7.7±12.9mg/l, 4.5±0.74mg/l and 3.6±5.5mg/l in T1, T2 and T3 of ABPI, respectively (p=0.0006), and was independently associated with T1 of ABPI (odds ratio 1.40, 95%CI 1.07-1.83, p=0.013). Mean follow-up period was 71±27months. Adjusted hazard ratio (HR) of lower ABPI was 3.69 (95%CI 1.85-7.35, p=0.0006 for T1 vs. T3) for CV mortality and 2.23 (95%CI 1.38-3.61, p=0.0016 for T1 vs. T3) for all-cause mortality, respectively. Similarly, adjusted HR of elevated CRP levels was 2.58 (95%CI 1.33-5.01, p=0.019 for T3 vs. T1) for CV mortality and 2.52 (95%CI 1.56-4.09, p=0.0002 for T3 vs. T1) for all-cause mortality, respectively. In the joint setting of ABPI and CRP, the risk of CV and all-cause mortality was 15.1-fold (p=0.0024) and 11.2-fold (p=0.0009) in the T1 of ABPI with T3 of CRP compared to the T3 of ABPI with T1 of CRP even after adjustment, respectively.

**Conclusions:** Lower ABPI and elevated CRP levels might be closely associated, and the combination of these variables is more markedly related to increased mortality than either variable alone in HD patients. The measurement of ABPI and serum CRP is useful for the stratification of CV and all-cause mortality risk in this population.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC054

**Effects of Neutral pH and Low Glucose Degradation Product Peritoneal Dialysis Fluid on Systemic Markers of Inflammation and Endothelial Dysfunction: A Randomized Controlled 1-Year Follow-Up Study** Sun-Hee Park,[1] Jun-Young Do,[2] Yeong Hoon Kim,[3] Ho Yung Lee,[4] Beom Seok Kim,[4] Sug Kyun Shin,[5] Hyun Chul Kim,[6] Yoon-Kyung Chang,[7] Jong-Oh Yang,[8] Hyun Chul Chung,[9] Se-Hee Yoon,[1] Chan-Duck Kim,[1] Yong-Lim Kim.[1] *[1]Kyungpook National University Hospital; [2]Yeungnam University Hospital; [3]Inje University Busan Paik Hospital; [4]Yonsei University Severance Hospital; [5]NHIC Ilsan Hospital; [6]Keimyung University Dongsan Hospital; [7]Daejeon St. Mary's Hospital; [8]Soonchunhyang University Cheonan Hospital; [9]Ulsan University Hospital, Korea.*

**Purpose**: To examine the effects of neutral pH and low glucose degradation product (GDP) peritoneal dialysis fluid (PDF) on systemic inflammation and endothelial dysfunction in PD patients. **Methods**: A multicenter, open labeled, randomized controlled trial of new PD patients. Patients were randomly allocated into standard PDF (Stay safe®, Fresenius Medical Care, Germany) and low GDP PDF (Balance®) group and were followed up to 1 year. Primary efficacy variable was inflammation-endothelial-dysfunction index (IEDI), composite score from serum hs-CRP, soluble ICAM (sICAM)-1 and sVCAM-1. The individual markers of the IEDI, peritoneal clearance, ultrafiltration, residual renal function (RRF), nutritional indices, peritonitis rate and technique survival were secondary efficacy variables. Statistical comparisons between the groups were performed using analysis of covariance (ANCOVA) with baseline values as covariates. **Results:** Of 152 patients, 146 (low GDP: standard 79:67) patients entered this trial (Male 46%, Diabetes 53%). IEDI, sICAM-1 and sVCAM-1 were significantly lowered in low GDP compared to standard group at 12 month, whereas hs-CRP was not different. The change of peritoneal transport, RRF and nutritional indices were not different between the two groups. Moreover, the incidence of peritonitis and technique survival were not different. **Conclusions:** Neutral pH and low GDP PDF is likely to be beneficial on inflammation and endothelial dysfunction compared to standard PDF in incident PD patients. The effects of neutral pH and low GDP PDF on long-term clinical outcomes need to be answered.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC055

**Is Neutrophil-to-Lymphocyte Ratio a Marker of Inflammation in Chronic Hemodialysis Patients?** Georges Ouellet,[1,2] Laura Rosales,[1] Stephan Thijssen,[1,2] Nathan W. Levin,[1,2] Peter Kotanko.[1,2] *[1]Renal Research Institute; [2]Beth Israel Medical Center, New York, NY.*

**Background**

Neutrophil-to-lymphocyte ratio (NLR), defined as neutrophil count divided by lymphocyte count, is a marker of inflammation and is sometimes used as a proxy for C-reactive protein (CRP). However, its correlation with inflammatory markers has not been investigated in chronic hemodialysis (CHD) patients. We aimed to study the relationship between NLR and commonly used markers of inflammation (CRP, interleukin-6 (IL-6), and epoetin resistance index (ERI)) in CHD patients.

**Methods**

Stable CHD patients were recruited. Complete blood count and high sensitivity CRP were measured pre-dialysis by routine laboratory techniques. IL-6 was determined by quantitative sandwich enzyme immunoassay (Quantikine, R&D, Minneapolis, MN). Epoetin resistance index was computed as units of EPO per week per g/dL hemoglobin per kg body weight. Correlations were assessed by Spearman test.

**Results**

We studied 164 patients (age 61±14 years; median dialysis vintage 2.4 years; 52% male; 45% Hispanics, 45% Blacks; 8.5% HIV+; 21% HCV+; 12% catheters). The distributions of NLR, CRP and IL-6 were skewed. The median NLR was 2.88 [interquartile range: 2.15-4.00]; median CRP was 7.1 mg/L [interquartile range: 3.3-13.8], median ERI was 10.7 U EPO/ g/dl Hb /kg body weight [interquartile range: 5.2-22.8] and median IL-6 was 2.08 pg/mL [range: 2.08-78.2]. NLR was positively correlated with CRP, IL-6 and ERI (table 1), irrespective of HIV status.

Table 1. Spearman correlations of NLR with inflammatory markers

| | CRP | IL-6 | ERI |
|---|---|---|---|
| NLR | rho=0.238, P=0.002 | rho=0.288, P<0.001 | rho=0.159, P=0.043 |

**Conclusions**

In a group of stable CHD patients, NLR was positively correlated with CRP, IL-6 and ERI. NLR could therefore be used as a surrogate marker of inflammation when CRP and IL-6 are not available.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC056

**Proteinkinase C-alpha Mediates the Peritoneal Dialysis Related Peritoneal Membrane Changes** Hermann G. Haller, Joon-Keun Park, Vega Goedecke, Marcus Hiss, Nelli Shushakova, Jan Menne. *Clinic of Hypertension and Nephrology, Hannover Medical School, Hannover, Germany.*

Introduction: Peritoneal dialysis (PD) is a well established renal replacement therapy. One of its major limitations are the negative long-term effects of dialysis on the peritoneal membrane associated with mesothelial apoptosis, fibrosis and vascular changes. We have recently shown that effects of high glucose and glucose degradation products in diabetic kidneys are mediated by proteinkinase C isoform alpha (PKC-α). Using gen-deficient mice for PKC-α (PKCα$^{-/-}$) we tested the hypothesis that the deleterious effects of the high-glucose solution in PD are also mediated by PKC-α and that inhibition of PKC-α prevents glucose-induced peritoneal damage.

Material and Methods: We used (PKCα$^{-/-}$) (n=12) and SV 129 mice (n=12) and performed peritoneal dialysis for 4 weeks using twice daily intraperitoneal injections of a sterile pre-warmed dialysis solutions (Physioneal, 3,86%, Baxter). We used 75 ml/kg body weight, on average 1,5 ml, of dialysis fluid for each injection. After four weeks we analyzed peritoneal membrane thickness, expression of collagen IV, fibronectin, VEGF, VEGF receptors, and perlecan.

Results: Peritoneal membrane thickness was significantly reduced in (PKCα$^{-/-}$) as compared to controls. In the control group markers of peritoneal cell differentiation (perlecan) were lost and apoptosis was pronounced while in (PKCα$^{-/-}$) mice perlecan expression persisted and apoptosis was rare. Expression of VEGF and ist receptors in the peritoneum was also induced by dialysis in control animals while (PKCα$^{-/-}$) mice showed only minor changes.

Conclusion: Our results demonstrate that PKC-α is a major mediator of the glucose-induced changes in the peritoneal cavity and suggest a potential therapeutic use of PKC-alpha inhibition for the prevention of dialysis related changes of the peritoneal membrane

Disclosure of Financial Relationships: Consultancy: Daiichi Sankyo, Novartis, Genzyme, Roche, Bayer-Schering, Sanofi-Aventis, Noxxon, MedWiss, Phenos, MSD; Honoraria: Amgen, Astra-Zeneca, Recordati, Menarini, Pfizer.

### TH-FC057

**Impact of Continuous Veno-Venous Hemofiltration at Different Ultrafiltration Rate on Inflammatory Status and Immune Response of Porcine Endotoxemic Shock** Yimei Wang, Xiaoqiang Ding, Jianzhou Zou, Jie Teng, Yihong Zhong, Yi Fang. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

*Objective:* To study the impact of continuous veno-venous hemofiltration (CVVH) at different ultrafiltration rate on inflammation, immunology, hemodynamics, organ injury in porcine endotoxemic shock model, and to investigate the related mechanisms.

*methods:* Eighteen porcines received endotoxin infusion, then randomized into three groups. Control group received no further intervention, CVVH group and HVHF group received pre-dilution hemofiltration for 24 hours with a corresponding post-ultrafiltration rate of 45 ml/ kg· h and 70 ml/ kg· h respectively.

*Results:* Left ventricular stroke work index (LVSWI) and oxygen index (OI) were improved significantly in HVHF and CVVH group at T24($P < 0.05$). The survival time in CVVH group (21.4 ± 4.1) h, HVHF group (22 ± 6.7) h was significantly longer than that in control group (15.4 ± 5.2 h, $P < 0.05$). A significant decrease of plasma IL-10 levels was observed at T6, T12 and T24 in CVVH group compared with control group ($P < 0.05$). HVHF group accomplished a greater decrease in plasma TNF-α (T6) and IL-10 (T6,T12,T24) compared with control group and CVVH group. There was a significant negative correlation between IL-6 and survival time. HVHF increased PMN apoptosis and decreased the number of peripheral PMN ($P < 0.05$). The expression of ICAM-1 and p65, the degree of NF-κB/ DNA binding activity in lung and kidney were reduced in CVVH group and HVHF group ($P < 0.05$).

*Conclusion:* HVHF and CVVH can improve survival time, blood pumping and arterial oxygenation of porcine endotoxemic shock. IL-6 was a powerful independent predictive factor for survival time. HVHF can alleviate the decrease of PMN apoptosis, CVVH and HVHF can offer both renal and lung protection, which may be related to suppression of inflammation and NF-κB.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC058

**Cell Stretch during Peritoneal Dialysis Increases Mesothelial TGFβ and VEGF Production** Michael F. Flessner,[1] Zhi He,[2] Rebecca Potter,[2] Xiaorong Li.[1] *[1]Medicine, University of Mississippi Medical Center, Jackson, MS; [2]Pathology, University of Mississippi Medical Center, Jackson, MS.*

Peritoneal dialysis (PD) requires indwelling of large volumes of solution that increase the abdominal girth and stretch the mesothelial cells of the abdominal wall. To address the hypothesis that stretch stimulates these cells to increase synthesis and production of inflammatory cytokines, we grew MeT-5A human peritoneal mesothelial cells to confluence and then placed the cells in growth arrest (0.1% fetal bovine albumin) on Flexercell BioFlex membranes coated with Collagen in multiple duplicate wells. After 48 hours, cells were either left stationary (STA) or cycled with a sinusoidal stretch (STR) frequency of 10/ min and maximal amplitude of 30% with a Flexercell 3000T system. The supernatant and cells of individual wells were removed at 0, 4, 12, 24, 48, 72, and 96 hours. Supernatant was assayed with ELISA for TGFβ, IL-6 and VEGF. After trypsinization, the total number of viable cells in each well was estimated from their volume, cell count in a standard

hemacytometer, and lack of staining with methylene blue to determine the % viable cells. Total RNA from cells were extracted and Reverse Transcription - real time PCR (RT-PCR) was carried out to determine mRNA for IL-6, TGFβ, VEGF, TGFβ-R2, TGFβ-R3, KDR and FLT1. Results for STR were compared with STA for each time interval. Analysis was restricted to ≤ 72 hours due to decline of cell number and viability. mRNA for TGFβ, VEGF, TGFβ-R3 and KDR were significantly higher in the STR group throughout the 72 hours, while STR IL-6 mRNA declined in a non-significant way. Supernatant IL-6, TGFβ, and VEGF, normalized to the number of viable cells were not significantly different in the two groups. We conclude that mechanical stress due to mesothelial stretch does not enhance mesothelial cell secretion of IL-6, TGFβ or VEGF, but increases the expression of TGFβ, VEGF and their correspond receptors TGFβ-R3 and KDR. Upregulation of cytokine receptor may result in decreased cell secretion.

Disclosure of Financial Relationships: nothing to disclose

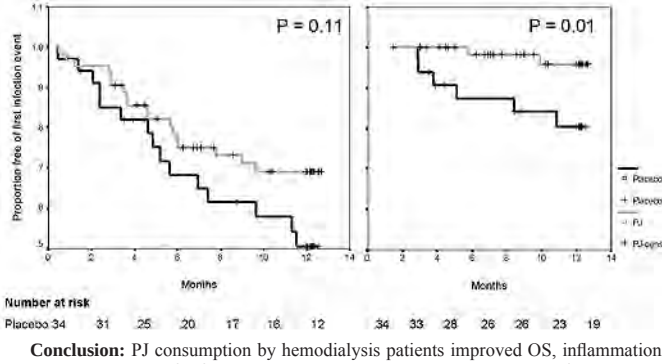
## TH-FC059

**One Year of Pomegranate Juice Consumption Decrease Oxidative Stress, Inflammation and Incidence of Infections in Hemodialysis Patients** Batya Kristal,[1] Lilach Shema,[1] Liora Ore,[2] Galina Shapiro,[3] Ronit Geron,[1] Shifra Sela.[3] *[1]Nephrology, Western Galilee Hospital, Nahariya; [2]School of Public Health, University of Haifa, Haifa; [3]Eliachar Research Lab, Western Galilee Hospital, Nahariya.*

**The aim** of the present study was to investigate the effect of Pomegranate juice (PJ) consumption, on oxidative stress, inflammation and incidence of infections after one year of intervention.

**Methods:** 101 HD patients were randomized to receive 100 cc of PJ or matching placebo, in the beginning of each dialysis session, three times a week for one year. The primary endpoints were levels of neutrophil priming expressed by CD11b, protein oxidation expressed by oxidized fibrinogen, IL-6 and albumin as inflammation markers. Secondary endpoints were hospitalization due to infections.

**Results:** PJ consumption, but not placebo intake, yields a significant time response improvment in CD11b (P=0.003), oxidized fibrinogen (P=0.001), IL-6 (P<0.001) and albumin (P=0.005). Incidence rate of first and second infection event were lower among PJ group compared to placebo (for first event: 32.5/1000pm vs. 54.9/1000pm, for second event: 3.1/1000pm vs. 17.9/1000pm). Survival analysis demonstrated a significant higher proportion free of second infection event among PJ group.




**Conclusion:** PJ consumption by hemodialysis patients improved OS, inflammation and reduce incidence of infections. Those results highlight the important of the relation between *OS & Inflammation & Innate immune dysfunction* originating from primed PMNLs. It apparent, that PJ consumption may have a beneficial effect on the above relation, and as such favor a reduction in the morbidity and mortality of HD patients

Disclosure of Financial Relationships: nothing to disclose

## TH-FC060

**Inflammation Affects Endothelial Progenitor Cells in Patients on Different Extracorporeal Dialysis Therapies** Detlef H. Krieter, Christoph Wanner. *Nephrology, University Hospital Würzburg, Würzburg, Germany.*

*Introduction:* Enhancing middle molecule removal of dialysis treatment is considered to exert beneficial effects on endothelial progenitor cells (EPCs) in maintenance dialysis patients.

*Methods:* In a prospective, randomized, cross-over trial, 18 maintenance dialysis patients were subjected to 4 weeks of each low-flux HD (LF-HD), high-flux HD (HF-HD), and online predilution hemodiafiltration (HDF). EPCs were determined at baseline and at the end of each 4 week period. 16 healthy volunteers served as control. EPCs derived from isolated PBMNCs were determined in colony assays after culture on fibronectin (CFU-Hill) and collagen-1 (ECFC), respectively, and were further analyzed for the coexpression of CD34, CD45, and VEGFR2-KDR.

*Results:* There was no difference in the effects of LF-HD, HF-HD, and HDF on EPCs. Differences were found between dialysis patients and healthy controls consisting of a lower total number of ECFCs (median from 5.4 x $10^3$ to 12.7 x $10^3$ (range 0.2 x $10^3$ to 101 x $10^3$) vs. controls 75.9 x $10^3$ (32.0 x $10^3$ to 318 x $10^3$) per $10^7$ MNC; $p<0.001$) and reduced fraction of vital ECFCs (mean between 54 ± 25% and 68 ± 20% vs. controls 86 ± 9%; $p<0.05$), whereas the formation of endothelial cell colonies (ECC) was increased in the dialysis patients (median between 0.0 and 0.8 (range 0.0 to 17.5) vs. controls 0.0 (0.0 to 0.3) per $10^7$ MNC; $p<0.05$). The number of prototypical EPCs (ECFC with the immunophenotype $CD34^+/VEGFR2\text{-}KDR^+/CD45^-$) was similar between patients and controls. Correlations of the CRP plasma level with the ECC count ($r=0.556$; $p<0.001$), the CFU-Hill colony count ($r=0.223$; $p=0.016$) and the $CD34^+/VEGFR2\text{-}KDR^+/CD45^-$ subpopulation of both ECFC ($r=0.364$; $p<0.001$) and CFU-Hill cells ($r=0.372$; $p<0.001$) were observed.

*Conclusions:* Enhanced middle molecule removal by dialysis therapy has no favorable effect on EPCs. A reduced vitality of EPCs and enhanced ECC formation in dialysis patients suggest growth induction of impaired EPCs in chronic renal failure. In this respect, inflammation seems to play an important role.

Disclosure of Financial Relationships: Consultancy: Membrana GmbHResearch Funding: Membrana GmbH.

## TH-FC061

**MiR-29 Regulate Collagen Expression by Target Sp1 in Tubular Epithelial Cells** Lei Jiang, Ruoyun Tan, Junwei Yang. *Center of Kidney Disease, the 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

**Objective**: Deposition of collagen in proximal tubular cells plays an important role during obstructive nephropathy, but the mechanism underlying excessive production of collagen remains poorly understood. MicroRNAs (miRNAs) have emerged rapidly as a major new direction in many fields of research including kidney disease. The aim of this study is to investigate the role of miR-29 family on collagen expression.

**Methods:** We analyzed the expression of miR-29 family in murine interstitial fibrotic kidneys induced by unilateral ureteral obstruction (UUO) using miRNAs array, and miR-29a, miR-29b, miR-29c expression in NRK-52E cells after TGF-β1 stimulation by Real-time Quantitative PCR. Then we transfected miR-29 mimics or miR-29 inhibitors to NRK-52E cells, and detected collagen I and collagen III expression though Real-time Quantitative PCR and western blot analysis.

**Results**: In obstructed kidneys induced by UUO, miR-29 family expression declined in miRNAs array. TGF-β1 significantly induced down-regulation of miR-29 expression. miR-29 mimics could reduce collagen I and collagen III mRNA and protein expression induced by TGF-β1, and miR-29 inhibitors alone could upregulate collagen I and collagen III mRNA and protein expression. MiR-29 mimics also could reduce smad2/3 phosphorylation and transcription factors Sp1 expression. Furthermore, transfection of Sp1 siRNA could decrease collagen I and collagen III expression and smad2/3 phosphorylation stimulated by TGF-β1, while downregulation of Sp1 also could ameliorate collagen I and collagen III induced by miR-29 inhibits.**Conclusions:** MiR-29 could negatively regulate collagen expression induced by TGF-b1 though target transcription factor Sp1 in tubular epithelial cell.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC062

**miR-29 Inhibits TGF-beta/Smad3-Mediated Renal Fibrosis In Vitro and In Vivo** Wei Qin,[1,2] Arthur Chi-Kong Chung,[1] Xiao Ru Huang,[1] Xiaoming Meng,[1] Hui Y. Lan.[1] *[1]Department of Medicine and Therapeutics, and Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, Hong Kong SAR, China; [2]Department of Medicine-Nephrology, West China Hospital, Sichuan University, Chengdu, Sichuan, China.*

TGF-beta acts by stimulating Smad3 to play opposite roles in fibrosis and immunity. Thus, strategies aimed to treat renal fibrosis need to precisely target the Smad3-specific regulatory gene(s) related to fibrosis. In the present study, we tested the hypothesis that **miR-29 may be a downstream inhibitor and therapeutic molecule specifically for Smad3-mediated renal fibrosis.** This hypothesis was tested in Smad3 KO kidney tubular cells (TEC)/fibroblasts and in a mouse model of unilateral ureteral obstructive kidney disease (UUO) induced in Smad3 KO mice or by overexpressing a doxycycline-inducible miR-29b mimic using the ultrasound-microbubble-mediated technique.

Using microRNA array and real-time PCR, we found that miR-29 was a downstream inhibitor of TGF-beta/Smad3-mediated fibrosis because TGF-beta1-mediated renal fibrosis in vitro and in the UUO kidney was associated with a loss of miR-29abc, which was prevented in Smad3 KO cells and Smad3 KO UUO kidney. In vitro, Smad3 bound miR-29 promoter physically as determined by the ChiP assay and regulated a loss of miR-29 in response to TGF-beta1. The protective role of miR-29 in TGF-beta/Smad3-mediated fibrosis was revealed by the findings that overexpression of miR-29b mimic blocked, but knockdown of miR-29 enhanced, TGF-beta1-induced collagen I and III expression. Importantly, in vivo overexpression of miR-29b mimic in the normal kidney was able to prevent UUO-induced renal fibrosis. Furthermore, in the established UUO kidney, restored miR-29b in the fibrotic kidney by ultrasound-microbubble technique on day 4 after UUO was able to block the progression of renal fibrosis at day 10, demonstrating the therapeutic effecacy of miR-29b mimic on renal fibrosis. In conclusion, miR-29 is a downstream inhibitor of TGF-beta/Smad3-mediated renal fibrosis. Targeting the Smad3-miR-29 axis may represent a novel and specific therapy for renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC063

**Role of Specific Phosphorylations outside the Carboxyl-Terminus of Smad3 in the Collagen Response to TGF-β** James A. Browne, Tomoko Hayashida, H. William Schnaper. *Division of Kidney Diseases, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

Regulation of TGF-β/Smad3-mediated fibrogenesis is a complex process. We previously described several non-canonical signaling pathways that contribute to renal cell collagen expression. Here, we report the importance of several potential phosphoacceptor sites outside the carboxyl-terminal serines targeted by the activated TGF-β receptor. Four of

these (Thr 179, Ser 204, Ser 208, and Ser 213) are within the Smad3 linker region (LR). Some groups propose that LR phosphorylation inhibits Smad3 signaling. However, we have previously described phosphorylation of the LR by ERK MAP kinase, and that inhibiting ERK blocked both LR phosphorylation and the collagen response to TGF-β. Therefore, we and others believe that the effect of LR phosphorylation is cell-context dependent. We propose that specific phosphorylations could enhance, inhibit or have no effect on TGF-β-induced type-I collagen expression; and different sites could have different and even opposing effects.

To dissect these possibilities, we evaluated COL1A2-luciferase activity induced by TGF-β1 in Smad3-null murine embryonic fibroblasts (MEFs) transfected with Smad3 constructs, mutated at Thr8 or at various LR sites (Thr179, Ser204, Ser208, and Ser213). A collagen response to TGF-β1 in wild type MEFs, but not in Smad3-null MEFs, was confirmed. Transfection of wild type Smad3 into Smad3-null MEFs reconstituted the collagen response. Smad3-null MEFS transfected with Smad3-EPSM (all four LR phosphoacceptor sites mutated) responded to TGF-β. Reconstitution with Smad3 mutated at Thr178, Ser204 and/or Ser212 maintained COL1A2 activity. However, Smad3-null MEFs reconstituted with Smad3 mutated only at Ser208 of the LR showed a significantly reduced collagen response. Therefore, although Ser208 in the LR of Smad3 may enhance the collagen response, the remaining sites (Thr178, Ser204 and Ser212) may have different and even opposing effects. Further investigation of the role of the Smad3 linker region in renal cell function may offer new approaches to selectively modifying TGF-β1-stimulated responses in progressive kidney disease.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC064

**Type I Collagen Induced by TGF-β1 Is Negatively Regulated through the Autophagic Pathway** Hee-Jun Na, Sung I. L. Kim, Yan Ding, Mary E. Choi. *Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Type I collagen (Col-I) is a major component of the extracellular matrix (ECM) whose regulated expression is important maintaining tissue homeostasis, and its relentless production and progressive accumulation is a hallmark of renal fibrosis in progressive kidney disease. Autophagy is a highly conserved cellular process regulating turnover of cytoplasmic proteins and organelles via a lysosome-dependent pathway. Here we show a novel role of autophagic process as a mechanism to negatively regulate and prevent excess collagen/ECM accumulation in the kidney. Kidneys from mice deficient in autophagic protein beclin 1 (*beclin 1*$^{+/-}$) exhibited pro-fibrotic phenotype, with increased ECM deposition as assessed by Masson's trichrome staining, compared to wild-type (*beclin 1*$^{+/+}$) littermates. Increased renal expression of Col-I protein was confirmed by western blot analysis of kidneys from *beclin 1*$^{+/-}$ mice and in mice with null mutation of microtubule-associated protein 1 light chain 3 (*LC3*$^{-/-}$), compared to their wild-type littermates. Moreover, mouse mesangial cells (MMC) deficient in autophagic proteins, either by targeted knockdown of beclin 1 with specific siRNA, or in MMC from *beclin 1*$^{+/-}$ and *LC3*$^{-/-}$ mice also expressed increased levels of Col-I protein. Interestingly, treatment with TGF-β1, a potent inducer of Col-I, induced both beclin 1 and LC3 expression in MMC, indicating dual nature of this multifunctional cytokine TGF-β1. Furthermore, inhibition of autophagy using bafilomycin A1, a potent inhibitor of lysosomal degradation pathway, did not alter the Col-I mRNA expression but led to increased Col-I protein levels, which was further enhanced by co-treatment with TGF-β1 and bafilomycin A1, suggesting an important role of autophagy in Col-I degradation. Blockade of MKK3-p38, a major TGF-β1 signaling pathway, either by using MMC from MKK3$^{-/-}$ null mice or p38 inhibitor SB203580 abrogated LC3 expression and beclin 1 induced by TGF-β1. Our findings are the first demonstration that, at least in part, renal Col-I protein levels are regulated by autophagy and that collagen degradation is dependent on the autophagy-lysosomal pathway.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC065

**Inhibition of β-Catenin Signaling Attenuates Renal Interstitial Fibrosis** Sha Hao,[1] Weichun He,[1] Hong Ding,[1] Michael Kahn,[2] Youhua Liu.[1] *[1]Department of Pathology, University of Pittsburgh, Pittsburgh, PA; [2]Center for Regenerative Medicine and Stem Cell Research, University of Southern California, Los Angeles, CA.*

Renal interstitial fibrosis is the common final outcome of a wide variety of progressive chronic kidney diseases, and current clinical therapy to halt and reverse renal fibrosis is ineffective. Recent studies demonstrate that Wnt/β-catenin signaling plays an important role in the pathogenesis of renal fibrosis, suggesting that this pathway could be exploited as a potential target for antifibrotic therapy. In this study, we examined the therapeutic efficacy of a highly specific small molecule β-catenin inhibitor (ICG-001) on renal interstitial fibrosis in obstructive nephropathy. In vitro, ICG-001 dose-dependently suppressed the β-catenin-driven gene transcription in a Topflash luciferase reporter assay. ICG-001 also abolished the expression of plasminogen activator inhibitor-1 (PAI-1), a direct target gene of the canonical Wnt/β-catenin signaling. In human proximal tubular epithelial cells (HKC-8), β-catenin was activated by TGF-β1, and ICG-001 abolished TGF-β1-induced α-smooth muscle actin (α-SMA), fibronection, type I collagen expression. This anti-fibrotic effect of ICG-001 was clearly independent of a disruption of Smad signaling, as it did not affect TGF-β1-triggered Smad2 and Smad3 phosphorylation in HKC-8 cells. In vivo, administration of ICG-001 (5 mg/kg body wt) through daily intraperitoneal injections significantly inhibited β-catenin signaling and attenuated renal fibrosis in the obstructed kidneys at 7 days after unilateral ureteral obstruction (UUO). Real-time RT-PCR, Western blotting and immunostaining revealed that ICG-001 significantly suppressed renal expression of α-SMA, fibronectin and type I collagen, as well as fibroblast-specific protein-1 (Fsp1). ICG-001 also repressed renal expression of Snail1, Snail2/Slug and Twist1, key transcriptional factors that regulate epithelial-mesenchymal transition (EMT). These results suggest that targeting β-catenin signaling by small molecule inhibitor could be a novel and effective approach for the treatment of fibrotic kidney diseases.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC066

**Vitronectin Accumulates in the Interstitium without Impacting Fibrosis Severity in Experimental Chronic Kidney Disease** Allison A. Eddy, Allen Rassa, Sarah J. Collins, Jesus M. Lopez-Guisa. *Pediatrics, Seattle Children's Research Institute & University of Washington, Seattle, WA.*

Vitronectin (VN) is a glycoprotein normally found in serum (also known as complement S-protein) and in the extracellular matrix during certain disease states. Given its known interactions with plasminogen activator inhibitor-1 (PAI-1) and VN cellular receptors – the αvβ3 integrin and the urokinase receptor (uPAR) – this study was designed to investigate its potential role in renal fibrogenesis using the mouse model of unilateral ureteral obstruction (UUO). We have previously reported increased uPAR expression and its anti-fibrotic function in this model (JASN 14:1254, 2003). By Western blotting, VN protein was increased 1.9-3.0x on days 7, 14 and 21 after UUO compared to sham kidneys levels. αvβ3 protein levels increased 6-8x after UUO (days 7 and 14; declining to near sham levels on day 21). By immunofluorescence confocal microscopy the VN protein was localized within glomeruli and the interstitium. Phenotypically normal VN knockout (-/-) mice on a C57BL/6 background were re-derived from cryopreserved embryos and the genotype confirmed by PCR and by the absence of a protein band by immunoblotting. Groups of age-matched C57BL/6 wild-type (VN+/+) and VN-/- mice (n = 10-11/group) were sacrificed 7, 14 or 21 days after UUO. Absence of VN resulted in significantly fewer αSMA+ interstitial myofibroblasts by immunostaining (0.53x) and significantly lower PAI-1 protein (0.23x) and αvβ3 protein (0.32x), but only on day 14. The number of CD68+ macrophages did not differ between the genotypes. Despite these differences, the absence of VN had only a modest effect on fibrosis severity based on both picrosirius red+ interstitial area and total kidney collagen measured by the hydroxyproline assay: only the day 21 total collagen levels were significantly lower in the VN-/- mice (9.8 ± 1.6 vs 11.9 ± 2.9 mm/mg wet kidney wt). These findings suggest that despite significant interstitial VN deposition in the UUO model of chronic kidney disease, its fibrogenic role is either non-essential or redundant. These data are remarkable given its strong affinity for the potent fibrogenic molecule PAI-1.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC067

**Progenitor Cell Secretory Products Are Renoprotective *In Vitro* and *In Vivo*: Efficacy of Cell Free Conditioned Medium in the Remnant Kidney Model** Darren A. Yuen, Andrew Advani, Yanling Zhang, Kim Connelly, Richard E. Gilbert. *St. Michael's Hospital, Toronto, Canada.*

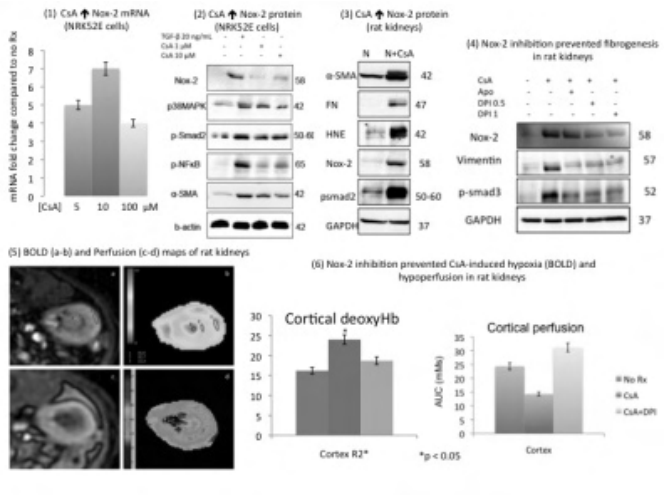
**Background:** The mechanisms underlying the beneficial effects of progenitor cells are poorly understood with most contemporary studies suggesting a paracrine mode of action. Recently, however, an endocrine effect has also been postulated in kidney disease models, wherein progenitor cells mediate their beneficial effects by secreting renoprotective factor(s) from a reticulo-endothelial system niche. To test this 'endocrine' hypothesis we examined the secretory products of early outgrowth endothelial progenitor cells (EPCs), focusing on their angiogenic and anti-fibrotic activities in both the *in vitro* and *in vivo* settings. **Methods:** EPCs were grown from donor F344 rat bone marrow. Cell free conditioned medium (CF-CM) was generated by incubating EPCs with serum-free EBM-2 medium to collect their secreted factor(s). *In vitro* antifibrotic and angiogenic activities of CF-CM were assessed using $^{3}$H-proline incorporation and capillary tube formation assays. For the *in vivo* studies, subtotally nephrectomised (SNX) F344 rats, 4 wks post-SNX, received thrice weekly tail vein injections of either 10 x concentrated CF-CM or EBM-2 medium for 2 wks. FITC-inulin GFR, urinary protein, and renal collagen IV deposition were measured 4 wks later (8 wks post-SNX). **Results:** CF-CM demonstrated robust antifibrotic activity *in vitro*, potently reducing TGF-β and angiotensin II-induced fibroblast collagen synthesis ($IC_{50}$ >1:1000 dilution). CF-CM also displayed potent angiogenic activity in an endothelial tube-formation assay. Compared with EBM-2, infusion of CF-CM into SNX rats attenuated disease progression, as evidenced by improved GFR (CF-CM vs EBM-2: 1.8 ± 0.1 vs 1.1 ± 0.2 μL/min/g body wt), reduced urinary protein (CF-CM vs EBM-2: 1.4 ±0.3 vs 2.6 ±0.4 g/mmol creatinine), and reduced mesangial collagen IV (CF-CM vs EBM-2: 0.14 ± 0.01 vs 0.26 ± 0.06 AU, $p < 0.05$ for all). **Conclusions:** The systemic administration of EPC-derived secreted factor(s) mimics the therapeutic effects of cell infusion. Identification of the responsible factor(s) and their subsequent synthesis may provide a new therapeutic strategy for CKD.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC068

**Nox-2 May Regulate CsA-Induced Kidney Injury** Lingjin Huang, Aparna Vidyasagar, Shannon Reese, Arjang Djamali. *Medicine and Surgery, UW Madison School of Medicine and Public Health, Madison, WI.*

We hypothesized that the classical phagocytic Nox-2 enzyme plays an important role in renal hypoxia and fibrosis mediated by CNIs. We tested this hypothesis in vitro, studying CsA-mediated injury of renal tubular epithelial cells (NRK52E) and in vivo, using the CsA-induced model of chronic nephrotoxicity. We first demonstrated that CsA (5, 10 and 100 M) increased tubular Nox-2 mRNA 3-7 fold (Figure 1).




Next we confirmed these studies by immunoblot analyses demonstrating that CsA increased Nox-2 protein levels and fibrogenesis (phospho-MAPK, smad2, NFκB and α-SMA) in NRK52E cells (Figure 2). We further examined the effects of CsA on Nox-2 and fibrogenesis in vivo. Fisher344 rats received CsA 15mg/kg/24h, vehicle or CsA + Diphenyleneiodonium (DPI) a flavoprotein inhibitor that inhibits Nox activity (n=6-8 in each group). All animals were treated for 1 month. Immunoblot analyses of whole kidney tissue lysates demonstrated that CsA therapy increased Nox-2, oxidative stress (HNE) and fibrogenesis (α-SMA, fibronectin and phospho-smad2) (Figure 3). DPI downregulated Nox-2 and fibrogenesis (vimentin and phospho-smad3) suggesting that Nox-2 is a mediator of CsA-induced renal fibrosis (Figure 4). To characterize the effects of CsA on intrarenal oxygenation and perfusion, rats underwent blood oxygen level dependent MRI (BOLD-MRI) and perfusion studies (Figures 5 and 6). BOLD-MRI is a non-invasive imaging method that uses hemoglobin as an endogenous contrast agent. These studies demonstrated that CsA therapy significantly decreased cortical oxygenation (increased R2* levels) while decreasing cortical perfusion. Nox inhibition by DPI prevented these changes. In conclusion, these studies indicate that Nox-2 is involved in the pathogenesis of CsA-induced fibrogenesis and hypoxia. Specific Nox-2 inhibition may prevent CNI nephrotoxicity.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC069

**Gadodiamide (Gd-OMN) Binds Circulating ASARM-Peptides: A Model for Nephrogenic Systemic Fibrosis (NSF)** Peter S. N. Rowe,[1] Anne-Marie Hedge,[1] Jennifer S. Laurence,[2] Sang-Pil Lee,[1] William M. Brooks,[1] Ellen T. McCarthy.[1] *[1]University of Kansas Medical Center, KS; [2]The University of Kansas, KS.*

Nephrogenic systemic fibrosis (NSF) is a devastating condition associated with gadolinium (Gd) containing contrast-agents and chronic kidney disease (CKD). The primary cause of NSF remains unknown. Bone matrix ASARM-peptides are linked with renal-handling defects and bone-mineralization abnormalities. We hypothesize that increased levels of these acidic, highly reactive and phosphorylated ASARM-peptides: 1. Bind to gadodiamide (Gd-OMN) and 2. Destabilize the Gd-OMN complex resulting in release of toxic Gd and thus NSF.

To test our hypothesis, injections of Gd-containing Omniscan (Gd-OMN) were given to, 1. Normal mice (WT): 2. PHEX defective, X-linked hypophosphatemic rickets mice (HYP) that have high circulating levels of ASARM-peptides: 3. HYP-mice treated with a 4.2 kDa synthetic PHEX-related peptide (SPR4). SPR4 binds with affinity to ASARM-peptides, neutralizing biological activity. Finally, we used magnetic resonance imaging (MRI), HPLC and $^{1}H/^{15}N$ nuclear-magnetic-resonance (NMR) to study Gd-OMN-ASARM binding and competitive-inhibition of this binding with SPR4.

Renal *ex vivo* MRI scans of WT-mice treated with Gd-OMN showed a strong signal indicative of a sequestered gadodiamide complex. In contrast, the signal was almost absent in Gd-OMN treated HYP mice but restored in SPR4 treated HYP mice. Using HPLC and solution NMR we showed ASARM-peptide binds to Gd-OMN *in vitro*. Also, $^{15}N$ labeled SPR4 and $^{1}H/^{15}N$ NMR confirmed Gd-OMN binds to SPR4. Finally, HPLC data indicated SPR4 disrupts Gd-OMN binding to ASARM-peptide.

In summary: 1. High levels of ASARM-peptide in HYP mice results in altered metabolism of a Gd-contrast agent, Gd-OMN; 2. ASARM-peptide binds to Gd-OMN *in vitro*; 3. The ASARM-Gd-OMN complex likely launches reactive de-sequestration and toxic release of Gd. 4. SPR4-peptide binds competitively to ASARM-peptide and thus indirectly stabilizes the Gd-OMN complex. In conclusion, SPR4-peptide might be an ideal adjuvant to prevent NSF. Specifically, such a protective agent could allow safe use of MRI in the care of patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC070

**Targeted Expression of Pan-Caspase Inhibitor Protein p35 in Tubular Epithelium Protects Mice Against Renal Fibrogenesis Following Nephrotoxic Serum Nephritis (NTN)** Tsutomu Inoue, Hiromichi Suzuki, Hirokazu Okada. *Department of Nephrology, Saitama Medical University, Irumagun, Saitama, Japan.*

Background: The caspase family of enzymes participates in cell apoptotic and proinflammatory reactions. Although caspase activation was known to be involved in renal fibrogenesis in NTN, in such a nephritic kidney how caspase activation plays a role remains to be determined.

Methods: Two lines of transgenic (tg) mice were used. p35 tg mice that bear the baculovirus pan-caspase inhibitor protein *p35* gene separated from a universal CAG promoter by a floxed STOP sequence were crossed with γGT.Cre tg mice that express Cre recombinase in tubular epithelium. Double-tg mice (γGT.Cre;p35) and wild-type mice were then challenged with NTN. Cell apoptosis was defined by in situ end labeling and the point-counting method. Fibrosis-related parameters in the kidneys were determined by real-time RT-PCR and immunohistochemistry.

Results: Either urinary protein excretion or glomerular damage such as crescent formation was significantly increased at the similar degree in the γGT.Cre;p35 and wild-type mice with Day14 NTN. In contrast, F4/80$^{+}$ monocyte infiltration and matrix deposition by Masson's trichrome staining in the peritubular interstitium were significantly decreased in the NTN kidneys of the γGT.Cre;p35 mice, compared to those of the wild-type mice, possibly due to cell-type specific caspase inhibition by p35 gene product (10.2±4.2 vs. 15.6±6.7 [monocytes/hpf], and 8.4±2.1 vs. 17.2±3.4 [% blue area], respectively). mRNA levels of IL-1β, MCP-1 and fibronectin-EIIIA were also significantly decreased in the NTN kidneys of the γGT.Cre;p35 mice, compared to those of the wild-type mice. Surprisingly, the number of apoptotic tubular cells in the NTN kidneys was small and not significantly different between the γGT.Cre;p35 and wild-type mice (0.5±0.7 vs. 0.3±0.5 [apoptotic tubular cells/hpf]).

Conclusion: Targeted expression of p35 in the tubular epithelium provides evidence of the critical role of caspase activation in the tubular epithelium in proinflammatory cell recruitment, but not apoptosis induction, and subsequent fibrogenesis in the NTN kidneys.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC071

**Downregulation of Hedgehog Signaling Prevents Renal Cystogenesis in Mouse Models of Ciliopathies** Pamela Vivian Tran, David Beier. *Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Cystic kidney disease (CKD), a leading cause of renal failure, is proposed to originate from an underlying ciliary defect. There are no proven therapies for CKD and molecular mechanisms remain unclear. In our characterization of the ENU mutant mouse *alien (aln)*, we identified a novel ciliary protein, THM1 (Tetratricopeptide Repeat Containing Hedgehog Modulator 1, also IFT139), which negatively regulates Hedgehog (Hh) signaling. Mutations in IFT139 have been identified in patients with several ciliopathies that feature renal cysts as a major clinical component, including nephronophthisis, Bardet-Biedl Syndrome and Meckel-Gruber Syndrome. By E16.5, the *aln* mutant also develops renal cysts. Importantly, *aln* cysts were prevented by genetic deletion of *Gli2*, the main transcriptional activator of Hh signaling, implicating a role for increased Hh signaling in the etiology of *aln* renal cystogenesis. To explore whether the Hh pathway might present a novel target for CKD, we examined the effects of small molecule Hh inhibitors in a cAMP-induced cystogenic kidney explant assay. In the presence of cAMP, cultured *aln* kidneys exhibited a three-fold greater cystogenic potential than wild-type. This was prevented by small molecule Hh inhibitors, Gant61 or Sant2, supporting a role for increased Hh signaling in *aln* renal cystogenesis. Surprisingly, the small molecules also abrogated cAMP-induced cysts in wild-type kidneys, indicating a beneficial role for Hh inhibitors in cAMP-mediated renal cystogenesis, which is proposed as a general mechanism in CKD. We thus questioned whether the preventive effects of Gant61 and Sant2 might extend to other models of CKD, such as the *jck* mutant, a mouse model for NPHP9. This mutant develops severe CKD by P21 and also showed a three-fold higher cystogenic potential than wild-type using the cAMP cystogenic assay. This increased cystogenesis was dramatically reduced by both Gant61 and Sant2. Our results indicate a protective role for small molecule Hh antagonists in CKD and demonstrate the potential of developing Hh inhibitors as targeted therapies.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC072**

**Exome Capture and Massively Parallel Sequencing Reveals Mutations in *SDCCAG8* as a New Cause of Nephronophthisis-Related Ciliopathy**
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Nephronophthisis-related ciliopathies (NPHP-RC) are recessive disorders featuring dysplasia or degeneration in kidney, retina and cerebellum. We combined homozygosity mapping with "ciliopathy candidate exome capture" of 828 candidate genes followed by massively parallel sequencing. Candidates were derived from ciliopathy animal models, the centrosomal and photoreceptor cilia proteomes. We detected 6 homozygous null mutations in the gene *SDCCAG8* in 5 NPHP-RC families. Subcellular localization studies revealed that SDCCAG8 occurs at centrosomes throughout the cell cycle and is located e.g. in mouse photoreceptor basal bodies and connecting cilia transition zone together with other NPHP-RC proteins including NPHP5. We identified OFD1, another cystic kidney disease associated NPHP-RC protein, as a direct interaction partner of SDCCAG8 by yeast-2-hybrid screening. Depletion of *sdccag8* in zebrafish resulted in kidney cysts and body axis defects. Furthermore, siRNA knockdown of *Sdccag8* disturbed lumen formation of renal epithelial cells in 3D spheroid cultures, indicative for a cell polarity defect. We observed SDCCAG8 at cell-cell junctions. Interestingly, SDCCAG8 abandons cell-cell junctions in response to increased intracellular cAMP levels, which have been described as a therapeutic target in cystic kidney disease. In summary, this work identifies *SDCCAG8* loss of function as a novel cause of NPHP-RC, thus contributing to the understanding of disease mechanisms of retinal-renal ciliopathies.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC073**

**How To Build a Primary Cilium with the Exocyst**
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The pathogenesis of cystic kidney disease is dependent on disruptions in primary cilia function in renal epithelial cells. This includes ADPKD, which is caused by mutations in genes that encode polycystins-1 and-2. Despite intense study of cilia and associated ciliopathies, it is not understood how ciliary proteins are targeted and delivered to cilia. We previously showed that the exocyst, a highly conserved eight-protein trafficking complex, is required for ciliogenesis. Based on our findings, we developed a "construction" model for primary cilia: the GTPase Cdc42 localizes the exocyst to primary cilia, the exocyst is stabilized by binding to the Par complex, and the exocyst then docks Rab8-positive vesicles carrying ciliary proteins. In support of this model, we show that Cdc42 co-localized with the exocyst at primary cilia; Cdc42 biochemically interacted with exocyst Sec10; and expression of dominant negative Cdc42 prevented ciliogenesis in MDCK cells. The exocyst co-localized and co-immunoprecipitated (co-IP) with Par3, and the exocyst also co-IP'd with Rab8, a GTPase found on vesicles necessary for ciliogenesis. We also show that exocyst Sec10 interacted with ciliary proteins polycystin-2, IFT20, and IFT88. To further support this model, knockdown of Sec10 in MDCK cells led to a cellular phenotype similar to ADPKD cells: loss off flow-generated calcium release; hyperproliferation; and activation of the MAPK pathway. In vivo Sec10 knockdown in zebrafish phenocopied many aspects of polycystin-2 knockdown, including: curly tail up; glomerular expansion; left-right patterning defects; and MAPK activation. Importantly, co-injection of small amounts of sec10 and pkd2 morpholinos, which individually had no effect, together resulted in a severe phenotype.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC074**

**Pkhd1 Deficiency Identifies a Novel Functional Relationship between Fibrocystin/Polyductin, SMURF-Mediated Endocytosis, and TGF-beta Signaling**
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Individuals with human autosomal recessive polycystic kidney disease (ARPKD) typically develop collecting duct cysts lined by flattened epithelial cells, and congenital hepatic fibrosis. These features have been attributed to altered cellular morphology and spatial orientation, and increased TGF-b signaling in the biliary tract, respectively. The pathogenic mechanisms underlying these abnormalities have not been determined. In characterizing a renal epithelial cell line with inducible expression of PKHD1, the gene mutated in ARPKD, we observed cytomorphic changes opposite those of cystic epithelial cells and corresponding changes in Rho protein expression. In characterizing these findings, we discovered by co-IP and by IF that PD1 and Smurf1 form a complex. PD1 also associates with Smurf2, and levels of the Smurf2 target, Rap1B, changed in parallel with those of RhoA. Smurf1, 2 and their respective targets RhoA and Rap1B were localized differently in Pkhd1-deficient cells (PDCs) compared to controls, and these changes were associated with differences in the F-actin cytoskeleton and trafficking of E-cadherin. We also found enhanced clathrin-mediated endocytosis (CME) and reduced raft-mediated endocytosis (RME) in PDCs, with large vacuole-like lysosomes and intracellular cholesterol accumulation. Consistent with the roles of Smurfs and RME in down-regulating TGF-b activity and the converse role of CME in sending TGF-b signals to the nucleus, we found increased TGF-b activation in Pkhd1-defecient cholangiocyte cells. Our results provide a mechanistic explanation for both the cellular effects of PKHD1and in vivo phenotypic abnormalities that result from its mutation.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC075**

**The gamma-Secretase Cleavage Product of Polycystin-1 Regulates Tcf and CHOP-Mediated Transcriptional Activation through a p300-Dependent Mechanism**
David Merrick,[1] Hannah Chapin,[1] Zhiheng Yu,[2] Stefan Somlo,[2] John B. Hogenesch,[3] Michael J. Caplan.[1] *[1]Cellular and Molecular Physiology, Yale University, New Haven, CT; [2]Department of Medicine, Section of Nephrology, Yale University, New Haven, CT; [3]Pharmacology, University of Pennsylvania, Philadelphia, PA.*

Mutations in the gene encoding Polycystin-1 cause Autosomal Dominant Polycystic Kidney Disease. We show that the carboxy-terminal tail of Polycystin-1 is released by a gamma-secretase-mediated cleavage, and translocates to the nucleus where it is involved in the regulation of the Wnt and CHOP signaling pathways. Loss of Polycytin-1 expression results in increased proliferation and apoptosis, while re-introduction of its C-terminal tail fragment into polycystin-1 null cells reestablishes normal growth rate and apoptosis, and prevents cyst formation in three dimensional culture. Inhibition of gamma-secretase impairs the growth and apoptosis suppressive effects produced by full length polycystin-1 expression. The released polycystin-1 C-terminal tail fragment interacts with the transcription factors Tcf and CHOP, which regulate proliferation and apoptosis, respectively. Thus, the shedding of the extracellular domain of PC1, together with the cleavage and nuclear translocation of its cytoplasmic domain, mark PC1 as a member of a growing collection of plasma membrane proteins that are cleaved by gamma-secretase and participate in direct signaling to the nucleus. We find that the polycystin-1 tail employs a convergent mechanistic pathway for the regulation of Tcf and CHOP by disrupting these protein's interactions with their common transcriptional co-activator, p300. Regulation of Tcf and CHOP by the cleaved polycystin-1 C-terminal tail fragment explains how mutation of the gene encoding polycystin-1 can lead to the increased proliferation and apoptosis seen in Autosomal Dominant Polycystic Kidney Disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC076**

**Regulation of Wnt Pathway Genes by the Transcription Factor HNF-1β**
Sachin S. Hajarnis, Zhendong Ma, Peter Igarashi. *Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX.*

Mutations in hepatocyte nuclear factor-1β (HNF-1β), an epithelial- specific transcription factor, produce kidney cysts in humans. Recent studies in three rodent models of cystic kidney disease, including kidney-specific inactivation of HNF-1β, have shown that cyst formation is preceded by randomization of the orientation of cell division, a hallmark of altered planar cell polarity (PCP) and non-canonical Wnt signaling. Canonical (β-catenin-dependent) Wnt signaling is upregulated in some forms of PKD. We used SuperTopFlash luciferase reporter assays to measure canonical Wnt signaling in renal epithelial cells that express dominant-negative mutant HNF-1β (DN-HNF-1β). Treatment with LiCl or Wnt3a, known activators of canonical Wnt signaling, produced higher luciferase activity in cells expressing DN-HNF-1β compared with control cells. Western blot analysis showed that the levels of activated β-catenin were higher in cells expressing DN-HNF-1β. ChIP-chip analysis of renal epithelial cells identified several components of the canonical and non-canonical Wnt signaling pathways as novel transcriptional targets of HNF-1β. Consensus HNF-1β binding sites were identified within the gene promoters and chromatin-immunoprecipitation (ChIP) confirmed that HNF-1β binds to the promoters in vivo. Quantitative RT-PCR (Q-RT-PCR) confirmed that the expression of the Wnt pathway genes was altered in kidney cells expressing DN-HNF-1β. An unbiased microarray-based approach identified additional Wnt pathway genes whose expression is altered in cells expressing DN-HNF-1β. These results indicate that HNF-1β directly and indirectly regulates the expression of genes that are involved in the canonical and non-canonical Wnt pathways. Further elucidation of the molecular mechanism may provide new avenues for therapy of PKD directed at correcting the abnormalities in Wnt signaling.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC077**

**PC2 Regulates Canonical Wnt Signaling Requiring PC1 Expression** Ao Li, Bo Hu, Dan Liang, Guanqing Wu. *Medicine and Cell & Developmental Biology, Vanderbilt University, Nashville, TN.*

Autosomal dominant polycystic kidney disease (ADPKD), one of the most common monogenic disorders, exhibits numerous fluid-filled cysts in both kidneys. ADPKD is caused by at least two genes, *PKD1* and *PKD2*, and is therefore considered a genetically heterogeneous disease. Although recent studies have suggested that the gene products of both polycystin-1 and -2 (PC1 and PC2) are linked to β-catenin-dependent signaling, the precise molecular basis by which PC1 and PC2 affect this signaling pathway remain largely unknown. We established immortalized renal collecting duct cell lines from the kidneys of Im::*Pkd1*$^{-/-}$ mice and their wildtype littermates. Using these cell lines we demonstrate that PC2 participates in the regulation of canonical Wnt signaling and PC2 interacts with the β-catenin/E-cadherin complex required PC1 expression. Thus, it appears that PC1 may mediate PC2-associated canonical Wnt signaling. Utilizing a previously established null-*Pkd2* cell lines, we also found that loss of PC2 significantly downregulates PC1 expression *in vitro* and *in vivo* and the PC2-dependent PC1 reduction may result from increased E3 ubiquitin ligase Siah-1, which has been demonstrated to physically interact with PC1 and regulate PC1 proteasomal degradation. Our findings indicate that polycystins are important participants in canonical Wnt signaling in ADPKD, and provide new insight into the precise molecular hierarchy between polycystins onto the β-catenin/E-cadherin complex.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC078**

**Identification of a Polycystin-1 Cleavage Product, P100, That Regulates Store Operated Ca2+ Entry through Interactions with STIM1** Owen M. Woodward,[1] Shengqiang Yu,[2] Alessandra Boletta,[3] William B. Guggino,[1] Feng Qian.[2] *[1]Dept. of Physiology, Johns Hopkins School of Medicine, Baltimore, MD; [2]Dept. of Medicine, Div. of Nephrology, Johns Hopkins School of Medicine, Baltimore, MD; [3]Dulbecco Telethon Institute, Div. Genetics & Cell Biology, San Raffaele Scientific Institute, Milan, Italy.*

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a genetic disorder resulting in large kidney cysts and eventual kidney failure. Mutations in either the PKD1 or PKD2 / TRPP2 genes and their respective protein products, polycystin-1 (PC1) and polycystin-2 (PC2) result in ADPKD. PC2 is known to function as a non-selective cation channel, but PC1's function, and the function of PC1 cleavage products are not well understood. Here we identify an endogenous PC1 cleavage product, P100, a 100kDa fragment found in both wild type and epitope tagged PKD1 knock-in mice. Expression of full length human PC1 (FL PC1) and the resulting P100 and c-Terminal Fragment (CTF) cleavage products in both MDCK and CHO cells significantly reduces the store operated Ca2+ entry (SOCE) resulting from thapsigargin induced store depletion. Exploration into the roles of P100 and CTF in SOCE inhibition reveal that P100, when expressed in *Xenopus laevis* oocytes, directly inhibits the SOCE currents but CTF does not, nor does P100 when containing the disease causing R4227X mutation. Interestingly, we also found that in PC1 expressing MDCK cells, translocation of the ER Ca2+ sensor protein STIM1 to the cell periphery was significantly altered. In addition, P100 Co-immunoprecipitates with STIM1 but CTF does not. The expression of P100 in CHO cells recapitulates the STIM1 translocation inhibition seen with FL PC1. These data describe a novel polycystin-1 cleavage product, P100, which functions to reduce SOCE via direct inhibition of STIM1 translocation; a function with consequences for ADPKD. This work was supported by NIDDK grants DK032753-25A1 (to W.B.G.) and DK062199 (to F.Q.).

Disclosure of Financial Relationships: nothing to disclose

**TH-FC079**

**Multi-Target Based Therapy by Curcumin Delays Cystogenesis in a *Pkd1*-Deletion Mouse Model for ADPKD** Wouter N. Leonhard,[1] Anne Marike Van der Wal,[2] Zlata Novalic,[1] Martijn H. Breuning,[1] Emile De Heer,[2] Dorien J. M. Peters.[1] *[1]Human Genetics, Leiden University Medical Center, Leiden, Netherlands; [2]Pathology, Leiden University Medical Center, Leiden, Netherlands.*

Autosomal Dominant Polycystic Kidney Disease (ADPKD) caused by mutations in either the *PKD1* or *PKD2* gene, is a major cause for end-stage renal failure resulting from progressive cyst formation and fibrosis. Few compounds targeting specific cellular signaling pathways were able to inhibit cystogenesis in rodent models and are currently being tested in clinical trials. However, given the complex signaling in ADPKD, an ideal therapy would likely have to comprise several pathways at once. Therefore, multi-target compounds may provide promising therapeutic interventions for treatment of ADPKD.

To test this hypothesis we treated tamoxifen-inducible kidney epithelium-specific *Pkd1*-deletion mice with Curcumin (diferuloylmethane), a compound with limited side effects which is known to modulate several pathways. Curcumin-mediated inhibition has been reported for AP-1, STAT3, NF-κβ, Wnt/β-catenin signaling, TNFα, MAPKs, EGR-1, HIF-1α, Notch-1, and mTOR-regulated signaling. Many of these signaling pathways are altered in kidneys from patients with ADPKD.

Cystic kidneys of inducible *Pkd1*-deletion mice indeed demonstrated increased expression of phosphorylated rpS6 acting downstream of mTOR/S6K ($p<0.05$). In addition, we observed activation of STAT3, which strongly correlated with cyst progression ($p<0.05$ and $R^2=0.86$). Curcumin treatment reduced both mTOR and STAT3 activation *in vitro* and *in vivo* ($p<0.05$ and $p<0.01$). Importantly, Curcumin reduced KW/BW ratio at an intermediate stage of PKD (from 2.59 to 1.95%; $p<0.01$; n=5 for both groups) and postponed renal failure (from 105 to 119 days after *Pkd1* disruption; $p<0.001$; untreated n=11, Curcumin treated n=12).

In conclusion, this study provides evidence that multi-target compounds like Curcumin are able to delay cyst progression without apparent side effects. Current developments in the field of Curcumin analogues and other compounds acting in a similar fashion are highly promising and should be further evaluated as possible therapeutics for ADPKD.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC080**

**Modulation of Glycosphingolipid Metabolism as a New and Effective Approach for the Treatment of PKD** Thomas A. Natoli,[1] Herve Husson,[1] Kelly A. Rogers,[1] Laurie A. Smith,[1] Bing H. Wang,[2] Svetlana Komarnitsky,[2] Yeva Budman,[2] Alexei Belenky,[2] Nikolay Bukanov,[1] William R. Dackowski,[1] Ryan J. Russo,[1] James A. Shayman,[3] Steven R. Ledbetter,[1] John P. Leonard,[4] Oxana Beskrovnaya.[1] *[1]Cell Biology, Genzyme Corp., Framingham, MA; [2]Analytical Research & Development, Genzyme Corp., Waltham, MA; [3]Internal Medicine, University of Michigan, Ann Arbor, MI; [4]Pharmacology, Genzyme Corp., Waltham, MA.*

Polycystic kidney diseases (PKD) are characterized by growth of fluid-filled cysts in kidneys and other organs ultimately leading to ESRD. Sphingolipids (SL) and glycosphingolipids (GSL) have recently been recognized as playing important roles in the regulation of numerous cellular processes, including proliferation, apoptosis, and modulation of mitogenic signaling pathways. Many of these processes are affected in multiple forms of PKD regardless of the causative mutation. We sought to evaluate the therapeutic potential for GSL modulation as a new approach to treat PKD. We demonstrate that glucosylceramide (GlcCer) and ganglioside GM3 levels are elevated in human PKD kidneys and in cystic kidneys of mouse models orthologous to ADPKD and nephronophthisis as compared to normal counterparts. To reduce the levels of GlcCer and its derivatives in cystic kidneys we used a highly specific inhibitor of GlcCer synthase that blocks the conversion of ceramide to GlcCer. Treatment with GlcCer synthase inhibitor reduced kidney GlcCer and GM3 levels and effectively attenuated PKD progression in mouse model with conditionally inactivated *Pkd1* gene as well as in two models of nephronophthisis: *pcy* and *jck*. Mechanistic studies showed that inhibition of GSL synthesis leads to effective cell cycle blockade and inhibition of Akt-mTOR signaling in cystic kidneys. Together, these data demonstrate for the first time that modulation of GSL metabolism is a new and effective approach to treat PKD.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC081**

**CKD-EPI and MDRD Study Equations for GFR Estimation and Mortality: The Third National Health and Nutrition Examination Survey (NHANES III)** Tariq Shafi,[1] Kunihiro Matsushita,[1] Brad C. Astor,[1] Elizabeth Selvin,[1] Lesley A. Stevens,[2] Josef Coresh.[1] *[1]Johns Hopkins University, Baltimore, MD; [2]Tufts Medical Center, Boston, MA.*

The Chronic Kidney Disease Epidemiology (CKD-EPI) equation improves glomerular filtration rate estimation (eGFR) compared with Modification of Diet in Renal Disease (MDRD) Study equation but its association with mortality in the general population has not been studied. We compared the associations between eGFR, estimated from serum creatinine by the CKD-EPI ($eGFR_{CKDEPI}$) and MDRD equations ($eGFR_{MDRD}$), and mortality in 15,818 NHANES III participants aged ≥17 yrs with 18yrs of mortality follow-up (till 12/31/2006). To evaluate the effect of reclassification by $eGFR_{CKDEPI}$, we calculated the net reclassification improvement (NRI). NRI=clinically correct classification-clinically incorrect classification. NRI ranges from 0 (no improvement) to 1 (perfect). $eGFR_{CKDEPI}$ reclassified 1,472 (20%), 1,819 (43%), 114 (24%) and 8 (4%) of the participants with $eGFR_{MDRD}$ categories 90-119, 60-89, 45-59 and 30-44 ml/min/1.73m$^2$, respectively, to a higher eGFR category. <5% participants were reclassified to a lower eGFR category. Participants with $eGFR_{MDRD}$ 45-59 ml/min/1.73m$^2$ who were reclassified upward had a 44% lower risk of all-cause mortality (adjusted incidence rate ratio [IRR], 0.56; 95% CI, 0.35-0.88) and a trend towards lower risk of cardiovascular disease (CVD) mortality (adjusted IRR, 0.55; 95% CI, 0.28-1.06) than those not reclassified. Those reclassified upwards were more likely to be younger, female, white, had less CVD and lower blood pressure. NRI was significantly improved across all sub-groups. In the general US population, the CKD-EPI equation improved risk stratification of persons at risk for mortality. These data support the adoption of CKD-EPI equation for GFR estimation.

NRI* by CKD-EPI eGFR

| | Mortality | |
|---|---|---|
| Group | All-Cause | CVD |
| All | 0.28 | 0.29 |
| Age ≥65 yrs | 0.15 | 0.10 |
| White | 0.29 | 0.31 |
| Black | 0.28 | 0.27 |
| Male | 0.27 | 0.29 |
| Female | 0.29 | 0.29 |
| ACR<30 | 0.26 | 0.27 |
| ACR 30-299 | 0.35 | 0.30 |
| ACR ≥300 | 0.39 | 0.23 |

ACR, albumin/creatinine (mg/g); *p<0.001 for all results

Disclosure of Financial Relationships: Honoraria: Novartis.

**TH-FC082**

**Risk Implications of the CKD-EPI Creatinine GFR Equation Compared to the MDRD Study Equation** Kunihiro Matsushita,[1] Marcello Tonelli,[2] Anita Lloyd,[2] Andrew S. Levey,[4] Josef Coresh,[1] Brenda Hemmelgarn.[3] *[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [2]Departments of Medicine, University of Alberta, Edmonton, AB, Canada; [3]Departments of Medicine, University of Calgary, Calgary, AB, Canada; [4]Division of Nephrology, Tufts Medical Center, Boston, MA.*

**Purpose:** To evaluate whether the CKD-EPI creatinine equation for estimated GFR (eGFR) predicts clinical outcomes better than MDRD Study equation in a large cohort derived from the Alberta Kidney Disease Network with mean follow-up time of 2.3 years.

**Methods:** We categorized 690,202 participants into five groups of eGFR (≥90, 60-89, 45-59, 30-44, 15-29 ml/min/1.73m$^2$) by both equations and assessed reclassification by CKD-EPI equation and its association with clinical outcomes.

**Results:** The CKD-EPI equation reclassified 33.5% of participants with $eGFR_{MDRD}$ 60-89, 39.1% with $eGFR_{MDRD}$ 45-59, and 15.5% with $eGFR_{MDRD}$ 30-44.

| eGFR (MDRD) | eGFR (CKD-EPI) ≥90 | 60-89 | 45-59 | 30-44 | 15-29 | Reclassification |
|---|---|---|---|---|---|---|
| ≥90, n | 182,502 | 3,106 | 0 | 0 | 0 | 1.7% |
| ESRD | 0.05 (0.0-0.1) | 0.14 (0.0-1.0) | - | - | - | |
| Death | 4.5 (4.3-4.7) | 67.5 (62-74) | - | - | - | |
| AMI | 0.7 (0.6-0.8) | 5.8 (4.3-7.9) | - | - | - | |
| 60-89, n | 143,284 | 286,305 | 1,019 | 0 | 0 | 33.5% |
| ESRD | 0.03 (0.0-0.1) | 0.05 (0.0-0.1) | 0.45 (0.1-3.2) | - | - | |
| Death | 2.0 (1.9-2.2) | 8.2 (8.0-8.4) | 94.0 (82-107) | - | - | |
| AMI | 0.7 (0.6-0.8) | 2.2 (2.1-2.3) | 12.2 (8.4-18) | - | - | |
| 45-59, n | 0 | 22,316 | 36,189 | 938 | 0 | 39.1% |
| ESRD | - | 0.02 (0.0-0.1) | 0.40 (0.3-0.6) | no event | | |
| Death | - | 5.0 (4.5-5.6) | 29.1 (28-30) | 127 (112-144) | | |
| AMI | - | 1.8 (1.4-2.1) | 5.9 (5.5-6.3) | 11.9 (8.0-18) | - | |
| 30-44, n | 0 | 0 | 1,475 | 9,993 | 359 | 15.5% |
| ESRD | - | - | 1.6 (0.7-3.6) | 3.4 (2.7-4.2) | 4.1 (1.3-13) | |
| Death | - | - | 16.0 (12-21) | 72.7 (69-76) | 182 (154-216) | |
| AMI | - | - | 3.8 (2.2-6.3) | 10.4 (9.2-12) | 15.3 (8.5-28) | |
| 15-29, n | 0 | 0 | 0 | 99 | 2,617 | 3.8% |
| ESRD | - | - | - | 12.9 (4.2-40) | 32.8 (26-38) | |
| Death | - | - | - | 21.0 (6.7-50) | 139 (130-150) | |
| AMI | - | - | - | 4.2 (0.6-30) | 15.2 (12-19) | |

The reclassification was mostly upward to a higher eGFR category, lowering the prevalence of CKD stage 3-4 from 10.7% to 7.6%. Participants who were reclassified upward had lower incidence rates per 1,000 person-years compared to those who were not reclassified (ESRD: 0.02 vs. 0.4, deaths: 5.0 vs. 29.1 and acute myocardial infarction [AMI]: 1.8 vs. 5.9 in $eGFR_{MDRD}$ 45-59, all P-values <0.001) (figure). Similar results were observed after the adjustment for age and sex. Significant net reclassification improvement by CKD-EPI equation was observed for all outcomes (ESRD: 0.191, deaths: 0.246, AMI: 0.162, all P-values <0.001). Similar results were observed across dipstick (negative, trace/1+, ≥2+) and age (<45, 45-54, 55-64, ≥65 y) categories.

**Conclusion:** The CKD-EPI equation more accurately categorized individuals as compared to MDRD Study equation regarding clinical risk, suggesting its clinical usefulness.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC083**

**Applicability of the MDRD and CKD-EPI Equations for Estimation of GFR in Older People** Hannah Kilbride,[1] Gillian Lorraine Eaglestone,[1] Sarah J. Knight,[1] Paul E. Stevens,[1] Michael Delaney,[1] Christopher K. T. Farmer,[1] Shelagh E. O'Riordan,[1] R. Neil Dalton,[2] Edmund J. Lamb.[3] *[1]Kent Kidney Care Centre, East Kent Hospitals, Canterbury, Kent, United Kingdom; [2]Well Child Laboratory, St Thomas' Hospital, London, United Kingdom; [3]Department of Clinical Biochemistry, East Kent Hospitals, Canterbury, Kent, United Kingdom.*

Introduction

Measurement of glomerular filtration rate (GFR) is an essential tool in the detection, management and evaluation of CKD. GFR is commonly estimated using the Modification of Diet in Renal Disease (MDRD) Study equation. Recently the CKD epidemiology collaboration (CKD-EPI) equation has been proposed, claiming reduced bias. Neither equation has been extensively validated in older people and it is frequently claimed that these equations under estimate true GFR in this population.

Aim

To examine the relationship between true GFR and estimated GFR in older people.

Methods

Using difference plot analysis on log transformed data we examined the relationship between GFR measured by a reference iohexol method and estimated GFR from the MDRD and CKD-EPI equations in people aged over 74 y. Subjects were asked to avoid meat consumption prior to the test and following a 5 mL IV bolus of Omnipaque 240 venous samples were taken at 5, 120, 180 and 240 mins post-injection. Iohexol was measured by isotope dilution mass spectrometry (IDMS) and iohexol clearance was calculated. Serum creatinine was measured by IDMS and the IDMS-related MDRD and CKD-EPI GFRs were calculated.

Results

To date 127 subjects with a median age of 80 y (range 74-97), 67 female and 61 male, have been studied.

Performance characteristics of the MDRD and CKD-EPI equations versus true GFR

| | Median GFR (mL/min/1.73m$^2$) | Bias, % (± 95% CI) | Percent within 30% | 95% limits of agreement |
|---|---|---|---|---|
| Iohexol | 43, IQR 30 to 62 | | | |
| MDRD | 48, IQR 30 to 71 | 7.0 (2.1 to 12.0) | 75 | 36 to 79 |
| CKD-EPI | 46, IQR 29 to 71 | 2.3 (-2.3 to 7.2) | 78 | 39 to 71 |

Conclusion

This study shows no evidence to support the contention that the MDRD equation over diagnoses CKD in older people. Our data suggests that the CKD-EPI equation may be slightly more accurate than the MDRD equation in this population.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC084**

**Relative Performance of the MDRD and CKD-EPI Equations for Estimating Glomerular Filtration Rate in Different Patient Populations** Kazunori Murata,[1] Timothy S. Larson,[1,2] Amy K. Saenger,[1] Andrew D. Rule,[2] Nikola A. Baumann,[1] John C. Lieske.[1,2] *[1]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [2]Department of Internal Medicine, Mayo Clinic, Rochester, MN.*

The objective of our study was to examine the ability of both the MDRD and CKD-EPI equations to estimate glomerular filtration rate (GFR) in a variety of patient populations, including the elderly, healthy patients, nephrectomized patients, and kidney transplant recipients.

A cross sectional study was conducted of patients who underwent renal function assessment for clinical purposes by simultaneous measurements of serum creatinine (IDMS-traceable method), estimation of GFR using the MDRD and CKD-EPI equations, and renal iothalamate clearance (n=5238). The study population consisted of CKD patients (n=2324), nephrectomized kidney donors (n=97), potential kidney donors (n=583), kidney transplant recipients (n=1375), and non-kidney transplant recipients (n=859); 16.6% (n=870) were > age 70.

The MDRD and CKD-EPI equations performed similarly in elderly and non-elderly patients regarding ability to correctly stage CKD patients. Overall, % bias (estimated GFR minus measured GFR) with either equation did not differ by age. Within the nephrectomized and healthy populations the CKD-EPI equation demonstrated less bias than the MDRD equation, and classified patients better into CKD stage. The CKD-EPI equation gave estimates that were biased by 6, 8, -8, and -7% in CKD patients, kidney transplant recipients, potential donors, and nephrectomized donors, respectively, while the MDRD equation gave estimates that were biased by 3, 1, -18, and -15%. Notably, among potential kidney donors the CKD-EPI equation had greater specificity for detecting measured GFR <60 ml/min/1.73m$^2$ than the MDRD equation (98 vs 94%) but lower sensitivity (50 vs 70%).

Therefore, the MDRD and CKD-EPI equations perform similarly in elderly and nonelderly patients with CKD and/or status post organ transplantation. Among people who present in good health, the CKD-EPI equation is more specific for measured GFR < 60 ml/min/1.73m$^2$, but also less sensitive.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC085**

**CKD, Metabolic Abnormalities and Outcomes in US Adults < 65: CKD-EPI or MDRD Formulas?** Yi Peng,[1] Robert N. Foley,[1,2] David T. Gilbertson,[1] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

**Background:**

It has recently been suggested that the CKD-EPI creatinine equation (Ann Intern Med. 2009;150:604-612.) is more accurate than the MDRD equation for GFR estimation and should replace it for routine clinical use. However, outcome comparisons in the setting of large administrative databases are lacking.

**Methods:**

This analysis considered 724,205 subjects aged 20-64, enrolled in fee-for-service plans, and had at least one serum creatinine measured in the U.S. throughout 2008 (Ingenix i3 database). eGFR < 60 was defined with the MDRD and CKD-EPI equations. ICD-9-CM claims were used to define comorbidity and hospitalization and metabolic abnormalities were identified from laboratory measurement files.

**Results:**

4.2 % had GFR < 60 with the CKD-EPI formula and 8.0% with the MDRD formula. For eGFR < 60, use of the CKD-EPI formula (vs. the MDRD formula) was associated (P < 0.05) with a higher prevalence (%) of WHO anemia (21.4/15.6), hypertension (63.2/52.9), cardiovascular disease (23.4/18.8), high PTH (33.8/29.8), low HDL (41.2/37.3), high triglycerides (33.8/29.8), high uric acid (35.3/28.1), low calcium (6.5/5.5) and high glucose (43.1/37.2). The corresponding values for hospitalization for 1 to 7 days, hospitalization for > 7 days, 1 admission and 2+ admissions were 9.0/7.6, 5.0/3.6, 9.4/7.8 and 4.7/3.4, respectively.

**Conclusions:**

Regarding metabolic abnormalities and hospitalization outcomes, low GFR defined with the CKD-EPI formula has substantially more sensitivity than with the MDRD formula.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC086

**Evaluation of Creatinine Based GFR Estimating Equation in an HIV Positive Population** Lesley A. Stevens,[1] Christina M. Wyatt,[2] Rebecca Creamer,[3] James Hellinger,[1] Mathew Hotta,[2] Maia Leppo,[1] Andrew S. Levey,[1] Aghogho A. Okparavero,[1] Sunila Reddy,[4] Martin Rhee,[1] Karen Savage,[3] Fran Wallach,[2] Zipporah Krishnasami.[3] *[1]Tufts Medical Center; [2]Mt. Sinai School of Medicine; [3]University of Alabama; [4]Gilead Sciences, Inc.*

**Background** Changes in muscle mass and fat distribution may occur with advanced HIV disease and with the use of some anti-retroviral agents. These changes may affect the accuracy of creatinine based GFR estimating equations.

**Methods** We will measure GFR using plasma clearance of iohexol in 200 HIV positive patients on stable antiretroviral therapy. Here we report preliminary results from the first 125 participants. Creatinine was assayed using standardized methods. GFR was estimated by the MDRD Study and CKD-EPI equations, and performance is described for the whole cohort and for subgroups defined by eGFR, age, sex, race, and body mass index (BMI).

**Results** Mean (SD) measured GFR was 86 (25) ml/min/1.73 m$^2$. The table shows median difference and interquartile range (IQR) overall and by subgroup.

**Conclusions** The CKD-EPI equation is more accurate than the MDRD Study equation in all subgroups except BMI less than 20 kg/m$^2$, although sample size was small. The relative improvement of tbe CKD-EPI equation compared to the MDRD study equation is similar to those reported in non-HIV populations. Creatinine based GFR estimating equations can be used in HIV positive patients.

Table: Performance by Subgroup

| | | MDRD | | | CKD-EPI | | |
|---|---|---|---|---|---|---|---|
| | | N | Difference | IQR | N | Difference | IQR |
| Overall | | 125 | 11.3 | 22.4 | 125 | 5 | 23.4 |
| eGFR (ml/min/1.73 m$^2$) | >90 | 33 | -1.7 | 17.9 | 49 | -1.3 | 22.6 |
| | 60-89 | 55 | 16.0 | 18.6 | 48 | 10.6 | 22.9 |
| | <60 | 37 | 11.9 | 20.9 | 28 | 7.4 | 20.7 |
| Age (years) | <40 | 20 | 14.3 | 16.4 | 20 | 9.1 | 15.4 |
| | 40-65 | 90 | 9.4 | 23.8 | 90 | 3.0 | 26.6 |
| | >65 | 15 | 7.5 | 22.4 | 15 | 5.8 | 23.1 |
| Male | Y | 84 | 12.9 | 23.7 | 84 | 5.9 | 23.8 |
| | N | 41 | 7.1 | 18.8 | 41 | -.06 | 23.0 |
| Black | Y | 55 | 13.7 | 18.7 | 55 | 5.9 | 18.2 |
| | N | 70 | 4.8 | 23.0 | 70 | 1.1 | 26.4 |
| BMI (kg/m$^2$) | <20 | 10 | -0.8 | 21.5 | 10 | 4.8 | 30.5 |
| | 20-25 | 39 | 11.9 | 19.5 | 39 | 5.0 | 22.0 |
| | 25-30 | 49 | 12.3 | 18.9 | 49 | 3.1 | 21.8 |
| | >35 | 27 | 15.5 | 36.9 | 27 | 10.9 | 31.1 |

Difference= Measured – estimated GFR

Disclosure of Financial Relationships: Consultancy: Orexigen Therapeutic Inc. Research Funding: Gilead Inc.

## TH-FC087

**Discordant Glomerular Filtration Rate Determinations between Iothalamate and Iohexol Renal Clearances** Jesse C. Seegmiller,[2] Bradley E. Burns,[2] John C. Lieske,[1,2] Timothy S. Larson.[1,2] *[1]Division of Nephrology and Department of Internal Medicine, Mayo Clinic; [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

***Purpose:*** Few studies have compared renal clearances of iothalamate with iohexol, and controversy exists as to their comparability. The purpose of this study was to directly compare the renal clearances of these analytes across a wide range of glomerular filtration rate (GFR).

***Methods:*** 150 study subjects received concurrent subcutaneous injections of iothalamate (iothalamate meglumine 600 mg/mL) and iohexol (iohexol 647.1 mg/mL) followed by timed urine and plasma collections. Iothalamate and iohexol were measured in urine and plasma specimens by a novel liquid chromatography tandem mass spectrometry method (LC-MS/MS; ABSCIEX 5500), and GFR calculated from the clearance of each analyte by standard formulae. Both iothalamate and iohexol were analyzed using multiple reaction monitoring (MRM) transitions. Simultaneous creatinine clearance was also measured. To evaluate for possible protein complexation by either analyte, plasma samples from 3 study subjects were dialyzed using a 2000 molecular weight cutoff membrane against a phosphate buffered saline dialysate (Slide-a-lyzer; ThermoFisher).

***Results:*** Among the 150 study subjects the range of iothalamate and iohexol renal clearances was 1-132 mL/min/1.73m$^2$. The renal clearance of iohexol was consistently lower than iothalamate clearance (%mean difference =-14.7% (95% CI -16.3% to -13.0%; p= <0.0001). The % difference was constant throughout the range of measured GFRs. As expected, creatinine clearances were significantly greater than both iothalamate and iohexol clearances by 24% (95% CI 19.57% to 28.29%) and 38% (95% CI 33.72% to 42.35%) respectively. Plasma dialysis experiments revealed significantly greater post dialysis concentration ratios for iohexol compared to iothalamate suggesting greater protein binding of iohexol.

***Conclusion:*** These results demonstrate that renal clearance of iohexol is significantly lower than renal clearance of iothalamate across a wide range of GFRs. This difference may be due to greater plasma protein binding of iohexol as compared to iothalalmate.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC088

**Drift in Dade Behring Cystatin C Assay 2003 to 2009** Lesley A. Stevens,[1] Jane Manzi,[2] Andrew S. Levey,[1] John H. Eckfeldt,[3] Frederick Van Lente,[0] Josef Coresh.[2] *[1]Nephrology, Tufts Medical Center, Boston, MA; [2]Johns Hopkins University; [3]University of Minnesota.*

**Background:** Variation in GFR estimating equation performance is in part due to measurement biases of filtration markers among laboratories. The CKD-EPI cystatin C (CysC) 2008 equation was developed in 2003 using samples assayed at the Cleveland Clinic (CC) (Stevens et al. AJKD 2008). We and others observed a drift in CysC concentrations. Magnitude of the drift was determined by comparing a calibration panel assayed in 2003 and 2009. An equation re-expressing 2009 values as 2003 values was developed.

**Methods:** Serum calibration panels were created in 2003 at CC and stored at -70$^o$. The panel includes 40 pooled donor sera covering serum reatinine concentrations from 0.5 to 5.0 mg/dL. The panel was assayed at CC in 2003 using Dade Behring (DB) PENIA reagents and a BNII nephelometer, and at the University of Minnesota (UMN) in 2009 using the same PENIA reagent on a DB ProSpec nephelometer. Conversion factors were determined using Deming linear regression. eGFR from CysC was calculated using '03 and '09 CysC values.

**Results:** Mean (SD) level of CysC was 2.01 (0.74) mg/L in CC'03 and 1.67 (0.58) mg/L in UMN '09. The mean (SD) difference was -0.34 (0.16) mg/L (p-value <0.01). Deming regression shows $\mathbf{CysC_{CC'03} = 1.267 * CysC_{UMN'09} - 0.105}$ ($R^2$=0.9951). The table shows the impact on eGFR.

**Conclusion:** CysC concentrations measured by DB methods have changed substantially. Without correction, the downward drift in CysC values from 2003 to 2009 would lead to substantially higher eGFR and underestimate CKD prevalence. CysC methods should be made traceable to international reference materials. In the interim, the equation above allows for conversion of DB 2009 CysC values to DB 2003 values.

| CysC (mg/L) | | | eGFR-cys 2003 Equation (ml/min/1.73 m$^2$) | | |
|---|---|---|---|---|---|
| 2009 | 2003 | Difference (2009-2003) | 2009 | 2003 | Difference (2009-2003) |
| 0.87 | 1.00 | -0.13 | 91 | 77 | 14 |
| 1.00 | 1.16 | -0.16 | 77 | 64 | 13 |
| 1.10 | 1.29 | -0.19 | 68 | 57 | 12 |
| 1.20 | 1.42 | -0.22 | 62 | 51 | 11 |
| 2.00 | 2.43 | -0.43 | 34 | 27 | 7 |

eGFR-Cys= $76.7 * CysC^{-1.19}$

Disclosure of Financial Relationships: Consultancy: Orexigen Therapeutic Inc.Research Funding: Gilead Inc.

## TH-FC089

**Estimating Progression Risk in a CKD Population in Hawaii** Brian J. Lee. *Nephrology Division, Hawaii Permanente Medical Group, Honolulu, HI.*

Current guidelines subdivide CKD based on degree of impaired renal function, rather than by risk of progression. Within a health maintenance organization in Hawaii with 220,000 patients, we noticed that proteinuria seemed to improve prediction of risk. We developed a model of GFR-proteinuria stratification and compared with GFR stratification.

Methods: a cohort of 8831 with baseline estimated GFR (MDRD 4 variable) between 20 and 59 mL/min/1.73m2 was followed for 2 years. Fewer than 5% were lost to follow-up. The GFR was divided into 20s, 30s, 40s and 50s; Proteinuria as urine protein/creatinine ratio was divided into 1, 1-2, 2-4 and over 4 mg/mg. The sixteen categories were ranked by rate of ESRD for purposes of ROC analysis.

Results:

2 year ESRD risk, by baseline GFR and urine protein/creatinine ratio

| | < 1 | 1-1.999 | 2-3.999 | 4+ | unknown | Total |
|---|---|---|---|---|---|---|
| 20-29 | 2.65% | 7.41% | 17.39% | 38.24% | 5.15% | 9.04% |
| 30-39 | 0.62% | 5.00% | 7.84% | 36.67% | 1.08% | 2.50% |
| 40-49 | 0.15% | 2.78% | 6.38% | 10.00% | 0.00% | 0.38% |
| 50-59 | 0.00% | 0.00% | 4.00% | 5.26% | 0.08% | 0.12% |
| Total | 0.26% | 2.51% | 8.76% | 24.78% | 0.28% | 0.84% |

ROC curve areas were 0.859 (continuous GFR) and 0.945 (GFR-proteinuria categories)

Conclusions: Progression risk was highly dependent on proteinuria, and estimating risk using both GFR and proteinuria was superior to using GFR alone. A two dimensional stratification system should guide selection of patients for referral and other intensive management. Our data supports efforts to create a new CKD staging system.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC090

**Racial Differences in Kidney Function Decline among Persons without Chronic Kidney Disease – The Multi-Ethnic Study of Atherosclerosis** Carmen A. Peralta,[1] Ronit Katz,[2] Ian H. de Boer,[2] Joachim H. Ix,[5] Holly J. Kramer,[3] Mark J. Sarnak,[4] Bryan R. Kestenbaum,[2] Moyses Szklo,[6] Steven Shea,[7] David Siscovick,[2] Michael Shlipak.[1] *[1]University of California San Francisco and SF VA Medical Center; [2]University of Washington; [3]Loyola Medical Center; [4]Tufts Medical Center; [5]University of California San Diego; [6]Johns Hopkins University; [7]Columbia University.*

It is currently unknown whether or not race/ethnic groups differ in their rate of kidney function decline prior to the onset of CKD (eGFR <60ml/min/1.73m$^2$).

We studied race/ethnic differences in kidney function decline among White, Black, Hispanic and Chinese participants in MESA using linear mixed models over a five year

follow up with with 2 to 3 repeated measures of kidney function. GFR was estimated by cystatin C (eGFRcys) and creatinine- CKD Epi (eGFRcreat). We evaluated potential mediators in staged models.

Among 5179 adults aged 60±10 years with eGFRcreat >60 ml/min/1.73m$^2$ at baseline, mean (SD) changes in eGFRcys and eGFRcreat were -1.1 (4.4) and -1.5 (2.8) ml/min/1.73m$^{2/}$ year, respectively. Blacks had higher rates of eGFRcys decline compared with whites: -1.4 (4.8) vs. -0.88 (4.3) ml/min/1.73m$^2$/year. This difference was not attenuated by adjustment for age, sex, income, education, HDL, LDL, BMI, C-reactive protein, smoking, diabetes, hypertension, or systolic blood pressure : β: 0.31 ml/min/1.73m$^2$/year faster decline for Blacks, p=0.001. Hispanics also had faster rates of decline, but these varied by country of origin. Dominicans declined the fastest compared with whites (β 0.55, p < 0.011), followed by Puerto Ricans (β 0.47, p 0.03) after full adjustment, whereas Mexicans, South American and Other Hispanics did not differ significantly from Whites. Chinese had similar rates of eGFRcys decline compared with Whites (β 0.16, p 0.18). Findings were similar when we used eGFRcreat as the outcome.

Race/Ethnic differences in kidney function decline occur early, prior to the onset of clinical CKD, and are not explained by differences in traditional mediators. Future studies should focus on identifying persons at highest risk in order to develop targeted prevention strategies.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC091**

**Modulation of Inflammatory Kidney Injury with Leukadherins** Vineet Gupta, Jochen Reiser, Dony Maiguel, Hafeez Faridi, Changli Wei. *Division of Nephrology & Hypertension, Dept of Medicine, Miller School of Medicine, University of Miami, Miami, FL.*

Inflammatory renal diseases classically affect the glomerulus or the interstitium. Leukocyte tissue accumulation is a hallmark of renal ischemia-reperfusion (I/R) injury and also key to the pathogenesis of anti-GBM nephritis. The integrin CD11b/CD18 (a.k.a αMβ2, Mac-1) is the predominant integrin receptor expressed on the surface of leukocytes and plays a central role in mediating pro-inflammatory functions of these cells. Current treatments include non-steroidal and steroidal medications to reduce inflammatory damage. Unfortunately, these treatments are not always effective and harbor potential serious side effects. While blocking integrin-mediated leukocyte adhesion provided some additional benefit in many experimental models of inflammatory diseases, the blocking agents have had little success in treating inflammatory/autoimmune diseases in humans and β2 integrin blockers have also shown unexpected side effects.

We have pursued an alternative approach that involves pharmacologic activation, rather than inhibition, of leukocytic integrin CD11b/CD18 as a mechanism for reducing inflammatory injury. By screening a chemical library of >100,000 compounds, we recently discovered three novel small molecules that we have termed leukadherins. Leukadherins bind to CD11b/CD18, whereby they increase CD11b/CD18-dependent cell-adhesion and decrease cell migration in vitro. Using murine models of I/R injury and anti-GBM nephritis, we show that leukadherin administration reduces leukocyte recruitment and tissue accumulation in vivo and preserves renal function upon kidney injury. Mechanistically, we find that leukadherin treatment decreases both, leukocyte migration as well as the secretion of soluble factors. Our results suggest that leukadherins constitute a novel class of anti-inflammatory compounds that offer a unique approach for modulating inflammatory diseases of the kidney and other organs, via pharmacological activation of CD11b/CD18 and modulating leukocyte infiltration and function.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC092**

**Ischemia-Reperfusion Induces Interferon-Regulatory-Factor-4 in Renal Dendritic Cells Which Suppresses Postischemic Inflammation and Prevents Acute Renal Failure** Maciej Lech, Saraswati Linda Lassen, Hans J. Anders. *Medizinische Poliklinik, University of Munich, Munich, Germany.*

Ischemia-reperfusion (IR) activates Toll-like receptors which fosters sterile inflammation, for example in postischemic acute renal failure. Unexpectedly, TLR signaling predominates in intrinsic renal cells and not in intrarenal antigen-presenting cells in the postischemic kidney. We hypothesized that certain factors suppress antigen-presenting cell activation and thereby limit sterile renal inflammation, e.g. interferon-regulatory-factor (IRF)-4, an inducible inhibitor of LPS signaling.

Oxidative stress was induced in vitro and found to be a trigger for IRF4 induction in myeloid cells in-vitro as well as in CD45+/CD11c+ cells in the postischemic kidney. Oxidative stress did not induce IRF4 in tubular epithelial cells. Lack of IRF4 aggravated acute renal failure 24 hours after renal artery clamping together with increased intrarenal expression of TNF-α, IL-6, CXCL2, and CCL2 as well as excessive tubular necrosis and peritubular neutrophil influx as compared to wildtype IR kidneys. This effect almost entirely depended on the role of IRF4 to suppress TNF-α release by intrarenal antigen-presenting cells because either clodronate liposome depletion of these cells or TNF-α blockade with etanercept entirely abrogated the aggravation of cytokine expression and acute renal failure in *Irf4*-deficient mice.

Thus, loss-of-function mutations in the IRF4 gene predispose to IR injury because the postischemic induction of IRF4 in resident antigen-presenting cells like CD11c+ dendritic cells, suppresses them to secrete TNF-α, and thereby limits inappropriate immunopathology.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC093**

**Slit2 Impairs Neutrophil Adhesion and Improves Renal Function in Ischemia Reperfusion Injury** Swasti Chaturvedi,[1] Soumitra Tole,[2] Liping Huang,[3] Ilya Mukovozov,[2] Guang Ying Liu,[2] Yi-Wei Huang,[2] Mark D. Okusa,[3] Lisa Robinson.[1] *[1]Nephrology, The Hospital for Sick Children, Toronto, Canada; [2]Research Institute, The Hospital for Sick Children, Toronto, Canada; [3]Medicine, University of Virginia Health System, Charlottesville, VA.*

Acute Kidney Injury (AKI) occurs in approximately 5% of hospitalized patients and leads to significant morbidity and mortality. Inflammation marked by recruitment of circulating leukocytes, particularly neutrophils, in the injured kidney is a key component of AKI caused by ischemia-reperfusion injury (IRI). Recruited leukocytes exacerbate injury by releasing inflammatory mediators. Therapies targeting different leukocyte subsets help in ameliorating injury. However no single therapy is entirely effective because of diversity of recruiting signals and the cells recruited. The neuronal guidance cue, Slit2 and its transmembrane receptor roundabout (Robo) prevents axonal migration during the central nervous system development. Recently it has also been shown that Slit2 inhibits chemotaxis of neutrophils and lymphocytes towards diverse chemoattractants by preventing activation of the small GTPases, Rac and Cdc42.

To determine whether Slit2 could be used to prevent AKI, we examined whether Slit2 prevents adhesion of human neutrophils to human vascular endothelial cells subjected to IRI. Slit2 significantly reduced neutrophil adhesion to endothelial cells subjected to hypoxia and periods of reperfusion ranging from 30-180 minutes ($p<0.05$). In an *in vivo* mouse model of renal IRI, where mouse kidneys were subjected to 28 min ischemia followed by 24 h reperfusion, Slit2 administered intraperitoneally 1 hr prior to reperfusion significantly prevented the rise in serum creatinine in a dose dependent manner (0.5-2.0 μg). Because activation of Rac and Cdc42 plays a role in protective immune functions of neutrophils, we determined the effects of Slit2 on these functions. Slit2 did not impair the ability of neutrophils to phagocytose opsonised particles, nor to produce superoxide production following inflammatory stimulation. These findings suggest that Slit2 could be used to prevent and treat the inflammatory cell recruitment in AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC094**

**TNFR2 Interposes the Proliferative and NF-κB-Mediated Inflammatory Response by Podocytes to TNF-α** Leslie A. Bruggeman,[1] Paul E. Drawz,[1] Nicole Kahoud,[3] Ke Lin,[2] Laura M. C. Barisoni,[2] Peter J. Nelson.[3] *[1]Divison of Nephrology, MetroHealth Medical Center, Case Western Reserve University, Cleveland, OH; [2]Department of Pathology, New York University, New York, NY; [3]Division of Nephrology, University of Washington, Seattle, WA.*

The development of proliferative podocytopathies has been linked to ligation of TNFR2 expressed on the renal parenchyma; however, the TNFR2 positive cells within the kidney responsible for podocyte injury are unknown. We detected de novo expression of TNFR2 on podocytes prior to hyperplastic injury in crescentic glomerulonephritis of mice with nephrotoxic nephritis, and in collapsing glomerulopathy of Tg26 mice (a model of HIVAN) and B6 *kd/kd* mice, as well as human biopsies with collapsing glomerulopathy, but not primary FSGS or normal transplant tissue. We further found that serum levels of soluble TNF-α and TNFR2 correlated significantly with renal injury in Tg26 mice. Thus, we asked whether ligand binding of TNFR2 on podocytes ex vivo precipitates the characteristic proliferative and pro-inflammatory diseased podocyte phenotypes. Soluble TNF-α activated NF-κB and dose-dependently induced podocyte proliferation, marked by expression of the podocyte G1 cyclin and the NF-κB target gene cyclin D1. Microarray gene and chemokine protein expression profiling showed a marked pro-inflammatory NF-κB signature, and activated podocytes secreted CCL2 and CCL5 and induced macrophage migration in transwell assays. Neutralization of TNFR2 on podocytes with blocking antibodies abrogated NF-κB activation and the induction of cyclin D1 by TNF-α. In similar studies, IκBα degradation, the initiating event in NF-κB activation, was dependent on TNFR2 signaling. These results suggest that TNFR2 expressed on podocytes and its canonical NF-κB signaling may directly interpose the compound pathogenic responses by podocytes to TNF-α.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC095**

**PKC-Theta Mediates Inflammatory Responses Via Activation of Resident Macrophages and Neutrophil Adhesion Strengthening** Anna Bertram,[1] Sibylle Von Vietinghoff,[2] Hermann G. Haller,[1] Klaus Ley,[2] Nelli Shushakova.[1] *[1]Nephrology and Hypertensiology, Hannover Medical School, Hannover, Germany; [2]Inflammation Biology, La Jolla Institute for Allergy and Immunology, La Jolla, CA.*

Activation of resident macrophages leading to proinflammatory mediator release and subsequent leukocyte trafficking into the site of inflammation are critical components of the host defence response. Here, we investigated the role of PKC-Θ in the development of inflammatory response in a murine model of acute peritonitis and LPS-induced lung injury. Thioglycollate-induced peritonitis in wild type SV129 mice resulted in increased production of TNF-a as well as CXCL-1 and -2 in peritoneal lavage fluid accompanied by strong neutrophil accumulation into the peritoneal cavity. PKC-Θ deficient mice showed a marked reduction in PMN infiltration, TNF-a, CXCL-1 and -2 levels. These results were confirmed *in vitro* using resident peritoneal WT or PKC-Θ deficient macrophages stimulated with LPS, demonstrating a significant contribution of PKC-Θ to activation of resident

macrophages and inflammatory mediator release. In mixed chimeric mice reconstituted with bone marrow cells from GFP$^+$ wildtype (WT) and GFP$^-$ PKC-Θ deficient (KO) mice, migration of KO neutrophils was strongly reduced in acute peritonitis and LPS-induced acute lung injury. In inflamed mouse cremaster postcapillary venules *in vivo*, CXCL-1 increased the number of firmly adherent cells to a similar extent for both WT and KO cells. However, while most adherent WT neutrophils remained adherent for at least 180 sec after CXCL1 injection, 50% of KO neutrophils were detached after 105 sec, and in the remaining cells adhesion and spreading were diminished. These data suggest that PKC-Θ may mediate divergent downstream effector pathways in inflammatory responses. In resident macrophages, PKC-Θ is required for their activation and proinflammatory mediator release. In neutrophils, PKC-Θ is required for sufficient migration to the site of inflammation by sustaining firm adhesion on the endothelium.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC096**

**Heme Oxygenase-1 (HO-1) Expression Regulates Immune Cell Infiltration in Renal Inflammation** Anjana Perianayagam,[1] Abolfazl Zarjou,[1] Reny Joseph,[1] James George,[2] Anupam Agarwal.[1] *[1]Medicine, Division of Nephrology, University of Alabama at Birmingham, Birmingham, AL; [2]Surgery, University of Alabama at Birmingham.*

HO-1 modulates innate and adaptive immune responses. Previous studies showed increased renal macrophage infiltration following unilateral ureteral obstruction (UUO) in HO-1$^{-/-}$ mice compared to wild-type (WT) mice. In WT mice, M1 (classically activated) macrophages predominate early, whereas M2 (alternatively activated) macrophages predominate at 72h after kidney injury. The purpose of this study was to determine the effect of HO-1 on the distribution of renal macrophage subtypes following UUO. Kidneys were harvested 48h following UUO and sham surgery in HO-1$^{-/-}$ and WT mice. Cell suspensions isolated from kidneys were stained using CD45, CD11b, CD11c, MHC II, Ly6C and Gr-1 antibodies and analyzed by flow cytometry. Compared to WT mice, UUO kidneys of HO-1$^{-/-}$ mice showed a significantly increased infiltration of macrophages (CD45$^+$, F4/80$^+$ and CD11b$^+$)(31.5±8% vs 56.9±16%, p= 0.02). A two-fold increase in inflammatory M1 macrophages (CD45$^+$, MHC II$^+$, Ly6C$^+$ and Gr-1$^+$) were observed in UUO kidneys of HO-1$^{-/-}$ mice (30±5.8%) compared to WT mice (17.2±5.3%, p= 0.008). The increase in M1 macrophages was also significant using another set of markers which included CD45$^+$, MHC II$^+$, CD11b$^+$ and Gr-1$^+$ (HO-1$^{-/-}$ vs WT, 33.5±5.1%, 22.3±6.6%, respectively, p= 0.02). Real time PCR analysis for M1 (TNFα) and M2 (mannose receptor and arginase-1) markers was also performed. There was a 1.54 fold increase in TNFα mRNA in HO-1$^{-/-}$ mice kidneys compared to WT mice. For M2 markers, a 2.3 fold increase in mannose receptor and 15.6 fold increase in arginase-1 mRNA were observed in HO-1$^{-/-}$ mice kidneys compared to WT mice with UUO. No difference in the proportion of dendritic cells (CD45$^+$, MHC II$^+$, CD11c$^+$ and Gr-1$^-$) were found between UUO kidneys of HO-1$^{-/-}$ (22.1±4.6%) and WT mice (26.43±4.2%, p=n.s). These data suggest that HO-1 expression modulates pro-inflammatory macrophage infiltration following UUO and that increased M1 macrophage infiltration maybe responsible for the increased renal fibrosis and inflammation observed in HO-1 deficient mice.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC097**

**The Influence of Sirolimus on Lipid Accumulation in Inflamed Apolipoprotein E Knockout Mice** Kun Ling Ma,[1] Xiong Zhong Ruan.[2] *[1]Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China; [2]Centre for Nephrology, Royal Free and University College Medical School, Royal Free Campus, University College London, United Kingdom.*

**Objective:** Inflammatory stress, combined with dyslipidemia, exacerbates the progression of atherosclerosis. Our *in vitro* studies have demonstrated that Sirolimus inhibits foam cell formation through the influence of intracellular cholesterol homeostasis. This study was to investigate the effect of Sirolimus on lipid accumulation in inflamed apolipoprotein E knockout mice and its underlying mechanisms.

**Methods:** Apolipoprotein E knockout (apoE KO) mice were randomly divided into four groups: phosphate saline injected mice (Control), Sirolimus injected mice (Sir), casein injected mice, Sir plus casein injected mice (Sir+casein). Serum levels of inflammatory cytokines and lipid profile were respectively measured by enzyme-linked immunosorbent assay and clinical biochemistry assay. The effects of Sirolimus on lipid accumulation in aorta, liver and kidney were evaluated by Oil Red O staining and intracellular cholesterol quantitative assay. The protein expression of low density lipoprotein receptor (LDLr), sterol regulatory element binding protein-2 (SREBP-2) and SREBP-cleavage-activating protein (SCAP) in tissues was checked by immunohistochemical staining or Western Blot.

**Results:** There is a significantly elevated serum levels of TNF-α and serum amyloid A in casein injected mice, suggesting the successful induction of inflamed model. Serum levels of lipid profile (total cholesterol and triglyceride, LDL and high density lipoprotein) in flamed mice were markedly decreased compared to controls. Inflammatory stress increased lipid accumulation in aorta, liver, and kidney, interestingly, which was significantly inhibited by Sirolimus. Further analysis showed that Sirolimus overrode inflammatory stress induced protein expression increase of LDLr, SREBP-2, and SCAP in aorta, liver and kidney of inflamed mice.

**Conclusion:** Sirolimus provided protective effects on the multiple-organ lipid accumulation induced by inflammatory stress, which was through reestablishing LDLr feedback regulation.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC098**

**Circulating and Intra-Renal CSF-1 Expression Distinctively Contribute to Macrophage-Rich Lupus Nephritis** Julia Menke,[1] Yasunori Iwata,[2] Vicki R. Kelley.[2] *[1]I. Dept Med, Johannes Gutenberg-Univ, Mainz, Rheinland-Pfalz, Germany; [2]Renal Division, Brigham&Women's Hosp, Boston, MA.*

CSF-1, the major macrophage (Mø) growth factor, is a potential therapeutic target for lupus since: 1) over-expressing CSF-1 hastens the onset of Mø-rich lupus nephritis in MRL-Faslpr mice, 2) deleting CSF-1 eliminates Mø-rich lupus nephritis in MRL-Faslpr mice and 3) CSF-1 is elevated in serum/urine and kidney in patients with active lupus nephritis. As CSF-1 is rises in the circulation and kidney in lupus MRL-Faslpr mice and patients, we hypothesized that CSF-1 in the circulation and kidney distinctively contribute to Mø-rich lupus nephritis. To test this hypothesis, we injected CSF-1 into the circulation of CSF-1-deficient MRL-Faslpr mice (sole source of CSF-1 is circulation). We detected an increase in circulating monocytes (Mo), seeded from the bone marrow, and a shift to circulating Mo phenotypes (Ly6Chi,CD69+) more readily recruited to the kidney that, in turn, initiate renal injury (TEC apoptosis). Conversely, using mutant TgCS/+;MRL-Faslpr mice that express only the cell surface(cs) CSF-1 isoform (CSF-1 in kidney, not circulation) we detected: 1) intra-renal recruitment of the Mo (GFP+) comparable to WT mice, 2) skewing of circulating Mo towards an "inflammatory" (Ly6Chi), activated Mo (CD69+) phenotype and 3) intra-renal proliferation of Ly6ChiMø. This suggests that intra-renal rogue Mø activated by csCSF-1 may initiate inflammation, thereby releasing chemokines from the kidney responsible for recruiting additional Mo and amplifying renal inflammation. In conclusion, circulating and intra-renal csCSF-1 distinctively contribute to Mø-rich lupus nephritis. This suggests that blocking CSF-1 in the circulation and kidney is required to halt lupus nephritis.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC099**

**IgA Mediated Proximal Tubular Cell Activation and Tubulointerstitial Scarring in IgA Nephropathy** Karen Molyneux,[1,2] Ravinder S. Chana,[1] Joanna Boyd,[2] Alice C. Smith,[1,2] Richard J. Baines,[1,2] Nigel J. Brunskill,[1,2] Jonathan Barratt.[1,2] *[1]Infection, Immunity & Inflammation, University of Leicester, Leicester, United Kingdom; [2]John Walls Renal Unit, Leicester General Hospital, Leicester, United Kingdom.*

One of the most powerful determinants of progressive kidney disease in IgAN is the development of tubulointerstitial inflammation, atrophy and scarring. The extent of tubulointerstitial damage does not however correlate with intensity of mesangial IgA deposition and is associated with the development of chronic kidney disease and ESRD independently of changes in glomerular morphology. Normally the glomerular barrier is impermeable to immunoglobulins but glomerular barrier pore size can significantly increase in glomerular disease and an increase in urinary IgA concentration has been reported in IgAN. We studied the interaction of IgA with cultured proximal tubule epithelial cells (PTEC) to establish whether IgA filtered into the proteinuric proximal tubule could independantly generate a proinflammatory environment in the renal tubulointerstitium that favours the development of fibrosis.

Growth arrested HK2 cells were exposed to IgA1 (100μg/ml) from healthy subjects or patients with IgAN (100μg/ml), IgM (100μg/ml) or medium alone for 0, 5, 10, 30 or 60 minutes. IgA1 (IgAN) activated extracellular signal-regulated protein kinase in PTEC to a much greater extent than IgM or IgA1 from healthy subjects. Similarly, IgA1 (IgAN) induced PTEC synthesis of fibronectin, TGF-beta and IL-6. Exposure to IgA1 also stimulated PTEC production of complement component C3 and activation of the complement cascade with generation of the chemotactic component C5a. We have also shown that IgA1 phosphorylates a megalin cytoplasmic tail-GST fusion protein in HK-2 cells suggesting that IgA1 binding to PTEC may trigger the same intracellular signalling pathways as those activated following exposure to albumin.

We provide clear evidence that exposure to IgA1 results in a profibrotic and proinflammatory phenotypic transformation of PTEC and that interaction between filtered IgA1 and PTEC in IgAN may be one of the principle factors in initiating and driving tubulointerstitial damage in IgAN.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC100**

**Pentraxin-2/Serum Amyloid P Inhibits Fibrosis through FcgR Dependent Monocyte/Macrophage Release of IL10 In Vivo** Jeremy S. Duffield. *Medicine, Brigham and Women's Hospital, Boston, MA.*

In models of kidney fibrosis, systemic administration of Serum amyloid P or Pentraxin-2 (PTX-2) significantly inhibits fibrosis through a monocyte/macrophage-dependent mechanism, and as such represents a potential novel therapy for the treatment of chronic inflammation with fibrosis. In transgenic Coll-GFP reporter mice, PTX-2 inhibited myofibroblast synthesis of collagen, but myofibroblast numbers were unaffected. PTX-2 is deposited on injured tissue and debris in the kidney, and is detected in endosomes/phagosomes of macrophages (Mφs). It does not bind to, or inhibit collagen production by, fibroblasts in vitro, but it binds with high affinity to activating Fcγ receptors on monocytes/Mφs. Purified Mφs from fibrotic kidney are less activated and produce 20 fold more IL10 in mice treated with PTX-2. To test the direct role of IL10 in the inhibition of fibrosis mediated by PTX-2, we systemically administered IL10 producing adenovirus (Ad-IL10 and control virus (Ad-mock) in models of kidney fibrosis. Ad-IL10 resulted in high levels of systemic IL10 and markedly attenuated fibrosis accumulation in the kidney. Systemic IL10 inhibited both monocyte/ Mφ activation and myofibroblast proliferation and activation in

vivo. We propose that the major mechanism of action of PTX-2 is to switch M2 or wound healing Mφs into regulatory Mφs. Local generation of IL10 blocks Mφs driven fibrosis and directly inhibits myofibroblasts.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC101

**Intrarenal Dopamine Deficiency Is Associated with Hypertension and Decreased Longevity** Mingzhi Zhang, Shilin Yang, Raymond C. Harris. *Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN.*

The kidney possesses an intrarenal dopaminergic system, and intrarenal dopamine levels can reach high nanomolar concentrations. Intrarenal dopamine is primarily biosynthesized through the actions of aromatic amino acid decarboxylase (AADC) in the proximal tubule. We have generated mice with kidney dopamine deficiency through selective deletion of AADC in proximal tubule by crossing AADCflox/flox mice with γ-GT Cre mice onto a 129/sv background (AADC KO). Here, we report our unexpected finding that AADC KO mice exhibit a significantly shorter life span compared to wild type mice. At 19 months of age, only 8 of 19 AADC KO mice still survived while 19 out of 20 wild type mice survived. Intrarenal dopamine may modulate components of the renal renin-angiotensin system (RAS) and antagonize Ang II actions in the kidney. Q-PCR and immunoblotting demonstrated that aged AADC KO mice exhibited increased renin and AT1b receptor expression and decreased AT2 and Mas (Ang1-7) receptor expression in the kidneys but not in aorta and heart. It has been reported that deletion of AT1a receptors promotes longevity in mice along with upregulation of the prosurvival genes nicotinamide phosphoribosyltransferase (Nampt) and sirtuin 3 (Sirt3) in the kidney. Aged AADC KO mice were found to have markedly decreased renal expression of Nampt and Sirt3. In addition there was increased expression of nitrotyrosylated protein, a marker of oxidative stress, and CTGF as well as increased renal expression of Kim-1 and α-smooth muscle cell actin, markers of kidney injury. Aged AADC KO mice also had increased lymphocyte and macrophage infiltration. Aged AADC KO mice were hypertensive (135 ± 3 vs. 105 ± 2 mmHg of wild type). These results indicate that the intrarenal dopamine system normally mediates increased renal expression of vasodilatory and cytoprotective components of the RAS, and inhibition of intrarenal dopamine production leads to increased oxidative stress, hypertension, renal injury and decreased longevity, which may be at least in part a result of the unopposed upregulation of vasoconstrictive, proinflammatory AT1 activity.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC102

**Human GRK4γ Wild-Type Gene Prevents Salt Sensitivity by Inhibition of Renal Proximal Tubular Sodium Transport in Transgenic Mice** Zheng Wang,[1] Laureano D. Asico,[1] Crisanto Escano,[1] Magali Araujo,[2] William J. Welch,[2] Pedro A. Jose.[1] *[1]Center for Molecular Physiology Research, Children's National Medical Center, Washington, DC; [2]Georgetown University, Washington, DC.*

High NaCl intake is associated with increased cardiovascular risk. Mortality and morbidity are higher in hypertensive and NaCl-sensitive normotensive subjects than in NaCl-resistant normotensive subjects. However, the genetic cause of salt sensitivity is not known. GRK4 variants (R65L, A142V, and A486V) are associated with human essential hypertension. Human(h) GRK4γ142V transgenic mice are hypertensive on normal NaCl intake while hGRK4γ486V transgenic mice develop hypertension on high NaCl intake. The role of hGRK4γ wild-type gene on sodium metabolism and blood pressure (BP) was studied in salt-sensitive C57BL/6J mice. On normal NaCl diet, BPs of hGRK4γ wild-type transgenic mice (WT-Tg) and non-transgenic littermates (NT-Tg) were similar. However, 3 wks of 6% NaCl diet increased the BP (aorta under anesthesia) in NT-Tg (102±2 to 122±1mmHg, n=8-11/group, P<0.001), but not in WT-Tg (101±2 to 103±2 mmHg, n=7/group). The 24 hr BP (telemetry) of conscious mice confirmed that 6% NaCl diet increased the BP in NT-Tg (+14±1%, n=4), but not in WT-Tg (+0.8±1%, n=4) mice. Therefore, GRK4γ wild-type prevents the salt sensitivity of C57BL/6J mice. We next studied the relationship between BP and $Na^+$ excretion. 6% NaCl diet increased urinary $Na^+$ excretion to a greater extent in WT-Tg than NT-Tg (5.2±0.7 vs. 2.8±0.4 mmol/mg creatinine, n=10, P<0.01). The pressure-natriuresis plot in WT-Tg was shifted to the left of the plot of salt-sensitive NT-Tg, indicating facilitation of $Na^+$ excretion. This involved the proximal nephron because the pressure-lithium excretion plot of WT-Tg mice was also shifted to the left of the plot of NT-Tg (n=5/group) on 6% NaCl diet. Furthermore, renal micropuncture studies (S2 segment, proximal tubule) showed that the tubular fluid to plasma inulin ratio was lower in NaCl-loaded WT-Tg than in NT-Tg (1.6±0.1 vs. 2.2±0.3, n=7, P<0.05) mice. We conclude that GRK4γ wild-type prevents salt sensitivity, in part, by decreasing renal proximal tubule $Na^+$ transport.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC103

**Catalase Overexpression Prevents Programming of Hypertension and Kidney Injury in Offspring of Diabetic Dams** Shao-Ling Zhang, Yun-Wen Chen, Isabelle Chenier, Shiao-Ying Chang. *Research Center, CRCHUM-Hotel-Dieu Hospital, Montreal, QC, Canada.*

We investigated whether overexpression of catalase (CAT) in renal proximal tubular cells (RPTCs) could prevent maternal diabetes-induced perinatal programming of hypertension and kidney injury in offspring and examined potential underlying mechanisms.

Offspring of non-diabetic and diabetic dams of Hoxb7-green fluorescence protein (GFP)-transgenic (Tg) and Hoxb7-GFP/Catalase (CAT)-Tg mice were studied. Systolic blood pressure, microalbuminuria, nephron number, glomerular filtration rate, glomerular tuft volume, renal morphology and reactive oxygen species (ROS) generation, as well as gene expression of transforming growth factor-beta 1 (TGF-β1), plasminogen activator inhibitor-1 (PAI-1), angiotensingen (Agt), angiotensin II type I receptor (AT1R), angiotensin converting enzyme (ACE) and angiotensin converting enzyme 2 (ACE2), were assessed.

Offspring of Hoxb7-GFP-Tg dams with maternal diabetes developed hypertension, renal hyperfiltration, microalbuminuria and kidney injury. Renal ROS generation and gene expression of TGF-β1, PAI-1, Agt, AT1R and ACE were markedly upregulated, but ACE2 gene expression was lower in kidneys of hypertensive Hoxb7-GFP-Tg offspring of diabetic dams. These changes were normalized in offspring of diabetic CAT-Tg dams.In conclusion, our data demonstrate that overexpression of CAT in RPTCs prevents perinatal programming of hypertension and renal injury in offspring of dams with maternal diabetes. Enhanced intrarenal oxidative stress and RAS activation with down-expression of ACE2 are intimately associated with this process.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC104

**Dec1 Regulates Circadian Variation of Blood Pressure** Ayumu Nakashima,[1,2] Takeshi Kawamoto,[3] Mitsuhide Noshiro,[3] Kiyomasa Honda,[3] Noritsugu Ozaki,[3] Toshinori Ueno,[2] Yoshihiko Taniguchi,[4] Junko Tanaka,[1] Noriaki Yorioka,[2] Yukio Kato.[3] *[1]Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Sciences, Hiroshima University; [2]Department of Advanced Nephrology, Graduate School of Biomedical Sciences, Hiroshima University; [3]Department of Dental and Medical Biochemistry, Graduate School of Biomedical Sciences, Hiroshima University; [4]Division of Clinical Pharmacotherapeutics, Hiroshima International University, Japan.*

All living things on the earth need to follow earth's rotation period of 24 hours. The molecular clock, which consists of many regulatory factors including DEC1, is a system to generate and maintain circadian rhythms of various physiological processes. DEC1 is a transcription factor that binds to E-boxes and suppresses the expression of clock-controlled genes.

To identifiy target genes of DEC1, we utilized genome-wide chromatin immunoprecipitation (ChIP)-on-chip assay and found that DEC1 bound to the regulatory region of one of Na/K-ATPase genes in human embryonic kidney 293 cells. ChIP and luciferase reporter assays revealed that an E-box on the Na/K-ATPase gene promoter is a functional element governed by clock components such as CLOCK, BMAL1, and DEC1. In addition, DEC1 deficiency resulted in the increased expression of Na/K-ATPase, whereas overexpression of DEC1 decreased the expression level of Na/K-ATPase.

Since Na/K-ATPases are known to be involved in the blood pressure regulation, we focused on the functional significance of DEC1 in homeostasis and circadian regulation of blood pressure. In both kidney and aorta, robust circadian rhythms of the Na/K-ATPase expression were detected at mRNA and protein levels. $DEC1^{-/-}$ mice showed higher expression of Na/K-ATPase than wild type mice, although the circadian rhythmicities still existed. In addition, $DEC1^{-/-}$ mice showed lower blood pressure and larger amplitude of circadian variation of blood pressure than wild-type mice. In contrast, in CLOCK mutant mice, the expression level of Na/K-ATPase was lower than wild-type mice, and lost circadian rhythmicities.

We conclude that DEC1 regulates circadian variation of blood pressure via Na/K-ATPase.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC105

**Impaired Na Pump Signaling Characterizes Hypertension in Dahl Salt Sensitive Rats** Yanling Yan,[1] Lijun Liu,[2] Zi-Jian Xie,[2,1] Deepak K. Malhotra,[1] Bina Joe,[2] Joseph I. Shapiro,[1,2] Jiang Liu.[1] *[1]Medicine, University of Toledo College of Medicine, Toledo, OH; [2]Physiology and Pharmacology, University of Toledo College of Medicine, Toledo, OH.*

Cardiotonic steroids (CTS) such as ouabain signal through the plasmalemmal Na/K-ATPase (NKA) and inhibit transcellular 22Na+ transport by inducing endocytosis of NKA and NHE3 in proximal tubule (PT) cells. This phenomenon was also observed in vivo using Sprague Dawley rats treated with high salt diet or ouabain-infusion for 7 days. When we examined age-matched male Dahl salt resistant (R) and sensitive (S) strains, we found that a high (HS 2% NaCl) compared to a low salt diet, (LS 0.3% NaCl, 7 days,n=12/strain/treatment) caused disparate effects on BP, renal sodium excretion, Src kinase activation, as well as surface expression and activity of the PT NKA and NHE3. Plasma Na+ and K+ remained the same in all groups. HS caused a significant increase in systolic BP in the S (ΔBP=23.6±4.5, n=12 rats/treatment/strain. P<0.01) but not the R rats (ΔBP=2.6±1.8, n=12, p=NS). HS had same effect on creatinine clearance on both R and S rats. In the R rats, HS significantly reduced the NKA enzymatic activity in PT plasma membrane fraction and NHE3 activity in PT brush-border membrane fraction (n=6, p<0.01), increased total and fractional urinary Na+ excretion (n=8, p<0.01), stimulated c-Src phosphorylation (n=4, p<0.01), and induced endocytosis of PT NKA and NHE3 (n=12, p<0.01). These HS-induced changes in the R rats were either markedly reduced or absent in the S rats. This differential regulation of endocytosis and NKA signaling was confirmed in PT primary cultures derived from R and S rats. In these PT primary cultures, ouabain (25μM, 1h) stimulated c-Src phosphorylation (n=4, p<0.01) and redistribution of NKA and NHE3 (from cell surface to intracellular compartments) in R but not in S rats (n=6, p<0.01). We conclude that CTS-induced "trafficking" regulation of the PT NKA

and NHE3 that observed in R rats is "impaired" in S rats. This "impaired" CTS-induced signaling and "trafficking" may characterize salt-sensitive hypertension in experimental animals and possibly clinical subjects.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC106**

**The Activity of the Thiazide Sensitive Na-Cl Cotransporter NCC Is Regulated by a Protein Phosphatase, PP4** Mark Glover, Kevin O'Shaughnessy. *Clinical Pharmacology Unit, University of Cambridge, Cambridge, England, United Kingdom.*

Regulation of NCC, the target of thiazide diuretics, is crucial for salt homeostasis and blood pressure regulation. It is clear that NCC transporter trafficking and membrane expression is orchestrated by a scaffold of interacting proteins including WNK1 and WNK4 that are mutated in Pseudohypoaldosteronism type II. There are two phosphorylation controlled regulatory pathways for NCC: *Type 1*, mediated by WNK4 affecting trafficking to the surface membrane; and *Type 2*, affecting intrinsic transporter kinetics by phosphorylation of conserved N-terminal S/T amino acids especially threonine 58. Dynamic regulation of NCC function by kinases must involve dephosphorylation by phosphatases as illustrated by the role of PP1 and PP2B in the regulation of KCC members of the SLC12 family, yet no such phosphatase has been described for NCC.

Site mutation was used to produce plasmids expressing ECFP-NCCT T58D or T58A and a kinase-dead phosphatase, PP4R235L. *Xenopus* oocytes were injected with cRNA for these plasmids or wild-type cRNAs. NCCT function was assessed by $^{22}Na^{+}$ flux and confocal microscopy of the blue fluorescent ECFP-NCCT protein. Immunohistochemistry was performed on murine kidney sections.

PP4 reduced the activity of NCC by 78% without affecting surface membrane expression. The inhibitory effect of PP4 on NCC activity was abolished by either phosphatase dead site mutation of PP4, incubation with the PP4 inhibitor Fostreicin or mutation of NCC T58. WNK4 reduced the surface membrane expression of NCC as previously reported and this was unaffected by PP4. Immunohistochemistry of murine kidney showed that PP4 was expressed in the distal convoluted tubule and collecting duct.

Thus PP4 inhibits NCC activity but not trafficking to the surface membrane by a mechanism that requires phosphatase activity and a conserved N-terminal amino acid of NCC, threonine 58. This action is distinct from WNK4 regulation of membrane trafficking. In the mouse kidney, PP4 is selectively expressed in the distal nephron including cells of the distal convoluted tubule cells suggesting that PP4 may have a physiological role in regulating NCC and hence NaCl reabsorption *in vivo*.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC107**

**Cryo-Denervation of the Clipped Kidney in Two-Kidney One-Clip (2K1C) Hypertensive Rats Decreases Systemic Blood Pressure (BP), Contralateral Renal Sympathetic Nerve Activity (RSNA) and Plasma Angiotensin II (Ang II)** Russell Pajewski,[1] Haiping Chen,[1] Peter John Littrup,[2] Jesse J. Veenstra,[1] Noreen F. Rossi.[1] *[1]Internal Medicine and Physiology, Wayne State University School of Medicine, Detroit, MI; [2]Intervential Oncology - Karmanos Cancer Center, Wayne State University School of Medicine, Detroit, MI.*

Besides an activated renin-angiotenin system, increased RSNA contributes to elevated BP in 2K1C hypertension. Previous studies indicate that renal denervation with transection of the renal nerve plus application of phenol decreases BP in this model. Ablation of the renal nerves with radiofrequency catheters was recently shown to be effective in refractory human hypertension but may result in thermal damage to the vasculature. We hypothesized that cryoablation of the renal nerve on the clipped side of 2K1C rats would decrease BP and serum ang II. Male Sprague Dawley rats (5 wk-old) underwent sham (SC) or renal artery clipping (2K1C) and placement of telemetry transmitters for continuous BP monitoring. After 6 wks, rats were randomly assigned to cryo (crDN) at -155°C x 30 sec x 3 freeze-thaw cycles or sham (shDN) denervation and pair fed a 0.4% Na diet. Mean BP was 141±4 mmHg in the 2K1C group and decreased by 14±3 mmHg 1 week after crDN ($P<0.025$). SC rats had a mean BP of 115±4 mmHg that decreased by 10±4 mmHg after crDN. BP did not change in either 2K1C or SC with shDN. Mean BP continued to remain significantly lower for up to 5 wks post crDN. Plasma ang II decreased by 66% in the 2K1C crDN groups to values not different from the SC rats. A limited number of rats had telemetric nerve electrodes placed on the contralateral renal nerve. Post crDN, contralateral RSNA decreased significantly. Urine output and electrolyte excretion was higher in the crDN groups. The present findings are comparable to our results with surgical denervation. We conclude that cryoablation of the renal nerve on the clipped side of the 2K1C model of renovascular hypertension is effective in decreasing BP. These results provide proof of principle that cryodenervation may be a viable modality for translation to treat refractory human renovascular hypertension.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC108**

**Podocyte Injury during Nephrotic Syndrome in Children Results in Secretion of Vesicles from Microvillus Tips** Masanori Hara, Toshio Yanagihara. *Department of Pediatrics, Yoshida Hospital, Tsubame, Niigata, Japan.*

Podocyte(s) (Pod) injury is involved in both the onset and progression of glomerular diseases. Our previous studies revealed that apical cell membranes of Pod are shed into the urine and that urinary podocalyxin (u-PCX) is a useful biomarker of Pod injury. In this study we examined the pathological process of urinary shedding of podocyte cell membranes in nephrotic syndrome. Urine samples and kidney specimens from normal children (N=120) and children with glomerular diseases (N=59) were analyzed by immunohistological methods. The renal diseases included: Idiopathic nephrotic syndrome (N=44) and nephrotic syndrome secondary to glomerular diseases such as lupus nephritis, IgA nephropathy and Schoenlein-Henoch purpura nephritis (N=15). To quantify u-PCX, an ELISA was developed using monoclonal antibodies to PCX, as reported previously.

Larger amounts of u-PCX were shed into the urine in idiopathic nephrotic syndrome (281.5±56.7 ng/mg creatinine) and secondary nephrotic syndrome (239.7±24.1 ng/mg creatinine), compared to normal controls (17.6±2.5 ng/mg creatinine). In idiopathic nephrotic syndrome, the level of u-PCX in steroid-resistant cases (444.2±126.0 ng/mg creatinine) was significantly higher than that in steroid-sensitive cases (77.8±13.2 ng/mg creatinine). Immuno-EM revealed that the urinary PCX from nephrotic patients was present in vesicles. IF studies indicated that the urine vesicles did not contain cytoskeletal components such as actin filaments. EM revealed vesiculation of apical Pod membranes at microvillar tips (tip vesiculation) and shedding of vesicles into Bowman's space. Membranes generating vesicles were devoid of electron-dense material, while microvilli at the trunk regions contained electron-dense material, including cytoskeletal components such as actin bundles. The size of the urinary vesicles were similar to that of Pod microvilli.

We conclude that significantly greater amounts of PCX were excreted into the urine of nephrotic vs. control children, and that they were excreted as small membrane vesicles originating from microvilli of Pod membrane surface by tip vesiculation which did not include apparent cytoskeletal components.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC109**

**Collapsing Glomerulopathy (CG) in 19 Patients with Systemic Lupus Erythematosus (SLE): Cause or Coincidence?** Steven P. Salvatore,[1] Laura M. C. Barisoni,[2] Andrew M. Herzenberg,[3] Praveen N. Chander,[4] Volker Nickeleit,[5] Surya V. Seshan.[1] *[1]Weill Cornell Med Coll, NY; [2]NYU Medical Center, NY; [3]University of Toronto; [4]NY Med Coll; [5]UNC School of Med.*

Purpose: CG is a podocytopathy characterized by segmental or global collapse of capillary walls with wrinkling of the GBM and overlying podocyte proliferation. Idiopathic CG is a distinct clinicopathologic entity with poor response to immunosuppressive therapy and rapid progression to renal failure. CG is associated with viral infections, autoimmune disease, and drugs. We present the largest group of CG in patients with SLE.

Methods: Clinicopathological features in renal biopsies were retrospectively studied from 19 patients with SLE (16) or SLE-like (3) disease diagnosed with CG.

Results: The patients ranged from 16-65 years, M:F-4:15, 89% of African descent. Initially, 95% of patients had nephrotic syndrome with mean proteinuria (7g/24hr +/- 3.5), creatinine (Cr) 4 +/- 2.7 and BUN 37 +/- 27, except in one patient (Cr/BUN was 26.5/155). Serologies: C3 92 (M), C4 22 (M), ANA+ 93%, anti-dsDNA 73%, and anti-Smith 33%. HIV (19), HCV (11) and Parvovirus (7) serologies were negative in patients tested. On morphology, globally collapsed glomeruli ranged from 1-65% (mean 19%), segmentally collapsed (0-40%), and globally sclerosed (0-50%). Varied tubular atrophy and interstitial fibrosis was seen in 35% with focal microcystic changes. Minimal glomerular mesangial deposits were noted by IF and/or EM in 70%, and extensive foot process effacement in 83%. Initial treatment was pulse/oral steroids and dialysis (12). Follow-up from 13 patients: 7 with end-stage renal disease, 0-21 months after biopsy;2 patients returned to normal cr and 4 had cr 1.2-3.6mg/dl.

Conclusions: Immunologic (antibody or cytokine) mediated injury may lead to mild to severe glomerular podocytopathy in SLE (minimal change disease, FSGS, and CG). However, other known causes of CG should be considered in this setting. The high predominance of patients of African descent in our study suggests that collapsing features could result from immunologic and/or environmental factors on a genetic predisposition to epithelial injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC110**

**Childhood Onset Lupus Nephritis Presents More Severe When Compared with Late Onset Disease** Victor Sato, Igor Marques, Patricia T. Goldenstein, Lilian P. F. Carmo, Lectícia Jorge, Rui Toledo Barros, Viktoria Woronik. *Nephrology, University of Sao Paulo, Sao Paulo, Brazil.*

The aim of this study was to evaluate the clinicopathologic features and response to treatment of patients with lupus nephritis (LN) in different ages of disease onset. We retrospectively analyzed clinical presentation, treatment and one year follow-up of patients with diagnosis of LN with age ≤ 18 (n=23) and ≥ 50 (n=13) years submitted to renal biopsy (Bx) between 1999 and 2008 in a single center. The clinical and laboratory features at the time of Bx in childhood onset LN and late onset LN were, respectively: female gender 87 *vs* 92% (ns), hypertension 82 *vs* 31% (p=.01), SLEDAI 29±9 *vs* 18±7 (p=.01), estimated glomerular filtration rate (eGFR) 75±48 *vs* 70±18 mL/min/1.73m² (ns), proteinuria 3.3±2.7 *vs* 2.0±1.7 g/24h (ns) and hematuria 81 *vs* 54 % (p=.007). LN was diagnosed simultaneously with SLE in 90% of the patients with childhood onset LN, compared to only 17% in late onset LN group (p<.001). Analyzing Bx data, adolescents, in relation to elderly, showed the following distribution of WHO classes: III + IV 81 *vs* 61% and V 19 *vs* 39% (ns). Crescents were more frequent in childhood onset LN patients (74 *vs* 39 %, p=.001), who also showed a higher activity index (4.8±2.6 *vs* 3.3±1.9, p=.007). Diffuse interstitial fibrosis (>50%) was present in 9% of the adolescents and in none in elderly. Treatment employed for one year consisted mainly of methylprednisolone, prednisone and cyclophosphamide in both groups, with similar average cumulative doses. The final eGFR and proteinuria were 103±41*vs* 78±21 mL/min/1,73m² (p=.02) and 0.6±0.6 *vs* 0.6±0.7 g/24h (ns), in adolescents and elderly,

respectively. In childhood onset LN group, 3 patients presented in hemodialysis and did not recover renal function. Fifteen percent of the remaining reached the composite end point of duplication of serum creatinine or dialysis. No patient in late onset LN group reached this end point, however they had a smaller increase in eGFR (8±13 *vs* 26±36 mL/min/1,73m², p=.06). In conclusion, childhood onset LN presents more severe when compared with late onset disease, however with good responses to intensive treatment regimens.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC111**

**Results of Aspreva Lupus Management Study (ALMS) Maintenance Phase** David R. W. Jayne,[1] Gerald B. Appel,[2] Mary Anne Dooley,[3] Ellen Ginzler,[4] David Isenberg,[5] David Wofsy,[6] Neil Solomons,[7] Laura J. Lisk.[8] *[1]Addenbrooke's Hospital, Cambridge, United Kingdom; [2]Columbia University, New York; [3]University of North Carolina, Chapel Hill; [4]State University of New York, New York; [5]University College London, London, United Kingdom; [6]University of California, San Francisco; [7]Vifor Pharma (formerly Aspreva Pharmaceuticals), Vancouver, Canada; [8]Vifor Pharma, Surrey, United Kingdom.*

Aim: The 36-month, maintenance phase of the ALMS study compared the efficacy and safety of mycophenolate mofetil (MMF) with azathioprine (AZA) in patients with lupus nephritis (LN) classes III, IV and V achieving partial or complete response during the 6-month induction phase with corticosteroids (CS) and either MMF or cyclophosphamide (IVC).

Methods: Patients were re-randomized 1:1 to a double-blind comparison of either oral MMF (2 g/d) or oral AZA (2 mg/kg/d) both with CS (predniso[lo]ne ≤10mg/day). The primary efficacy measure was time to treatment failure (death; end-stage renal disease [ESRD]; doubling of serum creatinine (x2 Cr); and/or renal flare [proteinuric/nephritic]). Secondary efficacy assessments included time to event for each component of treatment failure; complete renal remission; and adverse events (AEs).

Results: 227 patients were randomized (ITT population); 127 completed the study (MMF, 73/116 [62.9%]; AZA, 54/111 [48.6%]). MMF was superior (log-rank test) to AZA in time to treatment failure (P=0.003) and renal flare (P=0.027). Other elements of the primary efficacy parameter showed numerical benefit in favor of MMF: time to ESRD (P=0.069) and x2 Cr (P=0.073). The incidence of AEs was similar between MMF and AZA; the most common AEs in both groups were infections and GI disorders. Numerically fewer patients treated with MMF than AZA reported at least one serious AE (27/115 [23.5%] vs 37/111 [33.3%]. One death (AZA group, unrelated to treatment) occurred during the study.

Conclusions: MMF was superior to AZA in maintaining renal response and preventing relapse in subjects with active LN who had responded to induction therapy with CS and either MMF or IVC.

Disclosure of Financial Relationships: Consultancy: Roche, GSKResearch Funding: Roche, ASPREVA.

**TH-FC112**

**B Lymphocyte Related Signatures Are Associated with Active Lupus Nephritis** Worapot Treamtrakanpon,[1] Yingyos Avihingsanon,[1,2] Somchai Eiam-Ong.[1] *[1]Medicine, Chulalongkorn University, Pthumwan, Bangkok, Thailand; [2]Lupus Research Unit, Chulalongkorn University, Pthumwan, Bangkok, Thailand.*

BLyS and APRIL are tumor necrosis factor-family cytokines that play an important role in generating and maintaining the mature B-cell pool. BLyS and APRIL mRNA levels were associated with active disease both in serum of SLE patients and renal tissue of nephritis patients. BLyS blockade is a potential therapy for active SLE. We assessed correlation between blood levels of BLyS and APRIL with clinical and renal histology of lupus nephritis patients.

**Methods**: Fifty-two lupus nephritis patients that underwent kidney biopsy were evaluated prospectively for at least 6 months. Renal SLEDAI score, anti-dsDNA, complements, blood BLyS and APRIL on biopsy day and renal histology were assessed. All patients were treated with the standard therapy (mycophenolate or cyclophosphamide plus steroid). Response to treatment was determined at 6 months of treatment.

**Results:** All patients had biopsy-proven lupus nephritis (class 3 or 4 ISN/RPS classification). Mean(SD) age of patients was 33(9) and duration of disease was 6(4) years. Twelve of fifty-two patients had extra-renal symptoms at the time of study. Dosage of prednisolone was 22.5(10) mg/day. Serum creatinine was 1.06(0.55) mg/dL, proteinuria was 3.59(2.18) gm/day, and erythrocyturia was 50.85(85.5) cells per high power field. APRIL levels were correlated with renal histology score ($r = 0.35$; $p < 0.05$) and proteinuria ($r = 0.44$; $p < 0.01$) whereas BlyS levels were correlated with all serum complement levels ($r = 0.60$; $p < 0.01$), anti-dsDNA ($r = -0.36$; $p < 0.01$), and dose of steroid usage ($r = -0.54$; $p < 0.01$). Blood APRIL levels above 3.6 ng/mL could precisely predict failure of standard treatment treatment. (PPV 95.8 %; NPV 38.1%)

**Conclusion:** Blood APRIL and BLyS levels were correlated with activity of lupus nephritis. APRIL is a potential biomarker for predicting treatment failure.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC113**

**Dense Deposit Disease Associated with Monoclonal Gammopathy of Undetermined Significance** Sanjeev Sethi,[1] William R. Sukov,[1] Yuzhou Zhang,[2] Lynn D. Cornell,[1] Richard J. Smith.[2] *[1]Mayo Clinic; [2]University of Iowa.*

Dense Deposit Disease (DDD) is a rare glomerular disease that typically affects children and young adults and much less commonly older patients. The pathophysiology underlying DDD is uncontrolled activation of the alternative pathway (AP) of complement most frequently secondary to an autoantibody to C3 convertase called C3 nephritic factor, although mutations or autoantibodies to Factor H can impair its function and also cause DDD. Since 1995, we have diagnosed DDD in 14 patients 49 years of age or older; ten of these patients (71.4%) carry a concomitant diagnosis of monoclonal gammopathy of undetermined significance (MGUS). In all patients, a monoclonal IgG protein was identified by serum protein electrophoresis and one patient also had a monoclonal IgA (biclonal). None of the patients had progressed beyond MGUS at the time of kidney biopsy and therefore none of the patients were receiving chemotherapy for plasma cell dyscrasia. Six patients had progressed to ESRD; one patient received an allograft but developed recurrent disease within one month of transplant. In one of ten, the index case that prompted this study, we evaluated the AP and demonstrated low serum AP protein levels consistent with AP activity, heterozygosity for H402 allele of Factor H, and low levels of Factor H autoantibodies, which can affect the ability of Factor H to regulate AP activity.

Work up of AP in DDD with MGUS

| Assay | Result |
|---|---|
| C3 nephritic factor | Negative |
| AP functional assay | Low at 25.8% (normal 65-130). |
| Hemolytic assay | Negative |
| Factor H autoantibody | Positive (1:100) |
| Mutational analysis for Factor H | 1 copy of H402 allele and Y402 allele |
| Mutational analysis for Factor I | Normal alleles |
| Mutational analysis for CD46 | Normal alleles |

In aggregate, these findings suggest that DDD may develop in some adults with MGUS as a result of autoantibodies to Factor H (or other complement proteins) that on a permissive genetic background (H402 allele of Factor H) lead to dysregulation of the AP with subsequent glomerular injury. Thus DDD in some older patients may be a distinct clinicopathologic entity that represents an uncommon complication of MGUS.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC114**

**Laser Dissection and Mass Spectrometry Based Proteomics for Diagnosis and Typing of Renal Amyloid** Sanjeev Sethi, Samih H. Nasr, Julie A. Vrana, Jason David Theis, Lynn D. Cornell, Mary E. Fidler, Nelson Leung, Ahmet Dogan. *Pathology, Mayo Clinic, Rochester, MN.*

Amyloidosis is a group of disorders characterized by extracellular deposition of proteins as insoluble aggregates. The clinical management of amyloidosis is based on accurate typing of the amyloid. Typing of renal amyloidosis can be difficult when heavy chain amyloid (AH) or other rare forms of amyloid including hereditary and familial forms are present. In this study, we describe the use of laser microdissection (LMD) and mass spectrometry (MS) based proteomic analysis for typing of renal amyloid. Between 2-4 microdissections of Congo red positive areas in each case was performed. We compare the LMD/MS proteomic prolife (PP) of 16 cases of amyloidosis including 4 cases of immunoglobulin (Ig) AH, 4 cases of light chain (AL), 2 cases of SAA, 2 cases of fibrinogen A-α chain (Afib), 2 cases of leukocyte cell-derived chemotaxin-2 (LECT2) and 2 cases of TTR (non renal) amyloid. The PP of 3 AL amyloid cases showed Ig lambda chain constant regions, while case 4 showed kappa chain constant and variable III regions, all with >95% probability, with 8, 11, 12, and 14 spectra. The PP of 2 cases of Afib amyloid showed fibrinogen A α-chain, all with >95% probability, and 96 and 23 spectra. The PP of 2 cases of SAA amyloid showed serum amyloid protein A, all with >95% probability, and 24 and 57 spectra. The PP of 2 cases with TTR amyloid showed transthyretin, all with 100% probability, and 17 and 35 spectra. The PP of 2 cases of LECT2 amyloid showed LECT2, all with >95% probability, and 9 and 11 spectra. The PP of SAA, Afib, LECT-2 and TTR amyloid did not show Ig heavy or light chains. The PP of 4 cases of Ig AH renal amyloid showed Ig heavy chains (AH) with (2 cases) or without (2 cases) light chains. In addition, amyloid P component (SAP), Apolipoprotein E and A-IV were also present in all cases. The results by LMD/MS correlated with clinical findings of plasma cell disorders, genetic testing for LECT2 and TTR variants, and immunohistochemistry for TTR, SAA, Ig light chains and SAP. We conclude that LMD/MS is a sensitive and specific tool for diagnosis and accurate typing of renal amyloid.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC115**

**PR3-ANCA Positivity in Patients without Primary Systemic Vasculitis** Stephen Paul McAdoo, Angela E. Hall, Jeremy B. Levy, Charles D. Pusey, Alan D. Salama. *Imperial College, London.*

Background: We recently indentified 3 patients who presented with clinical features of small vessel vasculitis in association with PR3-ANCA positivity, who were subsequently diagnosed with bacterial endocarditis. The combination of cANCA on immunofluorescence (IIF) and anti-PR3 on ELISA is reported to be highly (99%) specific for Wegener's Granulomatosis versus diseased controls.

Aims: To identify the rate and clinical associations of PR3-ANCA positivity in patients without primary systemic vasculitis using current methods in our laboratory.

Methods: Retrospective review of all patients identified as cANCA and anti-PR3 antibody positive as determined by IIF and Luminex testing respectively in our laboratory over a 6 month period.

Results: 194 patients were positive for the combination of cANCA on IIF and anti-PR3 on Luminex. The majority (174 patients) had primary ANCA-associated vasculitis (AAV). Twenty patients (10%) who were PR3-ANCA positive but without a diagnosis of AAV were indentified. Clinical associations included bacterial endocarditis (2 patients), HIV (3), TB (2), other automimmune disorders (4), malignancies (3), osteoarthritis (2), cirrhosis (1), bronchiectasis (1), myelofibrosis (1), cyclical thrombocytopenia(1). Anti-PR3 titre ranged from 31-278 (normal range 0-25)

Conclusions: Clinicians should remember the rare but recognised association of bacterial endocarditis and PR3-ANCA. Our data suggests that PR3-ANCA may be found in association with other conditions, including infections, malignancy and autoimmune diseases.

The aetiology of these ANCA are unclear; a process of 'molecular mimicry' with microbial antigens has been suggested.

A recent study in healthy army recruits suggests the presence of PR3-ANCA may predate disease onset by 2 years. It remains to be seen if any of this cohort will develop AAV and ongoing follow-up is advised.

Our data suggest that application of ANCA testing to an unselected population, with a low clinical suspicion of vasculitis, is likely to increase "false-positive" rates. Reliance on detecting PR3-ANCA this population, without securing a tissue diagnosis, may lean to erroneous diagnoses and catastrophic treatment decisions.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC116

**Anti-PLA$_2$R Autoantibodies in Recurrent Membranous Nephropathy** Laurence H. Beck,[1] Fernando G. Cosio,[2] Fernando C. Fervenza,[2] Fahim Malik,[1] Jean M. Francis,[1] Joel M. Henderson,[3] William S. Asch,[4] Reginald Y. Gohh,[5] David J. Salant.[1] *[1]Nephrology, Boston University Medical Center, Boston, MA; [2]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [3]Pathology, Boston University Medical Center, Boston, MA; [4]Yale New Haven Transplantation Center, New Haven, CT; [5]Rhode Island Hospital, Providence, RI.*

Antibodies to the phospholipase A2 receptor (PLA2R) are present in up to 80% of patients with primary membranous nephropathy (MN) and are associated with clinical disease activity. The aim of this study was to determine, in patients with recurrent MN after renal tansplantation, the prevalance of anti-PLA2R and the association with disease activity after treatment with rituximab.

Human PLA2R was immunoblotted with patient serum (1:25) and IgG4 anti-PLA2R was detected with appropriate secondary antibodies. Levels were semi-quantitatively assessed by densitometry.

7 of 12 patients (58%) with recurrent MN had anti-PLA2R antibodies. In those positive for anti-PLA2R, there was a disappearance of anti-PLA2R in many of those who responded to rituximab. Several patients who were negative for anti-PLA2R did not completely respond to rituximab.

The significance of these findings is underscored by the case of a young male diagnosed with MN in 2004. He progressed to ESKD and ultimately received a kidney transplant in 2010. This patient was found to have circulating anti-PLA2R antibodies 1 week post-transplantation; subsequent testing showed that he had also been positive immediately pre-transplantation. Although initially non-proteinuric, he developed 1.8 g/d proteinuria 1 month after his transplant. A renal biopsy performed at this time showed early recurrent MN, with tiny subepithelial deposits by EM. He has been treated with rituximab with initial improvement in proteinuria.

The proportion of anti-PLA2R positivity in patients with recurrent MN is similar to that found in those with primary MN. A disappearance of anti-PLA2R is associated with a clinical response to treatment with rituximab. Detection of circulating anti-PLA2R in the peri-transplantation period may be a useful tool in the monitoring of these patients.

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## TH-FC117

**De Novo MGN Is Immunologically Distinct from Idiopathic MGN and Associated with C4d Deposition** A. Bernard Collins,[1] Laurence H. Beck,[3] Anjali A. Satoskar,[2] Daniel L. Karel,[3] Evan A. Farkash,[1] Tibor Nadasdy,[2] Robert B. Colvin.[1] *[1]Pathology, Massachusetts General Hospital, Boston, MA; [2]Pathology, Ohio State University Medical Center, Columbus, OH; [3]Nephrology, Boston University School of Medicine, Boston, MA.*

**Background**: De novo MGN is a significant complication of renal transplantation that occurs in HLA-identical and non-identical allografts. Beck et al have recently demonstrated that 70% of patients with idiopathic MGN have autoantibodies to phospholipase A2 receptor (PLA$_2$R). **Purpose:** To test whether de novo MGN is associated with autoantibodies to PLA$_2$R and features of alloantibody mediated rejection and whether IF studies can distinguish de novo and idiopathic MGN. **Methods:** Sera from 18 patients in which the diagnosis of de novo MGN was made at the MGH (n=4) or at OSU Medical Center (n=14) were tested for reactivity to PLA$_2$R by western blot analysis, using native or recombinant forms of the antigen. C4d was detected in frozen tissue sections using a sensitive 3-step IF technique to seek evidence of alloantibody reactivity. Double IF studies for IgG4-FITC and PLA$_2$R-Cy3 were performed on frozen sections for evaluation of colocalization by visual inspection and morphometry. **Results:** None of the sera (0/18) from de novo MGN were reactive to PLA$_2$R, in sharp contrast to the published prevalence of approximately 70% anti-PLA$_2$R in idiopathic MGN. The IF in renal biopsies revealed distinctive patterns in the two diseases. IgG4 and PLA$_2$R did not colocalize in glomeruli in de novo MGN, but did in idiopathic and recurrent MGN by both visual and morphometric analysis. C4d was positive in peritubular capillaries (PTC) in 72% (13/18) of the allograft biopsies from de novo MGN. Renal allograft biopsies with FSGS or IgA nephropathy had a low frequency C4d+ PTC (0/17)**. Conclusions:** De novo MGN has a distinct pathogenesis from idiopathic MGN. These morphologically similar conditions can be distinguished in renal biopsies by IF for IgG4 and PLA$_2$R. The high frequency of C4d$^+$ argues that de novo MGN is a form of chronic alloantibody mediated rejection, of unknown specificities, that affects PTC endothelium and glomerular podocytes.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC118

**Variants in the Aquaporin-1 Gene Influence the Baseline Permeability and the Outcome of Peritoneal Dialysis** Céline Maréchal,[1] Marion Verduijn,[2] Raymond T. Krediet,[3] Pieter Evenepoel,[4] Friedo W. Dekker,[2] Olivier Devuyst.[1] *[1]Nephrology, UCL, Brussels, Belgium; [2]Epidemiology, Leiden UMC, Leiden, Netherlands; [3]Nephrology, AMC, Amsterdam, Netherlands; [4]Nephrology, KUL, Leuven, Belgium.*

Patients starting peritoneal dialysis (PD) show a significant variability in water transport across the peritoneal membrane (PM). The water channel aquaporin-1 (AQP1), the molecular counterpart of the ultrasmall pore, plays an essential role in the osmotic water flow across the PM. In this study we investigated whether variants in the *AQP1* gene influence the permeability of the PM and the outcome of PD. We sequenced the four exons, 2000 bp in the promoter and in the 3'UTR in the *AQP1* gene and investigated the influence of variants on baseline peritoneal equilibration test (PET) parameters and on mortality and technique failure (TF) in 165 incident Caucasian PD patients treated in Belgium (UCL and KUL). The Cox regression analysis was also performed for death and TF in a second, independent replication cohort (NECOSAD and AMC) (N = 576). Four SNPs (rs1476597, rs2075574, rs10253374 and rs1049305), with a minor allele frequency higher than 10%, were studied. In the Belgian cohort, patients with the T/T genotype of the rs2075574 in the promoter showed a significantly lower ultrafiltration (UF) ($p = 0.01$) than the C/C and C/T, whereas the D/P creatinine at 4 h was similar. Patients with the T/T genotype had an increased risk of death and TF (HR = 2.28, 95% CI 1.05 - 4.96). In the replication cohort, we also observed an influence of the T/T genotype on survival and TF (HR = 1.71, 95% CI 1.17 - 2.51). In vitro analysis showed that this variant resulted in a 61 % loss of activity relative to the wild type sequence for a luciferase reporter construct containing the proximal 1053 nucleotides of the *AQP1* promoter transfected into human erythroid k562 cells. Our data show that a promoter variant in *AQP1* influences the UF and the outcome of PD, in relation with a strong influence on the promoter activity. These results substantiate the influence of genetic factors on the transport parameters and the outcome of PD.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC119

**High Glucose Levels Induce Endoplasmic Reticulum Stress in Peritoneal Mesothelial Cells** Junichi Nakamata,[1] Hiroyuki Morimoto,[2] Ryoko Baba,[2] Tetsu Miyamoto,[1] Tatsuya Shibata,[3] Ryota Serino,[1] Narutoshi Kabashima,[3] Yutaka Otsuji,[1] Yoshiaki Doi,[2] Masahito Tamura.[3] *[1]The 2nd Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyusyu, Japan; [2]Department of Anatomy, University of Occupational and Environmental Health, Kitakyusyu, Japan; [3]Kidney Center, University of Occupational and Environmental Health, Kitakyusyu, Japan.*

Background. It is important to maintain adequate peritoneal structure and function for long-term peritoneal dialysis (PD) therapy. Peritoneal dialysate, containing non-physiological materials such as glucose, is implicated in long-term damage of the peritoneal membrane. Endoplasmic reticulum (ER) stress is associated with the progression of hyperglycemia-associated atherosclerosis and kidney diseases; however, the involvement of ER stress in PD has yet to be elucidated. We investigated the role of high glucose as an inducer of ER stress in PD, and examined components of the ER stress pathways in rat peritoneal mesothelial cells (PMCs). Following the culture of primary PMCs obtained from the peritoneal parietal wall of Wistar rats, serum-starved PMCs were incubated with 2% d-glucose in culture medium for 0-72 hours. High glucose concentrations significantly increased both the expression and phosphorylation levels of eIF2 alpha, a key signaling molecule that attenuates general protein translation and induces apoptosis in the PERK pathway, in a time-dependent manner with a peak at 6 hours. The ratio of phosphorylated to total eIF2 alpha was also increased in the same manner. After a 6-hour incubation of PMCs with 0-4% glucose, the relative phosphorylation levels of eIF2 alpha were increased in a concentration-dependent manner up to 2% glucose. At concentrations greater than 2%, glucose suppressed both the cell viability examined by WST-1 assay. These results demonstrate that high glucose concentrations promote eIF2 alpha-mediated ER stress in PMCs, and suggest that ER stress might be involved in peritoneal damage in patients receiving PD therapy.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC120**

**Peritoneal Macrophage Infiltration Is Correlated with Baseline Peritoneal Solute Transport Rate in Peritoneal Dialysis Patients** Akiho Sawai, Yasuhiko Ito, Masashi Mizuno, Yasuhiro Suzuki, Susumu Toda, Isao Ito, Waichi Sato, Naotake Tsuboi, Shoichi Maruyama, Enyu Imai, Yoshifumi Takei, Yukio Yuzawa, Seiichi Matsuo. *Department of Nephrology and Renal Replacement Therapy and Biochemistry, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

**Purpose:** High baseline peritoneal solute transport rate is reportedly associated with reduced patient and technical survival in peritoneal dialysis patients. However, the determinants of baseline peritoneal solute transport rate remain uncertain. The aim of this study was to investigate the relationship between peritoneal local inflammation, angiogenesis and systemic inflammation, and baseline peritoneal permeability.

**Methods:** Peritoneal biopsy specimens from 42 pre-dialysis uremic patients and 11 control individuals were investigated. Immunohistochemistry for CD68-positive macrophages, chymase- and tryptase-positive mast cells, interleukin-6 (IL-6)-positive cells, CD3-positive T cells , CD20-positive B cells, myeloperoxidase-positive neutrophils, and CD31- and pathologische anatomie Leiden-endothelium (PAL-E)-positive blood vessels in the peritoneum were performed. Clinical and laboratory parameters were measured at the time of peritoneal biopsy. Baseline dialysate-to-plasma ratio for creatinine (D/P Cr) was assessed after PD induction.

**Results:** Pre-dialysis uremic peritoneum showed infiltration by CD68-positive macrophages, IL-6-positive cells and mast cells, as compared to controls. Baseline D/P Cr was correlated with density of macrophages ($p<0.001$), IL-6-positive cells ($p<0.001$), CD31-positive ($p<0.05$) and PAL-E-positive blood vessels ($p<0.05$), and serum albumin ($p<0.05$). However, baseline peritoneal permeability was not correlated with infiltration by mast cells, T cells, B cells, neutrophils, CRP, age, gender, diabetes or other co-morbidities. On multiple linear regression analysis, the number of CD68-positive macrophages in peritoneum was an independent determinant for baseline peritoneal permeability ($p=0.009$).

**Conclusions:** Peritoneal macrophage infiltration is predominant in uremic patients and is an important factor in determining baseline peritoneal permeability.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC121**

**Atrial Natriuretic Peptide Prevents Peritoneal Adhesion Formation with Inhibiting Macrophage and CD3-Positive Cell Infiltration** Hideki Yokoi, Masato Kasahara, Kiyoshi Mori, Takashige Kuwabara, Hirotaka Imamaki, Tomoko Kawanishi, Kenichi Koga, Akira Ishii, Keita Mori, Yukiko Kato, Akira Sugawara, Masashi Mukoyama, Kazuwa Nakao. *Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan.*

Encapsulating peritoneal sclerosis is an infrequent, but most serious and refractory complication on continuous ambulatory peritoneal dialysis (CAPD). EPS is associated with high mortality in patients on CAPD. EPS is an intestinal obstruction syndrome caused by peritoneal deterioration and inflammation leading to intestinal adhesions with fibrin deposition. Treatment of EPS is total parenteral nutrition, steroid and surgery. We have already reported that natriuretic peptide inhibits extracellular matrix deposition in the kidney. To evaluate the therapeutic potential of atrial natriuretic peptide (ANP) on EPS, we treated chlorhexidine gluconate (CG)-induced EPS model mice with continuous intraperitoneal infusion of human ANP (carperitide). Next, CG was administered to develop EPS in brain natriuretic peptide (BNP)-transgenic (BNP-Tg) mice which overexpress mouse BNP in the liver with elevated level of plasma BNP, and in natriuretic peptide receptor GC-A deficient (GC-A KO) mice. Continuous intraperitoneal ANP infusion significantly prevents peritoneal adhesion. Inflammatory cytokines, TNF-$\alpha$ and IL-1$\beta$ were significantly reduced in ANP-treated mice. Surprisingly, the thickness of peritoneal membrane was not changed between ANP- and vehicle-infused CG-treated mice. Fibrosis-related gene expressions such as TGF-$\beta$1, CTGF, fibronectin and COL1A1 were not different. Infiltration of macrophages and CD3-positive cells was reduced with ANP treatment. BNP-Tg mice also showed reduced peritoneal adhesion with decreased expression of inflammatory cytokines and with reduced infiltration of macrophages and CD3-positive cells. In contrast, GC-A deficient mice exhibited increased peritoneal adhesion with augmented inflammation. These results indicate that natriuretic peptides prevent peritoneal adhesion, and that natriuretic peptides can be therapeutic drugs against EPS and peritoneal adhesion.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC122**

**Clinical Benefits of a CAPD Technique with One Icodextrin-Containing and Two Glucose-Containing Dialysates a Day in Incident CAPD Patients** Hye Eun Yoon,[1] Hyun Gyung Kim,[2] Yoon-Kyung Chang,[3] Young Soo Kim,[2] Seok Joon Shin,[1] Ho-Cheol Song,[4] Yong-Soo Kim.[5] *[1]Internal Medicine, The Catholic University of Korea, Incheon, Republic of Korea; [2]Internal Medicine, The Catholic University of Korea, Uijeongbu, Gyeonggi-do, Republic of Korea; [3]Internal Medicine, The Catholic University of Korea, Daejeon, Republic of Korea; [4]Internal Medicine, The Catholic University of Korea, Bucheon, Gyeonggi-do, Republic of Korea; [5]Internal Medicine, The Catholic University of Korea, Seoul, Republic of Korea.*

Icodextrin-based solutions have advantages over glucose-based solutions in fluid and metabolic management in continuous ambulatory peritoneal dialysis (CAPD) patients. We observed the clinical benefits of a three-exchanges-per-day CAPD technique using one icodextrin-containing and two glucose-containing dialysates in incident CAPD patients.

Sixty-eight incident CAPD patients completed the 12-month multicenter prospective randomized controlled trial. After one month of a CAPD schedule using four exchanges of glucose dialysates, patients were randomized to two groups; GLU group using four glucose-containing dialysates (n=33) and ICO group using one icodextrin-containing and two glucose-containing dialysates (n=35). The ICO group used icodextrin for the long-dwell (12h). Variables related to residual renal function (RRF), biocompatibility and dialysis adequacy were measured.

Weekly renal creatinine clearance and urine volume were significantly reduced during the 6 and 12 months in the GLU group ($P<0.05$), but not in the ICO group ($P>0.05$). The peritoneal glucose absorption was significantly less in the ICO group than the GLU group during 6 and 12 months ($P<0.05$). The ICO group showed a significant decrease in LDL-cholesterol and the GLU group showed a significant decrease in HDL-cholesterol ($P<0.05$). The weekly KT/Vurea, weekly creatinine clearance, and blood pressure did not differ between the two groups.

In conclusion, the CAPD technique using once icodextrin-containing and twice glucose-containing dialysates daily better preserved the RRF, was more biocompatible and showed comparable dialysis adequacy compared to the conventional CAPD technique in incident CAPD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC123**

**A Randomized Controlled Trial Comparing Mupirocin to Polysporin Triple for the Prevention of Catheter Related Infections in Peritoneal Dialysis (PD) Patients** Rory F. McQuillan, Ernest Chiu, Charmaine E. Lok, Sarbjit Vanita Jassal. *Division of Nephrology, University Health Network, Toronto.*

**Aims**

Infectious complications remain a significant cause of PD technique failure. Topical ointments appear to reduce peritonitis, however concerns over resistance have led to a quest for alternative agents. We studied the effectiveness of Polysporin Triple ($P^3$) in a multi-centred, double blind randomized controlled trial.

**Methods**

PD patients aged ≥18 yrs were eligible. Exclusion criteria included recent infection or use of an antibiotic; known allergies to the study drugs; or planned transplant surgery. Ointments were prepared by a central trials pharmacy and dispensed in identical study jars. Stratified variable block randomization using computer-generated random-number list was used to allocate treatment. Patients were stratified on PD modality; PD vintage and study site. The primary outcome was defined as a catheter related infection (a composite endpoint of exit site infection, tunnel infection or peritonitis). All patients were followed for 18 months or until death, transplant or transfer to hemodialysis. The time to first catheter related infection was compared using time-dependent univariate analysis.

**Results**

201 of 304 eligible patients were recruited. Patients were mostly male (65%) aged 60.5+/-14.4 years and on PD for a median 9.5 months. 31% were incident PD patients. 4% had MRSA nasal carriage and 31% had >1 previous peritonitis episode. 43.6% were diabetic A total of 75 patients had an event (51 peritonitis episodes, 24 exit site infections). No difference in the time to first event was seen (13.2 ±0.7 and 14.0±0.7 months) for $P^3$ and mupirocin respectively p=NS). There were twice as many patients with redness at the exit site in the $P^3$ group (16 versus 8, p=NS). A higher number of patients using $P^3$ had positive fungal exit site cultures(7 vs 0, p= 0.01).

**Conclusion**

This study has shown that $P^3$ is not superior to mupirocin in the prophylaxis of catheter related infections. The higher risk of positive fungal cultures needs further investigation. As such we cannot advocate the use of $P^3$ over mupirocin in the prophylaxis of catheter related infections in peritoneal dialysis.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC124**

**A Costing Model for Use in Evaluating the Fiscal Impact of Home Versus In-Center Dialysis within Various Healthcare Systems** Paul Komenda,[1, 2] Susan Garfield,[3] Amy White Poret,[3] Manish M. Sood,[1, 2] Meghan B. Gavaghan.[3] *[1]Medicine, University of Manitoba, Winnipeg, MB, Canada; [2]Manitoba Renal Program, Seven Oaks General Hospital, Winnipeg, MB, Canada; [3]Bridgehead International, Boston, MA.*

**Purpose and Rationale:** An economic model was developed to calculate and contrast the costs to payers for providing three types of hemodialysis (HD) in Australia, Canada and the United States. The model was designed to provide decision-makers with a transparent tool for assessing the costs and cost drivers of hemodialysis modalities in the first year of treatment and beyond.

**Methods** Model inputs were derived from a literature review that yielded published cost input values as well as observational data from a clinical setting in Canada. The specific dialysis modalities used to develop the model comprised conventional in-center (ICHD), conventional home (CHHD), and nocturnal home hemodialysis (NHHD). Sensitivity analyses were conducted on all cost variables.

**Results** The model found that ICHD costs are stable over time and driven by staffing, renal medication, and infrastructure costs. CHHD and NHHD costs in year 1 are driven by renal medication costs, patient training costs, costs for machines, consumables, and home preparation. Subsequent year costs are driven by renal medication, consumables, and hospitalization costs. Costs for CHHD and NHHD were comparable to ICHD in year one and less than ICHD in subsequent years. (See table)

**Conclusions** These results reinforce findings from previous studies indicating that HHD modalities provide economic advantages over ICHD. As demand and associated costs for end-stage renal disease treatment increases in most countries, payers can use this model to better understand their hemodialysis costs. The model demonstrates the positive cost-impact from patients moving to home versus in-center hemodialysis modalities.

Costs for CHHD, NHHD and ICHD

| | Canada | | US | | Australia | |
|---|---|---|---|---|---|---|
| | Year 1 | Year 2 & Beyond | Year 1 | Year 2 & Beyond | Year 1 | Year 2 & Beyond |
| ICHD | $50,739 | $50,399 | $54,244 | $53,904 | $50,313 | $49,973 |
| HHD | $45,788 | $35,419 | $48,937 | $39,186 | $46,458 | $35,962 |
| NHHD | $47,174 | $36148 | $50,708 | $40,301 | $47,843 | $36,691 |

Disclosure of Financial Relationships: nothing to disclose

**TH-FC125**

**Determinants and Predictors of Successful Home Hemodialysis Training in a Multi-Center US Observational Cohort** Christopher T. Chan,[1] John E. Moran.[2] *[1]Toronto General Hospital, Torontao, Canada; [2]DaVita Inc., Denver, CO.*

**Background:** Frequent home hemodialysis (HHD) is a growing renal replacement modality in North America. Emerging data suggest that intensive hemodialysis offers multiple clinical advantages over conventional hemodialysis while providing improved quality of life. Yet, there has been a lack of studies examining the suitability and determinants of successful HHD candidates.

**Methods and Objectives:** This was a descriptive cohort study of all patients enrolled into an HHD program with a large, US dialysis provider from January 1 2004 to December 31 2009. Our primary objectives were to define the demographic characteristics of our HHD cohort and to assess the risk factors for patients unable to successfully undertake HHD therapy.

**Results:** From January 1 2004 to December 31 2009, 1222 were able to achieve HHD therapy after a mean training duration of 17.7 ± 0.2 days (mean ± SE). In contrast, 144 patients failed to graduate to HHD despite receiving 10.1 ± 0.6 training days. The demographics of the HHD cohort and the unsuccessful training group are summarized in Table 1. A multivariable model predicting HHD training success indicated that age (per 1 year increase) (odds ratio: 0.97, p<0.001) and diabetes status (odds ratio: 0.65, p=0.006) were significant risk factors for failure.

Table 1: Demographic Characteristics of Home Hemodialysis Patients vs. Patients unable to achieve HH

| | Home Hemodialysis (n=1222) | Training Failure (n=144) |
|---|---|---|
| Age (years)* | 52 ± 0.4 | 56 ± 1.1 |
| F:M | 421:801 | 49:95 |
| Diabetes (%)* | 29 | 40 |
| Body Mass Index (g/m$^2$) | 28.4 ± 0.2 | 28.7 ± 0.6 |
| Hypertension (%) | 24 | 16 |
| Charlson Co-morbidity Index* | 5.2 ± 0.1 | 5.6 ± 0.2 |
| Dialysis Vintage (years) | 3.1 ± 0.1 | 2.9 ± 0.3 |

**Conclusion:** This is the first US multi-center systematic analysis of the determinants of HHD patients. This study showed that advanced patient age and diabetes status are associated with HHD training failure. These data may be used to help predict which patients are likely to succeed or fail in training.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC126**

**A Longitudinal Follow-Up of Quality of Life amongst Patients on Nocturnal Haemodialysis** David T. Lau, John W. M. Agar. *Renal Medicine, Geelong Hospital, Barwon Health, Geelong, Victoria, Australia.*

Background: Studies have shown that nocturnal haemodialysis (NHD) improves quality of life (QOL) when compared with conventional dialysis regimens. However, to our knowledge, there is currently no published QOL data of NHD patients followed over a prolonged period. We report the QOL outcomes for NHD over an 8 year period.

Method: Using the KDQOL-36, a validated tool in assessing QOL in dialysis subgroups, QOL data was collected annually from 2001 to 2009; medical records were analyzed for factors confounding QOL. Patients were categorized by according to the number of years they had been on NHD.

Results: A total of 111 surveys were returned in the 8 years, with a total of 314 patient-years on NHD. The two QOL domains reflecting the 'prevalence of symptoms' and the 'individual restrictions placed on lifestyle by dialysis treatment' both scored similarly and were sustained at a consistently high level throughout the 8 year study. However, our NHD patients did still feel that the total burden of their kidney disease and its treatment requirements remained significant, as was reflected by their lower though also stable scores for the 'burden of kidney disease'. Physical and mental health composite scores compared favourably with the normal population.

Conclusion: This study reveals that the symptom impact and lifestyle limitations in the NHD group are broadly mild and do not deteriorate throughout an 8 year follow-up period. However, there was a pervasive perception of frustration at the interference kidney disease had on overall lifestyle. This impression persisted throughout the study period. This data suggests that despite the lack of major lifestyle limitations, and despite physical and mental stability, NHD patients are still dialysis-dependent – an unalterable, permanent, additional encumbrance in their daily lives.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC127**

**Caregiver Burden among Nocturnal Home Hemodialysis Patients** Jean-Philippe Rioux, Christopher T. Chan. *Department of Medicine, Division of Nephrology, University Health Network, Toronto, ON, Canada.*

**Background:** Recent studies have suggested improvements in quality of life (QOL) in patients on quotidian dialysis compared with conventional hemodialysis. Few studies have focused on the burden and QOL in caregivers of patients with end-stage renal disease (ESRD) on nocturnal home hemodialysis (NHD). We aim to assess the caregiver burden, QOL and depressive symptoms and to compare these parameters with their patient counterparts.

**Methods:** Cross-sectional surveys were sent to 61 prevalent NHD patients and their caregivers. Surveys assessed demographics, general self-perceived health using the 12-Item Short Form Health Survey (SF-12) and the presence of depression using the Beck Depression Inventory. Subjective burden on caregivers was assessed by the Caregiver Burden scale and was compared with the perceived caregiver burden among patients on NHD.

**Results:** Thirty six patients and 31 caregivers completed the survey. The majority of caregivers were female (66%), spouse (81%) with no comorbid illness (72%). Their mean age was 51 ± 11 years. Patients were mostly male (64%) with a median ESRD vintage of 60 months (IQR, 18-136 months) and a mean age of 52 ± 10 years. Compared to caregivers, patients had lower perceived physical health score but had similar mental health score. Depression criteria were present in 47% of patients and 25% of caregivers. Total global burden perceived by either caregivers or patients is relatively low.

**Conclusion:** Although there is a relatively low global burden perceived by caregivers and patients undergoing NHD, a significant proportion of both groups fulfilled criteria for depression. Further innovative approaches are needed to support caregivers and patients performing NHD to reduce the intrusion of caring for a chronic illness and their risk of depression.

Measures of health among patients and their caregivers

| | Patients | Caregivers | p-value |
|---|---|---|---|
| | (n=36) | (n=31) | |
| | | | |
| SF-12 | | | |
| Physical component score | 43.9 ± 10.2 | 49.4 ± 10.2 | 0.03 |
| Mental component score | 46.9 ± 10.7 | 46.1 ± 11.6 | 0.8 |
| Beck Depression Inventory | | | |
| Depression, % | 47 | 25 | 0.058 |
| Caregiver Burden Scale | | | |
| Global burden | 1.9 ± 0.8 | 1.7 ± 0.5 | 0.3 |

Disclosure of Financial Relationships: nothing to disclose

**TH-FC128**

**Bone Microarchitecture in Hemodialysis Patients: A Disorder of Cortical and Trabecular Bone** Justine Bacchetta,[2] Solenne Pelletier,[1,2] Nicolas Vilayphiou,[2] Stephanie Boutroy,[2] Roland Chapurlat,[2] Denis Fouque.[1] *[1]Nephrology, Lyon, France; [2]Inserm U831, Lyon, France.*

Dual X-ray absorptiometry is the usual technique to analyze bone mineral density. Yet, measuring bone mass is not sufficient to define bone impairment: bone microarchitecture is also a major determinant of bone fragility. Here we hypothesize that volumetric bone mineral density (vBMD) and microarchitecture were impaired in maintenance hemodialysis (MHD) patients compared with control subjects.

56 MHD patients since 2.6±2.8 yrs were matched for age (51±16 yrs), BMI (25±4 kg/m²), gender and menopausal status for women, with 56 healthy subjects from epidemiological cohorts (STRAMBO and OFELY). A high-resolution peripheral quantitative computed tomography (HR-pQCT, XtremeCT, Scanco Medical AG) was used to assess cortical (Ct) and trabecular (Tb) vBMD and microarchitecture at the distal radius and tibia.

Compared to healthy subjects, MHD patients had a significant lower total vBMD at radius and tibia (respectively; -14% and -16% p<0.01), Ct vBMD (-6% and -3 % p<0.05), and Tb vBMD (-11% and -17% p<0.05). MHD patients had thinner cortex than controls at both sites (-16% and -17% p<0.01). Tb microarchitecture was also impaired in MHD patients. They had lowered Tb number (respectively at radius and tibia; -1% p=NS and -7% p<0.01), increased Tb separation (7% p=NS and 11% p<0.01) and heterogeneity of Tb network (7% p=NS and 16% p<0.01).

MHD patients presented an impairment of total, Tb and Ct vBMD, and Ct thickness at both sites compared to controls. However, Tb microarchitecture was significantly impaired at the tibia, but not at the radius. This suggests that Tb bone damage associated with MHD is more severe at lower limb than at radius, as already observed in CKD II-IV patients to a lesser extent.

HR-pQCT is a new non-invasive 3D-bone imaging technique and seems of interest in MHD. In this study, MHD patients had a greater bone impairment compared to healthy subjects. Longitudinal studies are required to confirm the differences observed between Tb microarchitecture of tibia and radius, and that this non-invasive technique allows improvement in the bone fracture prediction in this population.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC129

**TGF-β Plays an Important Role in the Pathogenesis of High-Turnover Renal Osteodystrophy** Shiguang Liu, Joseph H. Boulanger, Wenping Song, Robert Fogle, Eric C. Roux, Mandy M. Smith, Hong Ling, Yves Sabbagh, Susan Schiavi. *Genzyme Corp., Framingham, MA.*

TGF-β1 plays a critical role in normal bone remodeling by regulating coupling of bone resorption and formation. In osteitis fibrosa, a high-turnover renal osteodystrophy (ROD), TGF-β1 protein is increased in both osteocytes and bone marrow. To assess the relevance of this increase on the pathogenesis of high-turnover ROD (HTO), we administrated a neutralizing anti-TGF-β antibody, 1D11, to adenine treated rats, a uremic model of HTO. Ten week-old Sprague Dawley rats were randomized into 3 groups (n> 6): (1) Control; (2) Adenine and (3) Adenine plus 1D11 (10 mg/kg, 3X /wk for wks 1-10). All adenine treated animals were fed standard chow containing 0.6% adenine for wk1, followed by 0.3% adenine for wks 2-6. Compared with the control group, adenine treatment was associated with a dramatic increase in serum BUN by week 3 (72.0 ± 4.6- vs. 19 ± 0.5 mg/dl) and serum creatinine (Cr) (0.31 ± 0.01 vs. 11 ± 0.12) that was sustained through the 10 week study. 1D11 had no significant influence on the progression of renal failure. Consistent with the presence of HTO, the serum bone formation marker, osteocalcin (OCN) and the bone resorption marker, C-terminal telopeptides of type I collagen (CTX) were significantly elevated in the adenine group at week 10 (1961 ± 341 vs. 272 ± 20.0 ng/ml and 239 ± 35.7 vs. 43 ± 9.1 ng/ml respectively). Importantly, 1D11 administration led to significant reductions in serum OCN (775 ± 68.2 ng/ml) and CTX (142 ± 11.6 ng/ml) independent of kidney function. MicroCT analysis revealed significant cortical porosity (typically associated with HTO) in femurs from adenine treated animals that were ameliorated by 1D11 treatment. Bone mineral density and volume were reduced in adenine treated relative to control animals.1D11 treatment promoted a trend toward increases in these parameters. These findings are consistent with a direct effect of 1D11 on bone rather than a secondary effect on improved renal function. Taken together, these data suggest that TGF-β1 contributes to the pathogenesis of renal osteodystrophy and anti-TGF- β1 antibody may provide a novel therapeutic approach to HTO.

Disclosure of Financial Relationships: Employer: Genzyme Corp.; Ownership: Genzyme stock.

### TH-FC130

**Indoxyl Sulfate as a Modulator of Bone Turnover in Pre-Dialysis Chronic Kidney Disease Patients** Fellype Barreto,[1,2] Daniela Veit Barreto,[1,2] Aluizio B. Carvalho,[3] Maria Eugenia F. Canziani,[3] Cristianne Tomiyama,[3] Andrea Higa,[3] Anais Mozar,[1] Griet Lrl Glorieux,[4] Raymond C. Vanholder,[4] Ziad Massy.[1,2] *[1]INSERM ERI-12 (EA 4292), Amiens, France; [2]Clinical Research Centre - Division of Clinical Pharmacology, Amiens University Hospital and the Jules Verne University of Picardie, Amiens, France; [3]Division of Nephrology, Department of Internal Medicine, Federal University of São Paulo, São Paulo, SP, Brazil; [4]The Nephrology Section, Department of Internal Medicine, University Hospital, Gent, Belgium.*

Pre-clinical evidence suggests that indoxyl sulfate (IS) may be involved in the development of low bone turnover in uremia. The present study sought to characterize renal osteodystrophy (ROD) in pre-dialysis chronic kidney disease (CKD) patients and evaluate the association between histomorphometric parameters and circulating IS levels. Bone biopsies were performed in 49 consecutive patients (mean±SD age: 52±10; 67% male; estimated glomerular filtration rate: 36±17 mL/min). Patients at CKD stages 2 and 3 presented remarkably low bone formation rate. Patients at CKD stages 4 and 5 presented significantly higher osteoid volume, osteoblast and osteoclast surface, bone fibrosis volume and bone formation rate and a lower mineralization lag time than CKD stage 2 and 3 patients. We observed a positive association between IS levels on one hand and the bone formation rate, osteoid volume, osteoblast surface and bone fibrosis volume on the other. Multivariate regression models confirmed that the associations between IS levels and osteoblast surface and bone fibrosis volume were both independent of demographic characteristics and other assayed circulating factors capable of affecting bone turnover (calcium, phosphorus, intact-PTH, FGF-23 and vitamin D). A similar trend was observed for the bone formation rate. Our findings suggest that the ROD in the initial phases of CKD may be characterized by a lower bone turnover state. Moreover, we demonstrated that IS is positively associated with bone formation rate, osteoid volume, osteoblast surface and fibrosis volume in a clinical CKD setting.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC131

**Novel Mechanisms of Regulating Bone Formation in Chronic Kidney Disease (CKD)** Yifu Fang,[1] Suresh Mathew,[1] Richard Lund,[2] Keith A. Hruska.[1] *[1]Division of Pediatric Nephrology, Washington University School of Medicine, St. Louis, MO; [2]Division of Nephrology, Creighton University, Omaha, NE.*

The serum phosphorus has been established as a cardiovascular risk factor in CKD. Prospective studies *in vivo* demonstrate phosphorus stimulated vascular calcification (VC), and the stimulation of aortic osterix expression, which if inhibited stops VC. In several interventional studies, successful treatment of VC is associated with stimulation of bone formation. Human observational studies show inverse relationships between VC and bone formation in CKD, osteoporosis, and diabetes. A hypothesis tested in the studies reported here is that heterotopic mineralization inhibits orthotopic bone formation since osterix is a transcription factor for a circulating inhibitor of bone formation, Dkk1. In our hyperphosphatemic animal model of CKD stimulated VC and renal osteodystrophy/osteoporosis, we utilized a non-absorbable phosphorus binder that has no known systemic effects besides inhibition of phosphate absorption, reduction of the serum phosphorus and secondarily, PTH levels. In our model CKD induced VC and aortic osteoblastic gene transcription including osterix. We demonstrated increased aortic expression and serum levels of Dkk1 in the CKD animals. Treatment with LaCO3 1 and 3% added to the diet resulted in significant stimulation of bone formation rates and inhibition of FGF23 and VC. As a result, the osteoporosis of our animal model was corrected through an increase in bone volume. Lowering if the serum Pi was associated with decreased aortic osterix and Dkk-1 gene expression, but serum Dkk-1 was not decreased. The mechanism of LaCO3 stimulated bone formation was discovered by examining intestinal hormonal factors. We found that CKD stimulated an inhibitor of bone formation, serotonin, which LaCO3 diminished. We conclude that CKD stimulated serotonin levels are inhibited by LaCO3 contributing to the stimulation of bone formation and the inhibition of VC in CKD. Furthermore, that CKD stimulated Dkk-1 levels are of renal origin and not affected by changes in VC. LaCO3 may be a drug affecting intestinal epithelial functions other than binding luminal Pi.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC132

**Bone Mass and Structure in Diabetic and Non-Diabetic CKD Versus Controls: A CRIC Study** Thomas L. Nickolas,[1] Matthew T. White,[2] Babette Zemel,[2] Takayuki Hamano,[3] Raymond R. Townsend,[3] Harold I. Feldman,[3] Lucy W. Kibe,[2] Michael Sulik,[2] Mary B. Leonard.[2] *[1]Medicine, Columbia Univeristy, NY, NY; [2]Pediatrics, Children's Hospital of Philadephia, Philadelphia, PA; [3]Medicine, University of Pennsylvania, Philadelphia, PA.*

CKD and diabetes mellitus (DM) increase fracture (FX) risk but effects on cortical (Ct) geometry and trabecular (Tb) and Ct volumetric bone mineral density (vBMD), determinants of bone strength, are not established.

305 CRIC subjects [MDRD eGFR median 46 (range 14-69)] were compared with 536 healthy, community controls. Tibia peripheral QCT (pQCT) scans were obtained for Tb/CtvBMD and Ct geometry [area, thickness, perimeter (CtArea, CtTh, CtPm)]. pQCT measures were converted to age-, sex-, race- specific Z-scores. iPTH and bone alk phos (BSAP; bone formation marker) were measured.

CKD vs. controls were older with higher BMI (Table). DM-CKD had higher BMI vs. NonDM-CKD. DM-CKD had greater TbvBMD, CtTh and CtArea vs. controls and NonDM-CKD, and greater CtPm vs. controls. After BMI adjustment group differences did not persist. CtvBMD was lowest in NonDM-CKD after BMI adjustment. CKD had higher iPTH levels than controls (both p<0.05). BSAP was highest in DM-CKD (p<0.05). CtvBMD was not associated with iPTH or BSAP.

DM-CKD was associated with greater TbvBMD and Ct dimensions, attributed to greater BMI. NonDM CKD was not associated with Tb deficits. NonDM-CKD was associated with lower CtvBMD. Mechanistically, increased bone turnover may result in lower CtvBMD. Greater bone formation (BSAP) in DM may offset this effect. Studies are needed to determine FX implications.

| | Controls | NonDM CKD | DM CKD |
|---|---|---|---|
| n | 536 | 144 | 161 |
| Age (yr) median (range) | 48 (21-79) | 60 (21-75)[a] | 62 (38-77)[a] |
| % Female | 54 | 33[a] | 32[a] |
| BMI (kg/m2) | 27±6 | 30±7[a] | 33±7[a,b] |
| Tb vBMD Z | 0.0±1.0 | 0.0±1.1 | 0.3±1.2[a,b] |
| CtTh Z | 0.0±1.0 | -0.08±1.3 | 0.3±1.1[a,b] |
| CtArea Z | 0.0±1.0 | 0.03±1.2 | 0.4±1.1[a,b] |
| CtPm Z | 0.0±1.0 | 0.1±1.1 | 0.3±1.0[a] |
| Ct vBMD Z | 0.0±1.0 | -0.4±1.1[a] | -0.2±1.1[a] |
| a: p<0.05 vs Controls | b: p<0.05 DM- vs NonDM- CKD | | |

Disclosure of Financial Relationships: Consultancy: Abbott DiagnosticsResearch Funding: Abbott Diagnostics: A study of NGAL in the Emergency Room; Patent: Columbia University has licensed NGAL to Abbott and Biosite.

**TH-FC133**

**VKORC1 Polymorphism Is Associated with CAC, CAC Progression and Survival in CKD Patients** Rachel M. Holden,[1,2] Alexander R. Morton,[1,2] Wilma M. Hopman,[3] Robert Louis Nolan,[4] Jocelyn S. Garland.[1,2] *[1]Medicine, Queen's University, Kingston, ON, Canada; [2]Queen's University Vascular Calcification Investigators; [3]Community Health and Epidemiology, Kingston General Hosptial; [4]Radiology, Queen's University.*

Polymorphisms in the vitamin K epoxide reductase complex subunit 1 (VKORC1), the enzyme target of warfarin, have been associated with an increase in cardiovascular disease (CVD) in the general population. Coronary artery calcification (CAC) is a prevalent form of CVD in the chronic kidney disease (CKD) population. We tested the hypothesis that the recessive *CC* VKOR genotype (G1542C SNP) would be associated with lower CAC, less CAC progression, and a lower incidence of mortality in a cohort of CKD patients over 5 years. In 2005, 171 stage 3-5 CKD patients had CAC quantified by MSCT scan. 12 % had *GG*, 49% had *GC* and 39% had *CC* genotypes. Considering the *CC* genotype versus the others, there were no differences according to sex, diabetes, age, or hypertension. Baseline CAC scores were lower in *CC* participants (median score 124 versus 302; p=0.051). In 2009, 86 patients had repeat CAC scores. Patients with the *CC* genotype were less likely to have progressive CAC. By logistic regression with increase of CAC score greater than 50 Agatston units over 5 years as the dependent variable, the *GG* or *GC* genotype was associated with an increased risk of progressive CAC (adjusted for age, diabetes, eGFR and hypertension). There was an interaction between baseline CAC score and VKOR genotype, such that the risk for progressive CAC was higher in individuals with the *GG* or *GC* genotype and baseline CAC score (OR 1.65; 95% CI 1.06 to 2.6; P=0.03). Survival analysis demonstrated increased all-cause mortality for patients with *GG* or *GC* genotype over five years, versus patients with the *CC* genotype (Log rank test p=0.001). By logistic regression mortality risk was four times higher for individuals with the *GG* or *GC* genotypes (OR 3.8; 95% CI 1.2 to 12.5; p=0.02) adjusted for age, gender, diabetes and micro-albuminuria. In summary, patients with the recessive VKOR *CC* genotype had lower baseline CAC levels, had a lower risk of CAC progression and a survival benefit over five years of follow-up.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC134**

**Bone Alkaline Phosphatase Is Strongly Associated with Mortality in Incident Dialysis Patients** Christiane Drechsler,[1,2] Marion Verduijn,[1] Stefan Pilz,[3] Raymond T. Krediet,[4] Friedo W. Dekker,[1] Christoph Wanner,[2] Markus Ketteler,[5] Elisabeth W. Boeschoten,[6] Vincent Brandenburg.[7] *[1]Dept of Clin Epidemiology, LUMC, Leiden, Netherlands; [2]Div of Nephrology, University of Wuerzburg, Germany; [3]Div of Endocrinology and Nuclear Medicine, Medical University of Graz, Austria; [4]Dept of Nephrology, AMC, Amsterdam, Netherlands; [5]Dept of Nephrology, Hospital Coburg, Germany; [6]Hans Mak Institute, Naarden, Netherlands; [7]Dept of Cardiology, RWTH University of Aachen, Germany.*

Background: Alkaline phosphatase (AP) has been found to be associated with vascular calcification and mortality in hemodialysis patients, but AP derives from various tissues of origin. The aim of this study was to assess the effect of bone-specific alkaline phosphatase (BAP) on morbidity and mortality in dialysis patients.

Methods: From a prospective cohort study of incident dialysis patients in the Netherlands (NECOSAD), we selected all patients with measured BAP at 12 months after the start of dialysis (baseline); n=800, mean age 59±15 years and BAP 18±13 U/l. By Cox regression analyses, we assessed the impact of BAP levels on mortality within 6 months (short term) and 4 years (longer term) of follow-up.

Results: Levels of BAP were positively correlated with levels of AP and parathyroid hormone. High levels of BAP strongly affected short-term mortality. After adjustment for confounders, patients of the highest BAP tertile had a 6 fold increased risk of death within 6 months (HR 6.1, 95% CI 2.2-17.0) compared to patients of the lowest tertile. The effects applied for both cardiovascular (HR 5.0, 95% CI 1.3-19.0) and non-cardiovascular mortality (HR 8.1, 95% CI 1.6-41.7). Furthermore, high levels of BAP were associated with increased cardiovascular mortality in the longer term (HR 1.7, 95% CI 1.1-2.6). In comparison with total AP, the effect sizes related to clinical outcomes were much higher for BAP.

Conclusions: High levels of BAP were strongly associated with short-term mortality in dialysis patients, pointing out the important impact of uncontrolled bone disease. Longitudinal assessments of BAP may be useful for the treatment monitoring in clinical practice in patients with end-stage renal disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC135**

**NFAT Activation Causes Podocyte Damage and Severe Proteinuria in Mice** Alexis J. Sloan,[1,2] Britta Sylvia Walter,[1] Ansel P. Amaral,[1,2] Wilhelm Kriz,[3] Jochen Reiser,[1] Christian Faul.[1,2] *[1]Department of Medicine, Division of Nephrology & Hypertension, University of Miami Miller School of Medicine, Miami, FL; [2]Department of Cell Biology & Anatomy, University of Miami Miller School of Medicine, Miami, FL; [3]Department of Anatomy and Developmental Biology, University of Heidelberg, Mannheim, Germany.*

Our previous work has shown that the activation of calcineurin, a calcium-dependent serine/threonine protein phosphatase, in podocytes causes the dephosphorylation of the actin-regulating protein synaptopodin. This is turn causes the cathepsin L-mediated degradation of synaptopodin leading to the loss of actin stress fibers in podocytes and the development of proteinuria in mice. In the present study we analyze the podocyte-specific function of NFAT (nuclear factor of activated T cells), a calcium/calcineurin-regulated transcription factor. To study consequences of NFAT activation in podocytes in vivo we utilized two mouse models. First, we expressed a constitutively active NFAT mutant form (NFATc1nuc) by generating an inducible, podocyte-specific double-transgenic mouse line (podocin-rtTA x tetO-CMV-NFATc1nuc-HA). Expression of NFATc1nuc led to severe proteinuria within 72 hours, which was homogenous and stable over several weeks. Ultra-structural analysis of podocyte morphology revealed podocyte damage which seemed non-progressive. Secondly, we used a transgenic NFAT/Luciferase reporter mouse line (NFAT-Luc) in order to identify stimuli that can induce NFAT activation in podocytes in vivo. Lipopolysaccharide (LPS) injections in mice have been previously used to generate rapid, reversible, calcineurin-dependent podocyte effacement resulting in proteinuria. When NFAT-Luc mice were injected with LPS, isolated glomeruli showed a significant increase in luciferase activity when compared to PBS injected animals. In summary, NFAT presents an additional important downstream target of calcineurin in podocytes. We hypothesize that NFAT is activated by certain stimuli that cause podocyte damage. Furthermore, once activated NFAT is sufficient to cause podocyte damage and proteinuria in mice.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC136**

**Functional Calcium Permeable NMDA Receptors Are Expressed in Podocytes: Upregulation in Diabetic Mice and Their Effects on Nephrin Expression** Stuart E. Dryer. *Biology and Biochemistry, University of Houston, Houston, TX.*

NMDA receptors have been extensively studied in brain, where their activation by L-glutamate is essential for many forms of synaptic plasticity, and where their hyperactivation causes neuronal death. Systemic administration of NMDA antagonists is reported to cause acute proteinuria[1]. Here we show that functional NMDA receptors are expressed in podocytes. Using whole-cell recordings we observed inward currents evoked by NMDA in mouse and human podocyte cell lines and in primary cultures of mouse podocytes. These currents reversed at 0 mV, were highly reproducible, and were seen in 100% of cells tested. They do not desensitize with sustained or repeated exposure to NMDA. Responses were blocked by elevated $Mg^{2+}$ or by the antagonists D-APV or MK-801. Similar currents were evoked by D-aspartate and L-homocysteic acid, but responses to L-glutamate were surprisingly small. NMDA-evoked currents were potentiated by the modulatory ligand D-serine, but not by glycine. Responses to NMDA were completely inhibited by the glycine modulatory site antagonist L689,560. Sustained (2 hr) activation of NMDA receptors causese activation of Erk, Akt, but not RhoA in podocyte cell lines. However higher concentrations of NMDA also cause RhoA activation and a loss of nephrin expression in podocyte cell lines, but did not cause cell death even after 72 hr. In streptozotocin-treated mice we observed marked up-regulation of the NR1 subunit of NMDA receptors in some glomeruli. In segments of glomeruli with markedly elevated NR1 expression, we observed almost complete loss of nephrin. We also observed that NMDA receptors interact with other slit diaphragm proteins such as synaptopodin, and that stable knockdown of synaptopodin in podocyte cell lines causes marked reduction in the amplitude of NMDA-evoked currents. It is possible that NMDA receptors play a role in glomerularphysiology and pathophysiology. The properties of podocyte NMDA receptors are similar to those of neurons in broad outline, but their pattern of agonist responsiveness is somewhat unusual, especially the weak responses to L-glutamate and L-aspartate. 1. L. Giardino et al., J Am Soc Nephrol. 20:1929-40 (2009).

Disclosure of Financial Relationships: nothing to disclose

**TH-FC137**

**Nephrin Endocytosis Is Mediated by a Tyrosine Based Endocytic Signal** Agnieszka Swiatecka-Urban. *Nephrology, Children's Hospital of Pittsburgh, Pittsburgh, PA.*

Nephrin is a transmembrane protein expressed at the slit diaphragm between the podocyte foot processes in the kidney glomerulus. Loss of nephrin expression reduces the integrity of the slit diaphragm and leads to proteinuria and nephrotic syndrome. The mechanisms that regulate the slit diaphragm integrity are incompletely understood. In particular, little is known about the role of nephrin endocytosis in the dynamic regulation of the slit diaphragm. The cytoplasmic tail of human nephrin contains several sequences that conform to the canonical tyrosine based endocytic motifs of the YxxØ type where x is any amino acid and Ø is an amino acid with a hydrophobic side chain. We hypothesized that the plasma membrane expression of nephrin is regulated by dynamic protein-protein interactions that control nephrin endocytosis. Studies were conducted in the T-SV40 immortalized human podocytes transiently transfected with human nephrin. YxxØ motifs

interact with the endocytic adaptor networks. Dynamin II provides intracellular vesicle transport by scission of the vesicles containing cargo proteins after endocytosis from the plasma membrane. Overexpression of the dominant negative, DynaminK44A increased the plasma membrane expression of nephrin indicating that nephrin undergoes endocytosis in our human podocyte cell model. Furthermore, nephrin co-immunoprecipitated with the small GTPases, Rab4 and Rab8 in the endocytic and recycling compartment. The Y1139RSL sequence in the nephrin cytoplasmic C-terminus is phosphorylated by Fyn and conforms to a canonical, tyrosine-based endocytic signal. Because the YxxØ motifs are activated as endocytic signals upon tyrosine de-phosphorylation we predicted that mutating the tyrosine to phenylalanine (non-phosphorylated tyrosine mimic) should activate nephrin endocytosis. The Y1139F mutant showed increased nephrin endocytosis compared to the WT-nephrin, examined by the glutathione protection assay and by immunofluorescence. Taken together our data demonstrate that the Y1139RSL sequence mediates nephrin endocytosis. Our data suggest that the Fyn mediated phosphorylation of Y1139 may control nephrin endocytosis and dynamically regulate the slit diaphragm integrity.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC138

**The Role of wnt/β-Catenin Signaling in Podocytes** Hideki Kato,[1] Laura M. C. Barisoni,[2] Lawrence B. Holzman,[3] Katalin Susztak.[1] *[1]Medicine/Nephrology, Albert Einstein College of Medicine, Bronx, NY; [2]Pathology, New York University School of Medicine, New York, NY; [3]Division of Nephrology, University of Pennsylvania School of Medicine, Philadelphia, PA.*

We previously reported increased activity of the Wnt/β-catenin signaling in glomeruli of DKD patients. We created mice with podocyte specific stabilized β-catenin (by deleting exon3 the phosphorylation site) (NPHS2Cre/Ctnnb1Ex3/Ex3) and found GBM abnormalities, mild albuminuria and increased susceptibility to DKD. Podocyte specific β-catenin knockout mice (NPHS2Cre/Ctnnb1KO/KO) showed GBM abnormalities and increased susceptibility to DKD.

To understand the role of Ctnnb1, we performed microarray analysis of isolated glomeruli of these mice. We found 411 differentially expressed genes between WT and stabilized β-catenin expressing mice and 642 differentially expressed genes between WT and knockout mice. 270 transcripts were regulated in both animal models.

Phenotype analysis showed that glomerular podocyte number was decreased and urinary WT1 level was increased in NPHS2Cre/Ctnnb1Ex3/Ex3 mice, indicating a defect in cell adhesion. Therefore we generated immortalized podocyte lines from these mice by mating with the Immortomouse. Clones were established by limiting dilution method and cells were infected with Cre adenovirus. We confirmed in vitro Cre mediated recombination of Ctnnb1. In podocyte at baseline, Ctnnb1 showed membrane and perinuclear expression pattern, however, after Ad-Cre infection Ctnnb1 was expressed in the nuclei of Ctnnb1Ex3/Ex3 cells while its expression was diminished in Ctnnb1KO/KO cells. We analyzed podocyte adhesion to collagen IV. Cells expressing stabilized Ctnnb1 showed lower adherence and cells lacking Ctnnb1 showed higher adherence. Similar results were obtained when cells were treated with recombinant wnt3a. Gene expression analysis of the podocyte clones indicated Ctnnb1 controls the expression of podocyte cell marker (Nephrin, Podocin, WT1), cell adhesion molecules (β1integrin) in addition to Ctnnb1 target genes (axin, dkk).

Our results indicate that Ctnnb1 plays a key role in podocytes by controlling their gene expression, adhesion and motility.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC139

**FSGS-Associated Mutations in TRPC6 Result in ERK Activation in a Calcineurin Dependent Manner** Johannes S. Schlondorff, Martin R. Pollak. *Renal Division, Beth Israel Deaconess Medical Center, Boston, MA.*

Gain-of-function mutations in the canonical transient receptor potential 6 (TRPC6) are a cause of autosomal dominant focal segmental glomerulosclerosis (FSGS), and increased TRPC6 expression has been linked to several acquired proteinuric renal diseases. The mechanism whereby abnormal TRPC6 activity results in proteinuria remains unknown. The ERK1/2 MAP kinases are activated in glomeruli and podocytes in several proteinuric disease models, including angiotensin II-mediated hypertension. We therefore examined whether FSGS-associated mutations in TRPC6 result in activation of these kinases.

In both 293T cells and cultured podocytes, overexpression of gain-of-function TRPC6 mutants resulted in increases in ERK1/2 phosphorylation, representing activation of these kinases. ERK1/2 activation was dependent on channel activity. Pharmacologic studies demonstrated that inhibition of either MEK1, the kinase responsible for activating ERK, or calcineurin abolished ERK phosphorylation due to mutant TRPC6 expression. Use of additional pharmacologic inibitors suggests that ERK activation downstream of mutant TRPC6 ALSO depends upon an atypical, not a calcium-dependent, protein kinase C (PKC), and is insensitive to inhibition of phospholipase C, Rho GTPase and Rho kinase (ROCK).

We next examined whether TRPC6 may form a complex with ERK. Endogenous ERK was found associated with immunoprecipitated TRPC6. This association was not appreciably altered by activation of TRPC6 via the muscarinic acetylcholine receptor, addition of the MEK inhibitor U0126 or calcineurin, or overexpression of Fyn, which induces the binding to PLC gamma to TRPC6.

In summary, we have found that FSGS-associated TRPC6 mutations lead to activation of the MEK-ERK signaling pathway, and that TRPC6 and ERK are capable of forming a complex. These results expand the list of potential downstream targets of TRPC6 and calcineurin to include ERK activation. These findings are intriguing as activation of the ERK MAP kinase pathway has been reported in several models of podocyte injury and proteinuria. The importance of ERK activation in TRPC6-mediated glomerular disease will require future studies in animal models.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC140

**The Cytoplasmic Tail of Polycystin-1 Activates STAT3 and Is Cleaved to Differentially Regulate the Transcriptional Activity of STAT1 and STAT3** Jeffrey Talbot, Jonathan M. Shillingford, Thomas Weimbs. *Molecular Cellular and Developmental Biology, University of California Santa Barbara, Santa Barbara, CA.*

Autosomal-dominant polycystic kidney disease (ADPKD) is a common genetic disorder that frequently leads to renal failure. Mutations in polycystin-1 (PC1) underlie most cases of ADPKD, but the function of PC1 has remained poorly understood. Here, we show that PC1 regulates the transcriptional activity of STAT3 and that this occurs – surprisingly - by two different mechanisms. First, PC1 activates STAT3 by increasing its tyrosine-phosphorylation. The membrane-anchored PC1 tail is sufficient for this activation, which is JAK2-dependent and regulated by SOCS3. Second, PC1 undergoes proteolytic cleavage that releases its cytoplasmic tail, which undergoes nuclear translocation. The cleaved PC1 tail then strongly co-activates STAT3 in a manner that requires prior tyrosine-phosphorylation by cytokine signaling. The cleaved PC1 tail can also co-activate STAT1 and STAT6 in the same manner but in response to different cytokines.

We show that the cleaved PC1 tail accumulates in ADPKD kidneys and that patient mutations in the PC1 tail affect its ability to activate STAT3. Remarkably, STAT3 is strongly activated in cyst-lining cells in polycystic mouse models and human ADPKD compared to normal kidney in which it is barely detectable.

Altogether, these results indicate that PC1 can differentially regulate STAT1, STAT3 and STAT6 signaling depending on the state of PC1 cleavage, and on the cytokine environment. The cleaved PC1 tail hypersensitizes cells to STAT-dependent cytokine signaling. PC1 may integrate mechanical and chemical signals and direct the appropriate cellular response of renal epithelial cells.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC141

**MicroRNA-21 (miR-21) Targets PTEN To Regulate Mesangial Cell Hypertrophy and Fibronectin Expression by High Glucose** Nirmalya Dey,[1] N. Ghosh-Choudhury,[2] Falguni Das,[1] Meenalakshmi M. Mariappan,[1] C. Mandal,[2] B. S. Kasinath,[1] Goutam Ghosh-Choudhury.[1] *[1]Medicine, UTHSCSA, San Antonio, TX; [2]Pathology, UTHSCSA, San Antonio, TX.*

We have shown recently that in mesangial cells (MC), decreased expression of the tumor suppressor protein PTEN contributes to hypertrophy and increased matrix protein fibronectin expression in response to high glucose (HG; 25 mM). The mechanism of PTEN expression by HG is not clear. MicroRNAs strategically control the mRNA translation. Analysis of 3'UTR of PTEN revealed the presence of a microRNA recognition element (MRE) for miR-21. HG significantly increased the expression of pre- and mature miR-21 in MC. Increased expression of pre- and mature miR-21 was also detected in the renal cortices of type 1 diabetic OVE26 transgenic mice. Plasmid-derived expression of miR-21 in MC reduced the expression of PTEN analogous to its downregulation by HG, resulting in increase in phosphorylation of Akt. In contrast, expression of miR-21 inhibitor (miR-in) significantly suppressed HG-induced decrease in PTEN expression, resulting in attenuation of Akt phosphorylation. To directly determine the involvement of miR-21 in PTEN regulation, we used a reporter containing 3'UTR of PTEN fused to luciferase. Expression of miR-21 significantly inhibited the luciferase activity similar to HG in MC. miR-21 additively reduced the HG-induced inhibition of luciferase activity. Conversely, miR-in significantly prevented the inhibition of luciferase activity in response to HG. Furthermore, miR-21 markedly increased the expression of fibronectin protein and its transcription similar to those with HG. In contrast, miR-in inhibited the HG-induced expression of fibronectin protein and its transcription. Finally, expression of miR-21 activated TORC1 kinase, resulting in protein synthesis and hypertrophy of MC, analogous to HG. miR-in significantly prevented HG-induced TORC1 activation, protein synthesis and hypertrophy. Together these results provide the first evidence for a functional role of miR-21 in targeting PTEN, which regulates matrix protein fibronectin expression and hypertrophy of MC, that contribute to complications of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC142

**Hyperactivation of WNT-β-Catenin and PI3K/Akt Pathways in Monocytes and B Lymphocytes of IgA Nephropathy (IgAN) Patients Could Lead to a Defect in Antigen Handling** Francesco Paolo Schena,[1] Sharon N. Cox,[1] Fabio Sallustio,[1] Grazia Serino,[1] Francesco Pesce,[1] Nicola Ancona,[2] Patrizia Stifanelli,[2] Gianluigi Zaza.[1] *[1]Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy; [2]ISSIA, CNR, Bari, Italy.*

A whole genomic screening was carried out to identify genes and pathways differently modulated in peripheral blood leukocytes isolated from 12 IgAN patients and 8 healthy subjects (HS). Bionformatic analysis revealed 210 genes discriminating IgAN pts from HS, these genes were also able to discriminate IgAN pts from other glomerular diseases. The identified set of genes generated highly significant networks involving WNT-b-catenin and PI3K/Akt. These pathways were further tested on peripheral blood mononuclear cells (PBMC) isolated from an independent group of 16 IgAN pts and 16 HS. Low protein levels

of inversin and phosphatase and tensin homolog, two modulators of the WNT-b-catenin and PI3K/Akt pathways, were found in IgAN pts suggesting a hyperactivation of these pathways. Furthermore, we found increased phospho-Akt protein levels, nuclear b-catenin accumulation with enhanced PBMC proliferation in IgAN pts.

Our next aim was to investigate which PBMC subpopulation of IgAN pts maybe principally involved in the WNT signaling alteration. We isolated T, B lymphocytes and monocytes and used a WNT pathway PCR Array to compare the transcript levels of 84 genes in IgAN pts and HS. Monocytes from IgAN pts showed an hyperactivation of the WNT pathway, in particular we found 24 significantly up regulated genes. B lymphocytes, showed a more blunt modulation since only 8 genes were found significantly up regulated, these genes overlapped with altered monocyte genes. T lymphocytes displayed only 5 deregulated genes. These findings highlight the emerging role of the WNT pathway in orchestrating the immune system in response to microbial stimulation of the immune cells in IgAN pts. Thus, the hyperactivation of this pathway could explain the abnormal systemic response to mucosal encountered antigens responsible for the onset of the disease. These newly identified pathways could be exploited for the identification of specific targets for the treatment.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC143

**$GABA_BR2$-C-Terminal Tail Is a Novel Modulator of Renal Fibrosis** Madhavi J. Rane,[1] Shunying Jin,[1] Paul Johnson,[1] Michelle T. Barati,[1] Jon B. Klein.[1, 2] *[1]Medicine, University of Louisville, Louisville, KY; [2]Veterans Affairs Medical Center, Louisville, KY.*

$GABA_BR2$ agonists show beneficial effects on renal function by inhibiting fibrosis. However, mechanisms underlying such effects are largely unknown. Baclofen treatment induces Akt and CREB phosphorylation in renal proximal tubular cells (RPTCs) in a $GABA_BR2$-dependent manner. We documented Akt-$GABA_BR2$ -C-terminal tail association in granulocytes. Therefore, we hypothesized that active Akt promotes cleavage and nuclear translocation of the $GABA_BR2$- C-term tail in RPTCs. Over-expression of the $GABA_BR2$ with constitutively active Akt (AktCA) or TGF-b treatment of RPTCs induced cleavage of the $GABA_BR2$-C-term tail which was blocked by LY294002 pretreatment. AktCA but not AktDN (dominant negative) induced expression of $GABA_BR2$-C-term tail construct in RPTCs and stimulated its nuclear translocation. Because $GABA_BR2$ has been shown to bind the transcription factor CREB and contains a bZIP transcription factor domain, we examined if C-term tail acts as a transcriptional regulator. Luciferase assays demonstrated a significant increase in cyclic-AMP response element (CRE) luciferase activity by co-expression of AktCA and C-term tail. Co-expression of AktCA with C-term tail construct or 24 hr TGF-b treatment of RPTCs induced fibronectin expression which was blocked by LY294002 pretreatment. Furthermore, 30 kDa cleaved C-terminal fragment of $GABA_BR2$ was detected in 3 months STZ-diabetic kidneys a condition in which renal fibrosis is documented. Collectively, these data indicate that Akt plays a critical role in cleavage, expression, nuclear localization of the $GABA_BR2$-C-term tail and its action as a transcriptional regulator. Cleavage of $GABA_BR2$-C-terminal tail may prevent intact $GABA_BR2$-G-protein mediated inhibition of adenylate cyclase activity and may promote CRE activity with concomitant increase in fibronectin expression. Further investigation into how the $GABA_BR2$ C-term tail promotes fibrosis will provide insight into the renoprotective effects of GABA agonists in damaged kidneys and may identify targets for pharmaceutical intervention in renal diseases.

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Disclosure of Financial Relationships: nothing to disclose

## TH-FC144

**Renal Fibrosis-Associated Gene IHG-1 Regulates Mitochondrial Biogenesis through Stabilisation of PGC-1a** Fionnuala B. Hickey,[1] James B. Corcoran,[1] Neil G. Docherty,[2] Brenda Griffin,[1] Fiona M. Furlong,[1] Finian Martin,[1] Catherine Godson,[1] Madeline Murphy.[1] *[1]Diabetes Research Centre, University College Dublin, Dublin, Ireland; [2]Department of Physiology, Trinity College Dublin, Dublin, Ireland.*

We have previously described IHG-1 as a highly conserved, glucose-regulated transcript associated with diabetic kidney disease. Here we report that IHG-1 is localised to the mitochondria. The transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1a) plays a central role in the coordination of mitochondrial biogenesis. PGC-1a is a tightly regulated protein and its dysregulation has been implicated in the pathogenesis of several human diseases. We report that IHG-1 overexpression is associated with increased mitochondrial mass and stabilisation of PGC-1a protein. Consistent with increased mitochondrial mass we observe upregulation of PGC-1a-regulated transcription factors, including nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (TFAM), a key activator of the transcription of mitochondrially encoded genes, along with increased expression of mitochondrial proteins. Conversely, inhibition of endogenous IHG-1 expression using shRNAmir resulted in reduced PGC-1a protein, decreased expression of NRF-1 and TFAM, and reduced expression of mitochondrial proteins. Mitochondrial dysfunction has recently been reported to be a major contributor to renal fibrosis. We demonstrate that PGC-1a protein is increased in an experimental model of tubulointerstitial fibrosis [rat unilateral ureteric obstruction] concomitant with increased expression of IHG-l. In summary these data identify IHG-1 as a novel regulator of PGC-1a and suggest that IHG-1-mediated stabilisation of PGC-1a may play a role in renal pathogenesis.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC145

**Absence of TIM-1 Leads to Th-17 Mediated Rejection in a Model of Chronic Allograft Vasculopathy** Melissa Y. Yeung, Takuya Ueno, Mohamed H. Sayegh, Nader Najafian. *Transplant Research Center, Brigham & Women's Hospital, Boston, MA.*

We have previously reported the key role of TIM-1 molecule in differentiation of T helper cells in a model of acute cardiac rejection using a blocking monoclonal antibody. In this study, we sought to determine the role of TIM-1 in a chronic allograft vasculopathy (CAV) model (bm12 into B6) using a novel TIM-1deficient recipient (TIM1KO).

The absence of TIM-1 in B6 recipient mice led to accelerated rejection (MST=29d vs >60d, $p<0.01$) and severe vasculopathy. Intriguingly, rejection occurred despite a decrease in frequency of CD4+ effector-memory T cells (21.81± 1.2 vs 28.9 ± 1.8%, $p<0.01$) and alloreactive IFNg production (IFNg: 958 ± 176 vs 4278 ± 320 pg/ml, $p<0.001$) but was accompanied by an increase in IL-17 production (10.2 ± 2.7 vs 2.1 ± 0.3 pg/ml, $p=0.02$), suggesting a Th17-mediated rejection. Additionally, we observed a decrease in regulatory T cells (Tregs) (11.2 ± 0.7 vs 14.3 ± 0.6%, $p=0.03$) and alloreactive IL-10 (7.3 ± 1.0 vs 43.2 ± 7.1 pg/ml, $p<0.01$). To evaluate proliferation of nTregs in th absence of TIM-1, we utilized a GVH model. Whilst proliferation of CD4 (% divided: 24.0 ± 0.6 vs 29.6 ± 0.5, $p<0.001$) and CD8 (24.1 ± 0.5 vs 28.4 ± 0.6, $p<0.001$) T cells was reduced when TIM1KO splenocytes were injected as compared to WT splenocytes, proliferation of nTregs were comparable in both groups (42.1 ± 0.7 vs 40.7 ± 0.7, p=NS). To determine the role of TIM-1 in nTreg function, we first compared in vitro suppression by nTregs from TIM1KO mice with that of B6 WT counterparts and found no difference (IFNg suppression: 34.7 ± 2.7 vs 36.0 ± 2.1%, p=NS). Similarly, depletion of nTregs prior to transplantation led to similar degree of accelerated rejection in TIMKO recipient as well as WT mice (MST shortened by 38% in TIM1ko vs 30% in WT recipients) confirming similar functionality of nTregs in vivo.

These data suggest that in a class II mismatched Treg-dependent CAV model, the absence of TIM-1 does not affect proliferation or function of nTregs, but may impair the development/differentiation of "inducible" Tregs and shift CD4 differentiation towards a pathogenic Th17 milieu.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC146

**Ratio of Th17 and Treg Is Significant Predictor for Renal Allograft Outcome after Acute Cellular Rejection** Byung Ha Chung, Hoon Suk Park, Jayoung Lee, Bumsoon Choi, Cheolwhee Park, Yong-Soo Kim, Chul Woo Yang. *Department of Internal Medicine, Seoul St. Mary's Hospital.*

**Background**: The aim of this study is to investigate whether the ratio between Th17 and regulatory T cell (Treg) in infiltration of allograft tissue could affect the allograft outcome after acute rejection episode.

**Patient and methods**: This study was done in cases with biopsy proven acute cellular rejection developed within 1 year after transplantation. We performed immunostaining for FoxP3 and IL-17 on infiltrating cells in renal allograft tissue and counted the number of stained cells. Treg rich group was defined when Treg / Th17 was more than 5 and the others were Th17 rich group. We compared both group in the frequency of steroid resistance, recovery after anti-rejection therapy, recurrent cellular rejection and renal allograft outcome.

**Results**: 57 cases of biopsy proven acute T cell mediated rejections from 33 patients were included in this study. Mean interval from transplantation to acute rejection episode was 3.2 ± 3.3 months. Treg rich group were 29 cases and Th17 rich group were 28 cases. Baseline characteristics such as age at transplantation or rejection episode, HLA mismatch number, gender, Serum creatinine level at rejection did not differ significantly between two groups. In Treg rich group, 89.7% responded well to steroid pulse therapy without secondary therapy. In contrast, only 32.1% responded to steroid therapy in Th17 rich group. ($p<0.05$) The proportion of recovered cases after acute rejection episodes was significantly higher in Treg rich group than in Th17 rich group.(93.1% vs. 74.1%, $P<0.05$) In recovered cases, the probability of recurrent rejection was significantly lower in Treg rich group compared to Th17 rich group. (28.7% vs. 48.7 %, $P<0.05$) The 1, 5 year graft survival rate after acute rejection was 81% and 62 % respectively in Treg rich group and those were only 48% and 37% in Th17 rich group. The graft outcome was significantly better in Treg rich group compared to Th17 group. ($P<0.05$)

**Conclusion**: Ratio of Th17 and Treg in infiltrating cell of renal graft tissue is significant predictor for allograft outcome after acute rejection episode.

Disclosure of Financial Relationships: nothing to disclose

## TH-FC147

**T-bet/Stat6 Double Knockout Recipients Experience Accelerated Rejection in a Chronic Rejection Model and Are Resistant to Transplant Tolerance Induction** Bara Sarraj,[1] Vishnupriya Samarendra,[1] Guodong Chen,[1] Omar A. Shah,[1] Zheng Jenny Zhang,[1] Mohamed H. Sayegh,[2] Mohammed Javeed Ansari.[1] *[1]Comprehensive Transplant Center, Northwestern University Feinberg School of Medicine, Chicago; [2]Brigham and Women's Hospital, Boston.*

INTRODUCTION: T-bet is the master regulator of Th1 immunity. We have previously shown T-bet-/- recipients experience accelerated rejection in a chronic rejection (CR) model and are resistant to tolerance (TOL) induction by costimulation blockade (CTLA4Ig+MR1). This resistance is believed to be due to excessive Th17 responses in the absence of Th1 responses in T-bet-/- recipients. The role of Th17 responses alone and the contribution of Th2 responses to resistance to TOL induction is unknown.

METHODS: We investigated the ability to induce TOL and mechanisms involved in the absence of Th1/Th2 immunity in T-bet/Stat6 double knockout (DKO) recipients, in 2 established models: 1) MHC-II mismatched (bm12-B6) CR model and 2) full MHC-mismatched (BALB/c into B6) cardiac allograft model of TOL with CTLA4Ig+MR1 treatment. Graft survival, histology, multi-parameter flow-cytomety (MFC) and cytokine bead array (CBA) assays were studied.

RESULTS: We generated DKO mice on B6 background and confirmed that DKO mice are immunologically normal except that they demonstrate attenuated Th1 and Th2 responses. In the CR model all grafts in DKO (n=6) and T-bet-/- (n=7) rejected in 14-28 days compared to graft survival of >42 in all WT (n=5) recipients ($p<0.05$). In TOL model all grafts in DKO (n=7) and T-bet-/- (n=7) recipients rejected in 10-14 days compared to graft survival of >30 days in all WT (n=5) recipients ($p<0.05$). Graft pathology showed predominantly granulocytic infiltration in DKO and T-bet-/- compared to WT. Increased RB6hi cells in grafts from DKO recipients. CD4 and CD8 memory T cells expressing IL-17 were increased in spleen and graft in DKO compared to WT and T-bet-/- recipients.

CONCLUSIONS: T-bet/Stat6 deficiency results in attenuated Th1/Th2 and Th17-skewed immunity which may be mediating resistance to tolerance induction by increased IL-17 CD4 and CD8 memory T cell generation and infiltration along with intense granulocytic infiltration in the grafts.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC148

**Complement (C) Mediated Endothelial Cell (EC) Activation in a Swine Model of Renal Ischemia/Reperfusion (I/R) Injury** G. Castellano,[1] C. Curci,[1] T. Tataranni,[1] A. Loverre,[1] G. Lucarelli,[1] P. Ditonno,[1] M. Battaglia,[1] Beatrijs D. Oortwijn,[2] Edwin S. Van Amersfoort,[2] Francesco Paolo Schena,[1] G. Grandaliano.[1] *[1]Renal Unit, University of Bari, Bari, Italy; [2]Pharming Technologies, Leiden, Netherlands.*

I/R injury in transplanted kidney is the major cause of delayed graft function. The involvement of tubular endothelial cells (EC) in I/R injury has been widely investigated in rodents, with Complement (C) playing a major role in the induction of apoptotic and necrotic processes. On the contrary, renal EC seem not to be primary involved in the pathogenesis of C-mediated I/R renal injury.

By using a swine model of renal warm I/R injury, we found that 30' of ischemia was able to induce C4d and C5b-9 deposition on peritubular and glomerular EC, contrary to what has been described in rodents. By confocal microscopy analysis, we found the occurrence of AKT and NIK phosphorylation at 30' from reperfusion, which co-localized with EC markers, at peritubular and glomerular level. Interestingly, pAKT and pNIK co-localized with C4d on EC. pAKT and pNIK returned to basal level after 24 h from reperfusion, when C4d was also not detectable. Next, we stimulated EC in vitro with C3a and C5a. Western blotting analysis showed that C3a induced AKT phosphorylation at 15' (3.46±1.85 pAKT/AKT pixel ratio, ImageJ software) and 30' (5.01±2.11; control 1.82 ±0.23; $p<0.05$). Moreover, C3a induced NIK phosphorylation at 15' (1.97±0.63; pNIK/NIK pixel ratio) and 30' (6.10±1.10; control 1.10 ±0.38; $p<0.05$). Interestingly, also C5a induced pAKT and pNIK in cultured EC. To test the hypothesis of C-mediated EC activation in vivo, we injected 5 pigs with a C inhibitor, recombinant human C1-inhibitor (rhC1INH; Pharming). Pigs treated with rhC1INH showed dramatic reduction of C4d deposition on peritubular and glomerular EC. Interestingly, pAKT and pNIK were completely abrogated by C inhibition in vivo on pig glomerular and peritubular EC.

These data first indicate that C is primary involved in activation of renal EC in vitro and in vivo in a model of I/R injury, probably via C3a and C5a. Therefore, the use of C inhibitors might significantly limit EC activation with positive effects on graft survival.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC149

**In Vitro Cytokine and HLA Antibody Stimulation of Glomerular Endothelial Cells Results in Complement Factor Synthesis** Rizwan A. Hamer,[1,4] Daniel Mitchell,[4] Simon C. Satchell,[2] David P. Lowe,[3] David Briggs,[3] Nithya Krishnan,[1] Simon Fletcher,[1] Robert Higgins,[1] Daniel Zehnder.[1,4] *[1]University Hospital Coventry and Warwickshire, United Kingdom; [2]University of Bristol, Bristol, United Kingdom; [3]NHS Blood and Transplant, Birmingham, United Kingdom; [4]University of Warwick, United Kingdom.*

Human microvascular endothelial cells (HMEC-1) and conditionally immortalised glomerular endothelial cells (ciGEnC), cultured under laboratory conditions, were stimulated for 12-48 h with IFN-γ (200-1000 IU/ml) and LPS (1-10 μg/ml). Western blot (WB) analysis of cell lysates showed C3 and C4 synthesis by both cell types on cytokine exposure. Amount of expression was proportional to both time and dose of cytokine stimulation. Unstimulated cells did not appear to synthesize complement. LPS was more effective than IFN-γ in causing expression of C3 (3 fold increase; $p<0.05$). C4 synthesis was seen only on treatment with IFN-γ (band density >30 times relative to control $p<0.05$). C4 mRNA and protein synthesis was inhibited by siRNA.

.After HLA typing both HMEC-1 and ciGEnC cells, effluent obtained during double filtration plasmapheresis (DFPP) treatment of our HLA antibody incompatible renal transplant patients containing HLA antibodies against cell HLA- type and "3rd party" antibodies were identified. Following extraction with sepharose columns, these antibodies were used to stimulate both HMEC-1 and ciGEnC cells. Confocal microscopy showed C4 on surface of cells treated with cell specific antibodies but not on cells treated with 3rd party antibodies. WB confirmed increased C4 expression by cells treated with cell-specific antibodies relative to cells treated with non cell-specific antibodies ( 89% increase; $p=0.08$).

We have shown, for the first time, C4 synthesis by glomerular endothelial cells and confirmed previously demonstrated C3 and C4 production by other endothelial cells. IFN-γ appeared to have the maximum stimulatory effect for C4 synthesis. Anti-HLA antibodies directed against the HLA type of these cells also resulted in C4 synthesis. These findings suggest the possibility of local complement synthesis playing a role in AMR of renal transplants.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC150

**Development of De Novo Alloantibodies Post Transplantation Is Associated with Antecedent Change in Serum Cytokines and Subsequent Poorer Allograft Function** Ciara N. Magee,[1] Bechara G. Mfarrej,[1] Sacha A. De Serres,[1] Maninder K. Singh,[2] Barbara T. Murphy,[2] Nader Najafian.[1] *[1]Transplantation Research Centre, Brigham & Women's Hospital, Harvard Medical School, Boston, MA; [2]Dept. of Renal Transplant Medicine, Mount Sinai School of Medicine, New York, NY.*

Despite improvement in early allograft outcomes, long-term allograft survival remains suboptimal. A significant proportion of graft losses can be attributed to immune-mediated processes, highlighting the importance of developing clinically applicable methods of immune monitoring. We hypothesised that a detectable change in serum cytokines would precede the development of alloantibodies, thereby permitting antecendent monitoring.

Serum samples from 112 patients enrolled in the Genomics of Chronic Allograft Rejection (GoCAR) trial, a prospective longitudinal multicentre observational study of renal transplant recipients, were collected at predetermined study timepoints (baseline, 3, 6, 12, 18, 24 months); additional samples were taken at times of clinical indication. These samples were analysed by Luminex® for the presence of alloantibodies, including MICA; serum cytokines were assayed at concurrent timepoints to investigate the cytokine milieu associated with development of alloantibodies, in an attempt to identify potential sentinel markers of alloantibody conversion. Correlation with clinical outcome was made by comparison with allograft function using serum creatinine as a marker.

Of 112 patients analyzed, 57 were shown to have detectable alloantibodies, 42 at the time of transplantation, while 15 patients developed de novo alloantibodies at various timepoints post-transplantation. Several serum cytokines were shown to be temporally associated with alloantibody conversion, with levels of VEGF, TNFα, GMCSF, IL8 and IL1Ra shown to rise prior to conversion. Patients who developed de novo HLA antibodies had a trend towards higher serum creatinine.

We identified several serum cytokines which may have the potential to act as sentinel markers of alloantibody conversion; development of de novo HLA antibodies was associated with a trend towards poorer allograft function. These results need to be validated in the remaining study population.

Disclosure of Financial Relationships: nothing to disclose

### TH-FC151

**Regulatory B Cells Are Identified by TIM1 and Can Be Induced through TIM1 Ligation in an IL-4 Dependent Manner, To Promote Allograft Survival** David M. Rothstein,[1] Melissa Y. Yeung,[2] Mohamed H. Sayegh,[2] Nader Najafian,[2] Qing Ding.[1] *[1]University of Pittsburgh, Pittsburgh, PA; [2]Harvard Medical School, Boston, MA.*

TIM1 regulates immune responses by controlling T effector cell responses. For example, α-TIM1 mAb (RMT1-10) promotes long-term murine allograft survival (GS) through Th2 deviation. Yet, in vivo, TIM1 is expressed on <2% of CD4 cells. In contrast, 8% of B cells in naive mice express TIM1, which increases to 12% in transplanted mice and 25% after α-TIM1 (TIM1 on CD4 cells remains <2%). In diabetic BALB/c recipients of B6 islets, α-TIM1 increases MST from 12d to 28d (30% >120d GS). Yet, after B cell depletion (α-CD20) or in B-deficient JHD (BALB/c) recipients, α-TIM1 actually accelerates islet rejection (MST from 12d to 6d). Moreover, the Th2 shift normally induced by α-TIM1 requires B cells. Reconstituting JHD mice with wt B cells restores both prolonged GS and Th2 deviation mediated by α-TIM1. This suggests B cells are critical targets for α-Tim1 and promote Th2 responses. Accordingly, TIM1+ B cells are 10-20-fold enriched for IL-4 and IL-10 expression vs. TIM1- B cells. α-TIM1 not only induces TIM1+ B cells but doubles the % of these cells expressing IL-4 and IL-10. Transfer of TIM1+, but not TIM1-, B cells from BALB/c allograft recipients into otherwise untreated JHD recipients, markedly prolongs GS (MST >60d), indicating that TIM1+ B cells are regulatory. Breg have been previously defined as rare IL-10+ B cells with various phenotypes. TIM1 identifies B cells highly enriched for IL-10 in all B cell subsets including CD1dHiCD5+ ("B10") cells. IL-10 is essential for TIM1+ Breg function. In addition, IL-4-/- B cells do not restore α-Tim1-mediated GS in JHD recipients. However, IL-4-/- B cells exhibit markedly reduced TIM1 and IL-10 expression which is not induced by α-Tim1. IL-4R-/- B cells exhibit further reduction in TIM1 and IL-10 expression (20% of wt) and are even less effective at restoring α-TIM1-mediated GS in JHD mice. Thus, IL-4 signaling is required for TIM1 and IL-10 expression by Breg, and B cell IL-4 plays a predominant role. TIM1 represents the most inclusive marker yet for Breg which can be induced by TIM1 ligation, leading to prolonged allograft survival.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC152**

**Rapamycin Induced Functionally Active Immunoregulatory CD8+Foxp3+ Cells Reside in Donor Skin Allograft and Facilitate Long-Term Engraftment**
Basset El Essawy,[1,2] Wenda Gao, Terry B. Strom.[1] *[1]Harvard Medical School, Boston, MA; [2]Medicine, Al-Azhar University, Egypt; [3]Beth Israel Deaconess Med Ctr, Boston, MA.*

Are the CD8+Foxp3+ regulatory T cells a component of the allograft protection? And home to long-term engrafted DBA/2 skin allografts? We generated a knock-in mouse(C57BL/6 background) (KI) with a Foxp3 promoter controlling down-stream bicistronic expression of Foxp3 and EGFP reporter introduced into the endogenous Foxp3 locus. Thus, EGFP(Foxp3)+ cells can be analyzed at the single cell level.

We injected 2x105 FACS-sorted CD8+ EGFP- T cells from the KI mouse into Rag-/- mice, followed by fully MHC-mismatched DBA/2 skin transplant. One group was treated with Rapamycin(RPM)(i.p.,3mg/kg) for 3 consecutive days, then alternate day for 2 weeks. The 2nd group was not-treated. FACS analysis at 3 months post-TX showed 2.8% GFP+(Foxp3+) cells in CD8+ population in the peripheral lymphoid organs in RPM-treated recipients as compared to 0.4% in the control(P 0.01). Histological analysis of skin grafts from RPM-treated recipients at 100 days post-TX (LTE) showed an abundance of graft-infiltrating CD8+ EGFP(Foxp3)+ cells. We investigated if the LTE graft-infiltrating CD8+EGFP+ T cells are active in protecting the allograft from rejection. DBA/2 skin grafts were harvested from (i) LTE RPM-treated Rag-/- mice(n=5) and (ii) autologous DBA/2 recipients(30 days after auto-transplantation)(n=5), and transplanted onto Rag-/- hosts to allow T cell expansion by homeostatic proliferation. Then we challenged the above 2 groups of mice with 0.2X106 CD8+ T cells from naive C57BL/6 mice. Survival of LTE DBA/2 skin allografts transplanted onto Rag-/- mice was significantly prolonged as compared to the control auto-transplanted DBA/2 skins onto Rag-/- mice(P 0.03).

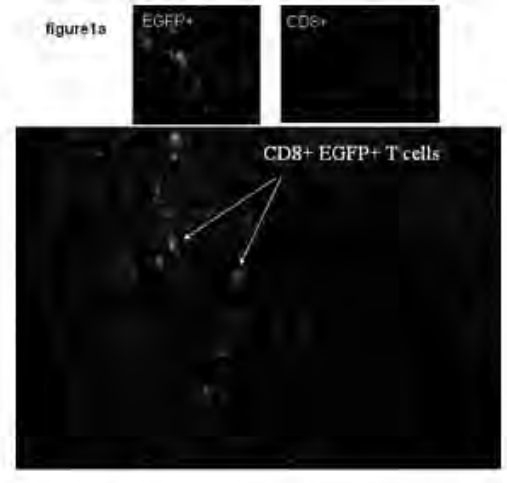
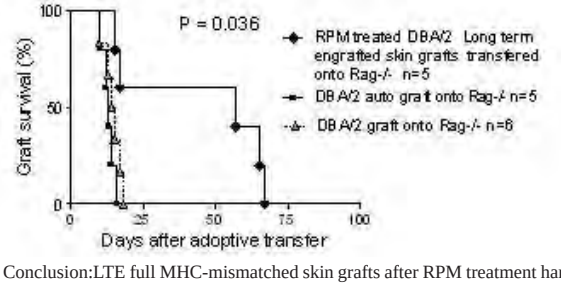



**figure 1b:** Survival after adoptive transfer of $2x10^5$ naive CD8+ cells onto Rag-/- bearing either the long term engrafted graft or the auto graft




Conclusion:LTE full MHC-mismatched skin grafts after RPM treatment harbor graft-residing CD8+Foxp3+ regulatory T cells that actively prevent CD8-mediated rejection.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC153**

**Conversion to Sirolimus Increases Regulatory T Cell and NK Cell Numbers in Transplant Patients with Previous Squamous Cell Skin Cancer: Results of a Randomized Controlled Trial**
Robert Carroll,[1] Paul N. Harden,[2] Kathryn J. Wood.[3] *[1]Renal and Transplantation Services, Central North Adelaide Health Service, Adelaide, SA, Australia; [2]Oxford Transplant Centre, Oxford Radcliffe Hospitals NHS Trust, Oxford, Oxfordshire, United Kingdom; [3]Nuffield Department of Surgery, Oxford University, Oxford, Oxfordshire, United Kingdom.*

Kidney Transplant Recipients (KTR) are at increased risk of squamous cell cancer (SCC). The mTOR inhibitor sirolimus has been associated with reduced cancer rates post solid organ transplantation. The RESCUE study aims to determine whether conversion to sirolimus in KTR with SCC reduces new SCC development. Subjects were randomized to stay on current therapy or convert to sirolimus (levels 5-10 ng/ml) and 5mg of predisolone.

Within this study 31 KTR were enrolled in an immune phenotyping substudy, 13 KTR were randomized to sirolimus and 18 stayed on current therapy. Lymphocyte subsets were assessed prior to randomization and at 3 and 6 months post randomization by multiparameter flow cytometry. The laboratory investigator was blinded to the randomization status and dermatological outcome of the KTR's.

In the control arm $FOXP3^+$ T cell, $CD56^{bright}$ NK cell and $CD3^-CD56^+CD16^+$ NK cell numbers (cells/ul) were stable over time. KTR converted to sirolimus experienced increases in these cell numbers over time and at six months these increases were statistically significant: median (range) $FOXP3^+$ T cell number increased 21(7-71) to 45(14-98) p=0.017; median NK cells number increased 52(1-315) to 109(14-320) p=0.028 and $CD56^{bright}$ NK cells increased 6(1-54) to 16(1-40) p=0.006. In those KTR who did not tolerate sirolimus the immune phenotype returned to baseline values.

This is the first report demonstrating that conversion to sirolimus is associated with increased numbers of NK cells and when sirolimus is removed, NK cell numbers fall to baseline values.

The anti proliferative effect of sirolimus is thought to mediate its anti-cancer effects. However high numbers of NK cells have been shown to be associated with reduced SCC accrual in KTR, therefore sirolimus may mediate its anti cancer effect by inducing protective changes in KTR immune phenotype.

Disclosure of Financial Relationships: nothing to disclose

**TH-FC154**

**Discovery and Validation of Differential Expression of MicroRNAs in Human Renal Allografts with Interstitial Fibrosis and Tubular Atrophy (IF/TA)**
Thangamani Muthukumar,[1] Franco Mueller,[2] Sylvia Ebalu,[1] Darshana M. Dadhania,[1] Manikkam Suthanthiran.[1] *[1]Cornell University; [2]The Foresight Group, New York.*

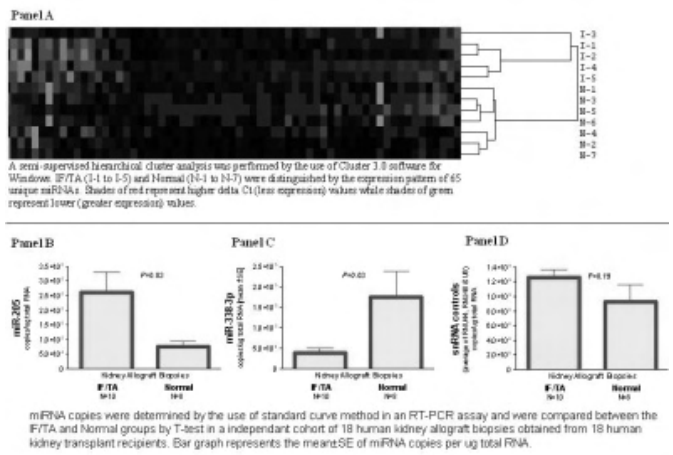
MicroRNAs (miRNA) are considered master regulators of adaptive & innate immunity. As both adaptive & innate immunity are implicated in the pathogenesis of IF/TA of human renal allografts, we tested the hypothesis that IF/TA is characterized by intragraft miRNA expression signatures.

In the discovery phase, we investigated the global expression pattern of miRNAs in human renal allograft biopsies using TaqMan® MicroRNA Array v2.0 containing primers/probes for the amplification of 667 human miRNAs. In the validation phase, we used our modified version of RT-PCR assay for absolute quantification of a subset of miRNAs found to be differentially expressed by global miRNA profiling. This selection was based on biological plausibility.

In the discovery phase we profiled 12 biopsies from 12 recipients; 5 IF/TA & 7 Normal. 70% of miRNAs were expressed in IF/TA & 69% in the Normal biopsies. 65 miRNAs were significantly different between the groups & a semi-supervised hierarchical clustering distinguished the two cohorts (Panel A).

Among the 65 miRNAs, we selected 2 based on their potential role in the pathogenesis of IF/TA for validation in an independent set of 18 biopsies from 18 recipients (10 IF/TA & 8 Normal).

miR-205, implicated in EMT was overexpressed in IF/TA (mean±SE 260103±70187 vs. 76650±16265 copies, P=0.03, Panel B). miR-338-3p, implicated in formation of epithelial cell polarity was under expressed in IF/TA (39144±10172 vs. 174395±63281, P=0.03, Panel C). The differential expression was miRNA specific as control snRNA was not different (Panel D).




A semi-supervised hierarchical cluster analysis was performed by the use of Cluster 3.0 software for Windows. IF/TA (I-1 to I-5) and Normal (N-1 to N-7) were distinguished by the expression pattern of 65 unique miRNAs. Shades of red represent higher delta Ct (less expression) values while shades of green represent lower (greater expression) values.

miRNA copies were determined by the use of standard curve method in an RT-PCR assay and were compared between the IF/TA and Normal groups by T-test in a independant cohort of 18 human kidney allograft biopsies obtained from 18 human kidney transplant recipients. Bar graph represents the mean±SE of miRNA copies per ug total RNA.

Our data demonstrates that intragraft miRNA expression patterns are diagnostic biomarkers of IF/TA & advance a new molecular basis for the pathogenesis of IF/TA.

Disclosure of Financial Relationships: nothing to disclose

**F-FC155**

**Macrophages Are Important Mediators of Recovery in a Novel Mouse Model of AKI with Targeted Injury of Proximal Tubule Cells** Bing Yao, Shilin Yang, Mingzhi Zhang, Raymond C. Harris. *Medicine, Vanderbilt University, Nashville, TN.*

We have previously reported the development of a model of selective proximal tubule injury in transgenic mice expressing the human diphtheria toxin receptor (hDTR) coupled to the γ-GT-I promoter. We determined that hDTR was selectively expressed in proximal tubules in adult kidney and was not expressed in adult liver. A single injection of DT caused reversible proximal tubule injury in DTR transgenic mice but not wild-type mice, and the injury was predominately due to tubular cell apoptosis, as indicated by increased TUNEL staining and caspase-3 and caspase-9 expression. Of note, during the recovery phase, there was marked infiltration of F4/80+ macrophages. In ischemia/reperfusion-induced AKI, numerous studies have indicated a detrimental role for infiltrating leukocytes, and previous studies have indicated that depletion of macrophages prior to ischemia lessens the degree of renal injury. Unexpectedly, in the DTR transgenic mice, macrophage depletion by clodronate either prior to, or after induction of injury by DT administration led to significantly greater histologic injury, increased and prolonged Kim-1 expression, increased apoptosis and delayed cell proliferation. At a concentration of DT that otherwise led to reversible injury without mortality in DTR-transgenic mice, clodronate-mediated macrophage depletion either before or after DT injection led to 60% mortality by 15 days (n=10 in each group). In response to DT, renal expression of markers of "M1" inflammatory macrophages (iNOS, CCl3, IL-23) were not changed, but "M2" wound healing macrophage markers (arginase, mannose receptor, IL4Ra) significantly increased; expression of these M2 markers was markedly inhibited by clodronate. In summary, these results demonstrate in this model of selective proximal tubule apoptosis induced by diphtheria toxin, M2 macrophage infiltration into the kidney increases in response to injury and is a crucial component of the reparative process.

Disclosure of Financial Relationships: nothing to disclose

**F-FC156**

**Flow Cytometric Characterization of Mitochondrial Subpopulations in the Kidney** Janet E. Saunders, Craig Cano Beeson, Rick G. Schnellmann. *Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Mitochondria in the kidney experience periodic bouts of oxidative stress from metabolic demand and xenobiotic metabolism resulting in persistent damage to mitochondria. We hypothesize that damaged mitochondria slowly accumulate making the kidney more susceptible to failure when stressed by disease or xenobiotic exposure. While flow cytometric techniques have been developed to quantify features of individual mitochondria related to size, calcium concentration, mtDNA content, respiratory capacity and oxidative damage, the identification and characterization of mitochondrial subpopulations in kidneys is lacking. Mitochondria from rabbit kidneys were stained with molecular probes for cardiolipin content (nonyl acridine orange, NAO) and membrane potential (tetramethylrhodamine, TMRM) and analyzed using flow cytometry. Mitochondrial subpopulations were identified using NAO gates. Upon titration with uncoupler, carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP), it was found that the high NAO content subpopulations were more resistant to uncoupling than lower NAO content populations. Calcium-induced swelling of mitochondria was measured as changes in light scattering at 540 nm and via flow cytometry. In cytometry, mitochondrial swelling was observed as changes in both SSC and FSC fluorescence. Swelling of distinct subpopulations was evaluated using probability binning (PB) analyses of SSC and FSC. Interestingly, when comparing controls to samples treated with calcium, the NAO-bright subpopulations are the most susceptible to swelling. When the mitochondria were pre-treated with cyclosporine A (CsA), less swelling was observed in all subpopulations. Our results demonstrate that flow cytometry combined with probability binning analyses can discriminate morphologically distinct mitochondrial subpopulations that have distinct stress-response characteristics.

Disclosure of Financial Relationships: nothing to disclose

**F-FC157**

**The Role of Proximal Tubular Socs-3 Expression in Acute Kidney Injury** Nathan D. Susnik,[1] Inga Soerensen,[1] Song Rong,[1] Lloyd G. Cantley,[2] Hermann G. Haller,[1] Roland Schmitt.[1] *[1]Hannover Medical School, Hannover, Germany; [2]Yale School of Medicine.*

In an attempt to identify new therapeutic targets for acute kidney injury (AKI), we found that suppressor of cytokine signaling 3 (Socs-3), a major negative regulator of the JAK/STAT and other receptor tyrosine kinase pathways, is up-regulated after ischemia reperfusion (IR) injury in mouse proximal tubules. To elucidate the specific role of Socs-3 in AKI, we created a proximal tubular conditional knockout mouse (Socs-3Δsglt2/Δsglt2). IR injury, induced through clamping of the renal pedicels for 20 min (medium damage) or 27 min(heavy damage), was used to induce AKI in Socs-3Δsglt2/Δsglt2 and Socs-3wt control mice. Renal function was monitored and kidneys were analysed by histology, immunohistochemistry, qPCR and immunoblot. Although there was no difference in mortality, Socs-3Δsglt2/Δsglt2 mice in the medium damage group did have significantly better renal function. Analysis of post-ischemic kidneys revealed marked differences in inflammation and repair processes. At 72 hours post clamping, when Socs-3 expression peaks in wild-type mice, Socs-3Δsglt2/Δsglt2 mice had less epithelial damage and Ki-67 staining revealed significantly more proximal tubular cell proliferation. Kidneys from Socs-3Δsglt2/Δsglt2 mice also had significantly higher expression of inflammatory markers such as interleukin-1 beta, greater TGF-β expression, and significantly more infiltrating leukocytes. In vitro analysis of migration and proliferation was performed by transfecting mIMCD3 cells with a Socs-3 plasmid or a GFP control plasmid. Proliferation assays were stimulated with either kidney protein homogenate from IR kidneys or from non-damaged control kidneys. These assays showed that IR kidney homogenate significantly increased cell proliferation but this effect was blocked by Socs-3 overexpression. Moreover, Socs-3 overexpression attenuated the rate of movement in migration assays. Our data illustrates the complex downstream effects of proximal tubular Socs-3 expression. While an increased Socs-3 level might attenuate post-ischemic inflammatory processes, it also suppresses reparative mechanisms such as epithelial proliferation and migration.

Disclosure of Financial Relationships: nothing to disclose

**F-FC158**

**MSCs Attenuate Ischemic Acute Kidney Injury by Modulating Dendritic Cells** Myung-Gyu Kim, Kichul Yoon, Eunjung Cho, Sang-Kyung Jo, Won-Yong Cho, Hyoung-Kyu Kim. *Division of Nephrology, Department of Internal Medicine, Korea University Hospital, Seoul, Republic of Korea.*

Previous studies demonstrated that inhibition of T cell activation or induction of immune tolerance by mesenchymal stem cells (MSCs) were partially mediated via monocytes or dendritic cells (DCs). The purpose of this study was to examine the role of DCs in MSCs induced beneficial effect in ischemia/reperfusion injury (IRI). C57/BL6 mice underwent bilateral ischemia and MSCs were administered before I/R injury. Twenty four hours after reperfusion, biochemical, histologic kidney damage as well as kidney inflammation were assessed. Percentage and immunophenotypes of DCs in kidney and spleen was performed by flow cytometry. Additional studies using in vitro splenocytes culture with or without MSC were also performed. To assess the effect of DC depletion on MSC induced in-vitro anti-proliferative effect, or also on MSC induced renoprotective effect, we used CD11c-DTR transgenic mice. Infused MSCs were not found in kidneys. However, pretreatment with MSCs attenuated kidney injury and also suppressed inflammation. In vitro MSC treated DC showed decrease of CD11c while increase of CD11b, and CD80 expression and in addition, relative expansion of spleen $CD11c^{low}$ $CD11b^{high}$ DC were also observed in MSC treated I/R mice, suggesting that MSCs might induce aberrant DC populations. In in-vitro experiment, MSCs showed IL-6 dependent antiproliferative property in PHA-ConA or anti-CD3 stimulation of CD4 cells from WT mice (MSC-lymphocytes). However, this immunosuppressive effect of MSCs was partially inhibited in lymphocytes from DC depleted mice (MSC-splenocytes with $DC^{dep}$; CD11c-DTR transgenic). Finally, systemic depletion of $CD11c^{+}$DCs using CD11c-DTR mice resulted in partial loss of the beneficial effect of MSCs on kidney injury and inflammation. These results suggest that effect of MSCs in suppressing inflammation and reducing kidney injury might be partially mediated by DC modulation. Further studies exploring precise immune-modulatory mechanisms of MSCs might be useful in developing various strategies that ultimately improve prognosis of AKI.

Disclosure of Financial Relationships: nothing to disclose

**F-FC159**

**HIF-1α Induction during Reperfusion Is Critical for Kidney Regeneration after Renal Ischemia** Elisa Conde,[1] Ignacio Blanco Sanchez,[1] Elia Aguado Fraile,[1] Marta Martinez,[1] Edurne Ramos,[1] Laura Alegre,[1] Jose-Antonio Sanchez-Tomero,[2] Rafael Selgas,[3] Laura Garcia-Bermejo.[1] *[1]Pathology, Hospital Univ Ramon y Cajal; [2]Nephrology, Hospital Univ La Princesa; [3]Nephrology, Hospital Univ La Paz.*

Mechanisms involved in kidney regeneration after ischemic damage are under deep investigation, with the aim to accelerate acute renal failure recovery and/or improve allograft outcome. Previous studies of our lab demonstrated a second induction of HIF-1α during

reperfusion (3-5days) suggesting that this accumulation contribute to tubular repair after renal ischemia. Here we injected a specific siRNA for HIF-1α to elucidate the role of HIF-1α in the response to renal ischemia by means of an in vivo model of ischemia/reperfusion (I/R) in rat. Initial experiments using 4 different siRNAs allowed us to choose the more effective one to inhibit HIF-1α in kidney. We demonstrate that rats interfered with HIF-1a agravated the proximal tubule damage, by PAS staining. In agreement, serum creatinine and urea levels were higher at 3 days of reperfusion in siRNA treated rats. Next, we assessed mechanisms underlying HIF-1α effects. Proximal tubular cells proliferation was evaluated by BRDU staining, finding a delay in cell proliferation in interfered rats in comparison with scramble rats. Furthermore, we evaluate HIF-1α relation with the inflammation response associated to ischemic injury, by qRT-PCR of proinflamatory mediators such as TNFα, IL-1β and MCP-1, finding higher levels of them in siRNA-treated rats vs scramble rats. Finally, since HIF-1α might regulate the expression of repairing genes, we studied the expression of EPO by RT-PCR and western blot, observing that EPO is only expressed at 3-5 days in scramble treated rats. Correlating with this, in human biopsies from renal transplantation, HIF-1α and EPO were expressed only in proximal tubules which exhibited non-damage structure, with a significant negative correlation between ATN and both gene expression. In summary, we demonstrate that HIF-1α is critical for proximal tubule epithelial cell recovery after ischemia, by promoting cell proliferation, expression of repairing genes and regulating inflammatory mediators.

Disclosure of Financial Relationships: nothing to disclose

### F-FC160

**Tubular Regeneration after Ischemia/Reperfusion (I/R) Injury Originates from Dedifferentiated Proximal Tubular Cells** Katja Berger, Bart Smeets, Peter Boor, Peggy Jirak, Jurgen Floege, Marcus J. Moeller. *Department of Nephrology and Clinical Immunology, University Hospital of RWTH Aachen University, Aachen, Germany.*

Tubular cells are regenerated from intrinsic renal cells after ischemia/reperfusion injury (I/R). However, it is unknown, whether the source of regeneration is a distinct population of intra-renal (intra-tubular) progenitor cells or if it involves any surviving tubular cell.

We found previously that rare, individual tubular cells are genetically labeled in adult triple-transgenic PEC-rtTA/LC1/R26R mice, which we have used to trace parietal progenitor cells. In young and still growing transgenic mice, significantly more tubular cells were genetically labeled by administration of doxycycline for 14 days compared to adult mice.

To test whether the triple-transgenic PEC-rtTA/LC1/R26R mouse labels a progenitor population of tubular cells, adult mice were treated with doxycycline for 14 days to induce the irreversible genetic label. After a washout phase of one week, the mice were subjected to I/R injury of the left kidney. Within 2 weeks after I/R injury, regeneration was completed but the number of genetically labeled cells had not increased in the injured kidney compared to the right control kidney (n=15). This suggested, that the cells labeled by the PEC-rtTA/LC1/R26R mouse were not a distinct tubular progenitor population.

Next we tested the alternative hypothesis that any surviving tubular cell participates in the regeneration process. For this purpose, doxycycline treatment was applied during (instead of prior to) the I/R injury and the subsequent regeneration process. After 14 days, significantly more tubular cells (50% versus 5%) were labeled within the injured left kidney compared to the right control kidney (n=15).

These experiments show for the first time, that regeneration after I/R occurs from the bulk of the remaining tubular cells and not from a distinct intratubular progenitor population.

Disclosure of Financial Relationships: nothing to disclose

### F-FC161

**Small Molecule Mediated Augmentation of Kidney Regeneration** Chiara Cianciolo Cosentino,[1] Eric Degroh,[1] Lisa M. Swanhart,[1] Mark P. De Caestecker,[2] Neil A. Hukriede.[1] *[1]Developmental Biology, University of Pittsburgh, Pittsburgh, PA; [2]Medicine, Vanderbilt University, Nashville, TN.*

One of the first molecular hallmarks of vertebrate renal tubular regeneration is the reactivation of embryonic genes normally required during organogenesis. To identify chemicals that could enhance this reactivation event, we developed a high-content screen using zebrafish embryos to identify compounds that cause expansion of the embryonic renal progenitor cell population. One compound identified from this screen, PTBA, expanded expression domains of markers of kidney organogenesis by increasing the number of renal progenitor cells through a proliferation-dependent mechanism. PTBA exhibits structural similarity to the HDAC inhibitors phenylbutanoic acid and trichostatin A. Treatment with either of these HDAC inhibitors also elicited renal progenitor cell expansion, but unlike PTBA, these responses were associated with abnormalities in embryonic patterning indicative of increased toxicity. In addition to its low toxicity, PTBA has a unique thioether moiety in the connecting unit that has not been described in other HDAC inhibitors. Consequently, we utilized an automated fluorescence-based secondary screen using transgenic cdh17:eGFP zebrafish embryos to identify PTBA analogues that cause expansion of the renal progenitor cell population at lower concentrations than PTBA. One of these compounds, m4PTB, promotes expansion of renal progenitor cells at nanomolar concentrations. Moreover, administration of m4PTB post-injury increases the rate of renal recovery in a gentamicin model of acute kidney injury (AKI) in zebrafish larvae. In a mouse model of diphtheria-toxin induced AKI, m4PTB also reduces the severity of renal injury and post-injury fibrosis. These findings indicate that PTBA analogues cause an expansion of renal progenitor cells during embryonic development and increase renal tubular regeneration following AKI. Moreover, while other HDAC inhibitors also promote expansion of renal progenitor cells in zebrafish embryos, favorable efficacy and toxicity profiles of these PTBA analogues suggest they have unique properties to augment renal regeneration following injury.

Disclosure of Financial Relationships: nothing to disclose

### F-FC162

**Temporal and Site-Specific Trafficking Receptors Expression on Regulatory T Cells during Repair from Ischemic Acute Kidney Injury** Yuhong Tao, Jinxiang Yu, Qianmei Sun, Elizabeth M. Higbee, Yanfei Huang, Nada Alachkar, Karl L. Womer, Manchang Liu, Hamid Rabb. *Department of Medicine, Division of Nephrology, Johns Hopkins University School of Medicine, Baltimore, MD.*

**Background:** Foxp3$^+$ regulatory T cells (Tregs) infiltrate into kidneys and directly participate in repair during ischemic acute kidney injury(AKI). Distinct trafficking receptors likely regulate Treg trafficking during kidney repair, which is important for understanding mechanisms and accelerating tissue repair.

**Objective:** To identify candidate trafficking receptors that mediate Treg trafficking into kidney during repair from AKI.

**Methods:** The left renal pedicle was clamped in C57BL6 mice for 45 min, followed by reperfusion. Animals were sacrificed at baseline, day 1,3,10,21 after IRI. Blood, kidney, spleen, and draining lymph nodes were evaluated. Flow cytometry was used to quantify CCR2,CCR5,CCR6, CCR7 and CD103 on Tregs.

**Results:** Expression of CCR6 and CCR7 on Tregs in kidney was upregulated significantly with time(%CCR6 on Tregs at baseline,day1,3,10,21: 2.74±2.04, 32.06±12.76, 58.18±12.46, 24.28±12.42 and 48.75±11.93,respectively. %CCR7: 4.28±1.95, 19.61±5.33, 28.59±7.96, 17.64±3.59 and 17.64±3.59). Expression of CCR6 and CCR7 on Tregs in blood at day1,3 ,21 was significantly higher than that at baseline. Expression of CCR2 and CCR5 on Tregs in kidney and blood at day 1,3 was significantly higher than that at baseline. Expression of CCR2 on Tregs in spleen and renal draining lymph nodes decreased with time. Increased expression of CCR6 on Tregs in spleen on day 1,3,21 was observed. There was no significant change of CCR5 and CCR7 on Treg in spleen or draining lymph node at all times. Unlike in other studied tissues, CD103 expression on Tregs in kidney significantly increased from baseline at all time points(%CD103 on Tregs in kidney at baseline,day1,3,10,21: 22.07±3.67, 38.47±15.43, 60.68±9.55, 56.82±2.87 and 69.24±4.38, respectively).

**Conclusion:** A specific temporal and site-specific expression of CD103, CCR6 and CCR7 on Tregs occurs during repair from AKI. These trafficking receptors may play a vital role in mediating Tregs trafficking and function in kidney repair from AKI.

Disclosure of Financial Relationships: nothing to disclose

### F-FC163

**Exploring the Origin of the Cells Responsible for Regeneration and Fibrosis in the Kidneys** Tomomi Endo,[1] Tomohiko Okuda,[1] Jin Nakamura,[1] Atsuko Y. Higashi,[1] Atsushi Fukatsu,[1] Toru Kita,[2] Motoko Yanagita.[1] *[1]Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]Kobe City Medical Center, Kobe, Japan.*

Kidneys repair after acute kidney injury (AKI) with a rapid proliferative response, which leads to the restoration of nephron structure and function, however the origin of the proliferating cells after AKI remains controversial. There are three hypotheses to explain the origin of the proliferating cells: the bone marrow cells, the stem/progenitor cells in the kidney, and the intrinsic tubule cells. We hypothesized that the intrinsic epithelial cells, possibly the mature proximal tubule cells, proliferate and repair kidney injury. To test the hypothesis, we generated proximal tubule-specific inducible Cre mice (NDRG1-CreERT2 knock-in mice), in which CreERT2 is activated only after the administration of tamoxifen.

First, to determine the specificity and efficiency of the Cre recombination, we bred NDRG1-CreERT2 knock-in mice to Rosa26R indicator mice. The X-Gal staining showed that the Cre recombination was specifically achieved in almost 100% of proximal tubules without any leakage.

Second, to explore the origin of the proliferating cells after AKI, we operated ischemia-reperfusion injury (I/R injury) to the labeled mice, and administered BrdU. At day1 after I/R injury, more than 60% of the proliferating cells expressed LacZ, indicating that mature proximal tubule cells indeed proliferated. Furthermore, no dilution of genetic label was observed in the proximal tubules of the repaired kidney at day45, despite of extensive proliferation after I/R injury, indicating that mature proximal tubule cells repaired themselves by their own proliferation.

We further analyzed the contribution of epithelial-mesenchymal transition (EMT) of proximal tubule cells to renal fibrosis, which also remains controversial. We performed kidney fibrosis model in the genetically labeled mice. After UUO, LacZ positive cells were hardly detectable in the interstitium, suggesting that EMT of proximal tubule cells did not significantly contribute to renal fibrosis in our study.

Taken together, NDRG1-CreERT2 knock-in mice may provide a powerful tool to analyze "Proximal Tubule Biology".

Disclosure of Financial Relationships: nothing to disclose

**F-FC164**

**Abstract Withdrawn**

**F-FC165**

**Regional Citrate Versus Systemic Heparin for Anticoagulation in Critically Ill Patients on Continuous Venovenous Hemofiltration: A Prospective Randomized Multicenter Trial** Gerd R. Hetzel,[1] Michael Schmitz,[1] Rainer Himmele,[3] Adelheid Gauly,[2] Bernd Grabensee,[1] Lars C. Rump.[1] *[1]Heinrich-Heine-University Duesseldorf; [2]Fresenius Medical Care Germany; [3]Fresenius Medical Care NA.*

Background:

Continuous veno-venous hemofiltration (CVVH) in the intensive care setting requires anticoagulation to prevent clotting of the extracorporeal circuit. Several protocols avoiding heparin and using regional citrate anticoagulation have been developed to diminish bleeding risks. However, data from randomized trials comparing citrate anticoagulation with systemic heparinzation are very limited.

Methods:

We randomly assigned 174 patients to either CVVH treatment with systemic heparinization or to CVVH treatment with a system that uses citrate as the only anticoagulant and buffering substance. All patients were on mechanical ventilation, therefore written informed consent was obtained from a patient's legal representative. The primary objective was to compare treatment efficacy represented by the patients' acid base status on day 3 and on each consecutive day. Several parameters of safety and efficacy were analyzed as secondary objectives.

Results:

Comparison of standard bicarbonate from day 3 until day 11 confirmed equal effectivity of both treatment modalities. Use of citrate resulted in less systemic anticoagulation, a lower risk of bleeding and a longer hemofilter patency. However, episodes of hypercalcemia, hypocalcemia as well as the need for additional bicarbonate infusions occurred more often under citrate. The patients' high mortality was not influenced by the mode of anticoagulation.

Conclusion:

Citrate may be used as a regional anticoagulant and as the only buffering agent in CVVH with adequate treatment efficacy and safety. However, neither citrate nor heparin anticoagulation should be regarded as a therapeutic standard, since there is no advantage of one of these substances with regard to the patients mortality.

Disclosure of Financial Relationships: nothing to disclose

**F-FC166**

**Regional Citrate Anticoagulation Reduces Polymorphonuclear Cell Degranulation in Critically Ill Patients Treated with Continuous Veno-Venous Hemofiltration (CVVH): A Randomized Controlled Trial** Khajohn Tiranathanagul,[1] Onanong Jearnsujitwimol,[1,2] Paweena Susantitaphong,[1] Narin Kijkriengkraikul,[3] Kearkiat Praditpornsilpa,[1] Somchai Eiam-Ong.[1] *[1]Division of Nephrology, Department of Medicine, Chulalongkorn Memorial Hospital, Thai Red Cross Society and Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [2]Phrapokklao Hospital, Chanthaburi, Thailand; [3]National Blood Center, Thai Red Cross Society, Bangkok, Thailand.*

Background: The high mortality in critical acute kidney injury (AKI) patients treated with CVVH is associated with increased oxidative stress and systemic inflammation which are induced by the blood-membrane reaction. This is the first study conducted to examine the effect of regional citrate anticoagulant (RCA) on PMN cells degranulation of myeloperoxidase (MPO) and inflammatory cytokine levels in patients with critical AKI undergoing CVVH treatment.

Methods: This prospective study was carried out in 20 critical AKI patients who were treated with pre-dilution CVVH and randomized into RCA group (n=10) and heparin group (n=10). The pre-filter and post-filter MPO and inflammatory cytokine (IL-6, IL-8, and TNF-$\alpha$) levels were measured at baseline, 6 hr and 24 hr after initiating CVVH.

Results: The baseline characteristics were similar between the two groups. In the heparin group, the pre-filter serum MPO levels were significantly increased at 6 hr (40.5 ± 21.3 vs. 66.0 ± 63.5 ng/mL, $p < 0.01$) and the post-filter serum MPO levels were also significantly higher than the pre-filter ($p<0.05$). Interestingly, citrate could significantly decrease pre-filter serum MPO (56.7 ± 51.0 vs. 27.7 ± 36.6 ng/mL, $p<0.01$) as well as TNF-$\alpha$ and IL-8 levels ($p<0.05$) at 6 hr. There were no significant differences between pre- and post-dialyzer MPO levels in the citrate group. The mean CVVH circuit survival in the citrate group was longer than the heparin group ($p=0.03$).

Conclusions: Treatment with CVVH caused PMN degranulation and increased oxidative stress which might be mediated by the blood-dialyzer membrane reaction. RCA could diminish the oxidative stress, prolonged the circuit survival time, and minimized bioincompatibility during on CVVH.

Disclosure of Financial Relationships: nothing to disclose

**F-FC167**

**Change in CRRT Dose Prescription after Recent Trials** Flavio Basso,[1] Zaccaria Ricci,[2] Claudio Ronco,[1] Dinna N. Cruz.[1] *[1]S Bortolo Hosp, Vicenza, Italy; [2]Bambino Gesù Hospital, Rome, Italy.*

Recent studies on RRT dose (RENAL, ATN, DoReMi) showed no difference in outcomes with higher CRRT dose. They also underlined that a delivered dose of at least 20 ml/kg/h was important, but to achieve this, a higher prescribed dose is usually needed. The impact of these studies on clinical practice is yet unknown.

**Methods**

We compared the results of practitioner surveys distributed during the International Course on Critical Care Nephrology held in Vicenza, Italy in 2004, 2007 and 2010 to evaluate trends in CRRT practice patterns. We limited this analysis to physician responses only.

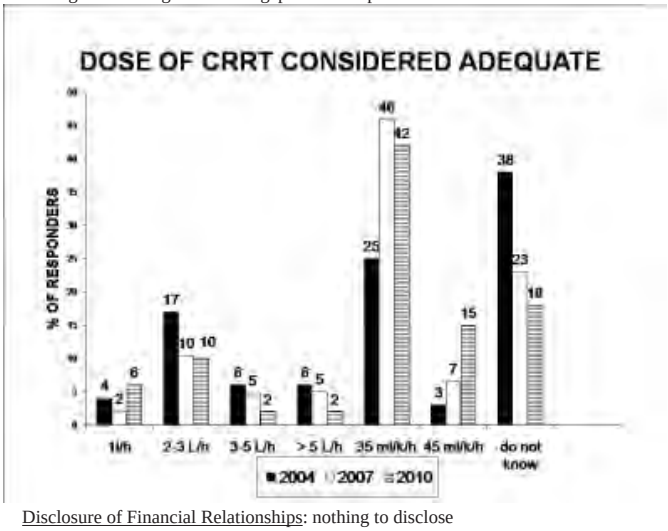
**Results**

In 2010, the overall response rate was 64%. Of 377 completed questionnaires, 338 were from physicians. Most were nephrologists (60%) followed by intensivists (36%); majority were from Europe (80%).

The use of individualized dose prescription based on body weight increased from 28% to 61.5% in 2010, while indiscriminate "by the liter dose" prescription decreased from 33% to 20%. The CRRT dose most commonly considered appropriate was 21-35ml/kg/h (42%), similar to 2007 (see Figure). The proportion of respondents who are "not sure" about adequate dose was fallen from 38% (2004) to 18% (2010). Fifty four percent of responders said they did not change their practice after publication of RENAL and ATN. Of the remainder, 28% increased their prescribed CRRT dose, while 22.5% decreased it.

**Conclusion**

Awareness regarding RRT dose has progressively improved over the past 6 years; in 2010, <20% expressed uncertainty about an "appropriate" CRRT dose. Less than half of surveyed physicians changed their practice after publication of recent landmark studies on CRRT dose. Interestingly, 28% actually increased their prescribed CRRT dose, perhaps indicating better recognition of a "gap" between prescribed and delivered CRRT dose.




Disclosure of Financial Relationships: nothing to disclose

**F-FC168**

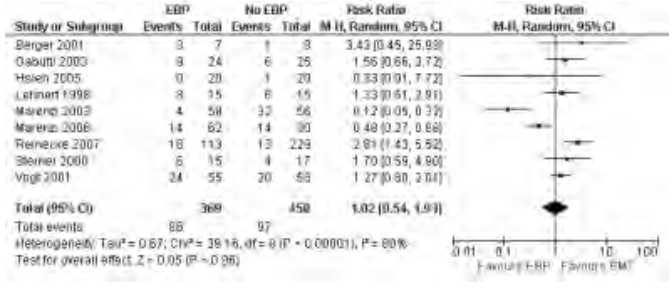
**An Updated Systematic Review of Extracorporeal Blood Purification in Prevention of Radiocontrast-Induced Nephropathy** Ching Yan Goh,[1,2] Valentina Corradi,[1] Claudio Ronco,[1] Dinna N. Cruz.[1] *[1]Nephrology Dialysis & Transplantation, S Bortolo Hosp., Vicenza, Italy; [2]Nephrology, Selayang Hosp., Selangor, Malaysia.*

**Background**: Radiocontrast induced nephropathy (RCIN) is an important cause of acute kidney injury, increasing in-hospital and long term mortality. It is controversial whether extracorporeal blood purification (EBP) reduces patient's risk of RCIN. A systematic review in 2006 showed that periprocedural EBP did not decrease the incidence of RCIN compared with standard medical therapy (SMT). We conducted an update of this review.

**Methods:** We searched through Pubmed and bibliographies of retrieved articles. Published studies of EBP for RCIN prevention in patients receiving radiocontrast were included. The primary endpoint was the incidence of RCIN, defined as an increase in serum creatinine ≥0.5mg/dL. Results were combined on the risk ratio (RR) scale. Random-effects models were used. Sensitivity analyses were defined *a priori* to evaluate the effects of EBP modality, study design, and sample size.

**Results**: Nine randomized controlled trials and 2 nonrandomized trials were included (n=1100 patients). Eight trials assessed IHD, 3 trials assessed CRRT. Nine studies had data for primary endpoint; RCIN incidence was 23.3% in the EBP group and 21.2% in the SMT group. EBP did not decrease the incidence of RCIN compared with SMT (RR1.02; 95%CI 0.54,1.93, see Figure); however, intertrial heterogeneity was high. In sensitivity analyses, limiting to only IHD studies significantly reduced heterogeneity. IHD appeared to increase RCIN risk (RR1.61; 95%CI 1.13,2.28) and had no effect on need for permanent RRT or progression to ESRD (RR1.47; 95%CI 0.56,3.89).

**Conclusion:** In this updated meta-analysis, periprocedural EBP does not decrease the incidence of RCIN compared with SMT. IHD appears to actually increase RCIN risk.




Disclosure of Financial Relationships: nothing to disclose

## F-FC169

**Cerebral Blood Flow Decreases during Hemodialysis in Patients with Acute Kidney Injury but Not in Patients with End-Stage Renal Disease** Carola Cademartiri,[1] Giuseppe Regolisti,[1] Aderville Cabassi,[1] Elena Cremaschi,[1] Stefano Tedeschi,[1] Elisabetta Parenti,[1] Caterina Maccari,[1] Alberto Guido Caiazza,[2] Enrico Fiaccadori.[1] *[1]Department of Internal Medicine and Nephrology, University of Parma, Parma, Italy; [2]Department of Diagnostic Medicine, AUSL Parma, Parma, Italy.*

**INTRODUCTION AND AIMS:** Hemodialysis (HD) can decrease cerebral blood flow (CBF) in patients (pts) with end-stage renal disease (ESRD). Pts with acute kidney injury (AKI) may be at higher risk for cerebral hypoperfusion during HD, as they often present with critical illness and hemodynamic instability. We studied CBF during HD in pts admitted for oliguric AKI and in pts with ESRD on regular HD thrice weekly. **METHODS:** CBF was examined by measuring middle cerebral artery mean flow velocity (mcaMFV, transcranial Doppler, 2-MHz probe, duplicate measurements) at start, at midtime and at the end of the first 4-h HD session *(Qb 200 ml/min, Qd 300 ml/min, co-current dialysis flow)* in 15 pts with AKI (median age 77 yrs, range 69-92, 8 males) and in 8 pts with ESRD (median age 81 yrs, range 36-89, 4 males), in whom mcaMFV was measured during the last HD session of the week *(Qb 300 ml/min, Qd 500 ml/min, counter-current dialysis flow)*. Data were analyzed using mixed models for repeated measurements. **RESULTS:** Mean blood pressure was similar at start HD in the pts with AKI or ESRD (87.8 [SD=20.1] *vs* 86.4 [SD=5.0] mmHg, P=0.799), and did not change significantly during the HD session in either group (group-averaged rate of change: P=0.368; time-averaged difference between groups: P=0.789). Mean weight loss at end HD (-1.5 [SD=0.8] *vs* -0.9 [SD=0.7] Kg, P=0.002) was greater in the pts with ESRD. Mean mcaMFV at start HD was higher in the pts with ESRD than in those with AKI (34.6 [SD 7.1] *vs* 26.4 [SD 9.0] cm/sec, P=0.037); after adjusting for different weight loss, mcaMFV decreased during the HD session in the pts with AKI (P=0.002) but not in those with ESRD (time-averaged difference between groups: P=0.026; rate of change difference between groups: P=0.045). **CONCLUSIONS:** Pts with AKI are at higher risk for cerebral hypoperfusion during HD than pts with ESRD, independently of changes in systemic arterial pressure.

Disclosure of Financial Relationships: nothing to disclose

## F-FC170

**Cefepime Clearance and Creatinine Generation during High Blood and Dialysate Flow Continuous Venovenous Hemodialysis (CVVHD)** Francis P. Wilson, Daniel A. Caroff, Becca Adler, Marcus A. Bachhuber, Jeffrey S. Berns. *Medicine, University of Pennsylvania Health System, Phialdelphia, PA.*

**Background**:

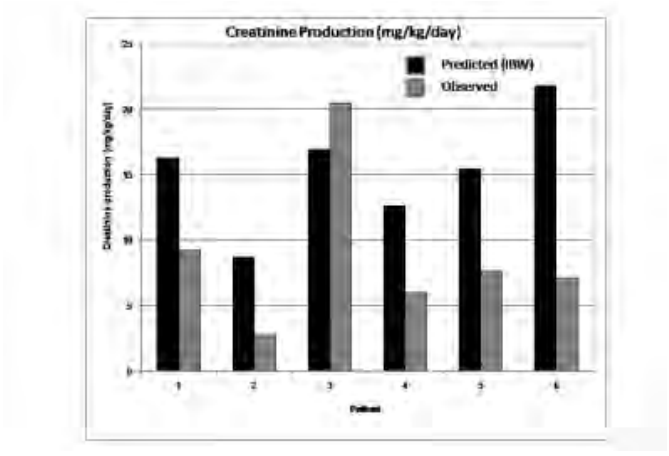
Guidelines for cefepime dosing during CVVHD were derived from studies using lower blood flow (Qb) and dialysate flow (Qd) than are used in our clinical practice. This study was designed to assess the pharmacokinetics of cefepime in CVVHD, and to determine creatinine generation rates in the critically ill population.

**Methods:**

All patients receiving CVVHD and cefepime at our institution from 12/2008 to 06/2009 were screened for potential enrollment. Six eligible patients gave informed consent and were included in the study. Pre- and post-filter blood and effluent samples were drawn at 0.5, 1, 2, 6, and 12 hrs after a cefepime dose and analyzed for cefepime concentration and creatinine concentration.

**Results:**

Cefepime dosages varied from 1-2g mg every 8 -12 hrs. Qb was 300 ml/min in all patients. Median Qd was 3000 ml/hr. All patients were anuric. The median sieving coefficient for cefepime was 0.34. Median half-life of cefepime was 7.55 hrs. With a cutoff of 8 ng/ml cefepime levels at 12 hrs after dosing were subtherapeutic in 3 of 6 patients, including both patients receiving 1000 mg every 12h. Median daily creatinine generation was 7.3 mg/kg. Estimated creatinine generation using the Jelliffe equation overestimated measured creatinine generation by a median 240%.




**Conclusions:**

There is significant clearance of cefepime during CVVHD using high blood and dialysate flow CRRT. We recommend dosing cefepime at least 2000 mg every 12 hrs to obtain sustained therapeutic serum levels of cefepime in this setting. Critically ill patients with oliguric AKI on CVVHD demonstrate reduced creatinine generation compared to expected levels for hospitalized patients. This finding has important implications for assessment of GFR in critically ill patients.

Disclosure of Financial Relationships: nothing to disclose

## F-FC171

**Fluid Overload (FO) Calculations in Pediatric Stem Cell Transplant (PSCT) Patients Requiring Continuous Renal Replacement Therapy (CRRT)** Rebecca M. Lombel,[1] Michael Heung,[2] Theresa Mottes,[1] Mallika Kommareddi,[2] Yong Y. Han,[1] Katherine L. Collins.[1] *[1]Pediatrics, University of Michigan, Ann Arbor, MI; [2]Internal Medicine, University of Michigan, Ann Arbor, MI.*

**Background:** FO>10% above baseline has been associated with poor outcome in PSCT patients who require CRRT. However, the optimal measure of %FO has not been established. We sought to compare published & novel methods for calculating %FO & to assess which method best correlates with patient outcomes.

**Methods:** We retrospectively analyzed all PSCT patients requiring CRRT from 2004-2009 at our hospital. We applied 7 different methods for calculating %FO that varied by "fluid balance" (weight difference or fluid [in - out]) & reference "dry weight" (pre-hospital clinic, hospital admission, PICU admission, or CRRT initiation). Differences between %FO calculation methods were determined by ANOVA-on-Ranks. The impact of %FO on mortality was assessed by logistic regression analysis. A mixed models approach was used to examine the relationship between %FO & Pediatric Logistic Organ Dysfunction (PELOD) scores (a surrogate outcome variable) over time. Data are presented as median [25%tile-75%tile].

**Results:** The median age of this study cohort (N=21) was 184 months [137-241]. Wide variations in %FO for the cohort were noted depending on the calculation method used (0.0% [-1.2%-2.9%] to 8.5% [0.5%-16.7%], $p$=0.170). %FO calculations among individual patients varied by a median of 11.3% [6.2%-17.1%] ($p$<0.001). Depending on the method used, as few as 3 (14%) patients & as many as 10 (48%) were identified as being FO>10% ($p$=0.109). No method was significantly associated with PICU or 6-month mortality. Increasing %FO was predictive of higher PELOD scores in 5 of 7 methods ($p$=0.003 to 0.038).

**Discussion**: Identification of patients with FO depends on the calculation method used. Although we did not see an association between %FO & mortality with any method (most likely due to limited cohort size), we observed that several %FO methods were predictive of PELOD scores over time. Future studies examining FO should include an assessment of multiple methods in order to determine the most clinically relevant approach.

Disclosure of Financial Relationships: nothing to disclose

## F-FC172

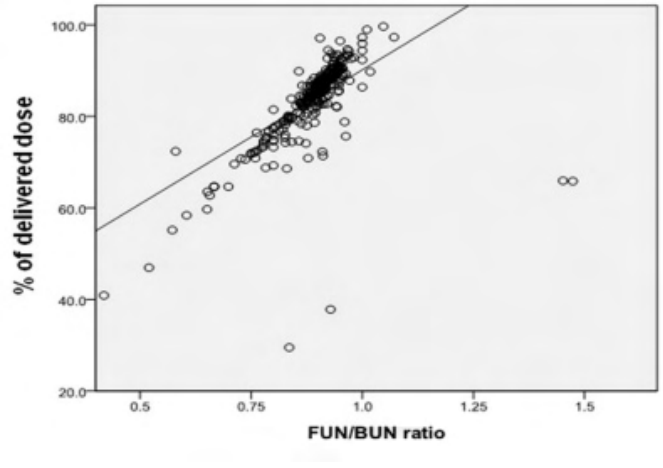
**The Impact of Down-Time and Filter Efficacy on Delivered Dose of Continuous Renal Replacement Therapy** Jiandong Wei, Rolando Claure-Del Granado, Rakesh Malhotra, Etienne Macedo, Yang Luo, Sharon Soroko, Ravindra L. Mehta. *Nephrology, University of California San Diego Medical Center, San Diego, CA.*

**Background:** Time off CRRT (down- time) and low filter efficacy due to clotting constitute important factors that lead to failure to deliver the prescribed dose in critically ill patients. We assessed the impact of down-time and filter efficacy on the difference between prescribed and delivered dose.

**Methods:** We prospectively analyzed data from 305 treatments in 54 critically ill patients treated with predilution continuous venovenous hemodiafiltration (CVVHDF), utilizing regional citrate anticoagulation, at the University of California San Diego Medical Center from September 2009 to March 2010. All patients were prescribed a 2700 ml/hr effluent rate. Delivered dose was calculated by actual mean urea nitrogen clearance with the formula (effluent urea nitrogen x effluent volume) / plasma urea nitrogen adjusted by

body weight. CRRT down-time was defined as the time off CRRT (hrs) per day. Filter efficacy is determined by filter clearance using effluent urea nitrogen (FUN)/plasma urea nitrogen (BUN) ratio. Percentage (%) of delivered dose was calculated by delivered dose/prescribed dose x 100%.

**Results:** 10% of all the treatments had down-time 2.0 (1.0-5.1) hrs/day. The mean FUN/BUN ratio was 0.9±0.1. Delivered dose by actual urea clearance was significantly lower than prescribed dose, 29.1±11.8 *vs* 34.5±13.2 ml/kg/hr, $p< 0.001$. A significant correlation was found between FUN/BUN ratio and % of delivered dose ($r =0.25$; $p < 0.001$) whereas the down-time did not affect % of delivered dose ($r =0.11$; $p = 0.062$).




**Conclusion:** Delivered dose of CRRT is lower than prescribed dose. Filter efficacy has significant impact on percentage of delivered to prescribed dose. We recommend monitoring solute clearances to optimize dose delivery in CRRT.

Disclosure of Financial Relationships: nothing to disclose

### F-FC173

**Comparison of Plasma Exchange and Hemodialysis Using a High Cut-Off Membrane (HCO) for Removal of Immunoglobulin Free Light Chains in Multiple Myeloma** Alain Wynckel, Vincent Vuiblet, Nathalie Schneider, Brigitte Kolb, Jean Pierre Melin, Julien Journet, Philippe Rieu. *University Hospital, Reims, France.*

Plasma exchange (PE) and hemodialysis with a high cut-off dialyzer (HCO-HD) have been tested for the treatment of acute renal failure (ARF) associated with multiple myeloma. Whether one technique is better than the other is not clearly evaluated in clinical practice. The aim of our study was to compare PE and HCO-HD for free light chains (FLC) removal in patients with dialysis-dependent ARF due to light chain multiple myeloma (LCMM). Seven patients with LCMM were included after informed consent. PE and HCO-HD (Gambro HCO 1100 dialyzer, surface area :1.1m²) were performed in each patient. Serum, plasma filtrate and dialysate FLC concentrations were measured by nephelometry (FREELITE, The Binding Site). Plasma filtrate was soustracted at a flow rate of 40 ml/min and collected entirely for measurement . During HCO-HD, serum and dialysate fluid samples were drawn simultaneously after 10 min (T10) then at T60, T120, T240 and T 360. Blood and dialysate flow rates were 250 ml/min and 750 ml/min respectively. Results: Mean plasma volume exchanged by PE session was 3842 ± 342 ml (46 ± 11 ml per kg of body weight). Mean PE duration was 94 ± 5 min. When considering serum FLC concentration before PE , cleared plasma volume was 3010 ± 423 ml per session; net FLC clearance was 31,4 ± 4,8 ml per min of PE. During HCO-HD, instantaneous clearances were 27,4 ± 11,1 ml/min, 29,3 ± 5,7 ml/min, 21,6 ± 8,2 ml/min , 21,0 ± 8,6 ml/min and 16,2 ± 5,2 ml/min at T10, T60, T120, T240 and T360 respectively. When considering serum FLC concentration before HCO-HD, cleared plasma volume was 1285 ± 534 ml between T0 and T60, 918 ± 348 ml between T60 and T120, 1272 ± 444 ml between T120 and T240, and finally 984 ± 288 ml between T240 and T360. In conclusion, 3.5 hours of HCO-HD (with a dialyzer surface area of 1.1 m²) are equivalent to one PE session. Prolonged HCO –HD session up to 6 h increased FLC removal by 50 %. The use of a dialyzer with a surface area of 2.1 m² should improve the performance of FLC epuration.

Disclosure of Financial Relationships: nothing to disclose

### F-FC174

**A Novel Program of Sustained Low Efficiency Dialysis (SLED) with Near-Automated Regional Citrate Anticoagulation (RCA) for 24-Hour ICU Renal Support: Improving Treatment Quality and Safety While Reducing Costs Compared to Traditional CRRT** Balazs Szamosfalvi, Stan Frinak, Lenar T. Yessayan, Jerry Yee. *Division of Nephrology and Hypertension, Henry Ford Health System, Detroit, MI.*

**Purpose:** Create an early start, large 24-h ICU renal support program of SLED-RCA to improve treatment quality and safety and lower costs.

**Methods:** We collected information from the literature and from dialysis personnel, renal and ICU physicians and nurses for desirable features of the new program. We developed a near-automated, simple RCA protocol for 24-h SLED to prevent blood clotting and to flexibly control dialysate Na, bicarbonate, potassium and phosphate. To eliminate errors and standardize therapy, RCA prescriptions are generated from a secure webserver which is also used to distribute protocol training materials and flowsheets. The delivered dose of effective ionic dialysance (OLC; small solute clearance) is monitored automatically. An optical hematocrit (Hct) sensor monitors blood volume and $O_2$ saturation. We use commercial dialysis equipment with available telemetry software.

**Results:** Standardized electronic order generation helped nephrologists write safe RCA prescriptions with a minimal learning curve. The RCA protocol completely prevented clotting and proved safe even in patients with severe liver dysfunction. Nurse satisfaction with modality effectiveness and simplicity has been very high. Nurse training was accelerated by the web-based document deployment and the simple, predictive calcium dosing. A 2:1 patient to nurse ratio could be maintained. Disposables cost savings are at least 200 dollars per day per patient compared to traditional CRRT. The delivered dose of dialysis monitor (OLC) and optical Hct and $O_2$ saturation sensors performed reliably and are quality improvements. Clearance with ultrapure dialysate is in the traditional CRRT range at 3 L/h with a convective component due to internal filtration on the dialyzers.

**Summary:** Our 24-hour SLED-RCA program has multiple quality and safety enhancements, costs less than traditional CRRT and helps expand the use of 24-h renal support, especially in combination with electronic order generation and telemetry.

Disclosure of Financial Relationships: Consultancy: Baxter, Inc.Research Funding: Fresenius; Honoraria: Renal Research Institute; Patent: Automated system for the delivery of regional citrate anticoagulation (patent application).

### F-FC175

**The Generation of a Novel Murine Model of B Cell-Mediated Proteinuria with Pathologic Changes Similar to Minimal Change Disease** Alfred Hyoungju Kim,[1] Andrey S. Shaw.[2, 3] *[1]Rheumatology, Washington University School of Medicine; [2]Howard Hughes Medical Institute; [3]Pathology & Immunology, Washington University School of Medicine, Saint Louis, MO.*

**Purpose**: Minimal change disease remains the most common etiology for pediatric nephrotic syndrome. B cell depletion therapy has been efficacious in the treatment of steroid-resistant disease, but contributions of B cells to proteinuria and podocyte effacement remain unknown. The development of a murine model of B-cell induced proteinuria with pathologic characteristics similar to minimal change disease would enhance our understanding of this disease.

**Methods**: The well-characterized B cell model antigen hen egg lysozyme (HEL) was biotinylated and complexed to avidin. Following intravenous injection in mice, naive HEL-specific B cells were adoptively transferred and proteinuria assessed. Kidneys were processed for immunofluorescence, H&E staining, and transmission electron microscopy. Intravital two-photon microscopy was performed on exteriorized kidneys from live, anesthetized mice.

**Results**: HEL was embedded within the glomerular basement membrane within 30 minutes following injection. Induction of proteinuria occurred only after the transfer of HEL-specific B cells. This was associated with podocyte effacement as visualized on transmission electron microscopy. The absence of periglomerular infiltrates on H&E stained sections were noted in both proteinuric and control mice, indicating the absence of inflammation. There were no immune complex or complement deposits in the glomeruli of proteinuric mice. Intravital two-photon microscopy demonstrated that HEL-specific B cells were retained only within the antigen-bearing glomeruli and became activated in situ as measured by calcium flux.

**Conclusion**: We have demonstrated that B cells were activated within antigen-bearing glomeruli, and induced proteinuria and podocyte foot process effacement in the absence of immune complexes, complement deposition, or inflammation. These are observations similar to those found in minimal change disease. We hypothesize that B cell-derived cytokines are directly responsible for this phenotype, and this is currently being tested.

Disclosure of Financial Relationships: nothing to disclose

### F-FC176

**Culprit and Victim: Distinct Roles of α3/4/5(IV) and α5/6/5(IV) Collagen Networks in the Pathogenesis of X-Linked Alport Post-Transplant Nephritis** Florina Olaru,[1] Wentian Luo,[1] Billy G. Hudson,[1] Clifford E. Kashtan,[2] Xu-Ping Wang,[1] Dorin-Bogdan Borza.[1] *[1]Dept of Medicine (Nephrology), Vanderbilt University School of Medicine, Nashville, TN; [2]Dept of Pediatrics, Univ. of Minnesota, Minneapolis, MN.*

Alport post-transplant nephritis (APTN), affecting ~3-5% of Alport patients after a kidney transplant, is mediated by alloantibodies to collagen IV chains present in the renal allograft but absent from Alport tissues. In X-linked Alport syndrome (XLAS), APTN alloantibodies target accessible sites in the noncollagenous (NC1) domain of α5(IV) collagen. Because α5(IV) collagen is a constituent of two supramolecular networks with different tissue distribution, we addressed the involvement of α3/4/5(IV) and α5/6/5(IV) collagen in the pathogenesis of APTN. APTN alloantibodies reactive with α5NC1 monomers also targeted α3/4/5NC1 and α5/6/5NC1 hexamers, likely sharing common alloepitopes. In addition, sera and allograft eluates from affected patients contained a novel subset of alloantibodies targeting specifically α3/4/5NC1 hexamers, but not α5/6/5NC1 hexamers, nor α5NC1 monomer subunits. Hence, neo-epitopes formed by supramolecular assembly of α3, α4 and α5 NC1 domains in the GBM are novel targets of APTN alloantibodies from XLAS patients. Murine alloantibodies to α3/4/5NC1 neo-epitopes were produced in Col4a5-null mice immunized with NC1 hexamers from human or mouse GBM but not with α5NC1 monomers. These results suggest that NC1 hexamers of α3/4/5(IV)

collagen are the culprit antigen initiating alloimmunity and the primary target of all APTN alloantibodies, whereas α5/6/5NC1 hexamers become secondary targets due to sharing tertiary α5NC1 epitopes.

Disclosure of Financial Relationships: nothing to disclose

**F-FC177**

**MPO-Specific Plasma Cell Depletion by Bortezomib Protects from ANCA-Induced Glomerulonephritis** Julia Bontscho,[1] Adrian Schreiber,[1] Rudolf A. Manz,[2] Wolfgang Schneider,[1] Friedrich C. Luft,[1] Ralph Kettritz.[1] *[1]Department of Nephrology and Hypertension and Experimental and Clinical Research Center, Medical Faculty of the Charite, Berlin, Germany; [2]Institute for Systemic Inflammation Research, Lubeck, Germany.*

ANCA cause small vessel vasculitis and necrotizing crescentic glomerulonephritis (NCGN). Steroids combined with cytotoxic drugs reduced mortality, but added substantial treatment-associated adverse events. We hypothesized that a MPO-ANCA NCGN mouse model allows to test novel treatment protocols and specifically tested the proteasome inhibitor bortezomib (BTZ). Anti-MPO IgG-induced NCGN was established by transplanting wild-type bone marrow (BM) into irradiated MPO-deficient mice immunized with MPO. Three weeks after BM transplantation actively treated mice received a steroid/cyclophosphamide (S/CYC) combination or BTZ, respectively. All untreated control mice developed hematuria, proteinuria, and NCGN, with 38±9% crescents and 13±3% necrosis. S/CYC and BTZ significantly reduced the urine abnormalities, crescents (3.9±2.5% and 2.8±2.8% respectively), necrosis (2.3±1.7% and 1.1±1.1%, respectively), and PMN and macrophage infiltration. BTZ, but not S/CYC, significantly reduced anti-MPO titers by ELISA. We analyzed total and MPO-specific splenic and BM plasma cells, MPO-specific plasma cells, and B lymphocytes by flow cytometry and ELISPOT. The data indicate that BTZ significantly diminished MPO-specific plasma cells in spleen and BM, reduced total plasma cells by approximately 50% (n.s.), and spared B lymphocytes. In contrast, S/CYC caused strong spleen shrinkage with reduction in total cells, B lymphocytes, plasma cells, including MPO-specific plasma cells. Two fatalities within 36 h after BTZ administration occurred.

Conclusion: An ANCA mouse model can be used to compare efficiency and adverse events of new drugs to standard protocols. More specifically, we show that BTZ depletes anti-MPO-specific plasma cells, reduces anti-MPO titers and prevents NCGN in mice.

Disclosure of Financial Relationships: nothing to disclose

**F-FC178**

**Serine Proteases Activate IL-1β To Produce ANCA NCGN** Adrian Schreiber,[1] Christine Pham,[2] Sylvia Krueger,[1] Friedrich C. Luft,[1] Ralph Kettritz.[1] *[1]Experimental and Clinical Research Center (ECRC) at the MDC Berlin, Berlin, Germany; [2]Division of Rheumatology, Washington University School of Medicine, St. Louis, MO.*

ANCA-activated neutrophils and monocytes cause necrotizing crescentic glomerulonephritis (NCGN). ANCA induce respiratory burst and release serine proteases. Serine proteases are generated as inactive pro-forms that are processed by the dipeptidyl-peptidase I (DPPI). We tested the hypothesis that serine proteases are essential to the ANCA response and NCGN. We immunized MPO-deficient mice with MPO to induce NCGN followed by irradiation and bone marrow transplantation from either wild-type (WT) mice or DPPI-deficient animals. WT mice developed NCGN with 37.4±8.2% glomerular crescents, whereas DPPI-/- mice were protected (1.3±0.7% crescents). Furthermore, inflammatory cytokine assessment in whole kidney lysates showed sharply reduced IL-1β generation in DPPI -/-mice (103.0 ± 29.6 pg/ml versus 5.9±1.1 pg/ml). Extracellular IL-1β release requires cytokine pro-form cleavage involving the inflammasome and caspase-1. To test whether or not serine proteases are involved in IL-1β activation, we stimulated murine monocytes and neutrophils with murine anti-MPO IgG, respectively. We observed a strong IL-1β release in WT monocytes that was reduced in DPPI-/- cells (152±9.0 vs. 47.5±15.9 pg/ml). We then tested whether or not human neutrophils and monocytes generate IL-1β after ANCA stimulation. We found strong IL-1β generation in monocytes and a smaller effect in neutrophils by immunoblotting and ELISA. We also observed that serine protease inhibitors reduced ANCA-triggered IL-1β generation, whereas caspase-1 inhibition had only small effects. Finally, we treated mice with the specific IL-1β antagonist, Anakinra®. In this set of experiments, untreated mice developed NCGN with 16.7±6.0% crescents, whereas Anakinra®-treated mice were protected (2.4±1.7%). Thus, we demonstrated that serine proteases are essential to ANCA-induced NCGN via IL-1β processing. Furthermore, IL-1 β is an effective therapeutic target.

Disclosure of Financial Relationships: nothing to disclose

**F-FC179**

**Robust Efficacy of C5aR Antagonist CCX168 in a Mouse Model of ANCA Glomerulonephritis** Hong Xiao,[1] J. Charles Jennette,[1] Daniel Dairaghi,[2] Jay P. Powers,[2] Yu Wang,[2] Linda Ertl,[2] Trageen Baumgart,[2] Shichang Miao,[2] Lisa C. Seitz,[2] Peiqi Hu,[1] Ronald J. Falk,[1] Thomas J. Schall,[2] Juan C. Jaen.[2] *[1]Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC; [2]ChemoCentryx, Inc, Mountain View, CA.*

**Purpose**

CCX168 is an orally active antagonist of human C5a receptor(C5aR) that has completed Phase 1 characterization. We evaluated CCX168 efficacy in a mouse model of antineutrophil cytoplasmic antibody (ANCA) glomerulonephritis (GN) known to involve C5a in pathogenesis. We used the biomarker of C5aR blockade used in the Phase 1 trial to establish pharmacokinetic, pharmacodynamic and therapeutic endpoints in C5aR-humanized mice.

**Methods**

A model of ANCA GN was iduced in human C5aR knock-in (hC5aR KI) mice by injection of anti-MPO IgG. The potency of CCX168 for C5aR in hC5aR KI mice was assessed by $^{125}$I-C5a binding, chemotaxis and other assays. C5a-induced neutrophil CD11b expression was used to assess CCX168 C5aR blockade in mice and humans. CCX168 doses ranged from 0.1-30 mg/kg qd.

**Results**

CCX168 had similar C5aR antagonist potency on human and hC5aR KI mouse neutrophils (inh. C5a-mediated chemotaxis in blood, $IC_{50}$ 2 nM; inh. C5a-induced CD11b upreg. in blood, $IC_{50}$ 4 nM). 30 mg/kg CCX168 markedly reduced the severity of anti-MPO induced GN with reduced crescents (vehicle 29.3%, CCX168 3.3%; $p<0.0001$) and necrosis (vehicle 8.2%, CCX168 1.1%; $p<0.0001$), which correlated with reduced urine protein, leukocytes and RBCs, and reduced serum BUN and creatinine. 30% reduction in crescents occurred with 0.1 mg/kg/d CCX168. 4 mg/kg CCX168 bid was the lowest dose that produced a near-maximal therapeutic benefit with plasma levels from 35 to 200 ng/mL throughout the day. At these doses C5aR blockade ranged from 95 to 99% with a time-averaged level of 97%. Plasma CCX168 levels of 197 ng/mL (~ 400 nM) were reached with a 100-mg dose in a human Phase 1 trial (see related meeting abstract).

**Conclusions**

Orally active hC5aR antagonist CCX168 has robust efficacy in a mouse model of ANCA GN. The lowest dose that induces a near-maximal therapeutic benefit produces CCX168 plasma levels and C5aR blockade attainable and well tolerated in humans.

Disclosure of Financial Relationships: Research Funding: ChemoCentryx Inc, Mountain View, CA.

**F-FC180**

**Tripartate Motif Containing Protein 27 (TRIM 27) Negatively Regulates CD4 T Cell Activation Via the Ubiquination and Inhibition of Phosphatidlyinositol 3 Kinase** β Xinjiang Cai,[1] Shekhar Srivastava,[1] Jon Backer,[2] Edward Y. Skolnik.[1] *[1]Skirball Institute, Department of Internal Medicine, New York University Langone Medical Center, New York, NY; [2]Department of Molecular Pharmacology, Albert Einstein College of Medicine, New York, NY.*

The Ca2+-activated K+ channel KCa3.1 is required for Ca2+ influx and the subsequent activation of T cells. We previously showed that the class 2 phosphatidylinositol 3 kinase C2β (PI3KC2β) is activated by the T cell receptor (TCR) and functions upstream of Nucleoside Diphosphate Kinase Beta to activate KCa3.1 channel activity. A yeast 2-hybrid screen was performed using a T cell cDNA library to identify PI3KC2β interacting proteins. One of the proteins identified was TRIM27. TRIM27 is a member of a large family of proteins that are characterized by the presence of a tripartite motif, which consists of a RING finger, a zinc-binding domain named a B box, and a coiled coil (CC) domain. TRIM family members have been shown to regulate a plethora of cellular pathways and recent evidence indicates that some TRIM family members function as a novel class of RING finger ubiquitin E3 ligases.

We now show that TRIM27 functions as an E3 ligase and ubiquitinates PI3KC2β. TRIM27 and PI3KC2β also co-immunoprecipitated in mammalian cells. Overexpression of wild type TRIM27, but not TRIM27 mutant for either the RING finger or CC domain, led to ubiquitination of PI3KC2β. siRNA knockdown of TRIM27 led to inhibition of PI3KC2β ubiquitination indicating that endogenous TRIM27 is the primary E3 ligase for PI3KC2β. TRIM27 mediated the polyubiquitination of PI3KC2β via lysine 48 (lys48) linkages. One function of lys48 polyubiquination is to inhibit proteins by targeting them for proteosomal mediated degradation. While TRIM27 inhibited PI3KC2β, this inhibition was mediated by inhibition of PI3K enzyme activity and not by protein degradation. Consistent with these findings, we found that TRIM27 also functions to negatively regulate CD4 T cells by inhibiting PI3KC2β leading to decreased KCa3.1 channel activity and TCR-stimulated Ca2+ influx. Thus, these finding identify TRIM27 as new negative regulator of CD4 T cells.

Disclosure of Financial Relationships: nothing to disclose

**F-FC181**

**The TH17-Defining Transcription Factor RORγt Mediates Severe Experimental Glomerulonephritis** Oliver M. Steinmetz, Shaun Andrew Summers, Poh-Yi Gan, Stephen R. Holdsworth, A. Richard Kitching. *Centre for Inflammatory Diseases, Monash Medical Centre, Clayton, Victoria, Australia.*

While Th17 responses may play a role in the pathogenesis of glomerulonephritis, the role of RORγt, the key transcription factor in Th17 cell development, is unknown. Glomerulonephritis (GN) was induced by planting sheep anti mouse anti glomerular basement membrane globulin in glomeruli of wild type (WT) and RORγt deficient ($RORγt^{-/-}$) mice. $RORγt^{-/-}$ mice were protected from disease, with reduced histological (crescents 18.7±1.8 vs. 6.4±1.1%, $p<0.0001$; glomerular necrosis 35.6±6.1 vs. 12.4±6.0%, $p<0.05$) and functional injury, diminished renal leukocyte infiltration and decreased IL-17A and F production, but intact lymphocyte activation and proliferative responses. As $RORγt^{-/-}$ mice lack lymph nodes which may influence the development of nephritis, cell transfer studies were performed. $Rag1^{-/-}$ mice (lacking B and T cells) were reconstituted with different leukocyte subsets from naïve WT or $RORγt^{-/-}$ mice and GN was induced. Mice receiving unfractionated WT splenocytes showed high mortality caused by renal failure while $RORγt^{-/-}$ cell recipients were protected (mortality 6/9 vs. 2/12, $p<0.05$). Likewise, recipients

of WT CD4$^+$ T cells developed severe glomerulonephritis whereas disease was attenuated in RORγt$^{-/-}$ cell recipients (crescents 21.4±3.6 vs. 8.7±1.1%, p=0.0011; necrosis 48.5±9.2 vs. 5.7±3.6%, p<0.001; no cell transfer controls 0.3±0.2% crescents and 1.3±0.8% necrosis). To exclude effects of altered regulatory T cell (Treg) development caused by RORγt deficiency, naïve CD4$^+$ T cells depleted of Tregs were transferred into Rag1$^{-/-}$ mice. GN was severe in recipients of WT, Treg depleted, CD4$^+$ T cells, but mice receiving RORγt$^{-/-}$, Treg depleted, CD4$^+$T cells were protected (crescents 3.6±1.0 vs. 0.3±0.3%, p<0.01; necrosis 82.9±3.1 vs. 14.3±14.3% p<0.001; no cell transfer controls: 0% crescents and necrosis). Both systemic and renal Th1 responses were intact in recipients of RORγt$^{-/-}$ cells. These studies therefore show an important role for RORγt in the pathogenesis of severe glomerulonephritis by directing nephritogenic Th17 responses.

Disclosure of Financial Relationships: nothing to disclose

## F-FC182

**Accumulation and Activation of T-Helper 17 (Th17) Cells in Obstructive Uropathy** Jana Pindjakova, Shirley Hanley, Michelle M. Duffy, Rhodri Ceredig, Matthew D. Griffin. *Regenerative Medicine Institute, College of Medicine, National University of Ireland, Galway, Galway, Ireland.*

**Background and Aims:** Intra-renal Th17 cells participate in experimental AKI and glomerulonephritis but remain incompletely characterized. Intra-renal Th17 cells were profiled in mouse unilateral ureteral obstruction (UUO) for chemokine receptor expression and activation. Th17-activating factors and their cellular sources were examined. **Methods:** Obstructed (Obs) and control (Ctr) kidneys were procured following 24-72 hr UUO. Th17 cells were identified by intracellular IL-17 staining of renal digests following brief activation *in vitro* with counterstaining for chemokine receptors. Renal CD4 T-cell subsets, dendritic cells (DC), monocytes and macrophages were purified by FACS for qRT-PCR. Conditioned media were prepared from MACS-enriched renal cell populations, analyzed by ELISA and added to *in vitro*-generated Th17 cells to detect Th17-activating factors. Blocking experiments were carried out with anti-IL-1R and/or IL-23R. **Results:** IL-17 mRNA increased progressively in cortex of Obs but not Ctr kidneys from 24-72 hrs. CD4+CCR6+ T cells ± CCR4 were identified as the primary source of IL-17. Numbers of these cells increased almost 100-fold in Obs vs Ctr kidneys. Ligands for CCR6 and CCR4 (CCL20, CCL17, CCL22) were selectively secreted by DCs from Obs kidneys. Conditioned media from the total leukocytes (CD45+) and DCs (CD11c+) but not CD45- cells of Obs kidneys augmented IL-17 production by *in vitro*-generated Th17 cells. This effect was inhibited by anti-IL-1R but not anti-IL-23R. IL-1α, IL-1β and IL-23 were detected in Obs kidneys and were secreted both by DCs and non-DC leukocyte fractions. FACS purification of multiple myeloid populations from 72-hr obstructed kidneys revealed a hierarchy of IL-1α/β expression: Ly6C-hi monocytes > F4/80+ DCs > F4/80- DCs > Ly6C-lo/CD11c- macrophages. **Conclusions:** Th17 cells are progressively activated following UUO and preferentially express CCR6 ± CCR4. During UUO renal DCs are a potent source of chemokine ligands for CCR6 and CCR4. Monocytes and DCs are the primary source of Th17-activating factors of which IL-1α and/or IL-1β are the most significant.

Disclosure of Financial Relationships: nothing to disclose

## F-FC183

**CD14 Mediates Inflammation and Kidney Injury of MPGN in the TSLP Model Cryoglobulinemic Glomerulonephritis Independent of TLR4** Takahisa Kobayashi, Tomasz A. Wietecha, Kelly L. Hudkins, Warangkana Pichaiwong, Kelly D. Smith, Shunhua Guo, Ichiro Kojima, Charles E. Alpers. *Dept. of Pathology, Univ. of Washington, Seattle, WA.*

Toll-like receptors (TLRs) are key proteins in innate immunity. We have reported upregulation of TLR4 in podocytes of TSLPtg (thymic stromal lymphopoietin transgenic) mice, a model of cryoglobulinemia associated MPGN (JASN,19:704,2008). To investigate the role of TLR4 in this model, we crossed the TSLPtg mice with mice deficient for TLR4 or CD14 (a major accessory molecule for TLR4). Renal disease was assessed in female mice after 50 days.

TSLPtg/TLR4-/- mice had a high mortality rate of 52.4% compared to 7.7% for TSLPtg/CD14-/- at 50 days. TSLPtg/CD14-/- mice had significantly reduced glomerular macrophage infiltration compared with TSLPtg and TSLPtg/TLR4-/- (% staining area/ glomerular tuft area (%GTA): TSLPtg 1.76±0.35, TSLPtg/CD14-/- 0.67±0.29 (p<0.05 vs TSLPtg), TSLPtg/TLR4 1.88±0.24). Despite similar levels of cryoglobulinemia and glomerular immune complex deposition, TSLPtg/CD14-/- mice had less glomerular hypercellularity, mesangial matrix expansion as measured by silver stained matrix (%GTA: TSLPtg 12.90±0.83, TSLPtg/CD14-/- 8.82±1.19 (p<0.05 vs TSLPtg), TSLPtg/ TLR4-/- 14.84±0.45), and mesangial cell activation as measured by smooth muscle actin (%GTA:TSLPtg 0.75±0.17, TSLPtg/CD14-/- 0.19±0.06 (p<0.05 vs TSLPtg), TSLPtg/ TLR4-/- 0.58±0.17). Proteinuria decreased in TSLPtg/CD14-/- mice compared with TSLPtg while it was not changed in TSLPtg/TLR4-/- (albumin/creatinine ratio (ug/mg): TSLPtg 100.4±15.3, TSLPtg/CD14-/- 43.9±17.6 (p=0.053)).

CD14 deficiency results in significant improvement in renal structure and function in TSLPtg mice, whereas deficiency of TLR4 does not. CD14 appears to mediate glomerular monocyte/macrophage infiltration, which may contribute to the development of kidney injury. In addition to LPS recognition by TLR4, CD14 also has been demonstrated to enhance recognition of some TLR2 ligands, and the TLR3 ligand, dsRNA. The clear beneficial effects of CD14 deficiency in the TSLPtg mice demonstrates TLR4 independent pathways of activity previously unrecognized as mediators of glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

## F-FC184

**Regulatory T Cells Suppress Kidney Ischemia Reperfusion Injury through Autocrine and Paracrine Adenosine Signaling** Gilbert R. Kinsey, Liping Huang, Mark D. Okusa. *Division of Nephrology and Center for Immunity, Inflammation and Regenerative Medicine, University of Virginia Health System.*

Regulatory T cells (Tregs) suppress the inflammation and injury associated with kidney ischemia-reperfusion injury (IRI). Activation of adenosine $A_{2A}$ receptors ($A_{2A}$Rs) on bone marrow-derived cells also blocks inflammation and prevents renal injury after IR. Tregs express $A_{2A}$Rs and CD39 and CD73, which are the membrane bound enzymes necessary for conversion of extracellular ATP to adenosine (ADO). Production of ADO is a recognized mechanism of suppression of adaptive immune responses by Tregs, but the contribution to suppression of innate immune pathology, such as early kidney IRI is unknown. We hypothesized that both the production of adenosine by, and $A_{2A}$R signaling in Tregs are required to block innate immune responses in kidney IRI. The ability of Tregs from WT, CD73KO and $A_{2A}$RKO mice to protect WT mice from kidney IRI was compared in terms of renal function, tubular necrosis and kidney infiltrating leukocytes. WT Treg cells protected WT mice from kidney IRI, but in the absence of adenosine generation (CD73KO) or $A_{2A}$Rs ($A_{2A}$RKO), the functional capacity of Tregs to block kidney IRI was impaired (plasma creatinine 18hr post ischemia: +non-Tregs: 1.3±0.3; +WT Tregs: 0.4±0.1*; +CD73KO Tregs: 1.2±0.4; +$A_{2A}$RKO Tregs: 0.8±0.3; *p<0.05 vs. IRI+non-Tregs). Furthermore, WT Tregs offered significant protection to $A_{2A}$RKO mice, although protection was not complete. Real-time PCR analysis revealed no difference in activation-induced expression of FoxP3, IL-10, TGF-β or CD73 in WT vs. $A_{2A}$RKO Tregs; however CD39 and CTLA-4 expression was significantly inhibited in $A_{2A}$RKO Tregs. Taken together these findings demonstrate that ADO production is vital for the protective effects of Tregs in IRI and suggest Treg-generated ADO acts in an autocrine manner to enhance the suppressive function of Tregs and paracrine manner to inhibit innate immune cell responses in kidney IRI.

Disclosure of Financial Relationships: nothing to disclose

## F-FC185

**Low Dose Atrasentan Safely Reduces Albuminuria in Subjects with Type 2 Diabetic Nephropathy (DN) on Renin-Angiotensin System (RAS) Inhibitors** Donald E. Kohan,[1] Mark E. Molitch,[2] Yili Pritchett,[3] Utpaul Audhya,[3] Shihua Wen,[3] Bo Yan,[3] Dennis L. Andress.[3] *[1]Univesity of Utah; [2]Northwestern University Feinberg School of Medicine; [3]Abbott.*

INTRODUCTION: DN is the leading cause of ESRD. RAS inhibitors only slow CKD progression due to incomplete inhibition and/or RAS-independent mediators of disease. Endothelin (ET)-1 is elevated in patients with DN and promotes glomerulosclerosis by activation of the ETA receptor. This study tested if low dose atrasentan, a highly selective ETA antagonist, could safely reduce residual albuminuria in subjects with DN on RAS inhibitors.

METHODS: A double-blind, placebo-controlled study of 89 subjects with DN on stable doses of RAS inhibitors for ≥2 months with UACR 100-3000 mg/g, eGFR >20 mL/min/1.73m$^2$, and NT-pro-BNP ≤500 pg/mL who were equally randomized to placebo, atrasentan 0.25, 0.75, or 1.75 mg daily for 8 weeks. The primary endpoint was change from baseline (BL) in first morning void UACR.

RESULTS: Mean age (64 years), mean eGFR (range 48-60 mL/min/1.73m$^2$) and median BL UACR (range 351-514 mg/g) were similar among groups. Repeated measures analysis showed early and sustained UACR reductions for the 0.75 mg (P=0.001) and 1.75 mg (P=0.011) groups, while the 0.25 mg dose had no significant effect. Change in UACR, SBP, DBP, and weight are shown in Table 1. ~15%-20% of the UACR lowering effect was related to SBP reduction. There were no significant changes in eGFR from BL. Peripheral edema (primarily mild) was the most common adverse event (14%, 18% and 46% for 0.25, 0.75 and 1.75 mg with p=0.007 for 1.75 mg vs. 9% in placebo). No deaths and only 1 treatment-related serious adverse event were reported.

CONCLUSIONS: Low dose atrasentan safely and effectively reduces residual albuminuria in subjects with type 2 diabetes receiving treatment with RAS inhibitors, mostly independent of reductions in SBP.

| | | Low Dose Atrasentan (P-values vs. Placebo) | | | | |
|---|---|---|---|---|---|---|
| Change from Baseline to Last Observation | Placebo (n = 23) | 0.25 mg (n = 22) | P-value | 0.75 mg (n = 22) | P-value | 1.75 mg (n = 22) | P-value |
| UACR, % reduction | 11% | 21% | 0.292 | 42% | 0.023 | 34% | 0.080 |
| Subjects with ≥ 40% Reduction UACR | 17% | 30% | 0.423 | 50% | 0.029 | 38% | 0.179 |
| Systolic Blood Pressure, mm Hg | 0.0 | -0.9 | 0.751 | -8.6 | 0.047 | -8.1 | 0.065 |
| Diastolic Blood Pressure, mm Hg | -0.4 | -0.8 | 0.902 | -6.0 | 0.050 | -8.1 | 0.007 |
| Weight, lb | -0.1 | -1.0 | 0.507 | 1.8 | 0.140 | 1.4 | 0.264 |

Disclosure of Financial Relationships: Consultancy: AbbottResearch Funding: Gilead Sciences; Honoraria: Several visiting professorships; Scientific Advisor: AJP Renal Editorial Board.

**F-FC186**

**Long-Term Renal Outcomes of Patients with Type 1 Diabetes and Microalbuminuria** Ian H. de Boer,[1] Tessa Rue,[1] Patricia A. Cleary,[2] John M. Lachin,[2] Mark E. Molitch,[3] Michael Steffes,[4] Wanjie Sun,[2] Bernard Zinman,[5] John D. Brunzell.[1] *[1]University of Washington; [2]The George Washington University; [3]Northwestern University; [4]University of Minnesota; [5]University of Toronto.*

In type 1 diabetes, long-term clinical outcomes after the development of microalbuminuria are variable. Frequencies of and risk factors for subsequent renal events are not clear. We evaluated long-term renal outcomes of 325 persons with type 1 diabetes who developed incident persistent microalbuminuria (albumin excretion rate [AER] ≥ 30 mg/24hr on two consecutive study visits) at a clearly defined time point in the Diabetes Complications and Control Trial (DCCT) and its observational follow-up (EDIC Study). In this group, we quantified the incidence of and risk factors for subsequent long-term renal events, including progression to macroalbuminuria (sustained AER ≥ 300 mg/24hr), impaired glomerular filtration rate (sustained estimated GFR < 60 mL/min/1.73m²), ESRD, and regression to normoalbuminuria (sustained AER < 30 mg/24hr). Median follow-up after microalbuminuria diagnosis was 13 years (maximum 23 years). 10-year cumulative incidences of macroalbuminuria, impaired GFR, and ESRD were 28%, 15% and 3% respectively, whereas 40% regressed to normoalbuminuria. Albuminuria outcomes were more favorable (decreased risk of progression to macroalbuminuria and increased probability of regression to normoalbuminuria) with randomly assigned intensive diabetes therapy, lower hemoglobin A1c, lack of retinopathy, female gender, lower blood pressure, and lower concentrations of LDL cholesterol and triglyceride (each p<0.05). Lower hemoglobin A1c, lack of retinopathy, and lower blood pressure were also associated with decreased risk of impaired GFR (p<0.05). In conclusion, the development of persistent microalbuminuria does not necessarily represent the start of an inexorable downhill course for diabetic nephropathy, since regression is as common as progression of kidney disease. Intensive glycemic control, lower blood pressure, and a more favorable lipid profile are associated with improved long-term renal outcomes after the development of persistent microalbuminuria.

Disclosure of Financial Relationships: Research Funding: Abbot Laboratories.

**F-FC187**

**Serum Placental Growth Factor (PlGF) Predicts All-Cause and Cardiovascular Mortality but Not Deterioration of Kidney Function in Type 1 Diabetic Patients with Diabetic Nephropathy** Simone Theilade,[1] Maria Lajer,[1] Anders Jorsal,[1] Lise Tarnow,[1] Hans-Henrik Parving,[2] Peter Rossing.[1] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]Dept. of Medical Endocrinology, Copenhagen University Hospital, Rigshospitalet, Denmark.*

**Objective**

Placental growth factor is a vascular endothelial growth factor involved in angiogenesis, vascular inflammation and plaque formation. The aim of this analysis is to evaluate the predictive value of PlGF in relation to all-cause and cardiovascular mortality, and decline in kidney function in type 1 diabetic patients.

**Design**

A prospective, observational follow-up study with 8(0-13) years (median(range)) of follow-up.

422 patients with longstanding type 1 diabetes and normoalbuminuria (223 men; age 45±11 years[mean±SD], diabetes duration 28±10 years) and 440 type 1 diabetic patients with diabetic nephropathy (DN) (267 men; age 42±10 years, diabetes duration 28±9 years, glomerular filtration rate ((GFR) 76±34 ml/min/1.73m²).

**Results**

PlGF levels were lower in normoalbuminuric patients vs. patients with DN (median(range) 11(7-131) vs. 15(4-68)pg/L (p<0.001).

In the normoalbuminuric group the patients were divided according to the median PlGF level. PlGF levels did not predict all-cause mortality (adjusted for sex, age and systolic BP) hazard ratio (HR) 1.0[0.5–1.9].

Patients with DN were divided according to tertiles of PlGF levels, and 61(41%) patients with the highest vs. 29(20%) patients with the lowest PlGF levels died during follow-up ($p$<0.01).

High levels of PlGF was predictive of all-cause mortality HR 1.9[1.1-3.2] (p=0.018) and cardiovascular mortality HR 2.9[1.5-5.9] ($p$<0.01), (adjusted for sex, age, smoking, systolic BP, $HbA_{1c}$, cholesterol, GFR, UAER, antihypertensive treatment and previous CVD).

High levels of PlGF did not predict development of end stage renal disease or rate of decline in GFR, $p$=0.49 and $p$=0.56, respectively.

Evaluating PlGF as a continuous variable introduced only minor changes in the statistical output.

**Conclusion**

In type 1 diabetic patients with DN higher levels of PlGF predict all-cause and cardiovascular mortality, but not deterioration of kidney function.

PlGF was measured by Roche Diagnostics, George Hess and Dietmar Zdunek

Disclosure of Financial Relationships: nothing to disclose

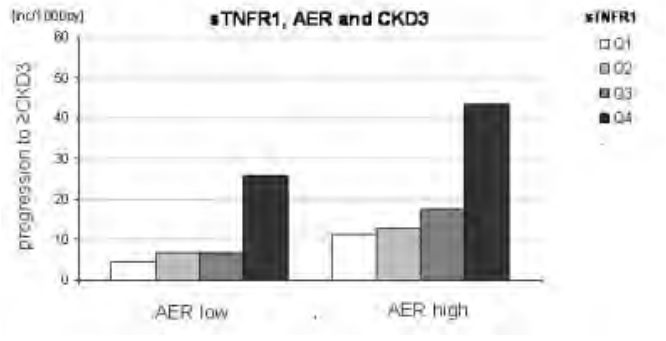
**F-FC188**

**Tumor Necrosis Factor Receptors 1 and 2 Are Early Serum Markers of Progressive Loss of Renal Function in Type 1 Diabetes** Tomohito Gohda, Monika A. Niewczas, Linda Hanna Ficociello, William Walker, Amanda Johnson, Gordon S. Crabtree, Jan Skupien, Adam Smiles, James Warram, Andrzej S. Krolewski. *Research Division, Joslin Diabetes Center, Boston, MA.*

Objective: To examine serum markers of the TNF pathway as determinants of early progressive renal function loss in patients with type 1 diabetes (T1D).

Methods: The study group comprises 629 patients with T1D and microalbuminura (MA) or high-normal albuminuria recruited between 1991 and 2005 and followed for 4-12 years. Concentrations of TNFα, soluble TNF receptor 1 (sTNFR1) and receptor 2 (sTNFR2), soluble Fas (sFas) were measured in baseline serum, and cystatin C was measured in serial samples for estimating the glomerular filtration rate (GFRcystatin) and determining two renal outcomes: "early GFR loss" (at least 3.3% decline in GFRcystatin per year) and onset of chronic kidney disease (CKD) ≥3 (GFRcystatin<60 ml/min/1.73 m²).

Results: While GFRcystatin was normal in all patients at baseline, both outcomes were significantly associated with baseline TNF receptors' concentrations, grouped into quartiles (p<0.0001). The odds ratios of early GFR loss were 1.4 [95% CI:1.1, 1.8] per quartile of sTNFR1 and 1.7 [95% CI:1.3, 2.3] per quartile of sTNFR2 after adjustment for HbA1c, urinary albumin excretion and initial GFRcystatin. Similarly, the hazard ratios for onset of CKD ≥3 were 1.8 [95% CI:1.3, 2.3] and 1.7 [95% CI:1.3, 2.3] per quartile. This effect was independent from albuminuria.




Renal outcomes were unrelated to TNFα and sFas.

Conclusions: The risk of early progressive renal function loss in patients with T1D and MA is strongly and independently associated with serum concentrations of sTNFR1 and sTNFR2. While the underlying mechanisms are unclear, a single determination of one of these receptors in serum may identify patients at high risk of early progressive renal function loss.

Disclosure of Financial Relationships: nothing to disclose

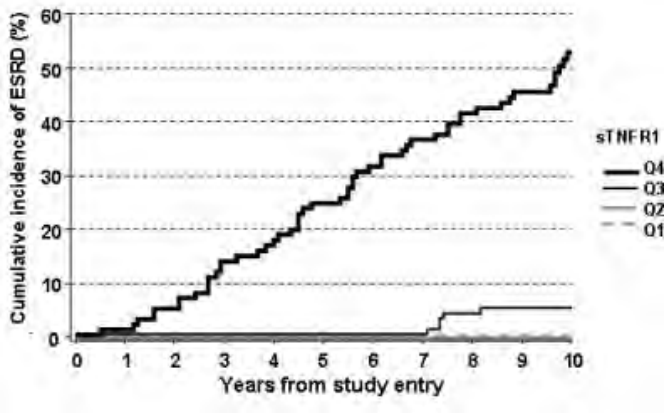
**F-FC189**

**Serum Markers of Tumor Necrosis Factor Pathway and Risk of End-Stage Renal Disease in Type 2 Diabetes** Monika A. Niewczas,[1,2,] Tomohito Gohda,[1,2,] Jan Skupien,[1,2,] Adam Smiles,[1] William Walker,[1] Linda Hanna Ficociello,[1,2] James Warram,[1] Andrzej S. Krolewski.[1,2] *[1]Research Division, Joslin Diabetes Center, Boston, MA; [2]Department of Medicine, Harvard Medical School, Boston, MA.*

Objectives: Incidence of end-stage renal disease (ESRD) due to type 2 diabetes (T2D) has increased during the last 20 years in the US. Better understanding of its etiology is needed to arrest this trend. In this study we explore the role of serum markers of inflammation as determinants of ESRD in T2D.

Methods: 410 patients attending the Joslin Clinic (211 normoalbuminuria, 119 microalbuminuria, 80 proteinuria) were recruited in the 1990s. We recorded characteristics at enrollment and measured markers of the TNF pathway, endothelial dysfunction and systemic inflammation in baseline serum. Onset of ESRD and deaths within 12 years of follow-up were ascertained.

Results: ESRD developed in 59 patients (1.7/100 p-years), and 84 patients died without ESRD (2.4/100 p-years). The risk of ESRD was associated with all TNF pathway markers and none of the others. Association with sTNFR1 was strongest. Ninety percent of ESRD cases were in the highest quartile of its distribution, giving that quartile a 10-year cumulative risk of 52% and leaving a risk of only 5% for the remaining quartiles (P<0.0001).




This effect of sTNFR1 was independent of other clinical characteristics. Deaths unrelated to ESRD were only moderately associated with TNF receptors. When examined as a predictor of ESRD, sTNFR1 outperformed estimated glomerular filtration rate, urinary albumin excretion, glycohemoglobin and their combination.

Conclusions: The activation of TNF pathway plays a major role in development of ESRD in T2D. A single determination of serum sTNFR1 predicts accurately the subsequent 10-year risk of ESRD.

Disclosure of Financial Relationships: nothing to disclose

**F-FC190**

**Systemic Transcriptome Analysis for Extraction of Specific Gene Clusters in Diabetic Nephropathy – Reduced Expression of Insulin Regulated Aminopeptidase Angiotensin IV Receptor (IRAP/AT4) in Diabetic Renal Tissue** Tadashi Konoshita. *Third Department of Internal Medicine, Fukui University School of Medicine, Eiheiji, Fukui, Japan.*

The renin-angiotensin system (RAS) plays pivotal roles on progression of diabetic nephropathy. However, much remains unknown about the molecular mechanisms involved. The aim of the study was to obtain novel insights into processes by systemic transcriptome analysis by DNA microarray technology. RNA was extracted from a small part of renal cortical biopsy specimens. A total number of 54,675 transcripts expression levels were analyzed systemically with GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix). Specific gene clusters involved in diabetic nephropathy was analyzed by hierarchical clustering method. Extracted candidate genes were analyzed by real-time PCR method with LightCycler (Roche). Hierarchical clustering analysis with 18 samples showed cluster formation considerably distinct by each original renal condition. By a hierarchical clustering analysis with 3 samples each from minor abnormality and diabetic nephropathy, we divided the transcripts to 12 clusters. By ontology and pathway analysis from the 11th cluster, the renin-angiotensin system was selected as a diabetic nephropathy specific pathway implying the reduction of ACE2, C9orf3, ENPEP and IRAP/AT4, which are thought to exert the effects for degradation of angiotensin II. Further analysis of 78 subjects with real-time PCR method revealed a significant reduction of IRAP/AT4 expression in diabetics renal tissues compared to non-diabetics (p=0.01). Recently AT4 (angiotensin IV receptor) was identified as IRAP (insulin regulated aminopeptidase). The IRAP/AT4 was originally identified from GLUT4 vesicles and thought to be involved in insulin sensitivity. On the other hands, IRAP/AT4 is thought to degrade angiotensin III to angiotensin IV and to further degraded fragments. Thus, the results suggest that reduction of IRAP/AT4 in renal tissue might be involved in formation and progression of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**F-FC191**

**Progression of Kidney Dysfunction and Its Association with Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus and Coronary Artery Disease in the BARI 2D Study** Barry M. Wall,[1] Phyllis August,[2] Mark E. Molitch,[3] Regina Hardison,[4] Janet B. McGill,[5] Michael Steffes,[7] Fadi G. Hage,[9] Oscar C. Marroquin,[6] Yves Rosenberg.[8] *[1]VAMC/Univ TN; [2]New York Hosp Queens; [3]Northwestern Univ. Feinberg School of Med; [4]Univ of Pittsburgh; [5]Washington Univ/Barnes Jewish Hosp; [6]Univ. of Pittsburgh Med Ctr; [7]Univ of MN; [8]NHLBI; [9]Univ of AL.*

We determined the progression of kidney disease and its association with cardiovascular outcomes Death\MI\Stroke (DMS) in 1937 patients with type 2 DM and coronary artery disease enrolled in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D), a trial comparing 5 yr outcomes in patients randomized to revascularization and medical therapy vs medical therapy, and 2 glycemic control strategies: insulin providing (IP) vs insulin sensitizing (IS). Definitions: Reduced eGFR (< 60 ml/min/1.73m$^2$ ) and eGFR progression (reduction of ≥15 ml/min/1.73m$^2$ or ≥25%); albuminuria: micro-(ACR>30, ≤ 300 mg/g,), or macro (ACR>300 mg/g) and progression as ≥ doubling of ACR to > 100 mg/g or to ≥ 2 visits with worsened ACR status. eGFR decreased over time. Patients with baseline macro or micro albuminuria had larger decreases in eGFR than those with normal albuminuria. During the study, the prevalence of eGFR's < 60 doubled, from 21.9 to 39.8%. The prevalence of micro- and macroalbuminuria increased minimally. The IS strategy had increased risk for albuminuria progression (IS vs IP OR 1.27, p=0.021). Other significant factors for ACR progression were being Hispanic and elevated HbA1c. Significant factors for eGFR progression were baseline ACR, female sex, systolic and diastolic BP. Progression of eGFR did not differ between randomized treatment groups. Reduced eGFR was related to higher risk of DMS (HR 1.7, p < 0.001). Macro (HR 2.2, p <0.001) and micro albuminuria(HR 1.4, p 0.006) also increased risk of DMS compared to normal albuminuria. We conclude that in subjects enrolled in BARI 2D 1) reduced eGFR and albuminuria were prevalent, 2) worsening of eGFR was more common than progression of albuminuria, and 3) eGFR and ACR were significant predictors of Death/MI/Stroke.

Disclosure of Financial Relationships: nothing to disclose

**F-FC192**

**Hypophosphatemic Effect of Niacin in Patients with Type 2 Diabetes: A Randomized Trial** Andrew G. Bostom,[1] Alexandra Maclean,[2] Darbie Maccubbin,[2] Diane Tipping,[2] Hilde Giezek,[2] William A. Hanlon.[2] *[1]Division of Kidney Diseases and Hypertension, Rhode Island Hospital, Providence, RI; [2]Merck and Company, Rahway, NJ.*

Niacin lowers serum phosphorus (P) concentrations in patients with stage 5 chronic kidney disease (CKD), an estimated glomerular filtration rate [eGFR] < 15 ml/min/1.73 m$^2$. We expanded upon these data with a post-hoc evaluation of the impact of extended release niacin (ERN), in fixed-dose combination with laropiprant (L), a specific inhibitor of prostaglandin-mediated, niacin-induced flushing, versus placebo (PBO), on serum P concentrations measured serially (at weeks 0,4,8,12,18,24,30, and 36) during a 36-week randomized, placebo-controlled trial. All subjects had a confirmed diagnosis of type 2 diabetes (n=446 niacin/laropiprant; n=339 placebo). Their eGFR ranged from 36-184 ml/min/1.73 m$^2$, with n=111 (14.1%) having an eGFR <60 ml/min/1.73 m$^2$. Subjects received 1 tablet daily of ERN-L (ERN 1g/ L 20 mg) for the first 4-weeks, and 2 tablets once daily, thereafter, or matched PBO. Repeated measures analysis demonstrated ERN-L lowered serum P concentrations by 0.36 mg/dl (95% CI: -0.40, -0.31; p < 0.001), relative to PBO, expressed as the treatment difference between the Week 12-36 average changes from baseline values of 3.57 and 3.56 mg/dl in the ERN-L and PBO groups, respectively. There was no evidence for P-lowering effect modification by these baseline variables: eGFR < 60 (n=111; 14.1%) vs. ≥ 60 ml/min/m$^2$ (n=674; 85.9%); P ≤ 3.5 mg/dl (n=392; 49.9%) vs. >3.5 mg/dl (n=393; 50.1%); or prior statin use (n=618; 78.7%) vs. non-use (n=167; 21.3%). Specifically, among the large (i.e., 50%) subgroup of patients (n=224 ERN-L; n=169 PBO) whose baseline serum P was > 3.5 mg/dl, ERN-L lowered serum P concentrations from baseline means of 3.98 and 3.97 mg/dl (for ERN-L and PBO, respectively) by 0.39 mg/dl (95% CI: -0.46, -0.31; p <0.001), relative to PBO.These data confirm that niacin's phosphorus-lowering effects—which may have therapeutic implications for the management of hyperphosphatemia in renal disease—extend across a broad spectrum of renal function (eGFR) in type 2 diabetics *without* stage 4 or 5 CKD (an eGFR ≥ 30).

Disclosure of Financial Relationships: nothing to disclose

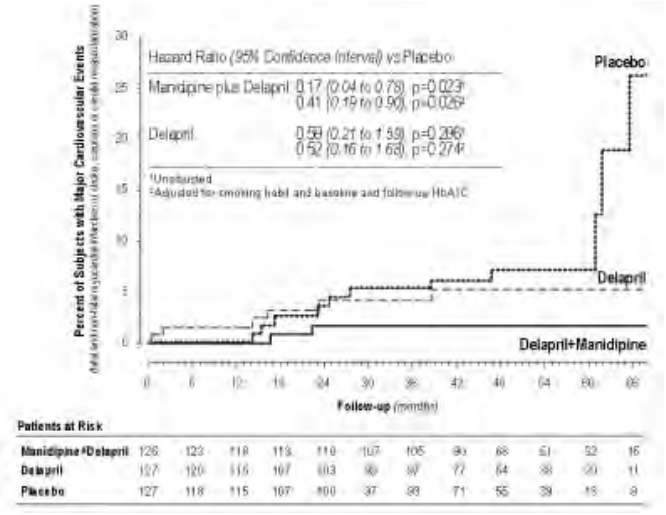
**F-FC193**

**Effects of Manidipine and Delapril on Chronic Complications of Type 2 Diabetes: DEMAND Study** Piero Ruggenenti,[1] Giuseppe Lauria,[2] Jelka Zaletel,[3] Giuseppe Remuzzi.[1] *[1]Mario Negri Institute for Pharmacological Research and Ospedali Riuniti di Bergamo, Italy; [2]Carlo Besta Neurological Institute, Milan, Italy; [3]University Medical Center, Ljubljana, Slovenia.*

Background: DElapril and MAnidipine for Nephroprotection in Diabetes (DEMAND) was a multicenter, double-blind, placebo-controlled, randomized trial to assess whether ACE inhibitors and third-generation dihydropyridine CCBs ameliorate complications of type 2 diabetes.

Methods: 380 hypertensive patients with albuminuria <200 µg/min were randomized to >3-year treatment with manidipine (10 mg/day) plus delapril (30 mg/day), delapril (30 mg/day), or placebo. Glomerular filtration rate (GFR, primary outcome) and glucose disposal rate (GDR) were measured by iohexol plasma clearance and hyperinsulinemic euglycemic clamps.

Results: Median monthly GFR decline (interquartile range) was 0.32 (0.16-0.50), 0.36 (0.18-0.53) and 0.30 (0.12-0.50) ml/min/1.73m$^2$ on combined therapy, delapril or placebo, respectively. Combined therapy decreased the incidence of major cardiovascular events vs placebo. Delapril had no significant effect.




Among 192 subjects without retinopathy at inclusion, three on combined therapy developed retinopathy vs nine on placebo [HR (95%CI): 0.27 (0.07-0.99), p=0.048]. Among 200 subjects with centralized neurological evaluation, 24 on combined therapy and 26 on delapril had peripheral neuropathy at 3 years vs 39 on placebo. Odd Ratios (95%CI): 0.45 (0.24-0.87), p=0.017, and 0.52 (0.27-0.99), p=0.048, respectively. GDR decreased on delapril or placebo, but was stable on combined therapy. No patient withdrew for treatment adverse events.

Conclusions: In hypertensive type 2 diabetic patients combined manidipine and delapril therapy failed to slow renal function loss, but ameliorated cardiovascular disease, retinopathy and peripheral neuropathy, stabilized insulin resistance, and was safe.

Disclosure of Financial Relationships: nothing to disclose

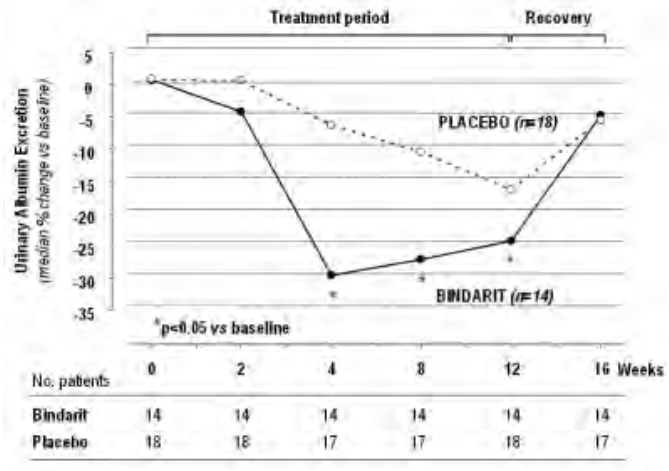
**F-FC194**

**Effects of MCP-1 Inhibition by Bindarit Therapy in Type 2 Diabetes Subjects with Micro- or Macro-Albuminuria** Piero Ruggenenti. *Mario Negri Institute and Azienda Ospedaliera Ospedali Riuniti di Bergamo, Italy.*

Background: Monocyte chemoattractant protein-1 (MCP-1 or, more recently, CCL2) is a cytokine with chemotactic activity toward monocytes involved in the development and progression of albuminuria in subjects with diabetes. Whether MCP-1 inhibition may reduce albuminuria in this population is not known

Methods: In this prospective, randomized, double-blind, placebo-controlled trial we evaluated urinary albumin excretion (UAE) in 41 micro (UAE: 20 to 200 µg/min) and 32 macro- (UAE: 200 to 2000 µg/min) albuminuric type 2 diabetes patients on stable irbesartan (300 mg/day) therapy randomized to 12-week therapy with the MCP-1 synthesis inhibitor bindarit (600 mg twice daily) or placebo and followed-up to 4 weeks after treatment withdrawal. Treatment was titrated to systolic/diastolic blood pressure <120/80 mmHg and HbA1C<7%. Within patient changes were assessed by paired t-test.

Results: Baseline characteristics of the two treatment groups were comparable. In those with macroalbuminuria at inclusion, UAE decreased significantly at 4, 8 and 12 weeks of bindarit therapy, but did not change appreciably on placebo.




UAE recovered to baseline values after treatment withdrawal. Blood pressure and metabolic control were similar throughout the study in both treatment arms. No significant treatment effect was observed in the 21 subjects on bindarit and the 20 on placebo who were microalbuminuric at inclusion. Treatment was well tolerated in all patients

Conclusions: Bindarit significantly and safely reduced albuminuria compared to placebo in type 2 diabetes patients with residual macroalbuminuria despite optimized blood pressure and metabolic control and background full-dose angiotensin II receptor blocker therapy. Prospective trials may help addressing the long-term renoprotective effect of bindarit therapy in this population.

Disclosure of Financial Relationships: nothing to disclose

**F-FC195**

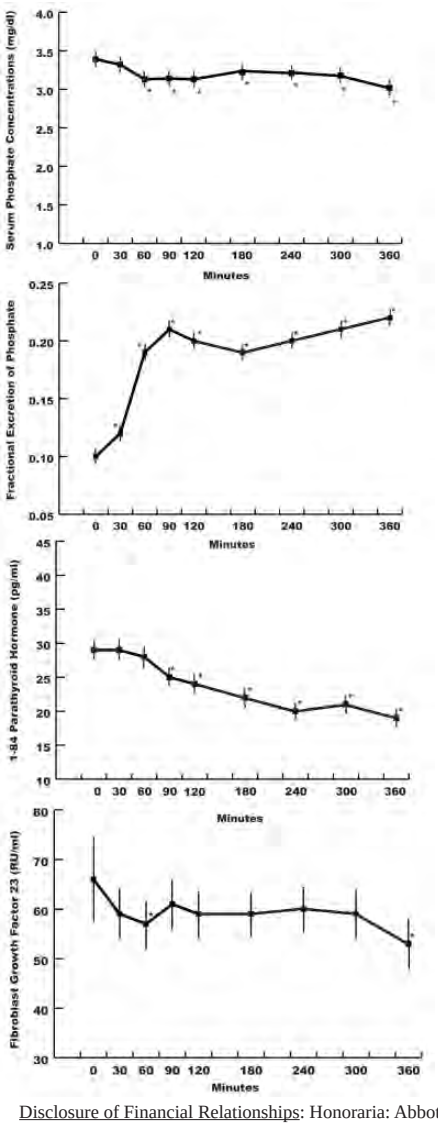
**(1-34) PTH Infusion Acutely Lowers FGF23 Levels in Healthy Volunteers** Orlando M. Gutierrez, Tamara Isakova, Myles S. Wolf. *University of Miami.*

**Purpose:** Previous studies showed that (1-34) parathyroid hormone (PTH) infusions stimulate increased fibroblast growth factor 23 (FGF23) secretion in healthy volunteers and dialysis patients over 24-48 hours, however the acute effects of (1-34) PTH infusion on FGF23 are less clear. Specifically, little is known about the change in FGF23 levels in relation to decreases in serum phosphate levels that occur early after the initiation of (1-34) PTH infusion and prior to the rise in calcitriol levels. Accordingly, we examined the acute changes in FGF23 levels in response to (1-34) PTH infusion.

**Methods**: 26 healthy volunteers underwent (1-34) PTH infusion at a rate of 0.055 mcg/kg/hr for six hours. Serum phosphate, (1-84) PTH, FGF23, and urinary $FE_{Pi}$ were measured every 30 minutes for the first two hours and than every hour for the remaining 4 hours.

**Results:** The mean age of the population was 36 ± 11, 19% (5/26) were female and 38% (10/26) were black. As depicted in the figure, mean serum phosphate levels decreased while mean $FE_{Pi}$ increased during the course of the 6 hour infusion. In addition, (1-84) PTH levels gradually decreased over 6 hours. FGF23 levels decreased early after initiating the infusion in parallel with the decrease in serum phosphate, with both analytes reaching their nadir after 6 hours.

**Conclusion:** In healthy volunteers, FGF23 levels acutely decrease after initiation of (1-34) PTH infusion in parallel with concurrent decreases in serum phosphate levels. Whether this reflects an effect of decreasing serum phosphate levels on FGF23 synthesis or a direct inhibitory effect of (1-34) PTH on FGF23 requires further study.




Disclosure of Financial Relationships: Honoraria: Abbott, Genzyme.

**F-FC196**

**PTH Increases FGF23 Gene Expression through PKA-Wnt Signaling and Mediates the High FGF23 Levels of CKD: A Bone Parathyroid Feedback Loop** Tomer Meir, Vardit Lavi-Moshayoff, Gilad Wasserman, Tally Naveh, Justin Silver. *Nephrology, Hadassah Hebrew University Medical Center, Jerusalem, Israel.*

PTH and FGF23 target the kidney to cause a phosphaturia. FGF23 also acts on the parathyroid to decrease PTH expression, but in CKD there are high serum PTH and FGF23 levels and resistance of the parathyroid to FGF23. We now report that PTH acts on bone to increase FGF23 expression and characterize the signal transduction pathway whereby PTH increases FGF23 expression. Remarkably, we show that PTH is necessary for the high FGF23 levels of early CKD. In CKD rats due to an adenine high phosphorus diet, parathyroidectomy totally prevented the 5-fold increase in FGF23 levels. Moreover, in rats with established experimental CKD, PTX corrected the high FGF23 levels. Therefore, in CKD the high FGF23 levels are dependent on the high PTH levels. PTH infusion for 3 days to mice with normal renal function increased serum FGF23 and calvaria FGF23 mRNA levels. To demonstrate a direct effect of PTH on FGF23, we added PTH to rat osteoblast like UMR106 cells. PTH ($1x10^{-7}$ M) increased FGF23 mRNA levels (4-fold) and this effect was mimicked by a PKA activator, forskolin. Both PTH and forskolin also decreased SOST mRNA levels (3-fold). SOST codes for sclerostin, a Wnt pathway inhibitor, which is a PTH receptor (PTH1R) target. The effect of PTH was prevented by added sclerostin (5 microg/ml). Therefore, PTH increases FGF23 expression in vivo and in vitro through the PKA and Wnt pathways. The effect of PTH on FGF23 completes a novel bone-parathyroid endocrine feedback loop whereby FGF23 decreases PTH expression and PTH in turn increases FGF23 expression in bone. Importantly, we demonstrate that secondary hyperparathyroidism is essential for the high FGF23 levels in CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-FC197**

**Fibroblast Growth Factor 23 Predicts Cardiovascular Mortality in Elderly Men** Per-Anton Westerberg,[1] Claes Ohlsson,[2] Åsa Tivesten,[3] Tobias Larsson,[4] Torbjorn Linde,[1] Osten Ljunggren.[1] *[1]Department of Medical Sciences, Uppsala University, Uppsala, Sweden; [2]Center for Bone Research at the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [3]Wallenberg Laboratory for Cardiovascular Research, University of Gothenburg, Gothenburg, Sweden; [4]Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden.*

Objectives. Fibroblast growth factor 23 (FGF23) decreases renal phosphate (Pi) reabsorption and vitamin D activation, and the level of circulating FGF23 is associated with atherosclerosis, endothelial dysfunction and left ventricular hypertrophy in the general population, and with mortality in dialysis patients. It is unknown if FGF23 is associated with cardiovascular mortality when the renal function is normal or slightly reduced.

Methods. The prospective population-based MrOS Sweden study included 3014 Swedish men (age 69 -81 years). On inclusion, among other factors related to mineral metabolism, intact FGF23 was measured using an ELISA (Kainos, Japan) in 2837 of the participants. Estimated glomerular filtration rate (eGFR) was calculated from cystatin C levels. 763 (27%) had eGFR< 60 ml/min. Mortality data for the whole cohort were collected after a mean of 4.5 years follow-up, 352 deaths occurred, 132 of cardiovascular cause. Using Cox proportional hazards regression, we examined if FGF23 was independently associated with all-cause and cardiovascular death.

Results. There was a significant association between FGF23 level and all-cause mortality with a hazard ratio (HR) of 1.13; 95.0 % confidence interval (CI) 1.01-1.27 per standard deviation increase in FGF23. For cardiovascular death HR was 1.44, CI 1.20-1.75. In multivariate analysis, including age, eGFR, 25OH vitamin D, albumin-corrected calcium, Pi and intact PTH, the association remained significant only for cardiovascular death: HR 1.26, CI 1.02-1.56. Conclusion. The serum level of FGF23 is associated with increased risk of cardiovascular death in elderly men. Whether FGF23 is a useful marker or a modifiable risk factor for cardiovascular mortality that can be affected by Pi-reduction or other methods are areas of further research.

Disclosure of Financial Relationships: nothing to disclose

**F-FC198**

**Genetic Variants That Modify the Association of Vitamin D Deficiency with Clinical Outcomes: A Candidate Gene Study** Gregory Levin,[1] Cassianne Robinson-Cohen,[1] Bryan R. Kestenbaum,[1,2] Ian H. de Boer,[1,2] Thomas J. Wang,[3] Bruce M. Psaty,[1] David Siscovick.[1] *[1]University of Washington; [2]Kidney Research Institute; [3]Massachusetts General Hospital Heart Center.*

Consistent data from prospective health studies link lower serum 25-hydroxyvitamin D concentrations with incident cardiovascular disease, fracture, cancer, and premature death. 25-hydroxyvitamin D represents available vitamin D substrate, which requires a series of metabolic steps for activation. In this study, we investigated whether variation within 6 genes involved in 25-hydroxyvitamin D metabolism modifies the association of 25-hydroxyvitamin D deficiency with the composite outcome of myocardial infarction, hip fracture, cancer, or death (gene x environment interaction). Our cohort consisted of 1,509 Caucasian participants (mean age 74 years) from the Cardiovascular Health Study. We tested 141 single nucleotide polymorphisms (SNPs) genotyped by the Illumina 370CNV BeadChip platform from genes encoding the vitamin D receptor (VDR), cubilin (CUBN), 1-alpha hydroxylase (CYP27B1), 24-alpha hydroxylase (CYP24A1), megalin (LRP2), and the vitamin D-binding protein (GC). We used Cox proportional hazards models to test for multiplicative interactions of 25-hydroxyvitamin D and SNP on the risk of composite outcome, using a false discovery rate threshold of 0.25 to account for multiple testing (no more than 25% of our reported discoveries are expected to be false positives). There were a total of 944 events during 14 years of follow-up (median 11 years). We found 6 SNPs located within CYP27B1, CUBN, and VDR that significantly modified the association of 25-hydroxyvitamin D with the combined endpoint. Each additional copy of the minor allele in these 6 SNPs was associated with an estimated 30-40% difference in the hazard ratio (all q-values <0.25, p-values between 0.002 and 0.009). These findings suggest that known associations of vitamin D deficiency with clinical outcomes vary according to genetic differences in 25-hydroxyvitamin D metabolism. If confirmed, specific genetic variants may identify individuals for whom vitamin D deficiency is associated with greatest risk of adverse events.

Disclosure of Financial Relationships: nothing to disclose

**F-FC199**

**The Use of Inhibitors of CYP24 To Control Secondary Hyperparathyroidism in an Adenine Induced Rat Model of CKD** Martin P. Petkovich,[1] Dominic Cuerrier,[1] Gary H. Posner,[2] Aza Kharebov,[1] Christopher Hosfield,[1] Christian Helvig.[1] *[1]Research and Development, Cytochroma Inc., Markham, ON, Canada; [2]Department of Chemistry, The Johns Hopkins University, Baltimore, MD.*

Recently, we have shown that CYP24, the cytochrome P450 enzyme responsible for catabolism of both 1,25-dihydroxyvitamin $D_3$ ($1,25(OH)_2D_3$) and its prohormonal form, 25-hydroxyvitamin $D_3$ ($25(OH)D_3$), is aberrantly over-expressed in kidney from adenine induced uremic rats, as well as, in kidney biopsy specimens from patients with chronic kidney disease (CKD) (1). These findings strongly indicate that CYP24 over-expression may contribute to the prevalence of vitamin D deficiency in CKD. Furthermore, they raise the possibility that such elevated expression of CYP24 may block kidney exposure

to vitamin D, thereby depriving it of crucial anti-inflammatory and anti-fibrotic activities, leading to accelerated progression of CKD. Current therapies, which are readily catabolized by CYP24, may be impaired in their ability to reach tissues expressing high levels of CYP24. Alternatively, we have developed and characterized a series of vitamin D-based inhibitors which target CYP24. *In vitro* studies indicate that these vitamin D analogs are potent CYP24 inhibitors ($IC_{50}$< 23 nM), that they are highly specific for CYP24 and do not activate or antagonize the vitamin D receptor. Several promising compounds, CTA091, CTA056, CTA102, CTA112 and CTA156 were evaluated in a rat adenine induced model of CKD. In single or repeat dose studies with these inhibitors, efficient reductions in parathyroid hormone could be achieved without significant increases in blood calcium levels. These studies demonstrated that blocking CYP24 activity effectively facilitated 1) the activity in target tissues of existing $1,25(OH)_2D_3$ and/or 2) the activation of $25(OH)D_3$ by residual kidney 1α-hydroxylase (CYP27B1) by increasing substrate availability. These studies show that CYP24 is a feasible therapeutic target for the treatment of secondary hyperparathyroidism associated with CKD.

(1) Helvig et al., (2010) Kidney International, in press.

Disclosure of Financial Relationships: nothing to disclose

**F-FC200**

**Regional Up-Regulation of 25-Hydroxyvitamin D 1a-Hydroxylase (CYP27B1) Gene Is Associated with the Pathogenesis of Ectopic Calcification in the alpha Klotho Mutant Mice** Ayako Otani, Hironori Yamamoto, Yuichiro Takei, Mina Kozai, Masashi Masuda, Shoko Ikeda, Otoki Nakahashi, Yutaka Taketani, Eiji Takeda. *Dept. Clinical Nutrition, Inst. Health Biosciences, Univ. Tokushima, Tokushima, Japan.*

Fibroblast growth factor (FGF) 23 is a critical regulator in the phosphate and vitamin D metabolism. Recent studies have revealed that alpha klotho plays an essential role in FGF23 signaling. Alpha klotho mutant (kl/kl) mice have hyperphosphatemia, hypercalcemia and hypervitaminosis D, and develop arteriosclerosis, osteoporosis and ectopic calcifications, together with short lifespan and infertility. It has been thought that these disorders are caused by high level of serum 1, 25-dihydroxyvitamin D through the up-regulation of key active vitamin D-metabolizing enzymes, 25-hydroxyvitamin D 1 alpha-hydroxylase (CYP27B1), in the kidney of kl/kl mice because these phenotypes are reversed by normalizing vitamin D homeostasis. However, it is still unclear how high-expressed CYP27B1 gene associate with the pathogenesis of ectopic calcification in kl/kl mice. In present study, we investigated the relationship between the expression of CYP27B1 and the pathogenesis of ectopic calcification in kl/kl mice. Real-time PCR and western blot analysis indicated renal cortex CYP27B1 mRNA and protein expressions were increased in 6 week old kl/kl mice compared with wild-type mice. Interestingly, immunohistochemical analysis and von Kossa staining using kl/kl tissue serial sections identified the regional high-expressed CYP27B1 in kidney cortex from 3 week old mice without ectopic calcification, in other hand, in 6 week old mice, its localization merged with calcifying renal arterioles and tubular cells in kl/kl mice. Importantly, we also found that the CYP27B1 protein accumulates in cardiac and aortic calcifying cells. In conclusion, the expression of CYP27B1 was regionally up-regulated and co-localized with calcified lesions in kl/kl mice. These results suggest that the regional overproduction of 1, 25-dihydroxyvitamin D through the up-regulation of CYP27B1 gene expression by abnormality of FGF23-klotho signaling may be implicated in the pathogenesis of ectopic calcification.

Disclosure of Financial Relationships: nothing to disclose

**F-FC201**

**Lack of PTH Receptor Phosphorylation Alters the Temporal Profile of the Acute Calcemic Response to PTH Analogs** Akira Maeda,[1] Makoto Okazaki,[1] Hiroko Segawa,[1] Abdul Abou-Samra,[2] Harald Jueppner,[1] John T. Potts,[1] Thomas J. Gardella.[1] *[1]Endocrine Unit, Massachusetts General Hospital, Boston, MA; [2]Wayne State University, Detroit, MI.*

Serine phosphorylation of the C-terminal of the PTHR1 plays a key role in controlling the receptor signaling and internalization responses induced by PTH ligands. A phosphorylation-deficient mutant PTHR1 (PD) exhibits defective internalization in cells, and PD-knock-in mice exhibit prolonged increases in blood cAMP after single PTH(1-34) injection, as well as frank hypercalcemia upon continuous PTH infusion (Bounoutous, 2006, Endo.). In separate work, we have developed high-affinity, N-terminally modified PTH analogs, e.g. M-PTH(1-28), that induce prolonged cAMP and robust internalization responses via the wild-type (WT) PTHR1 in cells, and prolonged calcemic and phosphaturic responses upon single injection in WT mice (Okazaki, 2008, PNAS). To explore further the relationships between PTHR1 phosphorylation/internalization and down-stream functional responses, we assessed the acute effects of PTH(1-34) and M-PTH(1-28) in PD knock-in mice. As expected, PD mice, vs. WT controls, exhibited enhanced/prolonged blood cAMP responses to both ligands. Surprisingly, however, the ionized(i)Ca response induced by either ligand was not prolonged, as compared to that induced in WT mice, and, in fact, was blunted, in that the onset of the response was delayed by 1-2h, vs. WT, and the maximum level attained was about half that attained in WT mice. For each ligand, the later-phase, 4-8h, of the iCa response induced in PD mice was similar to that observed in WT mice. The results suggest that PTHR1 phosphorylation, and potentially internalization, play key roles in mediating the rapid-phase of the iCa response induced by PTH ligands. The apparent discrepancy between the PTH-induced increases in blood iCa and cAMP, as revealed in PD mice, may be a manifestation of altered receptor internalization responses. The acute, calcium-homeostatic actions of PTH thus appear to involve multi-phasic and multi-component mechanisms of receptor signaling and trafficking, which may differ in different target tissues.

Disclosure of Financial Relationships: Employer: Chugai Pharmaceutical Co., Ltd.

**F-FC202**

**History of Parathyroidectomy and Survival among Hemodialysis Patients with Secondary Hyperparathyroidism: Results from a Nationwide Registry in Japan** Hirotaka Komaba,[1] Masatomo Taniguchi,[2] Atsushi Wada,[2] Masafumi Fukagawa.[1] *[1]Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine, Isehara, Kanagawa, Japan; [2]Patient Registration Committee, Japanese Society for Dialysis Therapy, Tokyo, Japan.*

**Background.** Parathyroidectomy (PTx) for severe secondary hyperparathyroidism (SHPT) can dramatically reduce PTH levels and ameliorate clinical symptoms related to the disease. However, the effect of PTx on future mortality has not been adequately investigated.

**Methods.** We compared 1-yr all-cause and cardiovascular (CV) mortality between patients who underwent PTx for severe SHPT and those who did not undergo PTx despite severe SHPT (>500 pg/ml), using data on 108,151 patients on hemodialysis on December 31, 2004 obtained from the nationwide registry of the Japanese Society for Dialysis Therapy (JRDR-08001). Analysis was done using multivariate Cox regression model, adjusted for patient demographics, body mass index, serum albumin, calcium, phosphate, CRP, history of CV disease, Kt/V, dialysate calcium, and use of active vitamin D.

**Results.** Among patients without a history of PTx, higher intact PTH levels were associated with an increased risk for all-cause and CV mortality. By contrast, patients with a history of PTx (N = 5104) had a significantly lower risk for all-cause and CV mortality as compared with those who had not underwent PTx despite severe SHPT (N = 5455) (hazard ratio [HR], 0.58 [95% CI, 0.42 to 0.80] and 0.46 [95% CI, 0.29 to 0.74], respectively). The trend for lower non-CV mortality was not statistically significant (HR, 0.70 [95% CI, 0.45 to 1.08]). We also compared survival in a subcohort of patients who did (N = 3041) and did not undergo PTx despite severe SHPT (N = 3041) individually matched by patient demographics. Compared with the matched controls, patients with a history of PTx had a significantly lower risk for all-cause and CV mortality (HR, 0.68 [95% CI, 0.54 to 0.85] and 0.61 [95% CI, 0.44 to 0.85], respectively), but not significant for non-CV mortality (HR, 0.76 [95% CI, 0.55 to 1.04]).

**Conclusion.** PTx for severe SHPT may reduce the risk for all-cause and CV mortality among patients undergoing hemodialysis.

Disclosure of Financial Relationships: nothing to disclose

**F-FC203**

**Serum Mineral Metabolism Markers and Risk of Incident Hip Fracture: The Cardiovascular Health Study** Cassianne Robinson-Cohen,[1] Ronit Katz,[2] Ian H. de Boer,[3] David Siscovick,[4] Bryan R. Kestenbaum.[3] *[1]Epidemiology, University of Washington, Seattle, WA; [2]Biostatistics, University of Washington, Seattle, WA; [3]Nephrology, University of Washington, Seattle, WA; [4]Medicine and Epidemiology, University of Washington, Seattle, WA.*

Mineral metabolism disturbances are highly prevalent among people with chronic kidney disease. Vitamin D deficiency and hyperparathyroidism are associated with fractures in the general population. However, associations of these disturbances with long-term fracture risk are unknown in the setting of CKD. We evaluated associations of intact parathyroid hormone (PTH), 25-hydroxyvitamin D (25(OH)D) and bone-specific alkaline phosphatase (BAP, an enzyme anchored to osteoblasts that indicates bone formation), with incident hip fracture among older adults with and without CKD. We studied 2284 ambulatory participants from the Cardiovascular Health Study (mean age 74 years) who were free of known cardiovascular disease and hip fracture at baseline; 413 subjects (18.1%) had CKD defined by an estimated glomerular filtration rate <60 ml/min/1.73m2. We measured serum 25(OH)D, PTH, and BAP concentrations from previously collected samples. We used Cox proportional hazards models to evaluate associations of mineral markers with incident hip fracture. There were a total of 242 incident hip fractures during up to 14 years of follow-up (9.7 fractures per 1000 person-years). After adjustment for traditional fracture risk factors and kidney function, 25(OH)D levels <15 ng/ml were associated with increased risks of incident hip fracture among participants who had CKD (HR 2.8, 95% CI 1.2, 7.0) and those who did not (HR 1.6, 95% CI 1.0, 2.4); p-for-interaction = 0.28. PTH concentration >65pg/ml was associated with a trend to increased risk of hip fracture only among participants who had CKD (HR 1.8, 95% CI 0.8, 4.0). BAP was not associated with hip fracture. Lower 25(OH)D levels are associated with increased long-term fracture risk among older adults with CKD, suggesting that supplementation with substrate forms of vitamin D may reduce fracture risk in this population.

Disclosure of Financial Relationships: nothing to disclose

**F-FC204**

**Molecular Mechanism of Vitamin D Regulation of Nephrin Gene Expression** Dilip K. Deb, Zhongyi Zhang, Youli Wang, Yan Chun Li. *Medicine, The University of Chicago, Chicago, IL.*

Prolonged diabetes often damages the glomerular filtration barrier leading to proteinuria. Nephrin plays a key role in maintaining the structure of the slit diaphragm, and reduction of nephrin leads to the development of proteinuria. Our previous studies demonstrated that 1,25-dihydroxyvitamin D (1,25-VD) has renoprotective activity and is able to up-regulate nephrin expression. The aim of this study is to understand the molecular mechanism. Nephrin mRNA expression was down-regulated in podocytes exposed to high glucose (HG, 30 mM), but 1,25-VD treatment increased nephrin expression by 8-10 fold over the basal level. Vitamin D up-regulation of nephrin was confirmed by immunostaining with anti-nephrin antibodies in podocyte cultures and in kidney sections from diabetic mice

treated with vitamin D analog. In mouse nephrin gene promoter, a putative VDRE was identified at -313 bp. EMSA showed that this putative VDRE could be bound by VDR/RXR. ChIP assays with anti-VDR antibody confirmed that VDR binds to the VDRE in podocytes. Transfection of podocytes with a luciferase reporter construct containing the nephrin promoter region from -0.427 to -0.127 kb showed induction of luciferase activity upon 1,25-VD treatment, but luciferase activity was not induced by 1,25-VD with the construct containing a mutant VDRE. Together these data demonstrate that 1,25-VD up-regulates nephrin gene expression in podocytes by acting on a VDRE in the nephrin promoter. Up-regulation of nephrin contributes to the renoprotective activity of vitamin D against renal injury in diabetes.

Disclosure of Financial Relationships: nothing to disclose

## F-FC205

**National Kidney Foundation Education Survey Results** Michael J. Choi,[1] Kerri L. Cavanaugh,[2] Chester H. Fox,[3] Mark A. Perazella,[4] Sandeep S. Soman,[5] Bernard G. Jaar.[1] *[1]Johns Hopkins University; [2]Vanderbilt University; [3]State University of New York at Buffalo; [4]Yale University; [5]Henry Ford Hospital.*

The National Kidney Foundation (NKF) is recognized for disseminating best available evidence-based clinical practice guidelines. The purpose of the Kidney Disease Outcomes Quality Initiative (KDOQI) Education Committee is to disseminate of kidney disease guidelines by determining educational needs, developing effective tools and identifying barriers to guideline implementation. The KDOQI Education Committee conducted an online survey to physicians and allied health professionals to assess use and opinions regarding current KDOQI guidelines, NKF educational tools, and Clinical Action Plans. Responders were asked demographic questions including profession (physician, physician extender, nurse, social worker, dietitian, pharmacist, dialysis technician, or other) and specialty. **Results:** A total of 607 nephrology providers completed the survey. Main results are outlined in the table. More non-physicians acknowledged using KDOQI guidelines in practice ($p<0.001$) and also reported satisfaction with the current density of information ($p=0.001$) than physicians/extenders. Few respondents were aware of the NKF Clinical Action Plans. **Conclusion:** This is the first national survey to assess the impact and utilization of the KDOQI guidelines by all health care professionals. Specific barriers to implementation, especially among physicians, need to be identified and addressed. A prospective study is needed to determine if improved dissemination and application of KDOQI guidelines translate into better clinical care of CKD patients.

Survey results

| | All Professionals (n=607) | Physicians and Extenders (n=251) | Non-physicians (n=356) |
|---|---|---|---|
| Use Guidelines | 96% | 84% | 97% |
| Look up Guidelines <once a month | 53% | 50% | 54% |
| Which Guidelines have you used most in past 3 months? | Bone-73% Anemia-67% HD Adequacy-49% | Bone-74% Anemia-67% CKD Classification-57% | Bone-73% HD Adequacy-53% Anemia-52% |
| Guidelines are presented with correct amount of information | 64% | 56% | 69% |
| Guidelines are easily adapted to my practice | 69% | 69% | 69% |
| Aware of NKF online Clinical Action Plans | 26% | 32% | 21% |

Disclosure of Financial Relationships: Research Funding: Otsuka Pharmaceuticals; Honoraria: ASN Renal Weekends – co-chair for Washington DC and speaker – Clinical Nephrology, Mid Atlantic Young Investigator's Forum co-chair; Scientific Advisor: Deputy editor, Advances in Chronic Kidney Diseases, National Kidney Foundation KDOQI education committee vice-chair.

## F-FC206

**Chronic Kidney Disease Education in the Primary Care Setting Improves Disease Prevention, Detection and Management** Cynthia R. Christiano, Melanie I. Hames, Tejas P. Desai, Hsiao L. Lai, Rachel Ward, Jessica Caroline Collins, Matthew Hojatzadeh, Karen R. Parker, Paul Bolin, Jr. *Division of Nephrology and Hypertension, Brody School of Medicine at East Carolina University, Greenville, NC.*

**Background**: The increasing prevalence of chronic kidney disease (CKD) and risk factors for CKD in the southeastern U.S. demands strategic efforts for addressing prevention and management. We hypothesize that an educational intervention targeting primary care providers will result in improved management of patients at risk or diagnosed with CKD, with the ultimate goal of reducing the number of patients who progress to end-stage renal disease (ESRD).

**Methods:** A federally funded primary care clinic was selected for educational intervention addressing kidney disease screening, prevention, and management. Nephrologists from a regional academic medical center provided educational sessions covering topics based on KDOQI clinical practice guidelines to clinic staff. Site visits were conducted weekly for 6-months and were tapered over a 3-year period. Outcome measures consisted of kidney-related prescription filling and lab ordering practices. Relevant prescriptions and laboratory tests were categorized based on their role in CKD treatment and detection. Electronic medical records were queried for data regarding the outcome measures over 18-months. Descriptive statistics were used to highlight trends in prescription filling and lab ordering practices. Paired sample t-tests were used to compare the mean filling and ordering rates between the pre-intervention and post-intervention periods.

**Results:** Preliminary data indicated a statistically significant ($p<0.05$) increase in prescription filling and in lab tests ordered for anemia, vitamin D and diabetes. Further analysis is needed to determine the impact of confounding factors on the selected outcome measures. Longitudinal observation is needed to demonstrate whether the impact on CKD prevention and management is sustainable.

Disclosure of Financial Relationships: nothing to disclose

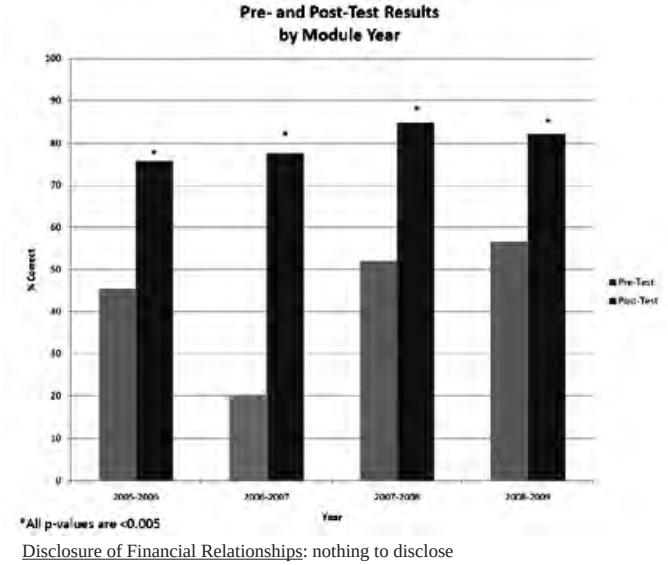
## F-FC207

**Effective Chronic Kidney Disease Training Tool for Physicians** Michelle M. Estrella, Stephen Sisson, Jennifer Roth, Michael J. Choi. *Medicine, Johns Hopkins School of Medicine, Baltimore, MD.*

**Purpose:** To determine if an online module used by several U.S. training programs improved knowledge in recognition and management of chronic kidney disease (CKD). **Methods:** An online education intervention was administered yearly from July 1, 2006 to June 31, 2009 to participating U.S. internal medicine training programs. To assess baseline skills in CKD recognition and management, participants completed a pre-test. A didactic module reviewing the diagnoses and management of CKD based on the National Kidney Foundation K/DOQI guidelines and more than 30 publications was completed. The effectiveness of this module was evaluated by a post-test made available only if the didactic module was completed. Participants who did not complete the module were excluded. Pretest scores by level of training were compared using the $X^2$ test while the pre- and post-test scores were compared by paired t-tests. Two-sided P-values were calculated. **Results:** The number of participating programs and residents increased annually (see Table 1). The baseline knowledge of CKD was poor and did not differ significantly by training level (Figure 1A). Completion of the online didactic module led to an average improvement of 25-33% each year (Figure 1B). **Conclusion:** Physicians are unfamiliar with the diagnosis and management of CKD. Web-based training can be effective in the education of physicians on CKD recognition and management. A prospective study is needed to determine if knowledge gained from such an intervention translates to improved clinical care of CKD patients

Table1. Study population

| | 2005-2006 | 2006-2007 | 2007-2008 | 2008-2009 |
|---|---|---|---|---|
| Programs, n | 23 | 63 | 80 | 88 |
| PGY1, n | 161 | 446 | 503 | 698 |
| PGY2, n | 176 | 399 | 783 | 482 |
| PGY3, n | 172 | 388 | 365 | 429 |
| Attending, n | 30 | 38 | 29 | 42 |




Disclosure of Financial Relationships: nothing to disclose

## F-FC208

**Validating Peer Chart Audit in a Nephrology Fellow Continuity Clinic Setting** Leslie Thomas, Suzanne Norby. *Nephrology & Hypertension, Mayo Clinic, Rochester, MN.*

**Background:** Peer chart audit is a method of assessing the Practice-Based Learning and Improvement competency used in our nephrology training program to evaluate the ability of nephrology fellows to identify complications of chronic kidney disease (CKD). However, the accuracy of data collection by fellows had not been systematically evaluated. The purpose of this study was to determine the accuracy of data collected by nephrology fellows performing peer chart audits of continuity clinic patient records. **Methods:** Each of 12 nephrology fellows was assigned to review medical records of 10 consecutive non-dialysis patients with CKD stage 3, 4, or 5 cared for by another fellow. Seven data points were abstracted from laboratory data and visit notes: presence of a medication list and BP measurement; whether a plan was documented for BP >130/80 mmHg; whether Hgb and PTH were measured within 6 months or at the time of the visit; whether iron studies were checked if Hgb <11 g/dL; and whether total 25(OH)D2+3 levels were checked if PTH > normal. Chart audits were then repeated by the program director. **Results:** Of 120 patient records reviewed, 3 patients treated with dialysis were excluded. Collections yielded 819 data points from 117 patient records. Overall, 91% of fellow data points were accurate

although individual fellow accuracy ranged from 77-100%. Mean accuracy did not differ among 1st year fellows (90%), 2nd year fellows (93%), and 3rd year fellows (89%). Accuracy varied depending on the type of data abstracted. Accuracy of data collected on documentation of a medication list (99%) and BP measurement (100%) as well as recent Hgb measurement (100%) was high. Data abstraction for presence of a plan for BP >130/80 and recent PTH measurement were each 92% accurate. The least valid data points were determination of whether iron studies were checked if Hgb <11 g/dL and whether total 25(OH)D2+3 were checked if PTH > normal, with accuracies of 83% and 70% respectively. **Conclusions:** Use of peer chart audit appears to yield valid results (91% mean accuracy), and validity was not affected by year of training. Validity differed among the parameters assessed, with accuracy rates ranging from 70% to 100%.

Disclosure of Financial Relationships: nothing to disclose

**F-FC209**

**A Description of Nephrologist Training, Beliefs, and Practices from the National Nephrologist Dialysis Practice Survey (2010)** Dorian R. Schatell,[1] Jennifer L. Bragg-Gresham,[1] Rajnish Mehrotra,[2] Joseph R. Merighi,[3] Beth Witten.[1] *[1]Medical Education Institute, Inc., Madison, WI; [2]Los Angeles Bio Medical Research Unit, CA; [3]Boston University, MA.*

Large variation exists in nephrologists' beliefs, training, and practices for treating dialysis patients. The National Nephrologist Dialysis Practice Survey was sent to all ASN member nephrologists to better understand and quantify this variation. One goal was to elucidate areas (such as Nephrology Board exam items, training, and modality preference) where educating nephrologists could lead to better care of dialysis patients.

A 1-page fax-back survey was mailed to 6,800 U.S. nephrologists and 669 responded. An incentive of $2 per survey was donated to the American Kidney Fund. Ninety-six percent (629) of responding nephrologists reported that clinical practice as their major focus; these respondents were used in the analysis. All analysis was done using SAS 9.2.

Most (79.8%) respondents were male. Other characteristics and responses from this sample were as follows:

| | | | |
|---|---|---|---|
| *Years in nephrology practice (%):* | | *Received additional training in dialysis after completing fellowship (%)* | 16.0 |
| <4 | 12.4 | | |
| 5 - 10 | 24.0 | | |
| 11 - 20 | 25.0 | *To prepare NEW nephrologists for dialysis, how many Board exam items should focus on:* | |
| 21+ | 38.6 | Hemodialysis | 20 |
| | | PD | 12 |
| *% of week dealing with these concerns:* | | CKD | 24 |
| Acute kidney injury | 18.8 | Diabetes | 12 |
| Fluid & electrolyte disorders | 14.3 | | |
| Predialysis CKD | 31.2 | | |
| Dialysis (all types) | 28.2 | *Dialysis Modality Nephrologists would choose for themselves if they had kidney failure and a 5-year wait for transplant (%):* | |
| Transplant | 7.5 | | |
| | | Standard *in-center* HD | 6.4 |
| *How prepared did nephrologists feel to care for patients after passing Nephrology Boards (%)?* | | Nocturnal *in-center* HD | 3.0 |
| | | PD | **45.4** |
| Not at all | 2.3 | Daily *home* HD | **24.8** |
| Somewhat | 35.8 | Nocturnal *home* HD | **17.5** |
| Very well | 61.1 | Standard *home* HD | 2.9 |

Approximately 60% of nephrologists' work week was spent on dialysis and pre-dialysis CKD care. 38.1% of respondents felt somewhat or not at all prepared to care for dialysis patients after passing the Board exam; respondents recommended, on average, that a total of 68 items (28%) of Board exam items cover clinical CKD or dialysis care. Just 6.4% of nephrologists stated a preference for standard in-center HD for themselves if they had kidney failure and expected to wait 5 years for a transplant. PD was their preferred modality, though only 7.1% of US patients currently receive it (US Renal Data System: USRDS 2009 Annual Data Report. The National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2009).

Disclosure of Financial Relationships: Employer: Medical Education Institute, Inc.; Honoraria: The Medical Education Institute (not me personally) has received honoraria from the ANNA, NKF, Annual Dialysis Conference, ESRD Networks, and others for my speaking engagements; Patent: The Medical Education Institute (not me personally) built the KDQOL COMPLETE scoring tool for the KDQOL-36 survey, and receives licensing fees for its use; Scientific Advisor: Member – Performance Excellence and Accountability in Kidney Care (PEAK) Technical Guidance & Curriculum Panel (2009 to present), member – Dialysis Outcomes and Practice Patterns (DOPPS) Patient Quality of Life Task Force (2008 to present), member – National Kidney Disease Education Program (NKDEP) steering committee (2007 to present).

**F-FC210**

**Correlates with Nephrologist Beliefs about Patient Job Retention from the National Nephrologist Dialysis Practice Survey (2010)** Jennifer L. Bragg-Gresham,[1] Dorian R. Schatell,[1] Rajnish Mehrotra,[2] Joseph R. Merighi,[3] Beth Witten.[1] *[1]MEI, Inc., Madison, WI; [2]Los Angeles Bio Medical Research Unit, CA; [3]Boston University, MA.*

The Medicare ESRD Program was funded in 1972 based on the promise that dialysis patients would be employed, tax-paying citizens. This promise remains largely unfulfilled. The ESRD Networks reported that only 23% of prevalent patients ages 18-54 were employed in 2007. Nephrologists' knowledge of and attitudes toward maintaining patient employment has rarely been studied. This study was conducted to provide insights that may help to improve patient job retention.

The National Nephrologist Dialysis Practice Survey is a 1-page fax-back survey and was mailed to 6,800 U.S. nephrologists with 669 respondents. An incentive of $2 per survey was donated to the American Kidney Fund. After limiting the responses to Nephrologists in clinical practice (n=629) and removing 40 surveys with missing data, a total of 589 responses were analyzed. Logistic regression was used to assess the association between nephrologists' beliefs about job retention and: gender, years of practice, patient modalities treated, knowledge of income replaced by Social Security disability insurance (SSDI), and who requests disability forms. Analyses were done using SAS 9.2.

Just over 80% of respondents were male, and more than 99% treat in-center hemodialysis (HD) patients, either solely or in addition to other modalities. Only 6.5% of respondents knew that SSDI replaces on average 35% of prior income.

| Measure | N (%) | Agree job retention is "Very Important" (%) | Odds Ratio (p) | |
|---|---|---|---|---|
| | | | Unadjusted | Adjusted |
| *Gender (F/M)* | 117 (19.9) | 76.1 / 63.1 | 1.86 (**0.009**) | 2.08 (**0.004**) |
| *Practice Years:* | | | | |
| < 4 years | 75 (12.7) | 49.3 | 1.00 (ref) | 1.00 (ref) |
| 5 to 10 years | 143 (24.3) | 67.1 | 2.10(**0. 011**) | 1.85 (**0.04**) |
| 11-20 years | 149 (25.0) | 66.7 | 2.05(**0. 013**) | 1.89 (**0.03**) |
| 21 + years | 231 (38.0) | 69.6 | 2.36 (**0.002**) | 2.33 (**0.003**) |
| *Nephrologist sees patients with Treatment Modality (Y/N):* | | | | |
| Daily Home HD | 240 (40.7) | 68.3 / 63.9 | 1.04 (0.26) | 1.15 (0.46) |
| Nightly Home HD | 48 (8.1) | 66.7 / 65.2 | 1.01 (0.88) | 0.97 (0.93) |
| PD (all) | 498 (84.6) | 67.1 / 58.2 | 1.09 (0.10) | 1.27 (0.34) |
| Standard Home HD | 139 (23.6) | 67.3 / 65.1 | 1.03 (0.59) | 1.06 (0.79) |
| In-center HD | 585 (99.3) | 65.5 / 100.0 | 0.71 (0.15) | 0.00 (na) |
| Nightly in-center HD | 126 (21.4) | 66.7 / 65.4 | 1.01 (0.80) | 0.97 (0.91) |
| *Knew SSDI $ (Y/N)* | 38 (6.5) | 79.0 / 64.8 | 2.04 (0.08) | 1.84 (0.15) |
| *Disability forms requested by:* | | | | |
| Don't sign | 71 (12.1) | 64.8 | 0.56 (0.24) | 0.65 (0.40) |
| Patient | 333 (56.5) | 65.1 | 0.57 (0.21) | 0.55 (0.19) |
| Social Worker | 155 (26.3) | 64.2 | 0.57 (0.22) | 0.60 (0.27) |
| Other | 30 (5.1) | 76.7 | 1.00 (ref) | 1.00 (ref) |

Female nephrologists and nephrologists with more years in practice had significantly higher odds of seeing job retention as important; dialysis modalities seen and who requested disability forms were not significant. Teaching the value of job retention during nephrologist training may help dialysis patients maintain their income and lifestyle.

Disclosure of Financial Relationships: nothing to disclose

**F-FC211**

**Kidney Disease Screening and Awareness Program (KDSAP): A Model for Potentially Increasing Workforce in Nephrology** Eric C. Shieh,[1] Jingshing Wu,[1] Albert C. Yeh,[2] Ang Li,[1] Li-Li Hsiao.[3] *[1]Harvard College, Cambridge, MA; [2]Harvard Medical School, Boston, MA; [3]Renal Division, Brigham and Women's Hospital, Boston, MA.*

The high prevalence of chronic kidney disease (CKD) in underserved populations and the shortage of nephrologists nationwide create a gap in health care provisioning that demands attention. Solutions must aim at raising community awareness of kidney disease and increasing the renal health care workforce. Kidney Disease Screening and Awareness Program (KDSAP), a kidney health initiative started by Harvard University undergraduates, offers a promising solution. With the key objectives of providing free health screenings, increasing awareness of CKD, and providing mentorship, career, and leadership opportunities for college students, KDSAP recruits undergraduate volunteers to organize health screenings free of charge to at-risk populations. Since its founding in Nov. 2007, KDSAP has screened 1000+ people in 13 Greater Boston neighborhoods. Under the guidance of nephrologists and in collaboration with medical and high school students, KDSAP provides a needed service to local communities, while offering college students an opportunity to explore medicine and the field of nephrology. The study's aim was to assess KDSAP's potential to impact the field of nephrology from a recruitment perspective. A one-year follow-up survey completed by 27 of KDSAP's current membership of 36 students indicated that the program significantly increased students' exposure and knowledge of nephrology ($p<0.008$, 0.001, respectively). KDSAP also had a strong influence on students' perceptions of nephrology, medicine, research, public health, clinical practice, and serving the underserved. Personal interviews with members showed the program's highly positive influence on personal development in areas of leadership and teamwork. The results suggest that KDSAP can serve as a model organization to increase interest in nephrology among college students, develop student leadership skills, and enhance public awareness of CKD. Our hope is that the KDSAP model of service learning can be replicated in universities nationwide to increase workforce in nephrology and improve community renal health.

Disclosure of Financial Relationships: nothing to disclose

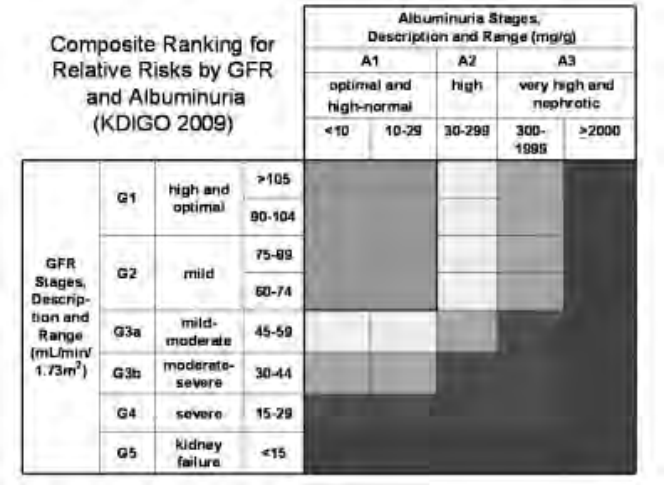
**F-FC212**

**Grouping eGFR and Albuminuria Stages into "Risk Categories" for CKD Clinical Practice Guidelines: Summary of Initial Results for the CKD Prognosis Consortium** Andrew S. Levey,[1] Ron T. Gansevoort,[2] Kunihiro Matsushita,[3] Brad C. Astor,[3] Josef Coresh,[3] Paul E. de Jong.[2] *[1]Nephrology, Tufts Medical Center, Boston, MA; [2]Nephrology, University Medical Center, Groningen, Netherlands; [3]Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

**Background:** Risk categories are often used to communicate risk and guide clinical management for chronic conditions. KDIGO initiated a collaborative meta-analysis and sponsored a Controversies Conference in October 2009 to examine the relationship of

eGFR and albuminuria to mortality and kidney outcomes. Based on analyses of 45 cohorts including 1,555,332 participants from general, high-risk and CKD populations, Conference attendees reached consensus to retain the current definition of CKD as GFR <60 ml/min/1.73 m² or ACR ≥30 mg/g, and to modify the classification by adding albuminuria stages, subdividing CKD stage 3, and emphasizing clinical diagnosis in addition to stage. Prognosis of CKD could then be assigned based on the clinical diagnosis, stage, and other key factors relevant to specific outcomes.

**Results:** Relative risks for all-cause mortality, cardiovascular disease (CVD) mortality, ESRD, AKI, and CKD progression were adjusted for demographic factors, CVD risk factors and history of CVD, and were ranked from lowest to highest. The rank was then grouped into four risk categories. Distribution of eGFR and albuminuria stages among risk categories was similar across five outcomes and three populations. The composite average ranking is shown below. Categories are identified by color (dark red for data from CKD cohorts).




**Conclusions:** The usefulness of risk categories in communication of risk and clinical management in CKD should be evaluated. Other methods of aggregating risks across outcomes and including additional outcomes of CKD should be considered.

Disclosure of Financial Relationships: Research Funding: Amgen (clinical trial agreement); Scientific Advisor: American Journal of Kidney Disease (NKF).

## F-FC213

**Differences in the Prevalence of Concurrent CKD Complications by Estimated GFR Using the CKD-EPI and MDRD Study Equations: The National Health and Nutrition Examination Survey III and 1999-2006** Lesley A. Stevens,[1] Josef Coresh,[2] Andrew S. Levey,[1] Paul Muntner.[3] *[1]Tufts Medical Center; [2]Johns Hopkins University; [3]University of Alabama at Birmingham.*

**Background** The CKD-EPI equation improves accuracy of estimation of measured GFR and mortality risk prediction compared to the Modification of Diet in Renal Disease (MDRD) Study equation. However, whether it better discriminates individuals with and without concurrent CKD complications is not well established.

**Methods** Using data from 30,528 participants, ≥ 20 years of age and eGFR 15-120 ml/min per 1.73 m² in the National Health and Nutrition Examination Survey 1988-1994 and 1999-2006, we compared the prevalence of anemia (hemoglobin < 12 g/dL for women, <13.5 g/dL for men), acidosis (bicarbonate < 22 mmol/L), hyperphosphatemia (phosphate ≥ 4.5 mg/dL) , hyperuricemia (uric acid > 8.3 mg/dl), hypoalbuminemia (albumin < 3.5 mg/dl) and hypertension ( ≥ 140/90 mmHg or antihypertensive medications) using eGFR categories from the CKD-EPI ($eGFR_{CKDEPI}$) versus the MDRD Study ($eGFR_{MDRD}$) equations .

**Results** Using $eGFR_{CKD-EPI}$ 39.1%, 26.5%, 8,4% and 3.6% participants with $eGFR_{MDRD}$ levels of 60-89, 45-59, 30-44, and < 30 ml/min/1.73m², respectively, were re-classified into higher $eGFR_{CKD-EPI}$ levels. Analogously, 1.6%, 0.4%, 1,5%, and 4.3% were re-classified into lower $eGFR_{CKD-EPI}$ levels. The table shows the prevalence of CKD complications in people with $eGFR_{MDRD}$ of 45-59 ml/min per 1.73 m² who were reclassified into a higher or lower $eGFR_{CKD-EPI}$ category. Results were consistent for reclassification from other categories.

**Conclusion** Compared to the MDRD Study equation, the CKD-EPI equation was better able to discriminate individuals with anemia, hyperuricemia, hypoalbuminemia and hypertension, but not hyperphosphatemia or acidosis.

Reclassification from $eGFR_{MDRD}$ 45-59 ml/min/1.73m²

| | eGFR -CKD-EPI ml/min/1.73m² | | | |
|---|---|---|---|---|
| | 60–89 | 45–59 | 30–44 | p-trend |
| Anemia, % | 7.7 | 13.9 | 34.7 | 0.008 |
| Hyperuricemia % | 4.5 | 9.1 | 28.1 | 0.003 |
| Hypoalbuminemia % | 0.8 | 2.6 | 6.3 | 0.013 |
| Hypertension % | 45.8 | 72.1 | 76.2 | <0.001 |
| Hyperphosphatemia% | 9.5 | 6.8 | 1.7 | 0.123 |
| Acidosis % | 10.2 | 6.4 | 1.8 | 0.063 |

Disclosure of Financial Relationships: Consultancy: Orexigen Therapeutic Inc. Research Funding: Gilead Inc.

## F-FC214

**NGAL Is a Differential Marker of Pediatric Nephrotic Syndrome and Its Severity** Michael R. Bennett, Kimberly Czech, Mark Mitsnefes, Prasad Devarajan. *Cincinnati Children's Hospital.*

Idiopathic nephrotic syndrome (NS) is the most common glomerular disorder of childhood. Pathology on invasive biopsy, including minimal change disease and focal segmental glomerulosclerosis (FSGS), remains the diagnostic method of choice for NS. Prognosis correlates with steroid responsiveness, from sensitive (SSNS) to resistant (SRNS). SRNS is the most common acquired cause of end stage renal disease (ESRD) in children. NGAL has shown great promise as an early diagnostic marker of AKI and has been demonstrated to be a powerful risk marker of CKD progression.

Objective: We set out to determine if urine NGAL can distinguish between patients with SRNS (FSGS), SSNS and healthy controls.

Methods: Urine and clinical data were collected from patients at Cincinnati Children's Hospital that were recently diagnosed with active nephrotic syndrome as well as healthy controls. Patients with a history of gross hematuria, active/recurrent UTI or nephrotic syndrome secondary to systemic disease were excluded. Urinary NGAL measurements were performed with a commercially available ELISA kit and normalized to urine creatinine.

Results: This study included subjects in the following three groups: biopsy-proven FSGS and steroid resistant clinical course (n=16), steroid sensitive clinical course (n=18), and normal controls (n=10). Median NGAL levels were calculated and subjected to Kruskal-Wallis One Way Analysis of Variance on Ranks and Dunn's multiple comparison test. Median NGAL was significantly ($p < 0.001$) higher in FSGS (114 ng/ml, IQR 25-571) than both SSNS (5.9 ng/ml, IQR 4.9-9.3) and healthy controls (6.5 ng/ml, IQR 4.9-9.1). NGAL was not different between SSNS and healthy controls. In FSGS patients, NGAL also demonstrated a significant ($p < 0.001$) negative correlation with glomerular filtration rate (GFR – R= -0.5), though NGAL was similar in SSNS whether patients were in relapse (n=8; NGAL 5.5 ng/ml, IQR 3.9-6.6) or remission (n=10; NGAL 7.8 ng/ml, IQR 4.8-12.6). Results did not change with NGAL corrected for urine creatinine.

Conclusion: Urine NGAL levels reliably differentiate SSNS from SRNS patients and correlate with disease severity in SRNS, but not with remission/relapse in SSNS.

Disclosure of Financial Relationships: nothing to disclose

## F-FC215

**Effecting Whole Population Improvement in Blood Pressure Control in CKD in the UK** Hannah Kilbride, Paul E. Stevens, Kushan Karunaratne, Jean Irving, Helen Hobbs, Richard W. C. Kingston, Christopher K. T. Farmer. *Kent Kidney Care Centre, East Kent Hospitals, Canterbury, Kent, United Kingdom.*

Introduction

The aims of implementation of national eGFR reporting and the inclusion of renal specific indicators in primary care incentive payments, the Quality and Outcomes Framework (QOF), were to promote identification of CKD and improved management of risk factors, especially hypertension.

Aim

To evaluate the effectiveness of renal indicators in the QOF on the management of hypertension in patients with CKD in primary care in the UK.

Methods

The study cohort comprised 7474 patients with stage 3-5 CKD identified from a primary care population of 36519 people with serum creatinine data 2 y pre- and 2 y post-QOF. Patients were separated into pre- and post-QOF groups (Pre-QOF: 1/4/2004-1/4/2006; Post-QOF: 1/4/2006-1/4/2008). Mean systolic (SBP) and diastolic (DBP) blood pressure (BP) together with antihypertensive medication were analysed in each group.

Results

Mean age was 74.5 y, 62.1% were female. 78.6% (n=5871) of the cohort were hypertensive. Diabetes mellitus and cardiovascular disease was present in 10.9% (n=811) and 16.9% (n=1264) of patients respectively. The table details BP control and prescribed antihypertensives.

| | All (n=7474) | | Hypertensive (n=5871) | |
|---|---|---|---|---|
| | Pre-QOF | Post-QOF | Pre-QOF | Post-QOF |
| Mean SBP (mmHg) | 139 ± 29 | 137 ± 25* | 147 ± 14 | 140 ± 22* |
| Mean DBP (mmHg) | 76 ± 16 | 75 ± 14* | 79 ± 8 | 76 ± 12* |
| ACE-I/ARBs (n,%) | 3766 (50) | 4697 (63)† | 3766 (64) | 4242 (72)† |
| Diuretics (n,%) | 630 (8) | 2075 (28)† | 630 (11) | 1712 (29)† |
| Betablockers (n,%) | 394 (5) | 1441 (19)† | 394 (7) | 1135 (19)† |
| Ca channel blockers (n,%) | 408 (5) | 1244 (17)† | 408 (7%) | 1059 (18)† |

* p<0.001, Student's T-Test. †p <0.001, Chi-squared test. Hypertension = BP>140/85 mmHg or on antihypertensive medication (during the pre-QOF period)

Conclusion

Population BP control markedly improved following introduction of renal indicators in the QOF, associated with a significant increase in the use of antihypertensives. Longer term follow up will establish whether or not this translates to improved outcomes in terms of progression of CKD, cardiovascular disease and mortality.

Disclosure of Financial Relationships: nothing to disclose

**F-FC216**

**Vitamin D Deficiency Is Associated with Renal Function Decline and with Circulating Markers of Bone and Mineral Metabolism in Chronic Kidney Disease Patients** Pablo A. Urena,[1] Marie Metzger,[2] Pascal Houillier,[3] Alexandre Karras,[3] Benedicte Stengel,[2] Marc C. Froissart.[2,3] *[1]Clinique du Landy, Saint Ouen, France; [2]INSERM U1018, Villejuif, France; [3]HEGP, Paris, France.*

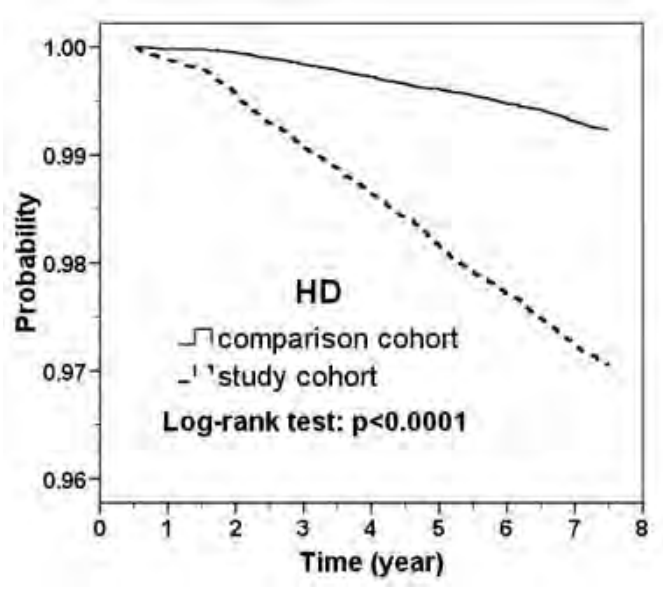
Several epidemiological studies have pointed out the high incidence of vitamin D deficiency in CKD patients. However, none of these studies have assessed the factors associated with serum vitamin D levels using measured glomerular filtration rate (mGFR) in CKD patients, nor in an European population of CKD patients. We therefore used data from the NephroTest cohort, including 1026 adult patients with nondialysis-dependent CKD stages 1 to 5 and free from vitamin D supplementation, to assess occurrence and determinants of vitamin D deficiency (<15 ng/ml), as well as its association with circulating markers of bone and mineral metabolism. GFR was measured by the renal clearance of 51Cr-EDTA. As mGFR decreased from ≥60 ml/mn/1.73 m2, to 45-59, 30-44, 15-29 and <15, the percentage of vitamin D deficiency progressively increased from 28% to 39%, 39%, 46%, and 51%, respectively. The percentage of vitamin D deficiency in CKD stages 1-2 was twice as high that of a referent Parisian population. Obesity, African origin, diabetes, macroalbuminuria, hypoalbuminemia, systolic hypertension, and winter season were strongly associated with vitamin D deficiency. After ajusting for age, sexe and the above confounders, the OR(95%CI) for vitamin D deficiency significatively increased from 1.4(0.9-2.3) to 1.4(0.9-2.1), 1.7(1.1-2.7), and 2.0(1.1-3.7) with decreasing mGFR from 45-59, 30-44, 15-29, and <15 as compared with ≥60 ml/mn/1.73 m2. After excluding patients using vitamin D analogs, those with deficiency were at higher risk of ionized hypocalcemia, hypocalcitriolemia, secondary hyperparathyroidism, and high CrossLaps® levels, with OR of 2.6(1.2-5.9), 1.8(1.3-2.4), 2.8(2.0-3.9), 1.6(1.0-2.6), respectively, independent of age, sex, center, and mGFR. In conclusion, in this cohort of European CKD patients, there was a high prevalence of vitamin D deficiency, which was independently associated with mGFR levels and with higher risk of abnormalities in circulating markers of mineral metabolism.

Disclosure of Financial Relationships: nothing to disclose

**F-FC217**

**The Risk for Advanced Chronic Kidney Disease in Patients with Heart Diseases: A 7-Year Follow-Up in a Cohort Study in Taiwan** Jiung-Hsiun Liu,[1] Chiu-Ching Huang,[1] Shih-Yi Lin,[1] Chung-Lin Hsieh,[1] Fung-Chang Sung.[2] *[1]Divison of Nephrology and Kidney Institute, Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan; [2]Department of Public Health, China Medical University, Taichung, Taiwan.*

The incidence of chronic kidney disease (CKD) is constantly increasing in the world. There is limited information of incidence of new CKD onset with a longitudinal follow-up in patients with heart diseases. This study investigated the risk of developing CKD among patients with heart disease. From universal insurance claims data, we retrospectively identified a cohort of 26005 insured people with newly diagnosed heart disease and 52010 people with no such disease both in the 2000-2001 claims. We observed prospectively both cohorts until the end of 2007 to measure CKD incidences in both cohorts and hazard ratios (HRs) by comparison of event rates. The incidence of CKD in the cohort with heart disease was 4.1 times greater than that in the comparison cohort (39.5 vs. 9.65 per 10,000 person-years). However, the HR changed into 2.37 (95% confidence interval (CI) = 2.05 - 2.74) in the multivariate Cox proportional hazard model after controlling for sociodemographic characteristics and comorbidities. Compared with individuals aged < 40 years, the HRs for CKD ranges 2.70 to 4.99 in other age groups. Significant estimated relative risks of CKD observed in heart disease patients independently associated with hypertension (HR=2.26, 95% CI = 1.94 - 2.63) and diabetes mellitus (HR=2.44, 95% CI = 2.13 - 2.80), but not with hyperlipidemia (HR=1.13, 95% CI =0.99-1.30). This population study provides strong evidence that patients with heart disease are at an elevated risk of developing CKD. Hypertension and diabetes mellitus are also comorbidities associated with increased risk of CKD independently.




Disclosure of Financial Relationships: nothing to disclose

**F-FC218**

**Novel Risk Factors for Peripheral Arterial Disease (PAD) among Patients with Chronic Kidney Disease: Results from the CRIC Study** Jing Chen, Emile Mohler, Dawei Xie, Michael Shlipak, Raymond R. Townsend, Lawrence J. Appel, Dominic S. Raj, Akinlolu O. Ojo, Martin J. Schreiber, Louise Frances Strauss, Xin Wang, Jiang He, L. Lee Hamm. *Medicine, Tulane School of Medicine, New Orleans, LA.*

Patients with chronic kidney disease (CKD) have an increased risk of developing PAD compared to those with normal kidney function. We examined the cross-sectional association between novel risk factors and PAD among 3,747 participants in the Chronic Renal Insufficiency Cohort (CRIC) study. CKD patients aged 21 to 74 years with an estimated glomerular filtration rate (eGFR) of 20-70 mL/min/1.73 $m^2$ were recruited from 7 clinical centers in the US. PAD was defined as an ankle-brachial index <0.9 or history of arm or leg revascularization. Compared to those without PAD, patients with PAD (n=694) were older (62.4 vs. 57.2 years, p<0.0001), more likely to be black (49.1% vs. 40.8%, p=0.0002) and less likely to have graduated from high school (70.5% vs. 81.8%, <0.0001). PAD patients were also more likely to be current smokers (19% vs. 12%, p<0.0001), to have hypertension (93.4% vs. 84.3%, p<0.0001) and diabetes (64.7% vs. 42.5%, p<0.0001), and less likely to be alcohol drinkers (28.1% vs. 40.7%, p<0.0001) or physically active (163.1 vs. 206.8 METs/week, <0.0001). PAD patients on average had lower levels of HDL-cholesterol (45.2 vs. 48.1 mg/dL, p<0.0001) and eGFR (38.4 vs. 44.0, ml/min/1.73$m^2$, p<0.0001), but higher pulse pressure (65.6 vs. 54.5, mmHg, p<0.0001). After adjustment for these traditional risk factors, the following novel risk factors were associated with PAD (odds ratios and 95% confidence interval for one standard deviation higher level): hemoglobin A1c (1.16, 1.04-1.29), HOMA-insulin resistance (1.11, 1.02-1.20), high-sensitivity C-reactive protein (1.11, 1.03-1.20), uric acid (1.14, 1.03-1.25), fibrinogen (1.19, 1.08-1.08), and cystatin C (1.37, 1.19-1.57). These data indicate that novel cardiovascular risk factors are associated with PAD independent of traditional risk factors among patients with CKD.

*Chen and Mohler contributed equally to this work

Disclosure of Financial Relationships: nothing to disclose

**F-FC219**

**Intima and Media Thickening, Calcification and Inflammation of Different Vascular Beds in Patients with Early and Late Stages of Renal Failure** Kerstin Benz,[1] Ildiko Varga,[2] Daniel Neureiter,[3] Kerstin U. Amann.[2] *[1]Pediatrics, University of Erlangen-Nürnberg, Erlangen, Germany; [2]Pathology, University of Erlangen-Nürnberg, Erlangen, Germany; [3]Pathology, Paracelsus Medizinische Privatuniversität, Salzburg, Austria.*

Introduction:

Death from cardiovascular causes is the major cause of death in chronic renal failure (CRF) patients. While these patients have a high prevalence of classical risk factors, there is increasing evidence that atherosclerosis is different in CRF than in non-renal patients. There is a lack of data concerning the morphological characteristics of vascular changes in patients with different stages of CRF. The present study compares changes in different arterial and venous vessels between patients with early and late stages of CRF and non-renal control patients.

Methods:

Fifty patients undergoing cardiac bypass surgery were divided into 3 groups: (i) 24 control patients (creatinine <1.3 mg/dl), (ii) 14 patients with moderate CRF (creatinine 1.3-2.0 mg/dl, CKD1/2), (iii) 12 patients with endsatge CRF (creatinine >2.0 mg/dl, CKD3-5). Different vessels (A. mammaria int., aorta, subcutaneous arterioles, V. saphena) were

analysed using morphometry and immunohistochemistry for markers of inflammation (CRP,CD40,CD154) followed by univariate analysis and correlation analyses.

Results:

Media thickness and inflammation score of A. mammaria int. and aorta were sig. higher in CKD3-5 patients than in controls. Significant vascular inflammation and aortic media calcification was already present in CKD1/2 whereas calcification of aortic intima and of V. saphena was significantly more pronounced in CKD3-5 patients than in and controls. Of note, CaxP product correlated well with markers of inflammation, but not with calcification itself.

Discussion:

Early stages of CRF are already associated with local upregulation of proinflammatory molecules in the vascular wall and calcification of aortic media. These findings point to the importance of microinflammation and may shed new light on the possibly overestimated role of the CaxP for vessel calcification in CRF .

Disclosure of Financial Relationships: nothing to disclose

## F-FC220

**Additive Value of Renal Risk Markers in the Decision To Initiate Cardiovascular Protective Treatment** Paul Smink, Hiddo Jan Lambers Heerspink, Ron T. Gansevoort, Stephan J. L. Bakker, Dick De Zeeuw. *Clinical Pharmacology and Nephrology, University Medical Center, Groningen, Netherlands.*

*Introduction*

Guidelines recommend treatment of individuals if 10 yrs risk for cardiovascular (CV) disease is >20%, based on traditional CV risk factors. However, renal risk markers albuminuria and eGFR also predict CV disease. Aim of this study was to assess the additive value of renal risk markers beyond classical, as well as recent novel CV risk factors.

*Method*

Data from the prospective community based cohort study PREVEND, comprising 8592 subjects, were used. Risk factors were measured at baseline. Endpoints, first occurrence of CV morbidity and all cause mortality, were measured during median 7.5 years follow up. Calculated risks were extrapolated to 10 years predictions.

*Results*

During follow-up 917 events occurred. Micoalbuminuria and eGFR <60 ml/min/1.73m$^2$ predicted CV disease (HR 3.61 (95%CI 3.02-4.31) and 2.41 (95%CI 1.79-3.26), respectively). Microalbuminuria identified subjects above the threshold for treatment, irrespective of normal or elevated classical or even novel (CRP and proBNP) CV risk factors.

| | Total N=8592 | alb≤30 mg/day N=8062 | alb>30 mg/day N=503 | eGFR>60 ml/min/ 1.73m2 N=8037 | eGFR≤60 ml/min/ 1.73m2 N=464 |
|---|---|---|---|---|---|
| Total | 10.5 | 9.3 | **31.1** | 9.9 | **22.6** |
| systolic BP | | | | | |
| ≤140 | 8.1 | 7.6 | **22.9** | 7.8 | 17.6 |
| >140 | **20.3** | 17.1 | **38.6** | 19.3 | **29.4** |
| Cholesterol | | | | | |
| ≤6.5 | 9.0 | 7.9 | **29.5** | 8.2 | **26.2** |
| >6.5 | 17.2 | 15.9 | **35.5** | 17.5 | 14.9 |
| BMI | | | | | |
| ≤30 | 9.8 | 8.7 | **30.3** | 9.3 | **20.6** |
| >30 | 15.3 | 13.1 | **33.2** | 13.9 | **30.1** |
| smoking habits | | | | | |
| non smoker | 5.5 | 4.8 | **23.4** | 5.1 | 14.4 |
| smoker | 13.0 | 11.5 | **33.4** | 12.2 | **26.4** |
| proBNP | | | | | |
| ≤125 | 8.3 | 7.7 | **21.3** | 8.2 | 12.9 |
| >125 | **29.7** | **24.9** | **60.9** | **27.7** | **41.7** |
| CRP | | | | | |
| ≤3 | 8.1 | 7.4 | **24.3** | 7.6 | 18.7 |
| >3 | 18.5 | 15.9 | **42.5** | 17.8 | **27.8** |

The additive value of low eGFR was limited to normal cholesterol ranges, BMI, smokers and elevated CRP levels.

*Conclusion*

Measurement of renal biomarkers, especially albuminuria, has additive value beyond the set of current classical and even novel biomarkers to identify those who have indication for CV protective treatment.

Disclosure of Financial Relationships: nothing to disclose

## F-FC221

**Elevated Urinary Markers of Glomerular, Proximal Tubular and Distal Tubular Injury during RAAS Inhibition Can Be Reduced by Dietary Sodium Restriction in Proteinuric Renal Disease** Maartje C. J. Slagman,[1] Ferdinand L. Nauta,[1] Femke Waanders,[1] Marc H. Hemmelder,[2] Gerjan Navis,[1] Ron T. Gansevoort,[1] Gozewijn Dirk Laverman.[1] *[1]Nephrology, UMCG, Groningen; [2]Nephrology, MCL, Leeuwarden, Netherlands.*

**Background:** High dietary sodium intake blunts the antiproteinuric response to renin-angiotensin-aldosterone-system inhibition (RAASi). This results in residual proteinuria (UP) and consequent proteinuria-induced tubulo-interstitial damage, allegedly elicited by proximal tubular uptake of leaked proteins. Dietary sodium restriction can reduce residual UP during RAASi. Whether this is accompanied by a reduction of urinary markers of glomerular, proximal tubular and distal tubular injury, is unknown.

**Methods:** In a cross-over RCT we treated 52 non-diabetic CKD patients with glomerular nephropathies (83% male, age 51±2 years, CrCl 83±6 mL/min) during two 6-week periods with lisinopril 40 mg/d (LIS), combined with a normal (189±8 mmol $Na^+$/d) and a low sodium diet (LS; 106±7 mmol $Na^+$/d; p<0.001), in random order.

**Results:** During LIS with a normal diet, UP was 1.7 [1.3-2.1] g/24h. Albuminuria and urinary excretion of glomerular markers (IgG, IgG4), proximal tubular markers (β2-microglobulin, NAG), and a distal tubular marker (H-FABP) were elevated compared to age- and sex-matched controls (n=52), and positively correlated with UP (UAE: ρ 0.98, p<0.001; IgG: ρ 0.76, p<0.001; IgG-4: ρ 0.59, p<0.001; β2MG ρ 0.42, p=0.003; NAG: ρ 0.66, p<0.001; H-FABP: ρ 0.58, p<0.001). Addition of LS diet to LIS significantly reduced UP (1.9-fold), albuminuria (2.0-fold), and all of the glomerular markers (13-23 fold), proximal tubular markers (1.2-2.9 fold), and the distal tubular marker (1.9 fold).

**Discussion:** In glomerular nephropathy patients on RAASi, UP is paralleled by elevated urinary markers of glomerular, proximal tubular and, remarkably, also of distal tubular injury. All markers decreased when dietary sodium restriction was added to RAASi. This suggests that not only the glomerulus and the proximal tubule, but also the distal tubule benefits from antiproteinuric intervention by sodium restriction.

Disclosure of Financial Relationships: nothing to disclose

## F-FC222

**Relationship between Waist Circumference and Pulse Pressure in Children: National Health and Nutrition Survey (NHANES) 1988-1994 Data** Gangadarshni Chandramohan,[1,2] Kamyar Kalantar-Zadeh,[1,2] Keith C. Norris,[3] Sheena Cecille Marie Go,[1] Dulcie Kermah.[3] *[1]Pediatric Nephrology, Harbor-UCLA Medical Center, Torrance, CA; [2]Los Angeles Bio-Medical Research Institute, Torrance, CA; [3]RCMI, Charles Drew University School of Medicine, Los Angeles, CA.*

Pulse pressure (PP) is an independent predictor of cardiovascular (CV) disorders. Prevalence of obesity has been increasing steadily over the past few decades along with various CV risk factors in children. This study evaluated the association between waist circumference (WC) and pulse pressure (PP) in children. Methods: High waist circumference: WC >75th percentiles for age and gender. Wide PP: 4th quartile PP. Results: We studied clinical and laboratory data in 4667 children in the third NHANES; 6 -17 years of age, 48% males, 74% White, 36% Hispanic, 36% Black, 11% obese and 27% had high WC. PP varied with age, gender and blood pressure. Mean PP was significantly high in children with high WC compared to those with normal WC (49cm Vs. 46cm, p=<.001). Odds ratio for wide PP was significantly increased in high WC, but not with obesity as assessed by body mass index (>90th percentile for age and gender).

Odd Ratio for Wide (4th Quartile) Pulse Pressure

| Variables | Odd Ratio | 95% Confidence Interval |
|---|---|---|
| Sex (Female=1.0) | | |
| Male | 1.4 | 1.1 - 1.8 |
| Races (White=1.0) | | |
| Black | 1.2 | 1.0 - 1.6 |
| Hispanic | 0.9 | 0.7 - 1.3 |
| Waist Circumference (Normal - 1.0) | | |
| High | 1.5 | 1. 2- 1.9 |
| BMI (<95th percentile = 1.0) | | |
| ≥95th percentile | 1.2 | 1.0 - 2.2 |
| Height (<50th percentile = 1.0) | | |
| ≥50th percentile | 0.9 | 0.7 - 1.2 |
| Blood pressure (Normal = 1.0) | | |
| *High | 2.5 | 1.6 - 3.8 |

* Systolic and/or Diastolic blood pressure > 90th percentile

Conclusion: There was a significant association between high WC and PP. Since WC has a better correlation to CV risk factors, the standard guidelines for routine physical examination in children might incorporate this parameter to identify those who are at risk for CV disease. Further studies needed to substantiate this finding and its long-term cardiovascular consequences in children as they age.

Disclosure of Financial Relationships: nothing to disclose

## F-FC223

**An Early Pregnancy Urinary Proteomic Fingerprint Accurately Predicts Later Pre-Eclampsia** Matt Hall,[1,2] Paul M. Bosio,[3] Jonathan Barratt,[1,2] Karen Molyneux,[2] Susan Carr,[1] Nigel J. Brunskill.[1,2] *[1]John Walls Renal Unit, Leicester General Hospital, Leicester, United Kingdom; [2]Department of Inflammation, Infection and Immunity, University of Leicester, Leicester, United Kingdom; [3]Department of Maternal Medicine, Sandwell and West Birmingham NHS Trust, Birmingham, United Kingdom.*

**Background**

Pre-eclampsia affects approximately 5% of pregnancies and is a leading cause of maternal and fetal morbidity and mortality worldwide. Abnormal placentation or placental insufficiency is central to the disease pathogenesis. Given that placentation is complete by 18 weeks gestation and proteinuria is a diagnostic criterion for the disease, we hypothesised that changes in the urinary proteomic profile early in pregnancy may be predictive of disease development.

**Methods**

We performed a prospective longitudinal study of urinary proteomics in pregnancy. Pregnant women were recruited prior to 20 weeks gestation from a high risk obstetric outpatient clinic. Urine samples were centrifuged to remove cellular debris within 30 minutes of micturition, and placed on ice until analysed by SELDI TOF MS the same day. Following delivery, patients were categorised as pre-eclampsia or normal pregnancy according to ISSHP 2001 criteria. A training sample of spectra obtained prior to 20 weeks

gestation were analysed between pre-eclampsia and normal pregnancies using an artificial neural network algorithm and multivariate non-linear regression. The model was validated by random sampling of 50 test datasets.

**Results**

Of 145 patients recruited to the study, 11 (7.6%) developed pre-eclampsia; 10 at >37 weeks gestation and one at 31 weeks. Spectral analysis of samples obtained prior to 20 weeks gestation identified a panel of 5 peaks which correctly predicted pre-eclampsia with 92% accuracy. Validation of the test datasets revealed a sensitivity of 87% and specificity of 82%.

**Conclusion**

Pre-eclampsia can be predicted by urinary proteomic profiling of spot urine samples in early pregnancy with high accuracy, before the development of any of the usual diagnostic criteria or symptoms. Early prediction of pre-eclampsia will allow focussed monitoring and tailoring of therapy to optimise maternal and fetal outcomes.

Disclosure of Financial Relationships: nothing to disclose

## F-FC224

**The Significance of Urinary Podocyte and Renin Angiotensin System in Preeclampsia Patients's Proteinuria** Gu Yong,[1] Chen Guixiang,[2] Niu Jianying.[2] *[1]Division of Nephrology, Huashan Hospital, Fudan University, Shanghai, China; [2]Division of Nephrology, The Fifth People Hospital of Shanghai, Fudan University, Shanghai, China.*

The mechanisms of proteinuria in preeclampsia is still enigmatic. We hypothesized that RAS may play an role in the regulation of podocyte injury in preeclampsia.

**Methods:** 14 preeclampsia and 14 gestational hypertension as well as 13 normal pregnant women were enrolled. Immunofluorescence microscopy using anti-podocalyxin(PCX) monoclonal antibody quantificated the urinary podocyte numbers. RAS components including Ang(1-7), Ang II and AGT in maternal serum and urine were quantified by enzyme-linked immunosorbent assay (ELISA). Urine albumin/creatinine ratio (ACR) was examined .The correlation among podocyturia, RAS components, and proteinuria were investigated. Receiver operating characteristic (ROC) curves were used to indicate reciprocal impact on sensitivity and specificity.

**Results:** Urinary levels of podocytes were significantly higher in preeclampsia than gestational hypertension [3.093(2.367, 5.658) vs. 1.405(0.71, 3.728) ] and normal pregnant women [0.523(0.012, 1.489)cells/ml of urine]. In preeclamsia group, prepartal urinary podocytes was higher than postpartum[3.093(2.367, 5.658) vs. 0.45 (0, 1.794)]. Serum and urinary Ang(1-7) in preeclampsia(53.88 ± 17.97 vs 69.99 ± 19.09 pg/ml)was significantly decreased compared to gestational hypertension (70.96 ± 19.83 vs 98.78 ± 23.99 pg/ml) and normal pregnant women(72.50 ± 20.59 vs 92.91 ± 18.04 pg/ml).Serum AGT had no difference among three groups. Urinary AGT in preeclampsia and gestational hypertension was lower than normal control (212.37 ± 35.13,193.24 ± 21.61 vs 317.10 ± 77.63 ng/L). There were positive correlation among prepartal urinary podocyte number to ACR and blood pressure. Negative correlation between prepartal urinary podocyte number and serum Ang1-7 was found. ROC curves of serum and urinary Ang(1-7) as well as podocyturia confirmed that they were useful in identifying preeclampsia.

**Conclusions:** Direct injury of podocyte may lead to proteinuria development in preeclampsia. Decreased Ang(1-7) and the renal local RAS may contributed to podocyte injury.

Disclosure of Financial Relationships: nothing to disclose

## F-FC225

**Podocyte Specific mRNA Levels Measured in Urine of Patients with Preeclampsia Are Increased Compared to Healthy Pregnant Controls** Hans J. Baelde,[1] Tim Peter Kelder,[1] Maria Elisabeth Penning,[1] Sicco Scherjon,[2] Jan A. Bruijn.[1] *[1]Pathology, Leiden University Medical Center, Leiden, Netherlands; [2]Obstetricx and Gynaecology, Leiden University Medical Center, Leiden, Netherlands.*

Preeclampsia affects 5% of all pregnancies, and is an important cause of maternal and perinatal morbidity and mortality worldwide. Podocyturia, detected with immunohistochemistry, has been noted as a specific marker for preeclampsia. The aim of this study was to quantify podocyturia in preeclamptic women using Q-PCR to detect podocyte specific mRNA transcripts.

Clean catch urine samples were collected from preeclamptic (n=34), healthy pregnant (n=34), and healthy non-pregnant (n=12) women. Preeclampsia was defined as new-onset hypertension (>140/90 mmHg) with proteinuria (>300 mg/ 24u urine) after at least 20 weeks of gestation. Patients and controls were matched for parity, amenorrhea duration, and age. mRNA was isolated and Q-PCR reaction was carried out for nephrin, VEGF, podocin, GAPDH, and GP330. mRNA levels were corrected for urine creatinine concentration. A Receiver Operating Characteristic (ROC) curve analysis was performed.

Significantly elevated mRNA expression levels of nephrin ($p<0,01$), podocin ($p<0,01$) and GAPDH ($p<0,01$) were detected in preeclamptic women compared to healthy pregnant and non-pregnant controls. Nephrin, podocin and VEGF were significantly higher in pregnant women, compared to healthy non-pregnant controls. The areas under the curve, distinguishing between preeclamptic and non-preeclamptic patients, were 0.735 for nephrin, 0.689 for VEGF, and 0.735 for podocin. GP330 expression was very low and did not show significant differences between all three groups. A positive correlation was found between nephrin and VEGF in both preeclamptic (R=0,899) and healthy pregnant women (R=0.861).

The podocyt specific slit diaphragm molecules, nephrin and podocin, seem to be reliable markers to detect podocyturia in urine samples of preeclamptic women. A strong correlation between nephrin and VEGF suggests no differential expression in podocytes between both genes in preeclamptic women. Measuring podocyturia with qPCR constitutes a novel sensitive marker to predict and detect preeclampsia.

Disclosure of Financial Relationships: nothing to disclose

## F-FC226

**Expanded Medullary Kidney Volume in Hypertensive African-Americans Is Associated with Increased Blood Flow but Reduced Medullary Oxygenation Related to Solute Transport** Stephen C. Textor,[1] Monika L. Gloviczki,[1] Michael F. Flessner,[2] David A. Calhoun,[3] James Glockner,[1] Joseph P. Grande,[1] Michael A. Mckusick,[1] Lilach O. Lerman.[1] *[1]Mayo Clinic, Rochester, MN; [2]National Institutes of Health, Bethesda, MD; [3]University of Alabama, Birmingham, AL.*

**Background:** Hypertension develops earlier and is more frequently associated with kidney disease in African-Americans (AA) than in Caucasians (C), but little is known about regional kidney volumes and oxygen (O2) consumption for these groups. **Methods:** Hypertensive subjects were treated with ACE-I/ARB and 150 mEq Na+ intake. 21 C and 16 AA subjects underwent Blood O2 Level Dependent (BOLD) 3T MR to measure R2* (deoxyhemoglobin) before and after IV furosemide. Cortical and medullary volumes and blood flows were measured with multidetector CT. GFR (iothalamate), renin (PRA) and urinary isoprostanes were also determined. **Results:** AA were younger (53±3 vs 68±3, $p<.01$) and had higher BMI (31.1±1.8 vs 27.5±0.8, $p<.05$). BP, creatinine, urinary Na+ and BP medications did not differ.

| | AA (32 kidneys) | Caucasians (42 kidneys) | |
|---|---|---|---|
| GFR/kidney (ml/min/1.73m2) | 55±3 | 46±2 | p=.02 |
| Cortical volume (cc/m2) | 50.3±2.8 | 49±2 | NS |
| Medullary volume (cc/m2) | 35.6±1.9 | 25.2±1.4 | p<.001 |
| Cortical blood flow: ml/min | 394±24 | 315±21 | p<.01 |
| Medullary blood flow:ml/min | 98±6 | 62±4 | p<.01 |
| Cortical R2* (/sec) | 17.5±0.8 | 17.9±0.3 | NS |
| Medullary R2* (/sec) | 40.4±1.1 | 36.8±1 | p<0.02 |

Basal medullary R2* values were higher in AA, but fell to similar levels after furosemide in both groups. Isoprostanes for AA correlated directly with blood flow (R=.67, p<. 02). Venous O2 did not differ between ethnic groups. Increased medullary volume and R2* persisted after allowing for age and BMI. **Conclusion:** These data demonstrate for the first time expanded medullary volume in AA subjects with higher blood flow and O2 consumption. Higher basal R2* values in AA reflected increased medullary, but not cortical, deoxyhemoglobin due to O2 consumption from loop solute transport, since furosemide eliminated the R2* difference. Increased medullary volume and O2 consumption in hypertensive, obese AA subjects may predispose to oxidative stress injury.

Disclosure of Financial Relationships: nothing to disclose

## F-FC227

**Intrarenal RAAS Activity Can Modify Circadian Blood Pressure Rhythm in Patients with IgA Nephropathy** Michio Fukuda,[1] Maki Urushihara,[2] Tamaki Wakamatsu,[1] Hiroyuki Kobori,[2] Genjiro Kimura.[1] *[1]Department of Cardio-Renal Medicine and Hypertension, Nagoya City University, Japan; [2]Department of Physiology, and Hypertension and Renal Center of Excellence, Tulane University Health Sciences Center.*

To address renal mechanism for non-dipper circadian blood pressure (BP) rhythm, we have postulated that both reduced glomerular ultrafiltration coefficient and enhanced tubular sodium reabsorption cause high sodium-sensitivity resulting in the defect in sodium excretory capacity. This in turn makes BP elevated during the night (non-dipper) in order to compensate for diminished natriuresis during daytime, thereby causing enhanced pressure natriuresis during the night. In fact, for patients with insulin resistance, diabetes mellitus, metabolic syndrome, and primary aldosteronism, non-dipper BP rhythm was noted despite preserved renal function. We also reported in patients with biopsy-proved glomerular diseases, as renal function deteriorated, nighttime BP was elevated. However, among these patients, some showed non-dipper BP rhythm even with preserved renal function. We previously showed that the intrarenal activation of renin-angiotensin-aldosterone system (RAAS), which may stimulate tubular sodium reabsorption, could be caused by the augmentation of proximal tubular angiotensinogen (AGT) expression. Therefore, this study was performed to examine whether subjects with non-dipper BP rhythm showed increased AGT expression in renal proximal tubules in patients with IgA nephropathy (n = 41, 23 females/18 males, 31 ± 11 years old). AGT protein levels in biopsied kidney samples were quantified in a semi-automatic manner by a robotic system using immunohistochemistry. The AGT expression had significant relationships with the night/day ratios of BP ($r = 0.38$, $p = 0.02$) and natriuresis ($U_{Na}V$) ($r = 0.34$, $p = 0.03$). As AGT expression was augmented in patients with IgA nephropathy, sodium reabsorption, which was reflected as a decrease in $FE_{Na}$, was enhanced ($r = 0.22$, $p = 0.20$). These data suggest that in addition to the reduced filtration capacity, the enhanced tubular sodium reabsorption may also contribute to non-dipper circadian BP rhythm in patients with IgA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**F-FC228**

**Uric Acid Level and Hypertension in U.S. Adolescents** Lauren F. Loeffler,[1] Edgar R. Miller,[2] Tammy M. Brady,[1] Jeffrey J. Fadrowski.[1] *[1]Pediatric Nephrology, Johns Hopkins University; [2]Internal Medicine and Epidemiology, Johns Hopkins University, Baltimore, MD.*

**Background:** Multiple studies have demonstrated an association between uric acid and cardiovascular disease in adults, and a small number of studies have examined the relationship in children. We sought to examine the association between uric acid and hypertension in a large, nationally representative cohort of U.S. adolescents.

**Methods:** We examined 6,036 adolescents 12-17 years of age who participated in the National Health and Nutrition Examination Survey (NHANES), a cross-sectional survey of non-institutionalized U.S. civilians, from 1999-2006. Using logistic regression, we analyzed the association between uric acid and hypertension, defined as a systolic (SBP) or diastolic blood pressure (DBP) ≥ the 95$^{th}$ percentile for age, gender, and height. Age, gender, race/ethnicity, BMI percentile and glomerular filtration rate (eGFR) estimated by the Schwartz formula were also included in the regression analyses.

**Results:** Cohort charteristics: mean age (SD) 14.52 (0.04) years; 49% female; 63% non-Hispanic White, 14% non-Hispanic Black, 11% Mexican American, 6% other Hispanic; mean serum uric acid (SD) 5.03 (0.03) mg/dL; 17% obese (BMI ≥95$^{th}$ percentile); mean eGFR (SD) 144.7 (0.7) mL/min/1.73m$^2$; 2.5% SBP ≥95$^{th}$ percentile, and 0.8% DBP ≥95$^{th}$ percentile. Thirty-four percent of participants had a uric acid level ≥5.5 mg/dL, which has been shown in other studies to have a high predictive value for hypertension. In fully adjusted analyses, for each increase of 0.1 mg/dL in uric acid level, participants had a 1.39 times higher odds of being hypertensive (95% CI, 1.15 to 1.60). Participants with a uric acid level ≥5.5 mg/dL had a 2.02 times higher odds of being hypertensive (95% CI, 1.37 to 3.00).

**Conclusions:** Increasing levels of serum uric acid are associated with hypertension in this large, nationally representative sample of healthy adolescents in the U.S. Uric acid may play a role in the early development of hypertension in adolescents and young adults, which may have important implications for screening and treatment in this population to prevent future cardiovascular disease.

Disclosure of Financial Relationships: nothing to disclose

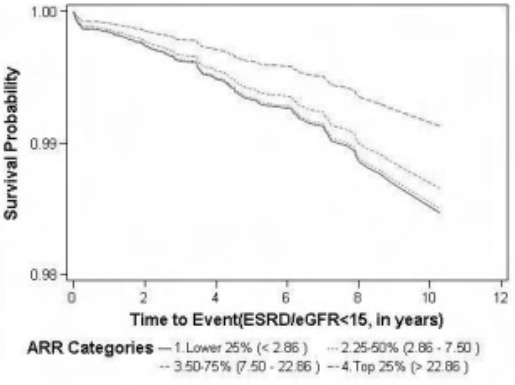
**F-FC229**

**Aldosterone to Renin Ratio (ARR) as a Predictor of Mortality and End Stage Renal Disease (ESRD)** John J. Sim,[1] Jiaxiao Shi,[1] Federico Calara,[2] Kamyar Kalantar-Zadeh,[3] Scott A. Rasgon.[1] *[1]Nephrology - Hypertension Research & Evaluation, Kaiser Permanente LAMC, Los Angeles, CA; [2]Novartis Corporation, Hanover, NJ; [3]Nephrology & Hypertension, Harbor UCLA Medical Center, Torrance, CA.*

BACKGROUND: Higher ARR is associated with resistant and refractory hypertension. We hypothesized that ARR is a predictor of death and ESRD.

METHODS: We conducted a historical cohort study within a large ethnically diverse outpatient population (>17 yrs) with minimum 1 yr follow-up and documented ARR calculated from plasma aldosterone and renin activity levels. Patients were categorized into 4 ARR quartiles based on population distribution. Medicare and hospitalization data were used to determine death, and ESRD defined as need for dialysis, renal transplantation, or eGFR<15 mL/min/ 1.73 m. Cox regression was employed to estimate hazard ratios (HR) at 2 and 5yrs after adjustment for age, gender, medication usage, hypertension, diabetes, and Charlson's comorbidity index.

RESULTS: 4,665 subjects with documented ARR had a median follow-up of 3.4yrs and an overall 10.0% mortality. Compared to the lowest quartile of ARR, the multivariate adjusted HR (95% CI) for mortality alone at 5 yr followup was 0.79 (0.61-1.02), 0.67 (0.51-0.87), and 0.71 (0.54-0.92); and HR (95% CI) for ESRD were 0.98 (0.61-1.58), 0.88 (0.55-1.42), and 0.57 (0.34-0.94) for quartiles 2, 3, and 4 respectively. Survival estimates showed a similar trend toward increased renal survival with higher ARR.




CONCLUSIONS: Higher ARR appears associated with lower mortality and a trend towards fewer ESRD outcomes. This was most pronounced in the population with high suspicion for hyperaldosteronism. Additional studies are to examine whether these associations are due to appropriate renin suppression or whether hyperaldosteronism is a disorder more amenable to treatment.

Disclosure of Financial Relationships: Research Funding: Novartis Corporation.

**F-FC230**

**Trends in Albuminuria under RAS Suppression** Julian Segura,[1] Cesar Cerezo,[1] Jose R. Banegas,[2] Juan J. De la Cruz,[2] Jose A. Garcia-Donaire,[1] Ton J. Rabelink,[3] Manuel Praga,[1] Luis M. Ruilope.[1] *[1]Hypertension Unit, Department of Nephrology, Hospital 12 de Octubre, Madrid, Spain; [2]Department of Preventive Medicine & Public Health, Universidad Autónoma, Madrid, Spain; [3]Department of Nephrology and Hypertension, Leiden University Medical Center, Leiden, Netherlands.*

Introduction- RAS suppression is considered as the therapy of choice, together with a strict BP control, to prevent the development and to impede the progression of albuminuria.

Objective- We have reviewed the evolution of albuminuria in a group of 1433 patients (mean age 60.5 yr; 50.3% male), arriving in our unit as a consequence of arterial hypertension with varying degrees of associated cardiovascular risk factors. All had in common the existence of previous therapy with an ACEi or an ARB for a minimum of two years before arrival in the Unit.

Results- When first seen 67.7% were normoalbuminuric (albumin-to-creatinine ratio [ACR] <10 mg/g for male, <15mg/g for female), 11.9% exhibited high-normal values of albuminuria (ACR 10-20 mg/g for male, 15-30 mg/g for female), 16.4% were microalbuminuric (ACR 20-200 mg/g for male, 30-300 mg/g for female) and 4% had macroalbuminuria (ACR >200 mg/g for male, >300 mg/g for female). At that time measured creatinine clearance was 96.8+/-49.6 and 54.1% had BP values below 140/90 mmHg. All of them were followed for three years during which RAS suppression was maintained, while BP control improved. At the end of follow-up, only 54.9% were normoalbuminuric, 16.1% presented high-normal albuminuria, 21.6% were microalbuminuric and 7.4% macroalbuminuric ($p<0.004$). The changes were seen in non-diabetic ($p<0.005$) but were more marked in diabetics with only 37.5% of patients being normoalbuminuric.

Conclusions- These results indicate that albuminuria develops in the presence of chronic RAS suppression at adequate doses and progresses continuously. Long-term RAS suppression needs to be revisited in order to control this alteration.

Disclosure of Financial Relationships: nothing to disclose

**F-FC231**

**Prevention of Microalbuminuria: Predictors for a Good Response to Olmesartan Treatment (ROADMAP Trial)** Hermann G. Haller,[1] Luis M. Ruilope,[4] Lars C. Rump,[3] Ton J. Rabelink,[5] Eberhard Ritz.[2] *[1]Medical School Hannover; [2]University of Heidelberg; [3]University of Düsseldorf; [4]Hospital 12 de Octubre; [5]Leiden University Medical Center.*

*Background* Microalbuminuria (MAU) is an early sign of diabetic nephropathy and increased cardiovascular risk. We investigated whether early treatment with an angiotensin receptor blocker (ARB) in diabetic subjects with normal albumin excretion delays the occurrence of MAU and analysed subgroups that would benefit most from treatment.

*Methods* We studied 4,447 subjects with type 2 diabetes and at least one additional cardiovascular risk factor in a randomized, double-blind, multicentre, controlled, and event-driven (MAU) trial. They received either 40 mg olmesartan medoxomil (OM) or placebo (Pb) od. for a median duration of 3.2 years. In both groups, additional antihypertensive treatment (except ACE inhibitors or ARBs) was used to reach the target BP of <130/80 mmHg.

*Results* Baseline UACR, eGFR, blood pressure and cardiovascular disease (CVD) risk profiles were comparable in both groups. During the double blind period, 178 (8.2%) subjects in the OM group and 210 (9.8 %) subjects in the Pb group developed MAU (time-to-onset: HR: 0.770; 95.1% CI: 0.630 to 0.941, p: 0.0104). To identify factors influencing the response to OM treatment, explorative post-hoc subgroup analysis using the corresponding median at baseline as a cut-off were performed. This analysis revealed that the treatment effect on time to onset of MAU was better in subjects with a SBP >135 mmHg than with SBP values ≤135 mmHg. The treatment effect in subjects with DBP < or > 80.3 mmHg was comparable. A baseline HbA1c ≤7.3% and an eGFR ≤83.79 ml/min. were predictors for a better response to OM treatment. Furthermore, less than 5% of patients with a baseline UACR ≤4 mg/g developed MAU during the study and the rate was similar between the OM and Pb treated patients.

*Conclusions* In subjects with type 2 diabetes olmesartan showed a significant 23% risk reduction regarding time to onset of microalbuminuria. Patients with a baseline eGFR ≤83.79 ml/min., or an UACR >4mg/g benefit most from olmesartan treatment.

ClinicalTrials.gov ID no.: NCT00185159

Disclosure of Financial Relationships: Consultancy: Daiichi Sankyo, Novartis, Genzyme, Roche, Bayer-Schering, Sanofi-Aventis, Noxxon, MedWiss, Phenos, MSD; Honoraria: Amgen, Astra-Zeneca, Recordati, Menarini, Pfizer.

**F-FC232**

**Mild Hyponatremia as a Risk Factor for Fractures: The Rotterdam Study** Ewout J. Hoorn, Robert Zietse, M. Carola Zillikens. *Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands.*

INTRODUCTION: Recent studies suggest that mild hyponatremia is associated with fractures, but prospective studies are lacking. Our aim was to study whether hyponatremia is associated with fractures and, if so, whether this is mediated through falls and/or bone mineral density (BMD).

METHODS: 5208 elderly (> 55 years) with available serum sodium at baseline were included from the prospective, population-based Rotterdam Study. The following data were analyzed: BMD, vertebral fractures (mean follow-up 6.4 years), non-vertebral fractures (mean follow-up 7.4 years), recent falling, co-morbidity, medication, and mortality.

RESULTS: Hyponatremia (serum sodium < 136 mmol/L) was detected in 399 community subjects (7.7%, 133.4 ± 2.0 mmol/L). Subjects with hyponatremia were older (73.5 ± 10.3 vs. 70.0 ± 9.0 years, P<0.001), had more recent falls (23.8% vs. 16.4%, P<0.01), higher type 2 diabetes mellitus prevalence (22.2 vs. 10.3%, P<0.001), and more often used diuretics (36.9% vs. 21.3%, P<0.001). Hyponatremia was not related to BMD, but was associated with increased risk of incident non-vertebral fractures (HR=1.39, 95% CI 1.11-1.73, P=0.004) after adjustment for age, sex and body mass index. Further adjustments for disability index, use of diuretics or psycholeptics, recent falls and prevalent diabetes did not modify results. Subjects with hyponatremia also had increased risk of vertebral fractures after adjustment for all covariates (OR=1.61, 95% CI 1.00-2.59, P=0.049). Finally, all-cause mortality was higher in subjects with hyponatremia (HR=1.21, 95% CI 1.03-1.43, P=0.022).

CONCLUSION: Mild hyponatremia in the elderly is associated with increased risk of vertebral fractures and incident non-vertebral fractures but not with BMD. Increased fracture risk in hyponatremia was also independent of recent falls, pointing towards a possible effect on bone quality. This study adds to the growing body of evidence that chronic hyponatremia is not a benign condition, and suggests the need for monitoring and treatment.

Disclosure of Financial Relationships: nothing to disclose

**F-FC233**

**Use of Vasopressin Receptor Antagonists for Treatment of Hyponatremia: A Meta-Analysis** Leena Almarzouqi,[1,2] Lea Borgi,[1,2] Victor F. Seabra,[1,2] Ethan M. Balk,[2,3] Bertrand L. Jaber,[1,2] Nicolaos E. Madias.[1,2] *[1]Department of Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Department of Medicine, Tufts University School of Medicine, Boston, MA; [3]Center for Clinical Evidence Synthesis, Tufts Clinical and Translational Science Institute, Tufts University, Boston, MA.*

**Background:** Several clinical trials have examined the efficacy of vasopressin receptor antagonists (VRAs) for the treatment of hyponatremia.

**Methods:** We performed a meta-analysis of randomized placebo-controlled trials (RCTs) to assess the efficacy and safety of VRAs among patients with normovolemic or hypervolemic hyponatremia. We searched MEDLINE for RCTs that evaluated the use of VRAs for treatment of hyponatremia. Using a random-effects model, the primary efficacy outcome of interest was the pooled net serum sodium correction on day 2 and day 4 in the treatment group compared to the placebo group. A pooled odds ratio was also compiled for the rate of overly rapid sodium correction.

**Results:** We identified 9 RCTs (4 conivaptan, 2 tolvaptan, 2 satavaptan, 1 lixivaptan) with 933 analyzable patients. Use of VRAs achieved a pooled net increase in serum sodium concentration of 4.6 mEq/L at day 2 (95% CI 3.9, 5.6) and of 5.4 mEq/L at day 4 (95% CI 4.3, 6.4) compared to placebo. The weighted incidence rate of overly rapid sodium correction among patients treated with VRAs was 11.5% (95% CI 8.3, 15.8). Use of VRAs was associated with a 2.9-fold increased odds of overly rapid sodium correction (95% CI 1.6, 5.3) compared to placebo.

**Conclusion:** This meta-analysis indicates that VRAs for the treatment of hyponatremia are effective at raising serum sodium concentration on day 2 and day 4, but are associated with a nearly 3-fold increased risk of overly rapid sodium correction.

Disclosure of Financial Relationships: nothing to disclose

**F-FC234**

**Comparison of Vaptans to Urea for Long-Term Treatment of Patients with SIADH. A Prospective Study** Alain Soupart, Guy Decaux. *General Internal Medicine, Erasme University Hospital, Bruxelles, Belgium.*

**Aim of the study**

Usual treatments of hyponatremia related to SIADH consist in water restriction, oral furosemide plus NaCl or Urea. A new therapeutic option is now available with selective AVP renal V2 receptors antagonists (Vaptans). We compare the efficacy and tolerance of Vaptans to Urea in patients with SIADH during a 2 years consecutive period. Twelve patients with chronic hyponatremia of were treated first with oral Vaptans (Satavaptan 5 to 25 mg/day or Tolvaptan 30-60 mg/day) for one year. After an 8 days drug-holiday, they received urea (15-30 g/day) for one additional year.

| | TREATMENT | DURATION | ONE | YEAR | 8 DAYS | ONE | YEAR |
|---|---|---|---|---|---|---|---|
| | Basal (n=2) | Basal | Vaptans | Vaptans | Drug Holiday | Urea | Urea |
| N | SNa | SUrea | SNa* | SUrea* | SNa | SNa* | SUrea* |
| 1 | 124 | 41 | 131 +/- 3 | 52 +/- 5 | 123 | 133 +/- 4 | 106 +/- 35 |
| 2 | 122 | 23 | 136 +/- 0.4 | 29 +/ - 2 | 120 | 138 +/- 2 | 93 +/- 15 |
| 3 | 125 | 23 | 133 +/- 1 | 48 +/- 5 | 131 | 134 +/- 2 | 52 +/- 9 |
| 4 | 121 | 25 | 139 +/- 1 | 34 +/- 2 | 130 | 135 +/- 1 | 41 +/- 9 |
| 5 | 120 | 23 | 134 +/- 2 | 40 +/- 8 | 116 | 131 +/- 1 | 101 +/- 25 |
| 6 | 131 | 41 | 136 +/- 1 | 63 +/- 2 | 126 | 137 +/- 1 | 56 +/- 6 |
| 7 | 123 | 18 | 134 +/- 1 | 22 +/- 4 | 125 | 131 +/- 6 | 42 +/- 3 |
| 8 | 129 | 28 | 137 +/- 1 | 52 +/- 4 | 130 | 138 +/- 2 | 74 +/- 5 |
| 9 | 130 | 28 | 140 +/- 2 | 36 +/- 6 | 132 | 134 +/- 4 | 49 +/- 6 |
| 10 | 128 | 21 | 141 +/- 1 | 24 +/- 1 | 133 | 137 +/- 2 | 44 +/- 17 |
| 11 | 125 | 25 | 129 +/- 0.8 | 31 +/- 1 | 131 | 133 +/- 2 | 48 +/- 6 |
| 12 | 124 | 19 | 141 +/- 1 | 23 +/- 4 | 124 | 135 +/- 4 | 35 +/- 2 |
| Mean values | 125 +/- 3 | 26 +/- 7 | 135 +/- 3 | 37 +/- 13 | 126 +/- 5 | 135 +/- 2 | 61 +/- 25 |

*Mean value of 6 data collected over one year. SUrea: mg/dl (+/-SD)

**Results**

One patient treated with Urea develops one episode of hypernatremia (155 mEq/l) when admitted for pneumonia and completely recovered. Another patient falled during the drug-holiday period and broke her wrist. One patient stopped Vaptan because of excessive thirst. Urea shows similar efficacy than Vaptans for treatment of chronic SIADH. Tolerance is good in both groups.

Disclosure of Financial Relationships: nothing to disclose

**F-FC235**

**KCNJ10: Report of a New Family with SeSAME Syndrome and Potential Role in the Maturing Tubule** Ute I. Scholl,[1] Haatal B. Dave,[2] Ming Lu,[1] James Listman,[2] Richard P. Lifton.[1] *[1]Genetics, Yale University School of Medicine, New Haven, CT; [2]Pediatrics, SUNY Upstate Medical University, Syracuse, NY.*

We have recently reported four families with a novel autosomal recessive Mendelian disorder characterized by seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME, Scholl et al. 2009). Independently, this disease was described as EAST syndrome in two kindreds (Bockenhauer et al. 2009). All patients had mutations in the *KCNJ10* gene, which encodes an inward rectifier potassium channel (Kir4.1) expressed in brain, inner ear, and kidney.

We here report a seventh family with four siblings clinically diagnosed with SeSAME syndrome. Using DNA sequencing, heterologous expression, electrophysiology and confocal microscopy, we identify and characterize a novel *KCNJ10* mutation (T57I). This residue is conserved from invertebrates to humans. Heterologous expression of the mutant channel in *Xenopus* oocytes revealed that is was non-functional despite adequate surface expression. We studied the clinical presentation of the four affected children to refine the spectrum of abnormalities resulting from *KCNJ10* mutations. Only two children showed evidence of sensorineural hearing loss. One subject developed brain MRI findings consistent with excitotoxicity following a seizure event.

The electrolyte abnormalities in SeSAME syndrome (hypokalemic alkalosis, hypomagnesemia) closely resemble that of Gitelman syndrome. This syndrome is caused by mutations in the NaCl cotransporter NCCT in the distal convoluted tubule (DCT). Kir4.1 is essential for DCT salt reabsorption; it recycles $K^+$ for the $Na^+$-$K^+$-ATPase. While patients with Gitelman syndrome typically manifest symptoms in early adolescence, it is unclear whether electrolyte imbalance is present earlier. Seizures in our SeSAME patients necessitated early electrolyte studies. Interestingly, hypokalemic alkalosis and hypomagnesemia were absent or mild early in life and deteriorated with age, raising the question whether the relative impact of DCT salt reabsorption increases with age.

Our report describes a novel *KCNJ10* mutation in SeSAME syndrome and points to a potential change of DCT function in the maturing tubule.

Disclosure of Financial Relationships: nothing to disclose

**F-FC236**

**Long-Term Neutralization of Diet-Induced Acid Load by Kcitrate Increases Bone Density in Elderly Subjects with Normal Bone Mass: Results of a 2 Year Placebo-Controlled Trial** Sigrid Jehle, Reto Krapf. *Department of Medicine, Kantonsspital Bruderholz, University of Basel, Bruderholz/Basel, Switzerland.*

The dietary acid-loads typical of the Western diet are postulated to decrease bone mass in humans and to contribute to the current osteoporosis epidemy. However, human intervention studies (long-term neutralization of dietary acid loads) on bone mineral density by DEXA (BMD) have yielded controversial results. To resolve this issue, 201 subjects healthy, elderly subjects [69.1 ± 3.3 yrs] of both genders [female 61 %, male 39 %)] with normal baseline BMD (t-score L2-L4 = -0.6) were enrolled in a DBPRT and received either 60mmol Kcitrate as base or placebo daily for 2 yrs. The 2 groups did not differ in any of the baseline parameters. Primary endpoint was the intergroup difference in bone density by DEXA (Lunar DPXL) and by quantitative CT (uCT, SCANCO Medical) at 24 mo. Results are summarized in table 1.

% Changes in bone density by DXA and μCT at different sites

| | Month 12 | | Month 18 | | Month 24 | |
|---|---|---|---|---|---|---|
| | %ΔPlacebo | %ΔKcitrate | %ΔPlacebo | %ΔKcitrate | %ΔPlacebo | %ΔKcitrate |
| Lumbar BMD | -0.4 | +1.2 | -0.2 | +1.5 | -0.04 | +1.8** |
| Hip BMD | -0.6 | +0.5* | -0.7 | +0.5* | -1.3 | +0.7** |
| Tot Body BMD | -0.5 | +0.3* | -0.7 | +0.3** | -0.8 | +0.6** |
| Radius R BMD | -0.8 | +1.0* | -1.0 | +1.4** | -1.0 | +1.6* |
| Radius L BMD | -1.2 | +1.0** | -1.2 | +1.1* | -1.3 | +1.5** |
| Radius R μCT | -0.8 | +1.1** | -0.7 | +1.1** | -0.7 | +1.4** |
| Radius L μCT | -0.02 | +1.1** | -0.1 | +1.2** | -0.1 | +1.3** |

*p<0.05, ** p<0.001 for intergroup comparison

Kcitrate induced a significant and sustained increase of bone density at all sites by either method. Furthermore, the increases in density analysed by the 2 methods were of the same magnitude at comparable sites (radius).

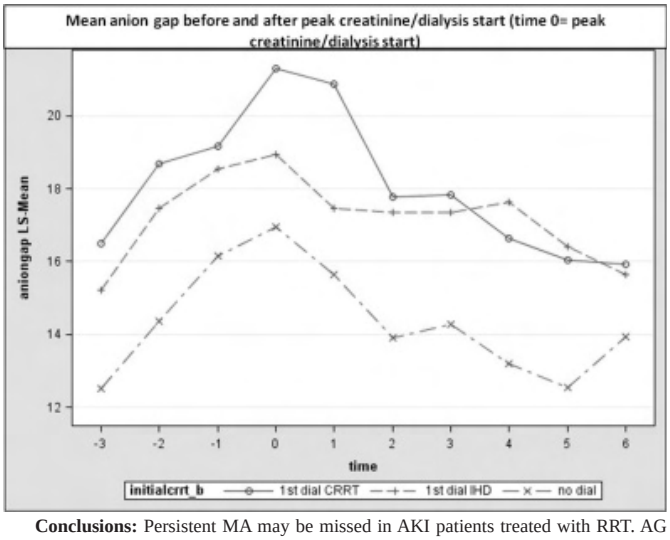
In conclusion, long-term neutralization of diet-induced acid loads by K citrate significantly increased bone density in this elderly population.The increase occurred in subjects with normal bone mass and, therefore, is evidence for a quantitatively important role of K citrate in preventing and even treating age-associated decreases in bone mass.

Disclosure of Financial Relationships: nothing to disclose

## F-FC237

**Assessment of Metabolic Acidosis in Critically Ill Patients with Acute Kidney Injury (AKI)** Rolando Claure-Del Granado,[1] Etienne Macedo,[1] Sharon Soroko,[1] Glenn M. Chertow,[2] Jonathan Himmelfarb,[3] T. Alp Ikizler,[4] Emil P. Paganini,[5] Ravindra L. Mehta.[1] *[1]University of California, San Diego; [2]Stanford University School of Medicine; [3]Kidney Research Institute, University of Washington; [4]Vanderbilt University Medical Center; [5]Cleveland Clinic Foundation.*

**Background:** RRT is often used to manage complex acid base disorders particularly metabolic acidosis (MA) in critically ill pts with AKI. $HCO^3$ and pH values are commonly used to assess response and evaluate effectiveness of RRT, however may not recognize persistence MA. We assessed the utility of anion gap (AG) to characterize MA in ICU pts with AKI. We hypothesized that AG would identify persistent MA despite correction of pH and $HCO^3$. **Methods:** We analyzed data from 363 critically ill pts with AKI from 5 centers included in the PICARD study. We assessed the presence of MA using the pH, $HCO^3$, the anion gap (AG) during the first 7 days of ICU admission. The course of MA was compared between patients who did and did not require RRT. **Results**: Day of peak creatinine (time 0) values of $HCO_3$ were 23.3±5.3 (no RRT) , 20.3±5.4 (CRRT), and 20.3±5.5 (IHD); pH 7.38±0.1 (no RRT), 7.34±0.1 (CRRT) and 7.34±0.1 (IHD); AG 17.1±5.5 (no RRT), 19.8±6.4 (CRRT), IHD 19.3±4.8 (IHD). CRRT and IHD were equally effective in correcting levels of pH, $HCO^3$, and AG at 48hrs, 72hrs and at day 6 after RRT initiation ($p > 0.05$) (Figure 1). pH and $HCO^3$ levels normalized at 48 and 72hrs respectively; while AG showed improvement after 24 hours of therapy, but still did not reach normal values after 72hrs of treatment.




**Conclusions:** Persistent MA may be missed in AKI patients treated with RRT. AG provides a simple method to evaluate the effectiveness of RRT to correct MA and should be calculated as part of the assessment of dialyzed patients.

Disclosure of Financial Relationships: nothing to disclose

## F-FC238

**Prevalence of Acidosis in Intensive Care Unit (ICU) Patient's on Continuous Renal Replacement Therapies (CRRT): Classical Versus Stewart's Approach?** Paolo Lentini,[1-2] Vincenzo Catena,[1] Monica Baccarin,[1] Luca Zanoli,[1] Alexandra Chronopoulos,[3] Massimo De Cal,[2-3] Valentina Pellanda,[1] Claudio Ronco,[3] Marco Baiocchi,[1] Roberto Dell'Aquila.[1] *[1]Nephrology-Intensive Care, St. Bassiano Hospital, Bassano Del Grappa, Italy; [2]University of Padua, Padua, Italy; [3]Nephrology, St Bortolo, Vicenza, Italy.*

Acid-base disorders are common in the ICU and are indications for CRRT. If the perturbations are unrecognized they may result in poor outcomes. The Stewart approach may be superior for acid-base analysis in the critically ill. AIM: Assessment of correlation and agreement between Classical versus Stewart approaches for the analysis of acid-base disturbances during CRRT. Materials and Methods: We enrolled 19 consecutive adult patients on CVVH and mechanical ventilation. All patients received a ≥35 ml/Kg/h infusion of a standard buffer [5 Litres (mmol/L): [HCO-3]35, [Na+]140, [K+]2, [Ca2+]1,75, [Mg+]0.5, pH 7,4]. We calculated [HCO-3] and SBE with the Henderson- Hasselbach and Siggaard-Andersen equations. Based on the SBE, metabolic status was classified as acidosis (SBE -5.0), normal (SBE -4.9 to 4.9) or alkalosis (SBE +5.0). Physicochemical analysis was performed using the Stewart equations modified by Figge et al. The apparent strong ion difference was calculated: SIDa =[Na+]+[K+]+[Mg+]+[Ca2+]-[Cl-]-[lactate](mEq/L). The normal variation for SIDa was defined as 40–42 mEq/L. The effective SID (SIDe) was then calculated: SIDe = 1000x 2.46 x 10-11 xPaCO2/(10-pH)+[alb]x(0.123 x pH-0.631)+[PO4-] x(0.309xpH-0.469)(mEq/L). Normal variation for SIDe was defined as 38–42 mEq/L. Chi square test was used at 0,6,12,and 24h after CVVH to compare the prevalence of acidosis detected with pH vs. SIDe and BE vs.SIDe. RESULTS: The prevalence of acidosis after CVVH, as assessed by pH vs. SIDe, was [36.8% vs. 94.7% ($p<0.001$)] at 6h, 21.1% vs. 73.7% ($p<0.05$)] at 12h, and [21.1% vs. 98.6% ($p<0.001$)] at 24h. The prevalence of acidosis after CVVH, as assessed by SBE vs.SIDe, was [57.9%vs.94.7%($p<0.05$)] at 6h,[63.2% vs.73.7% (p=NS)]at 12 h,and [63.2% vs.98.6%($p<0.05$] at 24h. CONCLUSION: Stewart approach seems to be more sensitive than Classical approach for detection of acidosis in ICU patients on CRRT.

Disclosure of Financial Relationships: nothing to disclose

## F-FC239

**Role of KLF15 in Podocyte Differentiation** Ruijie Liu,[2] Sandeep K. Mallipattu,[1] John C. He.[1, 2] *[1]Medicine/Nephrology, Mount Sinai School of Medicine, New York, NY; [2]Medicine, James J. Peters VA Medical Center, Bronx, NY.*

We have shown previously that retinoic acid (RA) induces podocyte differentiation via stimulation of cAMP/PKA/CREB pathway. However, it remains unknown what are the transcription factors mediating the effects of RA on the expression of podocyte differentiation markers. To address this, we performed microarray studies in podocytes incubated with or without RA for 6 hours. By computational analysis, we found that KLF15 was highly stimulated by RA in podocytes and is a CREB-target gene. KLF15 is known as a kidney-enriched Kruppel-like factor and was found to mediate differentiation of adipocytes. We confirmed that RA stimulates 2-3 fold increase of KLF15 by both real-time PCR and western blot in conditionally immortalized murine podocytes. We have reported previously that HIV infection induces podocyte dedifferentiation. Here, we found that HIV-infection suppressed KLF15 expression and over-expression of KLF15 stimulated expression of synaptopodin in HIV-infected murine podocytes. KLF15 expression was also suppressed in glomeruli of HIV-1 transgenic mice where podocytes undergo significant dedifferentiation. In addition, we confirmed these findings in human podocytes. We found that RA stimulated KLF15 expression in conditionally immortalized human podocytes with a peak level of 2 hours indicating that KLF15 is an early inducible gene. Expression of KLF15 was low at 33oC when podocytes were de-differentiated and high at 37oC when podocytes were differentiated. Finally, KLF15 knockout mice exhibited 2 fold increase of albuminuria with significant mesangial expansion and focal foot process effacements under EM. These data suggest a critical role of KLF15 in mediating podocyte differentiation.

Disclosure of Financial Relationships: nothing to disclose

## F-FC240

**Primary HIV Injury in a Fraction of Podocyte Population Triggers Secondary Injury in the Remaining Population throughout the Whole Glomerulus, Leading to Global Sclerosis: Evidence from a Mosaic Murine Model** Taiji Matsusaka,[1] Iekuni Ichikawa.[2,3] *[1]Department of Internal Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan; [2]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN; [3]Department of Bioethics, Tokai University School of Medicine, Isehara, Kanagawa, Japan.*

In female placental mammals, one of the two copies of X chromosome, either of paternal or maternal origin, is randomly inactivated. Using HIV-1 transgenic mice, which carry nephrin-vpr gene on X chromosome, we tested the hypothesis that progression of glomerulosclerosis involves spread of injury from podocytes targeted by a primary insult into non-targeted podocytes. As expected, all hemizygous male vpr mice (n=13) developed moderate to severe nephropathy at 8 weeks of age. Nephrin immunostaining was markedly downregulated with scores averaging 3.47±2.29 on 0 (complete loss) to 8 (normal) scale. Immunostaining revealed that all podocytes express the transgene in intact glomeruli. In contrast, biopsy specimens obtained from heterozygous female vpr mice at 6 weeks (n=27) showed no injury. At this time point, approximately 50% of podocytes randomly expressed the transgene within each glomerulus. At 9 weeks of age, heterozygous female

vpr mice (n=7), showed only mild nephropathy with nephrin scores averaging 7.18±0.97. Later, 17 out of 28 female heterozygotes died at 13-25 wks. Histological analysis in the remaining surviving mice showed that 6 had extensive global sclerosis with near complete loss of nephrin staining, and the other 5 showed only moderate sclerosis. In the non-sclerotic glomeruli, the number of WT-1 positive, i.e., intact, podocytes (7.7±3.0) was not largely changed from that at 6 wks of age (8.6±3.1); however, unlike at 6 wks, none of the podocytes showed transgene expression. These indicate that vpr expression in 50% of podocyte population is mostly sufficient to induce global sclerosis by spreading damage into the entire podocyte population. However, through mechanisms yet to be identified, under certain circumstances, the glomerulus, hence the mouse, is capable of escaping from sclerosis by suppressing transgene expression in podocytes.

Disclosure of Financial Relationships: nothing to disclose

**F-FC241**

**Activated PECs Are Crucially Involved in the Segmental Scar Formation in Classical FSGS** Bart Smeets,[1,2] Christoph Kuppe,[1] Eva Maria Sicking,[1] Peggy Jirak,[1] Wilhelm Kriz,[5] Jack F. Wetzels,[3] Jurgen Floege,[1] Marcus J. Moeller.[1] *[1]Nephrology and Clinical Immunology, Univerity Hospital Aachen, RWTH, Aachen, Germany; [2]Patholoy, RUNMC, Nijmegen, Netherlands; [3]Nephrology, RUNMC, Nijmegen, Netherlands; [4]Cellular and Molecular Pathology, DKFZ, Heidelberg, Germany; [5]Anatomy and Developmental Biology, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany.*

Focal and segmental glomerulosclerosis (FSGS) is a common histopathological finding in human biopsies and a major cause for end-stage renal disease. Loss of podocytes beyond a certain threshold triggers the formation of a classical FSGS lesion. So far, the pathogenesis of the formation of a classical FSGS lesion is not completely resolved.

Previously, we have made the observation that activated parietal epithelial cells (PECs) invade the glomerular tuft in a segmental pattern. To resolve whether PECs play a critical role in the pathogenesis of FSGS lesions, PECs or podocytes were specifically tagged in experimental mice in an irreversible fashion. Subsequently, the mice were uninephrectomised and treated with DOCA/salt to induce FSGS lesions within the remnant kidney.

Analysis of the histological findings revealed the following sequence of events: 1. A primary injury results in the formation of an adhesion between the glomerular tuft and Bowman's capsule. 2. PECs are focally activated. Subsequently, PECs proliferate and migrate onto the glomerular tuft via the adhesion. 3. PECs invading the glomerular tuft migrate onto the glomerular podocytes destroying the microanatomy of the affected glomerular segment and depositing extracellular matrix. Podocytes are displaced in this process and disappear from the lesion. The adhesion as an entry site for activated PECs also explains the focal and segmental character of the classical FSGS lesion.

Genetic tagging of PECs allowed to identify the origin of these cells even after prolonged periods of time or trans- or dedifferentiation. The sequence of events resulting in a segmental glomerular scar was also verified in other FSGS models as well as in human biopsy material.

In summary, we propose a novel theory for the pathogenesis of FSGS.

Disclosure of Financial Relationships: nothing to disclose

**F-FC242**

**Aggravated Phenotype of Preeclampsia in Mice Lacking eNOS** Nobuyuki Takahashi,[1] Feng None Li,[1] Nobuyo Maeda,[1] Oliver Smithies,[1] S. Ananth Karumanchi,[2] J. Charles Jennette.[1] *[1]Pathology, University of North Carolina at CH, Chapel Hill, NC; [2]Department of Medicine and the HHMI, Harvard Medical School, Boston, MA.*

Preeclampsia is pregnancy-associated hypertension with proteinuria. Excess sFlt-1, an endogenous VEGF inhibitor of placental origin has been implicated to cause hypertension, proteinuria and glomerular endotheliosis, all features of preeclampsia. sFlt-1 antagonizes VEGF and induces endothelial dysfunction via several pathways including decreased eNOS. Human eNOS polymorphisms leading to low NO production are associated with preeclampsia. We tested whether a decrease in eNOS causes aggravation of preeclampsia using eNOS knockout mice. eNOS-/- mice have higher systolic blood pressure (BP) than wild type (WT) mice (144.2 ± 3.3 mmHg vs. 126.0 ± 2.6 in WT). Adenoviral mediated sFlt-1 overexpression to the non-pregnant female mice increased BP of both eNOS-/- and WT mice (171.5 ± 7.9 mmHg vs. 153.2 ± 5.2 in WT). eNOS-/- sFlt-1 mice showed higher daily urinary albumin excretion (490.1 ± 73.1 mg/day vs. 180.4 ± 67.8 in WT sFlt-1) and lower GFR (129 ± 34 ml/min vs. 405 ± 125 in WT sFlt-1). eNOS-/- sFlt-1 mice had less glomerular open capillary volume (13.5 ± 2.4 % vs. 34.7 ± 8.5 % in WT sFlt-1 mice), and lost fenestration of glomerular capillary endothelial cells, suggesting eNOS-/- sFlt-1 mice have more severe endotheliosis than WT sFlt-1 mice. eNOS-/- sFlt-1 mice also show podocyte effacement. Expression of endothelin-1 and $ET_A$ receptor in the kidney was higher in eNOS-/- sFlt-1 mice than in WT sFlt-1 mice. An $ET_A$ receptor antagonist sulfisoxazole (1g/kg/day) decreased urinary albumin excretion, and increased glomerular open capillary volume more in eNOS-/- sFlt-1 mice than in WT sFlt-1 mice. Sulfisoxazole also improved endothelial fenestration and corrected podocyte effacement in eNOS-/- sFlt-1 mice. We conclude that reduced maternal eNOS/NO exacerbates preeclampsia through activation of ET system.

Disclosure of Financial Relationships: nothing to disclose

**F-FC243**

**Bacterial CpG-DNA Accelerates Alport Glomerulosclerosis by Inducing a M1 Macrophage Phenotype and TNF-α-Mediated Podocyte Loss** Mi Ryu,[1] Oliver Gross,[1] Hans J. Anders.[1] *[1]Medizinische Poliklinik, University of Munich, Munich, Germany; [2]Department of Nephrology, University of Goettingen, Goettingen, Germany.*

Loss of function mutations in the a3 or α4 chain of type IV collagen (COL4) cause Alport nephropathy which is characterized by progressive glomerulosclerosis. The mechanisms that determine disease progression remain unclear. We found that the progression of kidney disease in *Col4a3*-deficient mice is associated with an influx of immune cell subsets including non-activated macrophages. We hypothesized that immune recognition of bacterial products would accelerate Alport nephropathy by enhancing intrarenal inflammation.

Exposure to bacterial endotoxin from week 4 to 6 of age did not affect disease progression while an equipotent dose of cyrosin-guanosin (CpG)-DNA accelerated all aspects of Alport nephropathy and reduced the overall life span of *Col4a3*-deficient mice. This effect of CpG-DNA was associated with a significant increase of renal CD11b+/$Ly6C^{high}$ macrophages, intrarenal production of iNOS, TNF-α, IL-12, and CXCL10, and loss of podocytes. TNF-α was essential for acceleration of Alport nephropathy because etanercept entirely abrogated the CpG-DNA effect.

Together, non-activated macrophages accumulate during Alport nephropathy. Systemic exposure to CpG-DNA induces classically-activated (M1) macrophages which enhance intrarenal inflammation and disease progression, e.g. via the secretion of TNF. Thus, factors that modulate the phenotype of renal macrophages can affect the progression of Alport nephropathy, and potentially of other types of chronic kidney diseases.

Disclosure of Financial Relationships: nothing to disclose

**F-FC244**

**Inducible Expression of Constitutively Active TGF-beta Receptor Type 1 Transgene in Tubular Epithelia Causes Progressive Tubular Degeneration and Tubulointerstitial Fibrosis** Shaolin Shi, Taoran Zhang, Erwin P. Bottinger. *Nephrology/Medicine, Mount Sinai School of Medicine, New York, NY.*

TGF-β is considered a key mediator for renal tubular injury in various kidney diseases, however, the initiating pathomechanisms and cellular targets remain poorly understood. To define precisely the consequences of ligand-independent, epithelial-restricted activation of TGF-β receptor type 1 (Tgfbr1) signaling in mouse nephrons, we generated bi-transgenic Pax8-rtTA/Tgfbr1(AAD) mice carrying mutant Tgfbr1(AAD) (constitutively active form of Tgfbr1) and Pax8-rtTA (reverse tetracycline-depedent transactivator under control of Pax8 promoter) transgenes for doxycycline-inducible expression of Tgfbr1(AAD) in renal tubules. The bi-transgenic Pax8-rtTA/Tgfbr1(AAD) mice manifested rapid loss of body weight, anuria, and death within 10 days of doxycycline treatment. Renal histopathology revealed global loss of tubules and massive tubulointerstitial fibrosis, demonstrating that TGF-β signaling in tubular epithelia is sufficient to trigger progressive nephron loss and tubulointerstitial fibrosis. Detailed experiments to characterize the time course and reversibility of molecular signals and cellular responses in nephrons of Pax8-rtTA/Tgfbr1(AAD) mice are in progress and will provide novel insights into signaling networks and cellular mechanisms that mediate tubular injury and tubulointerstitial fibrosis induced by epithelial TGF-β signaling.

Disclosure of Financial Relationships: nothing to disclose

**F-FC245**

**Conditional Knock Down of Renal Epithelial ADAM17 Reduces Ischemia/Reperfusion Injury in Mice** Gemma M. Mulder,[1] Wynand B. W. H. Melenhorst,[1] Johanna Wam Celie,[2] Niels H. Huizing,[1] Jan-Luuk Hillebrands,[1] Marc Seelen,[3] Dorien J. M. Peters,[4] Lydia Visser,[1] Harry Van Goor.[1] *[1]Pathology, UMC Groningen, Groningen, Netherlands; [2]Pathology, AMC, Amsterdam, Netherlands; [3]Nephrology, UMC Groningen, Groningen, Netherlands; [4]Human Genetics, Leiden UMC, Leiden, Netherlands.*

Ischemia/reperfusion injury (IRI) plays a major role in the pathophysiology of interstitial fibrosis and tubular atrophy (IF/TA). EGF receptor is crucially involved in the response to ischemia, and knock down of its ligand HB-EGF protects against early IRI. ADAM17 (TACE) sheds various EGF receptor ligands. We investigated ADAM17 in human IF/TA and in experimental IRI.

We studied expression of ADAM17 mRNA in IF/TA and control kidneys by RT-PCR and in situ hybridization. Moreover, we assessed ADAM17-mediated HB-EGF shedding in cultured human kidney (HK2) cells. Using Cre-Lox technology, we selectively knock down ADAM17 in renal tubular epithelial cells to examine its contribution to IRI.

ADAM17 mRNA was seven-fold upregulated in IF/TA when compared with control kidneys ($P<0.001$). In normal kidneys, ADAM17 mRNA was weakly expressed in proximal tubules, peritubular capillaries, glomerular endothelium and parietal epithelium. In IF/TA, tubular, capillary and glomerular ADAM17 expression was strongly enhanced with *de novo* expression in the mesangium. Furthermore, we observed colocalization of ADAM17 protein with HB-EGF protein in interstitial fibrotic lesions.

*In vitro*, PMA stimulation induced a 4-fold increase in HB-EGF shedding compared with baseline conditions. Inhibition of ADAM17 with TNF484 resulted in a dose-dependent reduction of HB-EGF shedding, up to 82% in PMA-stimulated cells ($P<0.005$) and 53% in non-stimulated cells ($P<0.005$). Compared with WT mice, conditional ADAM17 KO

mice showed strong reduction in cortical tubular ischemic damage after 6h (4.05±1.55 vs 11.24±2.80, KO vs WT resp), 1d (6.25±3.67 vs 23.6±5.95, KO vs WT resp) and 3d of reperfusion (12.24±5.55 vs 30.97±5.62, KO vs WT resp, P<0.05).

In conclusion, ADAM17 inhibition reduces cortical tubular damage in experimental IRI, indicating a modulating role for ADAM17. Pharmacological ADAM17 inhibition might be a future therapy in renal transplantation.

Disclosure of Financial Relationships: nothing to disclose

**F-FC246**

**N-Terminal Truncated Intracellular Matrix Metalloproteinase-2 Induces Renal Injury** David H. Lovett,[1] Rajeev Mahimkar.[2] *[1]Medicine, University of California San Francisco, San Francisco, CA; [2]BioMarin Pharmaceuticals, Novato, CA.*

Modulation of renal structure and function by members of the matrix metalloproteinase (MMP) gene family is an area of intense interest. Much effort has been focused on the role of a specific MMP, matrix metalloproteinase-2 (MMP-2).Transgenic expression of active MMP-2 in the proximal tubule (PT) (Cheng, et al. FASEB J (2006) 20:1898) is sufficient to induce glomerulosclerosis, tubular atrophy and interstitial fibrosis. In this model PT cells acquire a mixed mesenchymal/epithelial phenotype with limited TBM penetration. To examine the mixed EMT phenotype further we developed an in vitro model by gradient expression of MEK in epithelial LLC-MK2 cells. Proteolytic targets of MMP-2 were assessed by in LLC-MK clones with a mixed phenotype by 2-D DIGE-LC/MS/MS and identified mitochondrial voltage-dependent anion channel-2 as a target. Western blot of isolated mitochondria identified a unique 65 kDa N-terminal isoform of MMP-2 generated by use of an alternative translational start site at $M^{77}$. This isoform lacks a secretory sequence and prodomain and is intracellular and enzymatically active. Microarray analysis after transfection with a N-terminal truncated MMP-2 (NTT-MMP2) cDNA indicated induction of genes characteristic of an innate immune response regulated primarily by NF-κB and NFAT. These genes included chemokines, pro-apoptotic factors and viral stress response genes, thereby defining a novel mitochondrial-nuclear signaling cascade. To assess the in vivo activity of NTT-MMP2 transgenic mice were developed with proximal tubule-specific expression of this isoform. NTT-MMP2 transgenic mice develop normally and have normal structure and function at 3 months: by six months there was evidence for PT cellular dedifferentiation, with mitochondrial structural abnormalities. By nine months there was severe glomerulosclerosis, tubular atrophy, TBM thickening and interstitial infiltration with mononuclear cells. We conclude that an NTT isoform of MMP-2 is generated during EMT, triggers mitochondrial-nuclear stress signaling and directly contributes to progressive renal injury independent of superimposed injury.

Disclosure of Financial Relationships: nothing to disclose

**F-FC247**

**Monoclonal Light Chain Deposition in Proximal Tubule Cells Induces Oxidative Stress and Defective Receptor-Mediated Endocytosis in Mouse** Sara Terryn,[1] Christophe Sirac,[2] Frank Bridoux,[3] Olivier Devuyst.[1] *[1]Nephrology, UCL, Brussels, Belgium; [2]Immunology, CNRS, Limoges, France; [3]Nephrology, CHU, Poitiers, France.*

Renal involvement is a common complication of multiple myeloma, associated with a worse prognosis. In particular, the storage of monoclonal immunoglobulin light chain (LC) within the endo-lysosomal compartment of proximal tubule cells (PTC) may lead to LC-associated Fanconi syndrome (FS). We have now used a transgenic mouse model overexpressing a pathogenic human monoclonal κ chain (CHEB mice) to investigate the cellular mechanisms involved in PTC dysfunction after LC-deposition. CHEB mice showed a low-molecular weight proteinuria as evidenced by the selective urinary loss of CC16 and transferrin, in absence of overt albuminuria. They also presented glucosuria and phosphaturia, indicating a general PTC dysfunction, whereas glomerular filtration rate, BUN and plasma creatinine were unchanged. Electron microscopy of CHEB kidneys revealed crystalline inclusions in PTC of predominantly the S3 segment. Immunostaining for megalin and cubilin showed a decreased expression of both receptors in S3 segments of CHEB kidneys. To gain insight in the cellular mechanisms, we established well-differentiated, polarized primary cultures of PTC from micro-dissected S3 segments. qRT-PCR analysis revealed a decreased expression of genes involved in endocytosis (megalin, cubilin, NHE3, amnionless), which resulted in a reduced endocytic uptake of transferrin but not of albumin in CHEB PTC. The defective endocytosis in CHEB PTC coincided with an increased expression of genes related to oxidative stress (HO-1, CAIII, SOD1/2, catalase, Trx) and proliferation (PCNA, Ki67). The state of oxidative stress of CHEB PTC was confirmed by carboxy-$H_2$DCFDA staining.

These results show that transgenic CHEB mice display a LC-associated FS which faithfully reflects the human pathology. The accumulation of LC within PTC results in defective endocytosis through the loss of megalin and cubilin and cellular responses including oxidative stress and proliferation. The interplay between lysosomal accumulation of LC, defective endocytosis and oxidative stress in PTC provides targets for intervention studies.

Disclosure of Financial Relationships: nothing to disclose

**F-FC248**

**MicroRNA Profiles of Tubular Cells under Hypoxia-Reoxygenation and Endoplasmic Reticulum Stress** Shiyo Muratsu, Masaomi Nangaku, Tetsuhiro Tanaka, Chih-Kang Chiang, Takehiko Wada, Toshiro Fujita, Reiko Inagi. *Div of Nephrol and Endocrinol, Univ of Tokyo Sch of Med, Tokyo, Japan.*

Both hypoxia and endoplasmic reticulum (ER) stress orchestrate the tubular damage via the stress signalings. Meanwhile, various physiologic and pathologic processes are regulated by microRNA (miR). We thus hypothesize that the crosstalk of these stress signalings in damaged tubules may be modulated by miRs at mRNA level.

To address this, we assessed the change in miR expression in cultured human tubular cells (HK-2) exposed to 16hr hypoxia (0.1% $O_2$) followed by 3hr (HR3) or 10hr (HR10) reoxygenation or ER stress inducers, tunicamycin (2ug/ml TUN) or thapsigargin (0.5ug/ml THG), by miR microarray analysis.

In 821 miRs we tested, we identified the miRs that showed the significant change in their expression under HR (11 miRs) or ER stress (10 miRs) (P<0.05). They included the miRs that are well-known to be regulated by oxygen tension (miR-21, miR-210), or that regulate the gene expression for cell proliferation or apoptosis (miR-886-3p, miR-923). Of note, we identified one miR, miR-205, whose expression was markedly decreased both under HR and ER stress. These findings were confirmed by real-time qRT-PCR followed by the sequence analysis: 0.73- in HR3, 0.45- in HR10, 0.55- in TUN, 0.57-fold in THG compared to control (P<0.05). In addition, the decrease in miR-205 expression was induced by hypoxia alone. While miR-205 is predicted to be a tumor-suppressor-miR or regulator for epithelial- mesenchymal differentiation (EMT), miR-205-overexpressed HK-2 did not change the cell morphology or cell growth. When these cells were exposed to 500uM $H_2O_2$ or 5ug/ml TUN, the cell survival was markedly increased (71.3 or 78.2 v.s. 46.4% in control miR transfectant). Augmented intracellular ROS induced by $H_2O_2$ was not declined by miR-205 overexpression, suggesting the contribution of miR-205 in modulation of these stress signals independent of oxidative stress.

These results strongly suggest that certain miRs may contribute to both signalings triggered by hypoxia-reoxygenation and ER stress in tubular cells and influence the tubular pathological phenotypes.

Disclosure of Financial Relationships: nothing to disclose

**F-FC249**

**A New Strategy for Preventing CKD-Induced Muscle Atrophy: Manipulation of Muscle Progenitor/Stem Cells Using a microRNA-29 Technique** Xiaonan H. Wang,[1] William E. Mitch.[2] *[1]Emory University, Atlanta, GA; [2]Baylor College of Medicine, Houston, TX.*

In a mouse model of CKD, we have found (JASN, 2010) that the functions of muscle progenitor cells (MPC) are impaired and since these cells are involved in regulating muscle mass, the impairment could contribute to muscle wasting. We investigated how CKD changed the function of MPC in a mouse model of CKD. We found: 1) increased expression of YY1, a protein that inhibits MPC differentiation; and 2) a 3-fold decrease in miR29, a microRNA that is complementary to a sequence in the 3' un-translated region (3'-UTR) of the YY1 mRNA. Therefore, we tested whether impaired differentiation of MPC results from an excess of YY1 because miR29 is suppressed. Alternatively, an increase in miR29 would suppress YY1 stimulating differentiation and increasing muscle mass when MPC grow. First, we used a lentivirus strategy to stimulate YY1 expression in cultured C2C12 myoblasts. In these myoblasts, embryonic myosin heavy chain (eMyHC) expression decreased sharply indicating that myoblast differentiation into myotubes was inhibited. Next, we transduced miR29 (Ad-miR29) into C2C12 myoblasts using adenovirus-mediated microRNA transfer. There was a significant (n=6, p<0.05) decrease in the YY1 protein. This response was accompanied by increased expression of markers of muscle cell maturation. Thus, miR29 regulates YY1 expression, promoting muscle cell maturation. Third, we cloned the YY1 3'-UTR sequence complementary for miR29 binding and inserted it into a pMir-Luciferase vector. C2C12 myoblasts expressing this pMir-luciferase-3UTR-YY1 were treated with an adenovirus expressing miR29 (Ad-miR29). Luciferase activity decreased (P<0.05; n=4) indicating that miR29 suppressed YY1 expression. Conclusions: expression of YY1 impairs muscle progenitor cell maturation and YY1 expression can be suppressed by miR29. These results identify a new mechanism regulating muscle mass and identify a mechanism for CKD-induced loss of muscle mass. Methods resulting in increased miR29 expression could lead to strategies for treating the muscle atrophy of CKD and possibly other catabolic conditions.

Disclosure of Financial Relationships: nothing to disclose

**F-FC250**

**Mechanisms Reducing Skeletal Muscle Mass in Chronic Kidney Disease (CKD): Studies in β1 Hom and Podocin Gene Knock-Out (β1 HOM-POD) Mice** Haiming Li,[1] Feng Sha,[1] Roy Zent,[1] Zhaoyong Hu,[2] William E. Mitch,[2] T. Alp Ikizler.[1] *[1]Vanderbilt; [2]Baylor.*

A substantial body of evidence implicates activation of the ubiquitin-proteasome system (UPS) as the principal cause of skeletal muscle atrophy. Triggers activating muscle catabolism in CKD include chronic inflammation. We found that β1 HOM-POD mice develop progressive CKD with proteinuria (Zent et al Development 2009) and used this model to examine the contributions of UPS and chronic inflammation in the development of skeletal muscle protein degradation in CKD. The β1 Hom-Pod mice were generated by crossing integrin $\beta\ 1^{flox/flox}$ with the podocin-Cre mice. Blood samples and muscle tissue were obtained from 6 HOM-POD and 6 control mice. IL-1, IL-6, and TNF-a were measured in serum by CBA and in muscle tissue by real-time PCR. Akt/pAKT, Ubiquitin (Ub)-

Conjugates and 20s proteasome subunits α1,2,3,5,6,&7 were measured by immunoblotting and mRNA expression of Ub, E1, E214K, E3aI, E3aII, atrogin-1 and MurF-1 were measured by real time PCR in muscles.

Results: β1 HOM-POD mice developed massive proteinuria and elevated BUN. They also had severe edema, smaller, pale kidneys, skeletal atrophy and weighed less (22.66+-8.9 g vs 17.96+-1.78 g) compared to HOM mice. Ub-conjugates and 20s proteasome subunits α1,2,3,5,6,&7 were significantly increased in muscle in HOM-POD mice and pAKT (a major regulator of UPS activity) was decreased. mRNA expressions of Ub and the E3 Ub-conjugating enzymes, E3aI, E3aII, atrogin-1 and MurF-1 were significantly elevated in β1 HOM-POD mice. Likewise, IL-6 and TNFa were elevated in serum and IL-1α and IL-6 were elevated in muscle of β1 HOM-POD mice (Data not shown).

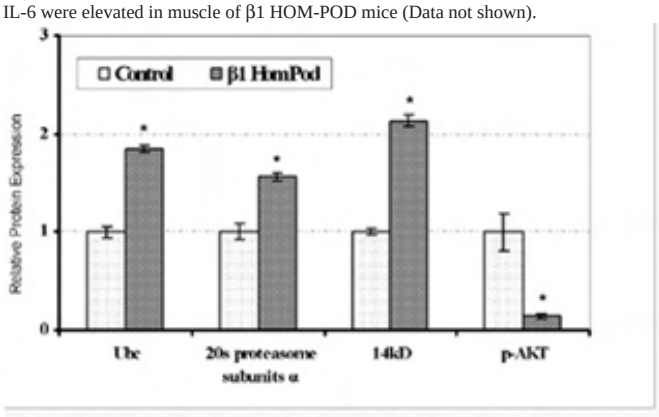



Figure 1:The Ub-Conjugates , 20s proteasome subunits <1,2,3,5,6,&7 and 14KD by immunoblotting show significant increase in Hom-Pod mice, while the pAKT decreased

*: p<0.05

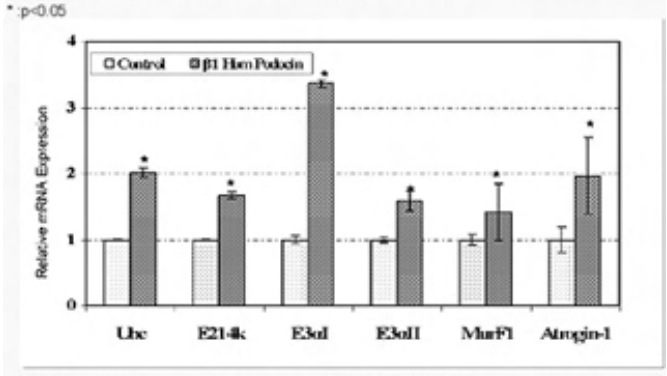



Figure 2: The mRNA expression of Ubc, E3aI, E3aII, atrogin-1 and MurF-1 are found to be significantly elevated in Hom-Pod mice

*: p<0.05

Conclusion: In the β1 HOM-POD mouse model of CKD, stimulation of the UPS causes loss of skeletal muscle. Besides depressed pAkt, stimulation of the UPS could be augmented by chronic inflammation.

Disclosure of Financial Relationships: nothing to disclose

## F-FC251

**Elucidation of the Anti-Fibrotic Mechanism of a Novel HIF-Regulated Globin, Cytoglobin, with Molecular Genetics** Imari Mimura, Hiroshi Nishi, Tetsuhiro Tanaka, Reiko Inagi, Toshiro Fujita, Masaomi Nangaku. *Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Tokyo, Japan.*

Cytoglobin (Cygb) is a novel member of the globin superfamily expressed by splanchnic fibroblasts in various organs under the control by HIF. In light of experimental evidence that tubulointerstitial hypoxia plays a critical role in chronic kidney disease, a role of Cygb was investigated in the rat remnant kidney (RK) model, focusing on its potential impact on fibrogenesis. Quantitative evaluation revealed an increase in expression of Cygb mRNA and protein, in parallel with the disease progression. Transgenic overexpression of Cygb significantly ameliorated fibrosis of the kidney, with improvement of renal functions. Histological improvement in the transgenic rat kidney was corroborated by immunohistochemical analysis of α-smooth muscle actin and ED-1 showing less severe myofibroblast changes and macrophage infiltration, respectively. Double-immunostaining studies clarified that Cygb was expressed in the interstitial area with collagen IV accumulation. Additionally, nitrotyrosine staining and urinary 8-OHdG measurement revealed that oxidative stress was less in Cygb-Tg rats. In vitro studies utilizing Cygb-overexpressing cells, as well as ex vivo studies using primary cultured fibroblasts from Cygb-Tg rats, demonstrated that overexpression of Cygb inhibited collagen synthesis at both mRNA and protein levels. Antifibrotic properties of Cygb were offset by introducing mutation in the heme moiety and disallowing its binding to molecular oxygen. Furthermore, flow cytmetric analysis clarified that the fibroblast cell line NRK49F overexpressing Cygb suffered from milder intracellular ROS, following acute challenge with hydrogen peroxide. Results of the present study identify Cygb as a critical antifibrotic molecule that plays an important role in protection against renal fibrogenesis, via reduction of oxidative stress.

Disclosure of Financial Relationships: nothing to disclose

## F-FC252

**Adipose-Specific Deletion of Angiotensin Type 1 Receptor Protects Against Obesity-Induced Proinflammatory Injury and Albuminuria** Li-Jun Ma,[1] Bridgette Corsa,[1] Dan Gao,[1,2] Valentina Kon,[3] Agnes B. Fogo.[1,3] *[1]Department of Pathology, Vanderbilt University; [2]Department of Nephrology, First Affiliated Hospital of ZhengZhou University, China; [3]Department of Pediatrics, Vanderbilt University.*

Background: Activation of the renin-angiotensin system is implicated in the pathogenesis of both obesity and CKD. We investigated the effects of deletion of angiotensin type 1a receptor (AT1a) in adipocytes on obesity and kidney injury.

Methods: AT1aflox/flox mice and aP2-Cre transgenic mice were crossed to generate adipose-specific AT1a knockout mice (KO). Littermates lacking the aP2-Cre transgene were used as wild-type controls (WT). Adult (age 8-10 wks) male KO and WT mice were fed with high-fat diet (HFD) for 24 weeks (KO+HFD, n=6; WT+HFD, n=6), and compared to baseline. Metabolic parameters, kidney morphology and albumin/creatinine ratio (ACR) were assessed. P-JNK protein expression was examined by Western blot. Data are expressed as mean±SE.

Results: Adipose tissue AT1 receptor protein level was markedly reduced by 82% in lean KO vs WT mice while AT1 levels in liver and kidney were not altered. When challenged with HFD, KO mice and WT mice showed similar body weight (47.2±1.2 vs 49.3±1.1 g) and adipose tissue mass (1.1±0.2 vs 1.1±0.1 g). Obese KO and WT mice had comparable blood glucose (187.5±4.0 vs 189.8±8.0 mg/dl) and blood pressure (BP, 102.3±3.6 vs 104.1±3.6 mmHg). Surprisingly, fat tissue p-JNK protein expression was dramatically inhibited in obese KO vs obese WT (p-JNK/GAPDH ratio 0.10±0.05 vs 0.42±0.05, p<0.01). Although obesity-induced renal structural injury (mesangial expansion, tubular vacuolization) was similar in KO vs WT, ACR was much less in obese KO vs obese WT (KO+HFD 42.4±8.8 vs baseline 6.6±1.5, WT+HFD 158.6±63.6 vs baseline 6.1±1.1 ug/mg, p<0.05 KO+HFD vs WT+HFD, p<0.01 WT+HFD vs WT baseline).

Conclusions: Adipose-specific AT1 deletion has no effect on obesity, BP, or glucose in response to HFD. However, adipose AT1 deletion resulted in decreased adipose p-JNK and albuminuria. These results suggest that AT1 is key for adipose tissue JNK activation, and that adipose-derived factors are crucial in driving obesity-induced kidney dysfunction.

Disclosure of Financial Relationships: nothing to disclose

## F-FC253

**Mechanisms of Uremia-Induced Down Regulation of ApoA-I Expression** Hamid Moradi,[1] Hamid M. Said,[2] Nosratola D. Vaziri.[1] *[1]Nephrology, Univ of California, Irvine, Irvine, CA; [2]Medicine, LBVA Med Ctr, Long Beach, CA.*

Atherosclerotic cardiovascular disease is a major cause of mortality in CKD patients. HDL deficiency and defective HDL-mediated reverse lipid transport contribute to atherogenic diathesis in this population. The primary cause of HDL deficiency in CKD is reduction of apoliprotein A-I which is the principal component of HDL. Plasma ApoA-I is reduced in dialysis patients and hepatic ApoA-1 mRNA expression is decreased in uremic rats. This study explored the mechanism/s by which uremia decreases ApoA-I expression. HepG2 cells were incubated for 48 hours in media containing whole serum or serum subfractionation (using Amicon filters with different size selectivities) from normal control subjects and ESRD patients before and after hemodialysis. Cells and culture media were then separated and used for measurement of ApoA-I protein (ELISA) and ApoA-I mRNA (real-time PCR). The effect of the test sera on ApoA-I promoter activity was measured using transfection with a luciferase promoter construct containing the -2096 to +293 segment of ApoA-I gene. RNA stability was assessed in presence of actinomycinD. Exposure to uremic serum resulted in a 5-6 folds reduction in the ApoA-I mRNA expression and significant reduction of ApoA-I level in the culture media (137±40 vs. 38±5).This effect was fully reversed when uremic serum was replaced with control serum. Transfection studies revealed no difference between uremic and normal sera on ApoA-I gene promoter activity. However exposure to uremic serum significantly reduced RNA stability. Fractionation studies revealed that the negative effect of uremic serum on ApoA-I mRNA abundance resides in the fraction containing molecules larger than 30 Kd. This supposition was supported by the observation that post-dialysis serum exerted equal inhibitory effect on ApoA-I mRNA abundance as that found with pre-dialysis serum samples. Uremia causes a significant reduction in ApoA-I mRNA abundance by lowering RNA stability without altering ApoA-I promoter activity. The negative effect of uremic milieu on ApoA-I mRNA stability resides in the fraction containing molecules larger than 30 Kd which are not removable by hemodialysis.

Disclosure of Financial Relationships: nothing to disclose

## F-FC254

**Fibrinogen Promotes Kidney Fibrosis by Activating Renal Interstitial Fibroblasts** Inga Soerensen, Nathan D. Susnik, Therese Inhester, Hermann G. Haller, Roland Schmitt. *Department of Nephrology, Hannover Medical School, Hannover, Germany.*

Studies in animal models have identified fibrinogen (fbg) as an important player in inflammation, tissue repair and regeneration. Here, we focused on the role of fbg in the development of renal tubulointerstitial fibrosis using the unilateral ureteral obstruction

(UUO) model. UUO was induced in fbg-/- and fbg +/- mice, which were sacrificed at 1 and 2 weeks to analyze kidneys using histology, immunohistochemistry, qPCR and immunoblot. For in vitro studies we used rat renal fibroblasts (NRK-49F) that were stimulated with fbg in the presence and absence of neutralizing antibodies and siRNA. We found that UUO kidneys from fbg +/- mice are characterized by massive deposition of tubulointerstitial fbg at 1 and 2 weeks. Fbg deficiency significantly attenuated UUO-dependent changes such as the deposition of collagen I and de novo expression of alpha smooth muscle actin. In parallel fbg-/- mice had significantly less S100A4 positive interstitial fibroblasts. In vitro we found that exposure of NRK-49F cells to fbg resulted in rapid ERK phosphorylation and in a significant increase in cell proliferation. As fbg can interact with a variety of cell surface receptors we first tested for integrin-dependent signalling. However, the mitogenic effect of fbg was unchanged in the presence of RGD or β1-integrin neutralizing antibody. Instead we found that NRK-49F cell proliferation was significantly reduced if binding of fbg to ICAM-1 was blocked using a neutralizing antibody and/or if TLR signalling was inhibited by siRNA knockdown of MyD88. The role of MyD88 was corroborated in primary renal fibroblasts from MyD88-/- mice where the proliferative response upon fbg was significantly reduced. Blocking of ICAM-1 receptor sites and knockdown of MyD88 reduced fbg-induced NRK-49F cell proliferation in an additive fashion. In summary, we demonstrate that fbg is an important activator of renal interstitial fibroblasts in vivo and in vitro. Its mitogenic effect is at least partially mediated through binding of ICAM-1 which is expressed on the surface of tubulointerstitial fibroblasts and secondly through the TLR signalling pathway as shown by MyD88 knockdown.

Disclosure of Financial Relationships: nothing to disclose

### F-FC255

**MYH9 Expression in Podocytes Is Reduced by HIV Infection** Thomas Hays, Marc E. Trubin, Deborah P. Hyink, Paul E. Klotman. *Department of Medicine, Mount Sinai School of Medicine, New York, NY.*

Recent genetic mapping studies demonstrated that MYH9 gene variants in African Americans are strongly linked to development of focal segmental glomerulosclerosis (FSGS), including HIV-associated nephropathy (HIVAN). Additionally, MYH9 mutations are known to cause rare autosomal dominant end stage renal disease with a variably penetrant glomerulosclerosis. To explore whether the pathogenesis of HIVAN involves a loss of MYH9 function, we analyzed the expression of MYH9 in several models of the disease. In the HIV transgenic mouse model, we observed by immunohistochemistry that MYH9 expression is dramatically reduced in the glomeruli. To determine what role podocytes played in the reduced glomerular expression, we next analyzed MYH9 levels in conditionally immortalized podocytes derived from the HIV transgenic mouse. Western analysis revealed that MYH9 expression was indeed reduced in these cells (75% reduction, p=0.02). Finally, to determine whether HIV infection directly reduced MYH9 expression, we infected wildtype conditionally immortalized podocytes with VSV.G pseudotyped HIV. Here too, western analysis showed that MYH9 expression was reduced (90%, reduction, p=0.01). These results demonstrate that a reduction in MYH9 expression is a component of the host response to HIV podocyte infection. Given the strong genetic link of MYH9 to HIVAN, these data provide an important insight into the mechanism of HIVAN and FSGS in African Americans.

Disclosure of Financial Relationships: nothing to disclose

### F-FC256

**The Transcription Factor ETS-1 Is a Critical Mediator of the Pro-Fibrotic and Pro-Inflammatory Effects of Ang II in the Glomerulus** Wenguang Feng,[1] Phillip H. Chumley,[1] Ping Hua,[1] Gabriel Rezonzew,[1] Dongqi Xing,[1] Edgar A. Jaimes.[1 2] *[1]Department of Medicine, University of Alabama at Birmingham, AL; [2]VA Medical Center, Birmingham, AL.*

The renin-angiotensin system (RAS) plays a major role in the pathogenesis of end-organ injury in hypertension. The transcription factor ETS-1 is an important mediator of growth-related responses and inflammation in different models of injury. We have previously shown that Angiotensin II (Ang II) increases cortical ETS-1 expression *in vivo* and that ETS-1 is required for fibronectin production in mesangial cells. In these studies we tested the hypothesis that ETS-1 mediates pro-inflammatory and pro-fibrotic effects of Ang II *in vivo*. C57B/L6 mice (n=6 per group) were infused with vehicle (Veh), Ang II (1.4mg/kg/day), Ang II and an ETS-1 dominant negative peptide (DN,10mg/kg/day) or Ang II and an ETS-1 mutant peptide (MU, 10 mg/kg/day) for 4 weeks. Mean blood pressure (MBP) was measured by radio-telemetry weekly until sacrifice. MBP (mmHg) was significantly increased by Ang II and was not modified by DN or MU: Veh 96±3.1, Ang II 168±3.8*, Ang II + DN 163±4.8*, Ang II + MU* 164±3.2 (* P <0.05 vs vehicle). By morphometric analysis we determined that Ang II induced mesangial expansion (% of glomerular PAS): Veh 15.4±0.3 vs Ang II 19.2±0.4, (p<0.05 vs control) which was partially prevented by DN: 17.1 ± 0.4 (P<0.05), but not by MU: 19.2 ± 0.6 (P =NS). Ang II increased macrophage infiltration (F4/80, cells/mm$^2$): Veh 25.8 ± 12.1 vs Ang II 52.1 ± 6.8, P<0.05) and cell proliferation (ki67, cells/mm$^2$): Veh 16.0 ± 7.2 vs Ang II 128.2 ± 23.9, P < 0.05) which were prevented by DN: 36.9 ± 4.3 (F4/80) , 76. 8± 10.2 (ki67) (P<0.05 vs Ang II). Ang II induced a 22 fold increase in nitrotyrosine expression as assessed by immunofluorescence (AFU): Veh 39 ± 35 vs Ang II 863 ± 194 (P<0.05) that was significantly reduced by DN: 206 ± 123 (P<0.05 vs Ang II) but not by MU: 579 ± 228 (P = NS). In conclusion, ETS-1 plays a major role as mediator of the pro-fibrotic and pro-inflammatory effects of Ang II in the kidney cortex. These findings may result in the development of novel strategies in the treatment and prevention of end-organ injury in hypertension.

Disclosure of Financial Relationships: nothing to disclose

### F-FC257

**Indoxyl Sulfate Reduces Klotho Expression and Promotes Senescence in the Kidney of Hypertensive Rats** Toshimitsu Niwa,[1] Ayinuer Adijiang,[1] Yuusuke Higuchi,[2] Fuyuhiko Nishijima,[2] Hidehisa Shimizu.[1] *[1]Department of Advanced Medicine for Uremia, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; [2]Biomedical Research Laboratories, Kureha Co., Tokyo, Japan.*

Administration of indoxyl sulfate, a uremic toxin, promotes progression of chronic kidney disease (CKD) in CKD rats. Klotho, an anti-aging gene, is expressed in the kidney, and its renal expression is decreased in CKD. This study aimed to clarify if indoxyl sulfate could reduce Klotho expression, and contribute to cell senescence in the kidney of hypertensive rats.

The rat groups consisted of 1) Dahl salt-resistant normotensive rats (DN), 2) Dahl salt-resistant normotensive indoxyl sulfate-administered rats (DN+IS), 3) Dahl salt-sensitive hypertensive rats (DH), and 4) Dahl salt-sensitive hypertensive indoxyl sulfate-administered rats (DH+IS). After 32 weeks, their kidneys were excised for histological and immunohistochemical analysis for Klotho, senescence-associated b-galactosidase (SA-beta-gal), p16$^{INK4a}$, p21$^{WAF1/CIP1}$, p53 and retinoblastoma protein (Rb).

DH+IS rats showed decreased expression of Klotho, increased expression of SA-beta-gal, p16$^{INK4a}$, p21$^{WAF1/CIP1}$, p53 and Rb in renal tubular cells, and increased tubulointerstitial fibrosis and mesangial expansion as compared with DH rats. Further, DN+IS rats showed decreased expression of Klotho as compared with DN rats.

Thus, administration of indoxyl sulfate to hypertensive rats reduced renal expression of Klotho, and promoted cell senescence with expression of senescence-related proteins such as p16$^{INK4a}$, p21$^{WAF1/CIP1}$, p53 and Rb accompanied by renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

### F-FC258

**Combined Losartan (L) and Hydrochlorothiazide (H) Arrests Renal Injury Consequent to L Consumption during Lactation** Camilla Fanelli, Bianca H. Ventura, Flavia G. Machado, Elizabete P. Poppi, Denise M. Malheiros, Claudia R. Sena, Clarice K. Fujihara, Roberto Zatz. *Renal Division, Dep Clin Med, Univ of Sao Paulo, Brazil.*

We previously described (AJP F1345) a model of severe CKD ($L_{LAC}$) based on L teratogenesis during lactation. Here we sought to 1-Verify if L+H treatment, highly effective in the remnant kidney (Nx), would afford equal renoprotection in $L_{LAC}$. 2-Gain further insight into injury mechanisms. Twenty Munich-Wistar dams, each nursing 6 pups, were divided into: Control, untreated, and $L_{LAC}$, given L, 250 mg/kg/d, until weaning. Male pups went untreated until 7 mo of age, when tail-cuff pressure (TCP, mmHg), albuminuria (ALB, mg/d), serum creatinine ($S_{Cr}$, mg/dL), glomerulosclerosis index (GSI) and interstitial expansion (INT), PCNA+ cells, α-actin (αACT) and matrix metalloproteinase 2 activity (MMP2) were measured in 17 rats ($L_{LAC}$Pre). The remaining 68 rats were divided into groups: $L_{LAC}$+V, untreated, $L_{LAC}$+L, given L, 50 mg/kg/d and $L_{LAC}$+LH, given L+H, 6mg/kg/d. Results after 3 months:

| | C | $L_{LAC}$Pre | $L_{LAC}$+V | $L_{LAC}$+L | $L_{LAC}$+LH |
|---|---|---|---|---|---|
| TCP | 149±4 | 146±6 | 187±4$^{abc}$ | 130±19$^{abc}$ | 126±3$^{abc}$ |
| ALB | 32±7 | 105±14$^{a}$ | 106±26$^{ab}$ | 59±9$^{c}$ | 32±5$^{bc}$ |
| $S_{Cr}$ | 0.68±0.04 | 0.78±0.07 | 1.12±0.07$^{ab}$ | 1.05±0.06$^{ab}$ | 0.96±0.05$^{a}$ |
| GSI | 6±2 | 35±17 | 94±26$^{ab}$ | 22±5$^{c}$ | 24±4$^{c}$ |
| INT | 0.4±0.1 | 0.6±0.6$^{a}$ | 5.8±0.9$^{ab}$ | 4.2±0.5$^{a}$ | 4.8±0.4$^{ab}$ |
| αACT | 0.5±0.1 | 1.9±0.3$^{a}$ | 4.1±0.6$^{ab}$ | 2.5±0.4$^{ac}$ | 2.1±0.2$^{ac}$ |
| PCNA | 20±3 | 71±12$^{a}$ | 107±12$^{ab}$ | 59±7$^{ac}$ | 50±6$^{c}$ |
| MMP2 | 1.0±0.1 | 1.1±0.3 | 2.7±0.4$^{ab}$ | 2.5±0.3$^{a}$ | 3.5±1.3$^{ab}$ |

$^{a}$p<0.05 vs. C, $^{b}$p<0.05 vs. $L_{LAC}$Pre, $^{c}$p<0.05 vs. $L_{LAC}$+V, $^{d}$p<0.05 vs. $L_{LAC}$+L

Progression of renal injury in $L_{LAC}$+V was associated with worsening of hyperplasia and αACT. No MMP deficiency was noted, suggesting that fibrosis resulted from enhanced matrix production. L and L+H arrested GS, INT and αACT but only L+H normalized TCP and ALB, prevented further rise of $S_{Cr}$, and increased MMP2 activity. In $L_{LAC}$ rats, L treatment provides renoprotection even if started at an advanced CKD stage, while L+H affords subtle but definite additional protection.

Disclosure of Financial Relationships: nothing to disclose

### F-FC259

**Reversibility of Diabetic Nephropathy and Podocyte Loss in the BTBR *ob/ob* Mouse** Warangkana Pichaiwong,[1] Kelly L. Hudkins,[1] Tomasz A. Wietecha,[1] Bardia Askari,[1] Takahisa Kobayashi,[1] Stuart J. Shankland,[2] Jeffrey W. Pippin,[2] Charles E. Alpers.[1] *[1]Pathology, Univ. of Washington, Seattle, WA; [2]Nephrology, Univ. of Washington, Seattle, WA.*

Podocytes (podos), with limited replicative capacity, are injured or lost early in diabetic nephropathy (DN). Inability of podos to regenerate may be a key limiting factor for reversal of DN.

We have developed a murine model of Type II DN, the BTBR mouse strain with the ob/ob leptin deficiency mutation (JASN 2010; in press). We tested whether interventions that cause restoration of a normoglycemic milieu (leptin replacement) or inhibit the renin angiotensin system (RAS) by angiotensin converting enzyme inhibitor (enalapril) or angiotensin II type 1 receptor blocker (losartan) can induce reversal of established DN and do so by restoration of podo density.

Development of DN was preceded by significantly diminished podo density quantified as WT-1 expressing cells divided by mean glomerular volume (cells/10$^6$ μm$^3$). Leptin replacement via minipump infusion, beginning at age 18 weeks and continued until 24

weeks resulted in reversal of morphologic features of DN and restored podo density (leptin 231.9±14 vs. 24wks untreated mice 129.2±15.5, $p<0.001$). Treatment with enalapril or losartan for 6 weeks resulted in no significant change in podo density vs. untreated mice. Leptin replacement resulted in return to normoglycemia, reduced body weight and reduced albuminuria (leptin 175±74.8 vs. 24wks untreated mice 740.7±167 µg/24hr., $p=0.01$). Normalization of glomerular morphology in leptin treated mice was characterized by significant reduction of extracellular matrix accumulation (% of Type IV collagen/ glomerulus: leptin 14.9±0.7 vs. 24wks untreated mice 22.5±1.2, $p<0.001$) and decreased mesangiolysis. Treatment with enalapril and losartan reduced mesangiolysis and decreased albuminuria, but had no effect on extracellular matrix accumulation.

We demonstrated structural and functional reversal of DN is accompanied by full restoration of podo density, proof of podo regeneration. The lack of podo restoration by RAS inhibition, despite improvement in albuminuria, may underlie limitations of this therapy for treating human DN.

Disclosure of Financial Relationships: nothing to disclose

## F-FC260

**Systems Analysis of Circulating Leucocytes of Patients with Type 1 Diabetes (T1D), Microalbuminuria and Early Progressive Renal Function Loss Suggests Activated Inflammatory Pathway** Wenjun Ju,[1] Monika A. Niewczas,[2] Viji Nair,[1] William Walker,[2] Felix H. Eichinger,[1] Ann Randolph,[1] James Warram,[2] Andrzej S. Krolewski,[2] Matthias Kretzler.[1] *[1]Internal Medicine/Nephrology, University of Michigan, Ann Arbor, MI; [2]Research Division of Joslin Diabetes Center, Harvard Medical School, Boston, MA.*

In patients with T1D, progressive loss of renal function leading to ESRD is associated with an intra-renal inflammatory response. We tested the hypothesis that presence of this inflammatory activity associated with progressive renal function loss is detectable in circulating leucocytes of patients with T1D and early diabetic nephropathy (DN). Leucocytes were harvested at study entry from 33 T1D patients with microalbuminuria. They were followed for 4-6 years to obtain serial measurements of serum cystatin C for tracing the trajectory of GFR loss using estimates of GFRcystatin. Changes over time were summarized as the percent change in GFRcystatin per year using a mixed effects model. All patients had GFRcystatin >60 ml/min at entry. During follow-up, the annual percent change in GFRcystatin varied among patients between -13.3 to +0.2 (median -2.32). The gene expression profile of leukocyte RNA was obtained with the GeneChip Human Genome U133 Plus 2.0 Array and correlated with percent change in GFRcystatin. The expression values of 1213 genes correlated significantly with percent change (Pearson correlation>0.4, FDR q<0.02). Transcripts correlated with GFRcystatin loss were significantly enriched in canonical pathways associated with DN, including: mitochondrial dysfunction, retinoic acid mediated apoptosis signaling, oxidative phosphorylation, and TNF-alpha /NF-KB. Interestingly, the gene expression of TNF receptors correlated with GFRcystatin loss (r=-0.4, P=0.02 for TNFRSF1B, and r=-0.33, p=0.06 for TNFRSF1A). In conclusion, inflammatory pathways are activated in circulating leucocytes in T1D patients who are at risk of early GFRcystatin loss, supporting the notion of a systemic inflammatory state being important for early as well late renal function loss in T1D.

Disclosure of Financial Relationships: nothing to disclose

## F-FC261

**Monocyte/Macrophage Chemokine Receptor CCR2 Mediates Diabetic Renal Injury** Alaa S. Awad,[1,2] Gilbert R. Kinsey,[2] Konstantine Khutsishvili,[2] Ting Gao,[1] Kline Bolton,[2] Mark D. Okusa.[2] *[1]Medicine, Penn State College of Medicine, Hershey, PA; [2]Medicine, University of Virginia, Charlottesville, VA.*

**Introduction:** Monocyte/macrophage recruitment correlates strongly with the progression of renal impairment in diabetic nephropathy (DN). CC chemokine receptor (CCR)2 regulates monocyte/macrophage migration into injured tissues. However, the direct role of CCR2 in the progression of DN remains unknown. We hypothesize that deficiency or blockade of CCR2 confers kidney protection in DN.

**Methods:** Experiments were conducted in CCR2 deficient mice ($CCR2^{-/-}$) and their wild type littermates ($CCR2^{+/+}$) following STZ induced diabetes (80 mg/kg iv injection; n=8 each group) or $Ins2^{Akita}$ mice (n=5-8 each group).

**Results:** $CCR2^{-/-}$ mice significantly attenuated diabetic albuminuria to normal range (5.5-fold reduction; $p<0.0001$) compared to $CCR2^{+/+}$ mice despite comparable blood glucose levels after 6 wks of diabetes. The kidneys of diabetic $CCR2^{-/-}$ had less diabetes-mediated histological changes (interstitial fibrosis: 5-fold; $p<0.005$ and mesangial expansion: 4-fold; $p<0.05$), kidney fibronectin mRNA expression (3-fold; $p<0.005$), and inflammatory cytokine production (TNF-α: 3-fold; $p<0.001$ and IFN-γ: 4-fold; $p<0.01$) reduction compared to $CCR2^{+/+}$ mice. Diabetic $CCR2^{+/+}$ mice showed significant increases in kidney macrophages ($p<0.05$) compared with control mice using flow cytometry and immunohistochemistry. In contrast, kidney macrophages in control and diabetic $CCR2^{-/-}$ mice were similar to control $CCR2^{+/+}$ mice. Bone-marrow derived monocytes from $CCR2^{+/+}$ mice adoptively transferred into $CCR2^{-/-}$ mice reversed the renal tissue protective effect in diabetic $CCR2^{-/-}$ mice. Furthermore, blocking CCR2 using the selective CCR2 antagonist, RS504393 (2 mg/kg/day via osmotic minipump) for 12 wks in $Ins2^{Akita}$ mice mimicked CCR2 deficiency by reducing diabetic albuminuria (3-fold; $p<0.005$) and kidney macrophage recruitment (2-fold; $p<0.005$) compared to $Ins2^{Akita}$ + vehicle.

**Conclusion:** These findings provide evidence that CCR2 is necessary for monocyte/ macrophage-induced diabetic renal injury and suggest that blocking CCR2 could be a novel therapeutic approach in the treatment of DN.

Disclosure of Financial Relationships: nothing to disclose

## F-FC262

**Kidney-Specific Sirt1 Tg Mice Protects Against Albuminuria in Diabetic Nephropathy by Inhibiting Claudin-1 Ectopic Overexpression** Kazuhiro Hasegawa, Koichi Hayashi. *Keio University School of Medicine.*

**Aims**

Although it has been reported that whole body Sirt1 induction by calorie restriction or specific Sirt1 activators increases insulin secretion or attenuates insulin resistance, the role of kidney Sirt1 in diabetic nephropathy has not been elucidated. We created kidney-Specific Sirt1 Tg mice and investigated its effect on diabetic nephropathy.

**Methods**

WT or Sirt1 Tg mice were subjected to intraperitoneal injection of saline (control) or streptozotocin with 50mg/kg/day for 5 days (WT+Sal, WT+STZ, Tg+Sal, Tg+STZ). After 2 or 6 months, we measured various blood and urine parameters, and kidney histology (PAS, PHA-E staining) focusing on the morphology of parietal (PECs) or visceral (VECs) Bowman's capsule epithelial cells.

**Results**

At 2 months, WT+STZ represented increased blood glucose, albuminuria, proliferation of flat PECs accompanied by decreased body weight and blunted cuboid PECs. At 6 months, WT+STZ mice manifested kidney hypertrophy and mesangial cell proliferation. Additionally, WT+STZ mice also manifested claudin-1 overepression in the PECs and VECs. All these findings were attenuated in Tg mice. The expressions of other molecules involved in tight junction, adherent junction, or foot process which are supposed to be related with albiminuria were not different between WT and Tg. Immunostaining of Sirt1 revealed that Sirt1 was expressed in proximal tubules and glomerulus in WT+Sal, which expressions were decreased in WT+STZ. In Tg+Sal, Sirt1 expressions were dominat in proximal tubles reflecting on promoter usage, Npt2, in Tg mice, which were retained also in Tg+STZ.

**Conclusions**

These results indicate that kidney Sirt1 rescues albminuria through the suppression of claudin-1 overproduction in PECs and VECs, which findings precedes typical findings of diabetic nephropathy, such as glomerular hypertrophy or mesangial cell proliferation. Since claudin-1 is expressed physiologically only in flat PECs and functions as a tight junction protein, it is postulated that DM-induced claudin-1overexpression may cause junctional imbalance in foot processes leading to albuminuria. Renal Sirt1 can be a potential therapeutic target for the treatment of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## F-FC263

**Identification of microRNA-93 as a Novel Regulator of Vascular Endothelial Growth Factor (VEGF) in Hyperglycemic Conditions** Jianyin Long, Yin Wang, Wenjian Wang, Farhad R. Danesh. *Department of Medicine/Nephrology, Baylor College of Medicine, Houston, TX.*

Vascular endothelial growth factor (VEGF) is a dimeric glycoprotein which plays a crucial role in microvascular complications of diabetes, including diabetic nephropathy. However, the precise regulatory mechanisms governing VEGF expression in the diabetic milieu are still poorly understood. Here we provide evidence that microRNA-93 (miR-93) regulates VEGF expression in experimental models of diabetes both *in vitro* and *in vivo*. Comparative miRNA arrays from high glucose-exposed (25 mM) podocytes and kidney microvascular endothelial cells, as well as in the kidney glomeruli obtained from diabetic db/db mice identified miR-93 as a "signature miRNA" in hyperglycemic conditions. We identified VEGF-A as a putative target of miR-93 in the kidney with a perfect complementarity between miR-93 and the 3'-UTR (untranslated region) of *vegfa* in several species. miR-93 repressed the transcription of luciferase reporter construct of the mouse *vegfa* 3'-UTR, but not the mutant form where the potential miR-93 binding site was abolished, indicating that miR-93 acts as a negative regulator of VEGF by binding to the *vegfa* 3'-UTR. Forced expression of miR-93 abrogated VEGF protein secretion. Conversely, anti-miR-93 inhibitors increased VEGF release. Transfection of miR-93 also prevented the effect of high glucose on VEGF downstream targets, including a3 collagen IV and fibronectin. Using transgenic mice containing VEGF/LacZ bicistronic transcripts, we found that inhibition of glomerular miR-93 by peptide-conjugated morpholino oligomers elicited increased expression of VEGF. Our findings also indicate that high glucose decreases miR-93 expression by downregulating the promoter of its host *MCM7* gene. Taken together, our findings provide new insights into the role of miR-93 in VEGF signaling pathway, and offer a potentially novel target in preventing the progression of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## F-FC264

**MicroRNA-29c Targets Sprouty1, Promotes Apoptosis and Fibrosis, and Its *In Vivo* Inhibition Prevents Progression of Diabetic Nephropathy** Jianyin Long, Yin Wang, Wenjian Wang, Farhad R. Danesh. *Department of Medicine/ Nephrology, Baylor College of Medicine, Houston, TX.*

While several recent publications have suggested that miRNAs contribute to the pathogenesis of diabetic nephropathy (DN), the role of these miRNAs in animal models of diabetic kidney disease in vivo remains poorly understood. To systemically explore the role of miRNAs in the diabetic milieu, we generated comparative miRNA expression profiles from high glucose-exposed (25 mM) podocytes and kidney microvascular endothelial cells for 24hrs, as well as in the kidney glomeruli obtained from diabetic *db/db* mice. We identified upregulated miR-29c expression as a "signature miRNA" in the diabetic environment. Following validation of miR-29c expression *in vivo* and *in vitro* by using

RT-PCR and Northern blot analysis, we injected miR-29c antisense oligos intraperitoneally into 8-weeks old *db/db* mice (115mg/kg body weight every other week for 8-10 weeks, n=5). Compared to control *db/db* mice, miR-29c-treated animals exhibited significant reduction in albuminuria (161.233±40.93 vs. 407.60±140.21, p<0.01). Furthermore, inhibition of miR-29c led to a significant decrease in glomerular apoptosis, as shown by TUNNEL assay and caspase-3 activity analyses. The *in vivo* miR-29c silencing also suppressed glomerular fibrosis as evident by immunohistochemistry staining of glomerular collagen IV, 24hr urinary collagen excretion, and decreased glomerular mesangial matrix index. *In vitro*, overexpression of miR-29c led to elevated apoptosis as shown by FACS analysis. We also identified Sprouty1 (Spry1) as a direct target of miR-29c. Expression of miR-29c repressed the luciferase activity of Spry1 3'-UTR (untranslated region), but not that of the mutant form where the miR-29c binding site was abolished. Importantly, we found that overexpression of miR-29c decreased the protein level of Spry1, which in turn led to increased Rho kinase activity. Taken together, these findings reveal a previously unrecognized signaling cascade involved in the pathogenesis of diabetic nephropathy, and highlights a novel molecular interaction between miR-29c, Spry1, and Rho kinase activation.

Disclosure of Financial Relationships: nothing to disclose

**F-FC265**

**MicroRNA Expression Analysis in Angiotensin II-Stimulated Human Podocytes and Its Glomerular Expression in Diabetic Mice** Kenichi Koga,[1] Masashi Mukoyama,[1] Hideki Yokoi,[1] Kiyoshi Mori,[1] Masato Kasahara,[1] Takashige Kuwabara,[1] Yoshihisa Ogawa,[1] Hirotaka Imamaki,[1] Tomoko Kawanishi,[1] Akira Ishii,[1] Keita Mori,[1] Yukiko Kato,[1] Moin Saleem,[2] Akira Sugawara,[1] Kazuwa Nakao.[1] *[1]Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Academic and Children's Renal Unit, University of Bristol, Bristol, United Kingdom.*

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by inhibiting protein translation or by inducing mRNA degradation of their target genes. Previous studies have shown that miRNAs regulate the expression of key genes relevant to the kidney disease. We have already reported that connective tissue growth factor (CTGF) is involved in the progression of diabetic nephropathy, and that the natriuretic peptide/guanylyl cyclase-A (GC-A) pathway has a protective role against diabetic nephropathy. In this study, we focused on the expression of miRNAs which target CTGF and GC-A genes. We studied expression of miRNAs in immortalized human podocytes with the stimulation of angiotensin II ($10^{-6}$ M). Using miRNA microarray analysis for 939 miRNAs, we revealed that 11 miRNAs showed > 2-fold change in its expression. Then we selected 7 miRNAs as candidate miRNAs in podocytes by using miRNA database, which target CTGF and/or GC-A genes. Three of them were miR-30c, miR-26a and miR-379. miR-30c expression was significantly increased by 3.5-fold after angiotensin II stimulation with real-time PCR analysis ($p = 0.029$). Next we examined these 7 miRNA expressions in glomeruli in type 2 diabetic db/db mice. In glomeruli of db/db mice, miR-30c, miR-26a and miR-379 were significantly increased by 9.2-, 20.8- and 10.6-fold compared with db/m mice, respectively ($p < 0.05$). Database shows that GC-A is a direct target of miR-379 and that CTGF is a target of miR-30c and miR-26a. These results suggest that these miRNAs may be involved in the progression of diabetic nephropathy and that function of GC-A and CTGF might be regulated by these miRNAs.

Disclosure of Financial Relationships: nothing to disclose

**F-FC266**

**Forced Deletion of ROCK1 Ameliorates Progression of Diabetic Nephropathy by Modulating Mitochondrial Fragmentation** Wenjian Wang,[1,2] Yin Wang,[1] Jianyin Long,[1] Farhad R. Danesh.[1] *[1]Medicine/Nephrology, Baylor College of Medicine, Houston, TX; [2]Nephrology, Guangdong General Hospital, Guangzhou, Guangdong, China.*

We have previously shown that pharmacological inhibition of Rho kinase (ROCK) activity ameliorates progression of diabetic nephropathy (DN). In the current study, we used a genetic approach to further examine the specific role of ROCK1 in the progression of DN. We observed a significant reduction of the urine albumin excretion and the mesangial matrix expansion in ROCK1$^{-/-}$ STZ-induced diabetic mice and ROCK1$^{-/-}$ *db/db* double gene knockout mice compared with wild-type (*wt*) diabetic mice. Furthermore, ROCK1$^{-/-}$ diabetic mice demonstrated a significant decrease in glomerular apoptosis compared to *wt* diabetic mice. The reduced frequency of apoptosis in ROCK1$^{-/-}$ diabetic mice correlated with a significant reduction in mitochondrial reactive oxygen species (ROS) and mitochondrial fragmentation as assessed by electron paramagnetic resonance spectroscopy and aspect ratio assay. *In vitro*, using a domain negative (dn) or constitutively activated (ca) mutants of ROCK1, we found that ca-ROCK1 significantly increased mitochondrial fragmentation, mitochondrial ROS generation, and apoptosis in podocytes and mouse kidney vascular endothelial cells. Interestingly, the use of dn-ROCK1 or si-ROCK1 reversed the increased mitochondrial fragmentation, mitochondrial ROS production and apoptosis induced by high glucose (25 mM). We further observed that ROCK1 modulated the mitochondrial fragmentation by phosphorylating the dynamin-related protein 1(Drp1) at S637. Our data indicate that deletion of ROCK1 ameliorates progression of DN by improving hyperglycemia-induced mitochondrial fragmentation.

Disclosure of Financial Relationships: nothing to disclose

**F-FC267**

**Bone Marrow Derived Endothelial Progenitor Cells Ameliorate Experimental Diabetic Nephropathy: Therapeutic and Mechanistic Implications** Yanling Zhang, Darren A. Yuen, Andrew Advani, Kim Connelly, Richard E. Gilbert. *St. Michael's Hospital, Toronto, Canada.*

Diabetic nephropathy is a leading cause of chronic kidney disease that continues to progress in a substantial proportion of patients despite the widespread use of agents that block the renin-angiotensin system. Given the reported beneficial effects of cell therapy in acute kidney injury, we considered whether bone marrow-derived progenitor cells, known to be reduced in patients with either diabetes or CKD, might also be useful in diabetic nephropathy. Methods: We used a well-established model of type 2 diabetic nephropathy, the db/db mouse, that develops albuminuria, mesangial expansion, glomerular hypertrophy and tubular apoptosis/atrophy. Eight week old BKS.Cg-m +/+ Leprdb/J (db/db) mice were randomised to receive a single injection of saline or 0.5 x $10^6$ endothelial progenitor cells (EPCs), administered intravenously. Cells were obtained from the bone marrow of db/m mice and cultured according to well-established techniques to yield a population enriched for so-called, early outgrowth EPCs. Outcome parameters, assessed 4 weeks after cell infusion, included urinary albumin, mesangial expansion, glomerular hypertrophy and tubular apoptosis. Results: Untreated db/db mice underwent a progressive increase in urinary albumin, mesangial sclerosis, glomerular hypertrophy and tubular epithelial cell apoptosis. In contrast, and without affecting blood glucose, EPC treatment not only attenuated the rate of rise in albuminuria but also significantly decreased mesangial type IV collagen deposition, glomerular enlargement, tubular basement membrane thickening and the number of apoptotic tubular epithelial cells (all p<0.05). In tracking experiments, exogenous EPCs were found predominantly within the reticulo-endothelial system, rather than in the kidney. Conclusions: This study shows firstly that early-outgrowth, culture modified, bone marrow derived cells (EPCs) preserve kidney structure and function in experimental diabetic nephropathy. Additionally, the benefits of this cell-type appear not to be a consequence of engraftment into the kidney, but rather suggest a systemic effect, as described in other disease settings.

Disclosure of Financial Relationships: nothing to disclose

**F-FC268**

**Inhibition of the Epidermal Growth Factor Receptor Preserves Podocytes and Attenuates Albuminuria in Experimental Diabetic Nephropathy** Andrew Advani,[1] Alison Joy Cox,[2] Yuan Zhang,[2] Richard E. Gilbert,[1] Darren J. Kelly.[2] *[1]Department of Medicine, St. Michael's Hospital, Toronto, ON, Canada; [2]Department of Medicine, St. Vincent's Hospital, Melbourne, Victoria, Australia.*

Epidemiological studies suggest an association between early renal enlargement and the subsequent development of nephropathy in patients with diabetes. The epidermal growth factor (EGF)-EGF receptor (EGFR) system plays a pivotal role in mediating renal hypertrophy, where it may act to regulate cell growth and proliferation and also to mediate the actions of angiotensin II through transactivation of the EGFR. The present study sought to investigate the effect of long-term (16 weeks) inhibition of the EGFR tyrosine kinase in a model of diabetes, the diabetic TGR(mRen-2)27 rat, that is characterized by angiotensin II dependent hypertension. Treatment of TGR(mRen-2)27 rats with the EGFR inhibitor, PKI 166 (Novartis), attenuated the increase in kidney size (kidney weight:body weight [%] control 0.55±0.02, diabetes 1.13±0.04, diabetes + PKI 166 1.01±0.03 [p<0.01]), glomerular hypertrophy ([volume x$10^6$μm$^3$] 1.18±0.06, diabetes 1.87±0.11, diabetes + PKI 166 1.41±0.05 [p<0.001]) and albuminuria (AER [mg/day, geometric mean ×/÷ tolerance factor] control 0.90×/÷1.39, diabetes 12.88×/÷1.42, diabetes + PKI 166 3.37×/÷1.36 [p<0.01]) that occurred with diabetes. The reduction in albuminuria in diabetic TGR(mRen-2)27 rats treated with PKI 166 was associated with preservation of the number of glomerular cells staining positively for the podocyte marker, WT1 (p<0.01). WT1 immunostaining inversely correlated with glomerular volume in diabetic rats ($r^2$=0.35, p<0.05). In contrast to agents that block the renin-angiotensin system (RAS), EGFR inhibition had no effect on either the quantity of mesangial matrix or the magnitude of tubular injury in diabetic animals. Taken together, these observations indicate that inhibition of the tyrosine kinase activity of the EGFR attenuates kidney and glomerular enlargement in association with podocyte preservation and reduction in proteinuria in diabetes. Targeting the EGF-EGFR pathway may represent a therapeutic strategy for patients who continue to progress despite RAS-blockade.

Disclosure of Financial Relationships: nothing to disclose

**F-FC269**

**Bone Morphogenetic Protein (BMP) Signaling Inhibitors for the Treatment of Anemia Due to Hepcidin Excess** Jodie L. Babitt, Elena Corradini, Delphine Meynard, Chia Chi Sun, Herbert Y. Lin. *Program in Membrane Biology, Nephrology Division, Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA.*

Dysregulated iron homeostasis contributes to the anemia of chronic kidney disease (CKD). In particular, many CKD patients have functional iron deficiency or reticuloendothelial cell iron blockade, characterized by low levels of circulating iron that limit erythropoiesis despite normal or high body iron stores. Recent research suggests that hepcidin excess may play a role in the dysregulated iron homeostasis and anemia of CKD. A peptide hormone secreted by the liver, hepcidin downregulates the iron exporter ferroportin, thereby inhibiting iron release into the bloodstream from dietary sources and body storage

sites. Hepcidin may accumulate in CKD patients due to reduced renal clearance and/or stimulation by inflammatory cytokines. Here, we explore the development of hepcidin lowering agents as a new therapeutic strategy to treat dysregulated iron homeostasis and anemia due to hepcidin excess.

We recently demonstrated that the BMP signaling pathway plays a key role in regulating hepcidin expression and systemic iron balance. Mutations in the BMP co-receptor hemojuvelin (HJV) lead to hepcidin deficiency and the iron overload disorder hereditary hemochromatosis. BMP ligands transcriptionally upregulate hepcidin expression and reduce serum iron levels, while BMP inhibitors decrease hepcidin expression, mobilize iron stores, and increase serum iron in normal mice. Notably, BMP inhibitors selective for BMP6 are the most effective hepcidin lowering agents *in vivo*, and *Bmp6* null mice develop hepcidin deficiency and iron overload similar to the phenotype of *Hjv* null mice, suggesting that BMP6 is a major endogenous ligand for hepcidin regulation *in vivo*. Here, we demonstrate that the small molecule BMP inhibitor LDN-193189 and a neutralizing BMP6 antibody inhibit hepcidin expression, mobilize iron stores, increase serum iron, and improve anemia in two different animal models of anemia due to hepcidin excess. These data support the possible utility of BMP inhibitors as hepcidin lowering agents to treat iron homeostasis disorders such as anemia of CKD.

Disclosure of Financial Relationships: Ownership: Ferrumax Pharmaceuticals.

### F-FC270

**Erythropoiesis Sustained 12 Months by the EPODURE Biopump in Patients with Chronic Kidney Disease: Further Results of Phase I/II Proof of Concept Trial** Anatole Besarab,[1] Allen R. Nissenson,[2] Doron Schwartz,[3] Andrew L. Pearlman,[4] Philip Ng,[5] Michal Elhalel.[6] *[1]Henry Ford Hospital, Detroit, MI; [2]UCLA School of Medicine, LA, CA; [3]Sourasky Medical Center, Tel Aviv, Israel; [4]Medgenics, Inc., Misgav, Israel; [5]Baylor College of Medicine, TX; [6]Hadassah Hospital, Jerusalem, Israel.*

The need for better hemoglobin (Hb) control during EPO therapy is paramount in view of studies linking hemoglobin variability with increased mortality, and concerns from supraphysiologic erythropoietin concentrations. Sustained delivery of EPO within the therapeutic window could reduce these risks and increase patient compliance. We are developing EPODURE to provide > 6 months of sustained EPO delivery from a single treatment. Autologous 30mm x 2mm dermis core biopsies excised from the patient's skin under local anesthetic are converted in days into "biopump" tissue EPO production units by introducing the EPO gene into cells of the intact explant. After a week the requisite dose is given by reimplanting the explants units subcutaneously into the patient. Dose can be varied by ablation or addition of more units.

We reported (ASN 2008) the first month's results of the first two patients following treatment using low dose EPODURE Biopumps delivering 20 IU/kg/day in an open label, single-center, Phase I-II study in anemic CKD patients.

We now report results in 7 patients treated up to 12 months: 4 EPO-naïve, 3 EPO-dependent (last EPO injection 4-6 wks before EPODURE treatment). 6 received EPODURE 20 IU/kg/day, 1 received 40 IU/kg/day: no adverse events reported to date. In 3 of 3 EPO-dependent, Hb decline following ESA cessation was reversed, Hb remained between 9.8-11.5 g/dl for > 3-12 months, some 1.7 – 2.4 g/dl above projected nadir. In 2 of 3 EPO-naïve, Hb was increased 0.5 – 0.7 g/dl above baseline and remained between 10-12 g/dl for > 5-7 months. One had minimal response. In the EPO-naïve treated at 40 IU/kg/day Hb remained 10-10.7 g/dl for 4.5 months.Conclusions: a single EPODURE treatment can provide up to 12 months sustained ESA therapy in CKD patients. Now treating additional patients at higher doses, results to be presented.

Disclosure of Financial Relationships: Consultancy: Amgen, Hoffman la Roche, Akebia, Affymax, Rockwell International; Ownership: Vasc AlertResearch Funding: Abbott, Roche, Fibrogen, Luitpold; Honoraria: Affymax, Amgen, ASN, Ash Access Technology, Bioconnect, FALLON MEDICA, FMC, Genentech, HemoSphere, Hoffman la Roche, Hospira, Indiana University, John Hopkins Univ, Luitpold Pharm, Merck and Co, National Kidney Fnd, NKF of Michigan, NKF of Georgia, New York Soc of Nephrology, QUINTILES, Renal Advantage, Rockwell Medical, Scientic Consulting Group (NIH) Soc of Nephrology of Puerto Rico, Speedel, St. Michael's Hosp. (Toronto), St. John's Hosp. (Detroit), Takeda, University of Cincinatti, University of Miami, University of Missouri, VascAlert, Walter Kluger (Publisher) Winthrop Univ., Watson Pharma; Scientific Advisor: Amgen, Affymax, Akebia, Rockwell International.

### F-FC271

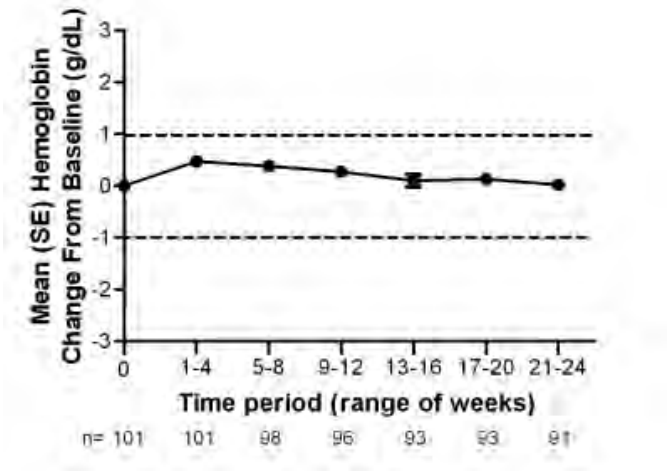
**A Phase 2 Study of the Safety and Effectiveness of Hematide™/Peginesatide for the Maintenance Treatment of Anemia in Patients Who Were Previously Treated with Darbepoetin Alfa** Steven Fishbane,[1] Francesco Locatelli,[1] Simon D. Roger,[1] Edouard R. Martin,[1] Grant S. Runyan,[2] Ping Qiu.[2] *[1]Peginesatide AFX01-202 Study Group; [2]Takeda Global Research & Development Center, Inc., IL.*

Background: Peginesatide is a PEGylated, investigational, peptide-based erythropoiesis-stimulating agent (ESA) that was designed and engineered to stimulate specifically the erythropoietin receptor that governs erythropoiesis.

Objective: To demonstrate that once-monthly peginesatide can maintain hemoglobin (Hb) levels in patients after conversion from darbepoetin alfa.

Methods: This phase 2 study included CKD patients who were receiving maintenance treatment with darbepoetin alfa and were either on hemodialysis (HD) or not on dialysis (CKD-ND). Starting peginesatide doses were determined with the use of a tiered weight-based darbepoetin alfa–to-peginesatide conversion table. Patients received Q4W peginesatide treatment for 24 weeks (Titration, Weeks 0-18; Evaluation, Weeks 19-24).

Results: A total of 101 patients were included (HD, n=52; CKD-ND, n=49). Mean (SD) baseline ferritin levels for the HD and CKD-ND groups were 448.1 (323.0) and 206.2 (132.9) ng/mL, respectively. Mean change in Hb from baseline by 4-week interval is presented in the Figure. Across both groups, 70.7% of patients maintained mean Hb within target (10-12 g/dL) during Evaluation. The most common adverse event (AE) was diarrhea (13.9% of patients). Four patients withdrew from the study because of AEs; 1 patient withdrawal was considered drug related (nausea, vomiting, decreased appetite).




Conclusions: In this study, once-monthly peginesatide treatment maintained mean Hb levels in CKD patients after converting from darbepoetin alfa, and the safety profile of peginesatide appeared consistent with those of marketed ESAs.

Disclosure of Financial Relationships: Consultancy: Roche, WatsonResearch Funding: Affymax, Takeda, Dynavax, Luitpold, Rockwell; Honoraria: AMAG, Abbott; Scientific Advisor: Affymax, AMAG, Rockwell.

### F-FC272

**Use of the Mayo Clinic Anemia Management System (MCAMS) Decreases Darbepoetin Use by 30% in Chronic Hemodialysis (CHD) Patients** James T. McCarthy,[1] James L. Rogers,[2] Craig L. Hocum,[1] Edward J. Gallaher,[2] Stephen F. Gudgell,[1] Robert C. Albright.[1] *[1]Division of Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [2]Advance Management Group, Rochester, MN.*

MCAMS is a proprietary application to determine therapeutic dosing regimens (TDR) for erythropoietic stimulating agents (ESA's) in CHD patients to keep Hgb in a target range. MCAMS determines TDR using an individual's historical Hgb response, physiologic and pharmacokinetic parameters, and conditions such as hemorrhage or illness. In 2007-2009, darbepoetin, given IV once weekly, was the only ESA used by Mayo Clinic Dialysis Services (MCDS). In 2007 (Control=C), ESA was administered following a protocol based on 2006 NKF K/DOQI Guidelines with target Hgb of 10.0-12.9 gm/dL. MCAMS was validated in 2007 by comparing actual Hgb values to those predicted by MCAMS, using doses of darbepoetin given with the 2007 protocol. In 2008 (Transition=T), MCAMS was used in a single facility of 60 patients. In 2009 (Implementation=I), MCAMS was introduced into all other MCDS facilities. Monthly, we recorded darbepoetin use, and the percentage of MCDS patients with Hgb values of 10.0-12.9. The years 2007-2009 were divided into 6 month intervals (1=Jan-Jun; 2=Jul-Dec.), and the average monthly values for each 6 month period was compared.

| **Darbepoetin** | **2007** | **2007** | **2008** | **2008** | **2009** | **2009** |
|---|---|---|---|---|---|---|
| **Administered** | **C-1** | **C-2** | **T-1** | **T-2** | **I-1** | **I-2** |
| **Total in MCDS (mcg)/ month** | 93214± 7783 | 94544± 10403 | 80553± 9011 a | 83985± 8983 | 74668± 6742 a | 61322± 7418 b c |
| **mg/patient/ month** | 301±25.5 | 300±29.6 | 251±24.2 a | 258±28.5 a | 228±21.2 b | 183±19.4 b c |
| N - all MCDS patients | 310 | 315 | 320 | 325 | 327 | 334 |
| **% of patients with Hgb 10.0-12.9** | 62.0±3.0% | 63.1±4.6% | 69.9±3.9%a | 70.8±3.3%a | 72.5±4.0%a | 77.9±2.0%b |

Mean ± SD. T-test. a=p<0.05 vs. C-1 & C-2; b=P <0.001 vs. C-1 & C-2; c=P <0.001 vs. I-1.

MCAMS decreased darbepoetin use by over 30% in MCDS and increased the percent of patients achieving Hgb of 10.0-12.9 gm/dL. MCAMS will be an important tool for dialysis providers as CMS introduces bundling of dialysis payments in 2011.

Disclosure of Financial Relationships: nothing to disclose

**F-FC273**

**Clearance of Circulating Hepcidin during Haemodialysis and after Transplantation** Damien Ashby,[1] Mitul Palan,[1] Georgina Henrietta Aldous,[1] Mark Busbridge,[2] Kevin G. Murphy,[2] Stephen R. Bloom,[2] Neill D. Duncan,[1] Frederick W. K. Tam,[1] Peter Choi.[1] *[1]Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom; [2]Investigative Medicine, Imperial College, London, United Kingdom.*

The hepatic peptide hepcidin is the master regulator of iron absorption and transport. Inappropriate elevation of hepcidin in renal failure is thought to contribute to anaemia and erythropoietin resistance. Reduced levels of hepcidin have been observed following haemodialysis, but the factors influencing hepcidin clearance are unknown.

Plasma hepcidin was measured by radioimmunoassay in a group of haemodialysis patients before and after a single dialysis session, and in a group of patients undergoing renal transplantation.

In 96 haemodialysis patients (aged 23-89) median plasma hepcidin was 138ng/ml (IQR 82-175ng/ml), and levels were strongly correlated with ferritin (R=0.531, p<0.001). Significant hepcidin removal occurred during haemodialysis, with a median reduction of 53.4% (IQR 33.4-64.8%, p<0.001). By multiple linear regression, hepcidin reduction was found to be independently predicted by albumin, litres processed during dialysis and weight (table 1). There was no correlation with Kt/V, and neither was any significant relationship between hepcidin reduction and high vs low membrane flux observed (45.9 vs 48.7%, p=0.6).

Predictors of hepcidin reduction

| | Adj R sq | Beta | Sig |
|---|---|---|---|
| Albumin | 0.272 | 0.355 | <0.001 |
| Litres | 0.309 | 0.340 | 0.001 |
| Weight | 0.366 | -0.281 | 0.005 |

In 4 patients undergoing renal transplantation, plasma hepcidin levels were measured before, and 2-4 weeks after surgery. Hepcidin levels after transplantation were lower in all patients (mean reduction 25.2%, p=0.02) despite higher ferritin levels (mean increase 155%).

Substantial reductions in hepcidin are achieved by haemodialysis or renal transplantation. Clearance of hepcidin by haemodialysis is related to litres processed during dialysis and weight, but not to small solute clearance as measured by Kt/V. Hepcidin reduction may be an important therapeutic target in renal patients.

Disclosure of Financial Relationships: nothing to disclose

**F-FC274**

**Implementation of a Novel IV Iron for Iron Deficiency Anemia in Patients Undergoing In-Center Hemodialysis (HD)** Brigitte Schiller, Andrea Neitzer, Sheila Doss, Sumi Sun. *Satellite Healthcare, Mountain View, CA.*

Feraheme (ferumoxytol) is a new iron product recommended as initial 510 mg rapid IV push followed by a repeat after 3-8 days to treat iron deficiency anemia in patients with CKD. Ferumoxytol was introduced in an iron maintenance protocol to be given after routine monthly lab draws as a one time 510 mg IV push administration if TSAT < 30% OR ferritin < 500 ng/mL in both incident and prevalent patients on in-center HD. Contraindications were iron hypersensitivity, ferritin ≥ 800 ng/mL, TSAT ≥ 50% or Hb > 12.5 g/dL.

A total of 3,222 patients were given 7,134 doses of ferumoxytol following the Dec 2009 monthly labs until the end of May 2010. 1.3% of patients had one or more adverse events (38 gastrointestinal symptoms, 8 allergic reactions, and 7 hypotension), reflecting a rate of 0.6% (41/7,134 doses). Four serious adverse events occurred - 2 anaphylactoid reactions and 2 adjudicated as unrelated (sepsis, stroke).

The 2,822 patients treated exclusively with ferumoxytol (no other iron products) required an average of 2.2 doses (range 1-7) over 6 months. 33% of patients received one dose only, 33% two doses, 20% three, and 14% 4+ doses. Laboratory results (mean ± SD) for these patients during the 5 months prior to and after ferumoxytol introduction are shown:

| | Pre-ferumoxytol | Ferumoxytol |
|---|---|---|
| Hb (g/dL) | 11.7 ± 0.9 | 11.5 ± 0.9 |
| TSAT (%) | 28.5 ± 9.4 | 28.6 ± 11.4 |
| Ferritin (ng/mL) | 697 ± 212 | 715 ± 264 |

Clinical performance measures showed that Hb target (10-12g/dL) was reached in 67%, TSAT (20-50%) in 78%, and ferritin (200-800 ng/mL) in 53% of the patients while on ferumoxytol therapy.

A simple iron replacement protocol adjusted to TSAT or ferritin with 510 mg ferumoxytol given once after monthly blood work achieved stable iron stores and confirmed the drug's published safety profile in this large patient cohort. A favorable utilization profile was noted; evaluation of its effect on ESA usage is pending due to recent anemia protocol changes. Less frequent and rapid IV push administration of iron with this protocol will allow nurses to spend more time on direct patient care.

Disclosure of Financial Relationships: Scientific Advisor: Affymax, Inc.; Other Relationship: Spouse employee at DaVita, Inc.

**F-FC275**

**Hepcidin Levels in Prevalent Non-Selected Hemodialysis Patients** Peter F. Barany,[1] Peter Stenvinkel,[1] Olof Heimburger,[1] Soheir Beshara.[2] *[1]Renal Medicine, CLINTEC, Karolinska Institutet, Stockholm, Sweden; [2]Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden.*

Hepcidin (Hep) is an important regulator of iron metabolism. Assays for assessment of plasma levels of Hep have recently been introduced but the role of Hep assays in clinical research and practice has not been established.

Hep-25 was measured in 219 prevalent hemodialysis (HD) patients using an ELISA method from Bachem (USA). Information about anemia treatment (210 treated with ESA), medications, comorbidity were recorded. Hb and ferritin, hypochromic RBC, CRP and IL-6 levels measured before a HD session. Results are presented as median (25-75 percentiles).

Hep levels in the HD patients, 111 (61-174) ng/ml, were higher than in healthy subjects (men 10-75 ng/ml, women 5-30 ng/ml). Age, sex and comorbidity did not affect Hep. Male patients with statin treatment had lower hepcidin 85 (31-144) ng/ml than those without statins 121 (73-218) ng/ml (p<0.01). Hep correlated strongly with ferritin levels (Spearman rho 0.58, p<0.001) but not with other anemia or inflammatory variables. In Table 1 the patients are grouped according to tertiles of Hep levels.

Anemia and inflammatory variables according to Hep tertile groups

| | Tertile 1 | Tertile 2 | Tertile 3 | p |
|---|---|---|---|---|
| Hep, ng/ml | 38 (22-61) | 110 (96-129) | 221 (170-267) | |
| Hb, g/dL | 11.9 (10.9-12.7) | 11.8 (11.0-12.7) | 12.0 (11.3-12.7) | 0.7 |
| Ferritin, µg/L | 216 (120-380) | 422 (296-610) | 630 (427-834) | <0.001 |
| Hypochromic RBC, % | 1.6 (0.75-4.1) | 1.1 (0.5-2.7) | 1.3 (0.8-4.0) | 0.12 |
| CRP, mg/L | 6.4 (2-18) | 6.3 (2.6-19) | 7.4 (3.0-24.3) | 0.5 |
| IL-6, pg/ml | 8.7 (4.7-14.5) | 8.8 (5.5-25.7) | 8.2 (5.5-47.7) | 0.8 |
| ESA, IU/kg per week | 168(106-240) | 119, (71-265) | 121 (78-209) | <0.05 |

Median (25-75 percentiles)

Unexpectedly, patients with the lowest Hep had the highest doses of ESA. However, ESA may down-regulate Hep expression. Several other factors such as iron overload, BMP, inflammation and anemia have been implicated in the regulation of Hep expression and the complexity of this signaling network affects the clinical usefulness of Hep assays.

Disclosure of Financial Relationships: Honoraria: Received honoraries for lectures and expert group meetings from Amgen, Roche, Vifor, Pharmacosmos.

**F-FC276**

**Efficacy of an Anemia Management Strategy Targeting Higher Transferrin Saturation (TSat) and Ferritin Levels Using Ferumoxytol** Premila Bhat, Joesan A. Gabaldon, Jodumutt Ganesh Bhat. *Atlantic Dialysis Management Services, LLC, Ridgewood, NY.*

Ferumoxytol, a novel intravenous iron formulation, was approved for treatment of anemia in chronic kidney disease (CKD) in June 2009. Ferumoxytol has been shown to improve iron indices in CKD and ESRD patients in comparison with oral iron. We report here our experience implementing an iron management protocol targeting TSat≥30% and ferritin≥500ng/mL using ferumoxytol. ESRD patients receiving hemodialysis at a regional chain of for-profit dialysis centers were treated with ferumoxytol (510 mg injection) if they had Hemoglobin<13 g/dL; TSat<30% or Ferritin<500ng/mL; and no documented allergies or intolerance to other intravenous irons. Prior to implementation of the ferumoxytol-based strategy, patients were treated with iron sucrose or sodium ferric gluconate at the discretion of their nephrologists. Primary outcome measures were changes in Hb and iron indices three months after ferumoxytol therapy was initiated. Secondary outcomes will include change in epoetin alfa dose, total iron dose, and overall costs. During the period from 9/15/2009 through 12/31/2009, 609 patients at 10 dialysis centers started treatment with ferumoxytol and 1379 doses of ferumoxytol were administered at three-month follow-up. Patients receiving ferumoxytol had a mean age of 64.07 years (SD 14.52); 57.7% were male and 42.2% were African-American. Patients were excluded from analysis if baseline or follow-up laboratory data was missing. Significantly improved Hb and iron parameters were observed with the ferumoxytol-based strategy (Table 1). In conclusion, a ferumoxytol-based anemia management strategy targeting TSat≥30% and Ferritin≥500ng/mL resulted in higher mean Hb values.

| | Baseline Mean (SD) | 3-Month Post Baseline (SD) | Change from Baseline (SD) |
|---|---|---|---|
| Hemoglobin (g/dL) (n=529) | 11.00 (1.318) | 11.33 (1.219) | 0.34 (1.662) p<0.0001 |
| Ferritin (ng/mL) (n=515) | 355.63 (267.16) | 541.25 (411.27) | 185.62(332.16) p<0.0001 |
| TSat (%)(n=530) | 23.82 (10.23) | 30.65 (13.07) | 6.83 (15.67) p<0.0001 |

Table 1. Hb and Iron Indices at baseline and 3 months after ferumoxytol.

Disclosure of Financial Relationships: nothing to disclose

**F-FC277**

**The Mayo Clinic Anemia Management System (MCAMS) Improves Achievement of Target Hemoglobin (Hgb) Values and Decreases High Hgb Values in Chronic Hemodialysis (CHD) Patients** James T. McCarthy,[1] James L. Rogers,[2] Craig L. Hocum,[1] Edward J. Gallaher,[2] Stephen F. Gudgell,[1] Robert C. Albright.[1] *[1]Division of Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [2]Advance Management Group, Rochester, MN.*

MCAMS is a proprietary application to determine therapeutic dosing regimens (TDR) for erythropoietic stimulating agents (ESA's) in CHD patients to keep Hgb in a target range. MCAMS determines TDR using an individual's historical Hgb response, with physiologic and pharmacokinetic parameters, and conditions such as hemorrhage or illness. We implemented MCAMS in Mayo Clinic Dialysis Services (MCDS) in 2008-2009. In 2007-2009, darbepoetin, given IV once weekly, was the only ESA used in MCDS. In 2007 (Control=C), ESA was administered according to a protocol based on 2006 NKF K/DOQI Guidelines with target Hgb of 10.0-12.9 gm/dL. MCAMS was validated in 2007 by comparing actual Hgb values to those predicted by MCAMS, using the doses of darbepoetin administered with the 2007 protocol. In 2008 (Transition=T), MCAMS was used in a single facility of 60 patients. In 2009 (Implementation=I), MCAMS was introduced into all other MCDS facilities. Monthly, we recorded the percentage of MCDS patients with Hgb < 10.0; Hgb 10.0-12.9; or Hgb > 12.9. The years 2007-2009 were divided into 6 month intervals (1=Jan-Jun; 2=Jul-Dec), and average monthly Hgb performance for each 6 month period was compared.

| | 2007 | 2007 | 2008 | 2008 | 2009 | 2009 |
|---|---|---|---|---|---|---|
| **Percent Patients** | **C-1** | **C-2** | **T-1 a** | **T-2 a** | **I-1 a** | **I-2 ab** |
| **Hgb < 10.0** | 7.6±0.8% | 6.3±1.2% | 9.2±1.3% | 9.1±1.3% | 11.3±2.4% | 11.1±2.1% |
| **Hgb 10.0-12.9** | 62.0±3.0% | 63.1±4.6% | 69.9±3.9% | 70.8±3.3% | 72.5±4.0% | 77.9±2.0% |
| **Hgb > 12.9** | 30.4±3.2% | 30.6±4.2% | 20.9±3.5% | 20.1±2.3% | 16.2±4.1% | 11.0±1.1% |
| N - all patients | 310 | 315 | 320 | 325 | 327 | 334 |

Mean ± SD. T-test. a=all p<0.05 vs. C-1 & C-2; b=all p<0.01 vs. T-1 & T-2

MCAMS decreased patients with Hgb > 12.9 gm/dL and increased patients with Hgb in range of 10.0-12.9 gm/dL. The use of MCAMS leads to significant improvement in anemia control when compared to the prior protocol.

Disclosure of Financial Relationships: nothing to disclose

**F-FC278**

**Oncostatin M Receptor β and Cysteine/Histidine-Rich 1 as Candidate Biomarkers of Erythropoietin Response** Michael Brier,[1,2] Michael Merchant,[2] Adam Gaweda,[2] Brad H. Rovin,[3] Jon B. Klein.[1,2] *[1]Robley Rex VA Medical Center, Louisville, KY; [2]University of Louisville, Louisville, KY; [3]The Ohio State University, Columbus, OH.*

Current biomarkers of hemoglobin (Hb) response to erythropoietic agents (ESA) primarily are measures of inflammation and iron availability. While these are important factors in modifying an individual's response to an ESA, they do not address all aspects of poor response. We tested the hypothesis that peptides present in the serum of hemodialysis patients are predictive of ESA response. Our objective was to identify biomarkers of ESA response. Serum from 15 good responders and 20 poor responders was collected and evaluated. Ninety-one candidate biomarker targets were identified and characterized using mass spectrometry (MS). Analysis of tandem MS data provided partial amino acid sequence information of 17 different peptides for 16 peptide masses whose serum abundance significantly differed between poor and good responders for further analysis.

| Peptide | m/z | p value | ROC |
|---|---|---|---|
| OSMR | 1273 | <0.0001 | 0.95 |
| OSMR | 1549 | <0.0001 | 0.96 |
| OSMR | 1664 | <0.0001 | 0.99 |
| CYHR1 | 1488 | <0.0001 | 0.089 |
| Fibrinogen B | 1552 | 0.005 | 0.20 |
| Factor XIII | 1210 | 0.015 | 0.25 |
| Complement C3 | 1504 | 0.07 | 0.26 |

ROC > 0.5 poor response, < 0.5 good response

The analysis concluded the 3 serum peptides associated with poor erythropoietin response were derived from the oncostatin M receptor β chain (OSMRβ). The 13 serum peptides associated with good erythropoietin response were derived from fibrinogen α chain, fibrinogen β chain, coagulation factor XIII A chain, complement C3 and cysteine/histidine rich 1(CYHR1). Poor response was most strongly associated with the largest molecular weight OSM fragment (ROC=0.99) while good response was most strongly associated with CYHR1 (ROC=0.91 (1-0.089)). Immunoblot experiments demonstrated that intact OSMRβ and CYHR1 abundance significantly differed between good and poor responders. Two novel biomarkers of ESA response are presented along with the first published association of CYHR1 and biological activity.

Disclosure of Financial Relationships: Consultancy: Fresenius USA Manufacturing, Inc., Hospira; Honoraria: Amgen.

**F-FC279**

**Insulin Is a Positive Modulator of NCC Activity through a PI3K Dependent Mechanism** María Chávez Canales,[1] Norma Hilda Vázquez,[1] Juan Pablo Arroyo,[1] Maria Castañeda-Bueno,[1] Benjamin S. Ko,[2] Norma Bobadilla,[1] Robert S. Hoover,[2] Gerardo Gamba.[1] *[1]Molecular Physiology Unit, INNSZ-INCICH-IIB, UNAM, Mexico City; [2]University of Chicago, Chicago.*

One potential mechanism that has been advanced to explain the obesity-induced hypertension is the effect that insulin may have upon renal salt reabsorption. In this study we assessed the effect of insulin on the activity of the renal NaCl cotransporter, NCC, using the heterologous expression system of *Xenopus* oocytes and mDCT15 cells. NCC activity was measured as the thiazide-sensitive tracer $^{22}Na^+$ uptake. Three days after injection of NCC cRNA, we observed that NCC activity was increased by 2 or 3-fold when oocytes were exposed to insulin for 15 minutes before the uptake. The positive effect of insulin on NCC activity was also observed in mDCT15 cells endogenously expressing NCC. In oocytes, addition of wortmannin, an inhibitor of phosphatydil-inositol 3-kinase (PI3K) completely abrogated the positive effect of insulin on the transporter's activity, without affecting the basal function. The effect of insulin was also abolished by the specific AKT inhibitor IV. MAP or mTOR kinases inhibitors had no effect. Coinjection of NCC cRNA with wild type or catalytically inactive WNK4, WNK3, or SPAK cRNA did not affect the activation of NCC by insulin. Western blot analysis using anti-FLAG monoclonal antibodies with proteins extracted from oocytes revealed that FLAG-NCC level of expression was not affected by insulin. We also assessed the phosphorylation of NCC at residue T58 using a specific phosphoantibody. Although NCC phosphorylation was increased by co-injection with WNK3 or by intracellular chloride depletion, no increased signal was observed after insulin treatment. We conclude that insulin is a powerful activator of NCC in *Xenopus* oocytes by its threonine/serine phosphorylation mediated by PI3K-AKT pathway. However, the WNK4-SPAK-T58 phosphorylation does not seem to be involved. The insulin effect on NCC is also present in mDCT15 cells. Our observations have implications to explain the association between obesity and diabetes mellitus with arterial hypertension.

Disclosure of Financial Relationships: nothing to disclose

**F-FC280**

**SPAK Knockout Causes a Gitelman-Like, but Not Bartter-Like, Phenotype** James A. McCormick,[1] Joshua H. Nelson,[1] Kerim Mutig,[2] Turgay Saritas,[2] Sebastian C. Bachmann,[2] Eric J. Delpire,[3] David H. Ellison.[1] *[1]Division of Nephrology & Hypertension, Oregon Health & Science University, Portland, OR; [2]Department of Anatomy, Charité Universitätsmedizin, Berlin, Germany; [3]Department of Anesthesiology, Vanderbilt University School of Medicine, Nashville, TN.*

SPAK (Ste20-related Proline Alanine rich Kinase) is a protein kinase that phosphorylates and activates members of the cation chloride cotransporter family (NCC, NKCC2, NKCC1). SPAK is expressed in neurons and in many epithelia. In kidney, its expression is highest along the thick ascending limb (TAL) and distal convoluted tubule (DCT). WNK (With No Lysine (K)) kinases phosphorylate SPAK, which would be expected to phosphorylate and activate transporters along the TAL and DCT. To test the physiological significance of SPAK interaction with renal ion transporters, we evaluated the renal phenotype of SPAK knockout (KO) mice. Immunofluorescence showed that SPAK was absent along both the TAL and DCT in KO mice. Baseline plasma electrolytes were similar in wild type (WT) and KO siblings, but the urine calcium/creatinine ratio was significantly lower (60% of WT; p<0.05). Dietary NaCl-loading stimulated kaliuresis and reduced serum $K^+$ concentration to a similar extent in both groups, but urinary calcium excretion increased only in WT mice. Systolic blood pressures did not differ between WT and KO mice on control diet; dietary NaCl restriction reduced blood pressure in KO (by 10mmHg) but not WT mice. Total NCC protein abundance was 60% lower in KO than in WT animals (p<0.001); the level of phosphorylated NCC (T53 and S71) was 80% lower in KO than WT mice, but cellular distribution of NCC did not differ. The fractional volume of the DCT was reduced by 46% in KO mice. The abundance of total NKCC2 was not different in KO versus WT mice, but phosphorylated NKCC2 was markedly increased. These results show that SPAK deletion abrogates calciuresis induced by extracellular fluid volume expansion, despite preserved flow-dependent $K^+$ excretion. In summary, SPAK has a non-redundant role in arterial pressure homeostasis by activating NCC in the DCT, but surprisingly they do not confirm such a role with respect to NKCC2.

Disclosure of Financial Relationships: nothing to disclose

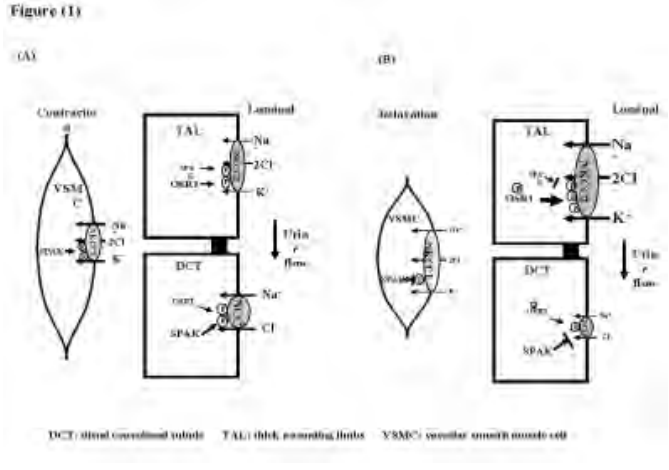
**F-FC281**

**SPAK Knockout Mice Manifest Gitelman's Syndrome and Impaired Vasoconstriction** Sung-Sen Yang,[1] Pauling Chu,[1] Shinichi Uchida,[2] Sei Sasaki,[2] Shih-Hua P. Lin.[1] *[1]Division of Nephrology, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; [2]Department of Nephrology, Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan.*

Polymorphisms in the STK39 gene encoding SPAK [STE20 (sterile 20)/SPS1-related proline/alanine-rich kinase] have been identified as hypertension susceptibility genes in humans. SPAK interacts with WNK 1 and 4 to regulate ionic transporters, $Na^+$-($K^+$)-(2)$Cl^-$ cotransproter [N(K)CC]. Mutations in WNK1/4 and N(K)CC can cause changes in blood pressure and dyskalemia in humans. To elucidate the physiologic role of SPAK *in vivo*, we generated and analyzed SPAK-null mice by targeting disruption of exons 9 and 10 of SPAK. Blood pressure, aortic contractility, blood and urine electrolytes and biochemistries,

and relevant protein expression in the kidneys and aortic tissues were examined. Compared with $SPAK^{+/+}$ mice littermates, $SPAK^{+/-}$ mice exhibited hypotension without significant electrolyte abnormalities while the $SPAK^{-/-}$ mice not only exhibited hypotension but also recapitulated Gitelman's syndrome (GS) with hypokalemia, hypomagnesemia, and hypocalciuria. In the kidney tissues of $SPAK^{-/-}$ mice, the expression of total and phosphor (p-)NCC was markedly decreased but that of p-OSR1, total NKCC2 and p-NKCC2 was significantly increased. Blunted response to thiazide but normal response to furosemide was also observed in $SPAK^{-/-}$ mice. In aortic tissues, both $SPAK^{+/-}$ and $SPAK^{-/-}$ mice had impaired response to phenylephrine (a selective $\alpha_1$-adrenergic agonist) and bumentanide (a NKCC1 inhibitor). While total NKCC1 expression was increased, p-NKCC1 was decreased. Thus, SPAK-null mice have defects of NCC in the kidneys and NKCC1 in the blood vessels, leading to hypotension through renal salt wasting and vasodilatation.SPAK may be a promising target for anti-hypertensive therapy.

Figure (1)




Disclosure of Financial Relationships: nothing to disclose

**F-FC282**

**Impaired $Na^+$ and $K^+$ Excretion in Inducible Renal Tubule-Specific Nedd4-2 Knockout Mice** Caroline Ronzaud,[1] Dominique Loffing,[2] Baoli Yang,[3] John B. Stokes,[4] Robert Koesters,[5] Johannes Loffing,[2] Olivier Staub.[1] *[1]Department of Pharmacology & Toxicology, Lausanne University, Switzerland; [2]Institute of Anatomy, Zurich University, Switzerland; [3]Obstetrics and Gynecology, Iowa University; [4]Department of Internal Medicine, Iowa University; [5]INSERM UMRS 702, UPMC, Tenon Hospital, Paris, France.*

Generation of Nedd4-2 total knockout (KO) mice revealed that Nedd4-2 inactivation leads to $Na^+$ retention and hypertension. To determine the role of renal Nedd4-2 in mediating salt-sensitive hypertension *in vivo*, inducible renal tubule-specific Nedd4-2 KO mice were generated by combining the TetOn and CreLoxP systems. Pax8-rtTA transgenic mice expressing the reverse tetracycline (Tet)-dependent transactivator (rtTA) along all renal tubules were bred with TRE-LC1 transgenic mice expressing the Cre recombinase under the control of a rtTA-response element (TRE). Double transgenic Pax8-rtTA/TRE-LC1 mice (Pax8/LC1) allowing Tet-inducible renal tubule-specific Cre-mediated recombination were bred with mice homozygous for the Nedd4-2 floxed allele to obtain the mutants ($Nedd4\text{-}2^{fl/fl}$/Pax8/LC1). Controls ($Nedd4\text{-}2^{fl/fl}$/Pax8 or $Nedd4\text{-}2^{fl/fl}$/LC1) and mutants were treated with doxycyclin (Dox, 2mg/ml) for 11d and fed with high-$Na^+$ diet for 8d. Western-blot on total kidney lysates revealed complete loss of Nedd4-2 in Dox-treated mutants. Daily urine volume was increased in mutants, whereas plasma aldosterone, urine $Na^+$ and absolute urinary $Na^+$ excretion were decreased. Interestingly, mutants showed impaired urinary $K^+$ excretion, paralleled with hyperkalemia. Western blot on kidneys showed increased α-, β-, γ-ENaC, and NCC protein abundance in mutants. In addition, immunofluorescence revealed increased ROMK protein expression in distal convoluted tubules and connecting tubules in mutants, which might be secondary to the hyperkalemia. Taken together, these data suggest that inducible Nedd4-2 ablation in renal tubules of adult mice leads to $Na^+$ retention, likewise via ENaC and NCC over-activation. The impaired $K^+$ excretion in mutants suggests that Nedd4-2 is important for maintaining $K^+$ balance. The mechanisms behind this regulation remain to be elucidated.

Disclosure of Financial Relationships: nothing to disclose

**F-FC283**

**Differential Regulation of NCC and ENaC by the Sgk1/Nedd4-2 Pathway** Juan Pablo Arroyo,[1] Dagmara Lagnaz,[2] Caroline Ronzaud,[2] Norma Hilda Vázquez,[1] Gerardo Gamba,[1] Olivier Staub.[2] *[1]INNSZ-INCICH-IIB, UNAM, Mexico; [2]Laussane University, Switzerland.*

Appropriate regulation of renal $Na^+$ transport is essential for control of blood pressure and $Na^+$ homeostasis. Aldosterone stimulates $Na^+$ reabsorption in the aldosterone-sensitive distal nephron (ASDN). It is known that aldosterone increases NCC expression at the protein level by an unknown mechanism, without increasing the NCC gene transcription rate or mRNA levels. In this regard, in the ASDN the ubiquitin-protein ligase Nedd4-2 regulates the sodium channel ENaC: under aldosterone induction, Sgk1 phosphorylates Nedd4-2, thus preventing the interaction of Nedd4-2 with ENaC and avoiding the ubiquitylation and degradation of the channel. In the present study we investigated if NCC is also regulated to this pathway. We obtained evidences that Nedd4-2 is indeed a regulator of NCC. In transfected Hek293 cells, Nedd4-2 co-immunoprecipitated with NCC, stimulated NCC ubiquitylation at the cell surface, and increased its turnover. In *Xenopus laevis* oocytes co-injection of cRNA of NCC with wild-type Nedd4-2, but not the catalytically inactive Nedd4-2, dramatically decreased NCC-induced thiazide-sensitive $^{22}Na^+$ uptake. This inhibition was prevented by Sgk1 in a kinase-dependent manner. In contrast to the regulation of ENaC by Sgk1/Nedd4-2, the modulation of NCC by Nedd4-2 is PY independent and mutation of Nedd4-2 Ser328 either to alanine or to aspartate did not interfere with Sgk1 action on Nedd4-2-NCC, implying a differential regulation of NCC and ENaC by Sgk1-Nedd4-2 pathway. Additionally, NCC expression is up-regulated in an inducible nephron-specific Nedd4-2 knockout mouse model, whereas NCC expression is reduced in Sgk1 knockout mice. These results strongly suggest that NCC activity is controlled by a regulatory pathway involving Nedd4-2 and Sgk1, provides an explanation for the well known aldosterone-induced increase in NCC protein expression and suggest a differential regulation of NCC-ENaC by Sgk1-Nedd4-2 pathway that has implications to understand the modulation of ion transport pathways during high aldosterone states like hyperkalemia.

Disclosure of Financial Relationships: nothing to disclose

**F-FC284**

**Insulin and IGF1 Enhance Endocytosis of ROMK Via a PI3 Kinase-Akt1/SGK1-WNK1 Dependent Pathway** Chih-Jen Cheng,[1,2] Chou-Long Huang.[1] *[1]Medicine, UT Southwestern Medical Center, Dallas, TX; [2]Medicine, Tri-Service General Hospital, NDMC, Taipei, Taiwan.*

WNK1 (with-no-lysine-1) kinase inhibits ROMK channel by stimulating its endocytosis, which contributes to hyperkalemia in patients with pseudohypoaldosteronism type 2 (PHA2) caused by overexpression of WNK1. Members of ACG protein kinase family, Akt1 and SGK1, phosphorylate WNK1 *in vitro*. In this study, we investigate the importance of this phosphorylation in the regulation of ROMK expressed in HEK cells using patch-clamp recording and biochemical studies. We find that serum deprivation increases ("dis-inhibits") whole-cell ROMK current. Application of insulin or insulin-like growth factor IGF1 (at physiological concentrations) after serum deprivation (re)inhibits ROMK within 30 minutes. The inhibition of ROMK by insulin/IGF1 is diminished by pre-treatment with phosphatidylinositol 3 (PI3)-kinase inhibitor wortmannin or by siRNA knockdown of endogenous WNK1, SGK1 or Akt1. The effect of knocking down both Akt1 and SGK1 is greater than that of knocking down Akt1 or SGK1 individually. Western blot analysis using respective residue-specific phospho-antibodies reveals that serum deprivation diminishes phosphorylation of Akt1 at the T-loop and the hydrophobic motif and of WNK1 at a threonine equivalent to T58 of rat WNK1. Addition of insulin thereafter increases respective phosphorylation of Akt1 and WNK1 in a wortmannin-sensitive manner. Overexpression of Akt1, SGK1 or WNK1 inhibits ROMK in serum-containing media. Co-expression with T58A mutant of WNK1 prevents the inhibition of ROMK caused by Akt1 or SGK1. The inhibition of ROMK by SGK1 is prevented by coexpression of a dominant-negative dynamin2 mutant, but not by mutation of S44 of ROMK1, a previously reported phosphorylation site for SGK1. We conclude that phosphorylation of WNK1 at T58 by Akt1/SGK1 enhances its ability to stimulate endocytosis of ROMK. This mechanism allows for regulation of ROMK and renal potassium secretion by PI3 kinase-activating hormones. One such condition is chronic dietary potassium restriction, in which IGF1 is upregulated and renal potassium secretion is inhibited to conserve potassium.

Disclosure of Financial Relationships: nothing to disclose

**F-FC285**

**Hypertension Resistance Polymorphisms in ROMK (Kir1.1) Alter Channel Function by Different Mechanisms** Liang Fang, Dimin Li, Paul A. Welling. *Physiology, University of Maryland School of Medicine, Baltimore, MD.*

The ROMK potassium channel plays a critical role in renal sodium handling. Recent genome sequencing efforts in the Framingham Heart Study offspring cohort (Ji et al, Nature Genet, '08) recently revealed an association between suspected loss-of-function polymorphisms in the ROMK channel and resistance to hypertension, suggesting that ROMK activity may also be a determinant of blood pressure control in the general population. Here, we examine whether these sequence variants do, in fact, alter ROMK channel function and explore the mechanisms. As assessed by two-microelectrode voltage clamp in Xenopus oocytes, 3/5 of the variants (R193P, H251Y and T313FS) displayed an almost complete attenuation of whole-cell ROMK channel activity. Surface antibody binding measurements of external epitope tagged channels and analysis of glycosylation-state maturation revealed that these variants prevent channel expression at the plasmalemma, likely as a consequence of retention in the endoplasmic reticulum. The other variants (P166S, R169H) had no obvious effects on the basal channel activity or surface expression but, instead, conferred a gain in regulated-inhibitory gating. As assessed in giant excised patch-clamp studies, apparent PIP2 binding affinity of the variants was reduced, causing channels to be more susceptible to inhibition upon PIP2 depletion. Unlike the protein product of the major ROMK allele, these two variants are sensitive to the inhibitory affects of G-protein coupled receptor stimulation of phospholipase C (PLC) and PIP2 hydrolysis. In summary, we have found that hypertension resistance sequence variants inhibit ROMK channel function by different mechanisms, providing new insights into the role of the channel in blood pressure control.

Disclosure of Financial Relationships: nothing to disclose

**F-FC286**

**Regulation of BK Channel-Mediated Net K Secretion (JK) by NKCC in the Cortical Collecting Duct (CCD)** Wen Liu,[1] Carlos Schreck,[1] Beth Zavilowitz,[1] Thomas R. Kleyman,[2] Lisa M. Satlin.[1] *[1]Mount Sinai School of Medicine, NY, NY; [2]University of Pittsburgh, Pittsburgh, PA.*

Apical SK/ROMK and Ca2+-activated BK channels mediate baseline and flow-induced JK (FIKS; pmol/min.mm), respectively, in the CCD. BK channels are detected in both principal (PCs) and intercalated (ICs) cells. Although the density of BK channels is greater in ICs than PCs, Na/K-ATPase activity in ICs is considered inadequate to sustain high rates of urinary K secretion. Immunodetectable basolateral 'secretory' Na-K-2Cl cotransporter (NKCC1) is present in ICs in rat medullary CD (Ginns et al, JASN 7:2533, 1996). Furthermore, microdissected rabbit CCDs express mRNA encoding NKCC1 (Liu et al, ASN Renal Week 2009: F-PO1167). To test whether basolateral NKCC in the CCD contributes to BK channel-mediated FIKS, we measured JK and net Na absorption (JNa) in NZW rabbit CCDs (n=3) microperfused in vitro at fast (~5 nl/min.mm) flow rates in the absence and presence of bumetanide (10 μM) added to the bath. Bumetanide did not affect JNa (73.7±4.6 to 70.1±8.5; p=NS), but inhibited FIKS (-15.8±2.1 to -8.4± 3.8; p<0.05); addition of luminal iberiotoxin (50 nM) to these same tubules did not further reduce JK (-6.3 ±2.1, p=NS). To confirm that FIKS requires basolateral Cl, we measured the effect of basolateral Cl removal (replaced with gluconate) on FIKS. Basolateral Cl removal reversibly inhibited FIKS (-22.7±5.6 to -8.2± 0.8; n=5, p<0.05) but, in CCDs perfused at a slow flow rate (~1 nl/min.mm), had no effect on JK (-10.4± 2.2 to -11.8± 1.9; p=NS) or JNa (p=NS). These results suggest that BK channel-mediated FIKS is dependent on a basolateral bumetanide-sensitive Cl-dependent transport pathway, proposed to be NKCC, in the CCD. Studies are underway to more precisely define the cell type to which this cotransporter is localized.

Disclosure of Financial Relationships: nothing to disclose

**F-FC287**

**Basolateral Kir2.3 and Kir 4.1 Form Heteromeric Potassium Channels That Are Disrupted by EAST/SeSAME Syndrome Mutations** Bernardo Ortega, Dimin Li, Boyoung Kim, James B. Wade, Paul A. Welling. *Physiology, University of Maryland School of Medicine, Baltimore, MD.*

Basolateral inwardly rectifying potassium channels (Kir) in the distal nephron are responsible for maintaining a negative membrane potential, essential for salt and mineral reabsorption. Although different Kir channel subunits are expressed in distal segments, the recent discovery that loss-of-function mutations in Kir 4.1 cause profound renal salt wasting (EAST/ SeSAME syndrome, Bockenhauer, NEngl J Med, 2009 and Scholl, Proc Natl Acad Sci, 2009) suggests Kir 4.1 may play an especially dominant role, possibly through assembly with the other Kir subunits. Kir 4.1 interacts with Kir 5.1, but the extent to which it may operate with the other basolateral Kir remains unknown. Here, we explore this issue. We found Kir2.3 co-localizes with Kir4.1 in the mouse distal nephron, and co-immunoprecipitation studies in a heterologous expression system revealed that Kir 2.3 is capable of interacting with Kir 4.1. As studied in Xenopus oocytes, co-expression of Kir 2.3 with Kir4.1 potentiated potassium current density and increased cell surface expression. Indicative of heteromultimeric assembly, a dominant negative mutation in the Kir4.1 potassium-selectivity filter blocked Kir2.3 activity, and a comparable mutation in Kir2.3 inhibited Kir4.1. At the single channel level, Kir2.3/4.1 channels exhibit properties different than Kir2.3 or Kir4.1 alone, more similar to the native 20pS channel in the CCD basolateral membrane. Interestingly, of the 6 described EAST/SeSAME syndrome mutations (R65P, C140R, T164I, A167V, R199X, R297C), 4 of them exerted a partial reduction on Kir4.1/5.1 function (R65P, C140R, T164I, R297C), and 3 of them (R65P, C140R, T164I) exerted a similar or even greater (C140R) dominant negative effect on Kir2.3. In conclusion: 1) basolateral K channels of the distal nephron can be formed through heterotetrameric assembly of Kir4.1/5.1 or Kir4.1/2.3 subunits; 2) Consequently, the inhibitory effects of EAST/SeSAME syndrome mutations can be broadly transmitted throughout the basolateral membrane conductance.

Disclosure of Financial Relationships: nothing to disclose

**F-FC288**

**The Novel Plasminogen Receptor, Plg-$R_{KT}$, Colocalizes with ENaC and Regulates Plasmin-Dependent ENaC Processing** Kevin W. Chen,[1,2] Hongdong Bai,[1,2] Stan Krajewski,[3] Volker Vallon,[1,2] William B. Kiosses,[4] Lindsey A. Miles,[4] Robert J. Parmer.[1,2] *[1]University of California, San Diego; [2]VA San Diego Healthcare System; [3]Sanford-Burnham Medical Research Institute; [4]The Scripps Research Institute, La Jolla, CA.*

Plasminogen (Plg) binding to cells markedly enhances its activation, and concentrates and localizes the proteolytic activity of plasmin on cell surfaces. Recent results suggest a key role for plasmin in the processing and activation of ENaC in nephrotic syndrome, in which increased Plg concentrations are present in urine. We recently identified a novel transmembrane Plg receptor, Plg-$R_{KT}$, from macrophages. Here, we tested the hypothesis that Plg-$R_{KT}$ regulates plasmin-mediated ENaC processing in renal epithelial cells. Immunohistochemistry with specific anti-Plg-$R_{KT}$ mAb demonstrated prominent expression of Plg-$R_{KT}$ in human kidney sections, particularly in distal tubular epithelium. Western blotting of MDCK and M1 mouse collecting duct cells revealed that Plg-$R_{KT}$ was highly expressed and localized to the cell membrane. In confocal microscopy Plg-$R_{KT}$ was immunodetected at the apical surface, an orientation allowing for interaction with urinary Plg. Moreover, merged images demonstrated prominent colocalization of Plg-$R_{KT}$ with ENaC and the urokinase receptor (uPAR); the extent of colocalization of Plg-$R_{KT}$ with ENaC was 79±3% and 75±3%, and with uPAR was 82±1% and 73±3% in MDCK and M1 cells, respectively. Thus, Plg-$R_{KT}$, uPAR and ENaC are present in very close proximity on the cell surface in an orientation to promote Plg activation and ENaC processing. When Plg activation was initiated by addition of Plg and urokinase to M1 cell membranes, plasmin-dependent γENaC processing was markedly inhibited by pre-incubation with anti-Plg-$R_{KT}$ mAb and by epsilon aminocaproic acid (a lysine analog that blocks the interaction of Plg with Plg-$R_{KT}$). These results demonstrate that Plg-$R_{KT}$ is prominently expressed in the kidney, and is colocalized with ENaC and with uPAR on the apical surface of distal nephron cells. The results also suggest that Plg-$R_{KT}$ is an important regulator of Plg activation and plasmin-dependent ENaC processing in renal epithelial cells.

Disclosure of Financial Relationships: nothing to disclose

**F-FC289**

**Pretransplant Risk Factors for Posttransplant Polyoma BK-Virus Nephropathy** Anke Schwarz,[1] Verena Broecker,[2] Hermann G. Haller,[1] Silvia Linnenweber-Held.[1] *[1]Nephrology, Hannover Medical School, Hannover, Germany; [2]Pathology, Hannover Medical School, Hannover, Germany.*

BK virus (BKV) replication threatens renal transplant function. - Methods: We tried to find out the source of BKV infection by testing blood and urine of 67 donor/recipient pairs before living transplantation by quantitative PCR (qPCR) (CE labeled in vitro diagnostic kit, Cepheid-Affigene); the recipient then was followed by regular posttransplant qPCR and protocol biopsies at 6, 12, and 26 weeks. Results: Ten of 67 recipients (15%) developed posttransplant BKV nephropathy (BKVN). In 6 of these BKVN patients, urine qPCR of either the donor (3) or the recipient (2) or both (1) as well as blood qPCR of the donor (1) or the recipient (1) had been positive before transplantation. However, in 4 of the 10 BKVN patients, pretransplant urine and blood PCRs had been negative. On the other hand, 4 donors and 1 recipient had a positive pretransplant urine (4) or blood (1) qPCR but the recipient did neither develop BKVN nor elevated BKV loads. In 1 other donor and recipient each, urine and blood PCR were positive before transplantation, but the recipients developed just a transient increase of blood and urine BKV loads without BKVN. Moreover, 7 other recipients with a negative pretransplant urine and blood qPCR developed transient urine and/or blood BKV loads. - Thus, in 13 of 67 donor/recipient pairs (19%), one of them or both showed pretransplant viral replication in urine and/or blood. Six of these developed BKVN and 2 nonsignificant BKV loads suggesting a BKV infection risk (8 of 13, 62%) and a BKVN risk (6 of 13, 46%); in comparison, only 12 of 54 recipients (22%) with negative pretransplant donor/recipient viral replication developed BKV infection (62% vs 22%, p=0.01) and only 4 of 54 recipients BKVN (46% vs 7%, p=0.001). We conclude that pretransplant viral replication of donor or recipient means a risk for later recipient BKVN. Only a minority of patients may develop BKVN by pretransplant low level BKV persistence of donor or recipient beyond the sensitivity threshold of qPCR. We are now going to sequence BKV DNA from pretransplant urine and blood samples and compare these with posttransplant viremia.

Disclosure of Financial Relationships: nothing to disclose

**F-FC290**

**The Association of HLA Epitopes with BK Virus Reactivation in Renal Transplant Recipients** Janna L. Huskey,[1] Michael T. Aubrey,[2] Ronald P. Schuyler,[3] Brian M. Freed,[2] Alexander C. Wiseman.[1] *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]ClinImmune Labs, University of Colorado Denver, Aurora, CO; [3]Computational Bioscience, University of Colorado, Aurora, CO.*

Background:BK virus (BKV) is a significant cause of renal allograft dysfunction and graft loss. However, only a fraction of patients develop BK reactivation despite similar degrees of immunosuppression, suggesting that additional individual factors such as HLA variations may contribute to the development of BK infection post-transplant (tx). We tested the hypothesis that there are HLA epitopes that may confer a protective or permissive state to BK reactivation.

Methods:We performed a molecular HLA analysis for Class I (HLA-A,B,C) and Class II (HLA-DRB1, DQB1) alleles to compare epitope frequencies in 52 kidney tx patients with BK viremia to a race-matched healthy control group of 891 umbilical cord blood donors. A validated R-based bioinformatics program capable of analyzing HLA data sets and identifying shared epitopes was used.

Results:HLA-A,B,C and DRB1 did not contain any epitopes that were associated with BK reactivation. However, the HLA-DQB1 analysis identified an epitope (EV--RGI$^{84\text{-}90}$) that was associated with BK resistance (p=0.028;corrected for multiple comparisons). EV--RGI$^{84\text{-}90}$ is found in DQ5 and DQ6 alleles, and amino acids in positions 86-87 appear to have no effect. Patients with BKV were more likely to be homozygous for the absence of EV--RGI$^{84\text{-}90}$ vs. controls (59.6% vs. 36.9%, p=0.001). The presence of a single DQB1 allele with the EV--RGI$^{84\text{-}90}$ epitope was more common in controls vs. those with BK infection (45.8% vs. 23.1%, p = 0.008) suggesting that being heterozygous for EV--RGI$^{84\text{-}90}$also confers BK resistance.

Conclusions:Individuals lacking the EV--RGI$^{84\text{-}90}$ epitope may be more susceptible to BK viremia after kidney tx, potentially due to less robust BK viral antigen presentation. This may help identify individuals who are at increased risk for BK reactivation and require more aggressive screening for BKV.

Disclosure of Financial Relationships: nothing to disclose

**F-FC291**

**A Multicenter Experience of Re-Transplantation for BK Virus Nephropathy**
Duvuru Geetha,[1] Stephen M. Sozio,[1] Mythili Ghanta,[2] Michelle A. Josephson,[3] Darshana Dadhania,[4] Ron Shapiro,[5] Sundaram Hariharan.[6] *[1]Johns Hopkins University; [2]Columbia; [3]University of Chicago; [4]Cornell; [5]Univ of Pittsburgh; [6]Medical College of Wisconsin.*

BK virus nephropathy (BKVAN) is a significant cause of renal graft loss. The aim of this multi center study is to describe the characteristics and outcomes of patients who have undergone re-transplantation after loss of their prior allograft to BKVAN. 25 patients from 5 centers underwent re-transplantation at a mean of 17 months after failure of the first allograft with 8/25 undergoing pre-emptive transplantation. 20/25 had clearance of viremia documented and 9 had transplant (Tx) nephrectomy performed prior to the re-transplant. 20/25 received induction therapy. 2 of 5 centers had changed immunosuppression protocols in the re-transplants. Post transplant, 22 had BKV screening with 9 patients having BKV replication in urine and/or plasma while none developed BKVAN. Only 4/9 had documented viremia clearance at the time of re-transplant. 7 of the 25 patients experienced 10 rejection episodes. In univariate analysis, documented viremia clearance was significantly associated with lack of BKV replication post transplant, while there was a trend toward more acute rejections in those that experienced BKV replication post transplant and a statistically significant higher creatinine at 1 year in those patients.

TABLE 1

| | BKV replication | No BKV replication | p-value* |
|---|---|---|---|
| Documented viremia clearance | 4 (44%) | 16 (100%) | 0.002 |
| Transplant nephrectomy | 2 (22%) | 7 (44%) | 0.4 |
| Induction Use | 7 (78%) | 13 (81%) | 0.9 |
| Acute Rejection | 6 (67%) | 4 (25%) | 0.09 |
| Creatinine at 1 year (mg/dl), median (IQR) n=13 | 1.6 (1.6, 2.2) | 1.45 (1.3, 1.5) | 0.03 |

*p-value by Fisher's exact or Wilcoxon rank-sum test

One graft loss occurred due to rejection. In this largest study to date examining risk of recurrent BKVAN, we conclude re- transplantation is safe and effective for patients with prior graft loss to BKVAN provided pretransplant BK viremia clearance is achieved. Screening and preemptive reduction in immunosuppression may be effective in preventing BKVAN after re-transplantation.

Disclosure of Financial Relationships: nothing to disclose

**F-FC292**

**Peripheral Reduction of γδ T Cell Numbers Is a Possible Risk Factor for Cytomegalovirus Activation in Kidney Transplant Recipients**
Maria Teresa Gandolfo,[1] Loredana Alberti,[1,2] Antonio Leoni,[1] Andrea Artoni,[3] Deborah Mattinzoli,[1,2] Maria Pia Rastaldi,[1,2] Piergiorgio Messa.[1] *[1]Nephrology, Fondazione IRCCS Cà Granda- Ospedale Maggiore Policlinico; [2]Nephrology Research Laboratory – Fondazione D'Amico; [3]Medicine and Medical Specialities, Fondazione IRCCS Cà Granda- Ospedale Maggiore Policlinico, Milan, MI, Italy.*

Recent data suggest that gamma-delta (γδ) T cells can play a role in renal transplantation (RT). The aim of the present study was to evaluate the behaviour of peripheral blood γδ T cells in the first 12 months after RT and the relationships between γδ T cell numbers and percentages and the main clinical events.

T cell phenotype was serially analyzed in the peripheral blood of 31 kidney transplant recipients; clinical and biochemical parameters, included CMV DNA assessment, were also collected. Total numbers of γδ T cells increased from day 90 onward, as compared with baseline ($p<0.01$-$0.05$). The expression of CD8α, one of the hallmarks of intraepithelial T cell activated state, increased transiently on γδ T cells as well, with higher percentages on day 60 and 90 ($p<0.05$ versus day 0). T cell numbers inversely correlated with serum creatinine values in renal transplant recipients during the first 12 months after RT ($p<0.001$).

Sixteen recipients developed CMV activation. These patients showed reduced γδ T cell numbers and percentages in the early period after RT, preceding CMV activation, compared to patients that never experienced it (γδ T cell numbers: day 15 $p<0.01$, day 30 $p<0.05$; percentages: day 0, 15, 30 and 45 $p<0.05$). After CMV activation had occurred, γδ T cells expanded, achieving the levels of patients without CMV activation; a transient increase in CD8α expression was also present in these recipients on day 90 compared to baseline ($p<0.05$) and on day 60 and 90 compared to patients that did not developed CMV activation ($p<0.05$). Three recipients suffered from an acute rejection episode; no unique behaviour of γδ T cells was detected.

In conclusion, these data suggest that reduced γδ T cell numbers and percentages in kidney transplant recipients may represent an important risk factor for developing CMV activation in the early period after RT.

Disclosure of Financial Relationships: nothing to disclose

**F-FC293**

**Everolimus Significantly Reduces Cytomegalovirus Infection Incidence Versus Mycophenolate in *De Novo* Renal Transplant Patients: Pooled Analysis of Three Prospective Studies**
Daniel C. Brennan,[1] Dharmesh Patel.[2] *[1]Washington University School of Medicine, Barnes-Jewish Hospital; [2]Novartis Pharmaceuticals Corporation.*

Cytomegalovirus (CMV) infections are associated with acute and chronic graft rejections. Everolimus (EVR) in heart and renal transplant (RTx) patients is known to decrease the incidence of CMV.

CMV data from 2004 *de novo* RTx from 3 EVR studies A2309 (N=833), B201(N=588) and B251(N=583) were analyzed to identify differences between two EVR dosing groups and mycophenolate (MPA) control groups. In all studies, EVR groups received 1.5 mg/day, or 3 mg/day with either standard (SD-CsA) or reduced dose cyclosporine (RD-CsA). All control groups received (mycophenolic acid) MPA with ST-CsA. Steroids and CMV prophylaxis were given as per center practice. CMV events (infection, syndrome, disease, viremia) were reported as per local center evaluations.

Donor and recipient (D/R) CMV status at baseline were comparable between the treatment groups. Incidences of any CMV event by 12 months were 2.10 and 1.95% for EVR 1.5 and 3.0mg vs MPA of 4.34% ($p<0.0001$).

**Incidence of CMV events by treatment group - n (%)**

| | EVR 1.5 mg/day, N=664 | **EVR 3.0 mg/day, N=671** | **MPA,N=669** |
|---|---|---|---|
| CMV prophylaxis at baseline | 201 (30.3) | 199 (29.7) | 205 (30.6) |
| CMV infection/syndrome - no prophylaxis | 25 (5.4)* | 22 (4.7)** | 66 (14.2) |
| CMV infection/syndrome with prophylaxis | 12 (6.0) | 13 (6.5) | 15 (7.3) |
| CMV viremia - no prophylaxis | 18 (3.9)# | 13 (2.8)* | 40 (8.6) |
| CMV viremia with prophylaxis | 10 (5.0) | 6 (3.0) | 12 (5.9) |
| CMV disease - no prophylaxis | 9 (1.9) | 5 (1.1) | 10 (2.2) |
| CMV disease with prophylaxis | 2 (1.0) | 5 (2.5) | 6 (2.9) |
| CMV infection/syndrome by serology status | | | |
| D+R-subgroup | 17 (6.2) | 19 (18.1) | 27 (25.2) |
| D+R+subgroup | 14 (4.9)^ | 10 (3.4)* | 33 (12.0) |
| D-R-subgroup | 1 (0.7) | 2 (1.7) | 6 (4.3) |
| D-R+subgroup | 3 (3.0) | 1 (0,8)*** | 8 (6.7) |

*p<0.001 vs MPA; **p<0.0001 vs MPA; #p=0.004 vs MPA; ***p=0.036 vs MPA; ^p=0.0034

This pooled analysis documents significant reductions in incidence of CMV infection/syndrome and viremia in EVR-treated *de novo* RTx recipients, primarily those who did not receive CMV prophylaxis compared with the MPA control group.

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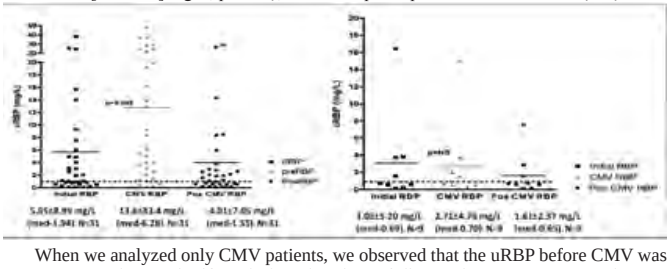
**F-FC294**

**Early Tubular Injury and Late Graft Dysfunction Associated with Cytomegalovirus Viremia in Kidney Transplants**
Lucio R. Requião-Moura, Erika A. Ferraz, Rogerio Chinen, Ana C. Matos, Thiago Corsi Filiponi, Eduardo J. Tonato, Mauricio Fregonesi, Marcelino Souza Durao, Alvaro Pacheco-Silva. *Transplant, Israeli Hospital Albert Einstein, São Paulo, São Paulo, Brazil.*

**Background:** Cytomegalovirus (CMV) is a risk factor for acute rejection and it could be associated with IF/AT as early as 3 month post kidney transplantation. A**im** of this study is assess the impact of CMV viremia in early predictor of tubular damage (urinary Retinol Binding Protein), and in 2 years graft function. **Methods:** We evaluated 195 renal transplants from deceased donor (Jan/02 to Jun/09). Patients received Thymoglobulin and preemptive therapy to CMV. A subgroup of 74 patients with uRBP serial measurement were analyze in separate and compared patients with or without CMV. **Results:** CMV viremia occurred in 63.5%. Patients with CMV used Sirolimus more frequently (7.2 vs 0.8%, p=0.04) than without. Time to CMV diagnose was 54.9±39.6 days. There no differences in acute rejection, loss and death among patients with or not CMV. Variables related with CMV risk were receptor age (OR=1.62, CI95% 1.11-2.38, p=0.01, when age > 45 yrs.) and Tacrolimo use (OR=2.03, CI95% 1.26-3.30, p=0.004). 2-years graft functions was 62.9±28.2 in no-CMV patients and 52.2±16.4 ml/min in CMV patients (p=0.028). CMV viremia was related with risk to chronic graft dysfunction (OR=1.42, CI95% 1.06-2.33, p=0.004). In similar time that occurred CMV diagnose, uRBP was higher in CMV patients (1.A) (17.4±50.5 [med=3.9] vs. 3.0±4.3 [med-1.1] mg/L, p=0.01), when compared patients without CMV (1.B).



When we analyzed only CMV patients, we observed that the uRBP before CMV was 5.65±8.99 [med-1.9], significantly less that time of diagnostic – 28.5±66.2 [med-6.28], p=0.005. **Conclusion:** CMV viremia was related with worse graft function and a profile of early tubular injury, measured by urinary RBP.

Disclosure of Financial Relationships: nothing to disclose

**F-FC295**

**Antiviral Therapy in Kidney Transplant Recipients with BK Virus Nephropathy** Billy Gilbert,[1] Abdul A. Abdellatif,[1] Luan D. Truong,[2] Aashish M. Pandya,[3] Venkataraman Ramanathan.[1] *[1]Nephrology, Baylor College of Medicine, Houston, TX; [2]Pathology, The Methodist Hospital and Baylor College of Medicine, Houston, TX; [3]Nephrology, Renal Specialists of Houston, PA, St. Lukes Episcopal Hospital Transplant Center, Houston, TX.*

**Background** The mainstay of therapy of BK virus nephropathy (BKVN) has been reduction in immunosuppressive medications. We report our experience with the use of adjunctive leflunomide or cidofovir in patients with BKVN and their influence on allograft function and outcome.

**Methods** - We identified kidney transplant recipients with BK virus in urine and / or blood between 2004 and 2009. BKVN was defined as (a) biopsy proven BKVN, i.e., at least one biopsy showing typical features of polyoma infection, or (b) elevated BK virus levels in blood (> 10,000 copies/ml).

**Results** We identified 52 patients with BKVN, translating to an incidence of 4.9%. Almost all patients had reduction in immunosuppressive medications. Thirty one (60%) and ten patients (19%) were started on i.v. cidofovir and oral leflunomide, respectively. Twelve patients (23%) had allograft failure, within a mean of 9.3 months after the diagnosis of BKVN. One-year allograft failure rate in leflunomide and cidofovir groups was 30% and 16%, respectively (p-value=ns) and serum creatinine for both groups at baseline and follow-up was similar.

When the groups of patients who did and did not have allograft failure after BKVN were compared, elevated serum creatinine at baseline (1.90.7 mg/dL vs. 1.40.5 mg/dL, p=0.003) and at the time of diagnosis of BKVN (2.80.9 vs. 1.70.6 mg/dL, p<0.0001) negatively influenced allograft outcome. Also, patients with allograft failure had significantly higher blood BK virus loads at the time of diagnosis (6.21.6 vs. 4.81.1 copies/mL, p<0.003). The presence of concomitant rejection, type of donor, induction or maintenance immunosuppressive drugs did not influence graft survival.

**Conclusion** Graft outcome and renal function after BKVN was similar between cidofovir and leflunomide. Higher serum creatinine values at baseline and at the time of BKVN diagnosis and higher blood BK viral load portend poor allograft outcome.

Disclosure of Financial Relationships: nothing to disclose

**F-FC296**

**The Determinants of the Evolution of Coronary and Aortic Calcification in Renal Transplant Recipients** Céline Maréchal,[1] Georg Schlieper,[2] Olivier Devuyst,[1] Michel Y. Jadoul.[1] *[1]Nephrology, UCL Medical School, Brussels, Belgium; [2]Nephrology, University Hospital Aachen, Aachen, Germany.*

**Background:** Vascular calcifications (VC) independently predict cardiovascular disease, the major cause of death in renal transplant recipients (RTR). We assessed the evolution of coronary artery and aortic calcification and its determinants in a published cohort of prevalent RTR. **Methods:** 281 RTR underwent a 16-slice spiral computerized tomography in order to measure the Agatston score at the time of inclusion. A second CT could be analyzed in 197 RTR 4.4 (0.2) years later. Indeed, the other 84 patients either had died (n = 40), refused to undergo a second CT (n = 37) or had a second CT that was not interpretable (n = 7). Demographic, clinical, biochemical and calcification parameters were recorded and DNA sampled at inclusion. The progression of coronary score was defined as an increase of the score by 90% or more or the development of coronary calcification in patients with a 0 score at baseline. **Results:** The coronary score increased significantly after 4.4 years follow up (616 mg (83) versus 957 mg (138); p = 0.001) whereas the aorta score did not change significantly (2384 mg (405) versus 2582 mg (411); p = 0.096). By univariate analysis, older age at inclusion, the absence of use of azathioprine, higher levels of homocysteine, of total cholesterol and lower levels of 25(OH)vit D3 were associated (p<0.05) with progression of coronary calcification. Higher phosphate (p = 0.07) and osteoprotegerin levels (p = 0.16) tended to be associated with progression. By multivariate logistic regression, a higher total cholesterol (HR = 1.01, 95% CI 1.01 - 1.02, p = 0.002), a lower 25 (OH)vitD3 (HR = 0.95, 95% CI 0.91 - 0.99, p = 0.013), and a higher phosphate level (HR = 1.74, CI 95% 1.12 - 2.72, p = 0.014) as well as the absence of use of azathioprine (HR = 0.36, 0.15 - 0.86, p = 0.021) are independent predictors of coronary calcification progression. **Conclusions:** In contrast to previous small sized short term studies, we demonstrate that coronary calcification progression is common within 4 years FU in prevalent RTR and associated with several potentially modifiable factors.

Disclosure of Financial Relationships: nothing to disclose

**F-FC297**

**Change in Mortality Risk over Follow-Up in Young Kidney Transplant Recipients** Bethany J. Foster,[1,2] Mourad Dahhou,[1] Xun Zhang,[1] Robert Platt,[1,2] James A. Hanley.[2] *[1]Pediatrics, McGill University, Montreal, QC, Canada; [2]Epidemiology and Biostatistics, McGill University, Montreal, QC, Canada.*

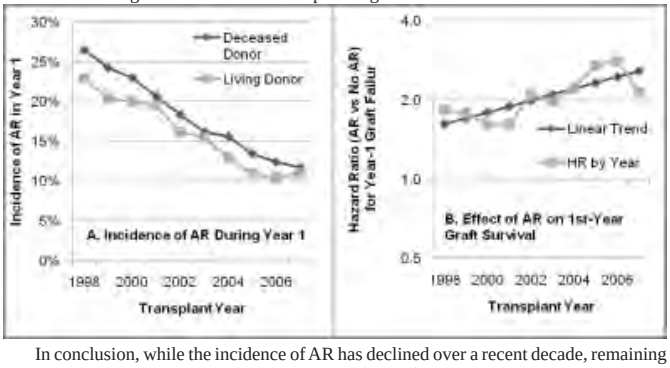
Mortality risk for young kidney transplant recipients (KTR) may change over follow-up and with change in renal replacement therapy (RRT) modality. We sought to characterize changes in mortality rates over follow-up in young KTR, and to identify time and RRT modality related risk factors for higher mortality. 18,911 patients who received a 1st transplant at <21 years of age (1983-2006), whose data were recorded in the USRDS were studied. There were 2713 deaths over a median follow-up of 10.8 y. Mortality was highest in the 1st post-transplant year, at 22/1000 py, then fell to 6.8/1000 py among those in whom the 1st graft was functioning, and varied little over time. Time-dependent Cox models with time-varying covariates (timescale age) were used to estimate the additional risk associated with each of the following RRT modality categories compared with the first transplant: dialysis, first year of re-transplant, and subsequent years of re-transplant. The following potential confounders were considered: sex, race, SES, donor source, primary disease, era (year) of transplant, and duration of dialysis before first transplant. Compared to KTR of the same age beyond the 1st year of the 1st transplant, mortality rates were higher by 4.4 times [95% CI 4.0, 4.9] for those who had returned to dialysis (p<0.001), 2.4 times [1.9, 3.1] for those in the 1st year of their 2nd transplant (p<0.001), and 1.2 times [1.0, 1.5] for those beyond the 1st year of their 2nd transplant (p=0.02). For every 5 y increment after the end of the 1st year of the 1st transplant, age-adjusted mortality rates fell by 10% [5%, 15%] (p=0.04). Mortality rates were lower in more recent eras: for every 5 y increment after 1983, mortality rates fell by 10% [5%, 15%] (p<0.001). Cause of death was cardiovascular disease for 37%, infection for 19%, malignancy for 4%, other for 22%, and unknown for 18%. The pattern of change in mortality risk over follow-up and by RRT modality was similar regardless of the cause of death. The most important risk factors for mortality were return to dialysis and temporal proximity to a transplant event.

Disclosure of Financial Relationships: nothing to disclose

**F-FC298**

**Association between Early Acute Rejection and All-Cause Kidney Allograft Failure Has Increased While the Incidence of AR Has Decreased over a Recent Decade in the US** Jon J. Snyder, Melissa Skeans, Bertram L. Kasiske. *United States Renal Data System, Minneapolis, MN.*

Acute rejection (AR) is a well known risk factor for allograft failure. We explored changes in the incidence of AR during the first post-transplant year over a ten-year period and concurrent changes in the hazard for all-cause graft failure associated with having an AR. We used USRDS data to construct a population of first-time kidney-alone transplants in patients age 18+, 1998-2007, who were discharged from the transplant hospitalization with a functioning allograft (N=121,055). AR, defined as at least one reported episode or indication of AR treatment in the first post-transplant year, were identified from Organ Procurement and Transplantation Network (OPTN) recipient follow-up data during year 1 post-transplant. For transplants in 1998, 26% of deceased donor transplants and 23% of living donor transplants reported AR in the first post-transplant year. These incidence rates declined steadily from 1998 to 2007, a decline of 52% in deceased donor transplants and 46% in living donor transplants (Figure, Panel A). The adjusted hazard ratio for all-cause graft failure during year one for those with AR vs. no AR increased from 1998 through 2007 (Panel C, test for linear trend p<0.0001), indicating that while the incidence of AR has declined, the risk associated with having an AR has increased, thereby offsetting the effect of declining incidence of AR on improved graft survival.




In conclusion, while the incidence of AR has declined over a recent decade, remaining AR episodes are associated with greater hazard for graft loss.

Disclosure of Financial Relationships: Research Funding: Bristol-Myers Squibb, Genzyme; Honoraria: Genzyme; Scientific Advisor: Genzyme.

**F-FC299**

**Alemtuzumab Versus Thymoglobulin and Interleukin 2 Inhibitor Induction in Deceased Donor Kidney Transplant, an Analysis of OPTN/UNOS Database** Hung-Tien Kuo,[1,2] Edmund Huang,[1] Marcelo Santos Sampaio,[1] Suphamai Bunnapradist.[1] *[1]UCLA Medical Center, Los Angeles, CA; [2]Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.*

**INTRODUCTION.** Induction with alemtuzumab (ALE) has been increasing in kidney transplantation. We aimed to compare post-transplant outcomes between ALE and the other induction agents in deceased donor kidney transplant recipients in the United States.

**METHODS.** Using OPTN/UNOS database (as of Nov 2009), we identified deceased donor kidney recipients [≥18 years] who received primary kidney transplant alone between 2001-2005. Those who received induction therapy with thymoglobulin (THY, n=10264), interleukin-2 antagonist (IL2A, n=10900), or ALE (n=1386) were included into the study population. The impacts of induction agent on acute rejection (AR) at 1 year post-transplant, death-censored graft failure (DCGF), and mortality were analyzed using multivariate analysis (logistic regression for AR at 1year; Cox regression for DCGF and mortality).

**RESULTS**. There were 3987 DCGF (17.7%) and 4030 mortality (17.9%) events in median follow up of 1508 days (interquartile range 1097-2138 days). The incidence of AR at 1 year in those with THY, IL2A, and ALE was 11.9%, 14.8%, and 18.2%, respectively (p<0.001). Unadjusted death-censored graft survival at 5 year in those with THY, IL2A, and ALE was 81.3%, 82.7%, and 76.0%, respectively (p<0.001). Compared to ALE, both THY and IL2A were associated with lower risks of AR at 1 year and DCGF after adjusting

for other confounding factors. There was no significant difference in the risk of mortality among recipients with different induction agent.

Adjusted Relative Risks for Outcomes

| | AR at 1 year | | DCGF | | Mortality | |
|---|---|---|---|---|---|---|
| THY | 0.54(0.44-0.65) | p<0.001 | 0.81 (0.68-0.96) | p=0.02 | 0.89 (0.75-1.07) | p=0.22 |
| IL2A | 0.73 (0.60-0.89) | p=0.001 | 0.83 (0.70-0.99) | p=0.03 | 0.93 (0.77-1.11) | p=0.40 |
| ALE | 1 | - | 1 | - | 1 | - |

**CONCLUSION.** Compared to ALE, induction with THY and IL2A were associated with lower risks for AR at 1 year post-transplant and DCGF in median follow up of 4 year post-transplant.

Disclosure of Financial Relationships: nothing to disclose

### F-FC300

**Mycophenolate Mofetil (MMF) vs. Azathioprine (AZA) as Adjunctive Therapy to Cyclosporine (CSA)-Based Immunosuppression in the Long-Term Rates of Renal Allograft Loss Due to Primary Renal Disease Recurrence** Phuong-Thu T. Pham,[2] Phuong-Chi T. Pham.[1] *[1]Nephrology Division, UCLA-Olive View Medical Center, Sylmar, CA; [2]Kidney and Pancreas Transplantation, David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Internal Medicine, Greater Los Angeles VA Medical Center, Sepulveda, CA.*

In the current study, we update the long-term follow-up of the impact of MMF on the rate of renal allograft loss due to glomerular disease recurrence compared to AZA as adjunctive therapy with CSA-based immunosuppression in both deceased and living primary kidney transplantation.

Kidney transplants performed during 1988-2003 (OPTN/UNOS database as of 2/05/2010) with the primary diagnoses of diabetes mellitus, IgA nephropathy, membranous glomerulonephropathy (MGN), membranoproliferative glomerulonephropathy (MPGN), lupus nephritis (LN), focal segmental glomerulonephropathy (FSGS), hypertensive nephrosclerosis, polycystic kidney diseases, and tubulointerstitial diseases (TIN) were included.

At 10-year follow-up, there was no statistically significant difference in Kaplan-Meier graft survival rates between AZA and MMF adjunctive therapy to cyclosporine-based immunosuppression in the rates of graft loss due to any of the glomerulonephropathies in either deceased or living renal transplants. There was however, a significantly worse outcome in the CSA+MMF group (n=1210) for recipients with the primary diagnosis of tubulointerstitial diseases compared to those on CSA+AZA (n= 1468), 2.45% vs 0.85%, log-rank p-value 0.048.

The rates of graft loss due to recurrence of the primary renal disease were highest for MPGN (types 1 & 2 not specified) at 6.5% vs 7.8% (CSA+AZA vs CSA+MMF respectively), FSGS (6.8%, same for either AZA or MMF), and MGN (6.8%, same for either AZA or MMF). The rates of graft loss due to recurrence of LN were only 3.2 vs. 4.2% (CSA+AZA vs CSA+MMF respectively).

The efficacy of MMF adjunctive therapy to CSA-based immunosuppression in reducing the recurrence of common glomerulonephropathies was comparable to that of AZA at 10-year follow-up. The rates of graft loss due to recurrence of primary renal disease were highest in those with MPGN, FSGS, and MGN.

Disclosure of Financial Relationships: nothing to disclose

### F-FC301

**Estimating Glomerular Filtration Rate in Renal Transplant Recipients with Cystatin C-Based Equations: A Systematic Review** Gavin Harman,[1] Greg A. Knoll,[1,3] Christine A. White.[2] *[1]Department of Medicine, University of Ottawa, Ottawa, ON, Canada; [2]Department of Medicine, Queen's University, Kingston, ON, Canada; [3]Kidney Research Centre, Ottawa Health Research Institute, Ottawa, ON, Canada.*

We performed a systematic review of diagnostic assessment studies evaluating the performance of Cystatin C (CysC)-based glomerular filtration rate (GFR) prediction equations in adult renal transplant recipients (RTRs).

We searched MEDLINE and EMBASE from 1966 and 1980 respectively to August 2009 for all entries related to renal disease or GFR and CysC, yielding 1,012 unique articles. Inclusion criteria were: subjects above 18 years of age, use of a GFR estimating equation and the direct measurement of GFR as a reference standard. Seven studies (475 GFR measures in 484 patients) were included in this review. Techniques used to measure GFR included 99mTc-DTPA (n=3), 125I-iothalamate (n=2) and inulin clearance (n=2). CysC was measured by particle enhanced nephelometric immuno-assay (n=4), turbidimetric immuno-assay (n=2) or ELISA (n=1). Prediction equations most commonly evaluated were those of Larsson (n=5), Hoek (n=4), Filler (n=4), LeBricon (n=3) and Rule (n=2). The analysis of each study reported bias (mean difference between measured GFR and GFR estimated by the prediction equation) and accuracy (percent of GFR estimates within 10%, 20%, or 30% of measured GFR) of each equation.

The range of bias among studies and the pooled weighted mean 30% accuracy (proportion of GFR estimates falling within 30% of the measured GFR) for each equation are reported in Table 1.

Disagreement between studies is likely due to differences in patient population, reference standard GFR measurement techniques and the immuno-assays used for serum CysC. The wide range of bias and poor accuracy of measured GFR suggests that CysC -based equations are not superior to those based on creatinine for estimating GFR in RTRs.

Table 1. Range of mean bias and weighted mean 30% accuracy from pooled data of included studies

| Equation | Bias (ml/min/1.73m2) | 30% accuracy |
|---|---|---|
| Hoek | -10.6 – -5.8 | 75 |
| LeBricon | -3.8 – +2.8 | 74 |
| Filler | -1.7 – +6.72 | 71 |
| Rule | -0.86 – -5 | 69 |
| Larson | -16.1 – -3.2 | 64 |

Disclosure of Financial Relationships: nothing to disclose

### F-FC302

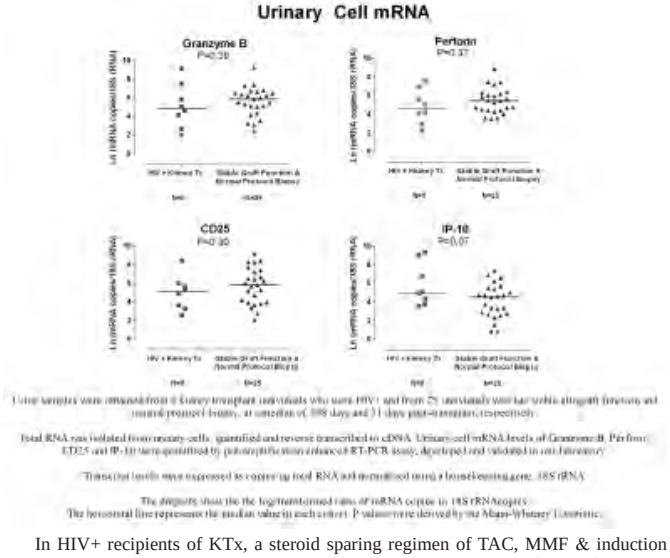
**HIV+ Individuals with Kidney Transplantation (KTx) on an Early Steroid Withdrawal Protocol Are Clinically Stable and Molecularly Quiescent** Thangamani Muthukumar, C. Afaneh, Choli Hartono, David Serur, Jun Lee, Darshana M. Dadhania, Roxana M. Bologa, D. Leeser, S. Kapur, Manikkam Suthanthiran. *Cornell University, New York.*

KTx is emerging as a viable option for individuals infected with HIV. Most receive IL-2 receptor antibody (IL-2R Ab)as induction agent & are on 3-drug immunosuppression. Increased incidence of acute rejection (AR) however has been observed in this cohort.

We studied 10 HIV+ individuals who received KTx on an early steroid withdrawal protocol (steroids stopped <7 d of KTx) & maintenance immunosuppression of tacrolimus (TAC) & mycophenolate mofetil (MMF). Induction was either Thymoglobulin® (N=8) or IL-2R Ab (N=2).

| | |
|---|---|
| N | 10 |
| Age, mean (SD) | 50 (9) |
| Gender (M:F) / Race (Black:Hispanic) | 9:1 / 7:3 |
| Dialysis duration, months, median (range) | 80 (6-227) |
| HBV/HCV pre KTx | 1/1 |
| HIV load, not detected, pre KTx | 9 |
| CD4 count in cu.mm, median (range) | 571 (134-938) |
| History of opportunistic infections | 0 |
| Donor type (deceased: living) | 7:3 |
| Cold ischemia time, hours, median (range) | 22 (12-40) |
| Delayed graft function | 5 |
| Acute rejection, N (months from transplant) | 2 (0,16) |
| Follow up, months, median (range) | 12 (7-34) |
| Graft loss, N (months from transplant) | 2 (0,25) |
| HIV load at last follow up, copies/ml | <48 in 8, 70 in 1 and not done in 1 |
| CD4 count in cu.mm at last follow up, median (range) | 183 (24-565) |
| Infections post KTx | UTI-3, Pneumonia 2, Bacterial diarrhea 1, Osteomyelitis 1 |
| Cancer post KTx | 0 |

We performed urinary cell mRNA profiling on them. Levels of granzyme B, perforin, CD25 & IP-10 were similar (P>0.05) to a cohort of stable transplants.




In HIV+ recipients of KTx, a steroid sparing regimen of TAC, MMF & induction with Thymoglobulin® or IL-2R Ab is associated with a low incidence of AR, safe & well-tolerated. These individuals remain clinically stable & are molecularly quiescent.

Disclosure of Financial Relationships: nothing to disclose

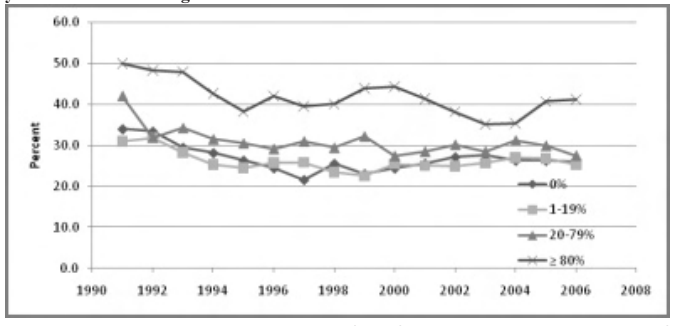
### F-FC303

**Continued Use of Transfusion in the Transplant Waitlist Population, Even among Non-Sensitized Candidates** Jon J. Snyder,[1] Hassan N. Ibrahim,[2] Bertram L. Kasiske,[2] Melissa Skeans,[1] Allan J. Collins.[1,2] *[1]Chronic Disease Research Group, Minneapolis, MN; [2]Department of Medicine, University of Minnesota, Minneapolis, MN.*

Sensitized patients spend more time on the kidney transplant waiting list than do non-sensitized patients. Exposure to blood products via transfusion may result in sensitization, thereby increasing the likelihood of a prolonged waiting time. We explored the 3-year

cumulative incidence of transfusion, stratified by PRA at the time of listing, to see if transfusions were used more sparingly in non-sensitized patients. We used USRDS data including adult wait-listed patients from 1991-2006 whose primary payer was Medicare (N=110,669). We examined Medicare inpatient and outpatient claims for evidence of transfusion in the first three years post-listing. We estimated 3-year cumulative incidence of transfusion, stratified by PRA at the time of listing, censoring at de-listing, death, and transplant. From 1991 to 1994, transfusion rates declined in patients at all levels of listing PRA. Patients with listing PRA > 80% had the highest incidence of post-listing transfusions. Patients with a PRA of 0% at listing had a similar incidence of transfusion on the waitlist as those with PRA of 1-19%, which ranged from 23% to 27% over the past decade. Overall, there was no difference in transfusion incidence in patients with PRA of 0% vs. patients with PRA of 1-19% (log-rank p-value=0.8066). Those with PRA of 20-79% had only slightly higher incidence of transfusion, which ranged from 27% to 32% over the last decade (Fig 1).

**Fig 1: Three-year cumulative incidence of transfusion in waitlisted patients, by year and PRA at listing**




Although one might expect the avoidance of transfusions in patients with listing PRA of 0%, use of the procedure remains common and is similar to the frequency seen in patients with low to mid levels of sensitization.

Disclosure of Financial Relationships: Research Funding: Bristol-Myers Squibb, Genzyme; Honoraria: Genzyme; Scientific Advisor: Genzyme.

### F-FC304

**Likelihood of Preemptive Transplant (Txpl) and Candidate Survival as Predicted by Renal Function at the Time of Listing for Renal Transplantation** Rachel B. Fissell,[1] Joseph V. Nally,[1] Sankar D. Navaneethan,[1] Emilio D. Poggio,[1] Titte Srinivas,[1] David A. Goldfarb,[2] Jesse D. Schold.[3] *[1]Department of Nephrology/Hypertension, Cleveland Clinic, Cleveland, OH; [2]Glickman Urological/Kidney Institute, Cleveland Clinic, Cleveland, OH; [3]Department of Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH.*

Purpose: This analysis evaluates whether renal function (eGFR or Creatinine Clearance (CrCl)) at listing is associated with likelihood of receiving a preemptive Txpl, and candidate survival prior to transplantation.

Methods: We included 61,020 solitary renal Txpl candidates from the national SRTR database listed preemptively from 2000-2009 with available renal function estimate. Multivariable linear regression was used to characterize associations between patient characteristics and renal function at listing. Multivariable logistic regression and survival models were used to assess likelihood of preemptive Txpl and candidate survival.

Results: Mean eGFR was 14.8 mL/min/1.73 kg/m$^2$(std=3.8) and CrCl was 14.7 mL/min/1.73 kg/m$^2$ (std=4.4) at listing. Factors associated with higher renal function at listing were male gender, Caucasian race/ethnicity, prior Txpl, presence of diabetes, and higher education level. A Txpl was received by 54% of the population over the study period (49% from a living donor and 51% from a deceased donor). Among those that received a Txpl, 51% had a preemptive Txpl prior to dialysis initiation. Higher renal function was significantly associated with likelihood of receiving a preemptive Txpl (AOR=1.34 per 5 ml/min, 1.29-1.40), for both deceased and living donor Txpls. Patients with higher renal function at listing had significantly better candidate survival prior to Txpl: 5-yr survival 82% with eGFR>15 vs. 79% with eGFR<15 (p<0.001).

Conclusions: Higher renal function at the time of preemptive listing was significantly associated with both higher likelihood of preemptive transplant and lower candidate mortality. Renal function at listing is a potentially modifiable factor. These results suggest that candidates for a preemptive Txpl should be listed at as high a level of renal function as possible.

Disclosure of Financial Relationships: nothing to disclose

### F-FC305

**Chronic Kidney Disease (CKD) Stage Progression in Liver Transplant Recipients** John C. Lamattina, David P. Foley, Alexandru Musat, Luis A. Fernandez, Joshua Mezrich, Anthony M. D'Alessandro, John D. Pirsch, Arjang Djamali. *Medicine and Surgery, UW Madison School of Medicine and Public Health, Madison, WI.*

There is little information on CKD stage progression rates and outcomes in liver transplant recipients. We performed a retrospective review of 1151 adult, deceased-donor, single-organ primary liver transplants between July 84 and December 07 and analyzed renal outcomes and risk factors for CKD stage progression. We included 729 patients in the study since they had available MDRD GFR levels at one year posttransplant, thus defining their baseline stage of CKD. Stage progression was calculated using all measured outpatient creatinine values during the follow-up interval. Mean recipient age at the time of transplant was 52.0±10.1 years. Recipients were more commonly male (61%) and Caucasian (96%). Mean recipient body-mass index (BMI) was 28.2±6.6 kg/m$^2$. The majority of liver transplants were from donation after brain death donors (95%). Mean donor age was 37.1±16.4. Donors were more often male (64%) and Caucasian (93%). Donor BMI was 26.2±6.0 kg/m$^2$. Mean cold ischemia time was 8.3±2.4 hours. At one year, 7%, 34%, 56%, 3% and 1% of patients were in stages 1, 2, 3, 4, and 5 CKD. The incidence of stage progression was 28%, 40%, and 53%, at 3, 5, and 10 years. The incidence of ESRD was 2.6%, 7.5%, and 18% at 5, 10, and 20 years. Pretransplant diabetes, hypercholesterolemia, and infectious complications during the first year were predictive of stage progression. Caucasian race and higher physiologic MELD at the time of transplant proved protective.

| | Univariate | | | Multivariable | | |
|---|---|---|---|---|---|---|
| Pretransplant diabetes | 1.4 | 1.06-1.81 | 0.01 | 1.90 | 1.38-2.63 | <0.0001 |
| Hypercholesterolemia | 1.44 | 1.05-1.98 | 0.02 | 1.46 | 1.04-2.05 | 0.02 |
| Infectious complications | 1.31 | 1.03-1.66 | 0.02 | | | |
| UTI | 1.38 | 1.06-1.81 | 0.01 | 1.39 | 1-1.93 | 0.04 |
| Caucasian | 0.6 | 0.41-0.88 | 0.008 | | | |
| MELD | 0.98 | 0.96-0.99 | 0.04 | | | |

In conclusion close monitoring and management of modifiable risk factors may prove critical to delay CKD progression in liver transplant recipients.

Disclosure of Financial Relationships: nothing to disclose

### F-FC306

**Characterization of Epithelial Oxalate Absorption** Felix Knauf,[1] Zhirong Jiang,[1] James M. Anderson,[2] Peter S. Aronson.[1] *[1]Nephrology, Yale University, New Haven, CT; [2]Physiology, UNC, Chapel Hill, NC.*

Calcium oxalate (CaOx) is the predominant constituent of kidney stones. Mice deficient for oxalate transporter SLC26A6 develop hyperoxaluria and CaOx stones on the basis of a defect in intestinal back secretion of oxalate. The goal of the current study was to characterize the mechanism(s) of epithelial oxalate absorption. We used native mouse intestine and measured unidirectional absorption of [14C]-oxalate simultaneously with flux of [3H]-mannitol as a marker of the paracellular pathway. Although absorptive fluxes of both oxalate and mannitol varied in parallel in different segments of small and large intestine, absorptive flux of oxalate was always the same as that of mannitol, indicating that it is predominantly passive and paracellular. In contrast, the secretory flux of oxalate exceeded that of mannitol in duodenum of wild type mice, indicating active transcellular secretion of oxalate. This excess of oxalate secretion compared to mannitol was abolished in duodenum of Slc26a6-null mice. Similarly, luminal addition of the anion transport inhibitor DIDS to wild-type duodenum reduced the secretory flux of oxalate to that of mannitol, but did not affect oxalate absorption. We then used cultured epithelial cells with gene silencing of tight junction proteins to alter paracellular permeability. Unidirectional transport of oxalate and mannitol increased in parallel when paracellular permeability to larger molecules was increased by knockdown of ZO-1. In contrast, induction of claudin-10a increased the anion permeability of the tight junction as shown by the change in dilution potential but had no effect on oxalate flux. Taken together, our studies in mouse intestine and cultured epithelial cells indicate that oxalate absorption is passive and paracellular, and that oxalate traverses the low capacity paracellular pathway for larger solutes but not the higher capacity claudin-based pores. We hypothesize that in the intestine the role of SLC26A6 in mediating active oxalate secretion is to counteract the passive absorption of ingested oxalate that takes place through the paracellular pathway.

Disclosure of Financial Relationships: nothing to disclose

### F-FC307

**Leptin Signaling Regulates Oxalate Transport by Intestinal Cells** Hatim A. Hassan, Zhen-Guo Wang, Ming Cheng. *Medicine, The University of Chicago, Chicago, IL.*

The vast majority of kidney stones are composed of calcium oxalate, and minor changes in urinary oxalate affect stone risk. Intestinal oxalate secretion mediated by anion exchanger Slc26a6 plays a major constitutive role in limiting net intestinal absorption of ingested oxalate. Leptin is emerging to play an important role in the regulation of intestinal transport through signaling pathways including PKC activation, which also regulates intestinal oxalate transport. We therefore examined whether leptin affects intestinal oxalate transport using the human intestinal Caco2 BBE cells, which express leptin receptors and in which ~50% of oxalate transport is mediated by SLC26A6. We measured apical [14C]oxalate uptake in the presence of an outward Cl gradient as an assay of Cl-oxalate exchange activity. Interestingly, we found that apical or basolateral lepin (0.2 or 100 nM x 6 hours) significantly inhibited [14C]oxalate uptake by ~ 20-30%, without affecting the transepithelial resistance. Importantly, Leptin also inhibits mouse duodenal oxalate secrtion. Following a 30-minute control period, the effects of mucosal or serosal leptin (100 nM) on transepithelial unidirectional 14C-oxalate fluxes (mucosa to serosa, JMS, and serosa to mucosa, JSM) was assessed over another 30-minute period. Mucosal leptin significantly inhibited oxalate secretion (by >29%), a process largely mediated by Slc26a6, without affecting absorption (Control: JMS = 32.11 ± 3.70, JSM = 41.95 ± 5.71, Jnet = -10.56 ± 6.59 pmol/cm2/h; Leptin: JMS = 35.22 ± 2.99, JSM = 29.73 ± 4.19, Jnet = 5.49 ± 5.13 pmol/cm2/h), resulting in conversion of oxalate transport from net secretion in control tissues to net absorption in leptin-treated tissues. Using RT-PCR, the observed leptin-induced inhibition of oxalate uptake by CaCo2 cells is not due to reduced SLC26A6 mRNA expression. However, we observed in preliminary experiments that leptin treatment led to

redistribution of some of the SLC26A6 protein to an intracellular space, suggesting that leptin inhibits oxalate uptake by CaCo2 cells by reducing SLC26A6 surface expression. We conclude that leptin negatively regulates intestinal oxalate transport.

Disclosure of Financial Relationships: nothing to disclose

**F-FC308**

***AGXT* Mutation Type Correlates with Phenotype in Primary Hyperoxaluria Type 1** Carla G. Monico,[1] Regina M. Herges,[2] Barbara M. Seide,[1] Julie B. Olson,[1] Andrea G. Cogal,[1] Eric J. Bergstralh,[2] Dawn S. Milliner.[1] *[1]Mayo Clinic Hyperoxaluria Center, Division of Nephrology, Mayo Clinic, Rochester, MN; [2]Division of Biostatistics & Epidemiology, Mayo Clinic, Rochester, MN; [3]International Primary Hyperoxaluria Registry Investigators, .*

Primary hyperoxaluria type 1 (PH1) is caused by deficiency or peroxisome-to-mitochondria mistargeting of hepatic alanine:glyoxylate aminotransferase (AGT) and characterized by markedly elevated urine oxalate excretion, recurrent calcium oxalate urolithiasis, nephrocalcinosis and renal failure. Previously, we showed an association between the most common mutation (G170R) and response to pyridoxine (VB6) treatment.

To further explore associations between genotype and phenotype, we classified PH1 patients in the International Primary Hyperoxaluria Registry by mutation type: mistargeting (M), missense (S) or truncating (T), where M=G170R or F152I, S=other single amino acid change, and T=splice site, nonsense or frameshift mutations. Associations between mutation type and clinical factors [age @ symptoms (Sx) and diagnosis (Dx), and urinary oxalate (Uox) @ Dx] were evaluated using non-parametric ANOVA and chi-square tests.

Clinical Characteristics According to Mutation Type in 129 PH1 Patients

| Genotype by Mutation Type | n | Age @ Sx* (yrs) | Age @ Dx* (yrs) | U ox/BSA @ Dx* |
|---|---|---|---|---|
| M/M | 25 | 12.7 (10) | 28.3 (18) | 1.3 (0.8) |
| S/S | 21 | 16.9 (13) | 25.8 (14) | 1.4 (0.7) |
| T/T | 10 | 10.2 (14) | 14.9 (17) | 2.4 (0.9) |
| M/S | 23 | 8.5 (12) | 17.3 (18) | 1.9 (1.4) |
| T/M or T/S | 50 | 7.8 (10) | 11.6 (13) | 2.8 (1.2) |
| p-value | | 0.01 | 0.0001 | 0.0004 |

*Mean (SD). Uox/BSA = mmol/1.73m2/24 hrs

Mutation type appears to correlate with PH1 phenotype. The presence of one truncating allele (T/M or T/S) was associated with younger age @ symptoms and diagnosis, and higher Uox @ Dx whereas M/M seemed to predict a lower Uox and older age @ diagnosis, suggesting a milder phenotype. Mistargeting and missense genotypes may portend greater mutant AGT dimer stability, perhaps due to more functional AGT protein. *AGXT* genotyping may hence be of value in optimizing treatment strategies in individual PH1 patients.

Disclosure of Financial Relationships: nothing to disclose

**F-FC309**

**Changes in Mitochondrial Proteome of Distal Renal Tubular Cells upon Calcium Oxalate Monohydrate Crystal Adhesion and Internalization** Visith Thongboonkerd,[1] Sakdithep Chaiyarit,[1] Supachok Sinchaikul,[2] Shui-Tein Chen.[2] *[1]Medical Proteomics Unit, Office for Research and Development, Siriraj Hospital, Mahidol University, Bangkok, Thailand; [2]Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan.*

Calcium oxalate monohydrate (COM), the major crystalline compound found in kidney stones, has been thought to trigger oxidative stress, which subsequently leads to renal tubular cell damage. Several reports have shown that COM crystals can induce overproduction of reactive oxygen species (ROS) followed by cellular injury. Our present study aimed to identify a set of altered mitochondrial proteins in distal renal tubular cells upon COM crystal adhesion and internalization. Madin-Darby Canine Kidney (MDCK) cells were maintained in the presence or absence of COM crystals. Crystal adhesion and internalization were investigated by immunofluorescence staining and laser-scanning confocal microscopy. Thereafter, mitochondria were isolated by differential centrifugation technique and the purity of mitochondrial isolation was confirmed by Janus Green B staining, Western blotting, and transmission electron microscopy. Subsequently, mitochondrial proteins were resolved by 2-DE (n=5 gels derived from 5 independent samples in each group; COM-treated vs. controlled) and visualized by Deep Purple dye. A total of 15 differentially expressed mitochondrial proteins (12 increased and 3 decreased) were successfully identified by Q-TOF MS and MS/MS analyses. The altered mitochondrial proteins included those involved in several biological processes; i.e., cellular metabolism, stress response, and cellular structure. Global protein network analysis of the altered mitochondrial proteins demonstrated that energy production and cellular development were affected by adhesion and internalization of COM crystals. Our data indicated that mitochondrial proteome of renal tubular cells was altered in response to COM crystal adhesion and internalization. The altered proteins were involved in important cellular processes, which might subsequently lead to cellular injury.

Disclosure of Financial Relationships: nothing to disclose

**F-FC310**

**Temporal Changes in the Expression of mRNA of Various NADPH Oxidase Subunits in Renal Epithelial Cells Exposed to Oxalate and Calcium Oxalate Crystals** Saeed R. Khan, Karen J. Byer. *Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL.*

Recent experimental and clinical studies suggest that kidneys may endure oxidative stress (OS), injury and inflammation during stone formation. It is our hypothesis that OS is caused by oxalate (Ox) and/or calcium oxalate (CaOx) crystals induced activation of NADPH oxidase and production of reactive oxygen species (ROS).

To investigate the activation of NADPH oxidase, we exposed HK2 human kidney epithelial cell line to 100 µmol Ox or 66.7 µgm/cm$^2$ CaOx monohydrate crystal for 6, 12, 24 or 48 hours. After the exposure media was collected to determine LDH, 8-isoprostane (IP) and extracellular SOD. cells were processed to determine NADPH oxidase activity. Expression of subunits, gp91, p22, p40, p47, p67 and RAC-GTPase was determined by real time PCR.

Exposure to both Ox and CaOx crystals resulted in significant LDH release and production of significant amounts of 8IP. SOD production also increased after both Ox and CaOx exposures. However NADPH oxidase activity increased significantly only after CaOx crystal exposure. Both Ox and CaOx crystals produced changes in the expressions of subunits in comparison to control as well as to each other.

Results indicate that exposure to both oxalate and CaOx crystals lead to changes in the expression of various NADPH oxidase subunits, lipid peroxidation and membrane injury. Oxalate and CaOx crystals appear to affect differently. Expression of a number of macromolecules such as monocyte chemoattaractant protein and osteopontin is increased when renal epithelial cells are exposed to high oxalate and CaOx crystals. While generation of large amount of ROS may play a major role in tissue injury or death, but regulated generation of low concentration of ROS, possibly by NADPH oxidase, may represent a second messenger system for generation of many of the upregulated macromolecules involved in biomineralization and inflammation.

Disclosure of Financial Relationships: nothing to disclose

**F-FC311**

**Effects of Calcium Oxalate Monohydrate Crystals on Expression and Function of Tight Junction of Renal Tubular Epithelial Cells** Visith Thongboonkerd, Paleerath Peerapen. *Medical Proteomics Unit, Office for Research and Development, Siriraj Hospital, Mahidol University, Bangkok, Thailand.*

Tight junction plays crucial role in regulating paracellular transports (as a barrier), and in separating apical from basolateral compartments to maintain cell polarity (as a fence). Tight junction can be disrupted by various stimuli, including oxidative stress, pathogens and proinflammatory cytokines. However, association of defective tight junction with kidney stone pathogenesis remains unknown. We therefore examined whether calcium oxalate monohydrate (COM) crystals, which are the major crystalline composition in kidney stones, have any effects on expression and function of tight junction of polarized renal tubular epithelial cells. Western blot analysis revealed marked decrease in levels of occludin and zonula occludens-1 (ZO-1) in COM-treated polarized MDCK cells. Immunofluorescence staining revealed not only the decline of these tight junction proteins but also their redistribution and dissociation in COM-treated cells. Additionally, transepithelial resistance (TER) was significantly decreased indicating impaired tight junction barrier and increased paracellular permeability in COM-treated cells. Subcellular fractionation followed by Western blot analysis of $Na^+/K^+$-ATPase-$\alpha$1 revealed that this basolateral membrane marker was also detectable in apical membrane fraction of COM-treated cells, but not in apical membrane fraction of controlled cells. These data provide the first evidence to demonstrate decreased expression and defective barrier and fence functions of tight junction of renal tubular epithelial cells exposed to COM crystals that may be fundamental for subsequent renal tubulointerstitial injury, which in turn enhances the stone pathogenesis.

Disclosure of Financial Relationships: nothing to disclose

**F-FC312**

**Oxalate Stimulates the Production of TNF-alpha Via NF-kappaB Activation in Renal Epithelial Cells** Vijayalakshmi Thamilselvan, Mani Menon, Sivagnanam Thamilselvan. *Vattikuti Urology Institute, Henry Ford Health System, Detroit, MI.*

INTRODUCTION: Hyperoxaluria is one of the major risk factors for calcium oxalate urolithiasis, and its intrinsic toxicity results in tubular dysfunction and interstitial inflammation. We have previously shown that oxalate induces free radical injury through activation of NADPH oxidase. NF-kB is an oxidative stress-responsive transcription factor that plays an important role in the regulation of pro-inflammatory mediators. Therefore, we explored the contribution of specific reactive oxygen species (ROS) to oxalate-induced NF-kB activation and subsequent TNF-$\alpha$ gene regulation in renal epithelial cells.

METHODS: LLCPK1 cells were exposed to 0.5-1.0 mM oxalate for different time periods with or without antioxidants (SOD, catalase, sodium formate, vitamin E and vitamin C) and NF-kB inhibitor peptide. Superoxide and H2O2 production were determined by colorimetry. NF-kB activation was determined by DNA binding activity using electrophoretic mobility shift assay combined ELISA and nuclear translocation by Western blot. TNF -$\alpha$ mRNA expression and secretion were determined by RT-PCR and ELISA respectively.

RESULTS: Oxalate treatment induced a time and dose dependent increase in ROS production. Oxalate significantly increased NF-kB activation as well as TNF-α mRNA expression and secretion in a time-dependent manner. Addition of SOD augmented oxalate-induced superoxide generation, NF-kB activation whereas catalase lowered H2O2 generation and NF-kB activation. Sodium formate significantly inhibited NF-kB activation. Sodium formate or NF-kB inhibitor peptide significantly blocked TNF-α mRNA expression and secretion. Treatment of vitamin E and C together significantly inhibited oxalate-induced NF kB activation and TNF-α gene upregulation.

CONCLUSION: We conclude that ROS dependent NF-kB activation is an important signaling pathway of oxalate toxicity and could be crucial for induction of inflammatory signals in renal epithelial cells. Free radical scavengers prevented oxalate-induced NF-kB mediated TNF-α secretion. Therefore these pathways may represent a novel therapeutic target for patients with kidney stones.

Source of Funding: NIH RO1 DK 56249

Disclosure of Financial Relationships: nothing to disclose

## F-FC313

**Nephrolithiasis in Brown Norway Wpk/+ Rats** Vincent H. Gattone,[1] James Williams,[1] Kenneth E. White,[1] Sharon M. Moe,[1] Elaine M. Worcester.[2] *[1]Indiana University School of Medicine, Indianapolis, IN; [2]University of Chicago, Chicago, IL.*

Nephrolithiasis is a relatively common clinical condition. Kidney stones come in many different forms and compositions. Kidney stones can start as outgrowths from the renal papilla and remain attached. Old (ages 12-18 months) Brown Norway(BN)- Wpk/+ rats develop spontaneous nephrolithiasis. They are heterozygote carriers of a mutation for Meckel Syndrome, a lethal autosomal recessive condition with renal cystic pathology. We evaluated renal function and morphology in old BN-Wpk/+ retired breeders versus retired BN breeder control rats. Metabolic cage studies were performed and at termination, rats were anesthetized and kidneys evaluated using X-ray, microCT, histology and stones were further evaluated using microCT and/or scanning electron microscopy followed by spectroscopic analysis. Calcifications were typically associated with the medullae and a hypertrophic papillary epithelium and were more prominent in males compared with female Wpk/+ rats. Kidney stones were also found in the pelvis and pelvic recess in many rats, some of which were adherent while others were free floating. MicroCT analysis identified a distinct attachment site while the remainder of the stone exhibited a laminated appearance. Urine had an increased concentration of phosphate while calcium, citrate and oxalate were all decreased. The urinary P/Cr ratio was increased suggesting an increased phosphate excretion in male Wpk/+ rats. There was misexpression of NPT2a mRNA in Wpk/+ renal cortex. The stones were composed of amorphous calcium phosphate. This laminated appearance is reminiscent of kidney stones found in humans and suggests a prolonged period of deposition. This is the first rodent model with spontaneous nephrolithiasis linked to a specific mutant gene and could be a clinical condition associated with carriers of Meckel Syndrome.

Disclosure of Financial Relationships: Research Funding: AMGEN funding; Patent: Licensing agreement with AMGEN.

## F-FC314

**Chlorthalidone Improves Bone Quality in Genetic Hypercalciuric Stone-Forming Rats** David A. Bushinsky,[1] Thomas Willett,[2] John R. Asplin,[3] Marc D. Grynpas.[2] *[1]U. Rochester, Rochester, NY; [2]Mt. Sinai Hospital, Toronto, ON, Canada; [3]U. Chicago, Chicago, IL.*

We have bred a strain of rats to maximize urine (U) calcium (Ca) excretion to model hypercalciuric nephrolithiasis. These GHS rats now excrete far more UCa than control Sprague-Dawley rats, uniformly form kidney stones and, similar to patients, demonstrate lower bone mineral density (BMD). Clinically thiazide diuretics are used to reduce UCa and prevent stone formation. Whether they have a beneficial effect on bone is not clear. We used GHS rats to test the hypothesis that the thiazide diuretic chlorthalidone (CTD) would favorably affect bone density and quality. Twenty GHS rats received a fixed amount of a standard 1.2% Ca diet and half were also fed CTD (4-5 mg/kg/day). U was collected weekly during the 18 wk study and then femurs and vertebrae were analyzed. Compared to vehicle, rats fed CTD had a marked reduction in UCa. Bone quality assessment techniques were used to study the effect of CTD treatment on the axial and appendicular skeleton. Compared to vehicle, CTD caused a significant increase in the bone mineral content and BMD of the lumbar vertebrae, measured by DEXA. An increase in the trabecular mineralization and architecture was observed with CTD. As indicated by the structural parameters obtained from micro CT, trabecular bone volume (BV/TV), trabecular thickness and trabecular number all increased significantly with CTD. Additionally, from static histomorphometry, there was a significant increase in trabecular thickness with CTD. Unsurprisingly therefore, CTD also improved the connectivity of trabecular bone. However, CTD did not alter formation parameters. CTD led to a significant improvement in vertebral strength and stiffness measured by vertebral compression. CTD treatment had a greater effect on trabecular bone than on cortical bone. These results obtained in hypercalciuric rats suggest that CTD can favorably influence vertebral fracture risk. CTD did not alter formation parameters suggesting that the improved bone strength was due to decreased bone resorption and retention of bone structure. Whether CTD will favorably affect human bone should be tested.

Disclosure of Financial Relationships: Employer: University of Rochester School of Medicine; Consultancy: Amgen, Genzyme, Cytochroma, Relypsa; Ownership: Amgen, Relypsa; Honoraria: Amgen, Genzyme, Cytochroma, Relypsa; Scientific Advisor: Amgen, Genzyme, Cytochroma, Relypsa.

## F-FC315

**Hydroclorthiazide & Spironolactone Decrease Urinary Calcium Oxalate Saturation in Premature Infants** Gregory Lee Braden,[1] Thomas Campfield,[2] Gary F. Rockwell.[2] *[1]Dept of Medicine, Baystate Medical Center/Tufts University School of Medicine, Springfield & Boston, MA; [2]Dept of Pediatrics, Baystate Medical Center/Tufts University School of Medicine, Springfield & Boston, MA.*

We have shown that hypercalciuria, hyperoxaluria & increased urinary calcium oxalate saturation ratios(CaOxRSR) may occur in premature infants(PI) at risk for nephrocalcinosis(NC). Furosemide may induce hypercalciuria & increased CaOxRSR in PIs with lung disease but there are no data on the effects of hydrochlorthiazide(HC) & spironolactone(SP) on CaOxRSR in PIs. We studied 39 PIs, 19 on HCTZ & SPIR for lung disease and 20 PI controls(C).Timed urine samples were obtained for Ca, Ox, uric acid, Na,K,Cl, phosphorus, creatinine & ph. Urine CaOxRSR was calculated with the EQUIL program. 19 PIs on HC&SP weighed less & were younger at birth(1185vs1414gms; gest age 26 vs 29 weeks), had 3 fold >Na intake(12.7 vs 4.3 mmol/d) but equal weight gain, serum Na, total calorie & Ca intake and daily urine output. Urinary ph and excretion of Ox, phosphorus,& uric acid were similar in both PIs. Na excretion was greater in PIs on HC&SP vs C (134mmol/L vs 47mmol/L,p<.01) & urine Ca less ( 1.2mmol/L vs 1.4mmol/L,p<.05). The mean CaOxRSR in PIs on HC&SP was less than C ( 7.8 vs 15.0,p<.01). There were no differences in CaPhos saturation (.62 vs .45)between PIs. CaOxRSR increased as urinary Ca increased while an inverse association occurred in CaOxRSR with Ox excretion in both groups. At any level of urinary Ca or Ox excretion, PIs on HC & SP had lower urine CaOxRSR p<.01. We conclude: In PIs, HC & SP lower urine CaOxRSR & may decrease the risk for NC. Lower urine Ca excretion despite a 3 fold > Na intake & 3 fold > Na excretion along with equal weight gain, serum Na & urine output in PIs on HC&SP vs C makes us speculate that Ca reabsorption may occur in the distal nephron via TRPV5 channels.

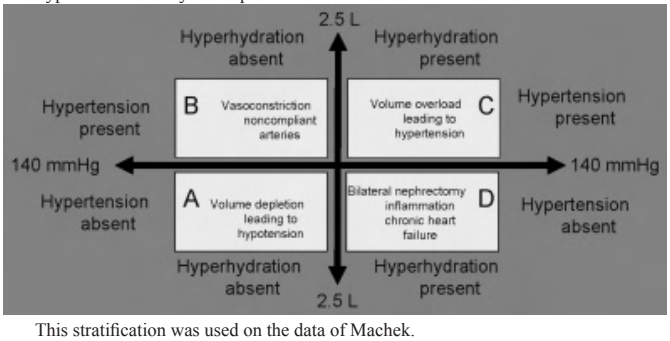
Disclosure of Financial Relationships: nothing to disclose

## F-FC316

**Hypertension & Fluid Status Management – Stratification of CKD Patients** Peter Wabel,[1] Petr Machek,[2] Paul William Chamney,[3] Tomas Jirka.[1] *[1]Fresenius Medical Care D GmbH; [2]NephroCare Czech; [3]Fresenius Medical Care UK.*

Introduction

Control of hypertension and management of fluid overload are closely linked. In the DRIP study, Agarwal (Hypertension 2009) demonstrated that management of hypertension by lowering fluid status can be successful. But not all hypertensive patients tolerated the reduction of post weight. Machek (NDT 2009) managed the fluid overload in a one year prospective trial. He showed a significant reduction in the fluid overload accompanied by an improvement of the hypertension status and the intradialytic adverse events. Agarwal (Sem Dial 2009) and Wabel (NDT 2008) proposed a separation of hypertensive patients into hyper- and normohydrated patients.




This stratification was used on the data of Machek.

Methods

The hypertensive patients were divided into a “hyperhydration present” and a “hyperhydration absent” group. We used a cut-off of 140mmHg for BPsys and 2.5 L for the hydration status (measured with the BCM-Body Composition Monitor).

Results

31 of 52 patients presented BPsys>140 mmHg. 39% of these patients could be assigned to group C (hypertension and hyperhydration). After the intervention (one year) period it was possible to reduce the BPsys by 25 mmHg (p=0.006) by reducing the fluid overload by 2 L (p<0.001). The antihypertensive medication was reduced significantly. Intradialytic adverse events reduced slightly.

Conclusion

For the successful management of hypertension it is essential to stratify the patients according to a measurement of their fluid status. In group C patients (hypertension and hyperhydration) control of hypertension can be achieved by sufficient removal of fluid overload. Not all hypertensive patients will benefit from a reduction in the fluid status. In some patients (group B) aggressive reduction of the hydration status can result in a higher incidence of intradialytic adverse events.

Disclosure of Financial Relationships: Employer: Fresenius Medical Care.

**F-FC317**

**Nocturnal Dysregulation of Heart Rate Is Associated with Cardiovascular and All-Cause Mortality in End-Stage Renal Disease Patient on Hemodialysis** Nobumasa Nakamura,[1] Hirotake Kasuga,[1] Keiko Kimura,[1] Ryo Takahashi,[1] Seiichi Matsuo.[2] *[1]Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Japan; [2]Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

**Background:** It has been established that elevated resting heart rate (HR) is associated with cardiovascular (CV) morbidity and mortality, besides, some reports have described that blunted nocturnal dipping of HR independently predicts the poor survival in general population. On the other hand, CV disease is a leading cause of death in patients on hemodialysis (HD), however, the association between CV mortality and the nocturnal HR regulation is little known in this population. We investigated whether the amplitudes of nocturnal HR dipping could predict CV and all-cause mortality in HD patients.

**Methods:** A total of 309 HD patients (male 55%, age 63±11years, diabetes 46%) underwent 24hr ambulatory Holter ECG monitoring during inter-dialysis day. From obtained mean diurnal HR and mean nocturnal HR during each periods based on ambulatory recording, diurnal-nocturnal HR dipping ratios were calculated as following; (mean diurnal HR – mean nocturnal HR) / mean diurnal HR x 100. They were divided into tertiles according to the percentage reduction of HR levels; <5.4% (T1, n=103), 5.4-11.7% (T2, n=103) and >11.7% (T3, n=103). They were followed up for 8years.

**Results:** During follow-up period (75±25months), 88 patients (28.6%) died including 36 CV death (11.7%). Eight-year event-free survival rates were 76.9%, 89.6% and 93.2% for CV mortality (p=0.0013), and were 60.4%, 68.1% and 81.0% for all-cause mortality in the groups of T1, T2 and T3 (p=0.0018), respectively. After adjustment for gender, age, diabetes, hypertension, hyperlipidemia, smoking, history of CV disease, hemoglobin, albumin and C-reactive protein, the reduction of HR dipping was an independent predictor for CV mortality (HR 3.31 for T1 vs. T3, p=0.011) and for all-cause mortality (HR 2.13 for T1 vs. T3, p=0.035), respectively. Neither diurnal HR nor nocturnal HR was associated with the mortality.

**Conclusion:** Nocturnal dysregulation of HR expressed as the reduction of HR dipping was strongly associated both CV and all-cause mortality in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**F-FC318**

**Higher Ultrafiltration Rate (UFR) Is Associated with Increased Cardiovascular (CV) Morbidity and Mortality on Hemodialysis (HD)** Jennifer E. Flythe, Steven M. Brunelli. *Brigham and Women's Hospital, Boston, MA.*

HD patients have very high rates of CV morbidity and mortality that may be related to the hemodynamic effects of rapid ultrafiltration.

**Aim:** This study was designed to test the hypothesis that higher UFRs are associated with greater all-cause and CV mortality, hospitalization for CV disease, and intra-dialytic hypotension.

**Methods:** Data were taken from the Hemodialysis Study, a randomized trial conducted between March 1995 and December 2001 that included 1,846 thrice-weekly chronic HD patients. UFR was considered as both a cubic spline and a categorical exposure: ≤10 ("low"; n=644), 10-13 ("intermediate"; n=517), and >13 ml/hr/kg ("high"; n=685).

**Results:** Cubic spline analysis revealed a steep rise in the risk for all-cause and CV mortality at UFR >10 ml/hr/kg. On categorical analysis, high UFRs were associated with increased all-cause and CV mortality: adjusted HRs (95% CIs) 1.36 (1.14- 1.61, p=0.001) and 1.53 (1.16- 2.03, p= 0.003), respectively, when compared to low UFRs.

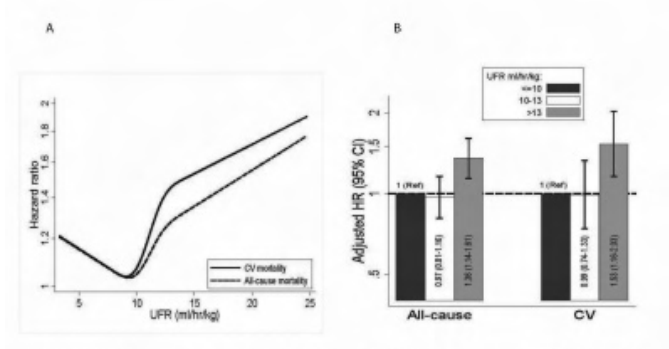



Figure. Association between UFR, considered as a cubic spline (A) and categorical (B) exposure, and all-cause and cardiovascular mortality.

Intermediate UFRs were not associated with mortality overall, but were among subjects with congestive heart failure: HR (95% CI) 0.73 (0.56-0.96) and 1.30 (1.02-1.66) among CHF- and CHF+, respectively (p-interaction=0.002). Secondary analyses considered time to: CV hospitalization or all-cause mortality (1081 events), CV hospitalization or CV mortality (843 events), and CV hospitalization alone (742 events); results followed a similar pattern to those of the primary analyses. Intermediate and high UFRs were significantly associated with intradialytic hypotension: adjusted ORs (95% CIs) 1.28 (1.18- 1.39) and 1.48 (1.35- 1.63), respectively.

**Conclusion:** UFRs >13 ml/hr/kg are associated with a higher risk of all-cause and CV death in chronic HD patients. The mechanism by which higher UFRs influence CV outcomes may relate to associated increases in intradialytic hypotension.

Disclosure of Financial Relationships: nothing to disclose

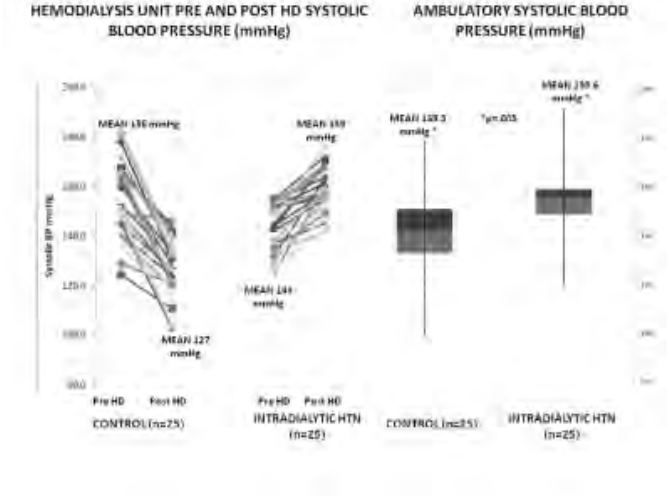
**F-FC319**

**Interdialytic Ambulatory Blood Pressure in Patients with Intradialytic Hypertension** Peter N. Van Buren, Robert D. Toto, Jula K. Inrig. *Internal Medicine-Division of Nephrology, University of Texas Southwestern Medical Center-Dallas, Dallas, TX.*

Purpose: Hemodialysis (HD) patients with intradialytic hypertension (IH) have higher risk for hospitalization and mortality compared to those in whom BP decreases during a routine HD session. The mechanism involved in IH is incompletelely understood. We hypothesized that those with IH have a higher time-integrated interdialytic BP burden as compared to those without IH.

Methods: We performed a case-control study including 25 subjects with IH (defined as systolic blood pressure [SBP] increase ≥ 10 mmHg in 4/6 consecutive HD treatments) and 25 HD control subjects (SBP decreased ≥ 10 mmHg during 4/6 consective HD treatments). Interdialytic ambulatory SBP (ASBP) was measured by 44-hour recording using a Spacelabs 90207 machine. SBP was recorded every 30 minutes during the daytime and hourly at night. ASBP were compared between groups using Wilcoxon Rank Sum test. Spearman Rank Correlation was used to compare ASBP with pre and post-HD SBP.

Results: The mean age of all subjects was 54 years; 80% were male, 38% were African American, and 82% were diabetic. Two-week time averaged pre and post HD unit SBP was 156 and 127 mmHg in controls and 140 and 159 mmHg in IH subjects (Figure). Mean 44 hour ASBP for the control and IH subjects was 140 and 156 mmHg, respectively (p=.005, Figure). There was a significant correlation with ASBP and post HD SBP (r=.61, p< 0.0001), but not pre HD SBP (r=.28, p=.05).




Conclusions: Patients with IH experience a greater inter-dialytic SBP burden compared to HD controls. The inter-dialytic ASBP correlates with post HD, but not pre HD SBP. Adverse outcomes associated with IH may partially be the result of increased interdialytic SBP burden.

Disclosure of Financial Relationships: nothing to disclose

**F-FC320**

**A Randomized Controlled Trial of Low Dialysate and Dietary Sodium on Blood Pressure in Hemodialysis** Albert J. Power, Seema Singh, Claire Edwards, Damir Tandaric, David Taube, Neill D. Duncan. *Imperial College Kidney & Transplant Institute, West London Renal & Transplant Centre, Hammersmith Hospital, London, United Kingdom.*

Up to 85% hemodialysis [HD] patients need antihypertensives as well as ultrafiltration for blood pressure [BP] control. Dietary sodium [Na] restriction with aggressive ultrafiltration and lowered dialysate Na can reduce interdialytic weight gain [IDWG] & antihypertensive use. We studied the combined effect of these measures which has not been prospectively examined to date.

Fifty stable maintenance HD patients entered an open-label prospective trial Aug 2008-Dec 2009 with a 12wk run-in [dialysate Na 140mM, unrestricted diet] and a 36wk intervention phase. Sodium gradient [NaG] is defined by the difference dialysate - serum Na. Groups consisted of controls, low dialysate Na alone [target 3mM below patient's mean serum level by graded reduction: 1mM / 2 wks], low dietary Na alone [2.3g/d with dietetic input], low dialysate & dietary Na. BP was recorded on HD and 24hr ambulatory records taken 6 weekly [midpoint of dialysis week]. Antihypertensive dose was recorded as defined daily dose [WHO]. A 22-point version of the Dialysis Symptom Index was completed 6 weekly.

Patients reaching the treatment phase were analysed [n=31, mean age 65.1±12.4yrs, 84% male, 40% diabetic]. Na restriction did not significantly alter predialysis Na. Low dialysate Na alone led to significant changes in NaG [+1.3 → -1.0mM, p<0.05]. Combined restriction led to reductions in NaG [+0.3 → -2.6mM, p<0.05] as well as IDWG [1.9 → 1.5kg, p<0.001] & improved intradialytic BP stability [p<0.05].

Any form of Na restriction caused more frequent but not severe cramps [+21%, p=0.04] vs controls. Isolated low dialysate Na reduced symptomatic intradialytic hypotension [27% → 4%, p=0.002].

Significant reductions in IDWG and antihypertensives require combined Na restriction which improves intradialytic hemodynamic stability. Na restriction (dialytic +/- dietary) leads to more muscle cramps but not of a degree to force treatment change. Findings from this small but prospective study need larger numbers and longer follow up to determine any long term impact on cardiovascular outcomes.

Disclosure of Financial Relationships: nothing to disclose

## F-FC321

**Heart Failure with Normal Ejection Fraction (HFNEF) – Why Defining This Entity in End-Stage Renal Disease Patients?** Angela Yee Moon Wang,[1,2(previous)] Mei Wang,[1,2(previous)] Christopher W. K. Lam,[3] Siu Fai Lui,[2] John E. Sanderson.[2] *[1]Medicine, Queen Mary Hospital, University of Hong Kong, Hong Kong, Hong Kong; [2]Medicine & Therapeutics, Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong, Hong Kong; [3]Chemical Pathology, Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong, Hong Kong.*

Heart failure (HF) is a frequent complication & powerfully predicts mortality in ESRD. This study aimed to determine the prevalence, severity, risk factors & prognostic importance of HF with normal ejection fraction (EF) (HFNEF) or diastolic dysfunction. We conducted a prospective study in 218 ESRD patients with 2D-echo & tissue Doppler imaging (TDI) done at baseline, then followed prospectively for 4 yrs. 100 had no HF, normal EF (EF ≥ 50%); 32 had no HF but EF < 50%; 47 (21.6%) had HFNEF; 39 had HF+ & EF < 50%. A significant increase in coronary artery disease (P<0.001), diabetes (P<0.001), & heart valve calcification (P=0.018) was observed across 4 groups. Hemoglobin (P=0.002) & serum albumin (P=0.002) showed significant decrease while systolic blood pressure (P=0.021), pulse pressure (P<0.001), C-reactive protein (P=0.037), troponin T (P<0.001) & NT-pro-BNP (P<0.001) showed significant increase across 4 groups. LV mass index & early mitral inflow velocity to peak mitral annulus velocity (E/Em ratio) was highest in HF+, EF < 50% group, followed by HFNEF group, then no HF group (P<0.001). The 4-yr cumulative cardiovascular (CV) & heart failure event-free survival probability were lowest in HF+, EF< 50% group, followed by HFNEF group, then no HF group (P<0.001). In multivariable Cox regression analysis, adjusted hazard ratios in relation to CV events were 2.9 in HF+, EF <50% group (P<0.001) & 2.2 in HFNEF group (P=0.002) using no HF & EF ≥ 50% as reference. These data clearly suggest the importance to define 'HFNEF' in ESRD patients & differentiate heart failure ESRD patients with normal & depressed EF as their clinical & cardiovascular profiles & outcomes are not the same. HFNEF patients had an increased risk of adverse CV outcomes though lower than HF+, EF <50% group. These results raise the need to differentiate treatment strategies in these two groups of patients.

Disclosure of Financial Relationships: Consultancy: International Medical Advisory Board Member of Baxter Renal Discovery Extramural Grant Program 2009 and 2010Research Funding: Abbott, Genzyme Corporation, Roche Diagnostics; Honoraria: Genzyme, Roche Diagnostics, Baxter Healthcare Corporation; Scientific Advisor: Editorial board member of J Am Soc Nephrol, Clin J Am Soc Nephrol, Am J Kidney Dis (associate editor), Am J Nephrol, J Nephrol, Nephrology (subject editor), J Diabetes, J Nephrol Renal Transplant, KDIGO CKD-MBD clinical practice guidelines workgroup member, board member of Faculty of 1000 Medicine, chair of Genzyme Asia-Pacific Renal Advisory Board Meeting 2009 and 2010.

## F-FC322

**A Prospective Randomized Controlled Open-Label Study on the Effect of Biocompatible Peritoneal Dialysis Fluids on Daily Urine Volume and Residual Renal Function** Sing-Leung Lui,[1] Wai Kei Lo,[1] Daniel Tak Mao Chan.[2] *[1]Medicine, Tung Wah Hospital, Hong Kong, China; [2]Medicine, Queen Mary Hospital, Hong Kong, China.*

The aim of this study was to examine the effect of biocompatible peritoneal dialysis fluids (PDF) on daily urine volume and residual renal function (RRF) in patients newly started on continuous ambulatory peritoneal dialysis (CAPD). One hundred and fifty patients were randomized to use either biocompatible PDF, consisting of neutral-pH low glucose degradation product fluid (Physioneal®), amino acid-based fluid (Nutrineal®) and icodextrin (Extraneal®) or conventional dextrose-based PDF (Dianeal®) for 48 weeks. The daily urine volume and RRF (mean of urea and creatinine clearance) were determined by 24-hour urine samples at baseline, 24 and 48 weeks. Seventy-seven and 73 patients were randomized to use biocompatible PDF and conventional PDF respectively. The demographics of both study groups were similar. There were no statistically significant differences in their daily urine volume (1087 ± 656 ml vs. 1067 ± 553 ml respectively, p=0.845) and RRF (4.02 ± 2.10 ml/min/1.73m² vs. 3.68 ± 2.09 ml/min/1.73m² respectively, p=0.377) at baseline. The biocompatible PDF group had significantly higher daily urine output at week 48 (959 ± 515 ml vs. 798 ± 615 ml, p=0.046), but the biocompatible and the conventional PDF groups showed similar RRF at week 24 (3.67 ± 2.17 ml/min/1.73m² vs. 3.43 ± 2.49 ml/min/1.73m² respectively, p=0.579) and at week 48 (3.24 ± 1.98 ml/min/1.73m² vs. 2.88 ± 2.43 ml/min/1.73m², p=0.379), and similar rates of RRF decline over 48 weeks (-0.76 ± 1.77 ml/min/1.73m² vs. -0.91 ± 1.92 ml/min/1.73m², p=0.568). KT/V values of the two groups at baseline, 24 and 48 weeks were similar. The two groups did not differ in diuretic and ACEI usage, peritonitis free survival, hospitalization rate, and patient or technique survival. We conclude that the use of a combination of Physioneal®, Extraneal® and Nurtrineal® for 48 weeks was associated with greater urine volume in CAPD patients, but did not affect RRF compared with conventional PDF after 48 weeks of treatment.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC323

**Alkaline Phosphatase Improves Sepsis-Induced Acute Kidney Injury: A Double Blind Prospective Randomized Placebo-Controlled Phase II Trial** Peter Pickkers,[1] Suzanne Heemskerk,[1,2] Johannes G. van der Hoeven.[1] *[1]Intensive Care Medicine, Radboud University Medical Centre Nijmegen, Nijmegen, Netherlands; [2]Pharmacology and Toxicology, Radboud University Medical Centre Nijmegen, Nijmegen, Netherlands.*

Rationale: Alkaline phosphatase (AP) is an endogenous detoxifying enzyme that is depleted in the kidney during an ischemic or inflammatory insult. Administration of AP improves outcomes in animal models and decreases urinary excretion of markers of tubular damage in a previous sepsis trial.

Objective: To evaluate whether AP treatment improves renal function in sepsis patients with acute kidney injury (AKI).

Methods: Thirty-six sepsis patients (27m/9f, mean age 66±14 yrs) with evidence for kidney injury (minimal AKIN stage 1) were included in a double-blind, randomized, placebo-controlled study on the safety and efficacy of AP. Patients on dialysis were excluded and during the study renal replacement therapy (RRT) was started according to the ADQI criteria. AP was administered intravenously as a bolus injection of 67.5U/kg followed by continuous infusion of 132.5U/kg for 48h and followed up for 28 days.

Measurements and Main Results: Progress in renal parameters was the primary endpoint (combined creatinine clearance, requirement and duration of RRT). Secondary endpoints were clinical improvement (duration of ventilator support and ICU LOS) and changes in the urinary excretion of biomarkers of renal injury.

AP treatment was well-tolerated by patients with sepsis and AKI. Creatinine clearance is restored to normal in the AP group within 7 days and remains impaired in the placebo group (p=0.02) and this improvement was sustained during the follow-up period (p<0.05). Fewer patients in the AP group tended to require RRT (19 vs. 36%, p=0.29) and the relative duration of RRT was shorter (12 vs. 34% of total time in study, p=0.04), supported by significant changes in biomarkers such as renal KIM-1. Furthermore, AP treatment reduced mean ICU LOS (10.9 vs 24.5 days, p=0.02) and duration of mechanical ventilation (8.0 vs 13.9 days, p=0.094).

Conclusions: Alkaline phosphatase treatment attenuates renal damage and improves renal function in sepsis patients with AKI.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC324

**Statin Use Is Associated with Less Acute Kidney Injury after Major Elective Surgery** Amber Molnar,[1] Steven G. Coca,[2] Arsh Jain,[1,3] Jin Luo,[4] Chirag R. Parikh,[2] John Michael Paterson,[4] Ron Wald,[5] Michael W. Walsh,[6] Amit X. Garg.[1,3,4,6] *[1]Division of Nephrology, University of Western Ontario, London, ON, Canada; [2]Section of Nephrology, Yale University, New Haven, CT; [3]Department of Epidemiology and Biostatistics, University of Western Ontario, London, ON, Canada; [4]Institute for Clinical Evaluative Sciences, Toronto, ON, Canada; [5]Division of Nephrology, University of Toronto, Toronto, ON, Canada; [6]Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, ON, Canada.*

Animal studies suggest statins protect the kidneys when administered prior to an ischemic insult. Whether a similar benefit occurs in humans is uncertain. We conducted a population-based retrospective cohort study of all older patients who underwent major elective surgery in the province of Ontario, Canada from 1995 to 2008 (cardiac, thoracic, vascular, intra-abdominal and retroperitoneal surgeries). The primary outcome was acute kidney injury within 14 days of surgery determined using administrative database codes. Secondary outcomes were acute dialysis within 14 days of surgery and mortality within 30 days of surgery. A total of 219,524 patients had major elective surgery. The incidence of postoperative acute kidney injury was 2.6% (5786 patients), acute dialysis 1.3% (2954 patients) and mortality 2.8% (6201 patients). Prior to surgery, 32% (70,485) of patients were taking a statin. After statistical adjustment for patient and surgical characteristics, statin use was associated with less acute kidney injury (odds ratio [OR] 0.88; 95% confidence interval [CI] 0.82 to 0.93), less acute dialysis (OR 0.91; 95% CI 0.84 to 0.99) and less mortality (OR 0.79; 95% CI 0.74 to 0.85). Similar results were seen with propensity score matching. Our data suggest statins prevent important renal complications after major elective surgery and reduce perioperative mortality. The hypothesis warrants testing in large multi-centre randomized controlled trials. However, conducting trials which establish a benefit on the most important renal outcomes will be logistically challenging.

Disclosure of Financial Relationships: nothing to disclose

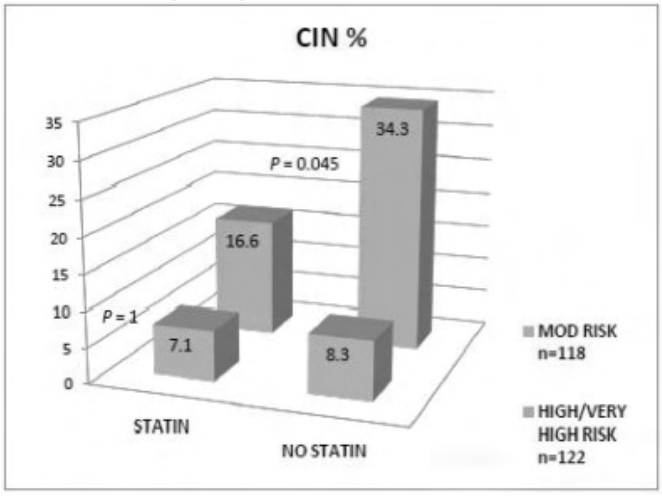
## SA-FC325

**Role of Statins in the Prevention of Contrast-Induced Nephropathy (CIN) in High Risk Patients** Hector Castro,[1] Ivonne Hernandez Schulman.[1,2] *[1]Nephrology Division, University of Miami, Miami, FL; [2]Nephrology and Hypertension Division, Miami VAMC, Miami, FL.*

*PURPOSE*: We investigated whether statin pretreatment may prevent CIN in high risk patients.

*METHODS*: 240 patients that underwent vascular procedures requiring the use of contrast media between 2007 and 2009, and that were at moderate, high or very high risk for CIN were studied. 160 patients received preprocedure statin, and 80 did not. CIN was defined as an increase in serum creatinine of ≥ 0.5 mg/dl or ≥ 25% from baseline 24 to 48 hs after contrast exposure. Multivariable regression analysis was used to determine the statistical significance of the findings.

*RESULTS*: The average risk score was similar in both groups (11.3 ± 3.2 and 10.5 ± 3.5, $p = 0.098$). There was a lower incidence of CIN in the statin group compared to the no-statin group (12.5% vs 18.75%, respectively), but it was not significant ($p = 0.24$). Sub-group analysis showed a significant reduction of CIN in high/very high risk patients in the statin group compared to the no-statin group ($p = 0.045$), whereas no difference was seen for moderate risk patients ($p = 1$).




In high/very high risk patients, multivariate analysis revealed that statin use was independently associated to the absence of CIN when adjusted for age, DM, CHF, eGFR and contrast volume (OR 0.3, 95% CI 0.10 – 0.91, $p = 0.03$). This association was not seen in the moderate risk group.

Multivariate association with CIN

| Variable | | High/very high risk patients | |
|---|---|---|---|
| | *p* | OR | 95% CI |
| Statin | 0.03 | 0.3 | 0.10 - 0.91 |
| Age | 0.13 | 0.96 | 0.91 - 1.01 |
| DM | 0.23 | 2.13 | 0.63 - 7.26 |
| CHF | 0.15 | 2.41 | 0.73 - 8.01 |
| eGFR | 0.004 | 0.96 | 0.94 - 0.99 |
| Contrast volume | 0.99 | 1 | 0.99 - 1.00 |

*CONCLUSIONS*: Statin pretreatment may be a useful strategy for CIN prevention in high risk populations.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC326

**Association of Pre-Operative ACEIs or ARBs with a Reduction in Post-Operative AKI after Elective CABG** Tao-Min Huang, Vincent Wu, Guang-Huar Young, Fan-Chi Chang, Pi-Ru Tsai, Wen-Je Ko, Kwan-Dun Wu. *Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan.*

Objectives: To examine the association of pre-operative ACEi/ARBs and renal outcome post CABG.

Background: The effect of ACEi/ARBs on renal outcomes in CABG is controversial. We evaluated the effect of pre-operative ACEi/ARBs on renal outcomes in this patient group.

Methods: We included non-dialysis patients undergoing CABG from 2002 to 2007. The primary endpoint was post-operative AKI, defined with RIFLE and the secondary endpoints were postoperative renal replacement therapy (RRT) and 30-day all-cause mortality.

Results: Total 1,172 patients were enrolled and 451 took ACEi/ARBs before surgery. AKI developed in 6.1% patients. Stepwise logistic regression revealed that pre-operative ACEi/ARB usage was associated with a lower incidence of AKI [OR 0.48, p =0.01]. After propensity score matching, pre-operative ACEi/ARBs were significantly associated with a lower incidence of post-operative AKI (3.7% vs. 9.1%, p=0.01). There was no significant difference of post-operative RRT or 30-day mortality.

Table 1. Demographic characteristics and outcomes of the propensity score matched cohort

| Variables | ACEi/ARBs (n = 298) | No ACEi/ARBs (n = 298) | P |
|---|---|---|---|
| | | | |
| Age | 65.6±10.2 | 65.6±10.9 | 0.988 |
| Gender (Male) | 78.2% | 75.5% | 0.497 |
| | | | |
| Recent MI | 18.8% | 18.8% | 1.000 |
| CHF | 12.8% | 11.4% | 0.706 |
| | | | |
| DM | 50.7% | 47.3% | 0.461 |
| HTN | 71.8% | 67.8% | 0.326 |
| | | | |
| Low LVEF | 47.7% | 46.3% | 0.806 |
| CPB | 13.1% | 11.1% | 0.053 |
| | | | |
| Cr (mg/dL) | 1.36±0.83 | 1.34±0.81 | 0.764 |
| | | | |
| Beta-blockers | 47.3% | 49.3% | 0.682 |
| CCB | 22.8% | 24.5% | 0.700 |
| Statins | 29.5% | 31.9% | 0.594 |
| Propensity scores | 0.434±0.209 | 0.430±0.207 | 0.802 |
| Outcome | | | |
| AKI | 3.7% | 9.1% | 0.011 |
| Need for RRT | 1.4% | 1.4% | 1.000 |
| 30-day mortality | 1.7% | 2.4% | 0.772 |
| Composite endpoint | 4.7% | 9.4% | 0.038 |

Composite endpoint: composite endpoint of acute kidney injury, renal replacement therapy, and 30-day mortality

Conclusions: We demonstrated an association between pre-operative ACEi/ARBs and reduced post-operative renal dysfunction in this patient group.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC327

**Risk of Acute Kidney Injury (AKI) in Hospitalized Medical Patients on ACE Inhibitors (ACE I) and Angiotensin Receptor Blockers (ARBs) Versus Other Antihypertensives** Etti Deborah Zeldis, David Schwarzbaum, Ladan Golestaneh, Michal L. Melamed. *Albert Einstein College of Medicine/ Montefiore Medical Center.*

AKI is a common hospital acquired event with substantial morbidity and mortality. ACE I and ARBs are inhibitors of the renin-angiotensin system and are associated with long term improved renal outcomes, but their effects in acutely ill hospitalized patients has not been established.

We studied all patients >18 years with no known end-stage renal disease on the medical services at two urban hospitals admitted in 2009 (n=12504). Use of ACE I/ ARBs and other antihypertensives (calcium channel blockers, beta blockers, diuretics) during admissions, baseline demographics, comorbidities, laboratory data, vital signs, and exposure to IV contrast and NSAIDs were obtained from electronic records. The primary outcome was the diagnosis of AKI risk using the RIFLE criteria, a rise in creatinine 50% from baseline (n=1602). Baseline creatinine was defined as the last creatinine value recorded within 1 year prior to admission.

Among the included patients, 4878 (39%) were on ACE I/ARBs (and potentially other anti-hypertensives) and 7626 (61%) were on other antihypertensives but not ACE I/ARBs. The mean age was 67 years, 58% were female, 36% were African-American, 36% were Hispanic, and 40% had diabetes mellitus. Mean baseline creatinine was 1.22 mg/dL for patients on ACE I/ARBs and 1.66 mg/dl for patients on other antihypertensives. Overall, 14.8% of the patients developed AKI risk. The presence of systolic blood pressures <90 mmHg (odds ratio (OR) 2.9, 95% CI: 2.4, 3.3), concomitant NSAID use (OR 1.4, (1.1,1.7)), and elevated baseline creatinine (OR 1.28, (1.24,1.32)) were associated with an increased AKI risk. Overall, 10.9% of those on ACE I/ARBs developed AKI risk compared to 17.2% on other anti-hypertensives. After multivariable adjustment, patients who were on ACE I/ ARBS during their hospitalization had an OR of 0.84 (0.74, 0.95) for the development of AKI compared to patients on other antihypertensives.

In summary, ACE I/ARB use was associated with a lower incidence of AKI risk in hospitalized medical patients. Future studies are needed to confirm these associations.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC328

**Acute Renal Events Associated with Fibrate Use: A Population-Based Study** Ying Y. Zhao,[1] Matthew A. Weir,[1] Michael Manno,[2] Peter E. Cordy,[1] Tara Gomes,[2] Daniel G. Hackam,[3] David N. Juurlink,[4] Muhammad Mamdani,[2,4] Louise M. Moist,[1] Chirag R. Parikh,[5] John Michael Paterson,[2] Ron Wald,[4] Amit X. Garg.[1,2] *[1]Division of Nephrology, Department of Medicine, University of Western Ontario, London, ON, Canada; [2]Institute for Clinical Evaluative Sciences, Toronto, ON, Canada; [3]Division of Clinical Pharmacology, University of Western Ontario, London, ON, Canada; [4]Department of Medicine, University of Toronto, Toronto, ON, Canada; [5]Section of Nephrology, Yale University School of Medicine, New Haven, CT.*

***Background***: Modest increases in serum creatinine associated with the use of fibric acid derivatives (fibrates) have been observed in randomized trials.

***Method***: We conducted a population-based cohort study of all patients 66 years or older with a new prescription for a fibrate or ezetimibe (comparator drug) in Ontario, Canada. Patients were accrued from January 2004 to December 2008. The main outcomes were

hospitalization for acute kidney injury (AKI) and consultation with a nephrologist within 90 days of a new prescription. We also compared characteristics of fibrate users in our cohort to those enrolled in three randomized trials.

***Results:*** Compared to ezetimibe users (n=61,831), fibrate users (n=19,072) had a higher risk of AKI requiring hospitalization (adjusted odds ratio [OR] 2.4, 95% CI 1.7 to 3.3) and were more likely to see a nephrologist in consultation (adjusted OR 1.3, 95% CI 1.1 to 1.6). Among those with serum creatinine measurements, 9.1% of fibrate users and 0.3% of ezetimibe users experienced a 50% or more increase in serum creatinine concentration (OR 29.6, 95% CI 8.7 to 100.5). The risk of AKI requiring hospitalization was greatest in patients with chronic kidney disease (CKD) (adjusted OR 3.4, 95% CI 2.2 to 5.1; test for interaction, p=0.043). Compared to patients in randomized trials, fibrate users in our cohort had a higher prevalence of CKD, were more likely to be on a higher dose of fibrate given their renal function, and had less routine renal monitoring in follow-up.

***Conclusion***: Fibrates are associated with adverse acute renal events. Their use warrants careful patient selection and close monitoring of renal function.

Disclosure of Financial Relationships: nothing to disclose

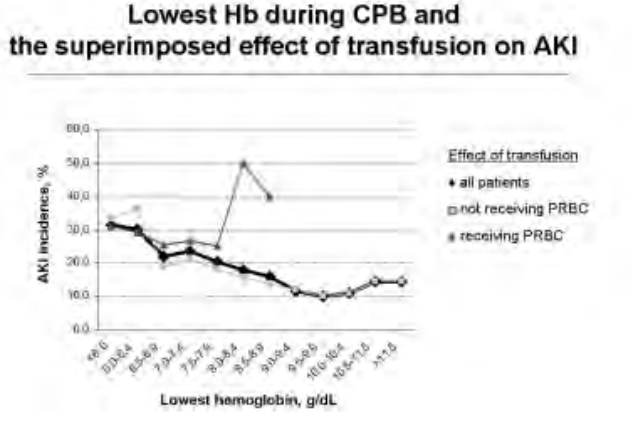
## SA-FC329

**Mean Arterial Pressure, Hemoglobin, and Blood Transfusion during Cardiopulmonary Bypass and Postoperative Acute Kidney Injury** Michael Haase. *Nephrology and Intensive Care, Charité, Berlin, Germany.*

*Purpose:* To investigate whether intraoperative hypotension, anemia, transfusion or their combination are independent risk factors for acute kidney injury (AKI).

*Methods:* We linked cardiac surgery and pathology databases with electronically stored systemic mean arterial pressure (MAP) data (three measurements/min) and blood gas analyses during cardiopulmonary bypass (CPB). We calculated indices of MAP (variability, time and area under the curve of MAP <50, <60 and <70 mmHg) and obtained hemoglobin, arterial oxygen saturation and pressure values. We tested the relationship of such variables and the need for red blood cell transfusion and vasopressor use in a mixed linear multivariable model with the development of AKI using RIFLE criteria.

*Results:* We analyzed 381,468 MAP measurements from 920 on-pump cardiac surgery patients of whom 19.5% developed AKI associated with 8.2-fold increased in-hospital mortality. Hemoglobin (Hb) concentration (OR 1.19 per g/dL decrease in concentration [95% CI 1.05-1.34]; *P*=0.006) was an independent risk factor for AKI with systemic arterial oxygen saturation and pressure values not adding further value. MAP alone or vasopressor administration was not independently associated with AKI but volume of red blood cell transfusion was with its impact being apparent at Hb levels >8.0 g/dL.




In patients with severe hypotension, the effect of anemia was more pronounced than in patients with minimal hypotension (OR 3.31 [95% CI 1.36-8.07]; *P*=0.007).

*Conclusions:* Intra-operative avoidance of the extremes of anemia, especially during severe hypotension and avoidance of transfusion in patients with Hb levels >8.0g/dL may help decrease AKI in patients undergoing cardiac surgery.

Disclosure of Financial Relationships: Honoraria: Abbott, Biosite.

## SA-FC330

**EArly RenaL Involvement in Hospital Acquired Acute Kidney Injury (EARLI)** Tarek M. Elachkar,[1] Geetha Balasubramanian,[1] Abdul Moiz,[1] Michael I. Rauchman,[1] Zhiwei Zhang,[1] Ziyad Al-Aly.[2] *[1]Department of Medicine-Nephrology, Saint Louis University and St. Louis VA Medical Center, St. Louis, MO; [2]Department of Medicine-Nephrology, St. Louis Veterans Affairs Medical Center, St. Louis, MO.*

**Background:** The optimal timing of nephrology consultation in hospital-acquired acute kidney injury (AKI) is unknown.

**Methods:** We examined the effect of Early Renal Involvement (EARLI) defined as a one-time Nephrology consultation at the onset of AKI on the risk of subsequent severe renal failure (≥250% increase in serum Creatinine (Cr)). We screened daily Cr of 4,296 patients admitted to the St Louis Veterans Affairs Medical Center between September 2008 and May 2009. 354 subjects (8.2%) met the definition of in-hospital AKI (Cr increase by 0.3mg/dl over 48 hours), of whom 176 patients met all inclusion criteria; 85 and 91 patients were enrolled in the control (standard care) and EARLI groups respectively.

**Results:** The two groups had similar baseline characteristics and at the time of AKI. Nephrology recommendations in the EARLI group included specific diagnostic, therapeutic and preventative components. Severe renal failure occurred in 12.9% of patients in the control group compared to 3.3% of patients in the EARLI group (p= 0.024). Patients in the EARLI group had a lower peak Cr of 1.8± 0.1 vs. 2.1± 0.2 mg/dl in control (p= 0.014). In a multivariate model adjusting for demographics and risk factors, both EARLI (OR= 0.08, CI= 0.13-0.47; p= 0.012) and statin use (OR= 0.04, CI= 0.01-0.25; p< 0.01) were associated with reduced risk of severe renal failure.

**Conclusion:** Early Nephrology involvement in AKI reduces the risk of severe subsequent renal failure. Our data also suggests that statin use may protect from worsening AKI.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC331

**Ang II-Induced TRPC6 Expression Is Cooperatively Regulated by NFAT, NF-κB, CREB, and Elk-1 in Cultured Mouse Podocytes** Gentzon Hall, Jason J. Eckel, Peter J. Lavin, Rasheed A. Gbadegesin, Guanghong Wu, Alison Byrd, Alison Homstad, Michelle P. Winn. *Department of Nephrology, Duke University Medical Center.*

TRPC6 is a non-selective $Ca^{2+}$ channel that has been found to be a cause of hereditary FSGS. Ang II is a potent inducer of TRPC6 activity and expression that has been associated with glomerulosclerosis and podocyte apoptosis. Nuclear Factor of Activated T-cells (NFAT), Nuclear Factor-κB (NF-κB), Cyclic AMP Response Element Binding Protein (CREB), and Elk-1 are transcriptional regulators involved in proinflammatory and proapoptotic signaling whose activity is induced by Ang II. We hypothesize that NFAT, NF-κB, CREB and Elk-1 cooperatively regulate the Ang II-induced expression of TRPC6 and that the activity of these transcription factors is modulated by the Mitogen and Stress Activated Protein Kinase (MAPK) and Phosphatidylinositol-3 Kinase (PI-3K) pathways, Inhibitor-κB Kinase (I-κK), $Ca^{2+}$/Calmodulin Kinase II (CamKII), and Calcineurin (Cn) in podocytes. Using a model system of immortalized mouse podocytes and a combination of immunoblot analyses, immunoflourescence staining, and chromatin immunoprecipitation (ChIP) we provide evidence that NFAT, NF-κB, CREB, and Elk-1 are activated in response to Ang II stimulation and that the activation of these transcription factors can be attenuated with inhibitors of the MAPK and PI-3K pathways, I-κK, CamKII, and Cn. Specifically, we demonstrate that Ang II induces phosphorylation of the p65 subunit of NF-κB, CREB, and Elk-1 at key serine residues associated with the activation of these transcription factors. This effect is attenuated with inhibitors of the MAPK and PI-3K pathways, I-κK, CamKII, and Cn. Additionally, we demonstrate that Ang II induces an upregulation of TRPC6 expression in podocytes and that this effect is also attenuated with inhibitors of the MAPK and PI-3K pathways, I-κK, CamKII, and Cn. Finally, using ChIP, we demonstrate the Ang II-induced recruitment of NFAT, NF-κB, CREB, and Elk-1 to sites within the upstream 450bp of the mouse *TRPC6* gene. Based on these findings, we propose a model of Ang II-induced TRPC6 expression governed by the cooperative activities of NFAT, NF-κB, CREB, and Elk-1.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC332

**miR-30 Protects Podocytes Against TGF-β Induced Apoptosis** Shaolin Shi, Taoran Zhang, Liping Yu, Haiying Qi, Erwin P. Bottinger. *Nephrology/Medicine, Mount Sinai School of Medicine, New York, NY.*

microRNAs control many biological processes by post-transcriptional regulation of gene expression. We have previously shown that podocyte-specific deletion of Dicer, the enzyme required for microRNA production, caused proteinuria, progressive glomerulosclerosis, and death, suggesting essential requirements for microRNAs in podocytes. We showed that miR-30 family was abundantly expressed in podocytes and miR-30 target genes were significantly enriched for apoptosis-associated genes [Shi et al, ASN abstract, 2008]. Based on these findings, we hypothesized that miR-30 members regulate apoptosis in podocytes. We have previously shown that TGF-β induces podocyte apoptosis *in vitro* and *in vivo* [Schiffer et al., JCI, 2001].

RESULTS: miR-30 family members (miR-30a, b, c, d, e) were strongly downregulated by TGF-β in conditionally-immortalized mouse podocytes within 24 hrs, and in glomeruli isolated from double transgenic (NPHS2-rTA/tet-O-TbRI(AAD)) mice with ligand-independent, doxycycline-inducible expression of constitutively active TGF-β receptor type I in podocytes, compared with controls. Lentivirus-mediated overexpression of miR-30d in podocytes significantly inhibited TGF-β-induced activation of caspase-3 and apoptosis (TUNEL-assay), compared to control scramble miRNA. We conclude that miR-30s protect against apoptosis and downregulation of miR-30 is required for TGF-β induced apoptosis of podocytes.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC333

**PAR-3 Activation by Activated Protein C: A Novel Podocyte Protective Signalling Pathway** Madhusudhan Thati, Hongjie Wang, Muhammed Kashif, Sandra Muller-Krebs, Vedat Schwenger, Martin G. Zeier, Peter Nawroth, Berend H. Isermann. *Internal Medicine-1 and Clinical Chemistry, University of Heidelberg, Heidelberg, Baden-Wuttenberg, Germany.*

Activated protein C (APC) has anti-coagulant and cytoprotective effects. The cytoprotecive role of APC has been well studied in endothelial cells but remains less well defined in non-endothelial cells. Here we used a rat model of puromycin aminonucleoside

(PAN) induced nephropathy and show that APC protects against PAN induced podocyte apoptosis and glomerular disease. *In vitro* experiments were performed using immortalized differentiated human podocytes to delineate the receptor mechanism.

PAN administration (150 mg/kg, i.p.) induced podocyte apoptosis and albuminuria by day-4, peaking at day 6. Administration of APC (5 mg/kg, i.v.day 1-6) protected against podocyte apoptosis and albuminuria. APC treatment after disease onset (day 4-6) significantly reduced podocyte apoptosis and albuminuria. Pre-incubation of APC with HAAPC antibody which blocks the anticoagulant, but not the cytoprotective, property of APC, did not abolish the cytoprotective effect, establishing that the protective effect of APC is independent of its anticoagulant property. To demonstrate the direct cytoprotective effect of APC on podocytes, FITC labelled APC was administered i.v. Within 3 min FITC-APC accumulated in the pericapillary space of rat glomeruli. In addition significantly elevated levels of PC were detected in urine samples of PAN treated rats as well as of diabetic patients or mice. These results suggest that APC can directly act on podocytes *in vivo*. *In vitro* APC (2 nM) prevented PAN induced apoptosis in podocytes. This anti-apoptotic effect is independent of endothelial protein C receptor (EPCR) but mediated by limited proteolysis of PAR-3 and cross-activation of either PAR-1 (mouse, rat) or PAR-2 (human).

In conclusion, we demonstrate a novel cyto-protective mechamism of APC in an acute model of nephropathy, which depends on APC mediated limited proteolysis of PAR-3. This novel podocyte protective signalling pathway may lay ground to the delineation of new pathophysiological concepts and therapeutic approaches.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC334

**Autophagy Induced by TGF-β1 Suppresses Apoptosis and Promotes Mesangial Cell Survival under Serum Deprivation Via Activation of the Akt Pathway** Yan Ding, Jin Kuk Kim, Sung I. L. Kim, Hee-Jun Na, Mary E. Choi. *Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Autophagy is a fundamental cellular homeostatic process that cells use to degrade and recycle proteins and remove damaged organelles. It can lead to cell death in response to stress, but it can also act as a protective mechanism for cell survival. We show that TGF-β1 induces autophagy and protects glomerular mesangial cells from undergoing apoptosis during serum deprivation. Serum withdrawal rapidly induced autophagy within 1 h in mouse mesangial cells (MMC) as determined by increased microtubule-associated protein 1 light chain 3 (LC3) levels and punctate distribution of the autophagic vesicle-associated-form LC3-II. We demonstrate that after 1 h there was a time-dependent decrease in LC3 levels, which was accompanied by induction of apoptosis evidenced by increases in cleaved caspase 3 and in the number of sub-G1 apoptotic cells. However, treatment with TGF-β1 resulted in an increase of mRNA and protein levels of the autophagy protein LC3 and increased autophagic flux, while suppressing caspase 3 activation and reduction in the number of sub-G1 apoptotic cells. TGF-β1 failed to rescue MMC from serum deprivation-induced apoptosis upon knockdown of LC3 by siRNA, and in MMC from LC3 null ($LC3^{-/-}$) mice. We show that TGF-β1 induced autophagy through Akt activation, and inhibition of PI3K-Akt-mTOR-S6K pathway by LY294002 or dominant-negative Akt suppressed LC3 levels and enhanced caspase 3 activation. TGF-β1 also up-regulated cyclin D1 and E protein levels, while down-regulating p27, thus stimulating cell cycle progression. Bafilomycin A1, but not MG132, blocked TGF-β1 down-regulation of p27, suggesting that p27 levels were regulated through autophagy. Taken together, our data indicate that TGF-β1 rescues MMC from serum deprivation-induced apoptosis via induction of autophagy through activation of the Akt pathway. Conversely, inhibition of autophagy led to mesangial cell apoptosis. The autophagic process may constitute a protective mechanism against glomerular injury by inhibiting apoptosis and promote mesangial cell survival.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC335

**Epithelial Notch Signaling Regulates Tubulointerstitial Fibrosis Development in the Kidney** Yasemin Sirin,[1] Bernhard O. Bielesz,[1] Volker H. Haase,[2] Katalin Susztak.[1] *[1]Medicine, Albert Einstein College of Medicine, Bronx, NY; [2]Medicine, Vanderbilt University, Nashville, TN.*

Tubulointerstitial fibrosis (TIF) is considered the final common pathway leading to end stage renal failure. Previously, we and others showed that the Notch pathway is activated in tubules of patients with TIF and in animal models thereof. Notch1, Jagged1 and HeyL were increased in Unilateral Ureteral Obstruction (UUO) and Folic Acid (FA) induced TIF models. Pharmacological inhibition of Notch activation with a γ-secretase inhibitor reduced the expression of profibrotic genes, such as collagen1, smooth muscle actin (SMA), vimentin, and fibronectin as well as TIF development in both models.

To better understand the mechanism of Notch induced TIF, we generated mice with a tubular defect for RBPj, the downstream transcriptional binding partner of Notch, by using the tubule specific PEPCK promoter (PEPCKCre-Rbpj flox/flox). These mice were significantly protected against fibrosis which was also reflected in a reduced expression of profibrotic genes after FA injection.

To further elucidate the role of Notch in TIF, we generated a doxycyclin inducible Notch1 over expressing mouse line using the tubule specific Pax8 promoter (Pax8rtTA-tetO-ICN1). Notch1 expression was induced at 4 weeks of age. We found that double transgenic animals develop severe TIF and die 5-6 weeks after induction with doxycyclin. We observed increased expression of markers of TIF including collagen1, 3, fibronectin, and SMA.

To get more insight into the involved pathways, we performed gene expression analysis using Affymetrix microarrays. We found 1784 differentially expressed transcripts (2-fold change, $p < 0.01$, Benjamini-Hochberg correction). Markers of proliferation were significantly increased. In accordance, we observed Notch induced proliferation both in tubular and interstitial cells on Ki67 immunostaining. Thus, we propose that epithelial Notch signaling induced interstitial cell proliferation and signaling might play a role in TIF development.

In summary, here we demonstrate in a proof of concept experiment that Notch activation in epithelial cells alone is sufficient to induce TIF.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC336

**AMP-Activated Protein Kinase Potentiates Hypertonicity-Induced Apoptosis by Blocking the NFκB/COX-2 Survival Pathway in Renal Medullary Interstitial Cells** Youfei Guan,[1,2] Hang Yang.[1] *[1]Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing, China; [2]Division of Nephrology, Vanderbilt University Medical Center, Nashville, TN.*

Renal medulla is characterized by extreme hypertonicity and severe hypoxia. Cells residing in this harsh environment depend on dynamic mechanisms that repeatedly reassess the status of amassed energy in order to adapt energy supply to demand. The AMP-activated protein kinase (AMPK) is a Ser/Thr protein kinase acting as a sensor of cellular energy status. The present study was designed to examine the role of AMPK in the survival of renal medullary interstitial cells (RMICs) under hyperosmotic condition both in vitro and in vivo. AMPK phosphorylation was decreased under hypertonic condition within 12 hours and then gradually returned to the baseline. Activation of AMPK by either an AMPK activator AICAR or a constitutively active AMPK construct markedly increased hypertonicity-induced RMIC apoptosis. This effect was associated with the suppression of hypertonicity-induced NFκB nuclear translocation and COX-2 expression and was abolished by overexpression of COX-2. AMPK activation also resulted in a marked reduction in the generation of reactive oxygen species (ROS) and expression of tonicity-responsive enhancer binding protein (TonEBP), which prevented up-regulation of osmoprotective genes in hypertonicity-treated RMICs. In vivo study further demonstrated that treatment of mice with AICAR caused a massive apoptosis of RMICs after a 12-hour water restriction. Taken together, these results identify AMPK as a critical factor involved in the maintenance of RMIC viability and suggest that AMPK is an important regulator of the NFkB-COX-2 survival pathway in renal medulla. Since tremendous efforts have been made in developing novel AMPK activator as potential anti-diabetic medicine, the present study raises safety concerns for such treatment in patients with dehydration.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC337

**HIF2α Is Sufficient for Rapid Generation of Renal Cysts in a Mouse Model of RCC** Nasir A. Shah,[1,2] Susan E. Quaggin.[1,3,4] *[1]Samuel Lunenfeld Research Institute, Toronto, ON, Canada; [2]Department of Physiology, University of Toronto, Toronto, ON, Canada; [3]Institute of Medical Science, University of Toronto, Toronto, ON, Canada; [4]Department of Nephrology, St. Michael's Hospital, Toronto, ON, Canada.*

Renal cell carcinoma is the most common urogenital tumor, accounting for 3% of all adult malignancies, 34,000 new cases, and a mortality of 13,000 each year in the United States. It has been shown that hypoxia inducible factors (HIFs) are upregulated in renal cell carcinoma (RCC). During normoxia the HIFα subunits are hydroxylated at specific proline residues, which allows for recognition by the VHL E3 ubiquitin ligase (composed of pVHL, Cullin2, elongin B, elongin C and Rbx1), and subsequent proteasomal degradation. In RCC, inherited or sporadic mutations in the VHL gene allow the HIFα subunits to escape degradation and translocate to the nucleus. Here they dimerize with HIF1β (ARNT), recruit transcriptional co-factors, and bind to consensus sequences in the promoter region of their downstream target genes. Although HIF-1α and HIF-2α are both upregulated in RCC, it has been suggested that HIF2α plays the more critical role. To further elucidate the role of HIF2α in RCC, we have generated an inducible transgenic mouse model that permits temporal stabilization of a transcriptionally active form of HIF2α in mature renal tubular epithelial cells under control of a Pax8rtTA driver. Induction of HIF2α results in the rapid development of renal cysts with concomitant loss of markers of tubular epithelial differentiation, which are both features of early RCC. Additionally, tubular-specific loss-of-function mutations in VHL and/or the tumor suppressor PTEN are predicted to result in full blown RCC and mice carrying these additional mutations have been generated and phenotyping is underway. Taken together, these results suggest that HIF2α is a key player in development of RCC and an excellent candidate target for therapy of this disorder.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC338

**Long Chain Acyl-CoA (LCA) Inhibition of NHE1 Plays a Role in Proximal Tubular Epithelial Cell (PTC) Deletion and Progressive Renal Disease** Bassam G. Abu Jawdeh, Shenaz Khan, Monu Goel, Satya P. Yadav, Raymond C. Harris, William P. Schilling, Eckhard K. Ficker, Jeffrey R. Schelling. *Case School of Medicine.*

Tubular atrophy caused by PTC apoptosis predicts CKD progression. Although free fatty acid (FFA) uptake by PTCs has been associated with accentuated apoptosis and tubulointerstitial disease, a pathophysiologic mechanism has not been delineated. We previously reported that NHE1 is regulated by a $PI(4,5)P_2/PIP_3$ dependent "on-off" switch, and defends against PTC apoptosis by $Na^+/H^+$ exchange-dependent increases in cell volume and pH. LCAs, the direct metabolites of FFAs, are structurally similar to phosphoinositides and regulate other exchangers. We hypothesize that LCAs accumulate in CKD and facilitate

lipotoxicity by inhibiting NHE1 activity. In ENOS$^{-/-}$db/db mice with diabetic nephropathy, triacylglycerol lipid droplets were observed in PTCs by Oil red O staining, reflecting FFA reabsorption. Kidney LCA content, determined by GC-MS, was significantly increased in ENOS$^{-/-}$db/db and ROP$^{Os/+}$(FSGS model) mice. The NHE1-LCA interaction was characterized by surface plasmon resonance, using purified, His-tagged wild-type (cNHE1) and point-mutant (KR/A) NHE1 peptides immobilized as ligands on a sensor chip, while flowing LCAs as analytes. LCAs bound cNHE1 with low affinity ($K_d$ ~10μM), which is similar to PI(4,5)$P_2$ affinity for cNHE1. LCA binding was attenuated by poly-L-lysine and $Mg^{2+}$ and to the KR/A mutant, suggesting an electrostatic interaction. To address the LCA effect on NHE1 function, LLC-PK1 cells were loaded with albumin-bound fatty acids, and inhibitors of FFA (triacsin C) and LCA (etomoxir) metabolism. Etomoxir inhibited NHE1-regulated $Na^+/H^+$ exchange (determined by BCECF fluorescence microscopy) and enhanced apoptosis (assessed by TUNEL). FFA-induced NHE1 inhibition and apoptosis were abrogated with triacsin C. Our data suggest that in proteinuric disease states PTCs reabsorb FFA-laden albumin, which leads to increased intracellular concentration of LCA. We conclude that accumulated LCA then inhibits NHE1, perhaps by competing with PI(4,5)$P_2$/$PIP_3$ binding, which enhances PTC apoptosis leading to tubular atrophy.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC339

**Defective Proteolysis of Albumin in the Proximal Convoluted Tubule of Limp2 Knockout Mice** Darren H. K. Lee,[1,2] Michael James Desmond,[1,2] Scott Andrew Fraser,[1] Marina Katerelos,[1] Kurt Gleich,[1] David A. Power.[1,2] *[1]Department of Nephrology, Austin Health, Heidelberg, Victoria, Australia; [2]Department of Medicine, University of Melbourne, Parkville, Victoria, Australia.*

Inactivating mutations of the lysosomal protein Limp2 cause Action Myoclonus Renal Failure Syndrome (AMRFS) in humans, which is associated with collapsing FSGS. Limp2 KO mice, by contrast, have a milder renal phenotype but develop tubular proteinuria and albuminuria. To identify the defect in tubular protein handling, Limp2 KO mice were injected with HRP and fluorescein-conjugated albumin. Uptake of HRP by the proximal tubule (PCT) at 7 mins showed no difference between KO and WT mice, indicating normal fluid phase endocytosis. Uptake of fluorescein-conjugated albumin at 7 mins was also similar in both groups. At 30 mins, however, there was persistence of albumin in the PCT of the KO mice with a change in distribution compared to the original uptake, indicating a failure of proteolysis. The presence of a normal tubular uptake system in the KO mice was confirmed by similar expression of the proteins taken up by the receptors megalin and cubilin along the brush border of the PCT. EM of perfusion fixed kidneys demonstrated an accumulation of large vacuoles in the PCT of KO mice, some apparently in contact with the luminal or basolateral surface of the cells. The appearance was similar to that seen in EBV-transformed lymphoblastoid B cells from 2 patients with AMRFS. This data suggests that the absence of Limp2 in the PCT leads to a lack of fusion of lysosomes with endosomes, persistence of the proteins reabsorbed by the PCT, and their eventual release from the surface of the cell into the urine. This data does not support the proposed ability of the PCT to reabsorb significant quantities of albumin without lysosomal degradation.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC340

**A Novel Protease-Activated Receptor-2 Antagonist Targeting Inflammation and Proliferation in Human Renal Tubular Epithelial Cells** David A. Vesey,[1] Jacky Yung Suen,[2] David W. Johnson,[1] David Fairlie.[2] *[1]Centre for Kidney Disease Research, The University of Queensland, Brisbane, QLD, Australia; [2]Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia.*

Protease-activated receptor-2 (**PAR2**) is a G-protein-coupled receptor (GPCR) activated by trypsin-like proteases and experimentally by hexapeptides corresponding to the receptors tethered ligand. Its ubiquitous expression has been linked to pro-inflammatory and proliferative conditions in a wide variety of tissues, including the kidney, and as such it is a highly desirable target for drug development. To date, little progress has been made in development of drug compounds targeting this receptor. Here we describe the actions of a non-peptidic specific **PAR-2** antagonist (GB88), which effectively inhibits these two responses in primary cultures of human kidney tubular epithelial cells (HTEC). In addition we reveal a potent non-peptidic **PAR2** agonist (GB110) which matches the peptidic **PAR2** agaonist, 2f-LIGRLO-$NH_2$, in its potency. Compared to the colon carcinoma cell line HT29, HTEC cells expressed 3-fold more **PAR2** mRNA (qRTPCR). In a calcium mobilisation assay, 2f-LIGRLO-$NH_2$ and GB110 were equally potent ($EC_{50}$ 1.5μM). GB88 reduced calcium release in response to these ligands and its natural ligand trypsin, with an $IC_{50}$ around 10μM. Exposure of these cells to either 2f-LIGRLO-$NH_2$ (0.5-2μM), or GB110 (0.5-5μM) induced a robust **DNA** synthetic response which was completely inhibited by 8μM GB88. In a human inflammation antibody array, more than 10 inflammatory cytokines were up-regulated by 2f-LIGRLO-$NH_2$ (2μM), or GB110 (2μM), including GM-CSF, MCP-1, IL-6, IL-8, RANTES, ICAM-1, MIP-1β, TIMP-2, IL-1α and TNFα. The 10-fold increase in IL-6 & IL-8 secretion induced by GB110 or 2f-LIGRLO-$NH_2$ was inhibited by GB88 ($IC_{50}$ <10μM). GB88 represents a novel druggable antagonist of **PAR2**, which will be invaluable in refining our understanding of the role of **PAR2** in disease processes. It also paves the way for the development of a new class of anti-inflammatory and anti-proliferative drugs with potential to treat inflammatory kidney diseases.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC341

**The Transmembrane Tyrosine Phosphatase CD148 (DEP-1) Functions as a Receptor for Thrombospondin-1** Keiko Takahashi,[1] Ray Mernaugh,[2] David Friedman,[2] Rebecca S. Weller,[1] Rosie T. Jiang,[1] Nobuo Tsuboi,[1] Takamune Takahashi.[1] *[1]Nephrology, Vanderbilt University Medical Center, Nashville, TN; [2]Biochemistry, Vanderbilt University School of Medicine, Nashville, TN.*

Anti-angiogenesis therapy is a promising strategy for a variety of human diseases, including diabetic vascular complications. Definition of the molecular determinants regulating angiogenic vessel growth promises a better anti-angiogenesis therapy. CD148 is a receptor-type tyrosine phosphatase which is abundantly expressed in vascular endothelial cells of many organs, including renal vasculature. Growing evidence demonstrates a prominent role for CD148 in negative regulation of endothelial cell growth. However, the regulatory mechanisms of CD148, including extracellular ligands, remain unknown.

To identify the extracellular proteins which bind to CD148, we introduced HA-tagged CD148 protein into cultured endothelial cells and isolated the CD148 interacting surface proteins by biotin-surface labeling, followed by affinity purifications using anti-HA affinity column and avidin beads. The binding proteins were identified by mass spectrometry. Here, we show that soluble thrombospondin-1 (TSP1) binds to the extracellular part of CD148 with high affinity and specificity (in vitro and in situ binding assays, Kd=13.03nM), increases its catalytic activity (PTP assay), and induces dephosphorylation of CD148 substrate proteins including VEGFR, EGFR, and ERK1/2. Furthermore, we show that introduction of wild-type, but not catalytically inactive, CD148 confers TSP1 cell growth inhibition to the CD148-deficient cells, whereas TSP1 increases cell proliferation when the cells lack CD148 expression. These TSP1 effects were antagonized by soluble CD148 ectodomain protein as well as by CD148 gene silencing. Lastly, we demonstrate that CD148 gene silencing reduces the anti-angiogenesis activity of TSP1 in endothelial cells. Taken together, these findings demonstrate that CD148 tyrosine phosphatase functions as a receptor for TSP1 and plays an important role in TSP1-mediated endothelial cell growth inhibition. Further investigation of this pathway would explore a novel strategy for anti-angiogenesis therapy.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC342

**Macrophage Angiotensin II Type 1a Receptor (AT1aR) Underlies Renal Injury-Induced Atherosclerosis by Modulating the Macrophage Phenotype** Suguru Yamamoto,[1] Yiqin Zuo,[1, 2] Patricia G. Yancey,[3] Zhi-Qi Xu,[1] Iekuni Ichikawa,[1] Valentina Kon.[1] *[1]Pedeiatrics, Vanderbilt University, Nashville, TN; [2]Pathology, Vanderbilt University, Nashville, TN; [3]Medicine, Vanderbilt University, Nashville, TN.*

**Background** We and others have shown that infusion of angiotensin II (AII) potentiates atherosclerosis. We also showed that reduction in renal parenchyma potentiates atherosclerosis and increases macrophage infiltration which are lessened by systemic antagonism of AII receptor. Although the macrophage is central to atherosclerosis and expresses AT1R, its role in atherogenesis remains uncertain because macrophage AT1aR (MacAT1aR) has a variable impact on atherosclerosis that depends on the level of circulating angiotensin II achieved by exogenous AII. Since renal injury is an AII-activated state, we examined the contribution of AT1aR on macrophages versus resident vascular cells in uninephrectomized (UNx)-induced amplification of atherosclerosis.

**Methods and results** Apolipoprotein E deficient mice (apoE-/-) were transplanted with bone marrow from apoE-/-:AT1aR-/- or apoE-/-:AT1aR+/+ mice and then underwent UNx or sham operation. Four groups were examined: Sham-MacAT1aR+/+ (n=10), UNx-MacAT1aR+/+ (n=15), Sham-MacAT1aR-/- (n=10), UNx-MacAT1aR-/- (n=15). There were no differences in body weight, blood pressure, lipid profile and creatinine clearance among the groups. In mice reconstituted with intact MacAT1aR, UNx significantly increased atherosclerosis (UNx-MacAT1aR+/+: 169071±21473 vs Sham-MacAT1aR+/+: 116071±8180μm$^2$, $p<0.05$) while reconstitution with MacAT1aR-/- prevented UNx-induced amplification in atherosclerosis (77174±9947 vs 75714±11333μm$^2$, *NS*). Further characterization of plaque lesions in UNx reconstituted with MacAT1aR-/- revealed greater number of macrophages with alternatively activated phenotype with anti-inflammatory/anti-atherogenic functions [Ym-1 positive macrophages (65.4±2.0 vs 38.7±3.5%, $p<0.05$)].

**Conclusions** AT1aR on bone marrow-derived macrophages modulates renal injury–induced acceleration of atherosclerosis by modifying the macrophage phenotype which in turn determines the extent and severity of disease.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC343

**Glyoxalase I Prevent Age-Related Aortic Medial Thickening by Reducing TGF-β and CTGF Signals Independent of Blood Pressure** Yoichiro Ikeda,[1] Reiko Inagi,[1] Airi Jo,[1] Takamoto Ohse,[1] Toshio Miyata,[2] Ichiro Manabe,[3] Ryozo Nagai,[3] Toshiro Fujita,[1] Masaomi Nangaku.[1] *[1]Nephrology and Endocrinology, University of Tokyo, School of Medicine, Tokyo, Japan; [2]Tohoku University, Japan; [3]Cardiology, University of Tokyo, School of Medicine, Japan.*

Background

Glyoxalase I (GLO1) decomposes precursors of AGEs, and we previously demonstrated that GLO1 retards age-related morphological and functional phenotypes of kidney. Atherosclerosis is the most important change related to senescence as well as chronic kidney disease. Aortic medial thickening is the hallmark of atherosclerotic changes, and TGF-β contributes to the histological changes. Here we investigated whether GLO1 prevents age-related atherosclerosis or not.

Methods

Aorta from young and aged rats of GLO1 transgenic and wild type was harvested, and morphological and functional changes were studied. Senescence status was evaluated by senescence-associated β-galactosidase (SABG) staining. The cDNA was isolated from smooth muscle cells of aortic medial wall. The expression levels of TGF-β1 and CTGF were measured to evaluate the profibrotic status *in vivo*.

Results

There were no significant differences among blood pressure and chow intake of young (10-week-old) and aged (14-month-old) rats of GLO1 transgenic and wild type (sBP; young WT 106±3, young GLO1-TG 105±6, aged WT 104±11, aged GLO1-TG 102±8 mmHg). Aortic media were thickened when aged, whereas in aged GLO1-TG rats, aortic thickening was attenuated (100±10, 97±15, 132±16, 111±11μm). Aortic structures showed age-dependent changes including extracellular matrix accumulation, which was reduced in aged GLO1-TG than aged WT. Positive staining of aorta from aged WT was observed in SABG staining, whereas no staining was observed in aorta of aged GLO1-TG. Expression levels in TGF-β and CTGF of aged WT were significantly increased 5.5 and 3.3 folds respectively compared to young WT, whereas those of young GLO1-TG were lower than those of WT (0.044 and 0.058 fold, respectively). We did not observe age-dependent increases of TGF-β and CTGF in GLO1-TG.

Conclusion

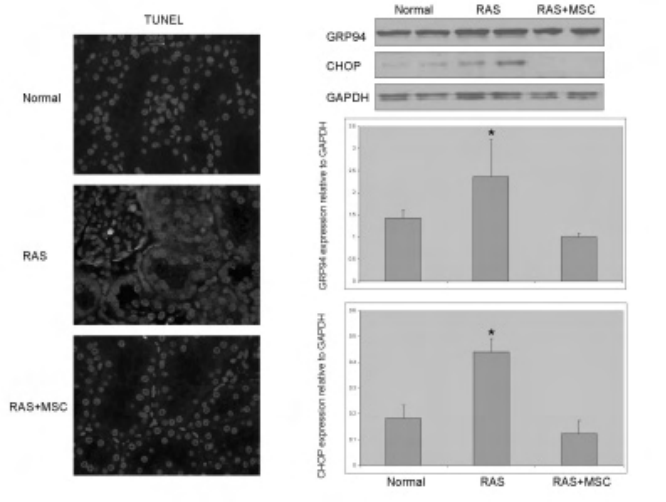
GLO1 prevents age-related aortic medial thickening by reducing TGF-β and CTGF singals independent of blood pressure.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC344

**Adipose-Derived Mesenchymal Stem Cells Blunt Endoplasmic Reticulum Stress and Apoptosis in Experimental Swine Renal Artery Stenosis** Xiang-Yang Zhu,[1] Victor Urbieta Caceres,[1] James Krier,[1] Stephen C. Textor,[1] Amir Lerman,[2] Lilach O. Lerman.[1] *[1]Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [2]Cardiovascular Diseases, Mayo Clinic, Rochester, MN.*

**Objective**: Endoplasmic reticulum (ER) stress has been recently recognized to play an important role in apoptosis and tissue damage. Adipose-derived mesenchymal stem cells (ad-MSC) can participate in tissue repair, but their effect on ER stress is unknown. This study tested the hypothesis that ad-MSC would reduce ER stress-induced apoptosis and protect the post-stenotic kidney. **Methods:** Allogenic Ad-MSCs were isolated from porcine omentum, expanded, and characterized by cell surface markers (e.g. CD44 and CD90). Single kidney hemodynamics and function were assessed using multi-detector CT in pigs after 10 weeks of renal artery stenosis (RAS) treated 4 weeks earlier with an intra-renal infusion of vehicle (n=7) or ad-MSC (10x10^6, RAS+MSC, n=6), and normal controls (n=6). Kidneys ER stress was evaluated by expression of the mediators GRP94 (in isolated ER) and CHOP, and apoptosis by TUNEL. **Results**: The degree of stenosis and hypertension were similar in RAS and RAS+MSC. Renal blood flow and glomerular filtration rate in RAS were lower than normal controls, but improved in RAS+MSC. Apoptotic tubular and interstitial cells were increased in RAS compared to normal (1.8±0.9 vs. 0.8±0.2 cells/field, p<0.05) and improved in RAS+MSC (1.1±0.9 cells/field, p=NS vs. normal). RAS produced increased expression of GRP94 and CHOP and fibrosis that all decreased with ad-MSC treatment.




**Conclusion**: Experimental chronic RAS was associated with increased ER stress and apoptosis in the injured kidney. Intra-renal delivery of ad-MSC improved renal function and attenuated ER stress, cell death, and fibrosis, demonstrating an anti-fibrotic therapeutic potential of ad-MSC in the ischemic kidney.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC345

**Liver-Specific and Kidney-Specific Angiotensinogen Gene Knockout Revealed That Renal Renin Activity, and JGA and Renal Vascular Morphology Are Dependent on Blood Pressure, Not Renal Angiotensin** Iekuni Ichikawa,[1] Fumio Niimura,[2] Akira Nishiyama,[3] Akihiro Shimizu,[4] Taiji Matsusaka.[4] *[1]Department of Pediatrics, Vanderbilt University Medical Center, TN; [2]Department of Pediatrics, Tokai University School of Medicine, Kanagawa, Japan; [3]Department of Pharmacology, Kagawa University School of Medicine, Kagawa, Japan; [4]Department of Internal Medicine, Tokai University School of Medicine, Kanagawa, Japan.*

Whole-body angiotensinogen gene (Agt) knockout (KO) mice are characterized by hypotension, polyuria, renal vascular hypertrophy and hypoplastic papilla. To gain insight into the mechanism of these abnormal phenotypes, we have generated kidney-specific and liver-specific Agt KO mouse strains. In the kidney Agt KO mice (n=15), renal Agt mRNA was decreased, on average, to 14.4% of that in control mice (n=9)(p<0.05), but renal Agt protein was unchanged. Renal renin activity (222±62 vs. 209±27 ng AngI/ml/h/g protein), JGA morphology, Ang II content in the kidney (175±62 vs. 177±48 fmol/g tissue) and systolic blood pressure (120.7±8.1 vs. 121.7±9.3) were also comparable to those of control mice. In the liver Agt KO mice (n=8), the levels of hepatic Agt mRNA, hepatic Agt protein and plasma Agt protein were all too low to detect. In the kidney of liver KO, although Agt mRNA was increased by 45% (p<0.05), Agt protein was undetectable by Western and immunostaining. However, renal renin activity was markedly increased (1821±557, p<0.05) with JGA hypertrophy, and renal Ang II content was maintained at levels similar to controls (147±39). The liver KO showed low systolic blood pressure (74.9±1.7, p<0.05), polyuria, renal arterial hypertrophy without hypoplastic papilla. These results indicate that Agt of liver origin is essential for maintaining normal blood pressure and urine concentration and that blood pressure, not renal Ang II, is the primary regulator for renal renin activity, and JGA and renal vascular morphology.

Disclosure of Financial Relationships: Research Funding: Daiichisankyo Co.

## SA-FC346

**Role of Farnesoid X Receptor (FXR) in Regulation of Vascular Calcification in Chronic Kidney Disease** Makoto Miyazaki,[1] Moshe Levi,[1, 2] Shinobu Miyazaki-Anzai.[1] *[1]Division of Renal Diseases and Hypertension, University of Colorado-Denver; [2]Denver Veterans Affairs Medical Center.*

**Rationale:** Vascular calcification is highly associated with cardiovascular morbidity and mortality, especially in patients with chronic kidney disease. The nuclear receptor farnesoid X receptor (FXR) has been implicated in the control of lipid, carbohydrate and bile acid metabolism in several cell types. Although recent studies have shown that FXR is also expressed in vascular smooth muscle cells, its physiological role in vasculature tissue remains obscure.

**Objective:** Here, we have examined the role of FXR in vascular calcification.

**Methods and Results:** The FXR gene, a bile acid nuclear receptor, was highly induced during osteogenic differentiation of bovine calcifying vascular cells (CVC) and in the aorta of apolipoprotein E ($ApoE)^{-/-}$ mice with chronic kidney disease which are common tissue culture and mouse model, respectively, for aortic calcification. FXR activation by a synthetic FXR agonist, 6a-ethyl chenodeoxycholic acid (INT-747) inhibited phosphate induced-mineralization and triglyceride accumulation in CVC. FXR dominant negative expression augmented mineralization of CVC and blocked the anti-calcific effect of INT-747 whereas VP16FXR that is a constitutively active form reduced mineralization of CVC. In addition, INT-747 ameliorates chronic kidney disease (CKD) induced-vascular calcification in 5/6 nephrectomized $ApoE^{-/-}$ mice. FXR deficiency on the other hand accelerates atherosclerosis and vascular calcification in both $ApoE^{-/-}$ and $LDLR^{-/-}$ mice with CKD.

**Conclusions:** These observations provide direct evidence for that FXR is a key signaling component in regulation of vascular calcification.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC347

**Ebselen Prevents Stress-Induced Premature Senescence (SIPS) of Endothelial and Endothelial Progenitor Cells (EPC) by Restoring Sirtuin-1 (SIRT1)** Jun Chen, Leonid Buryanovskyy, Eliza Moskowitz-Kassai, Connie Y. Lu, Robert Chen, Frank Fan Zhang, Alberto Nasjletti, Michael S. Goligorsky. *New York Medical College, Valhalla, NY.*

SIPS of endothelial cells has emerged as a notable contributor to endothelial cell dysfunction (ECD) in diseases. Endothelial progenitor cells (EPC) also undergo SIPS. Sirtuins have recently been recognized as potent regulators of longevity of cells and organisms. Chronic treatment with an antioxidant and peroxynitrite scavenger ebselen (Ebs) reversed SIPS in vivo and in vitro. In the renal vasculature of db/db mice, the expression of Sirt1 was depleted compared to age-matched db/m control mice. We inquired whether a combined antioxidant and peroxynitrite scavenging therapy with Ebs, to protect endothelial cells and EPC from SIPS, exerts its effect via SIRT 1 and what is the possible mechanism. Exposure of EPC and EC to cardiovascular stressors (ADMA, non-enzymatically glycated collagen, H2O2) resulted in depletion of SIRT1. The impact of SIRT1 on EPC viability (apoptosis, senescence) was studied by overexpressing or downregulating Sirt1 levels. The data showed that overexpression of exogenous Sirt1 in EPC prevented apoptosis and senescence induced by these stressors. In contrast, downregulation of endogenous Sirt1 by shRNA or using its inhibitor, Sitrinol, exaggerated apoptosis and senescence in EPC exposed to stressors. Pretreatment of EPC with Ebselen prevented apoptosis and senescence induced

by stressors and this effect was accompanied by the prevention of downregulation of Sirt1. This effect of Ebselen was mediated via modulation of autophagy in EPC. Exposure of EPC to the stressors increases the accumulation of intracellular p62, a marker of impaired autophagic process. Pretreatment with Ebselen prevented p62 accumulation under stress conditions in EPC. Disruption of autophagy process using chloroqine or 3-methyladenine increased p62 level and decreased Sirt1 expression in EPC. In conclusion, 1) ebselen is a potent inducer of SIRT 1 expression in EPC, 2) ebselen normalizes impaired autophagy and by doing so 3) prevents SIPS and apoptotic cell death induced by several cardiovascular stressors.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC348

**The Bone Marrow: A Novel Source of Smooth Muscle Cells Able To Undergo Osteoblastic Transformation** Rajesh Mohandas, Mark S. Segal. *Department of Medicine, University of Florida, Gainesville, FL.*

Though bone marrow derived progenitor cells, which are cells derived from the bone marrow that circulate in the blood stream, have definitively been shown to differentiate into endothelial cells, their ability to differentiate into a smooth muscle phenotype (smooth muscle progenitor cells or SMPCs) is less well documented. We hypothesized that SMPCs are derived from the bone marrow and because of their stem cell like characteristics these cells have an increased propensity to undergo osteoblastic transformation.

**Methods:** Monocyte rich plasma was isolated using Ficoll and plated in smooth muscle cell media supplemented with PDGF. Double immuno-fluorescent staining for actin and myosin was used to definitively demonstrate a smooth muscle phenotype. Wild-type mice transplanted with GFP bone marrow were used to confirm the bone marrow origin of the smooth muscle progenitor cells. Calcification was induced by supplementing media with inorganic phosphate and tendency to calcify quantified by measuring intracellular calcium normalized to protein content.

**Results:** Smooth muscle cell colonies isolated from mice appeared after one week of culture and those isolated from healthy adults appeared after two weeks. These cells demonstrate the characteristic spindle shaped cells arranged in a hill and valley pattern and stained positive for actin as well as myosin heavy chain. Smooth muscle progenitor cells isolated from the GFP chimeric mouse also expressed GFP confirming the bone marrow origin of these cells. In the presence of phosphate, these cells expressed the transcription factor cbfa1 suggesting transition to an osteoblastic phenotype and demonstrated evidence of intracellular calcium deposition.

**Conclusions:** Demonstration of cells that express two different smooth muscle markers, actin and myosin, definitively identify these cells as circulating smooth muscle progenitor cells. At least in mice, these cells are derived from the bone marrow on the basis of their GFP expression. In presence of phosphate these cells will undergo osteoblastic transformation. Studies are ongoing to examine the role of these cells in vascular calcification seen in chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC349

**Accelerated Vascular Lesion Formation in Mice Lacking All Nitric Oxide Synthases: Contribution of Bone Marrow Cells** Yumi Furuno,[1] Masato Tsutsui,[2] Tsuyoshi Morishita,[1] Kiyoko Shibata,[1] Yoko Fujimoto,[1] Tetsu Miyamoto,[1] Tatsuya Shibata,[3] Ryota Serino,[1] Narutoshi Kabashima,[3] Nobuyuki Yanagihara,[4] Yutaka Otsuji,[1] Masahito Tamura.[3] *[1]Second Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan; [2]Department of Pharmacology, University of the Ryukyus, Okinawa, Japan; [3]Kidney Center, University of Occupational and Environmental Health, Kitakyushu, Japan; [4]Department of Pharmacology, University of Occupational and Environmental Health, Kitakyushu, Japan.*

***Background*:** The precise role of nitric oxide synthases (NOSs) in vascular remodeling still remains to be fully elucidated. We addressed this issue in mice lacking all NOS genes. ***Methods and Results***: Permanent ligation of a unilateral carotid artery was performed in wild-type, singly nNOS$^{-/-}$, iNOS$^{-/-}$, and eNOS$^{-/-}$, and triply n/i/eNOS$^{-/-}$ mice. At 2 weeks after the carotid artery ligation, significant neointimal formation (NF), constrictive vascular remodeling (VR), and inflammatory leukocyte infiltration (LI) were noted in the ligated arteries of all genotypes. However, these vascular pathological alterations were most aggravated in n/i/eNOS$^{-/-}$ genotype. Although blood pressure was significantly elevated in eNOS$^{-/-}$ and n/i/eNOS$^{-/-}$ genotypes, those changes were not significantly correlated with the extents of the vascular lesion formation, indicating a minor role of hypertension. Finally, we studied the cell origin of vascular lesions. Bone marrow of WT, singly or triply NOS$^{-/-}$ genotype was transplanted into WT genotype, and 4 weeks later, the carotid artery ligation was performed. NI, VR, and LI were all similarly most exacerbated in the case of n/i/eNOS$^{-/-}$ transplantation, suggesting the contribution of bone marrow cells in the vascular lesion formation. ***Conclusions***: These results indicate that complete disruption of all NOS genes, especially in bone marrow cells, causes accelerated inflammatory vascular lesion formation caused by blood flow disruption in mice in vivo, demonstrating the critical role of the whole endogenous NOS system in preventing vascular remodeling.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC350

**Comparison of the Effects of Manumycin A and FTI-277 on Vascular Calcification** Arvind Ponnusamy,[1,2] Smeeta Sinha,[1] Colette A. Inkson,[1] Gareth D. Hyde,[1] Philip A. Kalra,[2] Ann E. Canfield.[1] *[1]University of Manchester, Manchester, United Kingdom; [2]Renal Department, Salford Royal NHS Trust Foundation, Salford, United Kingdom.*

Patients with chronic kidney disease have poor cardiovascular outcomes due to increased vascular calcification and atherosclerosis. Previous work from our laboratory has shown that nitrogen-containing bisphosphonates attenuate vascular calcification by inhibiting farnesylpyrophosphate synthase thereby depleting cells of farnesylpyrophosphate and geranylgeranylpyrophosphate which are essential for the prenylation and activation of small GTPases such as Ras and Rho.

This study compares the effects of 2 different farnesyl transferase inhibitors (FTI-277 and manumycin A) on vascular calcification using well validated in vitro and ex vivo model systems. Mineralisation was assessed by histological staining using alizarin red and quantified using a calcium assay. FTI-277 (10 μM) significantly inhibited β-glycerophosphate-induced calcification of vascular smooth muscle cells (VSMC) in vitro ($p<0.001$), whereas manumycin A (10 μM, 20 μM) either increased calcification or had no effect on this process. FTI-277 (10 μM) also inhibited phosphate-induced mineralisation of rat aortic rings ex vivo. Interestingly, manumycin A (10 μM) increased mineralisation ex vivo, although this was localised in the intima of the aortic ring, compared to the vessel media of phosphate-treated controls. Pull-down assays and westerns blots confirmed that both FTI-277 and manumycin A decreased Ras GTPase activation in VSMC.

To determine the effects of these farnesyl transferase inhibitors on signalling downstream of Ras, VSMCs were pre-incubated with FTI 277 (10 μM) or manumycin A (10 μM), and stimulated with serum-containing medium for 5 min. Cell lysates were collected and analysed for Akt phosphorylation by western blotting. Results showed that FTI-277, but not manumycin A, increased Akt phosphorylation in VSMC.

These studies demonstrate that farnesyl transferase inhibitors can inhibit vascular calcification. Intriguingly, however, manumycin A and FTI-277 exerted different effects on calcification and we suggest that this may be due to their differential effects on Akt phosphorylation.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC351

**Association of eGFR and Albuminuria with Kidney Outcomes. A Collaborative Meta-Analysis of General and High-Risk Population Cohorts on Behalf of the CKD Prognosis Consortium** Ron T. Gansevoort,[1] Kunihiro Matsushita,[2] Marije Van de Velde,[1] Brad C. Astor,[2] Mark Woodward,[4] Andrew S. Levey,[3] Paul E. de Jong,[1] Josef Coresh.[2] *[1]Dept. Nephrology, University Medical Center, Groningen, Netherlands; [2]Dept. Epidemiology, John Hopkins Institute, Baltimore; [3]Div. Nephrology, Tufts Medical Center, Boston; [4]George Inst. Int. Health, University of Sydney, Sydney, Australia.*

While both low eGFR and albuminuria are known risk factors for ESRD, data on their joint contribution to this and other kidney outcomes are limited.

We performed a collaborative meta-analysis of 9 general population cohorts with 845,125 participants and 8 cohorts with 173,892 participants selected because of high risk for CKD. Both eGFR (MDRD) and albuminuria (ACR or dipstick) were tested as risk factors for ESRD, acute kidney injury (AKI) and progressive CKD (pCKD).

In general population cohorts, the risk for ESRD was unrelated to eGFR at values 75-105 ml/min/1.73m$^2$ and increased exponentially at lower eGFR. Hazard ratios (HRs) (95% CI) at eGFR 60, 45, and 15 (versus 95) ml/min/1.73m$^2$ were 3.69 (2.36-5.76), 29.3 (19.5-44.1) and 454.9 (112.4-1840.2), respectively, after adjustment for ACR and CV risk factors. Albuminuria was associated with ESRD risk linearly without thresholds. Adjusted HRs at ACRs 30, 300 and 1000 (versus 5) mg/g were 4.87 (2.30-10.3), 13.4 (5.49-32.7) and 28.4 (14.9-54.2), respectively. eGFR and albuminuria were multiplicatively associated with ESRD, without evidence for interaction. Similar, but numerically less pronounced associations were observed for AKI and progressive CKD. The findings in high risk cohorts were generally comparable to those in general population cohorts. These pattern of increased HRs were seen for subjects above and below age 65 years, although HRs increased more steeply in younger subjects.

In conclusion, lower eGFR and higher albuminuria are risk factors for ESRD, AKI and pCKD independent of each other and of cardiovascular risk factors, both in the general population and high risk cohorts. These findings provide a quantitative basis for including these two kidney measures in CKD definition, staging and risk stratification.

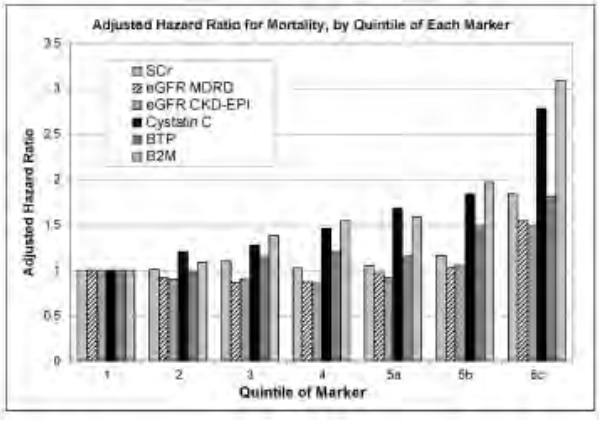
Disclosure of Financial Relationships: nothing to disclose

### SA-FC352

**Novel Markers of Kidney Function as Predictors of Mortality in the Atherosclerosis Risk in Communities (ARIC) Study** Brad C. Astor,[1] Tariq Shafi,[1] Ron C. Hoogeveen,[2] Yaping Wang,[1] Kunihiro Matsushita,[1] Christie M. Ballantyne,[2] Josef Coresh.[1] *[1]Johns Hopkins University, Baltimore, MD; [2]Baylor College of Medicine, Houston, TX.*

Serum Cystatin C predicts mortality more strongly than does serum creatinine (Shlipak et al., NEJM 2005), but it is unknown whether this is true for other novel filtration markers. We tested ß-trace protein (BTP), and $ß_2$-microglobulin (B2M) as risk factors for mortality and whether their association was independent of serum creatinine-based estimated GFR. We assessed the association between quintiles of each marker and incidence of death in 10,189

participants in the ARIC Study, a population-based study in 4 US communities (43% male; 22% African-American, ages 54-74 years at baseline). Quintile 5 was further divided into three tertiles (5a, 5b, 5c). Percentile cut-offs for $eGFR_{MDRD}$ were: Quintile 1>95.0, 83.4, 78.3, 68.2, 64.4, and subquintile 5c<59.6 mL/min/1.73m$^2$. A total of 1477 deaths occurred over a median of 9.8 years. Cox regression models were used to adjust for age, race, sex, diabetes, history of cardiovascular disease, smoking, systolic blood pressure, total cholesterol and urinary albumin:creatinine ratio. Other models further adjusted for eight categories of eGFR. Higher categories of BTP and B2M were strongly associated with greater mortality risk (Figure), even after adjustment for $eGFR_{MDRD}$ or $eGFR_{CKD\text{-}EPI}$ (all p-trend<0.001). Results were similar after further adjustment for high-sensitivity CRP.




These results suggest that additional filtration markers (B2M, and to a lesser extent BTP) share Cystatin C's advantage over serum creatinine as risk factors for mortality in a population based cohort. Translation into clinical practice will require balancing costs, benefits and implications for clinical decision making.

Disclosure of Financial Relationships: nothing to disclose

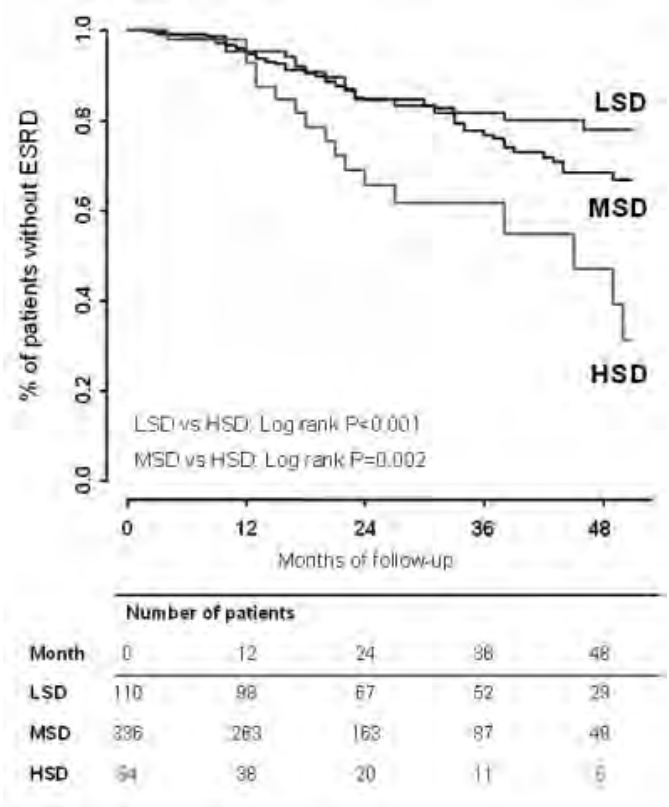
**SA-FC353**

**Sodium Intake, ACE Inhibition and Progression to ESRD: An Outcome Analysis of Five-Hundred REIN and REIN-2 Patients with Proteinuric Chronic Nephropathy** Stefan Vegter,[1, 2] Annalisa Perna,[3] Maarten J. Postma,[1] Gerjan Navis,[2] Giuseppe Remuzzi,[3, 4] Piero Ruggenenti.[3, 4] *[1]Unit of PharmacoEpidemiol & PharmacoEconomics, University of Groningen, Netherlands; [2]Division of Nephrology, University of Groningen, Netherlands; [3]Mario Negri Institute, Bergamo, Italy; [4]Ospedali Riuniti di Bergamo, Italy.*

Background: High sodium intake limits the antihypertensive and antiproteinuric effect of ACE inhibitors in chronic kidney disease (CKD) patients. Whether this translates into less renoprotection is not known

Methods: To assess the interactions between sodium intake, ACE inhibition and CKD progression, we compared by time-dependent Cox models progression to end stage renal disease (ESRD) over 4.25-year ramipril therapy in 500 subjects with proteinuric CKD retrieved from the Ramipril Efficacy In Nephropathy (REIN) and REIN 2 trials who were categorized to have low (LSD), medium (MSD) or high (HSD) sodium diet according to average follow-up 24-h urinary sodium/creatinine excretion <100, ≥100 and <200, or ≥200 mEq/g, respectively.

Results: 92 subjects (18.4%) developed ESRD. Among 110, 336 and 54 patients on LSD, MSD or HSD, ESRD incidence (95% CI) was 6.2 (3.9-9.9); 8.0 (6.2-10.4); and 18.1 (11.2-29.1) per 100 patients-years, respectively (P<0.001). Follow-up ESRD incidence and proteinuria were higher in HSD than LSD or MSD patients.




Number of patients

| Month | 0 | 12 | 24 | 36 | 48 |
|---|---|---|---|---|---|
| LSD | 110 | 98 | 67 | 52 | 29 |
| MSD | 336 | 283 | 163 | 87 | 40 |
| HSD | 54 | 38 | 20 | 11 | 5 |

Blood pressure (BP) was similar among groups. ESRD incidence increased by 1.53 (1.17-2.00) folds every 100 mEq/g increase in urinary sodium/creatinine excretion. This association was independent of BP, but was lost after adjustment for follow-up proteinuria.

Conclusions: In CKD patients, high sodium intake (>12 g/d of salt) blunts the antiproteinuric effect of ACE inhibition which, independent of BP control, translates into less protection against ESRD. Salt restriction is crucial to optimize renoprotection in this high-risk population.

Disclosure of Financial Relationships: Consultancy: Shire Pharmaceuticals plc. Research Funding: Shire Pharmaceuticals plc.

**SA-FC354**

**MYH9 Variations Are Associated with Chronic Kidney Disease (CKD) Progression in the African American Study of Hypertension and Kidney Diseases (AASK)** Brad C. Astor,[1] Michael S. Lipkowitz,[2] Wen Hong Linda Kao,[1] Rulan S. Parekh,[3] Michael J. Choi,[4] Jeffrey B. Kopp,[5] Lawrence J. Appel,[6] Cheryl Winkler,[7] George W. Nelson.[7] *[1]Epidemiology, Johns Hopkins University, Baltimore, MD; [2]Nephrology, Mount Sinai School of Medicine, New York, NY; [3]Nephrology, University of Toronto, Toronto, ON, Canada; [4]Nephrology, Johns Hopkins University, Baltimore, MD; [5]NIDDK, Bethesda, MD; [6]Medicine, Johns Hopkins University, Baltimore, MD; [7]SAIC Frederick Inc., Frederick, MD.*

Common variations in the gene MYH9 have been shown to be cross-sectionally associated with FSGS and non-diabetic ESRD. It is unknown whether these variations are also associated with chronic kidney disease (CKD) progression. We genotyped 706 participants with CKD from the African American Study of Kidney Disease and Hypertension (AASK) for six selected single nucleotide polymorphisms (SNPs) in MYH9. Time-to-event analysis was performed for both the AASK composite outcome of significant decline in GFR, ESRD, or death and the more specific outcome of only decline in GFR and ESRD. Several SNPs were associated with both outcomes as there was high correlations between the six SNPs. The strongest signal was observed for rs4821480. Individuals with the common genotype (55% with GG) at rs4821480 were about 1.5 times more likely to have the composite endpoint (p=0.004) and CKD progression (p=0.004) than their counterparts with the other genotypes irrespective of treatment groups, age, and sex. Common variations in MYH9 are associated with increased risk of CKD progression among African Americans with hypertensive nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC355**

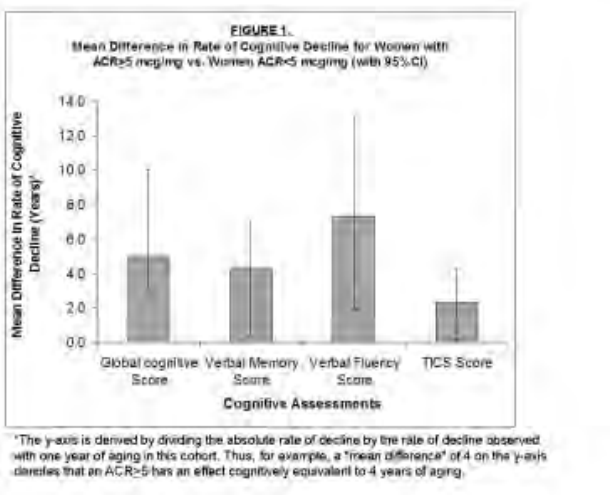
**A Prospective Study of Albuminuria and Cognitive Decline in Women** Julie Lin, Fran Grodstein, Gary C. Curhan, Jae Hee Kang. *Renal Division and Channing Laboratory, Brigham and Women's Hospital, Havard Medical School, Boston, MA.*

Background: End-stage renal disease is associated with substantial cognitive deficits but how early kidney damage such as presence of albuminuria may be related to subsequent cognitive decline is not well defined. In particular, increased levels of urinary albumin are consistently noted in older adults, who are at highest risk for cognitive decline.

Materials and Methods: Over 19,000 women aged ≥70 years in the Nurses' Health Study participated in a prospective study with repeated measures of cognitive function administered by phone every two years, including tests of general cognition (TICS), verbal memory, verbal fluency, and working memory. Of these, 1764 women had urinary albumin-to-creatinine ratios (ACR) measured in the year 2000, prior to their cognitive testing. Mixed effects regression analysis was applied to calculate mean differences in rate of cognitive decline between women with ACR≥5 vs. lower levels. Multivariable models were adjusted for numerous key potential confounding factors (including age, HTN, BMI, diabetes, smoking, physical activity, education level, and others).

Results: Median age was 74 years, 99% were Caucasian, 55% had HTN, 12.4% had diabetes, median BMI was 25 kg/m^2, median plasma creatinine was 0.84 mg/dL, and 25% had ACR≥5 mcg/mg. Main findings are in **Figure 1.** The apparent effect of ACR ≥ 5 mcg/mg was cognitively equivalent to that seen with 2 to 7 years of aging; that is the presence of higher ACR accelerated cognitive decline by 2 to 7 times faster than seen with aging alone (all p-values <0.05).

Conclusions: Presence of urinary ACR as low as 5 mcg/mg is independently associated with faster decline in cognitive function. As a non-invasive reflection of small vessel disease, screening for ACR ≥ 5 mcg/mg may be important as a prognostic marker for subsequent cognitive decline.




Disclosure of Financial Relationships: Employer: Brigham and Women's Hospital; Honoraria: Snell Publications; Scientific Advisor: Editorial board, CJASN, American Heart Association, grants peer reviewer.

**SA-FC356**

**Complete Recovery of Renal Function after Acute Kidney Injury Increases the Risk of Incident Chronic Kidney Disease in a Large Managed Care Organization** Jennifer A. DeGraauw,[1] John R. Holmen,[2] Jason Jones,[2] Sidney N. Thornton,[2] Michel B. Chonchol.[1] *[1]University of Colorado Denver Health Sciences Center, Aurora, CO; [2]Intermountain Health Care, Salt Lake City, UT.*

Purpose: Risk of incident chronic kidney disease (CKD) among patients with complete recovery of renal function after an episode of acute kidney injury (AKI) has not been studied. This study aimed to determine the risk of development of incident CKD in hospitalized patients who have an episode of AKI with complete recovery of renal function.

Methods: We conducted a population-based cohort study of adult patients with no history of CKD and who developed AKI requiring hospitalization and had complete renal function recovery between January 1 1999 and December 31 2009. AKI was defined by the RIFLE classification, which requires at least a 50% increase in serum creatinine. Complete recovery of renal function was defined if the serum creatinine returned to a level less than 50% above baseline serum creatinine within 7 days after discharge. The primary outcome of interest, incident CKD, was defined as an estimated glomerular filtration rate < 60 mL/min/1.73 m2 persisting for at least 3 months. Patients were tracked for outcomes beginning at discharge until March 31 2010. All subjects were required to have a minimum of 12 months follow-up data. These patients were matched for demographics and risk factors associated with kidney disease progression with individuals without AKI or CKD.

Results: We identified 1411 individuals with complete recovery of renal function after AKI and 1411 matched controls with no AKI during the index hospitalization. The median age of the enrolled participants was 65 years, 45% were women, and 56% had diabetes. After a median follow-up of 2.8 years, new cases of CKD were 14% and 2% among those with and without AKI, respectively, corresponding to an unadjusted HR of 7.65 (95% CI, 5.30-11.04). After adjusting for other potential confounders, the HR was 7.12 (95% CI, 4.89-10.37).

Conclusion: Subjects with an episode of AKI during hospitalization that have complete renal function recovery have an increased risk of kidney disease progression to incident CKD.

Disclosure of Financial Relationships: nothing to disclose

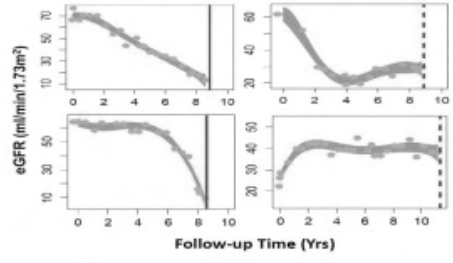
**SA-FC357**

**Long-Term Trajectories of Estimated GFR (eGFR) in CKD Patients Are Often Nonlinear** Liang Li,[1] Bo Hu,[1] Lawrence J. Appel,[2] Brad C. Astor,[2] Julia Lewis,[3] Michael S. Lipkowitz,[4] Robert D. Toto,[5] Xuelei Wang,[6] Jackson T. Wright,[6] Tom H. Greene.[7] *[1]Clev Clinic; [2]Johns Hopkins; [3]Vanderbilt; [4]MSSM; [5]UTSW; [6]CWRU; [7]Utah.*

**Introduction** Decline in GFR in CKD is often assumed to be linear with constant slope over time. We investigated long-term trajectories of eGFR in 1094 African American Study of Kidney Disease (AASK) pts over a planned follow-up of 9-12 yrs to determine if long-term progression is approximately linear in most.

**Methods** Penalized cubic splines were used to estimate smooth but possibly nonlinear eGFR trajectories while accounting for random variation in individual eGFR measures. For each pt, deviation from linearity was expressed as difference in slope between the 5/8ths of the follow-up period with the fastest decline vs. the 5/8ths with the slowest decline. Statistical simulation was used to determine if the maximum deviation of a given pt's trajectory from linearity exceeded 95% of such deviations generated under the assumption of linear decline.

**Results** 768 pts had ≥ 10 eGFRs measurements with no measurement gaps ≥ 2 yrs. The mean (SD) eGFR overall slope was -2.11 (2.30) ml/min/1.73m$^2$/yr. 180 pts (23%) exhibited extreme changes in slope ≥ 4 ml/min/1.73m$^2$ /yr between the slowest and fastest portions of their progression trajectory; 299 (39%) exhibited slope changes ≥ 3 ml/min/1.73m$^2$/yr. Deviations from linearity were too large to be explained by chance fluctuations for 232 (30%) pts. Trajectories of the 4 patients below exemplify common patterns. Solid and dashed lines indicate ESRD and censoring.




**Conclusion** With extended follow-up, trajectories of eGFR in CKD often exhibit major deviations from a linear decline. Investigation of sources of these deviations, which represent acceleration or deceleration in the rate of CKD progression, may yield useful insight into pathophysiology of CKD progression.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC358**

**Prenatal Risk Factors for Childhood Chronic Kidney Disease** Christine W. Hsu,[1, 2] Kalani T. Yamamoto,[3] Rohan K. Henry,[1, 4] Jordan M. Symons.[1, 2] *[1]Pediatrics, University of Washington, Seattle, WA; [2]Nephrology, Seattle Children's Hospital, Seattle, WA; [3]Nephrology, University of Washington, Seattle, WA; [4]Endocrinology, Seattle Children's Hospital, Seattle, WA.*

**BACKGROUND:** Development of childhood chronic kidney disease (CKD) may be programmed prenatally. To our knowledge, this is the first study to evaluate maternal diabetes mellitus (DM) and obesity/overweight as primary risk factors for CKD.

**PURPOSE:** To determine the association of childhood CKD with maternal DM and obesity/overweight in Washington State (WA).

**METHODS:** Population-based case control study of 4,063 childhood CKD cases (diagnosed ≤21 years of age) and 20,032 controls. Maternal and infant data were obtained by linking WA birth records to hospital discharge data for 1987-2008. We used ICD-9 codes to identify cases, defined by the most common childhood CKD diagnoses (NAPRTCS) and CKD stage (KDOQI). We estimated the associations of maternal DM and obesity/overweight with the risk of childhood CKD by stratified analysis using Mantel-Haenszel odds ratios (ORs) and 95% confidence intervals (CIs).

**RESULTS:** CKD prevalence was 258.1 per 100,000 births. After adjusting for gestational length, maternal pre-gestational DM (PDM) was associated with increased risk of CKD in offspring, with an OR of 1.69 (95% CI=1.09, 2.62). Maternal gestational DM (GDM) was associated with increased risk of CKD in offspring, with an OR of 1.28 (95% CI=1.07, 1.54). Both maternal obesity and overweight were associated with increased risk of CKD in offspring, with respective ORs of 1.22 (95% CI=1.08, 1.38) and 1.08 (95% CI=0.96, 1.22). In our subgroup analysis, maternal PDM was associated with increased risk of renal aplasia/dysplasia, with an OR of 6.81 (95% CI=3.54, 13.10). Maternal GDM, obesity and overweight were associated with obstructive uropathy, with respective ORs of 1.34 (95% CI=1.02, 1.75), 1.23 (95% CI=1.03, 1.47) and 1.21 (95% CI=1.03, 1.43).

**CONCLUSIONS:** Maternal PDM, GDM, obesity and overweight were associated

with development of CKD in offspring. Maternal PDM was associated with renal aplasia/dysplasia. Maternal GDM, obesity and overweight were associated with obstructive uropathy.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC359

**Albuminuria, Kidney Function and the Incidence of Cognitive Impairment in US Adults** Manjula Kurella Tamura,[1] Virginia G. Wadley,[2] Mary Cushman,[2] Frederick W. Unverzagt,[2] Neil A. Zakai,[2] Brett Kissela,[2] David G. Warnock,[2] William M. McClellan.[2] *[1]Medicine, Stanford, Palo Alto, CA; [2]For the REGARDS Study Group, UAB, Birmingham, AL.*

Objective: Albuminuria and low estimated glomerular filtration rate (eGFR) are each associated with an increased risk for cognitive impairment, however the joint associations of these kidney disease markers with cognitive impairment have not been studied.

Methods: We used data from 19,442 individuals participating in the Renal Reasons for Geographic and Racial Differences in Stroke (REGARDS) study. GFR was estimated using serum creatinine and albumin to creatinine ratio (ACR) was measured on a single-voided urine sample, both obtained at baseline. Cognitive function was assessed annually. Incident cognitive impairment was defined as a score ≤ 4 on the Six-item Screener at the last study follow-up visit among individuals without cognitive impairment at baseline.

Results: There were 6999 participants (36%) with albuminuria at baseline (ACR ≥10 mg/g) and 1884 (10%) with Stage 3-4 CKD (eGFR <60 ml/min/1.73m$^2$). There were 1575 participants (8.1%) who developed cognitive impairment over a mean follow-up time of 3.5 ± 1.3 years. In separate models that were adjusted for demographics, diabetes, hypertension, cardiovascular disease, and tobacco and alcohol use, albuminuria (odds ratio (OR) 1.23, 95% CI 1.11-1.37) and eGFR <45 ml/min/1.73m$^2$ (OR 1.36, 95% CI 1.08-1.71) were separately associated with incident cognitive impairment. Incident cognitive impairment occurred in 6.7% of individuals with neither condition; 10.1% with albuminuria; 18.8% with eGFR <45 ml/min/1.73m$^2$; and 13.4% with both. Compared to neither condition, the fully adjusted OR for cognitive impairment among participants with albuminuria was 1.25 (1.12-1.40); among those with an eGFR <45 ml/min/1.73m$^2$, 1.78 (1.23-2.57); and 1.41 (1.05-1.88) among those with both conditions. Similar relationships were noted when eGFR <60 ml/min/1.73m$^2$ was substituted.

Conclusions: Albuminuria predicts incident cognitive impairment independent of low eGFR.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC360

**Urinary Connective Tissue Growth Factor Levels Predict Incident Chronic Kidney Disease in the General Population** Conall M. O'Seaghdha,[1,2] Nrupen Anjan Bhavsar,[3] Anna Kottgen,[4] Josef Coresh,[3] Brad C. Astor,[3] Caroline S. Fox.[1,2] *[1]NHLBI's Framingham Heart Study; [2]Harvard Medical School; [3]Welch Center, Johns Hopkins University; [4]University Hospital Freiburg, Germany.*

Introduction: Connective tissue growth factor (CTGF) is an important cytokine involved in renal fibrosis, repair and diabetic nephropathy. It is unknown whether urinary CTGF levels are associated with risk for incident CKD in the general population.

Methods: We conducted an age-sex matched nested case-control study (n = 200) of incident CKD (eGFR < 60 ml/min per 1.73m2) within the Framingham Heart Study (FHS). Findings were externally replicated in participants from the Atherosclerosis Risk in Communities (ARIC) Study (n=276). Urinary CTGF concentrations were measured using the Human Kidney Tox 1 assay (Rules Based Medicine, TX). A paired t-test was used to assess for differences in baseline log CTGF concentrations between pairs of cases and controls. The odds ratio of CKD by quartile of baseline CTGF levels was modeled using conditional logistic regression.

Results: Baseline urinary CTGF concentrations were inversely related to case-control status 9.9 yr later in FHS. The median urinary CTGF concentration at baseline was lower among cases (1.42 ng/mL) than controls (2.32 ng/mL; paired t-test P<0.0001). We observed a significant trend of lower risk of incident CKD for each higher quartile of CTGF concentration in an unadjusted model and in models adjusted for CKD risk factors. Results in ARIC showed a similar trend (p=0.13) and were robust in meta-analysis: quartile 2 vs. quartile 1: OR for CKD 0.83; 95% confidence interval 0.49-1.40; quartile 3 vs. quartile 1: OR 0.30; 95% C.I. 0.16-0.57; quartile 4 vs. quartile 1: OR 0.34 (95% C.I. 0.18-0.64. P-value for trend across quartiles <0.001). Results were similar among those participants without diabetes.

Conclusions: Lower urinary CTGF concentrations precede the onset of CKD in the general population. These findings suggest that CTGF provides useful information on the balance of repair and fibrosis early in the development of CKD.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC361

**Activating and Inhibitory Roles of Ret-Docking Tyrosine Sites in Wolffian Duct Development and Maturation** Masato Hoshi, Sanjay Jain. *Internal Medicine (Renal), Washington University, St. Louis, MO.*

Several genes important in kidney development have been indentified from gene deletion studies, however, little is known about their specific attributes that are relevant to normal kidney development and malformations. Activation of receptor tyrosine kinase Ret signaling by Gdnf-Gfrα1 is essential for kidney formation. Mice harboring individual docking tyrosine mutations of Ret-Plcγ or Ret-Pi3k/Mapk pathways exhibit multiple kidney and ureter abnormalities (supernumerary ureters, hydroureters, hypoplasia and dysplasia) or agenesis, respectively. To elucidate early developmental mechanisms gone awry in these disparate phenotypic spectrums of CAKUT we generated Ret-EGFP reporter mice and bred them to Ret-Plcγ or Ret-Pi3k/Mapk mutant mice, and performed ontogenic characterization of their urinary systems. We discovered distinct abnormalities in Wolffiian duct (WD) maturation and development in both mutants. Analysis in vivo and exvivo by time-lapse microscopy demonstrates that supernumerary ureters in Ret-Plcγ mutants are not due to failure of mesonephros to degenerate but due to new outgrowths from the anterior mesonephric WD emerging as early as E9.5 and clearly evident by E10.5. Compared to UB budding site at a single somite level in controls, the budding site is markedly expanded over 3 somite levels in these mice leading to multiple UBs invading metanephric mesenchyme producing multiplexed kidneys. Gdnf haploinsufficiency in these mice normalizes these defects in a subset of Ret-Plcγ mutant mice suggesting overactive Ret signaling. Gdnf was abnormally expressed in the mesonephric mesenchyme indicating that it is normally repressed by Ret-Plcγ signals in the anterior WD. The common nephric duct in Ret-Plcγ mutants showed abnormal Ret expression and was deficient in normal degeneration. In contrast, Ret-Pi3k/Mapk mutants have normal anterior WD, show normal position of UB, but were deficient in UB induction. Further, Ret-Pi3k/Mapk distal WDs show a delay/arrest in growth towards the cloaca. These results delineate how specific pathways have activating and inactivating roles in sculpting a competent WD to make a normal kidney and ureter.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC362

**Cell Fate Determination in Zebrafish Pronephric Kidney by Wtip/Notch Signaling** Tomoko Obara. *Cell Biology, University of Oklahoma Health Science Center, Oklahoma City, OK.*

WT1 plays an important role for urogenital development. To further understand the function of WT1, yeast two-hybrid assay was performed with mice WT1 and WTIP was discovered. WTIP contains three LIM and one PDZ binding domain at the C-terminus. However, the precise in vivo function of the WTIP is still unknown.

Zebrafish wtip gene is a maternal gene, and ubiquitous expression is detected in the early embryonic stage. At 48 hpf, wtip expression is restricted to anterior brain, eye, inner ear, heart, and pronephros. Wtip protein express in the pronephros are in the glomerular, multiple ciliated cells (MCCs) in the early late segment and posterior segment. Next, we examined the effects of Wtip by loss- and gain-of-function experiments in zebrafish. Knock down of zebrafish wtip led to an increased number of MCCs in the early distal and also ectopic MCCs in the proximal region of the pronephros. Conversely, wtip mRNA injected embryos reduced the number of MCCs. Since Notch signaling has been shown to be a major regulator in MCC development, we next examined whether Wtip functions downstream of Notch signaling, by asking whether wtip mRNA could rescue the increased number of MCCs in the mind bomb (mib) (Notch signaling is impaired) and in the knocked down jagged2 morphants. Indeed, injection of wtip mRNA suppressed the mib and jagged2 morphant phenotype, suggesting that Notch signaling is epistatic to Wtip mediated cell signaling.

In addition to the MCCs effect, wtip morphants showed other pronephric defects. Gene expression in late distal pronephros was altered and cloaca was fused in the wtip morphants. Moreover, we also found glomerular defects similar to FSGS. These were probably due to the decrease in the heart rate caused by abnormal wt1a expression in the proepicardial organ (PEO). In wtip morphants and mibta52b, both podocytes and endothelial cells differentiate normally but remain unmerged and fail to terminally differentiate. In addition, the podocyte gene marker expression level were decreased by injection of wtip mRNA. Taken together, our data suggest that Wtip is an important regulator in the establishment of MCC fate, posterior pronephro and glomerulus development.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC363

**Disruption of Smad Signaling in the Lower Urinary Tract Causes UPJ Obstruction** Piyush Tripathi, Yinqiu Wang, Feng Chen. *Internal Medicine, Renal Division, Washington University School of Medicine, St Louis, MO.*

Ureteropelvic Junction (UPJ) Obstruction is a common congenital anomaly in pediatric population and is presumably caused by developmental defects in the ureter, a Wolffian ducts (WD) derivative. By E10.5, the ureteric bud (UB) emerges from the WD and invades the metanephric blastema for nephrogenesis. The proximal part of the UB undergoes branching and elongation to make the collecting duct system of kidney, the distal part of the UB develops into ureter for transporting urine to the bladder. Although collecting duct epithelia and ureteric epithelia share common embryonic origin, the ureter recruits smooth muscle progenitors and other cells from the tail-bud-derived mesenchyme instead of the metanephric mesenchyme that gives rise to the nephrons within the kidney proper. In this study, we have combined a *Tbx18-Cre* transgene with specific expression in lower urinary tract mesenchyme and a *floxed-Smad4* allele to disrupt Smad signaling in the ureteric and bladder mesenchyme during embryogenesis. *Tbx18-Cre/+, Smad4$^{flox/flox}$* mice have severe bilateral hydronephrosis at as early as E17.5. Indian ink injection experiment has confirmed UPJ obstruction as main cause of hydronephrosis, which is further evident by the absence of urine in the mutant bladders. We further determine that constriction within the ureteric lumen may be the main cause for UPJ obstruction. Although differentiation of the urothelium and ureteric smooth muscle occurred, disorganization of the urothelium was observed. Microarray study on E16.5 mutant and control ureters revealed transcriptional alterations in known Bmp targets, smooth muscle specific genes, and genes involved in extracellular matrix remodeling/maintenance. In particular, Id2 expression was significantly reduced in the mutants, consistent with previous finding that Id2 heterozygous mutants develop

UPJ obstruction. These data indicate an important functional role of Smad signaling in organizing the development of the lower urinary tract and provides a model to further study the pathogenesis of UPJ obstruction.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC364

**The Developmental Corepressor Groucho4, GRG 4, Associates with a Nuclear Phosphatase and Methyltransferase To Repress Transcription** Sanjeevkumar R. Patel,[1] Samina Bhumbra,[1] Lekshmi Nair,[1] Gregory R. Dressler.[2] *[1]Internal Medicine, University of Michigan, Ann Arbor, MI; [2]Pathology, University of Michigan, Ann Arbor, MI.*

In mammals, the Gro/TLE family consists of four proteins of similar molecular weight and structure, termed groucho gene-related protein 1-4 (Grg1-4) in mice. The Grg proteins modulate the process of transcription by physical interaction with DNA-binding transcription factors. These proteins can down regulate the expression of target genes of transcriptional activators, enhance the transcriptional repression effect of transcriptional repressors, or convert transcriptional activators into repressors. Grg/TLE co-repressors interact with multiple transcription factors including HES, RUNX and Pax proteins.

Expression of Grg1, 3 and 4 is widespread during development and overlaps partially with the Pax2/5/8 family in the neural tube and the kidney. Interaction of the Grg members and Pax proteins may repress Pax-regulated genes. To understand how Grg4 mediates repression, we identified Grg4 containing complexes from mammalian nuclear lysates by fractionation, immunoaffinity purification, and mass spectrometry. We show that Grg4 associates with a nuclear serine-threonine phosphatase and a histone methyltransferase. The associated methyltransferase activity was confirmed by immunoprecipiation of Grg4 and the methylation of various histone substrates. Using antibodies against specific histone H4 arginine residues, we have mapped the amino acid residues methylated by theGrg4 complex. Moreover, the phosphatase dephosphorylates Pax2 *in vitro*. At an integrated Pax2 responsive gene, co-expression of Pax2 and Grg4 results in gene silencing by a combination of mechanisms: displacement of a PTIP dependent activation complex by the Grg4 complex, possibly via dephosphorylation of DNA-bound Pax2, as well as deposition of repressive arginine methylation marks at the promoter. Our data suggest that Pax2 marks specific regions of chromatin by modification of histones. These epigenetic marks can delineate both active and inactive regions of the genomes in a stable and heritable manner through potential interactions with chromatin effectors.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC365

**Genetic Basis for the Iron Delivery in the Developing Kidney** Andong Qiu, Melanie Viltard, Neal A. Paragas, Kristen Mcnierney, Jonathan M. Barasch. *Columbia.*

Iron is a growth factor required for kidney development. Transferrin receptor 1 (TFRC) was thought to be the single, ubiquitous high affinity mechanism of iron delivery to mammalian cells for growth and development. Surprisingly, our recent study in chimeras of TFRC ko and wt kidneys suggested that TFRC was not required for the development of most organs or cell types of the embryo, except possibly for the development of ureteric and lung bud tips at late stages of morphogenesis. In contrast, TFRC was dispensable in both ureteric bud and lung stalks and in mesenchymal cells (Developmental Cell, 2009). To precisely dissect the functional role of TFRC in kidney development, we generated a conditional murine knockout model for the tissue-specific ablation of the TFRC gene. When the TFRC gene was deleted specifically in the ureteric bud using a Hoxb7-Cre mouse, we observed limited renal hypoplasia at E12.5, but after birth we found the TFRC-deleted kidneys demonstrated either severe hydronephrosis or severe hypoplasia, and histological analysis showed deranged cells particularly in the distal nephron and papilla, indicating that loss of TFRC in the UB led to kidney hypoplasia and damage in an accumulative process. Hence TFRC is required during late stages of the maturation of the ureteric bud, but additional iron delivery pathways may compensate for TFRC early in embryogenesis. In fact, iron starvation produced severe renal hypoplasia early in development, indicating that TFRC alone can not fully explain iron trafficking. Hence, using a functional screening approach, we have now identified a novel iron transporter, SPNS1 which increased ferrous iron uptake with an apparent Km of 1.6μM when expressed in Xenopus oocytes. SPNS1 was present both on the cell surface and in transferrin filled endosomes (recycling compartment). Over expression of SPNS1 stimulated transferrin-mediated iron uptake in Hek293 cells and SPNS1 showed a high pH optimum consistent with activity at the cell surface or in transferrin endosomes. In sum, TFRC is required for the normal function of the ureteric bud, but SPNS1 is an alternative pathway which conducts iron in the ureteric bud.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC366

**Notch Can Drive MET in the Developing Kidney Independently of Wnt** Scott C. Boyle, Raphael Kopan. *Developmental Biology, Washington University, St Louis, MO.*

Nephrons are formed from the metanephric mesenchyme (MM) by mesenchymal to epithelial transition (MET) through a stereotypical process, the early hallmarks of which are induction of the renal vesicle (RV) and progression to the S-shaped body (SSB). These structures are made up of undifferentiated epithelia but are polarized into proximal and distal domains that predict nephron segmentation in the adult. The Wnt and Notch signaling pathways are critical for this process. When Wnt4 is deleted, the RV does not form and epithelialization fails causing a complete loss of nephrons. Without Notch signaling, the RV forms but fails to segment into the SSB, resulting in non-functional nephrons that lack all proximal components, including glomeruli and tubules. Studies in the developing zebrafish kidney indicate that Notch is required for Wnt activity. We asked whether Notch and Wnt cooperate to drive MET in the mouse. Constitutive Notch activation in the MM drives MET, converting all progenitors to proximal fates at the expense of distal cell types. Contrary to observations in the zebrafish, Notch is not required for Wnt4 expression, and exogenous Wnt4 or LiCl do not rescue segmentation defects in Notch mutants. We then asked if developing nephrons must be exposed to Wnt4 to gain competence for Notch induced MET. To test this, we implemented a novel Tat-Cre based system to activate Notch in cultured metanephroi. Remarkably, when we activate Notch in Wnt4 null kidneys, MM cells still undergo MET. The epithelial cells that form express multiple markers of mature proximal tubule epithelia. A minority of cells make immature podocytes. These structures, however, do not develop apical basal polarity or form a lumen, instead adopting a tumor-like morphology. Furthermore, not all the cells in the metanephros are competent to form proximal epithelia in response to Notch: duct cells and mesenchyme lateral to the ureter are resistant. These data demonstrate that Notch can control a master program of proximal tubule differentiation, and show for the first time that MET of renal stem cells can be induced by pathways other than Wnt. Notch dependence on Wnt9b, the initial inductive factor in this process, is under investigation.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC367

**Growth-Arrest Specific 1 (Gas1) Is a Novel Target of the Wilms' Tumor Suppressor Gene Wt1 and Is Implicated in Nephron Progenitor Cell Maintenance** Martin Kann,[1] Eunnyung Bae,[1] Sunny Hartwig,[2] Mary E. Taglienti,[1] Jordan A. Kreidberg.[1] *[1]Department of Nephrology, Children's Hospital Boston, Boston, MA; [2]Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada.*

The transcription factor WT1 has long been known to be a key molecule in renal development as its homozygous deletion results in renal agenesis due to apoptosis of nephron progenitor cells (NPC). However, mechanisms by which WT1 maintains NPCs are largely unknown. Gas1 has been implicated in several signalling pathways relevant to apoptosis. We recently identified Gas1 as a putative WT1 target gene in a genome-wide siRNA approach in vitro.

Coexpression of Gas1 and Wt1 in NPC in vivo was shown by in situ hybridization (ISH). We confirmed binding of WT1 to the Gas1 gene DNA in vivo by chromatin immunoprecipitation followed by qPCR. Biological relevance of this interaction was established in vitro, ex vivo and in vivo: Knock-down of Wt1 by siRNA in a cell line expressing Gas1 and Wt1 resulted in downregulation of Gas1 mRNA. ISH signals for Gas1 were reduced in embryonic kidney organ cultures cultivated in the presence of a morpholino oligonucleotide (MO) that blocks Wt1 translation. Furthermore, we established a novel transgenic mouse line to generate hypomorphic Wt1 phenotypes by conditionally expressing an shRNA directed against Wt1 in NPC. By ISH, Gas1 was downregulated in NPC in mouse embryos hypomorphic for Wt1.

Functional relevance of Gas1 in NPC was investigated in embryonic kidney organ cultures treated with MO directed against Gas1. Efficient knockdown was confirmed by ISH. After 24 hours, branching morphogenesis arrested in Gas1 morphants, and NPC expression domains of Six2, Pax2, and Wt1 were abolished or significantly reduced as shown by ISH and immunofluorescence staining, respectively. TUNEL staining revealed increased apoptosis in the NPC pool. In summary we present a novel mechanism by which WT1 may protect NPCs from apoptosis.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC368

**DLG1 and CASK Cooperate To Influence Nephron Progenitor Proliferation and Migration** Sun-Young Ahn,[1] Sung Tae Kim,[1] Gloriosa K. Go,[1] Wojciech Swat,[2] Jeffrey H. Miner.[1] *[1]Renal Division, Washington University, St. Louis, MO; [2]Dept. of Pathology and Immunology, Washington University, St. Louis, MO.*

DLG1 (discs-large homolog 1), the mouse ortholog of the *Drosophila* discs-large tumor suppressor protein, functions to maintain epithelial cell polarity and synaptic function in neurons. We previously reported that DLG1-null mice demonstrate severe urogenital abnormalities, including hydronephrosis due to ureteric smooth muscle orientation defects and occasional renal dysplasia or renal agenesis. Due to its interaction with CASK (calcium/calmodulin-dependent serine protein kinase) at the membrane-cytoskeleton interface, we sought to further define the role of DLG1 in murine urogenital development by generating mice deficient in both DLG1 and CASK using the Cre-lox system. Whereas CASK null mice had normal-sized kidneys, kidneys deficient in both DLG1 and CASK were very small and dysplastic. DLG1+/-;CASK null kidneys were also small, though not as small as the double-null kidneys. We discovered several cellular and molecular defects in the double mutants. These include: reduced proliferation and increased apoptosis of cells in the nephrogenic zone; a decrease in the number of cells expressing Six2, a transcription factor essential in maintaining the nephron progenitor population; impaired aggregation of Six2-positive cells around the ureteric bud tips, indicating possible cell migration defects; and reduced nephrogenic zone expression of both Pax2, an upstream regulator of Six2, and Raldh2, an essential enzyme in retinoic acid synthesis and an important regulator of kidney development. To determine whether the impaired nephrogenesis was due to defects in the nephron progenitor pool or in the ureter, we deleted DLG1 and CASK in the urothelium and collecting ducts with HoxB7-Cre. These mice did not show a significant difference in kidney size compared to controls. Taken together, these results show that a DLG1 mutation

can synergize with a mutation in another gene to affect urogenital development and that scaffolding proteins play an important role in generating or maintaining the nephron progenitor population.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC369

**Isolation of Adult Renal Progenitors Capable of Nephron Formation in Zebrafish** Alan J. Davidson,[1] Cuong Diep,[1] Dongdong Ma,[2] Frank Bollig,[3] Christoph Englert,[3] Neil A. Hukriede,[4] Robert I. Handin.[2] *[1]Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA; [2]Hematology Division, Brigham and Women's Hospital, Boston, MA; [3]Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, Germany.*

Zebrafish have the ability to regenerate damaged tissues including organs such as the kidney. Using transgenic zebrafish where specific renal cell populations are fluorescently tagged, combined with gentamicin-induced nephron injury and transplantation experiments, we have identified a population of renal progenitors that when injected into the kidney can regenerate new functional nephrons. Time-lapse imaging in larval fish revealed that single renal progenitors coalesce together to form clusters that then differentiate into renal vesicle-like structures. These cells express a number of renal transcription factors including wilms' tumor suppressor-1 and pax2. Similar to nephrogenesis in the developing mammalian kidney, the renal vesicles grow into primitive nephrons that fuse with existing renal tubules and acquire blood filtration capabilities. These observations support the notion that adult renal regeneration recapitulates a developmental kidney program. Taken together, our results demonstrate that the zebrafish kidney contains a transplantable population of renal progenitors capable of de novo nephron formation. Our work provides new insights into the cellular mechanisms of renal regeneration and may lead to the development of novel therapies to treat kidney disease.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC370

**Oct4 Overexpression but Not Chromatin Modifying Agents Reprograms Adult Human Kidney Cells into a Renal Progenitor State** Dorit Omer,[1,2] Orit Harari-Steinberg,[1,2] Ella Buzhor,[1,2] Sally Metsuyanim,[1,2] Naomi Pode Shakked,[1,2] Benjamin Dekel.[1,2] *[1]Pediatric Stem Cell Research Institute, Sheba Medical Center, Tel Hashome, Sackler Faculty, Tel Aviv University, Tel Aviv, Israel; [2]Sheba Center of Regenerative Medicine, Sheba Medical Center, Tel Hashome, Sackler Faculty, Tel Aviv University, Tel Aviv, Israel.*

The nephrogenic cortex in which renal stem/progenitor cells are induced to form nephrons through mesenchymal to epithelial transition ceases to operate in the 34th week of human gestation, severely limiting the adult kidney's regenerative capacity. This cessation correlates with shutdown of *Six2* and *Osr1* which maintain responsible the nephron progenitor population. Here we show that human adult kidney (HAK) cells can be induced to highly re-express *Six2*/*Osr1* along with pluripotency markers (*Oct4*, *nanog*) following epigenetic modulation with chromatin-modifying agents, e.g. valproic acid (VPA) which induces epigenetic changes of the histones at multiple sites of the *Six2* promoter leading to gene activation. Nevertheless, VPA treated cells failed to undergo epithelial to mesenchymal transition (EMT), possibly due to activation of the Wnt pathway supporting epithelialization and cellular senescence appeared in early passages, all limiting cells developmental potency. Surprisingly, this could be overcome by lentiviral overexpression (OE) of *Oct4* and isolation by puromycin selection, generating HAK cells that re-express *Six2*/*Osr1*, appear uniform as round cells in culture and undergo EMT (high *vimentin*, low *E-cadherin*). *Oct4* OE HAK cells could be readily expanded and propagated into late passages while maintaining their traits. Finally, *in vitro* differentiation and colony-forming-unit assays of *Oct4* OE HAK cells showing epithelial re-differentiation, multipotentiality and clonogenic capabilities indicated enhanced developmental plasticity. Altogether, our results indicate that *Oct4* alone as opposed to chromatin-modifying agents is likely to be sufficient to induce HAK cells into a renal progenitor state.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC371

**Genotype Polymorphisms of Matrix Metalloproteinases Are Associated with Arteriovenous Fistula Patency in Hemodialysis Patients** Chih-Ching Lin.[1,2] *[1]Divison of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; [2]School of Medicine, National Yang-Ming University, Taipei, Taiwan.*

Matrix metalloproteinases (MMPs) are risk factors for cardiovascular diseases. This study evaluated the association of genotype polymorphisms of MMPs and tissue inhibitors of MMP (TIMPs) in hemodialysis patients (HD) with arteriovenous fistula (AVF) failure. Genotype polymorphism of MMP-1, -2, -3, -9 and TIMP-1, -2 as well as clinical and laboratory parameters were compared between Chinese HD patients with (n=170) and without AVF failure (n=426).

Cox regression model of factors associated with AVF failure in HD patients

| | Significance | Hazard ratio |
|---|---|---|
| HD duration (months) | <0.001 | 1.007 |
| Cardiovascular disease | 0.039 | 1.656 |
| Maximal venous pressure (mmHg) | <0.001 | 1.016 |
| MMP-1 -1607 genotypes (1G/1G vs. 1G/2G+1G/2G) | 0.007 | 2.315 |
| MMP-3 -1612 genotypes (6A/6A vs. 5A/6A+5A/5A) | 0.049 | 1.712 |
| MMP-9 -1562 genotypes (C/C vs. C/T+T/T) | 0.048 | 1.650 |

Significant associations were found between AVF failure and 1G/1G genotype of MMP-1 -1607 1G>2G SNP (2.315 vs. 1G/2G+2G/2G ), 6A/6A genotype of MMP-3 -1612 5A>6A SNP (1.712 vs. 5A/6A+5A/5A ), and C/C genotype of MMP-9 -1562 C>T SNP (1.650 vs. C/T+T/T ). The positive predictive rates for AVF failure were 63.0% and 6.7% for patients with the highest-risk (1G1G/6A6A/CC) and lowest-risk (2G2G or 2G1G/5A5A or 6A6A/TT or TC) composite MMP-1/-3/-9 genotype, respectively. The unassisted patency of AVF at 5 years decreased significantly from 72.2% to 55.2% for different MMP-1 genotypes (2G/2G + 1G/2G vs. 1G/1G, P=0.0038), from 77.3% to 67.9% for MMP-3 genotypes (5A/5A+5A/6A vs. 6A/6A, P=0.0094), from 79.0% to 67.3% for MMP-9 genotypes (C/T + T/T vs. C/C, P=0.0205), and from 93.3% to 38.4% for the composite MMP-1/-3/-9 genotypes (lowest vs. highest risk, P<0.001). In conclusion, specific genotypes of MMP-1, MMP-3 and MMP-9 with lower transcriptional activity are associated with higher frequencies of AVF failure, which may result from more extensive accumulation of intravascular extracellular matrix, thus leading to a higher risk of AVF stenosis.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC372

**Chymase Is Increased in Hyperplasic Veins Used for AVF Creation in Stage 4-5 CKD Patients** Monnie Wasse,[1] Rong Huang,[1] Angel Rivera,[2] Nawazish Naqvi,[2] Ahsan Husain.[2] *[1]Renal Division, Emory University, Atlanta, GA; [2]Cardiology Division, Emory University, Atlanta, GA.*

Angiotensin II and matrix metalloproteinases (MMPs) have been implicated in the development of venous neointimal hyperplasia (VNH) within arteriovenous fistulae (AVF). Angiotensin II causes vascular smooth muscle cells to proliferate and differentiate into a synthetic phenotype with upregulated α-smooth muscle actin (α-SMA) expression. MMPs, which degrade matrix, promote adverse vascular remodeling. Mast cell chymase converts angiotensin I to II and it activates several pro-MMPs. We studied chymase protein expression in veins used for AVF creation prior to AVF surgery in pre-dialysis Stage 4–5 CKD patients presenting for their first vascular access creation.

All 5 vein specimens exhibited VNH; average luminal stenosis and ratio of intima-to-media thickness and intima-to-media area were 84±7.3%, 0.39±0.23 and 0.3±0.16, respectively. The control vein had a normal thin intima and media (Figure.1A).Chymase and α-SMA protein (localized by immunofluorescence) were weakly expressed in these veins (Figure1.B). By contrast, the CKD veins demonstrated aggressive VNH, reflected by an intensely thickened intima (Figure.1C). Chymase expression was abundant in these veins (Fig.1D) and co-localized with α-SMA in the media and adventitia. These findings not only suggest a role for chymase in VNH, but also point to a therapeutic target for the treatment of VNH in the setting of dialysis vascular access.

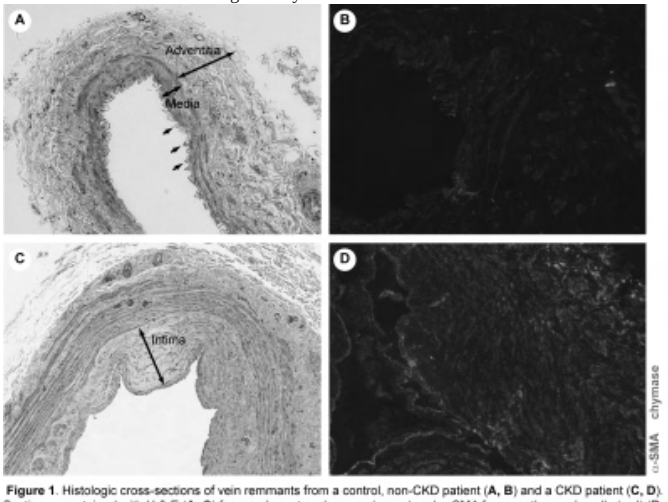



**Figure 1**. Histologic cross-sections of vein remnants from a control, non-CKD patient (**A, B**) and a CKD patient (**C, D**). Sections are stained with H & E (**A, C**) for morphometry, chymase (green) and α-SMA for smooth muscle cells (red) (**B, D**). **A**) Control vein with normal histology, a normal, thin intima (small arrows), and normal media and adventitial layers; **C**) CKD vein has a thickened intima and media; **B**) Control vein with little evidence of chymase and α-SMA, reflecting limited media layer; **D**) CKD patients has abundant chymase and colocalization with α-SMA, reflecting smooth muscle cells in the media layer.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC373**

**The Impact of Arterial Micro-Calcification on Aortic Stiffness and Endothelial Dysfunction in Patients with End-Stage Renal Disease** Hyun Gyung Kim,[#1] Young Soo Kim,[#1] Young Ok Kim,[#1] Hye Eun Yoon.[#2] *[1]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea Uijeongbu St. Mary's Hospital, Uijeonbu, Korea; [2]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea Incheon St. Mary's Hospital, Incheon, Korea.*

**Purpose**: We previously reported that arterial micro-calcification (AMC) at vascular access is associated with early failure of vascular access in patients with end-stage renal disease (ESRD) [ASN 2008]. Although vascular gross calcification is known as an independent risk factor for cardiovascular outcome, the role of AMC is not reported yet. **Methods**: Sixty-five ESRD patients awaiting vascular access operation were included in this study. Aortic stiffness and endothelial dysfunction were evaluated with brachial-ankle pulse wave velocity(baPWV) and flow mediated dilatation (FMD) of the brachial artery, respectively. Diagnosis of AMC was made by microscopic analysis with von Kossa staining. **Results**: Mean age of the patients was 60±12 years. The gross calcification by plain X-ray was detected in 29 out of the 65 aortic arches (44.6%) and 5 out of the 27 radial arteries (18.5%). The AMC was detected in 36 patients (55.4%). The AoAC score was higher in the positive AMC group compared to the negative AMC group (17.8±17.1% vs 5.1±12.3%, p=0.001). The baPWV was also higher in the positive AMC group (26.5±9.4 m/s vs 19.8±6.6 m/s, p=0.006). But there was no difference in FMD between the two groups (5.4±2.6% vs 5.7±3.5%, p=0.764). The positive AMC group had higher incidence of DM (91.6% vs 44.8%, p=0.001), systolic blood pressure (156±23 mmHg vs. 143±25 mmHg, p=0.036) and pulse pressure (67±9 mmHg vs 55±17 mmHg, p=0.014) than the negative AMC group. **Conclusion** : This data showed that AMC at vascular access site was related to baPWV but not to FMD in ESRD patients. Therefore we suggest that AMC is associated with cardiovascular morbidity and mortality via aortic stiffness in ESRD patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC374**

**Effect of Initial Blood Flow Rate (BFR) on Progression of Stenosis and Loss of Patency in New Arteriovenous (AV) Grafts** Bradley S. Dixon,[1] Bo Hu,[2] T. Alp Ikizler,[3] Gerald J. Beck,[2] Milena Radeva,[2] John W. Kusek,[4] Harold I. Feldman.[5] *[1]VA Med Cntr & U of IA, Iowa City, IA; [2]Biostatistics, Cleveland Clinic, Cleveland, OH; [3]Vanderbilt U, Nashville, TN; [4]NIDDK, NIH, Bethesda, MD; [5]U Penn, Philadelphia, PA; [6]for the DAC Study Group, .*

BFR contributes to blood vessel wall shear stress and may mediate the development of neointimal hyperplasia contributing to graft stenosis and failure. We examined whether baseline BFR (BBFR) in newly created AV grafts predicted the subsequent rate of development of stenosis or primary unassisted graft patency (PUGP). Development of stenosis was ascertained by the rate of decline in BFR after the baseline measurement. BFR was measured by the ultrasound dilution technique in a randomized clinical trial examining the effect of extended release dipyridamole plus aspirin (ERDP/ASA) compared to placebo on PUGP of newly created AV grafts for hemodialysis. BFR was measured twice within 4 weeks of first using the graft for dialysis and monthly thereafter until 1 month after loss of PUGP. Of 649 randomized patients, 308 had at least one BFR measured after baseline and were included in this analysis; the remaining 341 grafts failed before the required minimum 3 BFR measurements. Median BBFR was 1189 (IQR: 929, 1415) ml/min at a median of 9 (IQR: 6.86, 12.14) weeks after graft placement and was not statistically significantly different between patients taking ERDP/ASA (1214; 935-1476 ml/min) and placebo (1160; 919-1335 ml/min; p=0.09). High BBFR was significantly associated with younger age, male gender, upper arm graft, higher diastolic BP, less cardiovascular disease and diabetes, higher hemoglobin, less aspirin use, and more tobacco use. Log transformed data were used to normalize the skewed BFR distribution. Higher BBFR (>1189 ml/min) was associated with longer PUGP (adjusted HR= 0.52. 95% CI=0.35-0.77, p<0.001). The mean weekly decline in BFR (ΔBFR) at the median BBFR was 0.7% (0.6, 0.8; p<0.001), but ΔBFR was not significantly related to BBFR (p=0.44). The results suggest that overall patency is longer in grafts with higher BBFR but variation in BBFR over the observed range did not influence the rate of development of stenosis.

Disclosure of Financial Relationships: Consultancy: Proteon Therapeutics, Shire PharmaceuticalsResearch Funding: Proteon Therapeutics, Novartis Pharmaceuticals Corp., CardioKine Inc.

**SA-FC375**

**Evolution and Clinical Course of First (EVER) Fistulas and Grafts** Eduardo K. Lacson, Weiling Wang, J. Michael Lazarus, Raymond M. Hakim. *Fresenius Medical Care, North America, Waltham, MA.*

**Introduction:** The evolution and clinical course of arteriovenous (AV) access placement in patients new to hemodialysis (HD) are reported mainly as single center experiences. We describe results from a nationally distributed cohort for the first (ever) constructed AV accesses in the 1st year of HD.

**Methods:** We tracked 8,280 (55.8%) patients with newly constructed fistulas or grafts from 14,836 incident HD patients admitted solely with an HD catheter at Fresenius Medical Care North America facilities within 15 days of first-ever dialysis between January 1 and December 31, 2007. Three periods were carefully tracked: 1) Time to fistula or graft placement within one year of admission; 2) Time to maturation (i.e. catheter removal), for up to one year from placement; and 3) Time to AV access failure requiring a new access (predominantly catheter), for up to one year after maturation.

**Results**: The mean age was 62.0 ± 15.2 years, 55.4% male, 62.3% white and 33.3% black, with 57.5% having diabetes. Fistulas predominated at a ratio of 3:1 (see Table 1).

Table 1: Distribution of time to key access-related events (in days) for 8,280 patients.

| Fistula | N (%) | Median | Mean | SD |
|---|---|---|---|---|
| Time to placement | 6,364 (76.9) | 77 | 98 | 77 |
| Time to maturation | 3,736 (58.7)* | 111 | 122 | 78 |
| Time to failure | 666 (17.8)** | 87 | 120 | 102 |
| Graft | | | | |
| Time to placement | 1,916 (23.1) | 82 | 102 | 77 |
| Time to maturation | 1,364 (71.2)* | 49 | 57 | 46 |
| Time to failure | 357 (26.2)** | 119 | 146 | 106 |

* denominator consists of all placed fistulas or grafts;
** denominator consists of all matured fistulas or grafts.

Grafts had a better maturation rate (71.2% vs. 58.7%, p<0.0001) but worse failure rate (26.2% vs. 17.8%, p<0.0001) compared to fistulas. Though fewer, failure with abandonment occurred earlier in fistulas than in grafts.

**Conclusions:** Current surgical outcomes for AV access construction present major opportunities for improvement. Maintaining optimal AV access function may be another. More studies are needed to address low maturation and high failure rates as well as to determine cost-effectiveness of available interventions.

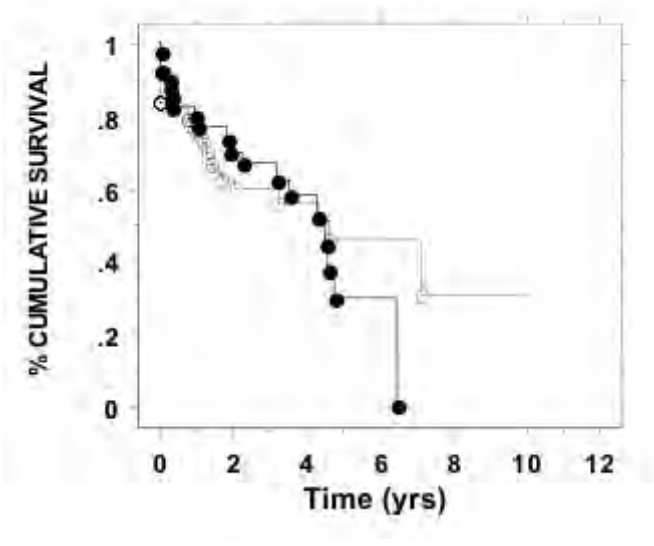
Disclosure of Financial Relationships: Employer: I am an employee of Fresenius Medical Care, North America.

**SA-FC376**

**Comparison of Primary and Secondary Arteriovenous Fistulas: A Nested Case-Control Study** Sunanda J. Ram, Bharat Sachdeva, Kenneth D. Abreo. *Medicine/Nephrology, LSU Health Sciences Center, Shreveport, LA.*

Background: We showed recently that secondary fistulas survive longer than prior ipsilateral forearm grafts. Upperarm fistulas are increasingly placed in patients with inadequate forearm vessels. Survival of these 'primary' brachiocephalic fistulas has not been compared to secondary fistulas, which may benefit from prior graft function. Methods: In this retrospective review of a prospective clinical database we determined whether secondary fistulas were superior to primary fistulas. Forty patients underwent secondary (type I) fistula creation following the loss of forearm synthetic grafts between 5/1/95 through 5/1/08. During this time, 267 upperarm fistulas were placed in patients without ipsilateral forearm grafts. As the sizes of the 2 groups were highly divergent, a sample of 80 patients was randomly selected from the 267 patients with primary fistulas, and matched for 2 characteristics known to influence fistula maturation and survival, namely, gender and diabetes. Results: Most patients were black, 50% were female and 40% had diabetes. Mean patient age was 52 yrs in both groups (NS). Primary fistulas did not have higher maturation failure rate than secondary fistulas (16% vs. 15%). Rates of infection, thrombosis, and failure were also similar. Cumulative survival at 1, 2, 3, and 5 yrs was 80, 70, 62 and 30% for secondary fistulas and 76, 61, 56 and 46%, respectively for primary fistulas. The figure confirms that the survival of primary and secondary fistulas was not significantly different.

Cumulative survival of primary (open circles) and secondary (closed circles) fistulas.



Conclusion: This study shows that both primary and secondary fistulas provide similar survival and confirms that fistulas are the access of choice. Differences in time to maturation and number of interventional procedures in the 2 groups needs further study.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC377

**Hospitalization and CKD: Are We Protecting Our Patients' Futures?** Rita L. McGill, Piyush S. Lohiya, Ankur A. Patel, Maria D. Vornicu, Mari Mori, Tomoki Tsukahara, Jeannie P. Co, Imran Dosani, Uzoamaka T. Nwaogwugwu, Rahul Bhardwaj, Richard J. Marcus. *Nephrology/Medicine, West Penn Allegheny Health System, Pittsburgh, PA.*

Fistula First recommendations for patients with CKD suggest that IV lines should not be placed in the non-dominant arm, and that PICC lines be avoided entirely, to avoid exhaustion of native veins in patients who may someday need a fistula (AVF). We prospectively examined the vein protection and access practices on a typical day in a referral hospital associated with an active fistula promotion program.

MDRD estimated GFR (eGFR) could not be determined for 8 of 391 patients. CKD, defined as eGFR<60, was present in 80 patients (21%), of whom 15 were dialyzing (CKD Stage 6), 15 were in CKD Stage 4, and 50 were in CKD Stage 3. Limb protection bracelets were found on 13/80 patients, of whom 7 had existing AVF (3 dialysis patients, and 4 transplant patients in stage 3 and 4 CKD), and 2 had CKD 3 and mastectomy. 4 patients dialyzing via permcath had bracelets, and 8 did not. No bracelets were found on transplant patients without fistulae.

Among patients with bracelets, no IV's or PICC lines were found in protected arms.

Access in Unprotected CKD patients (n=67)

| | PICC | IV's |
|---|---|---|
| non-dominant arm | 8 (12%) | 27 (40%) |
| dominant arm | 12 (18%) | 19 (28%) |

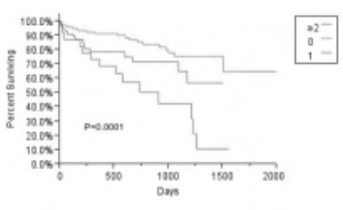
Limb protection bracelets effectively prevent undesirable exhaustion of future fistula veins during hospitalization, but are deployed infrequently among populations at risk, even in an institution with a proven commitment to fistula promotion. Unprotected inpatients with CKD have a 30% rate of PICC placement, and a 52% rate of having an intravenous device placed in the non-dominant arm; punctures for phlebotomy could not be assessed. Strategies that increase the use of simple limb protection devices during hospitalization, and protect future fistula vessel prospects by discouraging routine PICC and IV placement, may improve the outcomes of patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC378

**Decreased Cumulative Access Survival in Arteriovenous Fistulas Requiring Interventions To Promote Maturation** Timmy C. Lee,[1] Michael Allon,[2] Prabir Roy-Chaudhury.[1] [1]*Internal Medicine, University of Cincinnati, Cincinnati, OH;* [2]*Medicine, University of Alabama at Birmingham, Birmingham, AL.*

New arteriovenous fistulas (AVF) are frequently unsuitable for hemodialysis due to failure to mature. Aggressive endovascular (angioplasty) or surgical interventions are often undertaken to salvage non-maturing AVFs. The impact of early interventions to promote AVF maturation on subsequent long-term AVF outcomes is unknown. We evaluated 173 hemodialysis patients from two large academic centers, who received a new AVF. Of these, 96 (56%) required no further intervention, 54 (31%) required 1 intervention, and 23 (13%) required ≥2 interventions to achieve suitability for dialysis. We calculated AVF survival and frequency of post-maturation interventions in each group. Cumulative AVF survival (first cannulation to permanent failure) in patients with ≥2 vs 1 vs 0 interventions before maturation was 68 vs 78 vs 92% at 1 year, 57 vs 71 vs 85% at 2 years, and 42 vs 57 vs 75% at 3 years.




Using Cox regression analysis with interventions before maturation, age, sex, race, diabetes, peripheral vascular disease, access site, and obesity in the model, interventions before maturation (≥2) was the only factor associated with cumulative AVF survival (HR 1.67, 95% CI, 1.01 to 2.70). The number of interventions required to maintain patency after maturation was 3.51±2.20 vs 1.37±0.31 vs 0.76±0.10 per year in patients with ≥2 vs 1 vs 0 interventions before maturation (p=0.004 comparing ≥2 interventions with 0 interventions). Compared with AVF that mature without further interventions, AVF that require interventions have decreased cumulative survival and require more interventions to maintain their patency for hemodialysis.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC379

**Stent Grafts Decrease Risk for Restenosis Compared to Venous Angioplasty Alone in Non-Maturing Primary Arteriovenous Fistulas Secondary to Juxta-Anastomosis Stenosis** Saravanan Balamuthusamy,[1] Benjamin Estes,[1] Sasha Monty,[1] Mark Taylor,[1] William Mckee,[1] John Lucas.[2] [1]*Nephrology, Hypertension and Vascular Access, Northwest Mississippi Regional Medical Center, Clarksdale, MS;* [2]*Lucas Surgical Group, Greenwood Leflore Hospital, Greenwood, MS.*

Background: Over 40% of primary Arteriovenous fistulas do not mature in patients with end stage renal disease. The role of covered stents (stent grafts) in non-maturing AV fistulas secondary to juxta-anastamotic stenosis has not been well studied so far.

Methods:We performed a retrospective analysis of primary Arteriovenous fistulas over 1 year that wear considered non-maturing fistulas. Fistulas were considered non-maturing if they were less than 5mm in size after 8 weeks and if the blood pump could not be ran at 350ml/min despite using optimal sized needles. Stenosis at the juxta-anastomosis site was defined as greater than 60% narrowing of the luminal diameter on angiogram.

Results: We analyzed 46 new arteriovenous fistulas that were not suitable for cannulation after 8 weeks of its creation. Fistula angiograms revealed significant Juxta anastomosis stenosis as the reason for poor maturation in 37 fistulas. Fourteen patients had received a stent graft in the juxta-anastamosis site due to severe stenosis and the remaining 23 patients received angioplasty. Primary patency at 6 months was 100% for the patients with stent grafts vs 82% in patients receiving angioplasty alone (p=0.NS). The need for re-intervention in less than 3 months was 7% in the angioplasty group vs 39% in the stent group (p=NS). All 37 patients had follow-up angiograms in 3 months after the initial procedure. All the fourteen patients in the stent group met "single pool" Kt/V of >1.20 as opposed to 61% in the balloon angioplasty group (p=0.02). Restenosis occurred in 7% in the stent group as opposed to 43% in the angioplasty group (p=0.04).

Conclusion: Stent grafts are a safe and effective option for salvaging poorly maturing AV fistulas secondary to severe juxta-anastomosis stenosis. Stent graft placement decreased the risk for re-stenosis and enabled patients to achieve single pool Kt/V goals.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC380

**Long Term Vascular Access Survival and Complications with Daily Dialysis** Steven Achinger,[1] T. Alp Ikizler,[2] Aihua Bian,[2] Juan Carlos Ayus.[3] [1]*Nephrology, Watson Clinic, LLP, Lakeland, FL;* [2]*Nephrology, Vanderbilt University, Nashville, TN;* [3]*Renal Consultants of Houston, Houston, TX.*

**Background:** A potential complication of daily hemodialysis is repeated access cannulation (six times per week). There are no studies that examined long term effects of FHD on vascular access patency.

**Methods:** We conducted a non-randomized, controlled trial of the effect of daily (six sessions/week of three hours each) or conventional (three sessions/week of four hours each) hemodialysis on hemodialysis access outcomes. We enrolled 26 short daily hemodialysis and 51 matched conventional hemodialysis patients and collected vascular access procedures and treatment attendance. Baseline characteristics were similar between groups in terms of age, diabetes status and baseline vascular access.

**Results:** At 48-month follow-up, there were no significant differences between the two groups in the numbers of access procedure (thrombectomy or revision); 320.7/1000 person/year in the daily dialysis group versus 433.3/person/year in the conventional dialysis group ($P$ = 0.4). At 4 year follow-up, there were 7 access revisions in the daily dialysis group and one access revision in the conventional dialysis group. The numbers of treatments per month was significantly higher in the daily dialysis group compared with the conventional dialysis group (25 [24, 25] versus 14 [13, 22] $P$ = 0.008).

**Conclusion:** At four years of follow-up, 3 hour daily (six times a week) hemodialysis does not lead to increased vascular access complications and is associated with good long term fistula and graft survival.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC381

**Novel Insertion-Deletion Mutation in *Uromodulin* in a Large Kindred with Familial CKD and Nephrotic Range Proteinuria** Rasheed A. Gbadegesin,[1] Peter J. Lavin,[2] Alison Homstad,[1] Guanghong Wu,[2] Alison Byrd,[2] Jason J. Eckel,[2] Michelle P. Winn.[2] [1]*Pediatrics, Duke University, Durham, NC;* [2]*Medicine, Duke University, Durham, NC.*

***Background:*** Focal and segmental glomerulosclerosis (FSGS) remains an important cause of nephrotic syndrome (NS) and chronic kidney disease (CKD). The pathogenesis is not clearly defined; however current evidence from studies of large families with familial disease suggests that the podocyte plays a central role in this disorder.

***Objective:*** To positionally clone the gene mutated in a large kindred with familial CKD and proteinuria.

***Methods:*** We identified a large family with eight affected individuals spanning three generations. We performed genome-wide linkage analysis (GWLA) using the Illumina Infinum II HumanLinkage-12 beadchip genotyping assay and fine mapping with informative microsatellites.

***Results:*** Two of the affected individuals had 3g and 5g of proteinuria. Renal biopsy in four affected individuals showed foci of interstitial infiltrates and focal global glomerulosclerosis. GWLA and fine mapping yielded a multipoint parametric LOD score of 2.9 on chromosome 16p. Positional cloning of the genes within the locus yielded a novel

in-frame insertion deletion mutation 278_289delins T**CTGCCCCGAAG>CCGCCTCCT** in exon 3 of *uromodulin*. The in-frame change leads to loss of a highly conserved cysteine residue in position 94. The mutation segregates in affected individuals in the family.

***Conclusions:*** Phenotype previously associated with uromodulin defects include chronic interstitial nephritis, juvenile familial hyperurecimic syndrome and glomerulocystic disease. This finding in combination with the recent GWAS[1] reveal that uromodulin is a common disease locus in subjects with CKD and suggests that defective uromodulin may have wider deleterious effects on different compartments of the kidney and the podocyte. In conclusion, a novel insertion-deletion mutation in uromodulin in a large family with CKD and proteinuria expands the clinical spectrum of disease associated with defective uromodulin.

*1. Kottgen A et al. Multiple loci associated with indices of renal function and chronic kidney disease. Nature Genetics 2009, 41:712-717*

Disclosure of Financial Relationships: nothing to disclose

## SA-FC382

**ENU Mutagenesis Identifies a Mutation in Mouse Stk39 Causing a Gitelman's Syndrome-Like Phenotype with Albuminuria** Susan Marie Sheehan, Christina R. Caputo, Karen L. Svenson, Ron Korstanje. *The Jackson Laboratory, Bar Harbor, ME.*

In an ENU mutagenesis screen for albuminuria, 16 male C57BL/6J mice were identified with albuminuria at 20 weeks of age. A line was established from each mouse and a mapping cross was started to localize the underlying mutation. For our first mutant line (Rnl5), the mutation was mapped to a 6 Mb region on Chromosome 2. Next Generation Sequencing of the region identified 5 polymorphisms in the mutant line compared to the normal C57BL/6J sequence. Further validation showed a mutation in intron 10 of Stk39 as the most likely cause of the phenotype. This gene has recently been associated in human hypertension. It regulates NCC, in which mutations cause Gitelman's Syndrome, and Nkcc2, in which mutations cause proteinuria in mice. We phenotypically characterized animals with the mutation and determined that, in addition to albuminuria, mice carrying the mutation have polyuria, hypokalemia, reduced vascular resistance, and premature death in utero or perinatally. These phenotypes are consistent with Gitelman's Syndrome. Currently, we are using molecular genetic techniques to establish the effect of the intronic mutation on gene function. Additionally, mapping and gene identification of the other lines is in progress.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC383

**A Genome-Wide Screen for Novel Genomic Disorders of the Kidney and Urinary Tract Development** Simone Sanna-Cherchi,[1] Katelyn E. Burgess,[1] Monica Bodria,[2] Krzysztof Kiryluk,[1] Vladimir J. Lozanovski,[3] Anna Materna-Kiryluk,[4] Valentina Corbani,[5] Roel Sterken,[1] Nadica Ristoska-Bojkovska,[3] Patricia L. Weng,[1] Nilgun Kacak,[1] Gianluca Caridi,[2] Richard P. Lifton,[6] Landino Allegri,[5] Anna Latos-Bielenska,[4] Zoran Gucev,[3] Francesco Scolari,[7] Velibor Tasic,[3] Gian Marco Ghiggeri,[2] Ali G. Gharavi.[1] *[1]Columbia Univ, NY; [2]Gaslini Inst, Genoa, Italy; [3]Children Hospital of Skopje, Macedonia; [4]PRCM, Poznan, Poland; [5]Univ of Parma, Italy; [6]Yale Univ, CT; [7]Hospital of Montichiari, Italy.*

A novel approach to gene identification for congenital anomalies of the kidney and urinary tract (CAKUT) consists in assessing the whole genome for rare structural variants (copy number variants, CNVs). No adequately powered CNV studies for CAKUT have been conducted so far.

We performed a genome-wide search for CNVs using ILMN 610-Quad arrays in 163 CAKUT patients and 5,086 controls. We developed an analytic pipeline to identify rare pathogenic CNVs, which includes: annotation in public databases; testing for frequency in a large panel of controls; identification of recurrent CNVs or CNVs disrupting genes; prioritization based on linkage loci and gene expression.

Analysis of 163 patients yielded 2,532 unique CNVs. Comparison with 5,086 controls and cross-annotation with public databases led to 105 rare CNVs disrupting exons. Eleven patients (7%) carried CNVs diagnostic of known genomic disorders, indicating that our approach is robust for identification of pathogenic CNVs. Thirty patients (18%) carried unique CNVs with a high likelihood of pathogenicity: absence in > 5,000 controls; *de novo* occurrence in patients with unaffected parents; enrichment for genes expressed in embryonic kidney development; co-localization with linkage loci from our studies of familial CAKUT. We validated 92% of these CNVs and 13% were *de novo*.

In conclusion, we diagnosed known genomic disorders and identified unique CNVs highly suggestive of novel genomic disorders responsible for CAKUT. Our data indicate that rare genic CNVs play a major role in the pathogenesis of CAKUT, motivating larger CNV screens to confirm and discover novel genes underlying this common cause of kidney failure.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC384

**Targeted and Whole Exome Sequencing Reveals Multiple Deleterious Variants in CAKUT Patients** Rajshekhar Chatterjee, Enrique Ramos, Stanley P. Hmiel, Anne Beck, Keith A. Hruska, Douglas Coplen, Helen Liapis, Rob D. Mitra, Paul Austin, Todd E. Druley, Sanjay Jain. *Departments of Internal Medicine (Renal Division), Pediatrics, Genetics and Surgery (Urology), Washington University at St Louis, St Louis, MO.*

CAKUT are the most common cause of renal failure in children. Here we examined the association of mutations in developmentally important genes such as Gdnf, Ret, Spry1 and others in a subset of CAKUT using traditional and novel Whole Exome Next-generation sequencing.

Sanger sequencing of coding regions, splice sites and conserved promoter regions revealed non-synonymous polymorphisms/mutations in RET/GDNF in 21 of 84 patients. Of these 18 harbor RET-G691S polymorphism, previously associated with increased risk of CAKUT and predicted to alter MAPK signaling. In addition to RET-G691S, one of the patients harbored a mutation in GDNF between the predicted proteolytic processing site and canonical TGFβ cysteine residues (R93W), while Another had mutation in the RET tyrosine kinase domain (R982C, patient 2). The R982C is a rare variant with a potential to alter RET-SRC and RET-PLCγ signaling. Mutations in both these pathways in mice reveal a spectrum of CAKUT similar to this patient. We further established a pipeline to analyze capture based whole exome sequencing to investigate the possibility of other genetic changes in Patient 2 with features of renal dysplasia, dilated ureters and cryptorchidism. We found additional mutations in the GDNF-GFRα1-RET pathway and other developmentally important genes. Notably a novel ns mutation G438D in GFRα1 was discovered that is predicted to modulate anchoring of GFRα1 to the cell membrane. In this patient, the RET mutations were found to be inherited from father while the GFRα1 mutation from the mother. Interestingly, unaffected brother of patient 2 harbors the GFRα1 mutation but neither of the RET mutations.

Our studies for the first time provide evidence for presence of multiple deleterious rare variants in kidney development genes using a combination of traditional and Next-gen sequencing methods, and support the idea that increased deleterious rare variant burden plays a role in pathogenesis of CAKUT.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC385

**Identification of Two Novel Genetic Factors for CAKUT Origin: Results from the AGORA Project** Kirsten Y. Renkema,[1] Ernie M. H. F. Bongers,[1] Iris Van Rooij,[2] Loes F. M. Van der Zanden,[2] Albertien M. Van Eerde,[3] Jacques Giltay,[3] Wout F. Feitz,[4] Antoine Reginensi,[5] Andreas Schedl,[5] Franz S. Schaefer,[6] Barbara Franke,[1] Nine V. Knoers.[1] *[1]Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Epidemiology, Biostatistics, and HTA, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands; [4]Pediatric Urology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [5]Inserm U636, University of Nice, Nice, France; [6]Pediatric Nephrology, University of Heidelberg, Heidelberg, Germany.*

Congenital Anomalies of the Kidneys and Urinary Tract (CAKUT) occur frequently and comprise the most common cause of end-stage renal disease in children. Structural disorders belonging to the spectrum of these anomalies include renal agenesis, multicystic kidney dysplasia, ureteropelvic junction obstruction, and duplex collecting system. Not much is known about the origin of CAKUT. Alterations in genes expressed during nephrogenesis are considered to be important, with the final phenotypic outcome depending on additional modifying genetic and environmental factors. The aim of this study is to identify new genetic factors involved in CAKUT aetiology. From the AGORA biobank of the Radboud University Nijmegen Medical Centre over 700 well-documented CAKUT case-parent triads and 10 families with multiple affected members were recruited, comprising the largest CAKUT cohort world-wide. Mutation analysis of two novel CAKUT candidate genes was performed and revealed interesting genetic variants that were functionally tested *in vitro*. Genome-wide exome sequencing in CAKUT families identified variants possibly involved in CAKUT aetiology. In addition, linkage analysis of a large CAKUT family demonstrated suggestive linkage for loci on chromosomes 1, 2, 4, 7, and 11. The present identification of new genetic factors for CAKUT contributes to the understanding of the pathogenesis and the design of genetic diagnostic screening tests, facilitating early detection and recurrence risk estimations for CAKUT.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC386

**Rapid Genetic Diagnosis of Consanguineous Families with Histological Findings of FSGS by Homozygosity Mapping Coupled with Whole Exome Capture and Massively Parallel Sequencing** Khaldoun Al-Romaih,[1,2] Giulio Genovese,[1] Richard P. Lifton,[3] Martin R. Pollak.[1] *[1]Nephrology, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA; [2]Research Centre, King Faisal Specialist Hospital, Riyadh, Saudi Arabia; [3]Yale University School of Medicine, Yale University, CT.*

Focal segmental glomerulosclerosis is a histological glomerular phenotype that can be familial, primary, or secondary to a multitude of pathological processes including such tubulointerstitial diseases as nephronophthisis. Mutations in a number of distinct

nephronophthisis genes (NPHPs) have been described to date. We describe consanguineous unrelated Saudi Arabian families with NPHP1 deletion. Affected individuals in the two families presented with renal failure and clinical and histological features consistent with focal segmental glomerulosclerosis. Since FSGS patients may present atypical radiological findings, making the clinical diagnosis of the genetic syndrome difficult, we applied whole-genome single-nucleotide polymorphism analysis followed by state of the art sequence capture and exome sequencing on genomic DNA samples from these families. This analysis facilitated accurate diagnosis after isolation of homozygosity run of ~ 2 Mb. This homozygous run falls between rs6754115 (NCBI 36 genomic position 109,328,776) and rs17464100 (NCBI 36 genomic position 111,284,252), and is identical in affected subjects from the unrelated families. This provides evidence that this deletion is widely spread in the families' geographical regions, and implies its significant involvement in the development of chronic kidney disease in Saudi Arabia.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC387

**Genome-Wide Association Study (GWAS) for Albuminuria in Individuals of African and European Descent with and without Diabetes** Carsten A. Böger,[1] Ming-Huei Chen,[2] Adrienne Tin,[3] Matthias Olden,[1] Anna Kottgen,[3] Ian H. de Boer,[4] Conall M. O'Seaghdha,[5] Ching-Ti Liu,[2] L. Adrienne Cupples,[6] Jacques S. Beckmann,[7] Iris M. Heid,[1] Rainer Rettig,[8] Albert W. Dreisbach,[9] Murielle Bochud,[7] Caroline S. Fox,[6] Wen Hong Linda Kao.[3,10,11] *[1]Regensburg University Medical Center, Germany; [2]Boston University; [3]Johns Hopkins University; [4]University of Washington; [5]Brigham and Women's Hospital; [6]NHLBI's Framingham Heart Study; [7]Centre Hospitalier Vaudois, Switzerland; [8]Greifswald University, Germany; [9]Mississippi University; [10]For the CKDGen Consortium; [11]For the CARe Consortium.*

Background: Albuminuria is an independent predictor of chronic kidney disease progression and cardiovascular morbidity and mortality in the general population, but little is known about the influence of common genetic variants on albuminuria.

Methods: We performed a meta-analysis of data from 63,153 individuals of European ancestry with genotype data from GWAS (CKDGen consortium) and from a large candidate gene study (CARe consortium) to identify susceptibility loci for the quantitative trait urinary albumin-to-creatinine ratio (UACR) and the dichotomous trait microalbuminuria (MA). Findings were verified in 6,981 African Americans (CARe Consortium).

Results: A SNP in the CUBN gene (minor allele frequency=0.1) was associated with UACR (p=1.1*10-11) and MA (odds ratio=1.06, p=0.001). CUBN encodes cubilin, which is an integral protein of the complex responsible for receptor-mediated endocytotic albumin reabsorption in the kidney's proximal tubule. Similar associations were observed in CARe African Americans between the SNP's minor allele (frequency=0.03) and UACR (p=0.005) as well as MA (odds ratio=1.4, p=0.008). The associations were independent of diabetes or hypertension.

Conclusion: We identified a SNP in CUBN that is associated with albuminuria across different populations and irrespective of diabetes or hypertension status, thus highlighting the potential role of shared genetic susceptibility for albuminuria in diverse clinical settings.

Disclosure of Financial Relationships: Honoraria: Fresenius Medical Care Deutschland GmbH, Novartis Deutschland.

## SA-FC388

**Association of T2D Genetic Variants with Kidney Function: The Strong Heart Family Study** Nora Franceschini,[1] V. Saroja Voruganti,[2] Sandra L. Laston,[2] Karin Haack,[2] Jean W. Maccluer,[2] Barbara V. Howard,[3] Shelley A. Cole,[2] Kari North,[1] Jason G. Umans.[3] *[1]University of North Carolina, Chapel Hill, NC; [2]Southwest Foundation for Biomedical Research, San Antonio, TX; [3]MedStar Health Research Institute, Hyattsville, MD.*

Type 2 diabetes (T2D) is highly prevalent and is the major cause of progressive chronic kidney disease (CKD) in American Indians. Recent genome wide association (GWA) studies have identified several loci associated with T2D but their impact on susceptibility to T2D complications is largely unknown. We studied the association of 18 recently GWA-T2D identified single nucleotide polymorphisms (SNPs) in 16 loci with kidney function (estimated glomerular filtration rate: eGFR, MDRD) and urine albumin to creatinine ratio (ACR) in 3807 American Indian participants of the Strong Heart Family Study. Center-specific residuals of eGFR and of log ACR were obtained from linear regression models adjusted for age and sex. The residuals were regressed onto SNP dosage using variance component models to account for family relatedness and population history. Summary estimates across centers were combined using a weighted average of point estimates meta-analyses (alpha=0.002 for Bonferroni correction). T2D prevalence varied from 14 to 33% among centers. A SNP in the *WFS1* gene (rs10010131, minor allele frequency 7 to 20%), previously shown to associate with T2D in American Indians (p=0.01), was associated with eGFR (p=0.0005) and ACR (p=0.009). Each copy of the G allele (which confers increased risk of T2D in Caucasians) was associated with both increased eGFR (beta=0.13, se=0.04) and ACR (beta=0.09, se=0.04) residuals. Interestingly, the G allele was also associated with increased insulin levels among non-T2D American Indians (p<0.0001). These findings suggest that *WFS1* may be related to increased eGFR (hyperfiltration) and albuminuria in populations with high susceptibility to T2D.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC389

**Evaluation of SORCS1 as a Candidate Gene for Kidney Failure** Jozef Lazar,[1,2] Haiyan Xu,[2] Caitlin C. O'Meara,[3] Zelmira Lazarova,[1] Carol Patricia Moreno Quinn,[2,3] Howard J. Jacob.[2,3] *[1]Dermatology, Medical College of Wisconsin; [2]Human and Molecular Genetics Center, Medical College of Wisconsin; [3]Physiology, Medical College of Wisconsin, Milwaukee, WI.*

In 2010, Paterson et al. published that SORCS1 is a potential candidate gene for renal disease in humans. This linkage has confirmed our rat work. We have been investigating a quantitative trait locus, renal failure 1 (*Rf-1*) in a hypertension-associated renal disease model. Over the years, we have generated a series of congenics and sub-congenic animals in attempt to clone the gene by position. SORCS1 is one of the genes in our minimal congenic region. These data combined with Paterson's work warranted a more detailed analysis of this gene.

SORCS1 is large gene (26 exons spanning over 500Kb) and member of the type-I transmembrane receptors family containing a Vps10p-domain. To investigate its potential role, we used a cellular assay, sequence analysis and gene knock-out. The porcine proximal tubular cell line LLC-PK1 combined with shRNA against SORCS1 demonstrated that the reuptake of gold-labeled albumin was significantly reduced, demonstrating a potential role for this gene. We next sequenced the entire genomic region of SORCS1 utilizing 454 pyrosequencing technology. The results showed the presence of sequence variants in coding regions with potential functional impact. To further validate the role of this gene, we generated a SORCS1 knocked out rats. The gene was KO'ed using the zinc finger nuclease strategy previously published by our group. In conclusions, our results strongly indicate that SORCS1 plays a role in protein trafficking in kidney.

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Disclosure of Financial Relationships: nothing to disclose

## SA-FC390

**Non-Diabetic End-Stage Renal Disease (ESRD) in African Americans: Candidate Genes from Analysis of a Pooled DNA-Based Genome Wide Association Study (GWAS)** Meredith A. Bostrom,[1,4] Lingyi Lu,[2] Jeff W. Chou,[2] Pamela J. Hicks,[1] Jianzhao Xu,[4] Carl D. Langefeld,[2] Donald W. Bowden,[1,4] Barry I. Freedman.[3] *[1]Biochemistry, Wake Forest University School of Medicine; [2]Biostatistical Sciences, Wake Forest University School of Medicine; [3]Internal Medicine, Wake Forest University School of Medicine; [4]Centers for Human Genomics and Diabetes Research, Wake Forest University School of Medicine, Winston-Salem, NC.*

African Americans have increased susceptibility to non-diabetic (hypertension-attributed) ESRD compared to Caucasians and extensive evidence supports a genetic contribution. We performed a GWAS on 1000 African Americans, 500 cases with non-diabetic ESRD and 500 non-nephropathy controls, to identify genes associated with non-diabetic ESRD in African Americans. DNA was quantified using gel electrophoresis and spectrophotometric analysis. Samples were pooled to create 10 case pools and 10 control pools (50 samples/pool). Pools were genotyped in duplicate on the Illumina HumanHap550-Duo BeadChip. 1420 top scoring SNPs from the GWAS were genotyped individually on 962 African American non-DM ESRD cases and 947 non-nephropathy controls on the Illumina 1536 GoldenGate custom chip at the Center for Inherited Disease Research. SNPs were tested for association with non-DM ESRD with covariate adjustment for age, gender, logBMI, and percentage of African ancestry as determined by 70 admixture informative markers. The 5 most associated SNPs were located in the *MYH9* gene region on chromosome 22, previously associated with non-DM ESRD (p=5.91E-24 to 7.98E-5, odds ratio (OR) 0.33-0.65, various models). Other top SNPs included rs379489 in the *CFH* gene (p=2.05E-4, OR=0.73, confidence interval (CI) 0.62-0.86; additive) and rs12487085 located in an intergenic region on chromosome 3 (p=5.48E-4, OR=1.29, CI 1.12-1.50; additive). A case-only interaction analysis for *MYH9* risk status revealed association at rs9687184 on chromosome 5 in an intergenic region, (p=9.14E-4, OR=0.59, CI 0.43-0.80, additive). The first GWAS for non-DM ESRD in African Americans replicated association in the *MYH9* gene region and identified additional candidate loci for follow-up studies.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC391

**Identification of Soluble Urokinase Receptor as Circulating FSGS Factor** Changli Wei,[1] Shafic El Hindi,[1] Jing Li,[1] George William Burke,[1] Alessia Fornoni,[1] S. Ananth Karumanchi,[2] Hui Kim Yap,[3] Moin Saleem,[4] Pirouz M. Daftarian,[1] Phillip Ruiz,[1] Jochen Reiser.[1] *[1]University of Miami; [2]Beth Israel Deaconess Medical Center; [3]National University of Singapore, Singapore; [4]University of Bristol, United Kingdom.*

The identification of circulating FSGS factor(s) has haunted nephrologists for decades with limited success. Here we report the identification of a FSGS circulating factor as soluble urokinase receptor (suPAR), which is increased in the blood of FSGS patients and particularly high in transplant recurrent FSGS patients. Comparatively, suPAR is not elevated in the blood of healthy subjects or in patients with minimal change disease or preeclampsia. Biochemically, suPAR binds integrin β3 via a moiety on domain 2. This binding is sufficient to activate integrin β3, which then drives podocyte disease. We found increased integrin β3 activity in podocytes from failing kidney allograft due to recurrent FSGS. While suPAR-rich FSGS serum activates integrin β3 in human podocytes, co-incubation with uPAR antibody or cycloRGDfV reduces integrin activity. To define whether suPAR is cause or effect in FSGS, we set up three murine models. First,

we injected recombinant suPAR into uPAR knockout mice which led to dose-dependent suPAR deposits, β3 integrin activation and proteinuria. Second, we released large amounts of suPAR in wt mice that received one uPAR null kidney as a transplant (hybrid mouse). Interestingly, suPAR was deposited into uPAR null kidneys and caused foot process (FP) effacement showing that endogenous suPAR can cause podocyte injury. Third, we achieved overexpression of suPAR in the blood of mice by gene delivery. These mice had sustained elevation of serum and urine suPAR levels. Elevated wt suPAR but not the β3 integrin-binding mutant caused FP effacement and proteinuria. Moreover, the morphological changes after 4- 6 weeks are consistent with a progressive glomerulopathy reminiscent of FSGS. Both, proteinuria and histological glomerular changes could be ameliorated by co-administration of antibodies blocking suPAR. In conclusion, our studies identify suPAR as circulating proteinuria/FSGS factor.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC392

**Podocyte-Specific Deletion of NDST1, a Key Enzyme in the Sulfation of Heparan Sulfate Glycosaminoglycans, Leads to Abnormalities in Podocyte Organization, Adhesion, and Migration** Terrel D. Sugar,[2] Deborah J. McCarthy,[1] Jeffrey D. Esko,[3] Lawrence B. Holzman,[4] Kevin J. McCarthy.[1] *[1]Pathology, LSU Health Sciences Center, Shreveport, LA; [2]Cell Biology and Anatomy, LSU Health Sciences Center, Shreveport, LA; [3]Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA; [4]Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA.*

Previous work in our laboratory investigated alterations in the ability of heparan sulfate (HS) deficient podocytes to interact with pericellular matrices *in vivo* and *in vitro*. HS- podocytes develop foot process effacement *in vivo* and show compromised adhesion and migration *in vitro*. The present study reports the development of a novel mutant mouse (2.5P-Cre/NDST1$^{fl/fl}$) and immortalized cell lines (Immortomouse™/NDST1$^{fl/fl}$) in which HS glycosaminoglycan (GAG) assembly is preserved but sulfation of the HS glycosaminoglycan chains is greatly diminished via podocyte-specific deletion of NDST1 (N-deacetylase-N-sulfotransferase), a critical enzyme responsible for HS GAG sulfation . Both PCR for NDST1 and immunohistochemistry of tissue sections with a monoclonal antibody (10E4), which recognizes N-sulfated epitopes present on HS GAG chains, was used to confirm deletion of the *NDST1* gene. Comparison of H&E stained tissue sections from wild type and mutant animals showed no major glomerular changes but in older 2.5P-Cre/NDST1$^{fl/fl}$ cystic changes were observed in some of the renal tubules. Electron microscopy studies of thin section from mutant animals showed podocyte foot process effacement and the presence of autophagic vacuoles in podocytes. Deletion of the *NDST1* gene in immortalized podocytes having the NDST1$^{fl/fl}$ genotype was done using adenoviral delivery of Cre recombinase. The ability of the NDST1-null podocytes to attach, spread and migrate on fibronectin was significantly less ($p<0.01$) than NDST1+ podocytes. The data from both models are indicative that sulfation of the HS GAG chains is a key element in mediating normal podocyte behavior both *in vivo* and *in vitro*, further highlighting the importance of cell surface HS in mediating podocyte cell behavior.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC393

**Novel Therapeutics in Nephrotic Syndrome: Sialic Acid Precursor ManNAC Improves Sialylation of Angiopoietin-Like 4 (Angptl4) in Podocytes and Reduces Selective Proteinuria in Minimal Change Disease (MCD)** Lionel C. Clement,[1] Maria Carmen Avila-Casado,[2] Camille E. Mace,[1] Elizabeth Soria-Castro,[2] Sumant S. Chugh.[1] *[1]Medicine, University of Alabama at Birmingham, Birmingham, AL; [2]Pathology, Instituto Nacional de Cardiologia, Mexico City, Mexico.*

Studies from our lab show that upregulation of Angptl4, a secreted glycoprotein, in podocytes is a key event in the pathogenesis of experimental and human MCD (Clement *et al*, Nature Medicine, in revision). Angptl4 is upregulated up to 70 fold in vivo in podocytes in experimental MCD (PAN) and in biopsies from patients with glucocorticoid sensitive MCD. To study the consequences of this upregulation, podocyte specific NPHS2-Angptl4 transgenic rats were developed. These rats mimic all aspects of human MCD i.e. develop diffuse foot process effacement, selective proteinuria with over 500 fold increase in albuminuria, loss of Glomerular Basement Membrane (GBM) charge (assessed by alcian blue and PEI staining), and demonstrate clustering of podocyte secreted Angptl4 in the GBM. Proteomic analysis of glomerular Angptl4 in wild type rats using 2D gel electrophoresis revealed 2 distinct clusters of Angptl4 oligomers migrating either at neutral (major) or high isoelectric point (minor). By contrast, NPHS2-Angptl4 TG rats and rats with PAN show a reversal in this pattern. Using sialic acid binding lectins (MAA and SNAI), we showed that high isoelectric point Angptl4 lack normal sialylation present in neutral isoelectric point Angptl4 oligomers. Treatment of a stable cell line that secretes recombinant Angptl4 with a sialic acid precursor ManNAc (N-acetyl mannosamine) results in normalization of Angptl4 sialylation. Feeding NPHS2-Angptl4 TG rats ManNAc in tap water over a 12 day period resulted in 41% reduction in albuminuria from baseline, and increased sialylation of glomerular Angptl4. ManNAc, therefore, represents a novel non-immunomodulatory therapeutic option for MCD and other forms of nephrotic syndrome.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC394

**Divergent Functions of Rho GTPases in the Podocyte: Deletion of Cdc42 Results in Podocyte Failure, but Loss of Rac1 Protects Against Podocyte Injury** Jeffrey B. Hodgin,[1] Simone M. Blattner,[2] Courtenay M. Vining,[2] Abdul A. Soofi,[1] Matthias Kretzler.[2] *[1]Pathology, University of Michigan; [2]Nephrology, University of Michigan, Ann Arbor, MI.*

Injury to the podocyte results in characteristic morphological cellular changes, known as foot process effacement, and is closely correlated with the development of proteinuria. The changes in podocyte shape are dependent on rearrangement of the actin cytoskeleton and the Rho GTPases Cdc42 and Rac1 are best known for their roles in cytoskeleton assembly and organization. We hypothesize that Cdc42 and Rac1 are important for podocyte architecture and maintenance of the glomerular filtration barrier. Using NPHS2-Cre expression, floxed Cdc42 and Rac1 were conditionally deleted in mouse podocytes. Both podocyte-specific Cdc42 and Rac1 knockout mice (pod-Cdc42-/- and pod-Rac1-/- respectively) were born healthy and at expected Mendelian ratios. Pod-Cdc42-/- mice died within the first 5 weeks of life due to massive proteinuria and renal failure. By 4 weeks of age, pod-Cdc42-/- mice demonstrated extensive focal and global glomerulosclerosis accompanied by severe tubulointerstitial lesions and proteinaceous casts. Transmission and scanning electron microscopy revealed near total effacement of podocyte foot processes. Pod-Cdc42+/- mice (heterozygotes) showed no phenotype up to 16 months of age. In contrast to pod-Cdc42-/- mice, pod-Rac1-/- mice demonstrated no proteinuria or morphologic alterations. However, when challenged with protamine sulfate perfusion, pod-Rac1-/- kidneys were protected from foot process effacement, whereas control mice showed a 20% reduction of filtration slit frequency and broadening of podocyte foot processes. Our findings reveal podocyte-specific Cdc42 to be indispensible to podocyte architecture and maintenance of the glomerular filtration barrier. In contrast, Rac1 does not play a critical role in the establishment and homeostasis of podocyte shape and function, but appears to be required for podocyte response to injury, thus highlighting inhibition of Rac1 as a potential therapeutic target.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC395

**Central Role for TRPC6 in a Calcineurin/NFAT Feed-Forward Signaling Pathway Involved in Podocyte Injury and Proteinuria** Tom Nijenhuis,[1,2] Alexis J. Sloan,[3] Joost G. Hoenderop,[2] Harry Van Goor,[4] Jan Flesche,[3] Marinka Bakker,[1] Rene J. Bindels,[2] Rudolf Allert De Boer,[5] Gerjan Navis,[6] Jack F. Wetzels,[1] Jo H. M. Berden,[1] Jochen Reiser,[3] Christian Faul,[3] Johan Van der Vlag.[1] *[1]Nephrology, Radboud University Nijmegen Medical Centre (RUNMC), Netherlands; [2]Physiology, RUNMC, Netherlands; [3]Nephrology and Hypertension, Miami, FL; [4]Pathology, UMCG; [5]Cardiology, UMCG; [6]Nephrology, UMCG, Netherlands.*

TRPC6 is a slit diaphragm-associated ion channel expressed by podocytes. TRPC6 gain-of-function mutations and overexpression result in proteinuria. Angiotensin II (AngII) enhances TRPC6 expression in vitro as well as in vivo in glomerular injury models. We hypothesize that activation of the $Ca^{2+}$-dependent calcineurin/NFAT pathway is the intracellular route involved in TRPC6-mediated glomerular pathology.

AngII significantly enhanced NFAT activation and TRPC6 expression in cultured podocytes. PAN and adriamycin-induced TRPC6 expression was also AngII-dependent. Co-incubation with the calcineurin inhibitor ciclosporin A (CsA) prevented AngII and adriamycin-induced TRPC6 transcription. Calcineurin inhibition also blocked PAN-induced NFAT activation. $Ca^{2+}$ influx blockade by $LaCl_3$ or the TRP-channel blocker 2-APB inhibited AngII-induced TRPC6 transcription. Importantly, knockdown of TRPC6 expression by TRPC6 siRNA significantly reduced AngII-induced NFAT activation. This suggested that $Ca^{2+}$ influx through TRPC6 itself activates NFAT, which could result in a feed-forward signal constitutively activating the pathway promoting TRPC6 transcription and podocyte injury. We constructed a novel transgenic mouse model with podocyte-specific inducible expression of a constitutively active NFAT mutant, which indeed induced TRPC6 expression and proteinuria, showing that NFAT activation is sufficient to provoke these effects. Accordingly, in vivo interruption of this pathway by CsA treatment reduced adriamycin-induced and AngII-mediated glomerular TRPC6 expression and proteinuria.

In conclusion, our data show that AngII activates a TRPC6-dependent calcineurin/NFAT feed-forward signaling pathway that enhances TRPC6 expression and results in podocyte injury and proteinuria.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC396

**Podocyte-Specific Deletion of MYH9 Predisposes Mice to Nephropathy** Duncan B. Johnstone, Jidong Zhang, Britta George, Lawrence B. Holzman. *Dept of Medicine; Renal, Electrolyte and Hypertension Division, U of Pennsylvania, Philadelphia, PA.*

MYH9 mutations result in rare but severe CKD due to Epstein's/Fetchner's syndromes, and MYH9 non-coding polymorphisms or "risk alleles" correlate with common causes of CKD among African Americans and Hispanics, including non-diabetic CKD, FSGS and HIVAN. However, it remains unclear how these common MYH9 "risk alleles" result in altered MYH9 expression or regulation as, despite extensive sequencing, causative mutations remain elusive. Moreover, the mechanism by which both common MYH9 "risk alleles" and rare MYH9 mutations result in CKD remains unclear.

We hypothesize that rare MYH9 mutations and common MYH9 "risk alleles" result in CKD, including FSGS and HIVAN, by disrupting cytoskeletal dynamics in podocytes. To investigate the role of MYH9 in kidney disease we selectively deleted MYH9 in podocytes in C57BL/6 mice (Podocin::Cre/+; MYH9f/f). Surprisingly, we found that PodΔMYH9 mice survive in expected Mendelian ratios and do not develop overt CKD or proteinuria when compared to f/f control littermates, even in mice aged to 9 months (uP/C 0.13+/-0.1 PodΔMYH9; 0.24+/-0.2 control). Deletion of MYH9 was confirmed by PCR, and by loss of MYH9 staining only in podocytes in kidney sections of PodΔMYH9 mice using antibodies that we generated and purified. The absence of CKD was not due to MYH10 redundancy, which we find is not expressed in podocytes of either wild type or PodΔMYH9 mice. Thus, on the C57BL/6 background, PodΔMYH9 is not sufficient to cause kidney disease, but we hypothesized it might predispose mice to an additional provocation, which we tested using the Adriamycin nephropathy model. We found significantly more proteinuria in PodΔMYH9 mice from weeks 2 through 6 (wk6 uP/C: 26.5+/-27 in PodΔMYH9; 2.5+/-1.4 in controls; 0.2+/-.1 in PodΔMYH9 saline-injected). On histologic evaluation we found, only in Adriamycin-injected PodΔMYH9 mice, severe glomerulosclerosis by H+E and PAS, and severe FP effacement by SEM and TEM. Thus, MYH9 mutations may not be sufficient to cause CKD but may predispose mice, and possibly humans, to kidney disease by sensitizing podocytes to additional genetic or environmental provocation.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC397

**Targeting Activated Gα12 to Podocytes Leads to Proteinuria, Glomerulosclerosis (GS) and Changes in the Extracellular Matrix (ECM)** Ilene Boucher,[1,2] Sarah Beaudry,[1,2] Wanfeng Yu,[1,2] Bradley M. Denker.[1,2] *[1]Medicine, Renal Division, Brigham and Women's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA.*

Podocytes are specialized epithelial cells important in maintaining the glomerular filtration barrier. Due to their location, podocytes are uniquely susceptible to injury in numerous kidney diseases. Many pathways regulating podocyte function are mediated by G protein-coupled-receptors (GPCR). Gα12 couples to GPCRs important for vascular regulation (angiotensin II), inflammatory mediators (LPA, thrombin), and the actin cytoskeleton. Gα12 is upregulated in minimal change disease and is localized to the major podocyte processes and at the branch points proximal to the foot processes. We hypothesized that Gα12 regulates podocyte barrier function and the actin cytoskeleton and when activated could lead to increased permeability and podocyte injury. Transgenic mice were established with EE-tagged, constitutively active human Gα12 (QLα12) inserted after a floxed LacZstop. After crossing with podocin-Cre mice, podocyte expression of QLα12 was confirmed by confocal microscopy with EE immunofluorescence. QLα12$^{LacZ+/Cre+}$ mice developed proteinuria at 6m which increased with age. By 12-14m, GS was noted in some glomeruli and electron microscopy revealed focal thickened basement membranes and foot process fusion. Podocyte number was not altered in the QLα12$^{LacZ+/Cre+}$ mice compared to controls and increased apoptosis was not detected. Changes in podocyte and ECM gene expression were monitored by real-time PCR. No significant changes in nephrin, podocin, or CD2AP gene expression were found in 12-18m mice (n=7). However, nephrin was downregulated by 60% in QLα12$^{LacZ+/Cre+}$ mice at 6m (n=6). Analysis of ECM genes at 12-18m (n=6) revealed increased laminin α5 (3 fold), increased collagen IVα1 and α2 (2-4 fold), with no change in α3 and α4 chains, and decreased α5. Taken together, these findings indicate that activation of Gα12 in podocytes leads to proteinuria and changes characteristic of GS by affecting expression of slit diaphragm proteins and ECM composition. Gα12 may be a novel therapeutic target to delay or prevent progressive GS.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC398

**Podocyte-Specific Knockout of mTOR Results in Nephrotic-Range Proteinuria and Severe Glomerular Damage** Davide Pietro Cina,[1] Tuncer Onay,[1] Susan E. Quaggin.[1,2] *[1]Samuel Lunenfeld Res. Inst., University of Toronto, Toronto, Canada; [2]St. Michael's Hospital, Toronto, Canada.*

mTOR inhibitors (rapamycin and sirolimus) have been approved by the FDA for the treatment of renal transplants, renal cell carcinoma and are currently under investigation for the treatment of polycystic kidney disease. mTor is a serine-threonine kinase that controls cell growth, proliferation, and immune function. Despite the great promise for these drugs, glomerular toxicity including proteinuria have limited their use, especially in the transplant setting. We hypothesize that inhibition of mTor signaling in the podocyte is responsible for the observed renal toxicities. In vitro studies have suggested that mTOR may regulate slit diaphragm proteins and factors such as VEGF, but in vivo models have been lacking. To elucidate the mechanism underlying proteinuria and structural glomerular changes associated with mTOR inhibition in humans, we generated a mouse model with a podocyte-specific deletion of the mTor gene. mTor floxed mice were generated using a BAC recombineering approach to insert loxP sites around the first 3 exons of the mTor gene. The floxed mTOR allele was validated by global deletion using a pCaggs Cre deletor mouse strain. This resulted in embryonic death, identical to the standard KO mice. To generate a podocyte specific mTOR knock out, the floxed mTOR mouse was bred to podocin-Cre or pod-rtTA/TetOCre recombinase mice. Mice of all genotypes were born at the expected Mendelian frequency and appeared normal with no proteinuria up until 3 weeks of age. At 4 weeks, the mutant mice had growth restriction, and had developed nephrotic range proteinuria. Light microscopy of kidneys from 5 week-old mutants showed extensive glomerular lesions with Bowman capsular dilation, mesangial proliferation and tubular protein casts consistent with end stage renal failure. Deletion of mTor from mature podocytes also resulted in proteinuria and glomerular injury. Taken together, we have shown that disruption of mTOR signaling within the podocyte results in proteinuria and glomerular damage during development and in adulthood providing a model to dissect the glomerular toxicity observed in patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC399

**Robo2 Regulates Podocyte Actin Polymerization and Glomerular Permeability** Xueping Fan,[1] Qing-Gang Li,[1] Anna Pisarek-Horowitz,[1] Xiangling Wang,[1] Ramon G. Bonegio,[1] Hang Wang,[1] Margaret M. Mclaughlin,[2] Dennis Brown,[2] David J. Salant,[1] Weining Lu.[1] *[1]Renal Section, Department of Medicine, Boston University Medical Center, Boston, MA; [2]Nephrology Division, Massachusetts General Hospital, Boston, MA.*

Robo2 is the cell surface receptor for the repulsive guidance cue Slit and is involved in axon guidance and neuronal migration. It is also required for normal kidney induction and restricts outgrowth of the ureteric bud from the Wolffian duct to a single site. Disruptions of *ROBO2* are associated with congenital anomalies of the kidney and urinary tract (CAKUT) and vesicoureteral reflux (VUR) in humans and mice. Mouse mutants without either *Slit2* or *Robo2* develop supernumerary ureteric buds, abnormal ureterovesical junctions, and die after birth from severe hydronephrosis and kidney dysplasia. In addition to its role in the developing urinary tract, we have found that Robo2 is also expressed on the basal side of podocytes adjacent to the slit-diaphragm and co-localizes with nephrin and podocin. Yeast two-hybrid and protein co-precipitation assays revealed that Robo2 interacts directly with Nck SH3 domains and forms a complex with nephrin *in vivo* and *in vitro*. By analyzing the formation of F-actin tails in cells, we found that Slit-Robo2 signaling down-regulates actin polymerization induced by nephrin. Furthermore, podocyte-specific *Robo2* knockout mice develop significant albuminuria that is associated with podocyte foot-process effacement. These results suggest that Robo2 forms part of a signaling complex that regulates podocyte F-actin cytoskeleton and influences it structure and function. Our study thus identifies a novel level of regulation of Slit-Robo signaling to counterbalance nephrin-induced actin polymerization and maintain normal kidney glomerular permeability.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC400

**Rhophilin-1 Is a Podocyte-Specific Protein Required for Glomerular Filtration** Mark Lal, Karl Tryggvason. *Medical Biochemistry and Biophysics, Karolinska Insitutet, Sweden.*

We recently identified *Rhpn1* as a novel, highly expressed gene of the mouse glomerulus of the kidney. Here, we describe the generation of *Rhophilin-1* knockout mice and their resultant phenotype. Immunohistochemical analysis indicates that Rhophilin-1 is uniquely expressed in the podocytes of both mouse and human glomeruli. Kidney sections as well as protein extracts derived from isolated *Rhpn1*$^{-/-}$ glomeruli reveal that protein expression of the targeted gene was completely ablated in knockout mice. *Rhpn1* knockout mice were phenotypically normal at birth but developed progressive albuminuria from about two weeks of age into adulthood. Histological examination of kidneys from severely albuminuric mice revealed tubular protein casts and FSGS-like lesions as well as glomerular hypertrophy. Widespread podocyte foot process effacement as well as thickening of the glomerular basement membrane were readily detected upon electron microscopy. Immunochemical analysis of a host of podocyte-marker proteins indicated that glomerular dysfunction in *Rhpn1*$^{-/-}$ was not likely a result of any overt alterations in the pattern of their expression. In addition to yeast-2-hybrid identification of Rhophilin-1 interacting proteins, we have implemented a next-generation sequencing platform known as shotgun sequencing by hybridization to analyze the transcriptome profile of single glomeruli isolated from wild-type and knockout mouse kidneys. The development of this novel methodology has allowed us, for the first time, to describe glomerular gene expression at an individual glomerulus level. In summary *Rhpn1* is a glomerular gene necessary for maintaining proper kidney function in the mouse.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC401

**Abnormal O-Glycosylation of Serum IgA1 Early in the Disease Is Associated with Risk of Progression in IgA Nephropathy** Alice C. Smith,[1,2] Javeria Peracha,[1] Karen Molyneux,[1,2] Joanna Boyd,[2] John Feehally,[1,2] Jonathan Barratt.[1,2] *[1]Infection, Immunity & Inflammation, University of Leicester, Leicester, United Kingdom; [2]John Walls Renal Unit, Leicester General Hospital, Leicester, United Kingdom.*

IgA nephropathy (IgAN) is progressive in some but not all patients; as yet, there are no prognostic indicators specific to IgAN. Known abnormalities of serum IgA include raised IgA1 levels, altered IgA1 O-glycosylation, and increased usage of lambda light chain. However, it is not known whether occurrence of any of these abnormalities at an early stage of IgAN can predict long term outcome.

We studied sera from 76 patients with biopsy-proven IgAN obtained early in the course of their disease while renal function was normal, and 74 matched controls. The patients were classified as Progressors (serum creatinine increased by >100% during follow up), or Non-Progressors (serum creatinine remained normal after a minimum ten years follow up). We measured serum IgA1 and IgA1 kappa:lambda ratio by ELISA, and used Helix aspersa (HA) lectin binding assays to quantify IgA1 O-glycosylation. Raised HA binding to IgA1 indicates altered glycosylation.

Serum IgA1 and IgA1-HA binding were significantly higher in IgAN than controls, but we found no difference in kappa:lambda ratio. Of the 76 patients studied, 36 (47%) were Progressors and 40 were Non-Progressors. Neither serum IgA1 level nor kappa:lambda ratio correlated with outcome in IgAN. However, while the IgA1-HA binding of the Non-Progressors did not differ from controls (Non-Progressors 251.51AU, control 211.04AU, p=NS), the Progressors had significantly higher IgA1-HA binding than both Non-Progressors and controls (Progressors 312.09AU, p<0.005 vs Non-Progressors, p<0.0005 vs controls).

These results demonstrate a clear association between the presence of aberrantly O-glycosylated IgA1 in the serum at an early stage of IgAN and subsequent development of progressive renal disease. The findings lend further support to the importance of altered IgA1 O-glycosylation in the pathogenesis of IgAN, and suggest that it may be considered as a diagnostic marker and a possible therapeutic target.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC402

### Predictors of Progression in Idiopathic Membranous Nephropathy: A Comparison of Urinary $\beta_2$-Microglobulin and the Proteinuria Risk Score

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Outcome is variable in idiopathic membranous nephropathy (iMN). Accurate prediction of outcome allows optimized treatment for individual patients. We showed that urinary $\beta_2$-microglobulin ($U\beta_2m$) predicts progression in iMN. An alternative risk score based on the 6 month period of maximal proteinuria and creatinine clearance ($eC_{creatinine}$) is often used. We compared both predictors.

We included 102 patients with biopsy proven iMN, serum creatinine levels < 1.5mg/dl and nephrotic syndrome. We calculated risk scores using the first six months of follow up and the period of maximum proteinuria during the first two years of follow up. $U\beta_2m$ excretion was obtained by standardized measurement within three years of biopsy.

42% showed progression, defined as 50% rise in serum creatinine or a 25% rise and absolute level over 1.5 mg/dl. 25% and 15% went into spontaneous complete or partial remission respectively. When predicting progression, risk scores calculated in the first six months were comparable to scores during follow up, ROC-$AUC_{6\ months}$=0.788 (95% CI: 0.700-0.877) versus ROC-$AUC_{follow\ up}$=0.742 (0.642-0.841). $U\beta_2m$ yielded similar accuracy, ROC-AUC=0.798 (0.704-0.892). $\beta_2m$ was slightly more accurate in the prediction of spontaneous remission, ROC-$AUC_{U\beta2m}$=0.770 (0.632 – 0.908) versus ROC-$AUC_{6\ months}$=0.730 (0.589 – 0.871) for partial and ROC-AUC=0.704 (0.588 – 0.820) versus 0.611 (0.485 – 0.737) for complete remission. Adding $\beta_2m$ to the risk score did not lead to better prediction of progression, ROC-AUC=0.853 (0.776-0.930). A parsimonious model with change in $eC_{creatinine}$ during the first six months of follow up and $Ub_2m$ gave comparable results, ROC-AUC=0.833 (0.753 – 0.915).

Risk score calculated the first six months of follow up appears to be a sufficient predictor of prognosis. Evaluation during longer follow-up is no longer needed. Intensified ACEi/ARB use during follow up may explain these results. Risk scores and $U\beta_2m$ give similar accuracy when predicting progression. $Ub_2m$ seems slightly more accurate for spontaneous remission of proteinuria. A compound score including $U\beta_2m$ does not lead to better prediction of progression.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC403

### Influence of a Functional Polymorphism of Aldosterone Synthase Gene on Focal Segmental Glomerulosclerosis: A Clinicopathologic Study

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The C-344T polymorphism of the aldosterone synthase gene was associated with serum aldosterone levels and the development of arterial hypertension. In the present study we evaluated its influence on focal segmental glomerulosclerosis (FSGS).

Eighty-one patients with biopsy-proven primary FSGS were followed up for 8.0±12 years. Patients were classified according to the slope of reciprocal serum creatinine (≥or < -0.1 $dl*mg^{-1}*year^{-1}$) into groups A (slow progressors, n=57) and B (fast progressors, n=24). One hundred volunteers were analysed as controls. C-344T polymorphism was determined by PCR. The biopsies of 40 patients were reviewed by the same pathologist. Serum aldosterone levels were determined by ELISA in 57 patients with chronic glomerulonephritis.

C-344T polymorphism influenced the serum aldosterone levels (CC/CT: 106.8±70.4, TT: 243.2±323 pg/ml, p<0.05). The allele frequencies differed significantly between patients (C-allele: 0.55, T-allele: 0.45) and controls (C-allele: 0.45, T-allele: 0.55, p<0.05). The percentage of sclerosed glomeruli tended to be lower in patients with the TT genotype (segmental 19.8±14 and global 9.7±19 vs. 25.4±22 and 17.2±21% in CC/CT, ns). The degree of tubulointerstitial fibrosis tended also to be lower in the TT genotype (14.8±15 vs 19.4±17%, ns). C-344T polymorphism was associated with the progression of FSGS: 36.4% of the CC/CT genotype carriers and 0% of the patients with the TT genotype belonged to group B (fast progressors, p<0.01). There was also a significant difference in the rate of progression between the CC/CT and TT genotypes: -0.216±0.449 vs. -0.030±0.041$dl*mg^{-1}*year^{-1}$ (p<0.05). C-allele carriers had a worse kidney survival in the Kaplan Meier analysis compared to the TT genotype (p<0.05).

The functional aldosterone synthase gene C-344T polymorphism not only acts as a risk factor for the development of FSGS, but may influence its pathologic appearance and could serve as a progression marker.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC404

### Long-Term Outcomes for Patients Receiving Multiple Treatments of Rituximab for Anti-Neutrophil Cytoplasmic Antibody Associated Vasculitis

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Introduction: Anti-neutrophil cytoplasm antibody associated vasculitis (AAV) is a life threatening autoimmune inflammatory disease. Current treatment with cyclophosphamide and prednisolone is associated with significant morbidity and mortality. Rituximab (anti-CD20) is a promising alternative treatment for patients with relapsing AAV and cyclophosphamide related organ damage. Given the relapsing nature of AAV long-term efficacy and safety data for rituximab is required following multiple treatment courses. This study retrospectively evaluated all rituximab used to treat AAV at University Hospital Birmingham between November 2002 and February 2009.

Methods: Standardised case-note based data collection.

Results: 34 patients received 66 treatment courses. All patients became B cell deplete following treatment. 31/34 patients achieved remission following primary rituximab therapy. 8/31 did not relapse (median follow up 35 (4-71) months); 23/31 had one or more relapse (median time to relapse 13 (5-45) months. 31/32 rituximab re-treatment courses achieved further disease remission. 7/31 had sustained remission (median follow up 14 (9-43) months); 19/31 had further relapses (median time to relapse 12 (4-30**)** months); 5/31 had elective treatment before relapse. In patients receiving >1 treatment course there was no difference between the length of the first (13 (5-45) months) and subsequent (11 (4-30) months) periods of remission. 13/26 patients received concomitant immunosuppressive therapy with mycophenolate mofetil, azathioprine or methotrexate during remission. Concomitant immunosuppression did not affect the time to relapse (13 (3-45) vs 14 (4-30) months).

6 patients had infections that required hospitalization and 5 patients died during follow-up, in line with published adverse event rates for similar groups of patients. No significant adverse effects were directly attributable to Rituximab.

Conclusion: Rituximab is a safe and effective alternative treatment in patients with relapsing AAV. Treatment efficacy was not lost with multiple treatment courses.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC405

### Two Year Follow-Up Results from a Randomised Trial of Rituximab Versus Cyclophosphamide for ANCA-Associated Renal Vasculitis: RITUXVAS

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Purpose: Cyclophosphamide (CYC) based induction regimens are standard therapy for ANCA-associated renal vasculitis (AAVr); however, associated mortality and adverse event rates are high and safer regimens are required. Rituximab (RTX) based regimens are a potential alternative to CYC induction.

Methods: We report the two year results of a randomised trial comparing a RTX based induction regimen with a standard intravenous CYC regimen for new AAVr. All patients had newly diagnosed AAVr and ANCA positivity. 44 patients were randomised; 33 to RTX 4x375$mg/m^2$ & 2x15mg/kg intravenous (IV) CYC; and 11 to IV CYC 6-10x15mg/kg. Both groups received the same IV and oral prednisolone regimen.

Results: At entry: median age was 68 years, Wegener's granulomatosis 50%, microscopic polyangiitis 50%; CRP 28; BVAS 18; PR3-ANCA 57%, MPO-ANCA 43%, GFR 18ml/min, 20% required dialysis. At two years, the primary composite outcome of relapse, death or end stage renal failure (ESRF) occurred in 14/33 (42%) RTX versus 4/11 (36%) CYC (p=1.00). Relapse occurred in 7/33 (21%) RTX versus 2/11 (18%) CYC (p=1.00), death in 6/33 (18%) RTX versus 3/11 (27%) CYC (p=0.67) and ESRF in 2/33 (6%) RTX versus 0/11 CYC (p=0.57). Median estimated glomerular filtration rate was 20 & 44$ml/min/m^2$ in RTX patients at 0 and 24 months respectively compared to 12 & 31$ml/min/m^2$ in CYC patients. Serious adverse events (SAEs) occurred in 61% RTX (50 events, 20/33 patients) versus 36% CYC (15 events, 4/11 patients) (incidence rate ratio 1.16; 95% confidence interval 0.64-2.22) (p=0.64).

Conclusions: RTX based induction therapy is efficacious but is not superior to IV CYC at two years in terms of combined relapse, mortality and ESRF outcome. Further strategies to reduce mortality and SAEs and prevent relapse should be considered.

Disclosure of Financial Relationships: Honoraria: Lecture fees from Roche.

**SA-FC406**

**Safety & Efficacy of Eculizumab in aHUS Patients Resistant to Plasma Therapy: Interim Analysis from a Phase II Trial** Christophe M. Legendre,[1] Sunil Babu,[2] Richard R. Furman,[3] Neil S. Sheerin,[4] David J. Cohen,[5] A. Osama Gaber,[6] Frank Eitner,[7] Yahsou Delmas,[8] Chantal Loirat,[9] Laurence A. Greenbaum,[10] Lothar Bernd Zimmerhackl.[11] *[1]Hôpital Necker, France; [2]Fort Wayne Med, IN; [3]Weill Cornell Med College, NY; [4]Newcastle upon Tyne, United Kingdom; [5]Columbia Univ Med Ctr, NY; [6]Methodist Hospital, TX; [7]Univ Aachen, DE; [8]CHU Bordeaux - Pellegrin, France; [9]Hopital Robert Debré, France; [10]Emory Univ, GA; [11]Univ Innsbruck, AT.*

Atypical hemolytic uremic syndrome (aHUS) is a rare, chronic, life-threatening disease characterized by thrombotic microangiopathy (TMA) due to complement inhibitor (CI) deficiencies, constitutive complement activation and chronic inflammation. TMA results in platelet (plt) consumption, renal failure, hemolytic anemia, and 60% 1 year ESRD/death despite plasma therapy (PT). The safety/efficacy of terminal CI eculizumab (Ecu) to reduce TMA in 17 adult/adolescent aHUS patients (pts) with TMA exacerbation despite PT is evaluated in a 26-week (wk) single-arm trial. Ecu dosing: 900mg/wk x4;1200mg Q2 wk starting wk 5. Neisseria vaccine and prophylatic antibiotic x 14 days. Median age 28 yrs, 29% male, 71% CI mutation, 41% post transplant. Primary endpoint: Change in plt count, a measure of TMA. Interim 26 wk analysis was positive:Plts increased 80±64x10∧9/L vs baseline(P<0.0001), positive by Day 7 (P<0.05). Secondary endpoints: with Ecu, 80%[95%CI 52-96] TMA-Event Free (stable plt,no PT,no new dialysis), no observed TMA Intervention (TMAI;PT or dialysis), time to first TMAI (K-M analysis) not reached. CKD improved ≥1 stage in 59%[33-82]. Exploratory analyses: Complete Heme Response(CHR;normal LDH+plts) 76%[50-93], 90% (9/10) [56-100] with abnormal baseline plt/LDH. Complete TMA Response (CHR+sCr ↓≥25%) 65%[38-86]. eGFR improved ≥15mL/min/1.73*m∧2 in 53%[28-77];5/7 dialysis pts became dialysis-free(P<0.01). Hb improved ≥20g/L in 65% [38-86]. Similar response in pts with/without mutations. Ecu was well tolerated. Most common AEs: anemia (1 severe), diarrhea, headache;2 pts discontinued. All pts remain alive. These interim results indicate early and sustained Ecu therapy rapidly stops TMA and restores renal function without need for PT.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC407**

**Hypoalbuminemia Is a Risk Factor of Venous Thromboembolism in Membranous Nephropathy (MN)** Sophia Lionaki,[1,2] Vimal K. Derebail,[1] Susan L. Hogan,[1] Michelle A. Hladunewich,[3] Sean Barbour,[3] Yichun Hu,[1] Caroline E. Jennette,[1] J. Charles Jennette,[1] Ronald J. Falk,[1] Daniel C. Cattran,[3] Patrick H. Nachman,[1,4] Heather N. Reich.[3,4] *[1]UNC Kidney Center, Chapel Hill, NC; [2]Laiko Hopsital, Athens, Greece; [3]Univeristy of Toronto, Toronto, Canada; [4]Contributed Equally.*

**Objectives** To assess the frequency and risk factors of venous thromboembolic events (VTE) in patients with MN.

**Methods** Patients with biopsy-proven idiopathic MN from the Glomerular Disease Collaborative Network (n=412) & the Toronto Glomerulonephritis Registry (n=395) inception cohorts were reviewed. The cohorts were similar with respect to demographics, eGFR, proteinuria and serum albumin at biopsy, and were pooled. Cases with at least one VTE (pulmonary embolus, deep vein or renal vein thrombosis, or other) were identified. Groups were compared by Fisher's exact and Wilcoxon rank tests. Logistic regression models were used to estimate odds ratios (OR) for VTE.

**Results** Of 807 patients, 62(7.7%) had at least one VTE, and this rate did not differ between registries (7.04% vs. 8.35%, p>0.05). Mean time to VTE was 11±53 months from the first clinical assessment. Hypoalbuminemia was associated with a 2.5 fold increased risk of VTE with each g/dl decrease in serum concentration (OR= 2.5, 95% CI:1.5-4.1, p=.0003). At serum albumin levels of 2.0 to <2.5 mg/dl the adjusted OR of VTE was 2.50 (95% CI: 1.1-5, p=0.02) and at <2.0 mg/dl the adjusted OR of VTE was 3.8 (95% CI: 1.7-8.6, p=0.002), compared to albumin >2.5 g/dl after controlling for age, gender, proteinuria, and immunosuppressive therapy. Smoking status did not influence these results.

| | With VTE, N=62 | Without VTE, N=745 | p-value |
|---|---|---|---|
| Age at MN diagnosis (yrs) | 46±14 | 50±16 | 0.11 |
| Gender, males, N(%) | 45(73%) | 454(61%) | 0.08 |
| Race, Caucasian, N(%) | 44 (71%) | 496 (67%) | 0.22 |
| Serum albumin (g/dl) | 2.2±0.6 | 2.7±2.3 | <0.0001 |
| eGFR (ml/min) | 71±29 | 73±34 | 0.731 |
| 24hour proteinuria (g/day) | 8.6±5.1 | 7.7±5.5 | 0.07 |

**Conclusions** In a large cohort of patients with MN, the frequency of VTE was 7.7%. Hypoalbuminemia, particularly at levels below 2.5 mg/dL, was the strongest independent predictor of VTE risk.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC408**

**Disease-Specific Risk of Venous Thromboembolic Events in Idiopathic Glomerulonephritis** Sean Barbour,[1] Allen Greenwald,[3] Ognjenka Djurdjev,[1] Adeera Levin,[1] Michelle A. Hladunewich,[2] Daniel C. Cattran,[2] Heather N. Reich.[2] *[1]University of British Columbia; [2]University of Toronto; [3]Queen's University.*

The nephrotic syndrome is a known risk factor for the development of venous thromboembolic events (VTEs). While membranous glomerulonephritis (MGN) is thought to be associated with an increased risk of VTE compared to other forms of idiopathic glomerulonephritis (GN), this has not been confirmed in a large population of patients with only primary glomerular disease. Accordingly we sought to identify the disease-specific risk of VTE in patients with idiopathic GN.

We reviewed a large inception cohort of patients with biopsy proven idiopathic MGN (n=395), focal segmental glomerulosclerosis (FSGS n=370), and IgA nephropathy (IgAN n=548) followed prospectively for a minimum of 12 months in the Toronto GN Registry. All episodes of VTE recorded in the Registry were tabulated. Cox proportional hazards modelling was used to evaluate the association between VTE risk and type of idiopathic GN, adjusting for the degree of proteinuria, serum albumin, age and sex.

At baseline the cohort had a mean age of 42 years, was 63% male, had a creatinine clearance of 73ml/min/1.73m$^2$ with proteinuria of 3.1g/day, and 46% had proteinuria >3.5g/day. A total of 44 patients (3.35% of subjects) with idiopathic GN had a VTE during a median follow-up of 63 months. The risk of VTE was highest in patients with FSGS (11 events, HR 7.8, 95% CI 1.7-35.2, p<0.01) and MGN (31 events, HR 22.0, 95% CI 5.3-92.1, p<0.01) compared to patients with IgAN (2 events, reference group). Proteinuria at the time of first clinical assessment and during follow-up were also predictive of the risk of VTE (p<0.01 for trend test). After adjustment for the degree of proteinuria in the multivariate analysis, the underlying pathologic diagnosis remained an independent predictor of the risk of VTE.

In summary, in this large cohort of patients with idiopathic GN, the underlying pathologic diagnosis is an important predictor of the risk of VTE. Even after adjustment for the degree of proteinuria, MGN is associated with the highest risk of VTE, followed by FSGS and IgAN.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC409**

**The Treatment of Resistant Nephrotic Syndrome with Acthar Gel (ACTH)** Andrew S. Bomback,[1] James A. Tumlin,[2] Joel J. Baranski,[3] James E. Bourdeau,[4] Anatole Besarab,[5] Jai Radhakrishnan,[1] Gerald B. Appel.[1] *[1]Columbia University; [2]Southeast Renal Research Institute; [3]Balboa Nephrology; [4]Nephrology Specialists of Oklahoma; [5]Henry Ford Hospital.*

**Objective:** Synthetic ACTH has shown efficacy in European studies as primary and secondary therapy for nephrotic syndrome (NS). To date, there is no modern published experience on using the natural, highly-purified Acthar Gel (repository corticotropin, ACTH), available in America, for NS. We here summarize the initial use of Acthar for NS in non-research settings.

**Methods:** Participating centers evaluated all cases of NS treated with Acthar outside of research settings (i.e. by prescription) with therapy initiatied by 11/30/09, allowing at least 6 months of follow-up. Treating nephrologists collected data on pts' clinical response to therapy and adverse events. Complete remission was defined as stable or improved renal function with proteinuria <500 mg/day. Partial remission was defined as stable or improved renal function with ≥50% reduction in proteinuria and proteinuria 500-3500 mg/day.

**Results:** 21 pts with NS were treated with Acthar: 10 with MN, 2 with MPGN, 2 with FSGS, 1 with MCD, 1 with IgA nephropathy, 1 with class V SLE GN, 3 with DM nephropathy, and 1 with NS without biopsy. The most common treatment regimen was 80 units SC twice weekly for 6 months. Follow-up data was available for 19 pts. Acthar was used as primary therapy for 6 pts with diagnoses of diabetic nephropathy, IgA nephropathy, and non-biopsied NS. The remaining pts had failed a mean 2.7 therapies prior to Acthar therapy. Overall, 9 pts (47%) achieved a complete or partial remission, with 5 (26%) in complete remission. Of these 9 responders, 7 had MN, 1 had class V SLE GN, and 1 had IgA nephropathy. Of the 10 pts with MN, 3 achieved complete remission and 4 achieved partial remission despite having previously failed a mean 2.3 therapies. 5 pts reported steroid-like adverse effects with therapy; there were no infectious complications.

**Conclusion:** Acthar is a viable treatment option for resistant nephrotic syndrome (NS) due to MN. Short-term data suggests that for this disease, complete and partial remission rates may approach 70%.

Disclosure of Financial Relationships: Consultancy: QuestcorResearch Funding: Questcor, Novartis, Alexion, Teva; Honoraria: Questcor, Novartis, Alexion.

**SA-FC410**

**Outcome in Children with Steroid Sensitive Nephrotic Syndrome (SSNS)** Aditi Sinha,[1] Piyush Kumar Sharma,[1] Rahul Chanchlani,[1] Asha Moudgil,[2] Pankaj Hari,[1] Arvind Bagga.[1] *[1]Pediatrics, All India Institute of Medical Sciences, New Delhi, India; [2]Nephrology, Children National Medical Center, Washington, DC.*

**Objectives:** To review the course of SSNS & analyze the factors associated with its outcome

**Methods:** Records of all patients with SSNS, between 1990 & 2005, were reviewed. Outcome, evaluated only in those with ≥1 yr follow up at this center, was defined as infrequent relapses (IFR), frequent relapses or steroid dependence (FR) & late resistance (LR). Stata 9.0 was used for statistical calculations.

**Results:** Of 3070 patients with NS, 2603 (84.8%) had SSNS. The mean age at onset of NS was 4.1±2.8 yr; 75% were boys. Hematuria was seen in 242 (9%), 67 (3%) had allergies & 46 (1.8%) had family history of NS. The duration of initial steroid therapy was 10±8 weeks, with initial remission lasting 20±28 wk. Of 1071 patients followed for more than 1yr, 37.4% had IFR, 56.7% FR & 5.9% LR. Renal biopsy in patients with LR showed focal segmental glomerulosclerosis (67%) & minimal change disease (33%). During follow up of 21±30 months, the illness was complicated by peritonitis (9.7% patients), pneumonia (6.6%), urinary infections (5.7%), tuberculosis (2.4%), meningitis (0.4%) & thrombosis (0.5%). Six patients died due to infections. Cushingoid features, hypertension & cataract were seen in 32.5%, 8.2% & 3.8% patients respectively.

Patients with FR, compared to IFR, had an early age at onset (44±32 *vs.* 54±36 months) [diff of means (95% CI) 10.4 (6,15)] (P=0.00) & short initial remission (13±21 *vs.* 35±39 months) [diff of means 22 (17,27)] (P=0.00). The duration of initial steroid therapy in FR & IFR was 9.9±5.8 & 10±5.9 wk respectively [diff of mean (95% CI) 0.15 (-0.6,1)] (P=0.7). On univariate analysis, early age of onset (P=0.00), hematuria (P=0.057) and short duration of initial remission (P=0.000) were associated with FR, while hematuria (P=0.005) was associated with LR.

**Conclusions:** Over half of all patients with SSNS followed at this referral center show frequent relapses. Young age at onset & shorter duration of initial remission are associated with frequent relapses. Infectious and therapy associated complications constitute significant morbidity in these patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC411

**Caveolae on Glomerular Endothelial Cells Play a Pivotal Role for Urinary Albumin Excretion in Chronic Kidney Disease** Takahito Moriyama, Keiko Uchida, Kosaku Nitta. *Medicine, Kidney Center, Tokyo Women's Medical University, Shinjyuku-ku, Tokyo, Japan.*

Glomerular endothelial cells (GEC), together with the glomerular basement membrane and glomerular epithelial cells, play a pivotal role in glomerular permeability and selectivity. Caveolae are cell membrane invaginations on GEC. The role of caveolae on GEC remains unknown, though caveolae on vascular endothelial cells have been reported to play an important role in endocytosis, cell signaling, and the transport of macromolecules. We examined the expression of caveolin-1 (Cav-1), a main component of caveolae, on GEC and determined the relationship between Cav-1 expression and renal pathological findings and clinical findings in 104 patients with chronic kidney diseases (CKD) and 50 healthy controls. The control specimens were obtained from 0-hour biopsy tissues of donor kidneys intended for renal transplantation. Cav-1 was expressed very weakly in the control tissues, and the area of Cav-1 expression on the capillary loops relative to the total glomerular area was 0.57 ± 0.65%. However, the area of Cav-1 expression was significantly larger in biopsy tissues from patients with membranous nephritis (MN; 4.89 ± 3.11, P<0.001), membranoproliferative glomerulonephritis (MPGN; 2.95 ± 3.06, P<0.001), crescentic glomerulonephritis (Cre GN; 1.79 ± 1.05, P<0.01), focal segmental glomerulosclerosis (FSGS; 1.75 ± 1.12, P<0.01) and diabetic nephropathy (DMN; 2.86 ± 1.38, P<0.001), compared with the level in the control group. Cav-1 expression was positively correlated with the urinary albumin level (r=0.40, p=0.0009) and negatively correlated with the total protein (r=-0.30, p=0.001) and serum albumin (r=-0.27, p=0.009) level in the clinical findings but not in the histological findings. Electron microscopy revealed numerous caveolae in the GEC of the specimens from patients with MN. These results indicated that caveolae are rarely located on stable GEC but increase in number on the GEC of specimens from patients with MN, MPGN, Cre GN, and DMN; thus, caveolae might play an important role in the transportation of albumin in GEC, increasing urinary albumin excretion.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC412

**BOLD Kidney MRI in Patients with Chronic Kidney Disease and Healthy Volunteers before and during Anti-Hypertensive Treatment** Laima Siddiqi,[1] Hans Hoogduin,[2] William P. Th. M. Mali,[2] Peter J. Blankestijn.[1] *[1]Nephrology and Hypertension, University Medical Center Utrecht, Utrecht, Netherlands; [2]Radiology, Radiotherpay and Nuclear Medicine, University Medical Center Utrecht, Utrecht, Netherlands.*

Imaging of the kidney using blood oxygen level dependent MR presents a major opportunity to examine differences in tissue oxygenation within the cortex and medulla applicable to human disease. The aim of this study was to evaluate intra-renal oxygenation assessed by BOLD MRI (3.0 Tesla) before and after treatment with RAS-blockers in hypertensive CKD patients. Methods: 10 patients with stable CKD and 5 healthy volunteers are included. 5 CKD patients were subjected to kidney BOLD MRI scan before and after treatment with 300mg/day aliskiren for 6 weeks. 5 other CKD patients received BOLD before and 1 hour after acute treatment with 50mg captopril (oral). A group of controls (n =5) was scanned before and 1 hour after acute treatment with 50mg captopril (oral). Results: The ten patients (7 men) had a mean age of 61 ± 17 years and eGFR of 30 ± 11 ml/min per 1.73 $m^2$. Office systolic and diastolic blood pressures when on a RAS-blocker, were 130/86 ± 10/5 mmHg respectively. Controls had normal kidney function and were not on any medication. In untreated condition, systolic and diastolic blood pressure elevated, 145/95 ± 6/4 mmHg, respectively. After chronic treatment with aliskiren, arterial blood pressure decreased in all patients in this group, 122 ± 3 mmHg and 75 ± 3 mmHg. After acute treatment with captopril arterial blood pressure reduced to 125 ± 4 and 71 ± 8 mmHg. Tissue intensity signal (T2*) i.e. tissue oxygenation was increased in medulla after chronic treatment from 29 ± 6.5 to 34 ± 6.0 and after acute treatment from 34 ± 8.7 to 37 ± 11 in CKD patients. In addition, T2* ratio between cortex and medulla decreased in CKD patients after chronic treatment and acute treatment. This ratio remained stable in healthy volunteers before and after treatment with captopril 1.62 ± 0.1 and 1.65 ± 0.1, respectively. Conclusion: This study shows for the first time that RAS blockers improve intra-renal oxygenation in patients with stable CKD. Further investigation is required.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC413

**Vitamin D Receptor Activation Ameliorates Cachexia in Chronic Kidney Disease** Robert H. Mak,[1] Jianying Zhan,[2] Wai W. Cheung.[1] *[1]Pediatrics, University of California, San Diego, La Jolla, CA; [2]Pediatrics, ZheJiang University, HangZhou, ZheJiang, China.*

Cachexia, characterized by anorexia, increased basal metabolic rate and the pathologic loss of lean and fat mass, is prevalent in chronic kidney disease (CKD). We have previously shown that uremic cachexia is associated with maladaptive responses in energy homeostasis and body composition and that the pathogenesis may be mediated by inflammation(Cheung W et al JCI 2005). Vitamin D deficiency is prevalent in inflammatory conditions such as CKD and may influence cachexia. Using a mouse model of CKD-associated cachexia, 8-wk old c57BL/6J mice were subjected to 5/6 nephrectomy (N) or sham operation (S). Serum 25-hydroxyviamin D3 and 1,25-hydroxyvitamin D3 were significantly lower in N mice compared to S mice (p<0.01). We studied the effect of paracalcitol (PC), a vitamin D receptor activator, in this model. N mice received either PC(N-PC) (1.5 mg/kg, i.p., thrice/week) or vehicle. S mice received vehicle. Serum creatinine was higher in N (0.7 mg/d) and N-PC(0.6 mg/dl) compared with S mice(0.2 mg/dl, p<0.01). All 3 groups of mice were pair-fed and observed for 2 weeks. S and N-PC mice gained more weight than N mice (p<0.01) despite equal food consumption. Basal metabolic rate was elevated in N compared with S and N-PC mice (p<0.01). Efficiency of food consumption was decreased in N mice compared with S and N-PC mice. N mice lost lean and fat mass whereas S and N-PC mice gained lean and fat mass. mRNAs of uncoupling proteins(UCP)- 1 and 2, which control energy expenditure, were upregulated in skeletal muscle and adipose tissue in N compared with S and normalized in N-PC mice. mRNAs of myogenic pathway genes, IGF-I, MyoD and PAX3, were all downregulated in the skeletal muscles in N compared with S and normalized in N-PC mice. IL-6 mRNA in skeletal muscle and adipose tissue was upregulated in N compared with S and normalized in N-PC mice. In summary, vitamin D receptor activation ameliorated energy homeostasis and body composition abnormalities as well as reversed cytokine over-expression in CKD-associated cachexia. Vitamin D deficiency may be an important factor in the pathogenesis of cachexia and inflammation in CKD.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC414

**Rigorous Control of Blood Pressure Is Justified in Older People with Chronic Kidney Disease** Stephen G. John,[1] Paul J. Owen,[1] Jane H. Youde,[1] Chris W. McIntyre.[1,2] *[1]Royal Derby Hospital, Derby, United Kingdom; [2]University of Nottingham, Derby, United Kingdom.*

Chronic kidney disease (CKD) is highly prevalent in older people. Aggressive blood pressure (BP) control is the cornerstone of CKD management. Doubt exists concerning the optimal BP targets in this group, primarily due to a perceived risk of inducing additional CV instability and increasing falls risk. We performed a prospective, controlled study of the effects of antihypertensive therapy (AHT) on CV function, body composition, functional capacity and falls in older (>70 yrs) non-diabetic patients with CKD 3/4.

We recruited 61 subjects (including non-CKD controls). AHT was fully withdrawn for 2 weeks before initial assessment of body composition (bioimpedance analysis) and function (Timed get Up and Go test (TUG)), as well as CV function (pulse wave velocity (PWV) and baroreflex sensitivity (BRS)). AHT was restarted to target BP 130/80mmHg. We repeated assessment 4 weeks after full AHT titration (AHTr), and after a further 12 months follow-up (FU). Falls diaries were maintained.

Mean age was 76±4yrs, mean eGFR (CKD group) was 42±14ml/min/1.73$m^2$. AHT was in line with current guidelines, predominantly RAAS inhibition (mean achieved BP 128/69 mmHg). Improvements in PWV (13 to 12 m/s, p<0.001) and BRS (4.2 to 5.7 ms/mmHg, p=0.002) with AHTr were sustained over 12 months. Intra- and extra-cellular water fell slightly by around 0.3L. Muscle mass fell with AHTr and at FU (0.7, p=0.031; 1.0kg; p=0.020). A trend to bone mass reduction after AHTr (0.03kg; p=0.085) was confirmed at FU (0.6kg; p=0.021). TUG fell over the year by 8 to 9 s (p=0.001). Falls rates were low, with only 27 episodes (range 0-6 per individual). No associations were noted with AHT, BRS or BP. Increased arterial stiffness was associated with increased falls risk. Overall response to AHT was similar between patients with CKD or preserved renal function.

AHT use in older patients rapidly results in sustained improvement in CV function. Body composition and function decline, this does appear to be clinically significant. Concern that older patients with CKD may have a different risk/benefit profile for aggressive AHT than younger patients appears unfounded.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC415

**Trefoil Factor 3 Predicts Incident Chronic Kidney Disease in the General Population: The Atherosclerosis Risk in Communities (ARIC) Study and Framingham Heart Study** Brad C. Astor,[1] Anna Kottgen,[2] Shih-Jen Hwang,[3] Nrupen Anjan Bhavsar,[1] Caroline S. Fox,[3] Josef Coresh.[1] *[1]Department of Epidemiology, Johns Hopkins University, Baltimore; [2]Renal Division, University Hospital Freiburg, Germany; [3]Framingham Heart Study, National Heart, Lung and Blood Institute, Framingham.*

Chronic kidney disease (CKD) is associated with elevated risk for cardiovascular disease, end stage renal disease and death, but few factors that predict incident CKD have been identified. Urinary levels of trefoil factor 3 (TFF3) are associated with acute kidney injury in animal models, but the potential association of TFF3 levels with incident CKD in humans is unknown.

We conducted a case-control study nested within the Atherosclerosis Risk in Communities (ARIC) Study and ARIC Carotid MRI Study. Individuals with an estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73m$^2$ and with urinary albumin:creatinine ratio (ACR) <30 mg/g were eligible for selection. Incident CKD was defined as an eGFR decreasing by 25% to below 60 mL/min/1.73$^2$ after a median of 8.6 years of follow-up. The 143 cases that occurred were matched on age, sex and race to 143 non-cases. The median TFF3 level was 0.55 pg/mL (interquartile range: 0.27, 1.10). Higher TFF3 levels were seen in blacks, females, individuals with diabetes and those using anti-hypertensive medications. A doubling of TFF3 levels was significantly associated with higher incidence of CKD after adjustment for these factors, as well as for other CKD risk factors, including urinary ACR (adjusted odds ratio [OR] = 1.35; 95% confidence interval [CI]: 1.11, 1.64). Similar analyses were conducted in a case-control sample (n=200) nested within the Framingham Heart Study. The association between TFF3 level and incident CKD was positive, but substantially weaker and not statistically significant (adjusted OR=1.12, 95% CI: 0.80, 1.58). Combined analyses found that a doubling of TFF3 was associated with a 29% higher odds of incident CKD (p=0.004).

We hypothesize that higher urinary TFF3 levels indicate ongoing repair of damage in the kidney and may be useful as a marker of increased risk for CKD.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC416

**FG-4592, a Novel Oral HIF Prolyl Hydroxylase Inhibitor, Elevates Hemoglobin in Anemic Stage 3/4 CKD Patients** Anatole Besarab,[1] Henry N. Hulter,[2] Stephen Klaus,[2] Tyson T. Lee,[2] David E. Lilienfeld,[2] Thomas B. Neff,[2] Beth Anne Piper,[2] Robert Provenzano,[3] Kin-Hung Peony Yu.[2] *[1]Henry Ford Hospital; [2]FibroGen, Inc.; [3]St. Clair Specialty Physicians.*

Given heightened concerns about clinical outcomes arising from use of ESAs that cause supraphysiologic circulating EPO levels, use of agents producing transient, more physiologic increases in endogenous EPO (eEPO) may have significant benefit in CKD anemia. In this randomized, single-blind, placebo-controlled phase 2 study, 117 subjects were randomized with 116 (108 unique) treated: 88 to FG-4592 and 28 to placebo, in 4 dose cohorts (0.7, 1.0, 1.5, and 2.0 mg/kg FG-4592) administered 2 [BIW] or 3 [TIW] times weekly for 4 wks. 97 subjects who were anemic at baseline (BL Hb<11 g/dL) and treated for at least 2.5 wks were eligible for efficacy evaluation. BL Hb was 10.0 ± 0.7 g/dL (mean ± SD). Maximal Hb change from BL by wk 6 and Hb responder rate (% subjects with Hb increase ≥1 g/dL from BL at any time from wk 3-6) are shown in table.

Mean±SD Maximal ΔHb g/dL (%Hb Responders)

| Cohort | BIW [N] | TIW [N] |
|---|---|---|
| 0.7 mg/kg | 0.9±0.8 (33) [9] | 1.0±0.9(62) [13] |
| 1.0 mg/kg | 0.9±0.8 (60) [5] | 1.0±0.9 (60) [5] |
| 1.5 mg/kg | 1.7±1.0 (80) [10] | 2.0±0.9 (91) [11] |
| 2.0 mg/kg | 1.9±0.6 (100) [9] | 2.2±0.8(100) [11] |
| placebo | 0.4±0.5 (8) [24] | |

For responders, median time to Hb response was 22-43 and 15-22 days for BIW and TIW groups, respectively, generally faster than observed with ESA. FG-4592 administered for 4 wks was well-tolerated and led to dose-dependent Hb correction with observed peak eEPO levels 1-2 orders of magnitude lower than values reported for ESA. During treatment, one AE of increased BP was reported in the 0.7 mg/kg group; no significant BP change was observed across cohorts despite rapid rate of Hb rise with higher doses. There were no reports of thrombosis, sustained liver enzyme abnormality, or study drug related SAE. These results support a potentially unique mechanism of action permitting safer Hb increase and maintenance while avoiding thrombotic or hypertensive responses commonly observed with ESA.

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## SA-FC417

**Effect of Allopurinol in Chronic Kidney Disease (CKD) Progression and Cardiovascular Risk** Jose Luno, Marian Goicoechea, Soledad Garcia de Vinuesa, Ursula Verdalles, David Arroyo, Abraham Rincon, Caridad Ruiz Caro, Jara Ampuero. *Nephrology, Hospital General Universitario Gregorio Marañon, Madrid, Spain.*

**Background:**

Hyperuricemia is associated with hypertension, inflammation, renal disease progression and cardiovascular disease. However, no data are available about the effect of allopurinol in patients with chronic kidney disease.

**Design, setting, participants and measurements**

We conducted a prospective, randomized trial of 113 patients with estimated glomerular filtration rate (e-GFR) < 60 ml/min. Patients were randomly assigned to treatment with allopurinol 100 mg/day (n=57) or to continue the usual therapy (n=56). Clinical, biochemical and inflammatory parameters were measured baseline and at 6,12 and 24 months of treatment. The objectives of study were: 1) renal disease progression, 2) cardiovascular events and 3) hospitalizations of any causes.

**Results:**

Serum uric acid and C.reactive protein levels were significantly decreased in subjects treated with allopurinol (p=0.016 and p=0.018, respectively). In control group eGFR decreased 3.3±1.3 ml/min/1.73 m$^2$ and in allopurinol group increased 1.3±1.2 ml/min/1.73 m2 after 24 months (p=0.018). Allopurinol treatment slowed down renal disease progression independently of age, gender, diabetes, C-reactive protein, albuminuria and renin-angiotensin system blockers use. (HR:0,53, p=0.048)

After a mean follow-up time of 23.4±7.8 months, 22 patients suffered a cardiovascular event. Diabetes mellitus (HR: 4,38 p=0.004), previous coronary heart disease (HR:4,49 p=0.005) and CRP levels (HR: 2.83, p=0.031) increased cardiovascular risk. Allopurinol treatment reduces risk of cardiovascular events in 71% (HR: 0.29, p=0.026) compared with standard therapy.

**Conclusions:**

Allopurinol decreases C-reactive protein and slow down progression of renal disease in patients with chronic kidney disease. Besides, allopurinol reduces cardiovascular and hospitalization risk in these subjects.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC418

**Improved Renal Outcomes with a Calcium Channel Blocker Compared to a Thiazide Diuretic When Combined with an Angiotensin Converting Enzyme Inhibitor in Black Hypertensives** Matthew R. Weir, George L. Bakris, Michael A. Weber, Björn Lennart Dahlöf, Richard B. Devereux, Sverre E. Kjeldsen, Bertram Pitt, Jackson T. Wright, Roxzana Y. Kelly, Tsushung A. Hua, Robert Allen Hester, Eric J. Velazquez, Kenneth A. Jamerson. *Division of Nephrology, University of Maryland School of Medicine, Baltimore, MD.*

The Avoiding Cardiovascular events through Combination therapy in Patients Living with Systolic Hypertension (ACCOMPLISH) trial was a double-blind randomized event driven multi-center trial involving 11,506 patients with high cardiovascular risk randomized to benazepril + hydrochlorothiazide (B+H) or benazepril + amlodipine (B+A) and titrated in parallel to reach recommended blood pressure goals. There were 1,416 patients of self-described Black (AA) ethnicity. There was no overall difference in the primary composite cardiovascular outcome between treatment groups (B+H or B+A) in AA. The pre-specified kidney disease endpoint-defined as doubling in serum creatinine, endstage renal disease (ESRD), or death was also not different between treatment groups in AA patients. But, in view of the low event rates resulting from the early termination of the trial, this endpoint was modified to include a >50% sustained increased serum creatinine, ESRD or death. AA participants, despite having fewer primary cardiovascular events regardless of treatment assignment (7.8% vs 11.1%) than non-AA, were significantly more likely (12.5% vs 10.9% , p=.004) to develop a sustained >50% increase in serum creatinine. More AA (2.9 vs 1.0%, p=.001) had a greater than 50% increase in creatinine to a sustained level of 2.6 mg/dL or greater. Fewer patients on B+A compared to B+H developed sustained >50% increases in serum creatinine, both among AA (8.9% vs 16.0%) and non-AA (7.3% vs 14.5 %), both p<.0001). We conclude that in high risk patients with hypertension, AA patients have a much greater proclivity for renal disease progression even when having a lower rate of cardiovascular events. Combination antihypertensive treatment with B+A reduces kidney disease progression more effectively than B+H in both AA and non-AA patients.

Disclosure of Financial Relationships: Honoraria: Less than $10,000/year for each listed industry entity; Scientific Advisor: Amgen, Nicox, Novartis, Daichi-Sankyo.

## SA-FC419

**Dietary Sodium Restriction Is Superior to Dual Blockade in Enhancing the Antiproteinuric and Antihypertensive Effect of RAAS Blockade** Maartje C. J. Slagman,[1] Femke Waanders,[1] Marc H. Hemmelder,[2] Hiddo Jan Lambers Heerspink,[3] Gerjan Navis,[1] Gozewijn Dirk Laverman.[1] *[1]Nephrology, UMCG; [2]Nephrology, MCL; [3]Pharmacology, UMCG, Netherlands.*

**Background:** Persistent hypertension (HT) and residual proteinuria (UP) during single renin-angiotensin-aldosterone-system (RAAS) blockade predict unfavourable renal and cardiovascular outcome and thus implicate insufficient renoprotection. Dual blockade with ACE inhibition (ACEi) and angiotension receptor blockade (ARB) and/or dietary sodium restriction may be strategies to improve renoprotection. Their comparative efficacy, and the effects of their combination, were tested in the current study.

**Methods:** In a cross-over RCT 52 patients with non-diabetic nephropathies (83% male, 100% Caucasian, age 51±2 years) and residual proteinuria during single RAAS blockade at maximal dose (lisinopril 40 mg/d; LIS), were treated during four 6-week periods with LIS and dual blockade at maximal dose (lisinopril 40 mg/d and valsartan 320 mg/d; LIS+VAL), combined with consecutively a normal sodium diet (184±6 mmol $Na^+$/d) and a low sodium diet (LS; 104±5 mmol $Na^+$/d; p<0.001), in random order.

**Results:** At baseline (LIS) residual UP was 1.7 [1.3-2.1] g/24h. Addition of VAL reduced UP (-10±7%, p=0.003), but addition of LS diet was far more effective (-44±4%, p<0.001), while combination VAL+LS diet added to LIS was slightly but significantly more effective (-52±5%, p<0.001). Systolic blood pressure (SBP), which was high-normal (134±3 mmHg) at baseline (LIS), was not significantly altered by addition of VAL (131±3 mmHg, NS), whereas addition of LS diet normalized SBP (123±2 mmHg, p<0.001). Plasma $K^+$ levels (4.6±0.1 mmol/L) and creatinine clearance (83±6 mL/min) during LIS were unaffected by addition of VAL or LS diet, whereas both VAL+LS diet added to LIS increased plasma $K^+$ (5.0±0.1 mmol/L, p<0.001) and decreased creatinine clearance (69±6 mL/min, p=0.002).

**Discussion:** Dietary sodium restriction is superior to the addition of ARB for further reduction of HT and UP in renal patients already on ACEi. Our findings support the combined endeavours of patients and health professionals to accomplish satisfactory sodium restriction to enhance therapy response.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC420

**Add-On Aliskiren in Non-Dialysis CKD Patients Treated with Dual Blockade of Renin-Angiotensin System (RAS): A Prospective Pilot Study** Luca De Nicola,[1] Pasquale Zamboli,[1] Vincenzo Bellizzi,[2] Domenico Russo,[3] Felice Nappi,[4] Maristella Minco,[3] Giuseppe Conte,[1] Roberto Minutolo.[1] *[1]Nephrology Depts., Second Univ., Naples; [2]Hospital, Salerno; [3]Univ. Federico II, Napoli; [4]Hospital, Nola, Italy.*

The additive antiproteinuric effects of CEI+ARB are limited in most CKD pts possibly due to escape mechanisms. We evaluated antiproteinuric efficacy of direct renin inhibitor aliskiren in CKD pts with residual proteinuria after dual RAS blockade.

We selected consecutive adult CKD stage 1-4 (no transplant) pts with proteinuria >0.5 g/d treated with CEI+ ARB at maximal tolerated dose unchanged in the previous 3 months. Immunosuppressive therapy, sK >5.5 mEq/L and eGFR change >30% in the previous 6 months were exclusion criteria. Patients were evaluated before (baseline) and during 6 months of aliskiren at a dose titrated to 300 mg/d. Out of the 50 selected pts, 5 did not complete the study (immunosuppressive therapy, n=2; no assumption of aliskiren, n=3). Age was 57±15 yrs and BMI 30±6 kg/m$^2$; prevalence of males, diabetes and prior CV disease was 78%, 49% and 33%, respectively.

Main changes in the 45 pts studied

| | Basal | month 1 | month 3 | month 6 | P |
|---|---|---|---|---|---|
| Systolic BP (mmHg) | 146±13 | 139±13 | 139±16 | 141±19 | 0.0002 |
| Diastolic BP (mmHg) | 86±8 | 83±7 | 82±9 | 80±9 | 0.005 |
| eGFR (mL/min/1.73 m2) | 47.7±26.5 | 46.6±27.3 | 45.7±27.8 | 44.2±26.3 | 0.004 |
| sK (mEq/L) | 4.7±0.5 | 4.8±0.5 | 4.8±0.5 | 4.8±0.6 | 0.167 |
| Proteinuria (g/d) | 3.39±2.25 | 2.94±2.07 | 2.99±2.41 | 2.69±1.98 | 0.032 |

mean±SD

Median proteinuria decline (0.74 g/d, IQR 0-1.45) did not correlate with changes of either systolic (r=0.155, P=0.308) and diastolic (r=0.228, P=0.131) BP or eGFR (r=-0.015, P=0.922). No side effect requiring withdrawal of CEI/ARB or aliskiren occurred. Use of other antihypertensive drugs and UNaV did not differ from baseline (2.1±1.3 and 184±96 mEq/d, respectively).

Add-on aliskiren in CKD pts under dual RAS blockade allows an additional decline in proteinuria that is at least in part independent of its antihypertensive effect. While the drug appears to be safe in terms of hyperkalemia risk, long term evaluation of GFR is required.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC421

**Cyclin Dependent Kinases (Cdks): New Players Controlling AQP2 Trafficking** Grazia Tamma,[1] Domenica Lasorsa,[1] Christiane Trimpert,[2] Lisa Mastrofrancesco,[1] Maria Grazia Mola,[1] Maria Svelto,[1] Peter M. T. Deen,[2] Giovanna Valenti.[1] *[1]Department of General and Environmental Physiology, University of Bari, Bari, Italy; [2]Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Kidney water reabsorption is regulated by the hormone vasopressin (AVP) which exerts its effect on the water channel AQP2. AVP binds to its V2 receptors (V2R) and causes, via elevation of cAMP, activation of protein kinase A (PKA) and phosphorylation of AQP2 at serine 256 (pS256), AQP2 translocation to the plasma membrane. Besides S256, in vivo AVP action co-incides with phosphorylation of S264 and S269, and de-phosphoryation of S261, but the kinases involved are still unknown. Bio-informatical analysis suggests that cdk1/5 may phosphorylate S261 and their potential relevance in AQP2 regulation was investigated. Immunohistochemistry and immunoblot analysis indicated that cdk1 and cdk5 are both expressed in the AQP2-expressing principal cells. In *ex-vivo* kidney slices and MDCK-AQP2 cells, the cdk1/5 inhibitor roscovitine, increased pS256 and decreased pS261, while pS269 was unchanged. In MDCK-AQP2 cells, immunocytochemistry and cell surface biotinylation showed that roscovitine caused AQP2 translocation to the apical membrane in the absence of forskolin, resulting in increased osmotic water permeability (assessed by a FlexStation 3 fluorescence platereader). To investigate the possible signalling responsible for AQP2 relocation in response to roscovitine treatment, PKA activity was evaluated by FRET. MDCK-hAQP2 were co-transfeced with RII-CFP and Cat-YFP. Compared with non-treated cells, roscovitine treatment caused an increase in PKA activity, similar to that obtained with forskolin stimulation. Together, these data indicate that cdks are functionally involved in the regulation of AQP2 trafficking and suggest that inhibition of cyclin kinases may be an alternative approach for treatment of Nephrogenic diabetes insipidus due to vasopressin-2 receptor dysfunction.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC422

**Simvastatin Regulates Aquaporin 2 Trafficking and Urinary Concentration: Role of Actin Modulation Via Rho GTPase** Wei Li, Richard Bouley, Ying Chen, Toshiyuki Matsuzaki, Paula Nunes, Udo Hasler, Dennis Brown, Hua Ann Jenny Lu. *Medicine, Massachusetts General Hospital /Harvard Medical School, Boston, MA.*

Statins are 3-hydroxyl-3-methyglutaryl (HMG)-coA reductase inhibitors that are commonly used to inhibit cholesterol biosynthesis. Emerging data have suggested that they also have "pleotropic effects" including modulating actin cytoskeleton reorganization. Here, we report the role of simvastatin in the trafficking of aquaporin 2. Specifically, simvastatin induced the membrane accumulation of AQP2 in cell cultures and kidneys in situ. The effect of simvastatin was independent of protein kinase A activation and phosphorylation at AQP2-ser256, a critical event involved in vasopressin (VP) regulated AQP2 trafficking. Further investigation showed that simvastatin inhibited endocytosis and induced actin reorganization in parallel with down regulation of RhoA activity. Overexpression of active RhoA abolished simvastatin's effect on AQP2 trafficking, suggesting the involvement of this small GTPase in simvastatin mediated AQP2 trafficking. Finally, the effect of simvastatin on urinary concentration was investigated in VP-deficient Brattleboro rats. Simvastatin acutely (3-6 hours) increased urinary concentration and decreased urine output in these animals. In summary, simvastatin regulates AQP2 trafficking in vitro and urinary concentration in vivo via events involving down regulation of Rho GTPase activity, modulation of the actin cytoskeleton, and subsequent inhibition of endocytosis. Our study provides an alternative mechanism to regulate AQP2 trafficking, bypassing the VP/vasopressin receptor signaling pathway.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC423

**Reduced Rab5 Prenylation Might Explain the Lovastatin-Induced Accumulation of AQP2 at the Apical Plasma Membrane in Renal Cells** Giuseppe Procino, Claudia Barbieri, Monica Carmosino, Maria Grazia Mola, Giovanna Valenti, Maria Svelto. *Department of General and Environmental Physiology, University of Bari, Bari, Italy.*

We have previously shown that treatment with lovastatin accumulates the water channel AQP2 at the apical plasma membrane of cultured MCD4 renal cells by inhibiting its constitutive endocytosis. This observation might be of importance to rescue AQP2 apical expression in X-linked NDI. Here we propose a mechanism that might explain this effect.

In addition to inhibiting cholesterol synthesis, statins also inhibit the synthesis of other sterol and non-sterol intermediate compounds produced by the mevalonate pathway including the isoprenoids, farnesyl- (FP) and geranylgeranyl-pyrophosphate (GGP). Proteins of the Rab GTPase family must be post-translationally prenylated by addition of two geranylgeranyl moieties in order to be properly targeted to membranes and to be active. Members of the Rab family, including Rab5, were found associated with immunoisolated AQP2 storage vesicles. Rab5 is expressed in early endosomes and in chlatrin-coated endocytic vesicles suggesting a role in regulating AQP2 endocytosis at the plasma membrane.

In this study we found that, in MCD4 cells, mevalonate prevented apical accumulation of AQP2 induced by lovastatin incubation as demonstrated by confocal microscopy. Interestingly, similar results were obtained by provision of GGP, suggesting a role of prenylation in regulating AQP2 trafficking. Measurement of osmotic water permeability, obtained by a calcein-quenching-based assay on FlexStation, confirmed that the increase in water permeability induced by lovastatin treatment was completely abolished by mevalonate or GGP.

In agreement with these results, analysis of Rab5 prenylation under lovastatin treatment, showed that its electrophoretic mobility on SDS-PAGE was slightly reduced, consistent with a reduction of protein isoprenylation. Conversely, addition of mevalonate or GGP prevented Rab5 electrophoretic shift.

Taken together, these data suggest that statins might increase AQP2 apical expression by reducing Rab5 isoprenylation and its association to the apical plasma membrane where it regulates AQP2 endocytosis.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC424

**E3 Ubiquitin-Protein Ligases Associated with Regulation of AQP2 Expression in Rat Kidney** Yu-Jung Lee,[1] Jeong Eun Lee,[2] Moon-Chang Baek,[2] Tae-Hwan Kwon.[1] *[1]Dept of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Daegu, Republic of Korea; [2]Dept of Molecular Medicine, School of Medicine, Kyungpook National University, Daegu, Republic of Korea.*

Ubiquitination plays a role in the proteasomal degradation of AQP2. We aimed to identify E3 ubiquitin (Ub)-protein ligases in the kidney which are associated with AQP2 regulation. Sprague-Dawley rats were infused with vehicle (n=13), dDAVP for 5 d (n=13), or dDAVP was withdrawn for periods (15 min, 30 min, 1-, 3-, 6-, 12-, or 24-h) after 5 d-infusion (n=46). Total RNA was isolated from inner medulla (IM) for transcriptome analysis and plasma membrane (PM)- or intracellular vesicle (ICV)-enriched fractions of whole kidney were immunoisolated for LC-MS/MS analysis. dDAVP infusion for 5 d (D5d) significantly increased urine osmolality which was maintained during 3 h-withdrawal of dDAVP after 5 d-infusion (D5d-3h). Consistent with this, increased AQP2 expression was

seen in the PM-fractions of D5d and D5d-3h, whereas AQP2 expression in the ICV-fractions further increased after D5d-3h, indicating internalization of AQP2. Transcriptome analysis revealed eighty six genes of E3 ligases and LC-MS/MS analysis demonstrated 16 proteins of E3 ligases. Among them, seven E3 ligases (BRCA1, UBR4, BRE1B, UHRF1, NEDD4, CUL5, and FBX6) were shared. RT-PCR demonstrated mRNA expressions of all the seven E3 ligases in the kidney and immunoblotting demonstrated the changes in expression of the selected E3 ligases (BRE1B, NEDD4, and CUL5) in response to dDAVP stimulation/withdrawal or in lithium-induced NDI. Decreased rate of AQP2 degradation was observed in the mpkCCD cells with siRNA-mediated knockdown of CUL5 compared to control siRNA-transfected mpkCCD cells. Taken together, identified E3 ligases might play a role in the regulation of vasopressin-dependent AQP2 expression.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC425

**Vasopressin Independent Phosphorylation and Trafficking of Aquaporin-2 by Selective E-Prostanoid Receptor Agonists** Emma T. B. Olesen,[1] Helle A. Praetorius,[2] Robert A. Fenton.[1] *[1]The Water and Salt Research Center, Institute of Anatomy, Aarhus University, Denmark; [2]Institute of Physiology and Biophysics, Aarhus University, Denmark.*

Vasopressin increases the water permeability of the collecting duct (CD) acutely by $G_s$ protein-mediated targeting of aquaporin-2 (AQP2) to the apical membrane of principal cells. E-prostanoid receptor types 2 (EP2) and 4 (EP4) are alternative $G_s$ protein coupled receptors expressed in the CD. We investigated the effects of EP2 and EP4 stimulation on apical plasma membrane (APM) abundance and phosphorylation of AQP2 *in vitro*. MDCK cells stably transfected with AQP2 were stimulated with either PGE2, the EP2 receptor agonist butaprost (BUT) or the EP4 receptor agonist CAY10580 (CAY). The effects on AQP2 APM abundance were assessed by cell surface biotinylation. Compared to controls, PGE2 ($10^{-7}$M) increased abundance of AQP2 in the APM and phosphorylation at serine-264 (S264) and serine-269 (S269). The maximum effect on pS269 was reached after 5 minutes, whereas AQP2 accumulation in the APM and pS264 abundance further increased at later time points. The effect of BUT on AQP2 APM abundance and phosphorylation was concentration dependent. A significant increase in AQP2 targeting and phosphorylation occurred at $10^{-8}$M, both of which increased at higher doses of the agonist. Stimulation with either BUT or PGE2 (both $10^{-7}$M, 40 minutes) had similar effects on targeting, pS264 and pS269 abundance. Similarly, the effect of CAY on AQP2 targeting was concentration dependent in the range of $10^{-8}$M to $10^{-5}$M, with a significant increase in AQP2 membrane abundance observed at $10^{-7}$M. In conclusion, in MDCK cells, PGE2 increases phosphorylation of AQP2 at S264 and S269 and selective agonists for either EP2 or EP4 individually mimic the effect of PGE2 on AQP2 targeting. The EP2 selective agonist BUT additionally mimics the effect of PGE2 on pS264 and pS269 abundance. These results may provide further understanding of the concentrating mechanisms of the kidney collecting duct and could have implications for the treatment of nephrogenic diabetes insipidus.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC426

**mPGES-1 Deletion Potentiates Urine Concentrating Capability after Water Deprivation** Zhanjun Jia,[1,2] Gang Liu,[1,2] Tianxin Yang.[1,2] *[1]Internal Medicine, University of Utah, Salt Lake City, UT; [2]Medical Center, Veterans Affairs, Salt Lake City, UT.*

PGE2 plays an important role in regulation of fluid metabolism chiefly via antagonizing vasopressin-induced osmotic permeability in the distal nephron but its enzymatic sources remain uncertain. Among three prostaglandin E synthase (PGES) isoforms cloned thus far, including microsomal PGES-1 (mPGES-1), mPGES-2, and cytosolic PGES (cPGES), mPGES-1 is the only one processing the enzymatic activity in vivo. Present study was undertaken to investigate the potential role of mPGES-1 in regulation of urine concentrating ability after water deprivation (WD). After 24-h WD, wild-type (WT) mice exhibited a significant reduction in urine volume ($0.79 \pm 0.12$ vs.$1.22 \pm 0.22$ ml, $p<0.01$), accompanied by a significant elevation in urine osmolality ($2877.6 \pm 332.3$ vs. $2019.6 \pm 239.3$ mOsm/kg $H_2O$, $p<0.05$) as compared with control groups. In contrast, in response to WD, mPGES-1 KO mice had much less urine volume ($0.374 \pm 0.11$ $p<0.01$) and higher urine osmolality ($3836.3 \pm 523.3$ mOsm/kg $H_2O$, $p<0.05$). By qRT-PCR, renal medullary and cortical COX-2 mRNA in dehydrated WT mice was upregulated by 2.7- and 14-fold, respectively, contrasting to unaltered renal mRNA expression of COX-1 and mPGES-1. WD induced a 2-fold increase in urinary PGE2 output in WT mice ($p<0.05$ vs. WT control), which was completely blocked by mPGES-1 deletion. At baseline, the KO mice had a 30% increase in V2 receptor mRNA expression in the renal medulla but not the cortex as compared with WT controls; the expression was unaffected by WD irrespective of the genotype. In response to WD, renal medullary AQP2 mRNA exhibited a 60% increase in WT mice and an 110% increase in the KO mice ($p<0.05$ vs. WT) whereas the increases in renal cortical AQP2 mRNA were comparable between the genotypes so was NKCC2 mRNA. Immunoblotting confirmed increased renal medullary AQP2 protein abundance in the KO mice after WD. Taken together, these results suggest that mPGES-1-derived PGE2 via COX-2 activity inhibits urine concentrating ability during water deprivation likely through suppression of renal medullary expression of V2 receptors and AQP2.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC427

**Mice Lacking P2Y2 Receptor Are Resistant to Potassium (K)-Depletion Induced Polyuria** Bellamkonda K. Kishore,[1] Jeff M. Sands,[2] Yue Zhang,[1] Christopher F. Martin,[2] Janet D. Klein.[2] *[1]Medicine, Nephrology Div., VA Med Ctr & Univ. of Utah, Salt Lake City, UT; [2]Medicine, Renal Div., Emory Univ., Atlanta, GA.*

K-depletion and hypokalemia impairs urinary concentration ability. It is associated with decreased AQP2, NKCC2 and UT-B, but increased UT-A2 protein abundances in the medulla (Elkjaer et al, *AJP Renal* 283:F1376, 2002; Jung et al, *AJP Renal* 285: F1210, 2003). The P2Y2 receptor plays a significant overarching role in urinary concentration by apparently regulating the protein abundances of AQP2, NKCC2 and UT-A isoforms (Rieg et al, *FASEB J* 21:3717, 2007; Zhang et al, *AJP Renal* 295: F1715, 2008). Hence, we fed P2Y2 receptor knockout (KO) and wild type (WT) mice with normal or K-free diets for 2 weeks (N = 11/genotype/diet) with free access to drinking water. We assessed their sensitivity to K-depletion induced impairment of urinary concentration. There were no significant differences in the dietary K intake between the two groups. K-depleted WT mice showed a significant increase in urine output (5.2-fold) and decrease in osmolality (to 22%), associated with a marked increase in water intake. In K-depleted KO mice, these alterations were significantly less marked (urine output increased to 1.9-fold; urine osmolality decreased to 41%). Interestingly, K-depletion resulted in significant and comparable 136% increases in UT-A1 protein abundance in inner medulla in both groups, but did not alter the protein abundances of AQP1 in either group. However, K-depletion in KO mice caused a smaller decrease in NKCC2 (-28% in KO vs. -72% in WT; $P < 0.02$, N = 4) and AQP2 (-67% in KO vs. -80% in WT; $P$ = NS, N = 4) protein abundances. The apparent preservation of a K-depletion induced increase in UT-A1 coupled with lesser decreases in NKCC2 and AQP2 may be responsible for the observed resistance to polyuria in mice lacking P2Y2 receptor. Thus, these data suggest purinergic signaling may be involved in K-depletion-induced impairment of urinary concentrating ability.

Disclosure of Financial Relationships: Honoraria: JPS Health Network, Fort Worth, TX 76119; Patent: Holds a patent to develop purinergic-based drugs for nephrogenic diabetes insipidus and water balance disorders; Scientific Advisor: Member of editorial boards – American Journal of Physiology (Renal Physiol) and Open Urology and Nephrology (Bentham), member, Faculty of 1000 Biology (Renal Fluid and Electrolyte Physiology).

### SA-FC428

**Regulation of UT-A1 by Phosphatases** Titilayo O. Ilori, Mitsi A. Blount, Jeff M. Sands, Janet D. Klein. *Department of Medicine, Renal Division, Emory University School of Medicine, Atlanta, GA.*

UT-A1 the urea transporter present in the apical membrane of the inner medullary collecting duct (IMCD), are crucial to the kidney's ability to concentrate urine. Phosphorylation of UT-A1 on serines 486 and 499 is key to its ability to traffic to the plasma membrane, since phosphomutants without these sites are unable to traffic and are unable to transport urea. Equally important is the dephosphorylation process and its impact on trafficking and regulation of urea transporter activity. UT-A1 is hyperphosphorylated in IMCDs that are treated with phosphatase inhibitors. We investigated whether calcineurin (protein phosphatase 2B, PP2B) was able to dephosphorylate UT-A1. IMCDs from Sprague Dawley rats were metabolically labeled with $^{32}$P-orthophosphate and treated with calyculin to inhibit protein phosphatases 1 and 2A (PP1, PP2A) or tacrolimus (FK506) to inhibit calcineurin. UT-A1 was immunoprecipitated from the lysates and proteins were separated by SDS-PAGE. Total phosphorylation of UT-A1 was assessed by autoradiography. The phosphorylation per unit protein was assessed with parallel western blots probed for UT-A1. We also probed western blots with a phospho-specific antibody to pSer486-UT-A1 to determine if serine 486 is a site of hyperphosphorylation in response to inhibition of the different phosphatases. Our findings show that the inhibition of PP1 and 2A resulted in overall increased phosphorylation of UT-A1. However, using the phopho-specific antibody, we did not detect any significant increase in phosphorylation at serine 486. Inhibition of calcineurin with tacrolimus, on the other hand showed an increased phosphorylation per unit protein at serine 486 compared with controls or calyculin treatment. We conclude that UT-A1 is a substrate for multiple phosphatases and that the PKA-phosphorylated serine 486 is dephosphorylated by calcineurin. This is the first documentation of the role of phosphatases, and of the specific site of phosphorylation of the urea transporter, in response to treatment with tacrolimus.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC429

**Dissecting Microtubule-Dependent Vasopressin Type 2 Receptor Internalization and Trafficking Using a New High Affinity Fluorescent Vasopressin Ligand** Richard Bouley,[1] Sylvia Chen,[2] Matthew J. Webber,[1] Jean-Pierre Vilardaga,[2,3] Ashok Khatri,[2] Dennis Brown,[1] Dennis A. Ausiello.[1] *[1]Program in Membrane Biology, Nephrology Division, Massachusetts General Hospital, Boston, MA; [2]Endocrine Unit, Massachusetts General Hospital, Boston, MA; [3]Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA.*

The vasopressin receptor type 2 (V2R) is the major target of vasopressin (VP). Understanding molecular mechanisms involved in V2R downregulation is, thus, important for human biology. Previously we showed that VP induces V2R internalization and degradation, as well as accumulation in a perinuclear area. The identification of proteins

modulating V2R intracellular pathways remains elusive. To address this question, we synthesized 9 fluorescent VP analogues tagged by tetramethylrhodamine (TMR) in position 8 of VP. One of these VP-TMR compounds has a high binding affinity (Ki =157±52 nM, n=3) and mediated V2R internalization in LLC-PK1 cells expressing either a FLAG-tagged receptor (FLAG-V2R) or V2R C- terminally tagged with GFP (V2R-GFP). Internalized VP-TMR colocalized with V2R-GFP in the perinuclear area suggesting that the hormone and receptor traffic along the same pathway. VP-TMR and V2R colocalized initially with early endosome markers such as early endosome antigen 1 (EEA1), small G-protein (Rab5), and later, with recycling and late endosome markers such as Rab11 and Rab25. We then investigated the mechanism by which VP-TMR is distributed to these compartments using total internal reflection fluorescence (TIRF) microscopy and real-time spinning disk confocal microscopy on LLC-PK1 cells expressing GFP tagged microtubules (MT). These live cell studies showed that VP-TMR-containing vesicles travel along the MT network, and remain attached to microtubules during metaphase and anaphase of cell mitosis. Colchicine, a MT depolymerizing agent, abolished perinuclear accumulation of VP-TMR, and western blotting showed that VP-induced V2R-GFP degradation is greatly reduced by colchicine (10μM). We conclude that VP induced V2R trafficking and downregulation is a microtubule dependent mechanism.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC430

**Characterization of a Rat Model with Suppressed V2 Vasopressin Receptor Signalling in Thick Ascending Limb and Distal Convoluted Tubule** Kerim Mutig,[1] Michael Bader,[2] Turgay Saritas,[1] Tordis Ida Borowski,[1] Alexander Paliege,[1] Shinichi Uchida,[3] Sebastian C. Bachmann.[1] *[1]Department of Anatomy, Charité Universitätsmedizin, Berlin, Germany; [2]HELIOS Clinics and Max Delbrück Center for Molecular Medicine, Berlin, Germany; [3]Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan.*

Vasopressin (AVP) promotes urinary concentration by activating the vasopressin V2 receptor (V2R). Major expression of V2R was localized to the thick ascending limb (TAL), distal convoluted tubule (DCT), and collecting duct. Inactivating mutations in the V2R gene result in X-linked form of Nephrogenic Diabetes Insipidus (NDI) with loss of renal urine concentrating ability. To discriminate between the pathogenic mechanisms originating from the collecting duct and distal tubule a transgenic rat model with selective overexpression of a dominant-negative mutant of V2R (Glu242 stop; identified in human patients with NDI) in TAL and early DCT under the control of the Tamm-Horsfall protein promoter was generated. Overexpression of the mutated V2R was confirmed by immunohistochemistry and Western blot. Compared to control rats transgenic rats displayed polyuria (+50% at steady state and +250% under water deprivation for 18h; $p<0.05$); urine osmolality was decreased to half in both conditions ($p<0.05$). Kidneys had reduced medullary volume chiefly in inner stripe and revealed fibrotic foci within medullary rays. Transcriptional and protein levels of the major distal ion cotransporters were markedly reduced (NKCC2 -55% and –64% respectively; $p<0.05$) and NCC (-76% and -48%, respectively; $p<0.05$).

These data indicate that suppression of V2R signalling in TAL and early DCT results in impaired urine concentration and therefore contributes to the pathogenesis of X-linked NDI.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC431

**Is Treating Transplant Glomerulopathy Futile?** Jack W. Galliford,[1] Candice A. Roufosse,[2] Vassilis Filiopoulos,[1] Kakit Chan,[1] Michelle Willicombe,[1] Christopher Lawrence,[1] Adam Mclean,[1] H. Terence Cook,[2] David Taube.[1] *[1]West London Renal and Transplant Centre, Imperial College Kidney and Transplant Institute, London, United Kingdom; [2]Department of Histopathology, Imperial College NHS Trust, London, United Kingdom.*

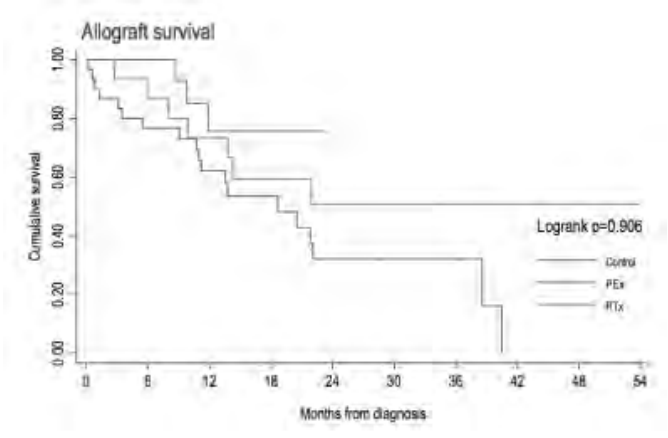
Transplant Glomerulopathy[TG] is a common cause of late allograft failure and there is no established treatment. Small studies have shown that enhanced immunosuppression, including Rituximab[Rtx], may slow progression of TG but with significant adverse events.

This study, the largest to date, describes our medium term experience of 3 treatment strategies for TG; defined after indication biopsy by double contours in gbm on light microscopy(cg1-3)without an immune complex GN or any other clear cause of TMA.

63 patients were diagnosed with TG [36M,27F;mean age 48±12 years].48% had class II DSAb,17% had class I+II and 8% class I alone.40/63 had a urinary PCR≥100;10/63≥300.

30 TG patients[19M,11F]receiving Tac[5-8ng/ml]and MMF[1.2-2.4 mg/l]without steroids acted as a control group. Fifteen patients[6M,9F]had Tac, MMF and Rtx[1g day 0&14]. A further 18 patients[11M,7F]had Tac, MMF and courses of plasma exchange[Pex] with ivIg.

Cumulative allograft survival shows none of the 3 strategies improved allograft survival; 15% overall at 4 years.




Control and Rtx groups had higher creatinines at diagnosis than Pex group [236±80&231±69vs169±53umol/l;p=0.002]but subsequent deterioration was the same in all groups at 3.3umol/l/month. There was no difference in outcome according to graft age, DSAb, proteinuria[logrank=0.63], %IFTA[logrank=0.35] or focal/diffuse C4d[logrank=0.28]. 1/30[3%]patients in control group had a serious infection,3/15[20%] in Rtx group with 1 death from sepsis and 2/18[11%]in Pex group.

This study shows that the addition of Rtx or Pex with ivIg does not alter the outcome of TG and may be associated with a significant risk of serious infection.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC432

**Interstitial Fibrosis Evolution on Early Sequential Routine Renal Allograft Biopsies Using Quantitative Analysis** Aude Servais,[1] Vannary Meas-Yedid,[2] Laure-Helene Noel,[1] Panterne Clarisse,[3] Julien Zuber,[1] Marc-Olivier Timsit,[1] Christophe M. Legendre,[1] Eric Thervet.[1] *[1]Necker Hospital; [2]Institut Pasteur; [3]CENTAURE.*

INTRODUCTION. Renal interstitial fibrosis (IF) is the main histopathological feature of chronic allograft injury (CAI). Whereas currently assessed by semi-quantitative analysis, automatic color image analysis is more reliable and reproducible. The aim of this study was to assess quantitative IF on sequential routine renal biopsies (RB) after transplantation.

METHODS. We analyzed RB performed at day 0 (D0), month (M) 3 and M12 from 141 renal transplant recipients. For each biopsy, stained by light green trichrome, a cortical section was analyzed by a program of color segmentation imaging which automatically extracts green color areas characteristic of IF. Clinical and biological data between D0 and M48 were collected including estimated glomerular filtration rate (eGFR) by MDRD.

RESULTS. Mean IF score is 19±9% at D0, 27±11% at M3 and 34±11% at M12. We observe a progression of 8% from D0 to M3 and of 6% from M3 to M12. Mean eGFR is 57.3±19.5 ml/min/1.73m2 at M3, 56.7±19.2 at M12, and 50.6 ±17.9 at M48. IF at M3 is correlated with eGFR at M3, 12, and 24 ($p<0.03$) and IF at M12 with eGFR at M12 and 48 ($p<0.04$). IF evolution between D0 and M3 (ΔIF D0-M3) is correlated with eGFR at M24, 36, and 48 ($p<0.03$).

IF at M12 is significantly correlated with blood concentration of tacrolimus at M6 and M9 (P=0.05). Risk factors of IF at M12 are donor age (P=0.04), donor sex (female 29% vs male 34%, P=0.02), acute rejection episode (36 vs 30%, P=0.01). ΔIF D0-M3 is correlated with donor age (P=0.01), donor sex (female 5% vs male 10%, P=0.04), delayed graft function (13 vs 6%, P=0.02), acute rejection episode (15 vs 6%, P=0.002), and diabetes (13% vs 7%, P=0.03).

CONCLUSION. Significant IF score is already present before transplantation. Evolution of IF is more important between D0 and M3 vs M3 and M12. Initial quantified IF progression is predictive of 4-year allograft function. IF scoring as a continuous variable allows the use of more sensitive approaches to statistical analysis than semi-quantitative analysis.

Disclosure of Financial Relationships: nothing to disclose

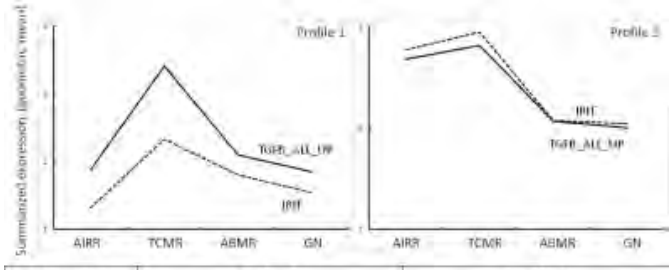
### SA-FC433

**Active Injury Repair Response in Human Kidney Transplants: Elevated Expression of Injury/Repair and Fibrogenesis Genes Associates with Good Recovery of Function and Prognosis** Konrad S. Famulski, Declan G. de Freitas, Philip F. Halloran. *Alberta Transplant Applied Genomics Centre, University of Alberta, Edmonton, AB, Canada.*

Repair of injured tissue reminisces the wound healing, where the pro-fibrotic processes and matrix remodeling play important role. We hypothesized that elevated expression of injury/repair induced transcripts will be accompanied by the emergence of TGFB-inducible transcripts and both will be the feature of healing grafts. Thus we studied expression of these genes and graft function in injured but non-rejecting human kidneys with early ATN (AIRR), in rejecting kidneys (acute TCMR, ABMR) and in non-rejecting with GN. Recovery of function and prognosis was best in the AIRR kidneys and kidneys with TCMR.

| Diagnosis | AIRR (ATN) | TCMR | ABMR | GN |
|---|---|---|---|---|
| Delta eGFR at 6 month post biopsy | 23.7 | 12.5 | -3.1 | -6.7 |
| Graft loss (censored) | 1/28 | 0/23 | 8/17 | 6/24 |

Gene expression studies revealed distinct expression patterns of genes involved in the injury/repair response (in house set: IRIT) and TGFB-inducible genes (MSigDB: TGFB_ALL_UP) across the four groups of transplants.




| Functions and genes | Profile 1 | Profile 2 |
|---|---|---|
| Diseases (IPA) | Cancer | Connective tissue disorder |
| Functions (IPA) | Cellular movement, cell death | Cell assembly and organization |
| Cellular component (GO) | Extracellular matrix | Collagen |
| Correlation with fibrosis | no | no |
| Top genes | C3, CP, MMP7, TNC , VCAN | COL1A1, COL1A2, COL3A1, COL6A3 |

Both profiles represent genes highly expressed in either TCMR or both AIRR and TCMR, but not in ABMR or GN. There was no correlation of the extent of fibrosis with the expression profiles. Thus similar active injury response takes place regardless of underlying insult and includes activation of genes related to cell death, matrix remodeling, TGFB effects and fibrogenesis. Importantly, restrained expression of these genes is observed in high risk kidneys with bad recovery, indicating stunned injury repair response in the presence of persistent stress. Thus elevated expression of injury/repair genes and profibrotic genes, and even of those associated with tissue disorders does not necessary lead to fibrosis and is beneficial in healing kidneys.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC434

**Results of a Prospective Screening Protocol for *De Novo* Donor-Specific Antibody Development after Kidney Transplant** James E. Cooper, Ryan J. Goldberg, Jane Gralla, Laurence Chan, Alexander C. Wiseman. *Renal Division, Transplant Center, University of Colorado at Denver and Health Sciences Center, Denver, CO.*

Background: Development of *de novo* donor-specific antibodies (DSA) after kidney transplantation has been associated with poor long-term outcomes. Our study is the first to utilize a prospective DSA screening protocol to evaluate the timing, characteristics, and clinical outcomes associated with DSA development in transplant recipients.

Methods: All recipients of living or cadaveric kidney or cadaveric kidney/pancreas transplants from 9/07 to 9/09 were screened for class I and II DSA at 1, 6, 12, and 24 months post-transplant, in addition to whenever clinically indicated, using single antigen beads and Luminex® technology (LABScreen beads, One Lamda Inc.). A mean florescence intensity (MFI) of >500 was defined as positive DSA.

Results: Of 246 patients without pre-existing DSA that underwent kidney or kidney/pancreas transplant at our center from 9/07 to 9/09, de novo DSA was detected in 61 (24.8%). 29% of all DSA were class I and 71% were class II. DSA was first detected by protocol screening in 80%, and by clinical suspicion in 20%. In those first detected by protocol, 87% of all new DSA occurred in the first 6 months. Baseline characteristics between DSA (+) and DSA (-) groups were similar. The mean follow up time was 1.5 years. Clinical outcomes are shown in the table below.

Clinical Outcomes in DSA (+) and (-) Groups

| Outcome | All Patients (n=246) | DSA (-) (n=185 ) | DSA (+) (n=61) | P |
|---|---|---|---|---|
| Patient Survival (%) | 98 | 97 | 98 | NS |
| Graft Survival (%) | 96 | 98 | 92 | .05 |
| Rejection (all) (%) | 14 | 9 | 28 | <.001 |
| Rejection (Cellular) (%) | 12 | 9 | 20 | .03 |
| Rejection (Humoral) (%) | 2 | 0 | 8 | <.001 |
| 12 mo MDRD-GFR | 64 | 67 | 59 | .04 |

Conclusions: Prospective screening for de novo donor-specific antibody production in kidney allograft recipients during the first 6-12 months post-transplant can identify patients at risk for poor graft outcomes. These data support the use of prospective DSA screening as a means of risk stratification in kidney transplant recipients.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC435

**Increase in Urinary IL-6 Levels after Kidney Transplantation Indicates Acute Rejection** Jens Gaedeke, Friederike Bachmann, Hans-Hellmut Neumayer, Klemens Budde. *Nephrology, Charité, Universitätsmedizin Berlin, Berlin, Germany.*

Increase in urinary IL-6 levels after kidney transplantation indicates acute rejection. Despite marked improvement in long term kidney transplant survival, acute rejection episodes remain an obstacle to longterm transplant survival.

Diagnosis (biopsy) and treatment can be delayed when the patient is taking oral anticoagulants (warfarin, aspirin, clopidogrel). Interleukin- 6 (IL-6) is a cytokine which is produced by infiltrating cells in the kidney. We investigated whether an increase in urinary IL-6 levels predicted an acute rejection.

We prospectively measured IL-6 levels in urine of 27 patients after de novo kidney transplantation. In addition, we analyzed patients referred from the outpatient clinic because of graft dysfunction (n= 73).

Twenty healthy individuals served as controls. Urine samples were collected as spot urine. All transplant patients received basic immunosuppressive therapy consisting of prednisolone, mycophenolic acid, cyclosporin or tacrolimus and induction with basiliximab.

The mean IL-6 concentration in healthy individuals was 2±4 pg/ml.

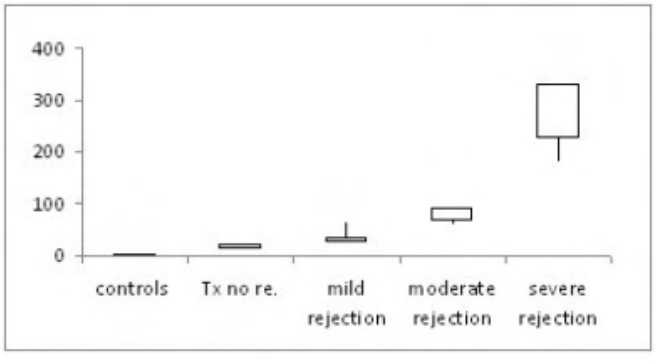
Patients with mild rejection (BANFF Ia) showed lower lower IL-6 concentrations (23,9 ±1,3 pg/ml) compared to moderate acute rejections (BANFF Ib / IIa 15.3±12.9 pg/ml ) and severe acute rejection (BANFF IIb/III, 363±303 pg/m).

After successful rejection treatment IL-6 concentrations returned towards baseline.

In case of therapy resistant rejection elevated IL-6 concentrations persisted.

Patients with non-rejecion-mediated graft dysfunction like CNI-toxicity had consistently low IL-6 concentrations (4.2±3.2 pg/ml).

Elevated urinary IL-6 concentrations are found in acute rejection. Persisting elevations indicated therapy- resistant rejection, while successful rejection treatment reduced IL-6 concentrations to normal. Urinary IL-6 is of diagnostic and prognostic value for acute rejections.




Disclosure of Financial Relationships: Honoraria: Shire.

## SA-FC436

**Protocol Biopsy Findings in Kidney Transplant Recepients in the Era of Modern Immunosuppression** Viresh Mohanlal, Emilio Ramos, Joseph M. Nogueira, David K. Klassen, Matthew R. Weir, Abdolreza Haririan. *Nephrology, University of Maryland Medical Center, Baltimore, MD.*

**PURPOSE**: The clinical utility of protocol biopsies in kidney transplant recipients remains controversial. The purpose of this study was to examine the prevalence of subclinical rejection and other histological features in renal allograft biopsies at 3 mo and 12 mo post-transplant with modern immunosuppresion (IS), and to assess the progression of histological changes within first year after transplant.

**METHODS:** We studied 114 patients who underwent protocol biopsies at 3.9±1.1 mo and 12.7± 2.7 mo. Biopsy findings were scored according to Banff 2005 criteria. Tacrolimus, MMF and early steroid withdrawal were used for maintenance IS. Biopsies performed for cause were not included in the analysis.

**RESULTS:** Baseline characteristics are summarized in Table 1.

Baseline characteristics

| | |
|---|---|
| Age (yrs) (mean ± SD) | 51.8 ± 13.0 |
| African American (%) | 61(53.5) |
| Male (%) | 70 (61.4) |
| Diabetes Mellitus (%) | 50 (43.8) |
| Preemptive transplant (%) | 39 (33.7) |
| Deceased donor (%) | 85 (74.6) |
| Alemtuzumab (%) | 63 (55.8) |
| Basiliximab (%) | 37 (32.7) |
| Thymoglobulin (%) | 13 (11.5) |
| PRA >40% | 14 (15.2) |
| HLA mismatch (mean ± SD) | 4.3± 1.6 |

Mean creatinine at 3 and 12 mo was 1.7 and 1.9 mg/dl respectively. The incidence of subclinical rejection was 15% at 3 mo and 9% at 12 mo respectively. IF/TA was noted in 60% of patients at 3 mo and 65% at 12 mo, with severe IF/TA in 5% at 3 and 12 mo. IF/TA progressed in 20% of patients and arteriolar sclerosis and hyalinosis progressed in 19% and 9% respectively. Glomerulitis was noted in 5 patients at 3 mo and 4 at 12 mo. 4

patients at 3mo and 3 at 12 mo had diffuse PTC C4D and DSA positivity. Interestingly. Transplant glomerulopathy was evident in 1 patient at 3mo and in 2 at 12mo. BKN was found in 2 patients at 3 mo and 12mo.

**CONCLUSIONS:** Our experience shows that even with modern IS, protocol biopsies reveal significant subclinical histological changes which could guide adjustment in maintainance IS. Further studies are needed to examine the impact of biopsy-guided IS modulation on long term renal allograft survival.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC437

**Complement Fixing Donor Specific Class II Antibodies Predict a High Rate of Rejection Episodes in Renal Retransplantation Patients** Anil Chandraker, Isabelle G. Wood, Nabil Mohsin, Nidyanandh Vadivel, Edgar L. Milford. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Aim: Retransplanted patients are at high risk for rejection. One known risk factor is the presence of anti-HLA antibodies. We investigated whether donor specific HLA antibodies (DSA) which bind complement predict rejection in these patients.

Method: We analyzed the likelihood of rejections during the first year in 103 renal retransplant patients between 1993 and 2000 in 13 centers in New England. Pretransplant sera were analyzed by standard solid phase screening for DSA class I and II (Luminex, One Lambda). Furthermore, we modified the single antigen (SA) solid phase screening technique (CmDSA I & II) to detect human complement binding. CmDSA was performed with the addition of a human complement source followed by mouse anti-C4d and anti-mouse phycoerythrin. The T cell anti-human globulin enhanced cytotoxic crossmatch (CDC) at the time of transplantation was the sole exclusionary test for transplantation. Rejection was defined as clinical or biopsy proven. Early loss of a previous transplant (PEL) was defined as graft failure within 3 months. Kaplan-Meier, Log-Rank was used for statistical analysis.

Results: Retranplant patients had significantly higher rates of rejection when their pretransplant sera contained complement binding anti-HLA class II (CompDSA II). Such difference was not noted for the CmDSA I.

% Likelihood of Rejection Episode at 1 Year

| | TFXM (+/-) | BFXM (+/-) | DSA ClassI (+/-) | DSA ClassII (+/-) | Cm DSA I (+/-) | Cm DSAII (+/-) |
|---|---|---|---|---|---|---|
| ALL (N=103) | 69/37* | 66/32** | 47/33* | 48/45* | 38/48 | 67/32* |
| PEL (N=41) | 100/41** | 89/36** | 52/42 | 66/36 | 50/50 | 89/40 |
| non-PEL (N=57) | 49/31 | 43/55 | 37/30 | 37/31 | 30/41 | 46/30 |

PEL:Previous early graft loss; **:p<0.01; *:p<0.05

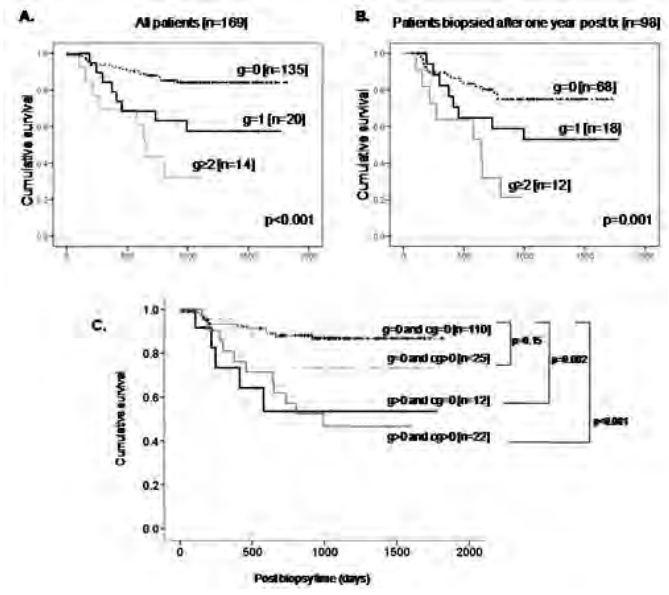
Conclusions: The modified CmDSA II may be useful in predicting increased risk of rejection in candidates for renal transplantation. Lack of significance in PEL patients p<.07 is likely due to small numbers. The CmDSA I was not significant most likely due to the exclusion of patients by CDC T cell crossmatches. The Flow crossmatch results TFXM and BFXM assay are also predictive of rejection.

Disclosure of Financial Relationships: Consultancy: T2 BiosystemsResearch Funding: Dialysis Clinic Incorporated; Other Relationship: Novartis – DSMB.

### SA-FC438

**Glomerulitis: Stronger Indicator of Molecular Rejection Than Tubulitis or Vasculitis, and Stronger Predictor of Outcome Than C4d or Transplant Glomerulopathy** Banu Sis,[1] Gian S. Jhangri,[1] Julie Riopel,[1] Jessica Chang,[1] Michael Mengel,[1] Declan G. de Freitas,[1] Luis G. Hidalgo,[1] Gunilla Einecke,[2] Bruce Kaplan,[3] Philip F. Halloran.[1] *[1]University of Alberta, Edmonton, AB, Canada; [2]Medizinishe Hohschule, Hannover, Germany; [3]University of Arizona, Tucson, AZ.*

The significance of glomerulitis in kidney transplants and its relationship to diseases remain obscure. We related glomerulitis to whole-genome microarrays, histopathology, and survival in 221 renal allograft biopsies for cause from 169 patients with a median follow-up of 32.1 months. We investigated the relationship between glomerulitis and probability of rejection assigned by a microarray-classifier. Glomerulitis was more frequent in late biopsies (>1 year), and in antibody-mediated rejection (ABMR), either C4d-positive (71%) or negative (52%), than in T cell-mediated rejection (TCMR) (25%) or other diseases (6-18%). IFNG-regulated transcript expression, capillaritis, and transplant glomerulopathy (TG) independently correlated with glomerulitis. Glomerulitis predicted poor survival if accompanied by alloantibody (C4d+/-), but not TCMR. Glomerulitis +/- TG predicted poor outcome, but TG without glomerulitis did not.




In a classification tree model, interstitial inflammation was the strongest predictor of molecular rejection and followed by glomerulitis within any given degree of interstitial inflammation, but not tubulitis or vasculitis. In multivariate logistic regression, interstitial inflammation (OR:3.01) and glomerulitis (OR:2.68) showed higher risk of molecular rejection compared to tubulitis (OR:1.79) or vasculitis (OR: 2.42). We conclude that glomerulitis is a stronger predictor of rejection than tubulitis or vasculitis and is better than C4d or TG to predict graft outcomes.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC439

**De Novo Donor Specific Antibodies Are Associated with Antibody Mediated Rejection in Patients Receiving Alemtuzumab Induction** Michelle Willicombe, Paul Brookes, Eva Santos, Jack W. Galliford, Adam Mclean, Candice A. Roufosse, Tom Cairns, David Taube. *Imperial College Kidney and Transplant Institute, London, United Kingdom.*

De novo donor specific antibodies [DSAbs] post renal transplant have been shown to be associated with rejection, allograft dysfunction and loss, in studies conducted in patients receiving a variety of induction and maintenance immunosuppressive agents with no reports referring to the use of Alemtuzumab. In this study we describe the significance of de novo DSAbs in patients receiving Alemtuzumab induction and Tacrolimus monotherapy.

435 patients [f:m=141:294, mean age: 47.44±13.14 yrs, mean HLA MM: 3.26±1.66, 1st graft: regrafts = 400:35] who were crossmatch and DSAb negative at the time of transplantation were included. Patients were screened for the development of DSAb post transplant using single antigen beads. Mean follow up was 1.69±1.16 yrs.

131/435 [30.11%] patients developed de novo anti-HLA antibodies post transplant, of which 78/435 [17.93%] developed DSAbs. Patient survival at 3 years was similar in the DSAb+ and DSAb- groups [98.0% vs 96.2%, p=0.42 (log rank)]. Patients with de novo DSAb were at increased risk of graft loss [RR DSA+ 3.5, p=0.0045]. 27/78 [34.62%] of DSAb+ patients experienced an episode of AMR. The relative risk of AMR following the development of DSAb was 17.13, p<0.0001. Allograft function was inferior in the DSAb+ group with a mean MDRD GFR at 12 months of 52.86 ±19.83mls/min and 58.49 ±17.51mls/min in the DSAb+ and DSAb- respectively [p=0.037].

Patients who developed both de novo anti-HLA Class I and Class II DSAb were at highest risk of developing AMR [RR 3.34, p=0.01] when compared with DSAb negative patients. Recipients who developed a DSAb with a mean fluorescence index [MFI] >1000 were also at higher risk of developing rejection [RR 1.74, p=0.01] when compared to patients with a DSAb with a MFI of <1000.

In patients receiving Alemtuzumab induction, the development of de novo DSAb is not uncommon and is associated with increased risk of AMR and inferior allograft survival. Patients should be screened post transplant for the presence of de novo DSAb and may benefit from augmented immunosuppression.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC440

**Pulsatile Preservation Reduces Apoptosis in a Porcine Model of Kidney Donation after Cardiac Death** Alkesh Jani, Jessica Martin, Kultigin Turkmen, Kameswaran Ravichandran, Danica Ljubanovic, Charles L. Edelstein. *U of Colorado.*

DCD kidneys suffer a high incidence of DGF due to warm ischemia (WI) and cold ischemia (CI). Clinical studies suggest pulsatile preservation (PP) of DCD kidneys may reduce injury. The mechanism of protection of PP is unknown. In a porcine model of DCD, we hypothesized that DCD kidneys have increased caspase-1 due to WI and increased caspase-3 and apoptosis due to CI. **Methods:** Male Yorkshire pigs subjected to cardiac death

were perfused with cold UW solution. Perfused kidneys were removed and stored in cold UW for 24 hrs. Kidney biopsies were obtained before cardiac death and at 0 and 24 hrs of CI. Apoptotic tubular cells/hpf were quantitated by a renal pathologist. Caspase activity was measured using fluorescent substrates. **Results:** Caspase-1 activity due to WI before cold preservation was significantly elevated vs normal (88.3±6.3 vs 59.3±3.6, $p<0.01$, n =5). CI was associated with a massive increase in apoptosis and caspase-3/7 activity.

| Time | 0 hrs | 24 hrs |
|---|---|---|
| Apoptotic tubular cells/hpf | 0.3±0.1 | 8.2±5.9* |
| Caspase 3/7 activity (nmol/min/mg) | 90.5±13.4 | 239.3±104.5* |

n=5; *P < 0.005 vs 24 hr

By immunoblot we determined that active caspase-3 was responsible for the caspase activity.Next we hypothesized that PP would protect against apoptosis.We compared DCD kidneys subjected to static preservation (SP) vs PP for 24 hrs CI. PP significantly reduced proximal tubular apoptosis and was associated with significantly increased anti-apoptotic BCL-XL and HIF-1α.Caspase-3/7 activity was not significantly different between SP and PP.

| | SP | | PP | |
|---|---|---|---|---|
| Time | 0 hrs | 24 hrs | 0 hrs | 24 hrs |
| Apoptotic tubular cells/hpf | 0.2 ±0.1 | 8±2.5 | 0.1 ±0.1 | 2.5 ±1.4* |
| Caspase 3/7 activity (nmol/min/mg) | 84.3 ±20.5 | 453.3 ±307.7 | 88.5 ±33.8 | 466.3 ±234.1 † |
| HIF-1 α activity | 283.8 ±14.7 | 345.9 ±28.2 | 301.8 ±12.6 | 494.4 ±23.4* |
| BCL-$X_L$ immunoblot | + | + | + | ++ |

n=5; *P < 0.05 vs 24 hr SP; †p = NS vs 24 hr SP

**Conclusion:** These findings suggest that in DCD kidneys, WI preferentially activates caspase-1 whereas CI activates caspase-3 and causes apoptosis. PP may protect DCD kidneys via activation of anti-apoptotic pathways involving BCL-XL and HIF-1α.

Disclosure of Financial Relationships: nothing to disclose

### SA-FC441

**Conversion to an Everolimus/Enteric-Coated Mycophenolate Sodium Regimen after Calcineurin Inhibitor Withdrawal in *De Novo* Renal Transplant Patients Improves Renal Function: 2 Years Follow-Up of the ZEUS Trial** Wolfgang Arns,[1] Thomas Becker,[1] Klemens Budde,[1] Ute Eisenberger,[1] Wolfgang Fischer,[2] Stefan Kramer,[2] Claudia Sommerer,[1] Petra Reinke,[1] Frank B. Pietruck.[1] *[1]ZEUS Study Group; [2]Novartis Pharma, Nuremberg, Germany.*

**Objective** In d*e novo* kidney allograft patients (pts) renal function, efficacy and safety was assessed after conversion to an Everolimus/Enteric-coated mycophenolate sodium (EC-MPS) regimen after Cyclosporine (CsA) withdrawal at M24 post-transplantation (tx)

**Methods** In this prospective, open-label, controlled, multi-center study renal allograft pts were randomized to a regimen consisting of either Everolimus/EC-MPS or CsA/EC-MPS at M4.5 after tx. After completion of the core study at M12, pts were included in an observational 12 Month follow-up study.

**Results** 300 pts were randomized to either Everolimus(n=155) or CsA(n=145), 258 (86.0%) pts completed the M24 visit. Renal function expressed as cGFR (Nankivell method) was similar in both groups at M4.5 post-Tx with an improvement by 7.84 mL/min in favor of the Everolimus regimen (p=0.0042) at M24 (61.3±17.2 vs. 69.2±19.0 mL/min). The observed GFR slope from M4.5 to M24 was +6.5[3.1,10.0] for Everolimus and -1.1[-4.8,2.5] mL/min for CsA pts. Similarly GFR slopes with MDRD(+9.0[4.2,13.8]) and Cockcroft-Gault formular (+6.7[3.2,10.3]) were significantly better ($p<0.001$) in the CNI-free regimen (CsA treated pts: MDRD: -1.3[-6.1,3.6]mL/min; Cockcroft-Gault: -1.4[-5.2,2.4]mL/min). Fewer pts in the Everolimus group had a decline of GFR compared to CsA pts (Nankivell: 31.7% vs 53.4%; p=0.0007). BPAR was reported in 17(11.0%) Everolimus vs. 7(4.8%) CsA pts between M4.5 and M24. After M12 two additional BPAR occurred in each group. Three death and one graft loss was observed in the CsA, none in the Everolimus group. Number of pts with infections (Everolimus 35(22.6%) vs. CsA 31(21.4%)) and hospitalization (Everolimus 45(29.0%) vs. CsA 52(35.9%)) in the follow-up period (M12-M24) was comparable.

**Conclusions** The conversion to Everolimus in *de novo* renal transplant pts after CNI withdrawal early after tx significantly maintains renal function over a period of 24 months without compromising efficacy and safety

Disclosure of Financial Relationships: nothing to disclose

### SA-FC442

**Safety Profile of Belatacept in Kidney Transplant Recipients from a Pooled Analysis of Phase II and Phase III Studies** Josep Grinyo,[1] Bernard Charpentier,[2] Jose Medina-Pestana,[3] Yves Vanrenterghem,[4] Flavio Vincenti,[5] Rebecca Shi,[6] Mamta Agarwal,[7] Dolca Thomas,[8] Christian Larsen.[9] *[1]Univ Hosp Bellvitge; [2]Bicetre Hosp; [3]Hosp do Rim; [4]Univ Hosp Leuv; [5]UCSF; [6]BMS; [7]BMS; [8]BMS; [9]Emory Univ.*

**Purpose:** This report focuses on safety outcomes of belatacept vs cyclosporine (CsA) in adult kidney transplant recipients.

**Methods:** Patients (pts) in 3 core studies (1 Phase II, 2 Phase III - BENEFIT & BENEFIT-EXT) were randomized to a more intensive (MI) or less intensive (LI) regimen of belatacept or CsA; all pts received basiliximab induction, and maintenance therapy with MMF and steroids. Safety data through 7/2009 from the 3 studies were pooled for the analysis.

**Results:** 1425 pts were included in the analysis (MI=477; LI=472; CsA=476). Median follow-up was ~2.4 yrs with some pts followed for ~7 yrs. Incidence of death (MI:7%; LI:5%; CsA:7%) and serious adverse events (MI:71%; LI:68%; CsA:69%) was lowest in the LI group. Overall incidence of malignancies remained low, but was slightly higher in the MI group (MI:10%; LI:6%; CsA:7%). Across the 3 studies, 15 PTLD cases occurred (n=8 MI; n=5 LI; n=2 CsA) including 8 involving the CNS (n=6 MI; n=2 LI). Excess PTLD risk was concentrated in EBV(-) recipients and in the MI group. No PTLD cases occurred after 18 mo in the belatacept groups. Frequency of serious infections was similar (MI:37%; LI:32%; CsA:36%). Rates of polyoma (MI:7%; LI:3%; CsA:6%) and fungal infections (MI:22%; LI:17%; CsA:21%) were lower in the LI vs MI or CsA groups. One case of progressive multifocal leukoencephalopathy was reported (MI group). Rates of herpes infections were higher with belatacept (MI:15%; LI:13%; CsA:10%). Tuberculosis occurred in 10 pts (n=5 MI; n=4 LI; n=1 CsA); mostly in endemic areas. No anaphylaxis or hypersensitivity reactions to belatacept were reported.

**Conclusions:** Longer-term treatment with belatacept-based regimens was generally safe. PTLD, including CNS presentation, was higher in belatacept vs CsA, especially in EBV(-) pts and the MI group. Incidence of death and serious infections was lowest in the LI group. The overall safety analysis favored the LI over MI regimen.

Disclosure of Financial Relationships: Scientific Advisor: BMS advisory board member.

### SA-FC443

**Belatacept vs Cyclosporine in Kidney Transplant Recipients: Two-Year Outcomes from the BENEFIT Study** Christian Larsen,[1] Josep Grinyo,[2] Bernard Charpentier,[3] Jose Medina-Pestana,[4] Nassim Kamar,[5] Yves Vanrenterghem,[6] Chen-Sheng Lin,[7] Gregory Dirusso,[7] Pushkal Garg,[7] Flavio Vincenti.[8] *[1]Emory Univ School of Medicine; [2]Univ Hosp Bellvitge; [3]Bicetre Hosp; [4]Hosp do Rim; [5]Univ Hosp Toulouse; [6]Univ Hosp Leuven; [7]BMS; [8]UCSF.*

**Purpose:** To report the efficacy and safety of belatacept vs cyclosporine (CsA) in adult kidney transplant recipients after 2 yrs of treatment in the BENEFIT study.

**Methods**: BENEFIT is a 3-yr, randomized, phase III study in adults receiving a kidney transplant from a living or standard criteria deceased donor. Patients were randomized 1:1:1 to a more intensive (MI) or less intensive (LI) regimen of belatacept or CsA; all patients received basiliximab induction, MMF, and corticosteroids.

**Results**: 666 patients were randomized and transplanted (ITT population); 493 completed 2 years on treatment (n=164 MI; n=176 LI; n=153 CsA). At Yr 2, patient/graft survival was similar across groups (94% MI; 95% LI; 91% CsA). The renal benefit of belatacept was sustained through Yr 2 as evidenced by a 15–17 mL/min higher measured GFR ($p<0.0001$ MI or LI vs CsA) or calculated GFR in the belatacept groups vs CsA. Eight patients experienced an episode of AR between Yrs 1 and 2 (n=4 MI; n=4 CsA). Improvements in the CV and metabolic risk profile seen in Yr 1 with belatacept vs CsA were sustained and an additional beneficial effect on LDL-cholesterol emerged at Yr 2 ($p\leq0.002$ MI or LI vs CsA). The overall incidence rate of malignancies and serious infections remained comparable across groups. Two cases of PTLD (previously reported) occurred between Yr 1 and Yr 2 in the MI group (total cases through July 2009: n=3 MI; n=2 LI; n=1 CsA). The overall safety profile remained similar across groups.

**Conclusion**: At 2 yrs, a belatacept-based regimen demonstrated sustained better renal function and similar patient/graft survival vs CsA. No incremental efficacy was gained by using the MI vs the LI regimen. No new safety signals emerged. Belatacept is a promising therapeutic option in kidney transplant patients.

Disclosure of Financial Relationships: Research Funding: Clinical trials supported by BMS and Genentech; Scientific Advisor: Consultant BMS advisory board.

### SA-FC444

**Belatacept vs Cyclosporine in ECD Kidney Transplants: Two-Year Outcomes from the BENEFIT-EXT Study** Antoine Durrbach,[1] Christian Larsen,[2] Jose Medina-Pestana,[3] Yves Vanrenterghem,[4] Flavio Vincenti,[5] Sander Florman,[6] Jun Xing,[7] Alan Block,[7] Pushkal Garg,[7] Josep Grinyo.[8] *[1]Bicetre Hosp; [2]Emory Univ; [3]Hospital do Rim; [4]Univ Hosp Leuven; [5]UCSF; [6]Mount Sinai Med Cntr; [7]BMS; [8]Univ Hosp Bellvitge.*

**Purpose**: To report the efficacy and safety of belatacept vs. cyclosporine (CsA) after 2 yrs of treatment in the BENEFIT-EXT study.

**Methods**: BENEFIT-EXT is a 3-yr, randomized, phase III study in adults receiving ECD kidney transplant. Patients were randomized 1:1:1 to a more intensive (MI) or less intensive (LI) regimen of belatacept or CsA; all pts received basiliximab induction, MMF, and corticosteroids.

**Results**: 543 pts were randomized and transplanted (ITT); 347 completed 2 yrs on treatment (MI=116, LI=119; CsA=112). Patient/graft survival was similar across groups (83% MI, 84% LI, 83% CsA) at 2 yrs. The renal benefit of belatacept was sustained at Year 2 as assessed by the measured GFR (52 mL/min MI, 50 mL/min LI, and 45 mL/min CsA; p=0.028 MI vs CsA; p=0.108 LI vs CsA) and by the calculated GFR (8-10 mL/min higher in the belatacept groups vs CsA). Three additional episodes of acute rejection occurred after between Yr 1 and Yr 2 (n=1 LI; n=2 CsA). The CV and metabolic risk profile benefits of belatacept vs CsA on serum lipids and blood pressure were sustained. The overall incidence rates of malignancies and serious infections remained comparable across groups. Two previously reported cases of PTLD occurred between yrs 1 and 2 (n=1 MI; n=1 LI; total cases through July 2009 in BENEFIT-EXT: n=2 MI; N=3 LI; n=0 CsA). The overall safety profile remained similar across groups.

**Conclusions**: A belatacept-based regimen maintained better renal function, a better cardiovascular/metabolic risk profile, and similar patient/graft survival vs CsA at 2 yrs. No incremental efficacy was gained by using the MI vs LI regimen. No new safety signals emerged. Belatacept is a promising option in pts receiving ECD kidneys.

Disclosure of Financial Relationships: Research Funding: Investigation grants from Wyeth; Scientific Advisor: Ad boards for: Novartis, Wyeth, Astellas, Roche.

## SA-FC445

**ABO Incompatible Renal Transplantation with Conventional Immunosuppression Alone** Rosemary Masterson. *Nephrology, Royal Melbourne Hospital, Melbourne, Victoria, Australia.*

INTRODUCTION

We & others have reported successful ABO incompatible renal transplantation (ABOi) using conventional immunosuppression and removal of anti-blood group antibodies. We report outcomes of 17 patients undergoing ABOi using conventional immunosuppression without antibody removal, including a range of donor/recipient blood group incompatibilities.

METHODS

Patients with low anti-blood group antibody titres at baseline (diamed gelcard ≤ 8 (median 4), orthomed ≤ 32 (median 8), tube ≤ 128 (median 32)) were included in the study. Immunosuppression consisted of Mycophenolate Mofetil (MMF) initiated at Day 7 pre transplant, Basiliximab induction, Tacrolimus & Prednisolone. Median Tacrolimus levels were 7ng/ml, 6ng/ml and 4ng/ml at 1,3 and 12mths respectively. Prednisolone was tapered to 5mg and MMF to 1-1.5g per day by 3 months. No patients had antibody removal, splenectomy or Rituxumab therapy.

RESULTS

17 patients met study criteria with a median follow up of 18 months (1 week-33 months) post transplantation. Incompatibilities were B to A (n=7), $A_1$ to O (n=3), B to O (n=2), A1 to B (n=2), $A_2$ to O (n=1), AB to A (n=1), AB to A (n=1).

Excellent (100%) patient & graft survival was observed. Median serum creatinine: 98umol/l (80 -132 umol/l); median eGFR 66mls/min (48 - 91mls/min). One episode of subclinical borderline cellular rejection was found on 3 month protocol biopsy with no episodes of antibody mediated rejection being detected. Protocol biopsies performed on 14 patients at 3 and 12 months respectively showed all to be histopathologically C4d negative with no evidence of transplant glomerulopathy.

No opportunistic infections or malignancies were diagnosed and only one patient developed diabetes post transplantation.

CONCLUSION: Excellent patient and graft outcome can be achieved in selected patients with low titre anti-blood group antibodies using standard immunosuppression alone. In addition to cost saving benefits, this strategy reduces the risk of over immunosuppression in this selected patient cohort.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC446

**Silent Development of *De Novo* Anti HLA Antibodies Following Transplantation Is Associated with Histological Changes in the Allograft. Interim Report on Behalf of the CTOT-02/CCTPT-02 Study** Sacha A. De Serres,[1] Bechara G. Mfarrej,[1] Indira Guleria,[1] Nader Najafian,[1] Yvonne R. Morrison,[3] Flavio G. Vincenti,[2] William E. Harmon,[1] Mohamed H. Sayegh,[1] Anil Chandraker.[1] *[1]Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital Boston, Harvard Medical School, Boston, MA; [2]UCSF, San Francisco, CA; [3]NIH/NIAID/DAIT, Bethesda, MD.*

The clinical relevance of monitoring solid organ recipients for the development of anti-HLA alloantibodies (Abs) is unknown and the importance of class I *vs.* II Abs remains unclear. Over 650 subjects have been enrolled in the screening phase of the NIH CTOT-02/CCTPT-02 study, a multi-center prospective trial where unsensitized kidney transplant recipients are screened for development of *de novo* anti-HLA Abs post transplant. The aim of this report is to look at possible associations between Abs and gender, donor type, induction, HLA mismatch (MM) and DGF and correlate development with concurrent histology. Abs were detected by Luminex and the analysis was performed using log-rank statistics and univariate cox model.

49 subjects developed Abs: 22, 35 and 8 subjects developing class I, class II or both class I & II Abs. Unexpectedly, among the 75 zero MM subjects, 6 developed Abs, but only against class I (HR 2.9; p=0.03 *vs.* at least 1MM). Female gender was associated with class II conversion (HR 1.9; p=0.05). There was a trend for an association between class II conversion and the use of any induction therapy (p=0.09). This association was not confounded by MM status; 94% of subjects with zero MM received induction therapy, similarly to MM subjects. There was no association between Ab development and donor type or DGF. Biopsy results were available for 12 subjects prior to randomization to the treatment phase of the study. 5/12 showed evidence of borderline/acute rejection; all positive for class II, none for class I Abs (p=0.01).

This interim analysis of CTOT-02 suggests important differences in the baseline characteristics and the clinical impact associated with anti-HLA Abs. Longitudinal and mechanistic studies are underway to characterize the immunological profile and clinical outcome post-Ab conversion.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC447

**The Influence of Co-Administered Omeprazole on the Bioavailability of Mycophenolate Mofetil (MMF) in Healthy Volunteers** Frieder Kees,[1] Korbinian Johannes Rupprecht,[1] Stefan Moritz,[2] Thomas Steinke,[2] Michael Bucher,[2] Lothar Faerber.[1,3] *[1]Dept. of Pharmacology, University of Regensburg; [2]Clinic of Anesthesiology and Surgical Intensive Care, University Hospital Halle; [3]Novartis Pharma, Nuremberg, Germany.*

**Aim** Prior studies showed co-administered pantoprazole decreases the bioavailability of mycophenolic acid (MPA) from MMF in healthy volunteers and in renal transplant patients. The aim was to investigate the interaction with omeprazole (OME), another member of proton pump inhibitors (PPIs), and to elucidate the mechanism of the drug-drug interaction

**Methods** In a cross-over study 12 healthy volunteers (6m/f) received one dose of 1000mg MMF with and without OME (20mg bid 4 days prior to MMF intake). Blood was sampled up to 48h and MPA concentrations were measured. PK parameters were evaluated for differences using the paired Student's t-test ($P<0.05$ significant) and treatments were tested for bioequivalence. Dissolution of MMF 500mg tablets was determined using an USP paddle apparatus (37°C, 50rpm) in 900ml aqueous buffer of pH1-7 and measured spectrophotometrically

**Results** *In vitro*, the dissolution of MMF decreased from 100% at pH1-4 to 47% at pH5, 25% at pH5.5, 15% at pH6 and 13% at pH7, respectively. Co-treatment of MMF with OME led to a significant decrease in MPA exposure. Mean $c_{max}$ of MPA decreased by 50% and $AUC_{12h}$ by 35%. Bioequivalence was not proven neither for $c_{max}$ nor for $AUC_{12h}$ of MPA. Due to remarkable enterohepatic cycling of MPA, resulting in long-lasting low MPA plasma concentrations, $AUC_{48h}$ were 10% lower and $AUC_{\infty}$ plus $c_{12h}$ were not altered.

| | MMF/OME | MMF | p |
|---|---|---|---|
| cmax(µg/ml) | 10.5±3.6 | 21.7±9.9 | 0.001 |
| tmax(h) | 1.2±0.5 | 0.6±0.2 | 0.003 |
| t1/2(h) | 17.7±5.4 | 12.2±2.8 | 0.006 |
| AUC12h(µg/ml*h) | 25.8±6.4 | 40.0±7.8 | 0.024 |
| AUC48h(µg/ml*h) | 51.3±17.7 | 56.0±16.8 | 0.052 |
| AUC∞(µg/ml*h) | 62.4±29.0 | 60.3±19.3 | 0.61 |
| c12h(µg/ml) | 1.1±0.5 | 1.0±0.4 | 0.54 |

**Conclusions** PPIs increase the intragastric pH resulting in poorer dissolution and reduced absorption of MMF. Administration of MMF with OME might result in insufficient MPA exposure in patients requiring dose adjustment

Disclosure of Financial Relationships: Research Funding: Novartis Pharma, Nuremberg, Germany; Honoraria: Novartis Pharma, Nuremberg, Germany.

## SA-FC448

**Cardiovascular Medication Use in Kidney Transplant Recipients** Eric D. Weinhandl,[1] Wendy L. St. Peter,[1,2] Jon J. Snyder,[1] Melissa Skeans,[1] Bertram L. Kasiske.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]University of Minnesota, Minneapolis, MN.*

**Background:** Pharmacological therapy for hypertension and hyperlipidemia in kidney transplant (tx) recipients may result in improved outcomes. Use of therapy is not well-characterized. **Methods:** We used data from the United States Renal Data System, including Medicare Part D event claims, to assess use of anti-hypertension (anti-HTN) agents (angiotensin-converting enzyme inhibitor [ACE-I] or angiotensin receptor blocker [ARB], beta blocker [βB], dihydropyridine calcium channel blocker [DP-CCB], diuretic) and lipid lowering (LL) agents (ezetimibe, fibrate, statin, other agent) during the first 6 mo post-tx. We analyzed adult US patients who received a kidney-only tx between Jan 1 and Jun 30, 2007, and who survived without graft failure and carried Part D coverage for 6 mo post-tx (N=3123). **Results:** Overall, 88% took ≥1 anti-HTN agent: 24, 73, 60, and 43% took an ACE-I/ARB, a βB, a DP-CCB, or a diuretic, respectively. Regarding combinations, 70% took at ≥2 agents, while 34% took ≥3 agents. Predictors of anti-HTN agent use included older age at transplant (odds ratio, 1.01 per yr), African-American (AA) *vs.* white race (1.33), both diabetes and HTN *vs.* other as primary end-stage renal disease (ESRD) cause (1.44 and 1.58, respectively), and living-donor status (0.76). Overall, 40% took ≥1 LL agent: 5, 3, 37, and 1% took ezetimibe, a fibrate, a statin, or another agent, respectively. Predictors of LL agent use included older age (1.03 per yr), AA *vs.* white race (0.74), and diabetes *vs.* other as primary ESRD cause (1.47). In those patients with 6 mo pre-tx Medicare coverage (N=2384), ischemic heart disease was positively associated with subsequent LL agent use (adjusted odds ratio, 1.61). **Conclusions:** Most recipients take at least one anti-hypertension agent post-transplant, and many take multiple; use of beta and calcium channel blockers is most common. In contrast, less than half of recipients take at least one lipid lowering agent; use of statins is predominant. Further research is needed to assess whether anti-hypertension regimens may be simplified without loss of efficacy, and whether use of lipid lowering agents is sub-optimal.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC449

**Cyclosporine (CYA) Very Low Dose with Everolimus (E) High Dose Is Associated with Better Outcomes in Renal Transplant Patients with Respect to Standard Treatment with EC-MPS (M)** Elisabetta Bertoni, Maurizio Salvadori. *Renal Unit, Careggi University Hospital, Florence, Italy.*

Aim of this study was to compare in cadaveric renal transplant recipients the efficacy and safety of CyA "very low dose" with everolimus "high dose"respect to CyA standard dose with EC-MPS.

In a randomized, prospective, monocenter, open study, patients were enrolled to receive either everolimus (C0 8-10 ng/ml) + CyA (C2 250-300 ng/ml) + steroids or EC-MPS (1440/day) + CyA (C2 500-700 ng/ml) + steroids. 56 patients were enrolled in group everolimus, 50 in group EC-MPS. Efficacy was eveluated at 24 months.

106 patients have been enrolled. After 2 years 41 EC-MPS patients and 46 E patients were evaluated.Biopsy proven acute rejection rate was higher in standard CyA therapy 24% vs 18.8% in CyA very low dose therapy (RR=1.4). CMV infection rate was 27% vs 26%. The lenght of first period hospitalization was similar (24.67±12.20 vs 24.77±11.13 days). We observed a trend towards a better 2-year graft survival rate in patients with CyA very low dose (95% vs 85%; p=ns). Cya dose at 2-year was lower in E group (1.35±0.49 vs 2.3±0.64 mg/kg, p<0.0001). At 2-year eGFR (Cockcroft-Gault) was higher in Everolimus group (77.78±31.9 vs 54.76±18.76 ml/min). The 2-year systolic blood pressure was lower in Everolimus group (125.3±13.96 vs 129.8±16.23 mmHg, p=0.03). The 2-year serum cholesterol levels were higher in everolimus group (229.7±47.08 vs 206.7±42.2 mg/dL, p<0.01), even if everolimus patients used more statins (RR=1.72). 24 hours proteinuria was low and similar in both groups (387.6±42.5 in E vs 409.3±76.8 mg/24 hours in EC-MPS, p=ns).

Examining our population per protocol we observed a 2-year significant higher eGFR in patients with CyA very low dose. We documented systolic blood pressure in patients with lower CyA levels. Our prospective study confirmed the safety and efficacy of CyA very low dose associated with high everolimus dose. We obtained an higher 2-year eGFR with reduction of acute rejection rate. Our data confirm the efficacy of CNI minimization using high everolimus blood levels.

Disclosure of Financial Relationships: nothing to disclose

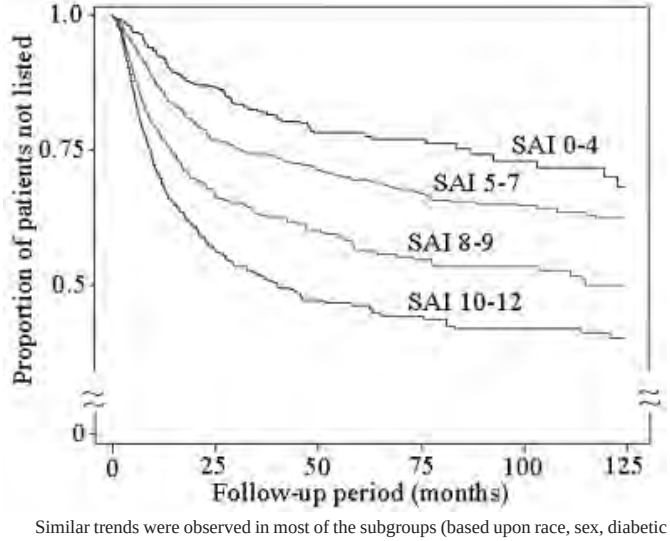
## SA-FC450

**Access to Kidney Transplantation: Role of Social Adaptability Index** Anna Barenbaum,[1] Gurprataap Singh Sandhu,[2] Martha Pavlakis,[2,3] Hongying Tang,[3] Preeti Rout,[2] Alexander S. Goldfarb-Rumyantzev.[2,3] *[1]Tel Aviv University, Tel Aviv, Israel; [2]Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [3]Transplant Institute, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.*

Background. Identifying the group of subjects prone to disparities in access to kidney transplantation is important for developing potential interventions.

Methods. Data from the United States Renal Data System (1/1/1990-9/1/2007; n=4,583) were used to study association between the Social Adaptability Index (SAI; based upon employment, marital status, education, income, and substance abuse) and outcomes (time to being placed on the waiting list and time to being transplanted once listed).

Results. Patients were 52.2±17.3 years old, 55.3% males, 64.9% White, and 44.1% had diabetes. SAI was higher in Whites (6.8±2.2) than African Americans (6.2±2.4) [ANOVA, p<0.001], greater in men (6.8±2.3) than in women (6.4±2.3) [T-test, p<0.001]. In multivaiate model greater SAI (range 0 to 12) was associated with increased likelihood of being placed on the waiting list (HR 1.19 [95%CI 1.15-1.23] per each point of increase in SAI, p<0.001); and greater likelihood of receiving a transplant once listed (HR of 1.06 [95%CI 1.03-1.09] per point of increase in SAI, p<0.001). When SAI was divided into quartiles we observed gradual improvement in access to transplantation with increase in the SAI.




Similar trends were observed in most of the subgroups (based upon race, sex, diabetic status, age, comorbidites and donor type).

Conclusion. SAI is associated with access to renal transplantation in patients with ESRD; it may be used to indentify individuals at risk for healthcare disparities.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC451

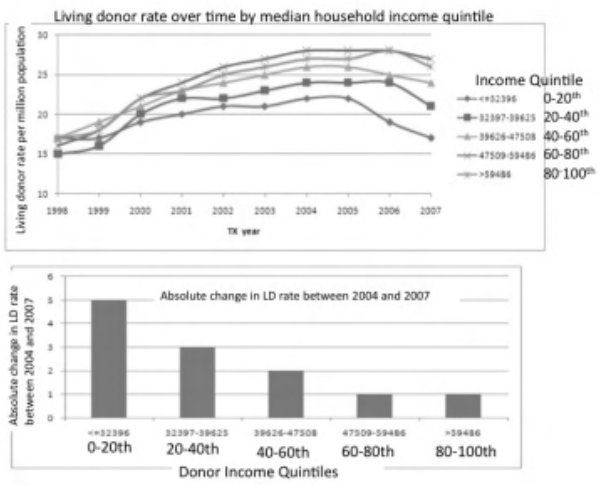
**The Retraction of Living Kidney Donation Is Most Marked among the Poor** Jagbir Gill, James Dong, Caren L. Rose, John S. Gill. *Nephrology, St. Paul's Hospital, University of BC, Vancouver, BC, Canada.*

Lower socioeconomic status is associated with a higher incidence of ESRD, yet reduced access to living donor kidney transplantation (Gore et al. AJT 2009). For reasons that are unclear, the number of living kidney donors in the US has decreased in recent years.

In this analysis we determine longitudinal changes in the rate of living donation (LD) as a function of median household income.

Methods: LD rates per million population, stratified by median household income, were calculated between 2000 and 2007 using data from OPTN/UNOS and the US 2000 Census.

Results: The figure shows that the rates of LD are inversely correlated with median household income. Rates of living kidney donation declined in all income groups over time, but the largest decline was seen in the lowest income group.




Conclusion: Living kidney donation decreased most rapidly among lower socioeconomic groups. Removing financial disincentives to living kidney donation may be an important strategy to prevent ongoing retraction of living kidney donation.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC452

**Race, Predialysis Transplant Discussion, and Preemptive Wait Listing in a National Cohort** Nancy G. Kutner,[1] Rebecca H. Zhang,[1] Yijian Huang,[1] Kirsten L. Johansen.[1,2] *[1]USRDS Rehabilitation/QoL Special Studies Ctr, Emory University, Atlanta, GA; [2]San Francisco VA Medical Ctr, UCSF, San Francisco, CA.*

Background. Preemptive listing for a kidney transplant can significantly reduce waiting time and improve patient outcomes, but previous research has shown that black patients are less likely than whites to be placed on the waiting list (WL) before beginning dialysis. It is not known if this race difference exists among patients with whom kidney transplantation has been discussed predialysis.

Methods. Incident dialysis patients aged ≥18 from 296 randomly selected clinics were surveyed in the USRDS Comprehensive Dialysis Study (CDS). Participants were asked "Was kidney transplantation discussed with you before you started your regular treatment for kidney failure?" and reported their education and employment status. Date of transplant WL, age, gender, race, medical insurance, diabetes, cardiovascular comorbidity, inability to ambulate or transfer, weight, and hemoglobin, serum creatinine and serum albumin values at treatment start were obtained from USRDS files.

Results. 813/1643 patients surveyed reported that transplantation was discussed with them predialysis, and within this group 7% were preemptively wait listed. In the preemptive WL group (n=58), 29.3% were black, compared to 26.6% of patients not preemptively WL (n=755). No gender, age or education differences were evident between the two groups, but preemptive WL patients were more often employed; had private insurance and higher average hemoglobin, serum albumin, and serum creatinine; and were less likely to have diabetes and cardiovascular comorbidity. Among the 813 patients reporting predialysis transplantation discussion, more preemptive WL patients had received early nephrology care (98.3% vs. 79.4%) and they were more likely than non-preemptive WL patients to begin renal replacement therapy on peritoneal dialysis (25.9% vs. 9.4%).

Conclusion. Black patients are frequently disadvantaged at multiple steps in the transplantation process. CDS data indicate that early discussion of transplant as a treatment option, linked with early nephrology care, may help remove barriers to preemptive WL for blacks.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC453**

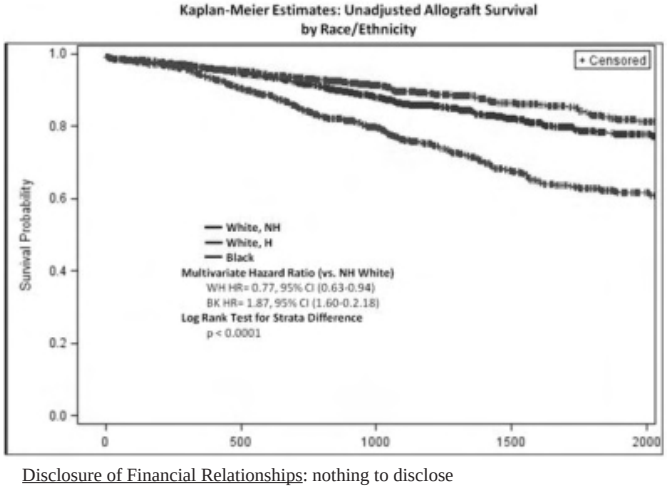
**Racial Disparities and Neighborhood Poverty in Pediatric Renal Allograft Survival** Rachel E. Patzer,[1] Nancy G. Kutner,[2] William M. McClellan,[1] Sandra Amaral.[3] *[1]Rollins School of Public Health, Emory University, Atlanta, GA; [2]Rehabilitation Medicine, Emory University; [3]Pediatrics, Emory University.*

**Background:** Racial and socioeconomic disparities exist in every aspect of kidney transplantation, from waitlisting to allograft survival. In pediatrics, the relationship of race and SES on graft survival remains poorly understood.

**Methods:** We explored this relationship among all pediatric (<21 yrs), incident ESRD patients in USRDS and UNOS who received a kidney transplant from Jan. 1, 2000 through Sept. 2006, and followed for transplant outcomes through Sept. 2008. Patients' residential zip codes were linked with poverty data from Census 2000. Cox models were utilized for multivariate analyses.

**Results:** Among 4,320 pediatric, incident ESRD patients, 37% received living donor kidney transplants. 18.4% experienced allograft loss within the median 3.6 yr follow-up time. In crude analyses, Blacks had nearly twice the risk of graft failure at any given time vs. White, Non-Hispanics (HR=1.87; 95% CI: 1.60-2.18). In contrast, Hispanic Whites were at a 23% reduced risk of graft failure vs. Non-Hispanic Whites (HR=0.77; 95% CI: 0.63-0.94). After controlling for demographic, clinical, and socioeconomic factors, this racial disparity remained highly significant, with Blacks at a 50% increased risk for allograft failure vs. White-NH. Increasing poverty was also associated with graft loss. Patients from the poorest neighborhoods had a risk of graft loss 20% greater than patients in the wealthiest neighborhoods (HR=1.20, 95% CI:1.01-1.43).

**Conclusion:** Racial and poverty disparities exist in pediatric kidney allograft survival, with Black pediatric ESRD patients and those living in poor neighborhoods having a shorter time to graft failure. These differences deserve further exploration to identify modifiable risk factors for graft loss and to ensure health equity.




Disclosure of Financial Relationships: nothing to disclose

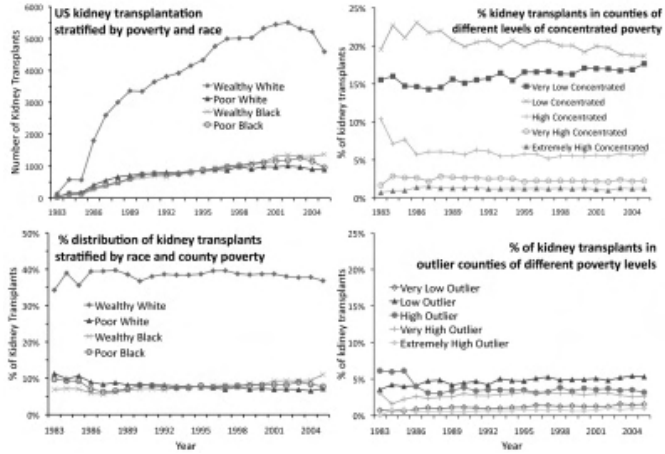
**SA-FC454**

**Transplantation and Intensity of Poverty in the United States** Sumit Mohan,[1] Richard Mutell,[3] James B. Holt,[4] David J. Cohen,[1] William M. McClellan.[2] *[1]Dept of Medicine, Div of Nephrology, Columbia University, New York, NY; [2]Dept of Medicine, Div of Nephrology, Emory University, Atlanta, GA; [3]DaVita Clinical Research, Minneapolis, MN; [4]Centers for Disease Control, Atlanta, GA.*

Introduction: Racial disparities are reported to persist in renal transplantation in the United States. We attempted to study the impact of local poverty rates on transplantation rates over time in the US.

Methods: Transplantation data and the location of patients receiving transplantation between 1983 and 2005 were obtained from the UNOS standard analytic files. The 2000 Census data was used to develop a previously published 10-category index of spatial topography of poverty in the US (Holt JB. Prev Chronic Dis. 2007 Oct;4(4):A111). Transplant recipient zip codes were used to match patients to counties. Analysis was performed with SPSS 16.0

Results: Over this period, the largest increase in the absolute number of kidney transplantations was seen among white recipients living in wealthy counties with concentration of low poverty (≥1 standard deviations below the national mean poverty level). However, the relative percentage of transplants did not change significantly. Also, rates of transplantation of black recipients in these same areas were similar to that of white and black recipients in poorer counties (Figure). These differences were attenuated in counties whose poverty rates were spatial outliers (significantly different from the rates of surrounding/nearby counties). Statistically significant ($\chi^2$= 15190, p<0.001) ethnic disparities persist with the greatest present in the rich counties.




Conclusions: These results suggest that both race and economic factors are associated with disparities in the prevalence of renal transplantation in US and that these disparities have remained largely unchanged despite the growth in the number of transplantations being performed each year.

Disclosure of Financial Relationships: Scientific Advisor: Amgen.

**SA-FC455**

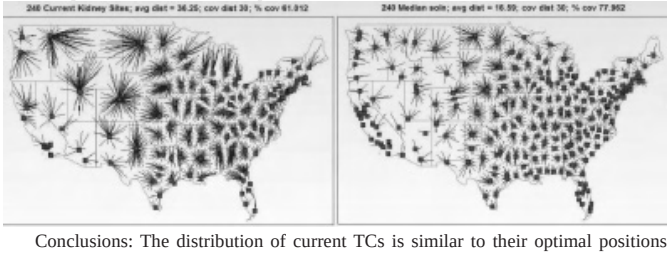
**Analysis of Kidney Transplant Center Locations in the United States** Daniela Ladner, Ashley E. Davis, John J. Friedewald, Mark S. Daskin, Sanjay Mehrotra, Vadim Lyuksemburg, Anton I. Skaro, Juan Carlos Caicedo, Michael Abecassis. *Northwestern University Transplant Outcomes Research Collaborative (NUTORC), Northwestern University Comprehensive Transplant Center, Chicago, IL.*

Purpose: The distance end stage renal disease (ESRD) patients must travel to a transplant center (TC) is predictive of listing and receiving a kidney transplant (KT). This study analyzes the geographic distance of the US and ESRD population to the existing 240 KT centers and compares the status quo to an optimal model maximizing geographic access to TCs.

Methods: Distribution of US and ESRD population was obtained from Census 2000 and ESRD Network data. US and ESRD population coverage within a 30 mile distance was computed for the current 240 KT centers. P-Median Facility Location Optimization (SITATION) was used to optimally locate 240 TCs with respect to every county's total and ESRD population. This technique locates TCs to minimize the total distance traveled by all individuals.

Results: Presently 61% of the country's population and 64% of ESRD patients live within 30 miles of a TC. Optimal TC distribution demonstrates that TCs are overall well distributed to serve either population with the exception of western and southern portions of the US.

Comparison of Current and Optimal Center Locations in the US.



Conclusions: The distribution of current TCs is similar to their optimal positions with underserved areas in the south and west. With approximately 40% of residents and patients living beyond 30 miles from a TC, this geographic disparity could be ameliorated by outreach clinics from existing TCs.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC456**

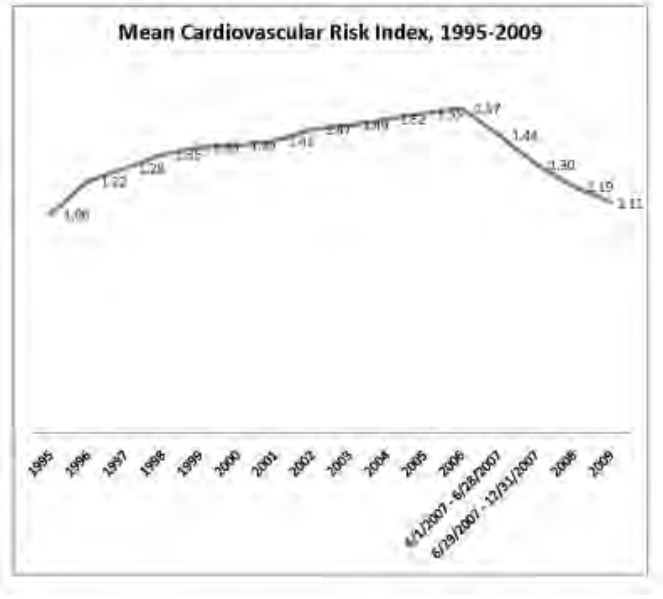
**Risk Aversion in Renal Transplantation – An Unintended Consequence of Regulatory Oversight** Anton I. Skaro, Edward Wang, Colleen Jay, Daniela Ladner, Yaojen Chang, Olivia A. Ross, Vadim Lyuksemburg, Jane L. Holl, Michael Abecassis, Lorenzo G. Gallon. *Northwestern University.*

**Purpose:** Conditions of Participation (CoP) issued by the Centers for Medicare and Medicaid Services (CMS) on June 28, 2007 mandated that transplant center-specific survival be in accordance with expected outcomes, which are inadequately risk-adjusted specifically for cardiovascular disease. However, quality improvement through performance measurement may act as a disincentive to perform renal transplantation in high cardiac risk patients. We developed a cardiovascular risk index (CRI) and examined trends before and after implementation of the CoP.

**Methods:** We retrospectively examined 176,739 kidney transplant recipients from the United Network for Organ Sharing database between 1995 and 2009. Patient survival according to CRI was analyzed using Kaplan-Meier and log-rank tests. Cox regression, stratified analysis and Rothman synergy index were used to evaluate covariates.

**Results:** Patient survival was inferior for high CRI (≥2) renal transplant recipients. After risk adjustment, high CRI remained a predictor of patient mortality (HR 2.89, 95% CI 2.75-3.04). Diabetes (HR 1.80, 95% CI 1.75-1.86) was the strongest and hypertension (HR 1.01, 95% CI 0.97-1.04) the weakest predictors of mortality. Combination of diabetes and hypertension augmented risk of mortality (synergy index 1.11, 95% CI 1.05-1.17). Interestingly, the mean CRI score increased from 1.06 in 1995 to a peak of 1.57 in 2006, but declined immediately prior to and after the implementation of the CMS CoP (Figure 1).

**Conclusions:** A contemporaneous decline in CRI with enforcement of the CMS CoP implicates risk aversion as an unintended consequence of regulatory oversight. Modification of risk adjustment is necessary to preserve equal access to renal transplantation among high cardiac risk patients.

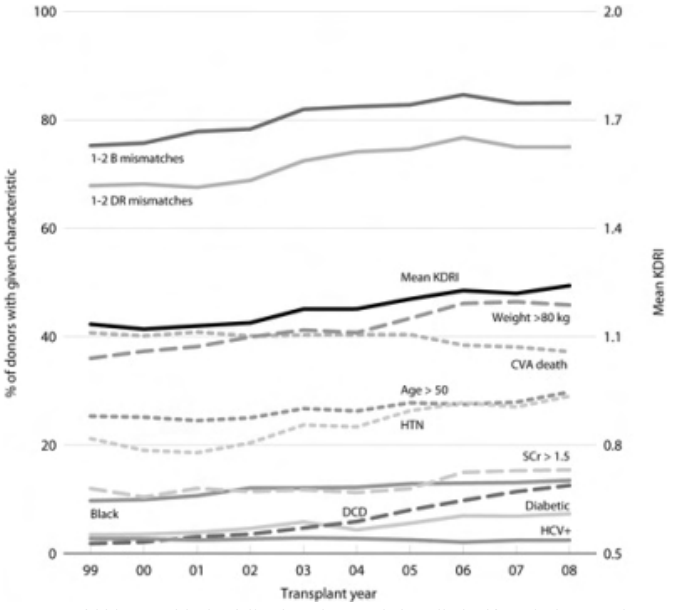



Disclosure of Financial Relationships: nothing to disclose

## SA-FC457

**Increasing Use of Riskier Deceased Kidney Donors in the US in the Past Decade** Jon J. Snyder, Sally K. Gustafson, Melissa Skeans, Bertram L. Kasiske. *United States Renal Data System, Minneapolis, MN.*

Survival of kidney transplants from deceased donors has remained fairly stable over the past decade, with a 5-year graft survival of 67% for transplants performed in 1999 and 69% in 2003, and first-year graft survival from 88% in 1999 to 91% in 2007. This is despite a 50% decline in the first-year incidence of acute rejection over the same 10-year period. Additionally, continued increases in the waiting list have lead to increased use of marginal kidney donors through the ECD program as well as increased use of DCD kidneys. We hypothesized that transplant programs were accepting higher risk deceased donor kidneys in many aspects of deceased donor quality. We examined trends in the kidney donor risk index (KDRI, Rao, Transplantation 2009) as well as trends in the individual components of the KDRI in first-time, adult, deceased donor kidney recipients in the US (N=83,901) using data from the United States Renal Data System (USRDS). Linear trends across transplant years were assessed using a logistic regression model. Mean KDRI increased significantly from 1.13 to 1.24 (Figure; p<0.0001).




Use of kidneys with the following characteristics all significantly increased over the 10-year period: black, diabetic, B/DR mismatches, age 50+, history of hypertension (HTN), DCD, weight 80+ kg, and terminal SCr > 1.5 mg/dL. Only a few risk characteristics decreased over the 10-year period: death due to CVA, HCV seropositive, and mean cold ischemia time (not shown, mean of 20 hours down to 19 hours). The KDRI supports the conclusion that use of higher risk deceased donors for kidney transplant has increased over the recent decade.

Disclosure of Financial Relationships: Research Funding: Bristol-Myers Squibb, Genzyme; Honoraria: Genzyme; Scientific Advisor: Genzyme.

## SA-FC458

**Living-Donor Kidney Volume as a Predictor of Graft Function: Is There a Role for Proteinuria?** Hadim Akoglu,[1] Tolga Yildirim,[1] Gonca Eldem,[2] Aysun Aybal Kutlugün,[1] Mahmut Altindal,[1] Rahmi Yilmaz,[1] Tuncay Hazirolan,[2] Fazil T. Aki,[3] Mustafa Arici,[1] Cetin Turgan.[1] *[1]Nephrology, Medical Faculty of Hacettepe University, Ankara, Turkey; [2]Radiology, Medical Faculty of Hacettepe University, Ankara, Turkey; [3]Urology, Medical Faculty of Hacettepe University, Ankara, Turkey.*

**Introduction:** The proposed mechanisms by which graft size affects graft outcome are intraglomerular hypertension and hyperfiltration leading to proteinuria and nephron loss. We aimed to evaluate whether proteinuria had an impact on relationship between graft size and graft outcome in living donor kidney transplantation.

**Methods:** We analyzed 69 living donors (25 male, 44 female, mean age 42.0±9.5 years) and their organ recipients (45 male, 24 female, mean age 29.8±10.1years) who underwent transplantation from 2003 to 2007. Transplanted kidney volumes were measured by 3D helical CT scanning. A transplant kidney volume-recipient body weight (Vol/Wt) ratio was calculated for each donor-recipient pair. Glomerular filtration rate (GFR) and 24-hour proteinuria at 6 months, 1 year and 2 years after transplantation were recorded from recipients' charts. The subjects were divided into two groups according to the median of Vol/Wt ratio: low Vol/Wt ratio group (≤2.33 $cm^3$/kg) and high Vol/Wt ratio group (>2.33 $cm^3$/kg).

**Results:** GFR was positively correlated with Vol/Wt ratio at 6 month, 1 year and 2 years (r=0.49, p<0.0001; r=0.48, *P*<0.0001; r=0.42, *P*=0.0001, respectively). Mean GFRs in high Vol/Wt ratio group were significantly higher compared to low Vol/Wt ratio group at 6 months, 1 year, and 2 years after transplantation (78.58±20.2 vs 61.72±17.8 ml/dk/1.73 $m^2$, *P*<0.001; 75.27±18.3 vs 61.5±17.9 ml/dk/1.73 $m^2$, *P*=0.001; and 71.36±18.4 vs 60.47±17.5 ml/dk/1.73 $m^2$, *P*=0.006; respectively). Proteinuria did not differ between the two groups at 6 months, 1 year and 2 years after transplantation (226±201 vs 208±232 mg/d, *P*=0.74; 203±158 vs 213±195 mg/d, *P*=0.80; and 158±77 vs 191±166 mg/d, *P*=0.29; respectively).

**Conclusion:** Living donor kidney transplantation with a low Vol/Wt ratio is associated with significantly worse graft function. This association is unlikely to be affected by the degree of proteinuria.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC459**

**Living Kidney Donation: Prediction of Glomerular Filtration Rate from Echographic Renal Dimensions** Carlo Donadio, Hesham Abdelkawy, Giulia Grassi. *Nephrology, Pisa University, Pisa, Italy.*

The gold standard to assess renal function is glomerular filtration rate (GFR). GFR is often estimated from serum creatinine (SCr), serum cystatin C (SCys) and creatinine clearance (CCr), or is predicted from SCr or SCys using formulas. Ultrasound scanning evaluates morphology and dimensions of kidneys.

Aim of this study was to evaluate the relationship among renal dimensions and renal function, and the possibility to predict GFR from echographic renal dimensions, in potential kidney donors (POTDON), in kidney donors (KDON) after donation, and in renal transplant recipients (RTR).

Patients. POTDON: 79 (52 females), SCr 0.5-1.3 mg/dL. KDON after donation: 33 KDON (28 females), SCr 0.81–1.90 mg/dL. RTR: 30 (8 Females), SCr 0.96 –2.42 mg/dL.

Methods. GFR was measured as $^{99m}$Tc-DTPA clearance. GFR was also estimated from SCr with Cockcroft&Gault (CG-CCr) and MDRD formula, and from SCys. Kidney dimensions were measured during bidimensional echography, and total and parenchymal kidney volumes were estimated with ellipsoid formula.

Results. Among sonographic measurements, in RTR and KDON, kidney length showed the best correlation with GFR, which was greater than that of SCr or SCys, and similar to that of CG-CCr or MDRD-GFR. Accuracy of kidney length as an indicator of GFR impairment was not different from laboratory tests. In POTDON the correlation with GFR was significant for SCys and for all prediction formulas, but not for Scr. The accuracy of CG-CCr and MDRD-GFR were better than Cys-GFR and CCr. However, their mean prediction errors versus GFR were relevant. Renal dimensions, particularly renal volume, showed a good correlation with GFR, higher than all prediction equations. Thus, we derived formulas to predict GFR from renal dimensions. These estimates of GFR were more closely correlated with true GFR than CG-CCr, MDRD-GFR, and Cys-GFR. GFR estimated from renal volumes had also a better agreement and a lower prediction error versus GFR than the other predictions.

In conclusion, renal echography provides also functional information, and it is possible to estimate GFR from renal volumes more accurately and with a lower prediction error than using formulas based on SCr and SCys or CCr.

Disclosure of Financial Relationships: nothing to disclose

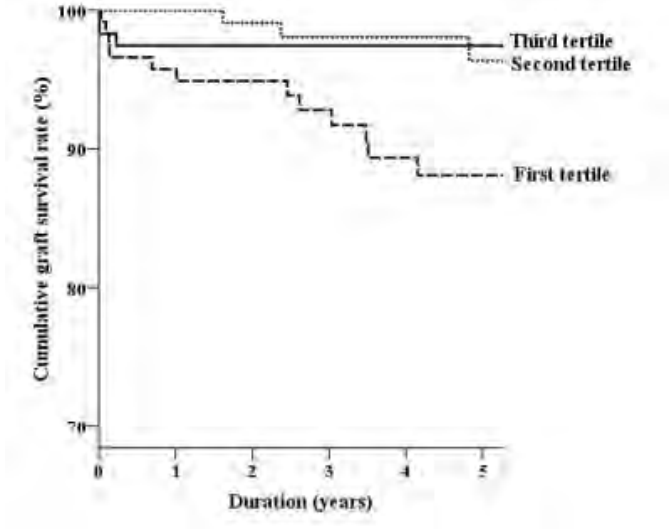
**SA-FC460**

**Influence of Graft Volume on the Long-Term Outcome of Kidney Transplantation** Seung Seok Han, Jin Suk Han, Suhnggwon Kim, Yon Su Kim. *Internal Medicine, Seoul National University Hospital, Seoul, Korea.*

Purpose: Although the short-term outcome of kidney transplantation has improved, the long-term outcome is suboptimal. This is because in addition to immune factors, nonimmune factors also influence the graft outcome. Herein, we assessed the graft volume as a nonimmune factor and studied its effect on the long-term outcome in kidney transplant recipients.

Methods: We studied 354 living transplant recipients between April 2001 and December 2008. In all donors, preoperative helical computed tomography (CT) with 3D reconstruction had been performed. Graft volume was estimated using the prolate ellipsoid method. The graft outcome (development of acute/chronic rejection and glomerulonephritis, and graft survival) was calculated by studying patients separated into 3 groups on the basis of the adjusted kidney volume to the body surface area of the donor.

Results: During the follow-up period (55.7 ± 27.20 months), 18 grafts stopped functioning. Graft survival rates were significantly different among tertile groups (second group vs. first group, OR = 0.25 (0.07–0.89), $P$ = .032; third group vs. first group, OR = 0.24 (0.07–0.86), $P$ = .028).




The differences in graft survival rates remained significant after adjustment for the recipient's age, sex, and body surface area; donor type (living related vs. living unrelated); and donor's age, sex, and degree of human leukocyte antigen (HLA) mismatch (second group vs. first group, OR = 0.21 (0.06–0.76), $P$ = .017; third group vs. first group, OR = 0.23 (0.06–0.81), $P$ = .022). The rates of acute/chronic rejections and glomerulonephritis were also significantly low in the groups with large kidney volume, irrespective of adjustment for other variables.

Conclusion: Recipients with large kidney volume have the benefit of graft survival as well as low incidence of rejection and glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC461**

**Therapeutic Potential of Locked Nucleic Acid Modified Anti-microRNA-192 in Mouse Models of Diabetic Nephropathy** Sumanth Putta, Mitsuo Kato, Linda L. Lanting, Guangdong Sun, Rama Natarajan. *Diabetes, Beckman Research Institute of City of Hope, Duarte, CA.*

Diabetic nephropathy (DN), a major complication of diabetes is characterized by mesangial cell (MC) hypertrophy and increased extra cellular matrix (ECM) deposition. Transforming Growth factor-β (TGF-β) has been implicated in these events. We recently reported that micro-RNA-192 (miR-192) was upregulated by TGF-β in MC and in diabetic mice glomeruli, and increased collagen expression by downregulating E-box repressors, ZEB1 and ZEB2. miR-192 appeared to be a key upstream regulator of other renal miRs and downstream genes related to DN. Therefore, we hypothesized that miR-192 could be a potential therapeutic target for DN. Locked nucleic acid (LNA) modified anti-miRs are effective in inhibiting target miRNAs in animals but have never been tested in kidney. Our aim was to evaluate the efficacy of LNA based anti-miR-192 oligos in mice as a therapeutic approach for DN. We first delivered LNA-antimiR-192 (LNA) subcutaneously in normal mice and evaluated efficacy at 6 and 24hr post injection. Next, we tested various parameters of DN in Streptozotocin (STZ) induced type 1 diabetic mice at 2, 7 and 17 wks post LNA treatment to evaluate early molecular and later functional indices of DN. In situ hybridization showed efficient accumulation of anti-miR in renal compartments of LNA injected mice. miR-192 levels were significantly inhibited in kidneys from LNA injected mice, but not miR-194, suggesting specificity of the LNA in vivo. Significant decrease in miR-192 and reciprocal increase in ZEB1/2 (miR-192 target) levels in renal tissues from diabetic and non-diabetic mice were observed in long term LNA delivery (2-17 weeks). Further, significant decrease in pro-fibrotic ECM gene expression (Col1A2, TGF-β, and Fibronectin) was detected in LNA injected diabetic mice and the reduction was confirmed by immunostaining of renal sections. In addition, functional indices of DN such as proteinuria and albuminuria were decreased in LNA injected diabetic mice. No toxicity was observed in mice injected with LNA-anti-miR-192 compared to controls. Thus, the LNA-antimiR-192 treatment could be a novel therapeutic strategy for DN.

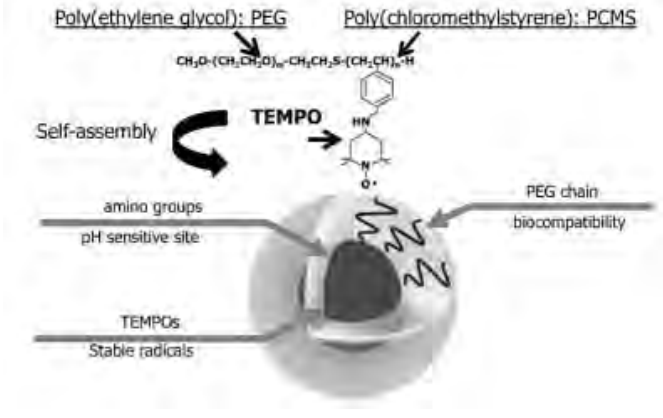
Disclosure of Financial Relationships: nothing to disclose

**SA-FC462**

**Development of a pH-Sensitive Reno-Protective Nano-Particle for Relief of Ischemia-Reperfusion Acute Kidney Injury** Aki Hirayama,[1] Toru Yoshitomi,[3] Shigeru Owada,[5] Atsushi Ueda,[2] Hirofumi Matsui,[3] Kazumasa Aoyagi,[1] Yukio Nagasaki.[3] *[1]Center for Integrative Medicine, Tsukuba University of Technology, Japan; [2]Namegata District General Hospital, Japan; [3]University of Tsukuba, Japan; [4]Asao Clinic, Japan.*

We have developed a TEMPO containing nano-particle (RNP: radical containing nano-particle) which possesses high stability and antioxidative effects as was reported in Renal Week 2009. We improve this RNP as a reno-protective agent and apply it in acute kidney injury (AKI).

The RNP was synthesized as a micelle of poly(ethelenglycol)-poly(chloromethylstyrene) in which the later were converted to TEMPO (PEG-PCTEMPO, Figure 1). Controlling the repeating units of PCTEMPO, we synthesized a pH-sensitive RNP (N-RNP) which releases the inner TEMPO in acidic circumstances (pH 6 or lower) by a self-disintegration and pH-insensitive RNP (O-RNP). The renal protective effects were evaluated in a murine one kidney-one clip ischemia-reperfusion AKI model. O-/N-RNP, TEMPOL, 4-amino-TEMPO ($NH_2$-TEMPO) or PBS (control) was intravenously injected before the reperfusion procedure.



Following 50 min of ischemia and subsequent reperfusion, 3 mg/kg of N-RNP (containing 0.25 µmol/kg TEMPO) reduced the elevation of serum creatinine (Cre), blood urea nitrogen (BUN), oxidative stress markers and improved histopathological changes compared to the equivalent dose of TEMPOL or $NH_2$TEMPO, or control. The renoprotective effect of O-RNP was similar to that of TEMPOL or $NH_2$TEMPO. When the TEMPO concentrations were increased to 2.5 µmol/kg (RNP 30 mg/kg), N-RNP and $NH_2$TEMPO showed similar inhibition of the elevation of Cre and BUN. $NH_2$TEMPO, however, caused remarkable hypotension.

These results indicate that the pH-sensitive RNP is an effective renoprotector. The nano-particle structure and induction of pH-sensitivity are the crucial strategies responsible for this renoprotection.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC463

**Assessment of Renal Perfusion in UUO Mice Using Retro-Orbital Injections of $^{99m}$Tc-MAG3 and NanoSPECT Imaging** Mohammed Noor Tantawy,[1] Rosie T. Jiang,[2] Feng Wang,[1] Keiko Takahashi,[2] Chuan-Ming Hao,[2] Raymond C. Harris,[2] Christopher Chad Quarles,[1] Takamune Takahashi.[2] *[1]Vanderbilt University Institute of Imaging Science, Nashville, TN; [2]Vanderbilt O'Brien Mouse Kidney Physiology and Disease Center, Nashville, TN.*

There is considerable interest and potential value in making available imaging methods that permit non-invasive assessments of renal function in mice. Here we optimized and evaluated the use of $^{99m}$Tc-MAG3 dynamic renal scintigraphy to detect decline in renal perfusion in a mouse model of unilateral ureteral obstruction (UUO). UUO mice were generated by complete ligation of the ureter of left kidney. The mice that underwent sham operation were used as a control. All the mice were imaged on days 0, 1, 3, and 6 post surgery using a NanoSPECT (Bioscan, Washington, DC) scan with planar dynamic mode and a parallel pin-hole collimator. On the days of imaging, the mice received ~37 MBq of $^{99m}$Tc-MAG3 retro-orbitally immediately before the start of a 45 min acquisition. Regions-of-interest were drawn in the left and right kidneys and time-activity curves were deduced. The peak activity of the kidneys and the time-to-peak were used as imaging metrics of renal perfusion. In UUO kidneys, the time-to-peak was significantly extended as early as day 0, exceeding 200 sec on any given day, while it was < 200 sec in the contra-lateral and sham kidneys. The peak activity of the UUO kidneys was equal to that in the contra-lateral kidneys on day 0 but was substantially reduced as the disease progressed (~3.5 times lower than the contra-lateral kidney at day 6). No significant differences were found in the peak activity between the contra-lateral kidney of the UUO mice and the kidneys of the sham operation mice.

In conclusion, our data demonstrate that reduction in renal perfusion in UUO mice can be reliably detected and quantified using retro-orbital injections of $^{99m}$Tc-MAG3. This method allows serial assessment of renal function in the same animals and could be effectively used for elucidating renal pathophysiology during the course of kidney disease.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC464

**Ultrasonographic 2D Strain Imaging, a New Approach to the Evaluation of Vascular Stiffness in Patients with Chronic Kidney Disease** Shirley Yumi Hayashi,[1,2] Anna Bjällmark,[2] Marcelo M. Nascimento,[1] Bengt Lindholm,[1] Astrid Seeberger,[1] Britta Lind,[2] Miguel C. Riella,[3] Lars-Åke Brodin.[2] *[1]Baxter Novum & Renal Medicine Karolinska Institute, Stockholm, Sweden; [2]Royal Institute of Technology (KTH), Stockholm, Sweden; [3]Pontifícia Universidade Católica do Paraná.*

Arteriosclerosis and atherosclerosis are common complications in patients with chronic kidney disease (CKD), being the leading cause of mortality in this group of patients. Ultrasonographic 2D strain imaging of common carotid artery using speckle tracking technique has been recently described and showed to be superior to elastic modulus in the evaluation of arterial stiffness. The aim of this study was to evaluate carotid strain (CS) and carotid strain rate (CSR) in CKD patients. 156 CKD patients stages 3, 4 and 5 (97 men, 58.3±14.5 years) and 9 healthy controls (8 men, 51.1±10.5 years) were evaluated. Measurements of circumferential strain (%) and strain rate (1/s) were performed in common carotid artery using ultrasonographic 2D strain imaging. CS and CSR were significantly correlated with age (r2 -0.49, p<0.0001). and significantly lower in CKD patients in comparison with controls (3.2±1.8 vs. 5.3±1.8, p<0.0001) and (0.4±0.2 vs. 0.6±0.2, p<0.01). The patients were divided into tertiles according to the CS values (group 1: < 2%, group 2: > 2% to 4%, group 3: > 4%). Intima media thickness was significantly higher in group 1 in comparison with the 2 other groups (0.8±0.4* vs. 0.7±0.2 vs. 0.5±0.1, p<0.0001). Cholesterol (5.2±1.6 vs. 4.7±1.2 vs. 4.0±1.3*, p<0.01), triglycerides (2.9±1.6 vs. 2.6±1.4 vs. 2.2±2.6*, p<0.001) and calcium levels (3.4±1.4 vs. 3.2±0.2 vs. 2.5±0.2*, p<0.05) were significantly higher in groups 1 and 2 in comparison with group 3. In conclusion, carotid circumferential strain and strain rate appear to be lower in CKD patients which could indicate the presence of increased arterial stiffness. The decreased values of CS correlate with increased IMT and higher levels of cholesterol, triglycerides and calcium These results might indicate that CS and CSR measurements may be a valuable tool in the evaluation of vascular stiffness in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC465

**Gene Expression Profiles in Different Venous Regions with Distinct Hemodynamic Environments in a Porcine Arteriovenous (AV) PTFE Graft Model** Li Li,[1] Randy Jay Christopherson,[2] Mary Carlson,[1] Donald Blumenthal,[3] Christi M. Terry,[1] Yan-Ting E. Shiu,[1,2] Alfred K. Cheung.[1,4] *[1]Internal Medicine, University of Utah; [2]Bioengineering, University of Utah; [3]Pharmacology, University of Utah; [4]Medical service, VASLC Healthcare System, SLC, UT.*

Implantation of a hemodialysis AV graft is often followed by the development of neointimal hyperplasia (NH) at the venous anastomosis (VA). The change of venous hemodynamic wall shear stress (WSS) caused by the graft likely plays a role in the NH formation. We monitored temporal changes in gene expression in different venous regions of different WSS levels through microarray analysis. Carotid-jugular PTFE grafts were placed in 4 pigs. Five days (n=2) or 14 days (n=2) after graft placement, the AV graft lumen geometry and blood flow data were obtained from MRI. These data were used as input for computational fluid dynamics (CFD) simulations of the AV graft flow field and contralateral non-operated jugular vein (JV) to identify average WSS in VA, proximal vein segments (PV, 2cm away from the VA) and JV. Subsequently, total RNA was isolated from these regions and analyzed by microarray. Statistical analysis (>2-fold change, p<0.001) was performed with GeneSifter software to identify altered gene expression in VA/or PV compared to JV. The average WSS in the VA, PV and JV regions at 5 post-operative days were 60 ± 28.3, 30 ± 14.1, and 7.5 ± 3.5 dyn/cm$^2$, respectively. Compared to JV, 55 genes were upregulated and 60 genes were downregulated at the VA. At 14 days, 13 genes were upregulated and 12 genes were downregulated at the VA. Using the same statistical criteria, 17 genes were upregulated and 14 genes were downregulated at the PV compared to JV at 5 days. At 14 days, 8 genes were upregulated and 3 genes were downregulated in PV compared to JV. These results showed a strong tendency for genes to be altered in VA which has the highest WSS environment. Detailed characterization of these genes and their individual associations with WSS over time are ongoing. Ultimately, these analyses may provide important insights into the role of mechanical force and molecular mechanisms leading to NH in hemodialysis vascular access.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC466

**Uremic Ovine Model of End Stage Renal Disease (ESRD) for the Evaluation of the Bioartificial Renal Epithelial Cell System (BRECS)** A. Westover,[1] D. Buffington,[1] J. Jung,[2,3] L. Lou,[1] A. Rojas,[2] L. Charles,[1] P. Smith,[1] K. Johnston,[1] C. Pino,[1] David Humes.[1,3] *[1]Innovative BioTherapies; [2]Chungnam Nat Univ; [3]Univ of Michigan.*

Renal cell therapy incorporated into conventional CRRT has shown metabolic, immunologic and survival benefits in renal failure in preclinical and clinical studies. However, maintenance of a blood extracorporeal circuit for chronic renal cell therapy in ESRD is limited due to clotting and infectivity risks. To this end, a wearable renal cell therapy device continues to be developed using an ovine model of ESRD. The model employs a continuous-flow peritoneal dialysis (CFPD) circuit incorporating a BRECS allowing for uremic control, maintenance of cell viability and communication between the device and the host animal.

**Methods:** Two in/out-flow PD catheters were placed 2 weeks before the start of CFPD and sequential nephrectomies performed 2 weeks and one day before initiation of CFPD with commercially available 4.25% glucose. BRECS containing $10^8$ renal epithelial cells were incorporated into the CFPD circuit post equilibration to physiologic pH. Cell viability and functionality within the device were monitored using $O_2$ consumption and γ-glutamyltranspeptidase (gGT) metabolism. Parameters including blood chemistry profiles, nutrition, inflammation (CD11b expression by neutrophils) and leukocyte function (oxidative burst) of the animal were monitored and compared to acellular sham devices. Cell therapy continued up to seven days and animals were evaluated for an additional 48 hours post therapy.

**Results:** BRECS remained viable with detectable $O_2$ consumption and gGT activity for up to 7 days of CFPD period and post therapy. Uremic control by CFPD measured by small-solute uremic parameters was within acceptable ranges. For neutrophils, CD11b expression was reduced while oxidative burst potential was maintained with BRECS therapy. **Conclusion:** The ovine model of ESRD is an acceptable model to evaluate the therapeutic functionality in the BRECS. Preliminary studies have identified possible renal tubule cell therapy targets in the amelioration of inflammation and neutrophil function known to be dysregulated in ESRD.

Disclosure of Financial Relationships: Employer: Innovative BioTherapies, Inc.; Ownership: Innovative BioTherapies, Inc.

## SA-FC467

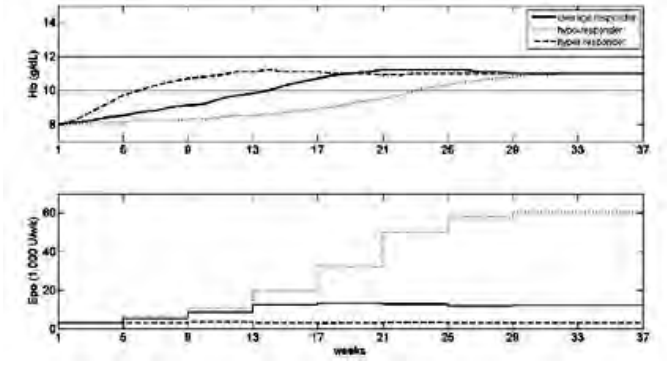
**Computer-Aided Personalized Anemia Management** Adam Gaweda,[1] Jordan Malof,[1] George R. Aronoff,[1] Alfred A. Jacobs,[1] Michael Brier.[2] *[1]Medicine, University of Louisville, Louisville, KY; [2]Department of Veterans Affairs.*

Erythropoiesis Stimulating Agent (ESA) dosing in ESRD anemia management is usually based on standard protocols applied to a diverse patient population. To enable dose individualization we developed software for ESA dosing, called Smart Anemia Manager™. We demonstrate its feasibility through *in silico* testing.

Using data from ESRD patients receiving Epoetin alfa (Epo) at University of Louisville, we developed three models representing typical pharmacodynamic (PD) Hemoglobin (Hb) response to Epo: hypo-, average, and hyper-responder. PD characteristics were represented by erythropoietic sensitivity (0.05, 0.25, and 1.0 g/dL per 1000 U/wk) and average

erythrocyte lifespan (60, 90, 120 days). Smart Anemia Manager™ is based on the concept of multiple Model Predictive Control (MPC). It uses multiple dose adjustment algorithms representing different PD profiles and automatically selects the correct algorithm for a given patient based on their response. Using the three response models, we simulated anemia management over a period of 37 weeks starting at baseline Hb=8g/dL.

Smart Anemia Manager™ drove Hb from the baseline value to the target range (10-12 g/dL) for all three test patients. The time to reach the target range was 23 (hypo-), 14 (average), and 7 (hyper-responder) weeks. The standard anemia management protocol used at our facility achieved the target Hb in 23 (hypo-) and 11 (average responder) weeks. For hyper-responder, the protocol resulted in Hb cycling and did not achieve a stable response.




Hb response (top) and Epo dose profiles (bottom) for the three representative patient models using Smart Anemia Manager™.

These test results demonstrate the feasibility of Smart Anemia Manager™ as a tool for personalized ESA dosing in anemia management, especially in reducing algorithmic Hemoglobin cycling.

Disclosure of Financial Relationships: nothing to disclose

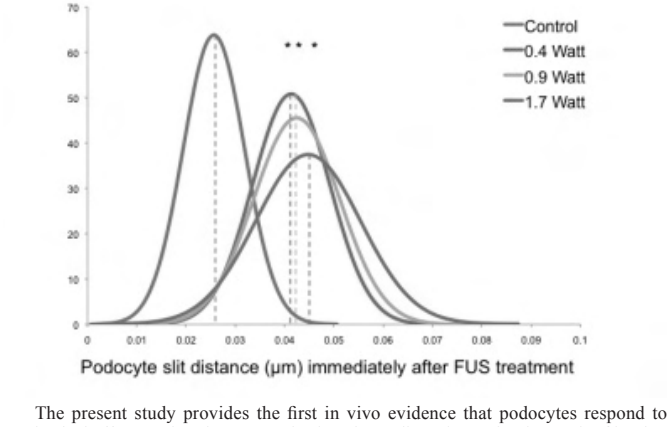
## SA-FC468

**Mechanical Stress Induced Functional and Structural Changes in the Glomerulus** Krisztina Fischer,[1,2] Nathan J. McDannold,[1] Gyorgy S. Reusz,[2] Ferenc A. Jolesz.[1] *[1]Radiology, Brigham and Women's Hospital/ Harvard Medical School, Boston, MA; [2]First Department of Pediatrics, Semmelweis University, Budapest, Hungary.*

Glomerular capillary pressure generates wall tension in glomerular capillaries that needs to be counterbalanced by the cellular layers. The podocytes are likely to predominantly stabilize the capillary wall and represent the dominant resistance to passage of large molecules. It is known that podocytes are sensitive to mechanical stress and fluidic shear stress in vitro. Focused ultrasound and microbubble contrast agents have proven to be able to change vascular permeability by mechanical effects on the vasculature.

In the present study we applied pulsed focused ultrasound exposures (10ms burst, 1Hz PRF, 30s duration) at three different power levels (0.4W;0.9W;1.7W) in the presence of microbubble contrast agent (Definity) as mechanical stress onto 19 healthy rabbits' kidneys. Five animals served as controls. The animals were either sacrificed immediately or one hour after the sonication treatment. Relative (treated versus non-treated kidney) creatinine clearance and protein to-creatinine ratios were calculated. Ultrafine structural analysis was performed using electronmicroscopy. Podocyte slit distance was measured using the AMT image capture engine software. A two-tailed t-test was applied to determine the statistical significance of the structural changes.

We have found a reversible, linear (proportional to the applied power level) increase in the relative creatinine clearance (R2= 0.998), protein to-creatinine ratio (R2= 0.983), and distance between podocyte foot processes (R2= 0.997, p<0.001) of the test subjects.




The present study provides the first in vivo evidence that podocytes respond to mechanical effects and such response leads to immediate change in glomerular filtration and proteinuria.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC469

**Vesicle Associated Membrane Protein, VAMP2, Mediates cAMP-Stimulated Renin Release from Mouse Juxtaglomerular (JG) Cells** Mariela Mendez. *Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Hospital, Detroit, MI.*

Exocytosis of renin containing granules is involved in renin release from juxtaglomerular (JG) cells, a process stimulated by cAMP. The molecular mechanism and proteins involved in renin exocytosis are unexplored. In other endocrine cells, granule exocytosis is mediated by proteins of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family. From this family, vesicle associated membrane protein 2 (VAMP2) mediates cAMP-stimulated exocytosis. We hypothesized that cAMP-stimulated renin release from JG cells is in part mediated by VAMP2. We measured: a) expression of VAMP2 by Western blot and immunofluorescence; and b) the effect of VAMP2 silencing *via* adenoviral delivery of short hairpin RNA (VAMP2-shRNA) on cAMP-stimulated renin release from primary cultures of mouse JG cells. Intracellular cAMP levels were increased by treatment with Forskolin plus IBMX. By Western blot we found that VAMP2 is expressed in JG cell lysates at the expected molecular mass (n=8). Immunofluorescence and confocal microscopy showed colocalization of VAMP2 with renin containing granules in JG cells. JG cells were transduced with adenoviruses carrying a scrambled sequence (control) or VAMP2-shRNA and found that 48 hrs after transduction VAMP2 expression was reduced by 75% of control (n=6). In control cells Forskolin/IBMX stimulated renin release by 259±26 % (p<0,05) however in JG cells transduced with VAMP2-shRNA, Forskolin/IBMX increased renin release by 107±25%, a 58% blockade (p<0.05). We concluded that VAMP2 is expressed in JG cells and mediates part of the cAMP-stimulated renin release from mouse JG cells. These data identify for the first time a vesicle associated protein that mediates renin release.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC470

**Kidney Damage-Induced Acceleration of Atherosclerosis Is Ameliorated by Pioglitazone Modulation of Macrophage Phenotype** Suguru Yamamoto,[1] Yiqin Zuo,[1, 2] Patricia G. Yancey,[3] Zhi-Qi Xu,[1] Iekuni Ichikawa,[1] Valentina Kon.[1] *[1]Vanderbilt University, Pediatrics, Nashville, TN; [2]Vanderbilt University, Pathology, Nashville, TN; [3]Vanderbilt University, Medicine, Nashville, TN.*

**Background:** We previously showed that angiotensin II (AII) has a role in uninephrectomy (UNx)-induced acceleration of atherosclerosis and that macrophage-specific deletion in peroxisome proliferator activated receptor g (PPARg) contributes to the proatherogenic effects of AII. We now examine if PPARg agonist benefits UNx-induced atherosclerosis and possible synergism with AII receptor antagonism.

**Methods and Results:** apoE-/- mice underwent UNx or sham operation (Sham). UNx were further subdivided into untreated UNx, UNx+Pioglitazone [Pio, n=10, 0.016% (w/w) in food], UNx+losartan (Los, n=10, 100mg/L in drinking water), or both (Pio+Los, n=10) for 10 weeks. There were no differences in body weight, creatinine clearance or triglycerides among the groups. Blood pressure was lower in Los and Pio+Los (90.0±3.3 and 87.1±2.8 vs UNx:108.1±4.4 mmHg, both $p<0.05$). Serum cholesterol was higher in Pio and Pio+Los (495.8±23.6 and 495.2±28.6 vs UNx 338.2±23.5 mg/dl, $p<0.05$). UNx significantly increased atherosclerosis assessed by Oil-red O (331385±25020 vs 197670±19131 $\mu m^2$ in Sham, $p<0.05$) which was lessened by Pio and Los (233408±17116 and 194250±25509 $\mu m^2$, p<0.05 vs UNx) but especially by the combination which was significantly less than Pio alone (Pio+Los: 146979±17046 $\mu m^2$, $p<0.05$). Assessment of plaque lesions revealed greater macrophage area in Pio+Los vs other treatments (80.7±11.4 vs Pio:50.3±4.2 and Los:57.2±6.5 %, $p<0.05$). The expanded macrophage area of Pio+Los had greater number of alternatively activated macrophages with anti-inflammatory/anti-atherogenic functions [Ym-1 positive macrophages (61.7±2.7 vs Pio:50.2±2.8 and Los:45.5±4.2 %, $p<0.05$)].

**Conclusion:** Despite elevated serum cholesterol, Pioglitazone lessens renal damage-induced amplification in atherosclerosis which is independent of blood pressure. This benefit is especially evident when combined with Losartan through an increase in the alternatively activated macrophages.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC471

**Salt Sensitivity of the Aberrant Tubuloglomerular Feedback Responses after Subtotal Nephrectomy** Prabhleen Singh, Ali Kashkouli, Roland C. Blantz, Scott C. Thomson. *Nephrology, UCSD & VASDHS.*

We have repeatedly confirmed preserved tubuloglomerular feedback (TGF) responses in normal rats fed a high NaCl diet. We recently reported anomalous TGF responses in 50% of nephrons in rats after subtotal nephrectomy (STN) on a normal diet with moderate NaCl (AJP2009). Presently, we examined the microvascular basis for the latter phenomenon and tested it for salt sensitivity.

Rats were begun on low (commercial NaCl deficient diet) or high (1% NaCl in drinking water) salt diets from the time of STN and underwent micropuncture after 7 days. Mean body weights were similar in the two groups (267 vs.274 g), as was the food intake (7 vs.8 g/day). STN rats on high salt drank 2-fold more water than those on low salt (72 vs. 33 ml/day, p=0.002).

Group mean GFR was 1.25 ml/min in high salt and 1.0 ml/min in low salt (p=ns). Urine flow rates were higher in the high salt group 19μl/min vs. 10 μl/min in the low salt group (p=0.05). SNGFR and tubular stop flow pressure (PSF) were measured during

orthograde perfusion of Henle's loop (LOH) at 0 or 50 nl/min to characterize the TGF response. Glomerular capillary pressure (PGC) was calculated from PSF and plasma oncotic pressure.

Table 1

| Groups | SNGFR (nl/min) | | Late proximal flow (nl/min) | | PGC (mm Hg) | |
|---|---|---|---|---|---|---|
| | No LOH perfusion | Max LOH perfusion | No LOH perfusion | Max LOH perfusion | No LOH perfusion | Max LOH perfusion |
| Low salt STN | 42±2 | 31±3* | 30±2 | 21±2* | 75±3 | 69±3* |
| High salt STN | 46±4 | 54±3* | 30±3 | 35±2* | 73±2 | 73±2 |
| 2-way ANOVA cross term | | p=0.002 | | p=0.004 | | p<0.0005 |

*P<0.05 vs. No LOH perfusion by paired t-test

Both groups exhibited glomerular capillary hypertension. SNGFR and PGC responses to LOH perfusion in low salt STN were mundane. In high salt STN, SNGFR responses were frankly anomalous while PGC responses were nil, on average.

In STN rats on a high salt diet, the combined SNGFR and PGC response to LOH perfusion implies vasodilation of both afferent and efferent arterioles in order for SNGFR to increase while PGC remains constant. To facilitate salt balance on a high salt diet, the remnant kidney sacrifices stability of the early distal delivery by converting TGF from negative to positive feedback.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC472

**A Mathematical Model of the Myogenic Response in the Rat Afferent Arteriole** Harold E. Layton,[1] Ioannis Sgouralis,[1] Leon C. Moore,[2] Anita T. Layton.[1] *[1]Department of Mathematics, Duke University, Durham, NC; [2]Deparatment of Physiology & Biophysics, SUNY Stony Brook, Stony Brook, NY.*

We have formulated a mathematical model of the rat afferent arteriole (AA). Our model consists of a series of arteriolar smooth muscle cells, each of which represents ion transport, cell membrane potential, cellular contraction, and wall mechanics. Blood flow through the AA lumen is described by Poiseuille flow. Model results suggest that interacting calcium and potassium fluxes, mediated by voltage-gated and voltage-calcium-gated channels, respectively, give rise to periodic oscillations in cytoplasmic calcium concentration, myosin light chain phosphorylation, and crossbridge formation with attending muscle stress mediating vasomotion. The AA model's representation of the myogenic response is based on the hypothesis that the voltage dependence of calcium channel openings responds to transmural pressure so that vessel diameter decreases with increasing pressure. With this configuration, the results of the AA model simulations agree well with findings in the experimental literature, notably those of Loutzenhiser et al., which showed that systolic blood pressure is an important determinant of renal vascular resistance (Am J Physiol Regul Integr Comp Physiol 290:R1153, 2006). The model can be incorporated into models of integrated renal hemodynamic regulation. This research was supported in part by NIH grant DK-42091 and by NSF grant DMS-0715021.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC473

**The Anion Transporter Slc26a9 Plays an Important Role in Systemic Blood Pressure Control by Regulating Renal Chloride Excretion** Hassane Amlal,[1, 2] Sharon L. Barone,[1, 2] Jie Xu,[1, 3] Kamyar A. Zahedi,[1, 2] Manoocher Soleimani.[1, 2, 3] *[1]Center on Genetics of Transport, University of Cincinnati, Cincinnati, OH; [2]Medicine, University of Cincinnati, Cincinnati, OH; [3]Research Services, Veterans Administration, Cincinnati, OH.*

Slc26a9 (PAT4 = Putative Anion Transporter 4) ) is an electrogenic chloride transporter and is expressed in several epithelial tissues. However, its localization and role in the kidney remain unknown. RT-PCR experiments demonstrated the expression of Slc26a9 in the cortex and medulla and in cultured CCD and IMCD cells, and double immunofluorescence labeling co-localized Slc26a9 and AQP2 to the apical membrane of principal cells in the collecting duct. To ascertain the role of Slc26a9 in renal chloride excretion, wt and Slc26a9 ko mice were examined, before and after 24 hours of water deprivation. Wild type and Slc26a9 KO mice were placed in metabolic cages and after acclimation, were subjected to 24 hrs of water deprivation. The results indicated that mice with genetic deletion of Slc26a9 showed significant reduction in renal chloride excretion relative to wild type littermates when subjected to water deprivation (0.37 mEq/l/24 hrs in wt Vs. 0.185 in Slc26a9 ko; p<0.02). Food intake was comparable (2.84 +/- 0.12 and 2.40 +/- 0.43 gm/day in wt and ko animals, respectively, p>0.05) and baseline urine chloride excretion was not significantly different between the wt and mutant mice (0.44 +/- 0.08 and 0.32 +/- 0.045 mEq/l/24 hrs in wt and ko animals, respectively; p>0.05). Urine volume was lower (1.6 ml/24 hrs in wt animals and 0.80 in PAT4 ko animals, p<0.001), urine osmolality higher, and reduction in body weight was significantly less in water deprived Slc26a9 ko mice. Systemic blood pressure measurements by tail-cuff method at basal state demonstrated that Slc26a9 mice have elevated blood pressure, with systolic blood pressure being 117 +/- 7 mmHg in wt and 142 +/- 7 in Slc26a9 ko mice (p<0.02). We conclude that Slc26a9 is expressed in the collecting duct and plays an important role in renal salt excretion. We propose that diminished Slc26a9 function can impair the excretion of excess salt and result in salt-sensitive hypertension.

Disclosure of Financial Relationships: nothing to disclose

## SA-FC474

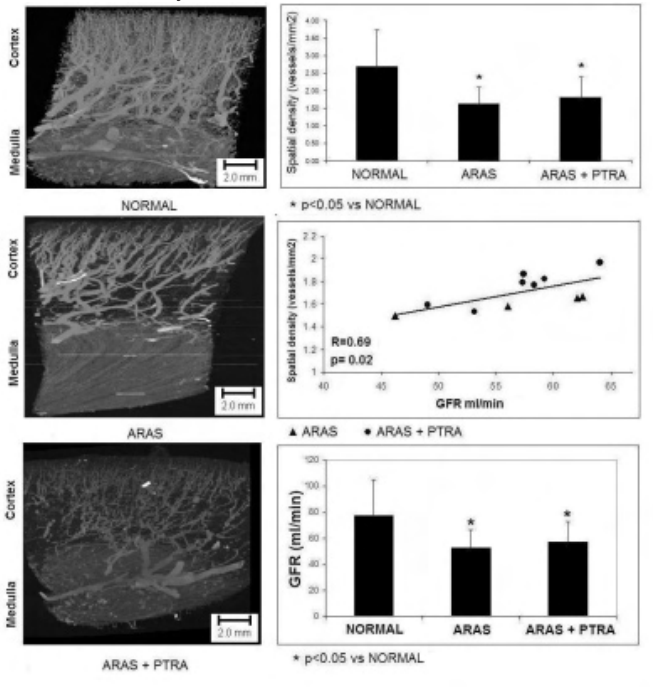
**Continued Renal Dysfunction after Percutaneous Transluminal Renal Angioplasty (PTRA) Is Associated with Microvascular Remodeling in Swine Atherosclerotic Renal Artery Stenosis (ARAS)** Alfonso Eirin,[1] Xiang-Yang Zhu,[1] Victor Urbieta Caceres,[1] James Krier,[1] John A. Crane,[1] Stephen C. Textor,[1] Amir Lerman,[2] Lilach O. Lerman.[1] *[1]Division of Nephrology and Hypertension, Mayo Clinic; [2]Cardiovascular Diseases, Mayo Clinic, Rochester, MN.*

Background: Revascularization fails to improve renal function in many patients with ARAS, but the mechanisms underlying irreversible renal injury have not been fully elucidated. This study tested the hypothesis that renal dysfunction after PTRA involves ongoing renal microvascular remodeling.

Methods: Pigs were studied after 10 weeks of ARAS, ARAS treated with PTRA and stenting 4 weeks earlier, and normal controls (n=10 each). Stenotic kidney blood flow and GFR were studied using multidetector CT. Renal microvascular architecture (micro-CT), fibrosis, oxidative stress, and angiogenic pathways were evaluated *ex-vivo*.

Results: Four weeks after successful PTRA mean arterial pressure decreased (from 179.116.48 to 105.216.5 mmHg, p<0.05 vs. ARAS, p=0.43 vs. Normal). However, stenotic kidney glomerular filtration rate (GFR) and renal blood flow remained decreased similar to untreated ARAS, and microvascular rarefaction and interstitial fibrosis did not change. The spatial density of outer cortical microvessels in ARAS and ARAS+PTRA correlated linearly with GFR (Figure). PTRA also failed to restore the expression of vascular endothelial growth factor or the fibrogenic factor Plasminogen Activator Inhibitor-1.

Conclusion: Tubulointerstitial injury in ARAS persisted 4 weeks after successful PTRA, and vessel loss correlated with the residual renal dysfunction. Microvascular loss and PAI-1-mediated fibrosis in swine ARAS, which might account for irreversible renal injury after PTRA, underscore the need for adjunct interventions beyond revascularization to restore stenotic kidney function.




Disclosure of Financial Relationships: nothing to disclose

## SA-FC475

**Succinate Activates the Collecting Duct Renin-Angiotensin System** Agnes Prokai, Anush Gevorgyan, Janos Peti-Peterdi. *University of Southern California, Los Angeles, CA.*

The TCA-cycle intermediate succinate is the ligand of the newly identified metabolic receptor GPR91, which is highly expressed in the juxtaglomerular apparatus (JGA) and in the collecting duct (CD). Tissue hypoxia and/or diabetic hyperglycemia causes mitochondrial stress, local accumulation of succinate in the kidney, GPR91-mediated glomerular hyperfiltration, JGA renin secretion, renin-angiotensin system (RAS) activation, and hypertension. CD renin is up-regulated in high angiotensin II states, and the CD is the major source of (pro)renin in diabetes (DM). In this study we investigated whether succinate and GPR91 signaling regulates the early elements of RAS in the CD.

M1 cells were treated with mannitol (5, 15, 25mM), glucose (5, 15, 25mM) and succinate (0.01, 0.1, 1mM) for 1 day. Acute treatment with 1mM succinate was performed for 6h. Western blot analyses and immunofluorescence were performed for extracellular signal-regulated kinases (ERK) 1/2 and p38, cyclooxygenase 2 (COX2), (pro)renin receptor [(P)RR], soluble (s) (P)RR, and (pro)renin. Urinary prostaglandin E2 (PGE2) excretion was measured in control and STZ-DM wild type (WT) and GPR91-/-mice.

Succinate treatment caused a significant, dose-dependent elevation in M1 cell pERK 1/2 levels. Similar increases in total (P)RR (1, 1.35, and 1.4-fold), s(P)RR (1, 1.5, and 2.65-

fold), pp38 (1, 4.6, and 1.75-fold), COX2 (1, 1.4, and 2.2-fold), prorenin (1, 2.1, 2.3-fold) and renin (1, 1.78, 2.73-fold) were observed 1 day after 25mM mannitol, glucose and 1mM succinate administration, respectively. Consistent with GPR91-mediated COX2-activation, the increased urinary PGE2 excretion (3 ng/24hr) observed in WT DM mice was abolished in DM GPR91-/-mice (1.3 ng/24hr, $p<0.05$).

These data support our hypothesis that succinate accumulation in diabetes activates the RAS in the CD, including the newly discovered (P)RR. Succinate and GPR91 signaling involves the activation MAP kinases and COX2, PGE2 release, and increased (pro)renin and (P)RR synthesis. Succinate-induced activation of the CD RAS may be important in controlling renal hemodynamics, systemic RAS, blood pressure in diabetes and in other kidney diseases that are associated with renal ischemia.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC476**

**Effects of GLP-1 Receptor Activation on Renal Hemodynamics** Ali Kashkouli, Prabhleen Singh, Scott C. Thomson. *UCSD and VASDHS.*

Glucagon-Like-Peptide -1 (GLP-1) is a hormone secreted by the gut. GLP-1 receptors in gastrium, brain, and pancreas serve at multiple levels to stabilize blood glucose. GLP-1 receptors are also expressed in kidney cortex, where their function is poorly defined.

We recently examined the effects of the long-acting GLP-1 mimetic, exenatide, on glomerular and tubular function in the rat. In awake rats, exenatide infusion (1 nmol/h) increased whole kidney GFR from 2.2±0.3 ml/min to 4.3±0.2ml/min, $p=0.002$ and urine flow rate from 12±3 to 34+4 µl/min, $p=0.06$. Micropuncture experiments confirmed that exenatide is both a potent renal vasodilator and a proximal tubular diuretic and that it causes a rightward shift in the tubuloglomerular feedback response (EB 2010). Since this pattern resembles a test for renal functional reserve, which depends on nitric oxide, we presently tested the renal hemodynamic effects of exenatide for senstitivity to nitric oxide synthase (NOS) blockade in anesthetized rats.

Inulin and PAH clearances were measured before and during exenatide infusion in control and NOS blocked rats with standard L-NMMA infusion (0.5 mg/kg/min) begun 30 minutes prior to the onset of data collection.

L-NMMA increased blood pressure by 15 mmHg on average. Exenatide increased RBF, GFR, and urine flow rate despite the presence of NOS blockade (Table 1). Filtration fraction was unaffected by exenatide, suggesting no effect on glomerular capillary pressure. The differences in these parameters were of similar magnitude to that obtained with exenatide in the presence of placebo (normal saline).

Table 1

| Groups | Filtration Fraction | RBF(mL/min) | GFR(mL/min) | Urine flow rate(µL/min) |
|---|---|---|---|---|
| L-NMMA | 0.55±0.05 | 7.34±0.7 | 2.24±0.1 | 10±0.6 |
| L-NMMA + Exenatide | 0.49±0.03 | 11.31±1.2 | 3.14±0.14 | 35±9 |
| p values (by paired t-test) | 0.419 | 0.06 | 0.0032 | 0.06 |

Exenatide remains a potent renal vasodilator and diuretic in the presence of LNMMA, suggesting that its effects on the kidney are independent of nitric oxide.

Disclosure of Financial Relationships: nothing to disclose

**SA-FC477**

**Attenuated Tubuloglomerular Feedback (TGF) in Equilibrative Nucleoside Transporter 1 (ENT1)-Deficient Mice** Jurgen B. Schnermann, Lingli Li, Christoph Eisner, Yuning George Huang. *NIDDK, NIH, Bethesda, MD.*

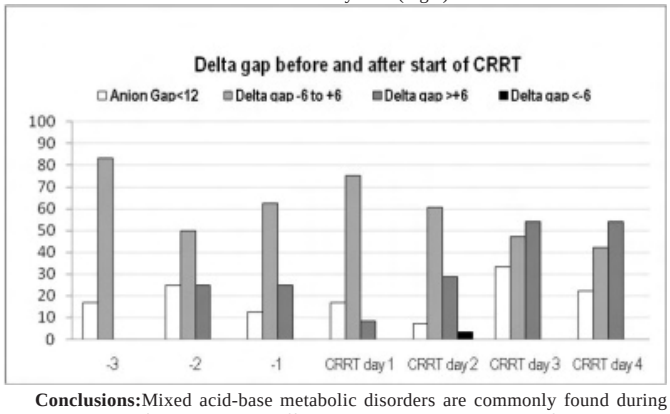
Previous evidence has indicated that adenosine 1 receptors (A1AR) are required for TGF responsiveness and that their ligand adenosine is at least in part derived from the extracellular metabolism of nucleotides. The present experiments were performed in ENT1-/- mice (C57BL/6 background) to test whether ENT1-mediated transmembrane movement of adenosine may also play a modifying role (breeder pairs of ENT1-/- mice were kindly provided by Doo-Sup Choi, Dept. Mol. Pharmacol. Psych, Mayo Clinic, Rochester, MN). Plasma concentrations of adenosine and inosine were markedly higher in ENT1-/- (1179±78 and 225±48 pmol/ml) than WT mice (179±24 and 47.5±9 pmol/ml). Telemetric measurements of 24-hour mean arterial blood pressure (mm Hg) and heart rates (bpm) were not significantly different between ENT1-/- and WT mice (114±14 and 557±39 vs. 114.3±8.1 and 594±46) despite the fact that spontaneous activity levels (6.5±5.7 vs. 9.3±8 movement cpm) as well as voluntary wheel running activity was lower in ENT1-/- animals. GFR of conscious female ENT1-/- mice averaged 347±59 ml/min (n=8; body weight 20.5±0.25 g) compared to 345±26 ml/min in WT mice (n=11; body weight 22.7±0.36 g). Responses of stop flow pressure (Psf) to maximum flow stimulation were significantly reduced in ENT1-/- compared to WT mice (1.63±0.4 mm Hg, n=28 vs. 5.8±1.1 mm Hg, n=17; $p<.0001$). Renal mRNA expression of A1AR, A2aAR, A2bAR, and A3AR were not significantly different between WT and ENT1-/- mice. In conclusion, TGF responsiveness is significantly attenuated in the absence of ENT1, and this effect may be related to A1AR saturation resulting from the marked increase of extracellular adenosine levels. The reduced spontaneous and voluntary locomotor activity is likely to result from the central inhibitory effect of adenosine.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO001**

**Clinical Utility of Delta Gap in Evaluating Mixed Metabolic Acid-Base Disorders in Acute Kidney Injury (AKI)** Rolando Claure-Del Granado,[1] Etienne Macedo,[1] Sharon Soroko,[1] Glenn M. Chertow,[2] Jonathan Himmelfarb,[3] T. Alp Ikizler,[4] Emil P. Paganini,[5] Ravindra L. Mehta.[1] *[1]University of California, San Diego; [2]Stanford University School of Medicine; [3]Kidney Research Institute, University of Washington; [4]Vanderbilt University Medical Center; [5]Cleveland Clinic Foundation.*

**Background:** Metabolic acid-base disorders are common in ICU pts with AKI however are often not well characterized. Mixed metabolic acid-base disorders are often unrecognized as they are influenced by multiple factors including the severity of AKI and the underlying disorders. We hypothesized that Δ gap would identify persistent alterations in acid-base balance. **Methods:** We analyzed data from 363 ICU pts with AKI who were dialyzed, from 5 centers included in the PICARD study. Mixed acid-base disorders were classified using the Δ gap (Δ anion gap- Δ bicarb), into the following groups: a) anion gap (AG) acidosis (Δ gap -6 to +6) b) AG acidosis + metabolic alkalosis (Δ gap >+6) c) non AG acidosis + AG acidosis (Δ<-6). Changes in Δ gap were assessed during consecutive days of IHD and CRRT. **Results:** At initiation of dialysis the mean values of $HCO_3$, pH and AG were similar for both modalities. After initiation of RRT, the % of pts with pure AG acidosis declined over consecutive days of RRT however there was an increase of metabolic mixed acid-base disorders which differed based on the modality used (Fig 1).




**Conclusions:** Mixed acid-base metabolic disorders are commonly found during consecutive days of RRT that can be effectively evaluated by Δgap. Use of saline solution and consequent hyperchloremia, citrate and other factors that are present during RRT could contribute to the persistence of mixed metabolic acid-base disorders. Therapy adjustments with dialysis should consider the effect on acid base status.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO002**

**Efficacy of Glucose Versus Glucose Plus Insulin in the Treatment of Hyperkalaemia** Mogamat-Yyazied Chothia,[1] Mitchell L. Halperin,[4] Megan A. Rensburg,[2] Mogamat Shafick Hassan,[3] Mogamat Razeen Davids.[1] *[1]Stellenbosch University, Cape Town, South Africa; [2]Stellenbosch University & NHLS, Cape Town, South Africa; [3]Cape Peninsula University of Technology, Cape Town, South Africa; [4]University of Toronto & St Michael's Hospital, Toronto, Canada.*

**Introduction**

The emergency treatment of hyperkalaemia remains controversial. Most authors recommend the use of insulin plus glucose as first-line therapy. However, symptomatic hypoglycaemia remains a common complication of this treatment. This study examined the ability of a glucose bolus to cause insulin release and potassium shift into cells without the risk of hypoglycaemia.

**Methods**

A randomised cross-over study compared an IV glucose bolus alone to glucose plus insulin in 7 chronic haemodialysis patients. The primary endpoint was a significant difference K at 60 minutes. Hypoglycaemic episodes and serum insulin concentrations were also recorded.

**Results**

Baseline K was similar at 5.86 ± 0.66 mM in the insulin group and 5.83 ± 0.70 mM in the glucose-only group. Mean serum potassium at 60 minutes was 4.91 ± 0.50 mM in the insulin group versus 5.40 ± 0.87 mM in the glucose-only group (p=0.022). The absolute fall in K was 0.94 ± 0.56 mM in the insulin group versus 0.43 ± 0.25 mM in the glucose-only group (p=0.063). There were 6/7 subjects in the insulin group and only 3/7 in the glucose-only group who demonstrated a fall of at least 0.5 mM at the 60-min time point.

The range of blood glucose concentrations recorded was 3.0-26.8 mM in the insulin group and 3.8-29.3 mM in the glucose group. At all time points after baseline, the insulin group had higher plasma insulin concentrations and lower glucose/insulin ratios.

**Conclusion**

This study confirmed the superiority of glucose plus insulin for the emergency treatment of hyperkalaemia. Clinicians must attempt to prevent hypoglycaemia by infusing adequate amounts of glucose. A glucose-only bolus has a moderate effect, similar to that seen in some studies of inhaled beta-agonists, and may be considered as another therapeutic option in a situation when insulin is not immediately available.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO003**

**Assessing and Tracking Impact of Use of Sodium Polystyrene Sulfonate in an Inner City Hospital** Zaher Hamadeh, Pu Zong, Manish Ramesh, Anjali Acharya. *Department of Nephrology, Albert Einstein College of Medicine (Jacobi), Bronx, NY.*

***Introduction:*** Sodium Polystyrene Sulfonate (SPS) is a cation-exchange resin commonly used for treatment of hyperkalemia.SPS releases sodium ions in the acidic stomach, binds hydrogen ions and exchanges hydrogen ions for potassium in the intestine. Originally SPS was given mixed in water which caused constipation and fecal impaction.It was then mixed with 70% sorbitol, an osmotic cathartic agent which has been implicated in the associated complications of intestinal necrosis and other serious gastrointestinal adverse events. The formulation was then changed to 33% Sorbitol. Recently, there has been an interest again in the safety and effectiveness of SPS. Since SPS is a commonly used drug at our institution we looked at our experience with it. ***Methods***: Retrospective chart review using Electronic Medical Record (EMR). We obtained from the pharmacy a list of all patients who received SPS from 05/01/2009 until 04/30/2010. Billing data was retrieved from the database using the ICD 9 codes *of interest and gastrointestinal symptoms during the same period of time. Records of the 28 patients who were cross referenced on the two lists were reviewed for complications following each SPS dose.

Table 1

| | |
|---|---|
| Total number of patients received SPS | 988 |
| Total number of SPS doses given. | 2023 |
| Total number of patients with ICD codes * | 26 |
| Average dose of SPS (gram) | 22.4 |
| Average number of SPS doses per patient | 2.04 |
| Mean pre SPS K (mEq/L) | 5.76 +/- 0.08 |
| Mean post SPS K (mEq/L) | 4.98 +/- 0.07 |
| Number of patient who had serious GI events | 0 |

*569.83 Perforation of Intestine, 560 Intestinal Obstruction,560.89 Acute pseudo-obstruction of intestine 560.81 Intestinal or peritoneal adhesions with obstruction 560.1 Paralytic Ileus.

***Results***: No serious gastrointestinal adverse effects occurred after SPS dose. Mean pre and post SPS potassium values were 5.76 +/- 0.08 mEq/L and 4.98 +/- 0.07 mEq/L respectively(p value<0.0001). ***Conclusion:*** In our experience SPS is effective in lowering potassium levels and has not been associated with any severe adverse gastrointestinal complications.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO004**

**A Comparison of Serum Bicarbonate and Potassium Concentration Measurements in Arterial and Venous Samples** William G. Herrington,[1] Helen Nye,[1] Peter Watkinson.[2] *[1]Renal and General (Internal) Medicine, Oxford Radcliffe Hospitals NHS Trust, Oxford, United Kingdom; [2]Kadoorie Centre for Critical Care Research and Education, Oxford Radcliffe Hospitals NHS Trust, Oxford, United Kingdom.*

**Introduction**

Blood gas analysis is an important tool for the rapid assessment of critically ill patients. Previous studies do not consistently suggest that arterial and venous sites can be used interchangeably. Lack of duplication of each analysis in these studies means it is unclear whether perceived differences between sample sites reflects instrument imprecision.

**Aim**

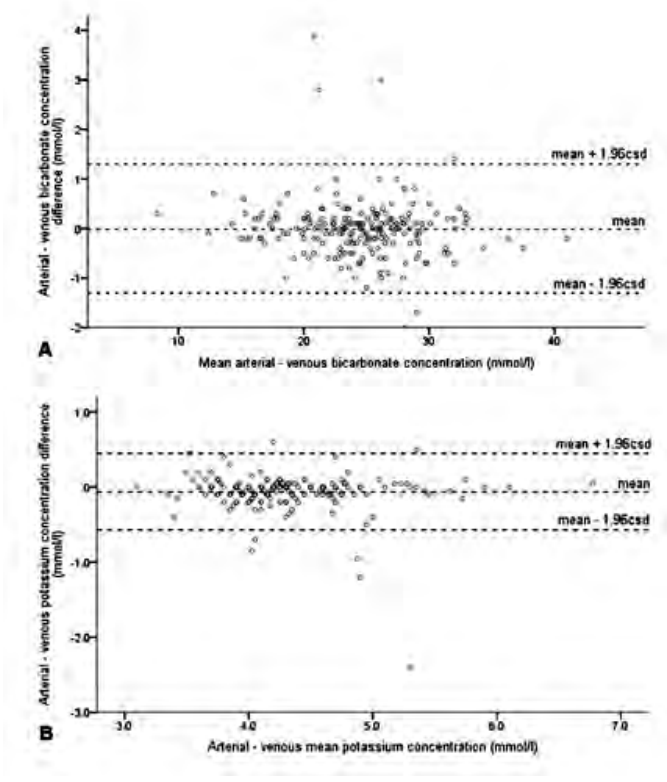
To assess the comparability of venous and arterial samples for bicarbonate and potassium measurements.

**Methods**

Simultaneous arterial and venous samples from 206 critically ill patients were analysed in duplicate. Coefficients of variation and 95% limits of agreement for duplicate measures were calculated for arterial and venous samples. Bland Altman plots (using the means of the two duplicates and the corrected standard deviation of the differences) were constructed to assess agreement between sampling sites.

**Results**

The median (range) of arterial bicarbonate concentrations and arterial potassium concentrations were 25 (9-41) mmol/l and 4.2 (3.1-6.8) mmol/l respectively. Coefficients of variation for arterial and venous bicarbonate results were 0.8 and 0.7% respectively, with bias (95% limits of agreement) of 0 (-0.5-0.5) mmol/l for both sample types (A). The bias between venous and arterial samples was 0 (-1.3-1.3) mmol/l. Coefficients of variation for arterial and venous potassium samples were 0.8 and 1.1% respectively, with bias of 0 (-0.1-0.1) for both sample types. The bias between venous and arterial samples was 0.1 (-0.4-0.6) mmol/l (B).




**Conclusions**

A venous blood sample is sufficiently accurate to assess bicarbonate and potassium concentrations in critically ill patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO005

**Clinicogenetic Features of Korean Patients with Gitelman Syndrome** Kwon Wook Joo,[1] Ki Young Na,[1] Sejoong Kim,[2] Hye Ryoun Jang,[3] Jay Wook Lee,[5] Hae Il Cheong,[4] Jin Suk Han.[1] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Korea; [2]Internal Medicine, Gachon University of Medicine and Science, Incheon, Korea; [3]Internal Medicine, Samsung Medical Center, Seoul, Korea; [4]Pediatrics, Seoul National University Hospital, Seoul, Korea; [5]Internal Medicine, Chung-Ang University Yong-San Hospital, Seoul, Korea.*

New classification of Bartter-like syndromes according to the underlying defective ion transporter is recently proposed due to diverse clinical features and genetic mutations of Gitelman syndrome(GS). Although mutations in *CLCNKB* as well as *SLC12A3* can also be the cause of GS, incidence and clinical differentiation in GS patients of the two mutations are still unclear. We investigated the clinical features and genetic variations of Korean patients with GS. 24 Korean GS patients(16 males and 8 females) were enrolled. Their clinical history, biochemical features, results of thiazide test, and mutation analysis of *SLC12A3* and *CLCNKB* were compared. Genes for *SLC12A3* and *CLCNKB* were directly sequenced and analyzed. All the subjects had hypokalemia, metabolic alkalosis, and normal blood pressure. Hypocalciuria was observed in 22 patients. 9 patients did not have hypomagnesemia. *SLC12A3* gene mutations were detected in 21 patients(9 compound heterozygous, 7 heterozygous, and 5 homozygous mutations). Mutations in *CLCNKB* were identified in 2 patients(1 heterozygous; W530L, 1 homozygous; W610X) with *SLC12A3* mutations and in 1 patient(homozygous; E199X) without *SLC12A3* mutations. Thiazide test was performed in 20 patients, of which results were compatible with GS except in 1 patient who had only homozygous *CLCNKB* mutation. The patient with mutations in *CLCNKB* showed relatively early onset and mild growth retardation, but biochemical abnormalities were not distinguishing. The patient with only homozygous *CLCNKB* mutation showed reduction of distal fractional chloride reabsorption in response to thiazide by more than 50%. Diagnosis of GS can depend primarily on the clinical criteria and thiazide test. The overlap between clinical presentations of *CLCNKB* and *SLC12A3* mutations was significant and mutation analysis of both *CLCNKB* as well as *SLC12A3* is necessary.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO006

**Unusual Presentation of Gitelman's Syndrome: A Case Report** Smitha Reddy Anam,[1] Yangming Cao.[2] *[1]Internal Medicine, UCSF Fresno, Fresno, CA; [2]Nephrology, UCSF Fresno, Fresno, CA.*

**Background**: Despite advancement of genetic tests, the clinical diagnosis of Bartter's syndrome (BS) and Gitelman's syndrome (GS) can be challenging. Here we describe an unusual case of GS with hypercalciuria and hypocalcemia.

**Case Report**: A 45 year-old Hispanic male presented to the emergency room with a 3 day history of generalized weakness and muscle cramps. He had no significant past medical history and denied taking any medications or recent alcohol use. On physical exam he was normotensive, but he developed bilateral carpopedal spasm immediately after admission. He had hypokalemia (serum $K^+$ 2.7 mEq/L), hypomagnesemia (serum Mg 0.8 mg/dL) and metabolic alkalosis. He also had hypocalcemia (serum Ca 6.7 mg/dL and ionized $Ca^{++}$ 4.05 mg/dL) with 25(OH)Vit D deficiency (serum 16 ng/ml). Serum renin and aldosterone levels were normal. Twenty-four hour urinary electrolytes were consistent with renal wasting of $Na^+$ (405 mEq), $K^+$ (86.8 mEq), $Cl^-$ (459 mEq), Ca (376 mg) and Mg (694 mg). Surreptitious use of diuretics (bumetanide and furosemide) was excluded by test. The hypercalciuria with hypocalcemia made diagnosis of GS difficult. Hence a a 50 mg hydrochlorothiazide (HCTZ) challenge test after adequate hydration was performed. Compared with baseline FeCl (1.5%), FeCl increased to 1.9% (Δ FeCl 0.4%) at 30 min and 2.1% (ΔFeCl 0.6%) at 60 min after HCTZ. This demonstrated a blunted response of FeCl to HCTZ. A diagnosis of GS was made. Over a few days, his serum electrolytes normalized with replacement. His symptoms resolved and was discharged on oral supplements of K, Mg, Ca, Vit D and spironolactone.

**Discussion:** Diagnosis of GS over BS was favored because of age at presentation, hypomagnesemia and absence of overt polyuria. HCTZ challenge test showed a blunted response of FeCl (Δ FeCl 0.6%). From literature review, maximal increase of FeCl (Δ FeCl) of <2.3% after HCTZ indicates GS. Even though GS typically has hypocalciuria, our patient had significant hypercalciuria despite hypocalcemia. The hypocalcemia may be explained by vitamin D deficiency and hypomagnesemia.

**Conclusion**: HCTZ challenge test aids the diagnosis of GS.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO007

**Lowe Syndrome-Like Phenotype Caused by a Large Deletion That Includes CLCN5: A Novel Contiguous Deletion Syndrome** Mathieu Lemaire,[1,2] Hae Il Cheong,[3] Weizhen Ji,[1,2] Hyewon Park,[4] Jae Il Shin,[5] Richard P. Lifton.[1,2] *[1]Genetics, Yale School of Medicine, New Haven, CT; [2]HHMI; [3]Pediatrics, Seoul National University, Children's Hospital, Seoul, Korea; [4]Pediatrics, Seoul National University, Bundang Hospital, Seongnam, Korea; [5]Department of Pediatrics, Yonsei University College of Medicine, Severamce Children's Hospital, Seoul, Korea.*

Lowe syndrome is characterized by renal proximal tubule dysfunction, congenital cataracts, cryptorchidism, stunted growth, mental retardation and seizures. It is caused by mutations in OCRL1. Proximal tubule dysfunction observed in Dent's disease is analogous to that of patients with Lowe syndrome: at a minimum, low molecular weight proteinuria (LMWP) and either hypercalciuria or nephrocalcinosis are documented, with or without other tubular defects. Dent's disease is mainly caused by mutations in CLCN5, but a small number of patients also harbor OCRL1 mutations. We report the case of an orphaned 5-year-old Korean male with clinical features consistent with a diagnosis of Lowe syndrome: LMWP with hypercalciuria and nephrocalcinosis, cryptorchidism, stunted growth (< 3rd percentile), developmental delay and recurrent seizures. However, a number of unusual characteristics for Lowe syndrome were noted, such as hypothyroidism, micropenis, cleft palate and absence of cataracts. Since genomic analysis revealed no mutation in OCRL1, analysis of CLCN5 was attempted: only exon 1 of CLCN5 was successfully amplified by PCR. SNP genotyping on the 650K Illumina platform suggested a 3.5 Mb deletion at Xp11.22, and long-range PCR was used for fine mapping. Apart from CLCN5, the deleted interval also contains 14 genes. Of these, only SHROOM4 has a well-described association to a male-specific condition, namely Stocco dos Santos syndrome. Its typical features are reminiscent of our patient's: mental retardation, seizures and stunted growth. We posit that the unusual Lowe syndrome-like phenotype exhibited by our patient is due to a novel contiguous deletion and is in major part explained by the simultaneous loss of function of CLCN5 and SHROOM4. The remaining 13 genes are prime candidates to explain the characteristics that remain unaccounted for.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO008

**The Relationship between Arginine Vasopressin Levels and Hyponatremia in Children Receiving Hypotonic or Isotonic Intravenous Fluids Following a Percutaneous Renal Biopsy** Michael L. Moritz,[1] Kandai Nozu,[2] Kazumoto Iijima.[2] *[1]Pediatrics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA; [2]Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan.*

**Background:** Post-operative hyponatremia is a common complication in children which results from hypotonic fluid administration in the presence of arginine vasopressin (AVP) excess. There is increasing evidence to suggest that 0.9% sodium chloride could prevent post-operative hyponatremia.

**Purpose:** To evaluate the relationship between the change in serum sodium and AVP levels following a percutaneous renal biopsy in children receiving either hypotonic or isotonic fluids.

**Methods:** A prospective observational study was conducted in 60 children, ages 2 – 21 yrs, undergoing a percutaneous renal biopsy (PRB) from July 2006 to September 2008 at Kobe University Medical Center, Japan. Thirty-three patients received hypotonic fluids (90 mEq/l NaCl) and 27 patients received isotonic fluids (154 mEq/L NaCl) for 5 hours post PRB. AVP levels and biochemistries were obtained prior to (T0) and 5 hrs following (T5) the PRB. Patients were further subdivided into two groups based on the AVP level at T5, those with normal T5 AVP level (< 3.5 pg/ml) and those with an elevated T5 AVP level (> 3.5 pg/ml). The relationship between change in serum sodium in patients with elevated AVP levels were evaluated.

**Results:** A similar proportion of patients, whether receiving hypotonic or isotonic fluids, had elevated AVP levels at T5 ( 30% vs 26%, p = 0.71). Patients receiving hypotonic fluids with elevated T5 AVP levels had a 1.9 ± 1.5 mEq/l fall in serum, whereas patient receiving isotonic fluids with elevated T5 AVP levels had a 0.85 ± 0.34 mEq/l rise in serum sodium. There were no significant changes in serum sodium in patients with normal T5 AVP levels in either group**.**

**Conclusions:** Elevated AVP levels are common following a percutaneous renal biopsy. 0.9% NaCl prevents a fall in serum sodium in the presence of elevated AVP levels while hypotonic fluids do not.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO009

**Maintenance Parenteral Fluid Prescribing Practices among Pediatric Residents** Michael A. Freeman,[1] Juan Carlos Ayus,[2] Michael L. Moritz.[1] *[1]Pediatrics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA; [2]Renal Consultants of Houston, Houston, TX.*

**Background:** The standard practice in pediatrics for over 50 years has been to prescribe hypotonic maintenance intravenous fluids (IVF) (Pediatrics 1957;19:123). In 2003, we questioned the safety of this practice (Pediatics 2003;111:2270) and since that time there has been mounting evidence that hypotonic IVF lead to hospital-acquired hyponatremia in states of AVP excess, whereas isotonic IVF effectively prevent it. There is no data evaluating whether IVF- prescribing practice has changed with increased awareness of this problem.

**Purpose:**To investigate sodium composition of IVF in use by pediatric residents throughout the United States in common clinical scenarios of AVP excess.

**Methods**: An internet survey was distributed to all pediatric residency programs in the US asking residents what type of IVF (0.2%, 0.45%, 0.9% NaCl or Lactated Ringers) they would administer in four common clinical scenarios (Table) in both a 6-mo-old and 13-yr-old child.

**Results:** Respondents selected a hypotonic IVF in78% of cases. The 0.2% NaCl solution was commonly used in infants (35.5%), yet rarely used in the older children (0.5%). The choice of IVF composition varied by both the age and illness of the child (Table), with isotonic IVF more likely to be used in children with meniningitis.

Percentage of Pediatric Residents Selecting Hypotonic Fluids

| Clinical Scenario | 13-yr-old pt | 6-mo-old pt |
|---|---|---|
| Gastroenteritis | 69% | 91% |
| Pneumonia | 75% | 92% |
| Meningitis | 51%* | 79%* |
| Post-surgical | 72% | 91% |

p < 0.01

Seventy-five percent acknowledged being aware of the clinical controversy regarding the use of isotonic IVF. Residents aware of the controversy were twice as likely to prescribe isotonic IVF than those unaware ( 24.5% vs 12.9%, P<0.001).

**Conclusions:** The majority of pediatric residents continue to use hypotonic IVF. However, a significant number of residents are using isotonic IVF. Awareness of this clinical controversy is prevalent and those residents who are aware of this controversy are more likely to use isotonic IVF.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO010

**Stanniocalcin-1 Protects from Ischemia/Reperfusion Kidney Injury** Luping Huang, Tatiana Belousova, David Sheikh-Hamad. *Medicine, Baylor College of Medicine, Houston, TX.*

Inflammation, endothelial dysfunction, ROS production and MAPKs play critical roles in the pathogenesis of Ischemia/reperfusion kidney injury (I/R). Published data from our lab suggest that stanniocalcin-1 (STC1): blocks cytokine-induced rise in endothelial permeability; inhibits macrophage function; and suppresses superoxide generation in many systems (including endothelial cells and macrophages). We hypothesized that STC1 protects from ischemia/reperfusion kidney injury through inhibition of key pathways in its pathogenesis. Using wild type and STC1 transgenic mice which display high circulating STC1 levels and preferential expression of STC1 transgene in macrophages and endothelial cells, we examined the following at baseline, 24h, 48h, 72h, and 8d after I/R kidney injury (generated by clamping bilateral renal arteries for 30 min; Sham-treated controls were subjected to the same surgical procedure without renal aa clamping): creatinine clearance (CrCl); kidney morphology and infiltration with macrophages and T-cells; trans-endothelial permeability, measured using evans blue; NOS activity, measured as production of nitrates and nitrites. Results: following I/R and compared to sham-treated controls: WT mice displayed >65% drop in creatinine clearance (at 72h), coupled with severe tubular vacuolization and cast formation (at 24h, 48h and 72h); increased macrophage and T-cell infiltration (from 24h through day 8); higher trans-endothelial permeability to Evans blue; lower NOS activity (24h and 48h); but, greater pERK/pJNK+p-p38 activity ratio (at 24h and 48h). On the other hand, STC1 Tg mice displayed: no drop in creatinine clearance; no morhpological changes; no increase in macrophage or T-cell infiltration; no change in pERK/pJNK+p-p38; while NOS activity was preserved. Conclusions: 1) Transgenic overexpression of STC1 protects from I/R kidney injury; 2) Protection by STC1 from I/R is associated with stable NOS activity, preservation of endothelial permeability, and inhibition of inflammation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO011

**Activator of G Protein Signaling 3 Promotes Renal Epithelial Cell Proliferation Following Ischemia-Reperfusion Injury** Kevin R. Regner,[1] Michelle J. Kwon,[1] Sarah M. White,[1] Vani Nilakantan,[2] Frank Park.[1] *[1]Nephrology, Medical College of Wisconsin; [2]Surgery, Medical College of Wisconsin, Milwaukee, WI.*

Renal tubular epithelial cell (RTEC) proliferation plays a critical role in the regeneration of the kidney following ischemia-reperfusion injury (IRI). The mechanisms that regulate RTEC proliferation following IRI remain poorly understood. Heterotrimeric G proteins are pivotal molecular switches that can regulate epithelial cell biology. In genetic models of abnormal RTEC proliferation, our lab has identified elevated expression of Activator of G protein signaling 3 (AGS3), a novel receptor-independent regulator of G proteins. We therefore reasoned that AGS3 may regulate RTEC proliferation during the regenerative phase following experimental renal IRI. Sprague-Dawley rats underwent 30 min bilateral renal ischemia and reperfusion for 24 to 168 hours. During the reperfusion phase, there was a temporal increase in renal AGS3 protein expression reaching a peak level at 72 hours (60-fold increase compared to time-control sham-operated kidneys). This increase in AGS3 expression lagged the changes in serum creatinine. By 168 hours, AGS3 expression decreased to levels found in sham-operated kidneys. The increased AGS3 expression was exclusively localized to outer medullary RTECs and ~50% of all AGS3-positive RTECs were also positive for Ki-67, a marker of proliferation. Genetic knock-down of endogenous AGS3 in normal rat kidney epithelial cells using specific AGS3 shRNA significantly reduced cell proliferation by 26% through a cAMP-independent mechanism. Dose-dependent decreases in epithelial cell proliferation were observed following the incubation of renal epithelial cells with gallein (1.3-6.5 mM), a selective inhibitor of Gβγ subunit activity. Similar reductions in epithelial cell number (~30%) were observed using lentiviral overexpression of GRK2ct, a scavenger of Gβγ subunits, which confirmed our pharmacologic experiments. In conclusion, our data suggest that AGS3 participates in the process of RTEC proliferation during renal tubular regeneration by activating Gβγ subunits through a novel receptor-independent mechanism.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO012

**PGC-1α Is Transcriptionally Up-Regulated in Renal Tubules of Ischemia/Reperfusion AKI Via Hypoxia and AMPK** Yoshio Terada. *Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Nankoku, Kochi, Japan.*

PGC-1α plays a central role in regulating the mitochondrial content and oxidative metabolism. Oxidative stress and mitochondrial damage were key factors in acute kidney injury (AKI). However, the regulation and functional roles of PGC-1α in renal tubules were poorly understood. Understanding the regulation and function of PGC-1α in renal tubules would provide important clues to the elucidation of the pathophysiology of AKI.

The aim of this study is to understand the roles of PGC-1α in AKI in vivo and in vitro, and the regulation of PGC-1α gene in renal tubular cells. To clarify the significance of PGC-1α and AMPK pathway in AKI, we used a rat AKI model in vivo and cultured renal tubular cells (NRK-52E cells) as an in vitro model. After clamping left rat renal artery for 1 h, kidney homogenate at 1-72 h after reperfusion was extracted. In Western blot analysis, PGC-1α expression and phosphorylation of AMPK were increased at 12-24h and 3-12h, respectively. In immunohistological examination, expression of PGC-1α and phosphorylation of AMPK were increased in proximal tubules. To understand the regulation of PGC-1α in renal tubular cells, we transfected PGC-1α-promoter-luciferase plasmid to NRK-52E cells. H2O2 and AICAR (AMPK activator) significantly increase promoter activity, mRNA and protein expression of PGC-1α. Overexpression of wild type of AMPK induced PGC-1α expression, and DN-AMPK reduced expression. Furthermore, overexpression of PGC-1α induced mitochondrial enzymes, such as UCP (uncoupling protein)-1.

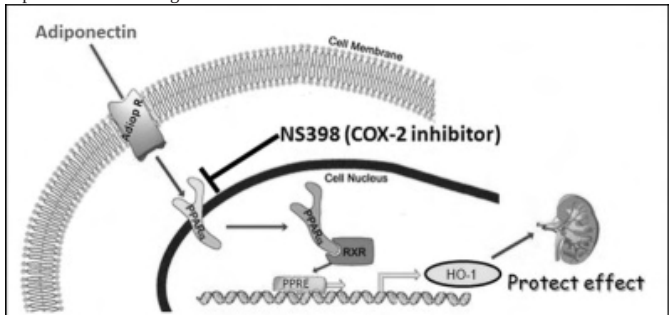
In conclusion, PGC-1α is transcriptionally up-regulated in hypoxic condition in renal tubules via oxidative stress and AMPK. PGC-1α may regulate mitochondrial content and oxidative metabolism in AKI. The current study therefore unravels both pathophysiological significance of PGC-1α in ROS-induced cell damages of renal tubular cells in vivo and in vitro.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO013**

**Protective Effects of Adiponectin Against Renal Ischemia-Reperfusion Injury Via Prostacyclin -PPARα- Heme Oxygenase-1 Signaling Pathway**
Wei-Shiung Lian, Hsiao-Fen Li, Ching-Feng Cheng, Heng Lin. *Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.*

Adiponectin (APN), a circulating adipose-derived hormone that regulates inflammation and energy metabolism, has beneficial effects on the cardio- and cerebro-vascular disorders. Serum APN levels are lower in patients with coronary artery disease and type II diabetes, while hyper-adiponectinemia is found in patients with chronic kidney disease. However, the precise role and molecular mechanism of APN in acute reno-vascular disease is not clear. Results of the present study show that the serum concentration of APN decreased after ischemia /reperfusion (I/R) injury in mice. In addition, I/R-induced renal dysfunction (elevated serum creatinine and urea levels), inflammation (number of infiltrating neutrophils, myeloperoxidase activity, induction of IL-6 and P-selectin) and apoptotic responses (apoptotic cell number and caspase-3 activation) were attenuated in APN-treated compared to control mice. Molecular and biochemical analysis revealed that APN up-regulates heme oxygenase-1 (HO-1) via peroxisome-proliferator- activated-receptor-α (PPARα) dependent pathway which is mediated through the enhancement of COX-2 and 6-keto PGF1α expression. Chromatin immune-precipitation assay demonstrated that APN increases the binding activity of PPARα to the PPRE region of HO-1 promoter. Furthermore, APN induced HO-1 expression only found in wild type but not in PPARα gene deleted mice. This provides in vivo evidence that APN mediated HO-1 expression depends on PPARα regulation.




In conclusion, our results provide a novel APN mediated prostacyclin-PPARα- HO-1 signaling pathway that mediates its protective effects on renal I/R injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO014**

**The Renoprotective Mechanism of Autophagy Against Ischemia-Reperfusion Injury Is UCP2 Protein Dependent**
Yang Zhou, Ruoyun Tan, Junwei Yang. *Center for Kidney Disease, 2nd Affiliated Hospital of Nanjing Medical University, Nangjing, China.*

Recently, autophagy has been identified as a major pathway that delivers damaged proteins and organelles to lysosomes in order to maintain cellular homeostasis. The renoprotective role of Autophagy during ischemia and reperfusion (I/R) is not clear and is currently under intense investigation. Uncoupling protein 2 (UCP2) is a ubiquitously expressed mitochondrial carrier protein. It mediates proton leak across the inner mitochondrial membrane to decrease the yield of ATP from glucose. Here, we found that UCP2 expression was up-regulated after renal I/R injury. Meanwhile, renal specific UCP2-knockout (UCP2-/-) mice had higher renal I/R-induced mortality, kidney dysfunction, oxidative stress, and apoptosis compared with wild-type mice. Furthermore, mice administered with genipin, a specific chemical inhibitor of UCP2, also had more severe kidney dysfunction compared with vehicle treated mice. Analysis of pathophysiological conditions indicated that autophagy-mediated abnormal protein and organelle degradation mechanism was largely impaired in UCP2-/- mice. These findings highlight the importance of UCP2 as a key regulator of autophagy-mediated homeostatic mechanism to maintain renal parenchyma integrity. We postulate that UCP2-dependent autophagy is a major protective mechanism against renal tubule injury, representing a putative target to ameliorate human acute renal injury and I/R-related loss of renal function.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO015**

**Proximal Tubular Cells Acquire the Progenitor Markers CD24 and CD133 during Tubular Repair**
Bart Smeets,[1,2] Jurgen Floege,[1] Johan Van der Vlag,[3] Jack F. Wetzels,[3] Marcus J. Moeller.[1] *[1]Div. of Nephrology and Immunology, University Hospital Aachen, RWTH, Aachen, Germany; [2]Pathology, RUNMC, Nijmegen, Netherlands; [3]Nephrology, RUNMC, Nijmegen, Netherlands.*

Acute tubular necrosis is followed by regeneration of the injured renal tubular epithelial cells. It is has been shown that proximal tubuli are regenerated from intrinsic cells. Nonetheless, it is still unresolved whether intratubular stem/progenitor cells or the entire cell population contribute to the regeneration.

In this study, we identified a cell population localized in the proximal tubuli expressing the progenitor markers CD24 and CD133 using immuno-electron microscopy and immunofluorescent staining of normal human kidney sections and of biopsies showing acute tubular necrosis (ATN). Within normal human kidneys CD24 positive cells were scattered as single cells or in small groups of 2-4 cells throughout the proximal tubule and co-expressed the stem cells marker CD133. The morphology of CD24 positive cells differed from that of normal proximal tubular cells and contained less cytoplasma, fewer mitochondria and the brush border was absent. In contrast to normal proximal tubular cells, the cells expressed CD24, CD133, CXCR4, and other markers that are associated with epithelial dedifferentiation.

In ATN biopsies, the number of CD24 positive tubular cells was much higher compared to normal human kidneys. To establish whether the CD24 positive cells were regenerative, we co-stained for the proliferation marker Ki-67. We observed that in both normal human kidneys and in the ATN biopsies the majority (~85%) of the Ki-67 positive cells was also CD24 positive.

In conclusion, CD24 positive proximal tubular cells represent a novel distinct subpopulation of cells, which apparently participate in the regeneration after ATN.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO016**

**Telomerase Is Expressed in Renal Epithelia in Kidney Medulla and Papilla and Is Upregulated with Injury**
Jie Song,[1,4] Suzanne Czerniak,[1] Teresa Wang,[1] Wendy Ying,[1] Diana Carlone,[2] David Breault,[2,3] Benjamin D. Humphreys.[1,3] *[1]Renal, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Endocrinology, Children's Hospital, Harvard Medical School, Boston, MA; [3]Harvard Stem Cell Institute, Cambridge, MA; [4]Renal, Huashan Hospital, Fudan University, Shanghai, China.*

Telomerase, the enzyme responsible for maintaining telomere length and thus preventing cellular senescence, is highly expressed in embryonic stem cells but downregulated in adults, except in certain adult stem cells including those in intestine, testis, and hematopoietic system. We generated transgenic reporter mice in which the Telomerase promoter drives either GFP or CreERt2 (*mTert*-GFP, *mTert*-CreERt2) to analyze kidney Telomerase expression during homeostasis, acute injury and repair.

We report that renal papilla and inner medulla, areas of high oxidative stress in kidney, express high levels of both *mTert* mRNA and Telomerase activity. Using *mTert*-GFP reporter mice, we observe mosaic *mTert* expression restricted to a subset of tubular epithelial cells, co-localizing predominantly with collecting duct marker (AQP2) but also with thin and thick loop of Henle markers (AQP1, NKCC2 and THP). There was no co-localization of *mTert*-GFP+ cells with interstitial markers CD31, F4/80, $NG_2$ or PDGFRβ. Since Telomerase expression marks stem cells in other adult tissues, we examined whether *mTert*+ cells are slow cycling. After a two-day BrdU pulse at birth followed by an 8 week chase, 5% of *mTert*-GFP cells were long term label retaining. Ischemia-reperfusion injury (IRI) was performed to investigate a role for mTert+ cells in kidney repair. Both mTert mRNA and telomerase activity are upregulated in papilla after IRI. To examine the fate of mTert+ kidney epithelia, adult mTert-CreERt2; R26LacZ bigenic mice received a single tamoxifen pulse, resulting in genetic labeling of 5% of epithelia in papilla and inner medulla, and the fate of these cells was tracked after IRI.

These studies show, for the first time, the cellular localization of Telomerase expression, demonstrate 5% of these cells are slow cycling and suggest a novel role for Telomerase activity in kidney repair.

Disclosure of Financial Relationships: nothing to disclose

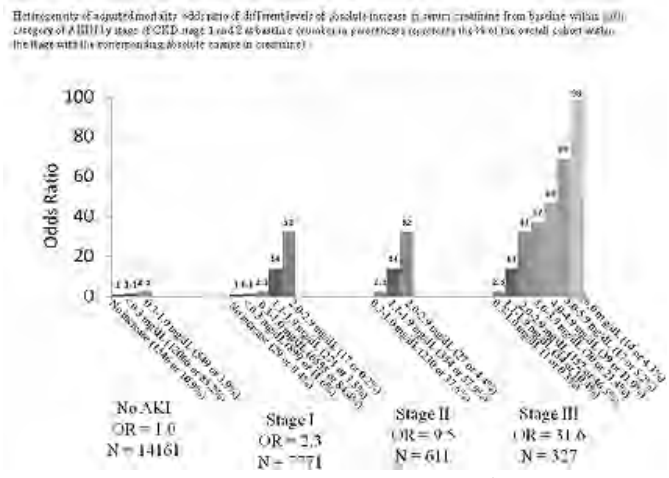
**TH-PO017**

**RIFLE and AKIN Misclassify the Severity of Acute Kidney Injury after Surgery**
Farsad Afshinnia,[1,2] Sean Nugent,[2] Tamara Schult,[2] Nancy Greer,[2] Areef Ishani.[1,2] *[1]University of Minnesota, Minneapolis, MN; [2]VA Medical Center, Minneapolis, MN.*

**Aims:** The prognostic improvement of RIFLE or AKIN compared to a continuous change in serum creatinine (Cr) to predict short-term mortality is unclear. Our study aim is to show the heterogeneity of risk of 30-day mortality after surgery within various stages of RIFLE and AKIN and their potential to misclassify the risk of mortality associated with acute kidney injury (AKI) when compared with continuous increase of Cr.

**Methods:** Using a nationwide VA database, 27204 patients with a baseline Cr corresponding to stages 1 to 3 of CKD who underwent surgery from 1999 to 2005 were identified. AKI after operation was defined according to RIFLE and AKIN. The outcome was 30-day mortality.

**Results:** In patients with CKD stage 1 or 2 at baseline, odds of mortality were 2.4, 9.7, and 29.3 in Risk, Injury and Failure, and 2.3, 9.5, 31.6 in stage 1 to 3 of AKIN, respectively, compared to no AKI ($P < 0.001$). There was also a graded increase in mortality from 2.3 to 98 by each 1 mg/dL increase of Cr from baseline when compared to no AKI in the same patients. As shown in the graph, there was a significant variability in odds of death within each stage of RIFLE or AKIN stratified by each 1 mg/dL increase of Cr, with the highest variability seen in category of Failure or stage 3 of AKIN. Similar results are noted in patients with CKD stage 3.



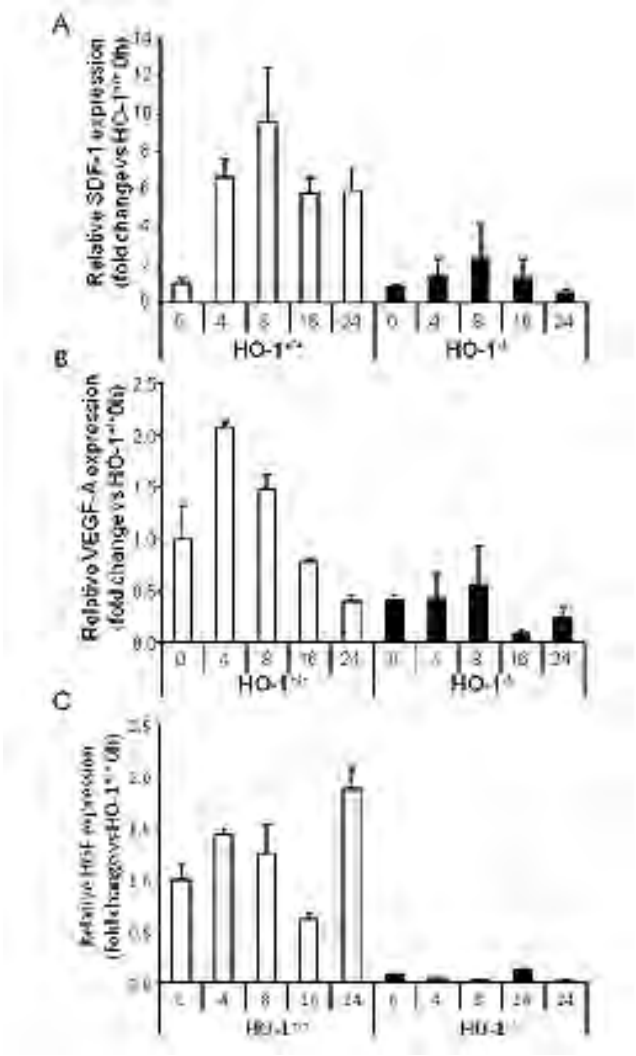

**Conclusion:** RIFLE and AKIN erroneously estimate the risk of mortality by collapsing wide range changes of Cr into similar categories. As a result, the risk of mortality is overestimated by lower levels of increase in Cr and underestimated by higher levels of increase in Cr within each stage of AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO018**

**Mesenchymal Stem Cells Induced Protection in Acute Kidney Injury (AKI) – Role of Heme Oxygenase-1 (HO-1)** Abolfazl Zarjou, Junghyun Kim, Carl A. Frizell, Amie Traylor, Anupam Agarwal, Lisa M. Curtis. *Medicine, University of Alabama at Birmingham, Birmingham, AL.*

Multipotent mesenchymal stem cells (MSCs) have become a popular and promising therapeutic approach in many clinical conditions. MSCs are beneficial in animal models of AKI, effects that are mediated by differentiation independent paracrine properties. MSCs have proven beneficial in cardiac surgery-related AKI and there is an ongoing clinical trial evaluating the safety and efficacy of MSCs. HO-1 is induced in response to stress and has important anti-apoptotic, anti-inflammatory and proagiogenic properties. We therefore examined whether HO-1 expression plays a role in the beneficial effects of MSCs. We isolated MSCs from bone marrow of age and sex matched HO-1$^{+/+}$ and HO-1$^{-/-}$ mice. Successful cultures were validated by differentiation into adipocytes, osteocytes and chondrocytes. Our studies indicate that differentiation of MSCs was not significantly different between cells isolated from HO-1$^{+/+}$ and HO-1$^{-/-}$ mice. However, when exposed to hypoxia MSCs derived from HO-1$^{-/-}$ mice demonstrate reduced expression and secretion of several important growth and proangiogenic factors when compared to MSCs derived from HO-1$^{+/+}$ cells. These factors include stromal cell-derived factor-1 (SDF-1), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and insulin like growth factor-1 (IGF-1).




Conditioned media from HO-1$^{-/-}$ MSCs exposed to 24 h of hypoxia showed significantly lower levels of SDF-1 compared to HO-1$^{+/+}$ MSCs (HO-1$^{-/-}$ vs HO-1$^{+/+}$, 5.3±1.6 vs 19.5±0.65 ng/mg protein, $p<0.05$, n=4/group). Our studies indicate that HO-1 expression in MSCs is required for their optimal protective function and provide a basis for further studies to explore the effects of modulating HO-1 expression in MSCs in in vivo models of AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO019**

**Adult Stem Cells from Foreign-Body Granulation Tissue Protect the Kidney and Accelerate Recovery from Acute Kidney Injury** Jilpa Patel,[2] Nishit Pancholi,[2] Krishnamurthy P. Gudehithlu,[1,2] George Dunea,[1,2,3] Jose A. L. Arruda,[3,1,2] Ashok K. Singh.[1,2] *[1]Nephrology, John H. Stroger Hospital of Cook County, Chicago, IL; [2]Nephrology, Hektoen Institute of Medicine, Chicago, IL; [3]Nephrology, University of Illinois at Chicago, Chicago, IL.*

In earlier studies we showed that granulation tissue induced by implanting polyvinyl tubes subcutaneously in rats is a source of mesenchymal stem cells (granulation tissue derived stem cells or GTSC). Here we tested the efficacy of GTSC to ameliorate acute kidney injury (AKI) in Fischer (F344) in-bred rats (baseline plasma creatinine 0.7 ± 0.06 mg/dL and plasma urea 50 ± 3.4 mg/dL). We induced AKI by unilateral (right) nephrectomy, occlusion of the left renal pedicle for 45 minutes followed by de-occlusion and reperfusion of the kidney. After inducing AKI, we divided the rats into two groups. Three hours after injury, group 1 (treated) rats received one intravenous injection of GTSC (2- 4 X $10^6$ cells in 0.7 ml saline) and group 2 (control) rats received vehicle. Twenty-four hours after injury, treated rats (versus controls) showed significantly lower plasma creatinine (1.0 ± 0.04 mg/dL vs. 1.6 ± 0.12 mg/dL) and plasma urea (71 ± 5.9 mg/dL vs. 138 ± 7.1 mg/dL) ($p<0.001$, N=8 in each group). In the treated group plasma creatinine and urea levels remained low, reaching baseline levels by day 4. In controls they did not reach baseline levels until day 7. On histology, there was less tubular dilatation, necrosis, congestion, and casts in the treated rats. PCNA immune-staining in the treated group showed a higher level of cell proliferation in the cortico-medullary region, suggesting that rapid cell renewal was responsible for the accelerated recovery in the treated rats. These results show that administration of GTSC protected the kidney and accelerated recovery from AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO020**

**P2Y Receptors Mediate EGFR Activation and Renal Tubular Cell Proliferation during Acute Kidney Injury** Monika Gupta,[1] Peifeng Deng,[1] Peter Green,[1] Rick G. Schnellmann.[2] *[1]Medicine, Medical University of South Carolina; [2]Pharmaceutical Sciences, Medical University of South Carolina, Charleston, SC.*

While it is known that epidermal growth factor receptor (EGFR) is critical for repair and regeneration of renal proximal tubular cells (RPTC) following acute kidney injury (AKI), the sequence of events that lead to activation of EGFR are not known. Using primary cultures of rabbit RPTC, the aim of this study was to examine the role of P2Y receptors in activation of EGFR and RPTC proliferation following injury. P2Y1, P2Y2, P2Y4, P2Y12 and P2Y14 were expressed in RPTC using RT-PCR analysis. RPTC monolayers were scrape wounded and incubated for 24 h in the presence or absence of AG1478 (EGFR antagonist), RB-2 (non-selective P2Y receptors antagonist), AR-C66096 (P2Y12 antagonist), and MRS 2279 (P2Y1 antagonist). Cell proliferation was determined using a BrdU incorporation assay. At 24 h, 19% of the injured cells were proliferating compared to 2% of the cells incubated with AG1478. While MRS 2279 and AR-C66096 had no effect on proliferation, RB-2 decreased proliferation to 11%. Addition of exogenous EGF to RB-2 treated cells reversed the inhibition produced by RB-2, providing evidence that P2Y receptors are upstream of EGFR. Hence, one or more of P2Y2, P2Y4 and P2Y14 are required for approximately 50% of the proliferative signal after cell injury. We then examined the activation of EGFR at tyrosine 845 by P2Y agonists ATPγS, MRS2690, and UTPγS using immunoblot analysis and observed that all three agonists activated EGFR. We then examined the role of Src and ADAM 17 in P2Y-stimulated RPTC proliferation following scrape wounding. Src inhibition (PP-1), P2Y (RB-2) and ADAM17 inhibition (TAPI-1) decreased proliferation to 9%, 11% and 13%, respectively compared to 19% in injured RPTC. The combination of PP-1 and TAPI-1 was additive, supporting Src and ADAM17 as major but distinct pathways for EGFR activation. While inhibition of ADAM17 and P2Y receptors was not additive, inhibition of Src and P2Y receptors was additive. Hence, P2Y receptors mediate EGFR activation and RPTC proliferation via ADAM17.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO021**

**VEGF-Modified Human Embroyonic Mesenchymal Stem Cell Implantation Enhances Protection Against Cisplatin-Induced Acute Kidney Injury** Li Yuan,[1] Changlin Mei.[2] *[1]Division of Nephrology, Changzheng Hospital, Second Military Medical University, Shanghai, China; [2]Division of Nephrology, Changzheng Hospital, Second Military Medical University, Shanghai, China.*

**Objective:** Vascular endothelial growth factor (VEGF) is an important factor in renoprotection produced by MSC. We investigated the effects of VEGF modified human embryonic mesenchymal stem cells (VEGF-hMSC) in a cisplatin-induced tubular cell damage model in vitro and a nude-mouse model of cisplatin-induced AKI in vivo.

**Methods:** TCMK-1 were pretreated with cisplatin for 24 h and cocultured with hMSC, VEGF-hMSC and Ad-hMSC. Three days after cisplatin pretreatment, PCNA expression, regeneration and apoptosis of TCMK-1 were tested. AKI was induced by subcutaneous injection of cisplatin (18 mg/kg). 24 h after cisplatin administration, nude mice were divided into four groups and received a tail intravenous injection as follows: saline, VEGF-hMSC, Ad-hMSC, and hMSC. Normal control animals did not undergo cisplatin injection. Serum creatinine (SCr), blood urea nitrogen (BUN),HE staining, PCNA, TUNEL staining and CD34 were tested 4 days after cisplatin administration.

**Result:** Compared with TCMK-1 pretreatment with cisplatin alone, co-culturing with every kind of hMSC ceased the inhibitory effect of cisplatin on TCMK-1 growth, reduced apoptosis, and increased PCNA expression. These effects were most pronounced in VEGF-transfected hMSC. VEGF transfecton improved the ability of hMSC to impair cisplatin induce TCMK-1 injury. In addition, in a nude-mice model of cisplatin-induced kidney injury, administration of VEGF-hMSC offered more protective effects on renal function, tubular structure and survival. Cell proliferation increased, apoptosis decreased, and peritubular capillary density in the kidney improved in animals treated with VEGF-hMSC.

**Conclusion:** Implantation of VEGF-modified hMSC could provide advanced benefits in protection against renal tubular injury by anti apoptosis, improving microcirculation and proliferation .

Disclosure of Financial Relationships: nothing to disclose

**TH-PO022**

**Impaired Proliferation and Mesenchymal Transition of Kidney Endothelial Cells Following Ischemic AKI: Role of VEGF** David P. Basile,[1] Jessica Friedrich,[1] Ellen Leonard,[1] Bruce A. Molitoris,[2] Robert L. Bacallao,[2] Timothy A. Sutton.[2] *[1]Cellular & Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN; [2]Medicine, Indiana University School of Medicine, Indianapolis, IN.*

AKI-induces the loss of renal microvessels and may lead to progressive CKD. Vascular rarefaction and progression to CKD is ameliorated by prior treatment with VEGF-121, but the fate of endothelial cells and the mechanism of VEGF protection is unknown. Endothelial proliferation was examined in kidneys of SD rats following 40 min of bilateral I/R injury and recovery for 1, 2, or 7 days post-ischemia. Cumulative cell proliferation was examined by repetitive administration of BrdU (twice daily) and colocalization in endothelial cells with CD31 or cablin. Regenerating tubules in the cortex and outer medulla accounted for >95% of BrdU + cells. Proliferating endothelial cells were not detected at 1 or 2 days post I/R and accounted for only ~ 1% BrdU+ cells at 7 days, which was not different than shams. VEGF-121 preserved vascular density post AKI at 7 days but did not affect BrdU incorporation in tubular, endothelial or perivascular cells. Potential endothelial mesenchymal transitions states were detected by localizing endothelial markers (CD 31, cablin or infused tomato lectin) with the fibroblast marker, S100A4. Endothelial transition states were prominent within 6 hours and sustained for at least 7 days following I/R and were 30-fold more prevalent than epithelial transition states. A transgenic mouse model with a Tie-2 promoter driving Cre crossed with a YFP reporter mouse was used to label endothelial cells and their progeny in response to kidney injury. Recovery from AKI for up to 14 days resulted in widespread distribution YFP+ interstitial cells, colocalized with S100A4 and SMA, further suggesting endothelial mesenchymal transition. Administration of VEGF-121 during the recovery period significantly attenuated the distributionYFP positive interstitial fibroblasts. These data indicate that capillary dropout post AKI results from low endothelial proliferative capacity and phenotypic transition, which can be minimized by VEGF activity.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO023**

**Attenuation of Folic Acid-Induced Renal Inflammatory Injury in Sphingosine Kinase 2-Deficient Mice** Amandeep Bajwa, Hong Ye, Diane L. Rosin, Mark D. Okusa. *Medicine and the Center for Immunity, Inflammation and Regenerative Medicine, University of Virginia, Charlottesville, VA.*

Epidemiological studies indicate some survivors of acute kidney injury (AKI) who regain renal function have a progressive decline in kidney function leading to end stage renal disease. Sphingosine 1-phosphate (S1P), a sphingolipid metabolite, is a mediator of numerous vital molecular processes, such as cell survival, differentiation, cytoskeletal rearrangements, and lymphocyte circulation. S1P is generated by phosphorylation of sphingosine by sphingosine kinases (SphK1 and SphK2). SphK1 and SphK2 have divergent cellular functions and intracellular localizations. SphK1, localized in the cytoplasm, promotes cell growth and survival, whereas SphK2, localized in the nucleus, induces apoptosis and hinders cell growth. The current study investigates the roles of SphK1 and SphK2 in folic acid (FA)-induced kidney fibrosis. WT, SphK1 KO and SphK2 KO mice were given FA (250mg/kg, ip) and followed for 14 days. SphK2KO mice exhibited significantly lower plasma creatinine at day 3 (48.54%, $p<0.01$) and fibrosis (Masson's trichrome staining) compared to WT and SphK1 KO mice at day 14 post FA administration. Compared to kidneys of SphK1 KO mice, kidneys of FA-treated SphK2 KO mice expressed lower mRNA levels of: TGF-β (15.79%, $p<0.001$), α-SMA (30.8%, $p<0.01$) and fibronectin (36.9%, $p<0.01$). Kidney sections of FA-treated SphK2 KO mice displayed lower levels of collagen, fibronectin, and α-SMA immunoreactivity compared to kidney sections of SphK1 KO mice. FA-treated SphK2 KO mice kidneys had reduced inflammatory leukocyte infiltration of macrophages (45.1%, $p<0.05$)) neutrophils (27.9%, $p<0.01$) and T cells (55.8%, $p<0.05$) compared to SphK1 KO mice. We conclude that: 1) FA induces fibrosis to a similar degree in WT and SphK1 mice, and 2) SphK2 deficiency attenuates FA-induced fibrosis. The discrete compartmentalization of SphK2 within the nucleus may contribute to its differential effect when compared to SphK1. Understanding the function of nuclear SphK2 may contribute further to our understanding of the pathogenesis of fibrosis of chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO024**

**Label-Retaining Cells Which Contribute to Tubular Regeneration after Renal Ischemia Are Not a Specific Minor Cell Population in the Kidney** Akito Maeshima, Masaaki Miya, Keiichiro Mishima, Noriyuki Sakurai, Hidekazu Ikeuchi, Takashi Kuroiwa, Keiju Hiromura, Yoshihisa Nojima. *Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.*

Using an in vivo BrdU labeling, we previously identified label-retaining cells (LRCs) with renal progenitor-like property in renal tubules of normal rat kidney (JASN 14: 3138–3146, 2003). However, it remains unknown whether label-retaining potential is limited to a specific cell population or not. To address this issue, we examined the presence of LRCs in normal rat kidney using three kinds of thymidine analogues, BrdU, IdU, and CldU. 1) labeling experiment: Using osmotic pomp, BrdU was continuously given into 7-week-old Wistar rats for two, three, and four weeks and the number of BrdU-positive cells were analyzed. 2) Triple labeling experiment: BrdU, IdU, and CldU, were sequentially administered for 7 days into rats (100 mg/kg/day each). Two-week chase intervals were placed between administrations of each thymidine analogue. After the last chase, ischemia/reperfusion injury was induced in these rats and kidneys were removed for histological analysis at 24h after reperfusion. Using anti-IdU Ab (that recognizes BrdU and IdU, but not CldU) and anti-CldU Ab (that recognizes BrdU and CldU, but not IdU), three LRC populations with different chase periods were identified and the numbers of them were analyzed. Long labeling experiment demonstrated that the number of BrdU-positive tubular cells was increased according to labeling period. Majority of cortical tubular cells became BrdU-positive after 4-week labeling. Triple labeling experiment showed that LRC-BrdU (IdU+CldU+), LRC-IdU and LRC-CldU were scattered in renal tubules and the numbers of each LRC was decreased as the chase period got longer (LRC-BrdU<LRC-IdU<LRC-CldU). While the number of each LRC was significantly increased after injury, there was no significant difference in the ratio of cell division among these LRCs. These findings suggest that tubular LRCs are not restricted to a specific minor cell population in the kidney, which equally contribute to tubular regeneration after renal ischemia.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO025**

**The Hormone Melatonine Augments Renoprotective Effects of Syngeneic Murine EPCs Via Stimulating Proangiogenic VEGF** Daniel Patschan, Susann Patschan, Johannes Wessels, Gerhard A. Mueller. *Department of Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany.*

Endothelial progenitor cells (EPCs) protect kidneys from acute ischemic damage. Recently, melatonine has been shown to agonize mesenchymal stem cells. Aim of the present study was (I) to evaluate whether melatonine would mediate beneficial effects on EPCs in the setting of acute ischemic renal failure, and, if so, (II) to analyze the cellular/molecular mechanisms involved herein.

EPCs, isolated from C57BL/6N mice were preincubated with melatonine and systemically injected into recipient animals with acute ischemic renal failure (40 minutes of ischemia). In some experiments cells were pretreated with combined melatonine and the melatonine antagonist luzindole. Two days later animals were sacrificed and renal function and histology were analyzed. In order to investigate melatonine-mediated cellular effects on EPCs, cultured murine EPCs were analyzed for apoptosis, necrosis, and production/secretion of VEGF in the presence of TGF-β with and without simultaneous melatonine treatment.

Systemic injection of $0.5\times10^6$ untreated EPCs did not protect mice from acute ischemic renal failure. Animals that were injected with melatonine-pretreated cells did not develop acute renal failure. These effects could be reversed by combined cell pretreatment with melatonine and luzindole. In vitro analysis did not show reduced TGF-β-induced EPCs apoptosis/necrosis in the presence of melatonine; nevertheless, the cells secreted significantly more VEGF into the culture medium if they had been preincubated with combined TGF-β and melatonine as oposed to pretreatment with TGF-β alone.

In summary, the data indicate melatonine as new therapeutic tool in stimulating EPC-mediated renoprotective effects in acute ischemic renal failure. In this process, increased production/secretion of vasostabilizing VEGF by EPCs is potentially pathogenetically relevant.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO026**

**EPC Regeneration in Sepsis with Impaired Renal Function** Susann Patschan, Daniel Patschan, Johannes Wessels, Gerhard A. Mueller. *Department of Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany.*

Endothelial progenitor cells (EPCs) are involved in vasculogenesis under various circumstances. Aim of the study was to evaluate EPC numbers and regeneration in patients with sepsis with and without impaired renal function.

Blood samples from sepsis patients (defined as infectious SIRS) were analyzed for total circulating EPCs (cytometric analysis) and EPC proliferation (colony forming unit-assay). For clinical evaluation, age, gender, mean serum creatinine, the AKIN score, necessity of dialysis treatment, and CRP were assessed.

Twenty-three patients (10 female [67.8 ±13.9 years] and 13 male [64.5 ±13.8 years]) were included into the study. The mean serum creatinine of all patients was 1.99 ±1.44 mg/dl, 15 patients underwent transient or permanent dialysis treatment during the course of the disease. In 17 patients the AKIN score was 3. Eight patients displayed 10 or more EPC colonies (CFU-ECs) (35.3 ±21.6). Patients with <10 EPC colonies (4.2 ±2.2, $p<0.0001$) showed significantly higher mean serum creatinine levels (2.4 ±1.5 mg/dl vs. 1.1 ±0.7 mg/dl, $p=0.04$) than patients with ≥10 colonies. There were no differences in the percentages of total circulating EPCs between the two groups.

In summary, the data indicate that EPC regeneration in sepsis is less affected in patients with lower creatinine levels. Thus, prevention of renal function or, if necessary, effective dialysis treatment in sepsis stabilizes the EPC system as an essential functional element of vascular homeostasis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO027**

**Tunicamycin, an Established Endoplasmic Reticulum (ER) Stress Inducer, Cause Causes a Proteinuric Form of Proximal Tubular Injury** Ling-Mei Chiang,[2] Pu Duann.[1] *[1]Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ; [2]Pediatrics, Chang-Gung Memorial Hospital, Keelung, Taiwan.*

The unfolded protein response (UPR) is a cellular recovery mechanism activated by endoplasmic reticulum (ER) stress. The UPR is coordinated with the ER-associated degradation (ERAD) to regulate the protein load at the ER. ER stress is currently believed to be an independent stress pathway which regulates inflammatory process and the autophagy/repair response to injury. The CHOP gene (encoding C/EBP homologousprotein-10, also known as GADD153) is regulated tightly by stress in a wide variety of cells. Studies on CHOP-knock mice point to CHOP as the core modulator molecule in the UPR pathway. Tunicamycin is a specific ER stress inducer ex vivo. Mice injected with tunicamycin (TN), develop tubular injury and renal dysfunction resembling human AKI. Using this model, we demonstrated (Nat Cell Biol 11(12), 1473-80, 2009) that CHOP was predominantly upregulated in proximal tubular cells (PTCs). PTC cell death and renal dysfunction in this model were ameliorated in CHOP knockout mice, suggesting that CHOP plays a critical role in ER stress-induced renal dysfunction. In this present studies we correlated tubular histopathological changes with proteinuria (Up/Uc) prior and 1, 2, 3 and 4 days after TN injection (intraperitoneal, 1 mg/Kg weight). We observed proteinuria, Up/Uc values: 0.69±0.23, 1.72±0.44, 3.46±0.98, 6.46±2.58, and 1.07± 0.33, prior and on days 1, 2, 3 and 4 after TN injection. Histologically, the extent of tubular injury, in terms of percentage of injured tubular area, correlated with changes in Up/Uc: 10%, 35%, 70% and 4% on days 1, 2, 3 and 4, respectively, after TN injection. Pretreatment with the TN chemical chaperone, 4-phenyl butyrate (4-PBA), reduced proteinuria and extent of tubular injury. These observations, coupled with our previous studies, suggest that TN-induced ER stress causes a proteinuric form of injury specific for proximal tubules and may provide a new model for ER stress-mediated AKI. TN chaperones may provide powerful tools to study mechanisms of ER stress-mediated AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO028**

**miR-127-3p Induced by HIF Is a Cytoskeleton Protector Mechanism in the Proximal Tubule Response to Ischemia/Reperfusion** Elia Aguado Fraile,[1] Edurne Ramos,[1] David Saenz Morales,[1] Elisa Conde,[1] Ignacio Blanco Sanchez,[1] Marta Martinez,[1] Angel M. Candela-Toha,[3] Fernando Liano,[2] Laura Garcia-Bermejo.[1] *[1]Pathology, Hospital Univ. Ramon y Cajal; [2]Nephrology, Hospital Univ. Ramon y Cajal; [3]Anesthesiology, Hospital Univ. Ramon y Cajal.*

microRNAs are small RNA molecules (20-25 nucleotides) that play important regulatory roles in the cell. Among other functions, they are regulators of cellular homeostasis, and they control cell response to different stress, such as ischemia. Ischemic injury is underlying most of the Acute Renal Failure (ARF) cases and contributes to kidney delayed graft function. Identification of microRNAs involved in renal ischemia/reperfusion (I/R) response could allow designing new therapeutic strategies to prevent organ injury or accelerate recovery.

Using and *in vitro* protocol of Hypoxia/Reoxygenation (H/R) in proximal tubule cell lines of Rat (NRK-52E) and Human (HK-2), and an *in vivo* protocol of Ischemia/Reperfusion (I/R) in Rat, we have found that miR-127-3p is up-regulated after ischemia but also in reperfusion. This induction is dependent on Hypoxia Inducible Factor alpha subunit (HIF-1α), a key regulator of cell response to low oxygen conditions, among other mechanisms. Knockdown of HIF-1α by siRNA transfection in HK-2 prevents miR-127-3p upregulation during hypoxia and also in reperfusion. Moreover HIF-1α overexpression in the same cell line, increases miR-127-3p levels.

H/R leads to cytoskeleton alterations, including actin and tubulin depolimerization. Modulation of miR-127-3p by transfection of specific Pre-miR and anti-miR molecules in NRK-52E submitted to H/R demonstrated that cells with overexpressed miR-127-3p present better microtubule and actin organization after hypoxia. Using different databases (Microcosm, Pictar and TargetScan 5) for target gene prediction, we have found that some of the putative targets for miR-127-3p are involved in microtubule organization and trafficking.

In summary, miR-127-3p is modulated during cell response to H/R regulating cytoskeleton dynamics. Maintenance of tubular cell structure through miR-127-3p would assure correct tubular function and might be contributing to ATN recovery.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO029**

**Urinary Biomarkers for the Early Detection of Recovery Phase in Patients with Acute Kidney Injury** Sang Choel Lee,[1] Eawha Kang,[2] Hyung Bok Park,[1] Soo Young Yoon.[1] *[1]Department of Internal Medicine, College of Medicine, Kwandong University, Goyang, Gyeonggi-do, Korea; [2]Department of Internal Medicine, NHIC Ilsan Hospital, Goyang, Gyeonggi-do, Korea.*

AKI is a common and serious complication in hospitalized patients. Several biomarkers including NGAL, KIM-1 and cystatin C have been suggested as useful markers for AKI. However, it is unknown whether such biomarkers are also helpful in detecting recovery of kidney function from established AKI. We measured urine NGAL and cystatin C levels every 2 days for 8 days in 30 patients with AKI. AKI was defined as a 50% or greater increase in serum creatinine from baseline. In addition, patients with a 50% or greater decrease in serum creatinine from peak level within 8 days after development of AKI were classified as recovery group. Exclusion criteria were pre-existing CKD or renal replacement therapy during admission. Urine NGAL and cystatin C levels were determined by ELISA. At day 0, there were no significant differences in serum creatinine, BUN, fractional excretion of sodium, urine NGAL and cystatin C levels between recovery (n=18) and non-recovery group (n=12). Compared to non-recovery group (4.6±0.6 mg/dL, mean±s.e.), serum creatinine concentrations in recovery group (3.9±0.6 mg/dL) started to decrease from day 2, but it did not reach statistical significance. The difference in serum creatinine levels between the two groups became evident only after day 6 (5.2±0.9 vs. 2.1±0.9 mg/dL, $p<0.001$). In contrast, urine NGAL (204.6±42.1 vs. 371.1±60.1 ng/ml, $p<0.05$) and cystatin C (0.15±0.03 vs. 0.26±0.07 mg/L, $p<0.05$) concentrations were significantly lower in recovery group than non-recovery group from day 2 until the end of the study. When urine NGAL and cystatin C were normalized with urine creatinine concentrations, these two biomarkers also decreased earlier than serum creatinine from day 2. In conclusion, this study showed that urine NGAL and cystatin C levels declined faster than serum creatinine levels in recovery phase in patients with AKI. Our findings suggest that urine NGAL and cystatin C may be more reliable biomarkers in the early detection of recovery from AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO030**

**Gentamicin (G) Nephrotoxicity: Prevention and Repair by Bone Marrow-Derived Mesenquimal Stem Cells (BMSCs)** Luciana Aparecida Reis,[1] Fernanda Teixeira Borges,[1] Manoel De Jesus Simoes,[2] Nestor Schor.[1] *[1]Medicine, UNIFESP/EPM, Sao Paulo, SP, Brazil; [2]Morphology, UNIFESP/EPM, Sao Paulo, SP, Brazil.*

The aim of this study is to investigate if BMSCs can prevent and or attenuate renal damage induced by G. Female Wistar rats received G (40mg/Kg/BW, *ip*) or water (CTL) for 10, 11, 12, 15 or 20 days. In the 10$^{th}$ day of G, the rats received IV male BMSCs (1X10$^6$). After 24, 48 hr or 5 days from BMSCs, blood and 24 hr urine were collected for creatinine (Cr), urea (U) and $FE_{Na}$. Prevention groups (P) rats received IV BMSCs 24 hr (P-24h) before or after the 5th day (P+5d) of G. Kidneys were evaluated for HE, KI67, caspase 3 and Y chromosome. It was observed that Cr, U and $FE_{Na}$ increased in all groups when compared to CTL. Rats that received BMSC, Cr and U as well as $FE_{Na}$ decreased when compared to G groups. There was no significant difference in Cr, U and $FE_{Na}$ in P groups, suggesting that renal injury homed BMSCs.

| GROUPS | Cr [mg/dl] | U [mg/dl] | FENa [%] |
|---|---|---|---|
| CTL | 0.5±0.03 | 24±2 | 0.6±0.02 |
| G10d | 1.9±0.07* | 98±2* | 1.5±0.04* |
| G11d | 2.3±0.04* | 104±1* | 1.6±0.02* |
| G12d | 2.7±0.03* | 104±1* | 1.7±0.01* |
| G15d | 2.9±0.04* | 119±1* | 1.7±0.03* |
| G20d | 3.4±0.08 | 128±5 | 2.1±0.04 |
| P-24h | 1.2±0.04 | 73±1 | 1.2±0.01 |
| P+5d | 1.8±0.03 | 87±1 | 2.0±0.03 |
| G11+BMSC10 | 1.0±0.04*,# | 64±2*,# | 1.0±0.02*,# |
| G12+BMSC10 | 0.7±0.03*,# | 54±1*,# | 0.7±0.01*,# |
| G15+BMSC10 | 0.6±0.01*,# | 41±1*,# | 0.7±0.03*,# |
| G20+BMSC10 | 0.9±0.02# | 68±2.8*,# | 1,0±0,01# |

*p<0.05 vs. CTL; #p<0.05 vs.G10, 11, 12 or 15d

G induced ATN and no KI67 marker. Animals treated with G+BMSCs did not present ATN, KI67 marker was observed . It was noted the presence of Y chromosome in the rats treated with BMSCs, suggesting additional mechanisms other than paracrine actions. This latter effect could indicate the permanence of BMSC in situations where the aggression persists, as in this model. These results suggest that BMSCs have a potential effect to minimize the nephrotoxicity induced by G.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO031**

**The Effect of Conditioned Medium (CM) of Mesenquimal Stem Cells Derived from Bone Marrow (BMSC) in Acute Kidney Injury (AKI) Induced by Gentamicin (G) in Rats** Luciana Aparecida Reis,[1] Fernanda Teixeira Borges,[1] Manoel De Jesus Simoes,[2] Nestor Schor.[1] *[1]Medicine, UNIFESP/EPM, Sao Paulo, SP, Brazil; [2]Morphology, UNIFESP/EPM, Sao Paulo, SP, Brazil.*

Cell therapy using BMSC seems to be a new alternative for the treatment of nephrology diseases, including AKI. Thus, we evaluate the effects of CM on AKI induced by G. Female Wistar rats received G (40mg/Kg/BW, *ip*, daily) or water (CTL) for 15 or 20 days. On 10$^{th}$ day with G, animals received 500µl, iv in only one dose of CM (BMSC cultured for 24 hr with DMEM) or CM+TPS (BMSC cultured for 24 hr with DMEM+tripsin, 100ug/ml) or CM+RNAse (BMSC cultured for 24 hr with DMEM+RNAse, 40 ug/ml). Blood and 24 hr urine were collected for creatinine (Cr), and $FE_{Na}$ dosages for all. Cytokines (IL10, TNFα and INTγ) were analyzed for the 15 days group. Kidneys were evaluated for HE, KI67 and caspase 3.

| GROUPS | Cr [mg/dl] | FENa [%] |
|---|---|---|
| CTL15d | 0.5±0.03 | 0.6±0.02 |
| CTL20d | 0.7±0.02 | 0.5±0.01 |
| G15d | 2.9±0.04* | 1.7±0.03* |
| G20d | 3.4±0.08* | 2.1±0.04* |
| G15+CM10 | 0.9±0.03# | 0.7±0.04# |
| G20+CM10 | 0.9±0.01# | 1.2±0.04# |
| G15+CMRNAse10 | 3.1±0.03* | 1.4±0.03* |
| G15+CMTPS10 | 1.0±0.1* | 1.2±0.02* |
| G15+RNAse10 | 3.4±0.08*,# | 2.2±0.03*,# |
| G15+TPS10 | 2.1±0.09*,# | 1.9±0.04*,# |

*p<.05 vs. CTL; #p<.05 vs.G15 or 20

G15 or G20 groups presented an important nephrotoxicity. CM induced decreases in Cr and $FE_{Na}$ indicating effect of CM at glomerular and tubular levels. G alone caused ATN and mild KI67. Surprisingly, CM showed no ATN and intensely KI67 observation. The association of CM and RNAse blunted the protective effects of CM alone. In groups $G_{15}$ ,$G_{15}$+$RNAse_{10}$ and $G_{15}$+$TPS_{10}$ caused pro-inflammatory cytokines increases, with decreases on IL10 when compared to $G_{15}$+$MC_{10}$. Results suggest that CM would act via paracrine effects, probably by releasing factors (among than mRNA and RNA) that activate resident cells. CM minimized inflammatory responses and the AKI induced by G, indicating CM is a potential tool to modify this nephrotoxicity.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO032**

**Effects of Bone Marrow Mesenchymal Stem Cells (BMSC) in Rats with Acute Kidney Injury (AKI) Due to Sepsis Induced by Lipopolysaccharide (LPS) Administration and E.coli Infection** Luciana Aparecida Reis,[1] Fernanda Teixeira Borges,[1] Manoel De Jesus Simoes,[2] Nestor Schor.[1] *[1]Medicine, UNIFESP/EPM, Sao Paulo, SP, Brazil; [2]Morphology, UNIFESP/EPM, Sao Paulo, SP, Brazil.*

Female Wistar rats received LPS (6mg/Kg/BW) (LPS group) or water (CTL) in a single dose *i.v.* (N=10). After 24, 48 or 72 hr, rats received a single dose iv of BMSC (1X10$^6$cells). For the E. coli protocol, both kidneys were punctured, one time each, with a human nephritogenic *E. coli* solution at a dose of 10$^9$ per 5 ml. Then, the animals received 1X10$^6$ BMSC iv, 24 hr after renal punction. Blood and urine were collected 24, 48 or 78 hr and measured creatinine (Cr), urea (U) and $FE_{Na}$. Kidneys were analyzed for HE, KI67, caspase 3 and for Y cromossome. In the LPS 24, 48 or 72 hr vs CTL the Cr (1.65±0.14; 2.4±0.05; 2.2±0.04 vs. 0.66±0.08; mg/dl), U (50.3±2.9; 70.3±2.9; 87.7±2.1; 146±465 vs. 15.4±3.1; mg/dl) and $FE_{Na}$ (2.18±0.01; 1.80±0.03; 2.0±0.03 vs. 0.6±0,2; %) increased when compared to CTL, respectively. The LPS+BMSC 24, 48 or 72 hr caused a decrease on Cr (0.75±0.01; 0.82±0.02; 0.80±0.03; mg/dl), U (42±3.7; 54±3.6; 51±1.8; mg/dl) and $FE_{Na}$ (0.58±0.01; 0.5±0.02; 0.53±0.03; %; p<0.05) comparing with LPS groups. In LPS-groups, the kidneys showed a small marked KI67 and intensive caspase 3 expression but differently, it was highly marked for KI67 and lower expression for caspase 3 in LPS+BMSC groups. However, a striking difference in the BMSC treated animals where the presence Y chromosomes were detected and no histological ATN lesions were observed. E. coli Protocol: After 24 hr we observed a significant increases (p<0.05) on Cr (1.3±0.1*vs.*0.9±0.1) and U (92.1±0.2*vs.*40.6±0.1), comparing with CTL groups. A significant protective effect (p<0.05) on the Cr and U were observed after 24hr (0.8±0.3 and 76.1±0.1) and 48hr (0.9±0.2 and 46.5±0.2). These results strongly suggest that BMSC minimize AKI in both sepsis models. The BMSC administration have a significant impact on renal function observed and thus, holds substantial promise for its use on sepsis for these protocols.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO033**

**Alterations in Mitochondrial Proteins, Dynamics, and Biogenesis Following Acute Kidney Injury** Jason A. Funk, Rick G. Schnellmann. *Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Mitochondrial dysfunction is a pathological process that occurs in a number of acute organ injuries, including acute kidney injury (AKI). In response to energy depletion or injury, mitochondrial biogenesis is initiated to aid in recovery of cell and organ function. We investigated mitochondrial biogenesis in a non-lethal rat model of myoglobinuric AKI. AKI was induced by glycerol injection (50% solution, 10 mL/kg, im) into the hind limbs of Sprague-Dawley rats. After glycerol injection, rats had reduced kidney function as determined by a rise in SCr levels from 0.5 to 2 mg/dL at 24h. Recovery of kidney function began around 72h with SCr levels decreasing to 1.3 mg/dL, and continued to decrease to control levels 120h post-injection. Cleaved caspase-3 expression, a marker of apoptosis, was observed in glycerol-treated rats between 24 and 120h post-injury. Mitochondrial respiratory proteins NDUFB8, ATP synthase β, and cytochrome c oxidase subunit I (COXI) were decreased 24h after glycerol injection and remained decreased throughout the study period, suggesting loss of mitochondrial function. Interestingly, expression of mitochondrial fission and fusion processes, including dynamin-related protein (Drp1) and mitofusin 2 (Mfn2), were elevated at 24h and throughout the course of the study. We observed increases in markers of mitochondrial biogenesis, including elevations in peroxisome proliferator-activated receptor coactivator (PGC)-1α, PGC-1 related coactivator (PRC), and nuclear respiratory factor (NRF)-1 mRNA and elevations in PGC-1α, NRF-1, and mitochondrial transcription factor A (mtTFA) proteins at 24h and throughout the study in glycerol treated rats. In summary, AKI with apoptosis occurred within 24h of glycerol injection and resulted in 1) loss of mitochondrial proteins/function, 2) increases in mitochondrial fission and fusion proteins and 3) initiation of mitochondrial biogenic signaling early after injury and was sustained throughout the study period. These findings reveal that persistent mitochondrial dysfunction occurs after AKI and mitochondrial biogenesis may play an essential role during recovery from injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO034**

**Adipose Tissue Contributes to Renal Lipotoxicity during Acute Kidney Injury** Kiran Nagothu, Syed M. Ali, Shenyang Li, Neriman Gokden, Gouri Ranganathan, Judit Megyesi, Didier Portilla. *Medicine, University of Arkansas for Medical Sciences, Little Rock, AR.*

We have shown increased accumulation of nonesterified fatty acids and triglycerides (TG) in serum and kidney tissue of mice undergoing cisplatin (CP)-mediated Acute Kidney Injury (AKI). We hypothesize that adipose tissue may contribute to the observed dyslipidemia during AKI. Epididymal fat tissue mass from CP-treated mice was significantly reduced (43±5%) when compared to controls. Adiponectin synthesis, adipose tissue mRNA levels of hormone sensitive lipase, lipoprotein lipase (LPL), and fatty acid synthase were also reduced. In contrast, there was a 10-fold increase in serum adiponectin levels in CP-treated mice measured by western blotting and ELISA. These results suggest: 1) that CP-mediated increased serum adiponectin levels relate to reduced degradation of circulating adiponectin rather than increased synthesis from adipose tissue, 2) that down-regulation of LPL in adipose tissue may account for the observed increase in serum TG levels in CP-

treated mice. Adiponectin receptors 1 and 2 are the major receptors for adiponectin in vivo and play an important role in the regulation of lipid metabolism. To better understand the effects of increased serum adiponectin levels in kidney tissue during AKI we examined the effects of CP and PPARα ligand like fibrate on the expression of renal adiponectin receptor-1 (AdipoR1). CP treatment caused a significant reduction (93%) in mRNA and protein levels of AdipoR1. Fibrates prevented CP-mediated reduced expression of renal AdipoR1, reduced renal lipotoxicity and ameliorated renal function. These changes were substantiated by immuno-histological studies that localized AdipoR1 to proximal tubules and thick ascending limb cells, with reduced expression of AdipoR1 in proximal tubules of CP-treated mice. We conclude that reduced expression of AdipoR1 in proximal tubules during AKI may account for reduced fatty acid oxidation and increased renal lipotoxicity. Increased serum adiponectin levels and reduced expression of renal AdipoR1 during AKI may represent a state of resistance to adiponectin previously described in patients with chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO035**

**Development of a Zebrafish Model of Acute Kidney Injury** Rebecca A. Wingert. *Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.*

Acute kidney injury (AKI) is a devastating and often-lethal condition, and patients who survive are at risk for developing numerous non-renal complications. In recent years it has become appreciated that the nephron can regenerate following epithelial cell damage incurred from ischemia or toxin exposure. However, there is only a rudimentary knowledge of the cellular events that transpire and the molecular controls are unknown. Determining how the regenerative process is accomplished will establish a foundation of core knowledge that will be vital for engineering new options for AKI treatment. The zebrafish model is an attractive system to study the molecular pathways responsible for nephron regeneration. Zebrafish nephrons share a number of similarities with mammals, and it is likely that essential mechanisms responsible for kidney regeneration will be conserved between these species. Zebrafish embryos possess a pair of nephrons that are anatomically situated such that they can be manipulated with laser ablation technology. Laser ablation was focally administered to a discrete patch of proximal tubule epithelial cells in one nephron, leaving the other nephron unperturbed to provide renal function. Following cell ablation, there was a rapid and robust regenerative response that restored epithelial intergrity of the tubule within several days. Regenerated nephrons display several hallmarks of the unperturbed control nephrons: for example, they showed expression of the proximal tubule specific solute transporter genes slc20a1a and nbc1. In addition, regenerated nephrons exhibited active uptake of fluorescent-labeled dextrans, suggesting restoration of epithelial function. These studies demonstrate that zebrafish nephrons can regenerate and that the zebrafish model can now be used to dissect the fundamental molecular signals that orchestrate kidney epithelial regeneration.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO036**

**Sirtinol, SIRT1 Inhibitor, Attenuates LPS-Induced Renal Inflammation in Mice** Yujin Jung,[1] Aesin Lee,[1] Duk Hoon Kim,[1] Jung Eun Lee,[1] Ki Dong Lee,[1] Mi Jeong Sung,[2] Kyung Pyo Kang,[1] Sik Lee,[1] Sung Kwang Park,[1] Won Kim.[1] *[1]Chonbuk National University Medical School, Jeonju, Republic of Korea; [2]Korea Food Research Institute, Songnam, Republic of Korea.*

Sepsis, characterized by depressed function in nearly all organs, has a high mortality when associated with acute kidney injury. Renal inflammation is characterized by activation of numerous common pro-inflammatory pathways.

SIRT1 is a mammalian $NAD^+$-dependent deacetylase that belongs to class III histone deacetylase. SIRT1-protein levels have been reported to be modulated during different cellular and disease conditions. There are controversies whether increased expression of SIRT1 might offer a protective mechanism or represent the disease progress. Recently, it has been reported that sirtinol, SIRT1 inhibitor, reduces antigen-induced airway inflammation and attenuates trauma-hemorrhage-induced lung inflammation. However, the role of sirtinol on kidney inflammation is not elucidated. Hence the present study was attempted to investigate the effect of sirtinol in preventing acute kidney injury during lipopolysaccharide (LPS)-induced endotoxemia.

In *in vivo* experiment, sirtinol suppressed LPS-induced increase of fractalkine and ICAM-1 expression in the kidney interstitial area and also decreased CD11b-positive macrophage infiltration. We further confirmed that, ICAM-1 protein level in the kidney tissue was significantly decreased after sirtinol treatment in the endotoxemic mice by immunoblot analysis. LPS-induced increase of pro-inflammatory cytokine such as TNF-α and IL-1β expression was significantly decreased by sirtinol treatment in the kidney tissue. In addition, we found that Sirtinol decreased TNF-α-induced increase of fractalkine, ICAM-1, and VCAM-1 in human umbilical vein endothelial cells (HUVECs).

In conclusion, sirtinol, SIRT1 inhibitor, has an anti-inflammatory effect on LPS-induced renal inflammation in endotoxemic mice.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO037**

**Factors Predicting the Severity of Acute Renal Failure Following Influenza A (H1N1) Infection** Rupesh Raina. *Department of Nephrology and Hypertension, Cleveland Clinic Foundation, Cleveland, OH.*

We describe the clinical course and risk factors for acute kidney injury (AKI) in patients with influenza A (H1N1) infection.

Between August 1st and December 5th of 2010, 2160 individual subjects were tested for influenza A infection; 1607 (74%) ambulatory, and 553 (26%) hospitalized, of which 203 (37%) required critical care. Among subjects who tested positive for influenza A, hospitalization was associated with older age (male gender (29 vs. 17%, $p$=0.01), but not pre-existing CKD (64 vs. 64%, $p$=0.99).

CKD or kidney transplant recipient status was a predisposing factor for kidney injury both by AKIN (acute kidney injury) diagnosis (43% vs. 9%, $p$=0.004), as well as severe renal failure requiring RRT (renal replacement therapy) (42% vs. 8%, $p$=0.05).

Critically ill patients, however, incurred greater injury reflected by higher peak creatinine (4.6±2.6 vs. 1.8±0.7 mg/dl, $p$=0.0001), creatinine change from baseline (3.5±2.6 vs. 0.8±0.4 mg/dl, p=0.0001), and need for dialysis (33% vs. none, $p$=0.0001), compared to regular nursing floor patients compared to those either on a regular nursing floor or treated as outpatients.

Critically ill patients with influenza A infection, had a higher likelihood of developing renal failure requiring dialysis if they were obese, had CKD, elevated CK and coagulopathy .CK levels ≥2000 U/L were noted only among obese patients (29% vs. none, $p$=0.01), but not severe hypoxia (38% vs. 50%, $p$=0.4). Provision of dialysis was associated with two-fold increase in mortality (50% vs. 24%, $p$=0.05).

The incidence of AKI among patients with influenza A infection was not higher than the general population matched per location of care. Obesity, elevated CK levels, and pre-existing CKD were associated with AKI requiring dialysis; the latter was associated with two-fold increase in hospital all-cause mortality. Information about the incidence of influenza A-related complications and their risk factors will aid in better care delivery, and resource allocation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO038**

**Is an Angiotensin-Converting Enzyme Inhibitor (ACE-I) or Angiotensin Receptor Blocker (ARB) an Independent Predictor for Post-Operative Acute Kidney Injury (AKI) in Non-Cardiac Surgery?** Miho Murashima, Ai Ogata. *Department of Nephrology, Kyoto Katsura Hospital, Kyoto, Japan.*

**Objective:** ACE-I or ARB might be an independent predictor for post-operative (post-op) AKI in cardiac surgery. This study is to evaluate whether ACE-I or ARB is an independent predictor for post-op AKI in non-cardiac surgery.

**Methods:** This is a retrospective cohort study of patients who underwent surgeries under general anesthesia from 2007 to 2009 at our hospital. The outcome variables is post-op AKI as defined by AKI network (increase in creatinine by >0.3mg/dL or >150% compared to baseline within 48 hours or urine output < 0.5ml/kg/hour for > 6 hours). The predictor variable is the pre-operative (pre-op) use of ACE-I and/or ARB. The covariates are age, sex, kinds of surgery (intrathorasic, intraperitoneal, and others), pre-op creatinine (Cre), body mass index (BMI), diabetes mellitus (DM), hypertension (HTN), chronic obstructive pulmonary disease, atrial fibrillation, peripheral arterial disease, cerebrovascular disease, coronary artery disease (CAD), prothrombin time (INR) >1.5, platelet <150X10$^3$/ml, emergent surgery, pre-op hematcrit (Hct), the use of vasopressors, diuretics, non-steroidal anti-inflammatory drugs or iodine contrast within 48 hours preoperatively, smoking and left ventricular ejection fraction < 40%.

**Results:** Interim analysis of 686 patients was performed. By univariate logistic regression analysis, age, sex, kinds of surgery, pre-op Cre, BMI, DM, HTN, emergent surgery, pre-op Hct, CAD, the use of vasopressors, diuretics, iodine contrast were significant predictors for post-op AKI. By multivariate logistic regression analysis, the use of ACE-I and/or ARB was not an independent predictor for post-op AKI.

| | Odds Ratio (95% CI) | p |
|---|---|---|
| Age | 1.02 (0.98-1.05) | 0.30 |
| Sex | 1.53 (0.69-3.42) | 0.29 |
| ACE-I/ARB | 0.55 (0.23-1.30) | 0.17 |
| Pre-operative creatinine | 2.83 (1.05-7.61) | 0.04 |
| BMI | 1.12 (1.02-1.24) | 0.02 |

**Conclusions:** In the interim analysis, only pre-op Cre and BMI were independent predictors for post-op AKI and the use of ACE-I and/or ARB is not an independent predictor. Further analysis may alter the results.

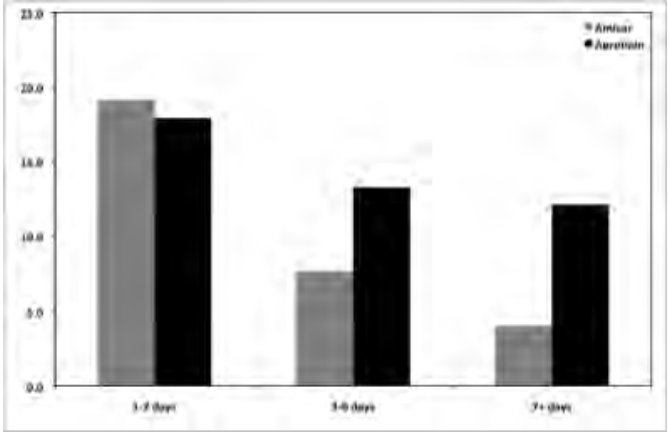
Disclosure of Financial Relationships: nothing to disclose

**TH-PO039**

**Acute Kidney Injury Duration Varies by Anti-Fibrinolytic Agent in Cardiac Surgery** Jeremiah R. Brown,[1] Robert Kramer,[2] Steven G. Coca,[3] Chirag R. Parikh.[3] *[1]The Dartmouth Institute for Health Policy and Clinical Practice, Dartmouth Medical School, Lebanon, NH; [2]Cardiothoracic Surgery, Maine Medical Center, Portland, ME; [3]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT.*

Background: Aprotinin has been shown to be nephrotoxic when given to patients undergoing cardiac surgery, however results have been inconsistent and variable. We hypothesized aprotinin results in longer acute kidney injury (AKI) duration than Amicar (Epsilon-aminocaproic acid).Methods: Consecutive patients undergoing cardiac surgery

between 2002-2007 were followed for post-surgery AKI. Patients with a history of chronic dialysis were excluded. Duration of AKI was calculated by the number of days AKI was present as defined as a ≥0.3 (mg/dL) or a ≥50% increase in serum creatinine from baseline, or new onset of acute dialysis. Kaplan-Meier and Cox's proportional hazard modeling was conducted to evaluate five year mortality. Results: Fifty-three percent of patients received Amicar (N=2,333) and 47% received high dose aprotinin (N=2,093). Patients receiving aprotinin were more likely to develop longer durations of AKI than Amicar (Figure, P<0.001): 7.0 ± 11.5 versus 3.8 ± 6.0 days (P<0.001). Nearest-neighbor propensity matching demonstrated aprotinin had significantly worse 5-year mortality compared to Amicar (relative risk [RR] 2.09, 95% CI 1.65-2.65). The findings were consistent among each duration category of AKI, as aprotinin was associated with increased risk of 5-year mortality compared to Amicar: AKI for 1-2 days (RR 2.47, 1.45-4.21); AKI for 3-6 days (RR 1.90; 0.98-3.67); and AKI for ≥7 days (RR 1.56; 0.88-2.76).




Conclusions: Aprotinin is associated with longer duration of AKI compared to Amicar. AKI duration may provide the sensitivity and specificity for evaluating renal outcomes in clinical trials.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO040

**Determinants for Duration of Acute Kidney Injury after Cardiac Surgery** Jeremiah R. Brown,[1] Robert Kramer,[2] Steven G. Coca,[3] Chirag R. Parikh.[3] *[1]The Dartmouth Institute for Health Policy and Clinical Practice, Dartmouth Medical School, Lebanon, NH; [2]Cardiothoracic Surgery, Maine Medical Center, Portland, ME; [3]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT.*

Background: Acute kidney injury (AKI) duration following cardiac surgery is associated with poor survival. However, it is not known what risk factors contribute to prolonged AKI and delayed recovery. We sought to identify perioperative risk factors that are associated with duration of AKI.

Methods: A cohort of 4,987 cardiac surgery patients from 2002 through 2007. AKI was defined as a ≥0.3 (mg/dL) or ≥50% increase in serum creatinine from baseline. We used multinomial logistic regression. Results: AKI developed in 39% of patients; 19% 1-2 days, 11% 3-6 days, 9% ≥7 days. Risk factors of AKI duration included older age, male sex, diabetes, hypertension, estimated glomerular filtration rate<60 (mL/min), preoperative red blood cell transfusion, blood prime, total heparin given on pump, nadir hematocrit<20 on pump, and pump time ≥120 minutes.

| Risk Factors | AKI 1-2 Days (OR) | AKI 1-2 Days (95%CI) | AKI 3-6 Days (OR) | AKI 3-6 Days (95%CI) | AKI 7+ Days (OR) | AKI 7+ Days (95%CI) |
|---|---|---|---|---|---|---|
| Age 60-69 | 1.25 | (1.03-1.53) | | | 2.11 | (1.47-3.04) |
| Age 70-79 | 1.61 | (1.31-1.97) | 2.54 | (1.95-3.32) | 4.40 | (3.10-6.23) |
| Age 80+ | 2.03 | (1.51-2.73) | 3.27 | (2.29-4.67) | 8.32 | (5.52-12.54) |
| Female | | | 0.64 | (0.51-0.80) | 0.68 | (0.53-0.88) |
| Diabetes | | | 1.57 | (1.28-1.91) | 1.39 | (1.10-1.74) |
| Hypertension | 1.22 | (1.04-1.44) | 1.78 | (1.43-2.23) | 1.27 | (1.00-1.61) |
| eGFR<60 (mL/min) | | | 2.39 | (1.93-2.96) | 2.82 | (2.23-3.56) |
| IABP use | | | | | 2.29 | (1.63-3.22) |
| Number of pRBCs | | | | | 1.41 | (1.14-1.73) |
| Blood prime | | | 1.22 | (1.04-1.43) | 1.43 | (1.23-1.66) |
| Cold cardioplegia | 0.74 | (0.63-0.86) | 0.69 | (0.56-0.84) | 0.75 | (0.60-0.94) |
| Heparin>= 50,000 (USP) | 1.21 | (1.02-1.43) | | | | |
| Nadir HCT<20 | | | 1.39 | (1.06-1.83) | 1.41 | (1.05-1.90) |

Conclusion: Risk-factors for AKI may be useful to evaluate a patient's risk for the rate of recovery from AKI after cardiac surgery and subsequent long-term survival.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO041

**Preventing AKI with a Hemoconcentration (HC) Strategy in Cardiac On-Pump Surgery (CS). A Risk-Adjusted Cohort Study** Alejandro Ferreiro,[1] Raul Lombardi.[2] *[1]Statistics, National Institute for Cardiac Surgery (INCC), Montevideo, Uruguay; [2]Medicina Crítica, SMI IMPASA, Montevideo, Uruguay.*

**Introduction:** The extracorporeal circulation is an etiologic factor for AKI in CS. Hemodilution (HD) is a newlly potentially modifiable risk factor for AKI. Hemoconcentration (HC) should protect kidneys in this setting. **Material and Methods:** to evaluate the impact of HD and the potential favorable effect of HC on AKI incidence, all patients submitted to CS between 1/1/2000 and 12/31/2009 were enrolled (n=7773; 6026 On-Pump (77.9%)). The INCC prospectively-collected database was used for analysis. Demographics, comorbidities, intraoperative and postoperative (PO) variables, and 30-day mortality (OM) were registered. A previously described priming redution strategy or in-pump ultrafiltration were used for HC. HD (preO - lowest on-pump Hct), AKI (RIFLE criteria), or RRT were registered. eGFR was calculated with the Cockroft-Gault formula. The PO RRT risk was estimated with the preO multivariable-adjusted Mehta score. ***Statistical analysis***. t test or Mann-Whitney test, $\chi 2$ and multivariate logistic regression analysis were used when appropriately, p<0.05 (*). **Results:** 6026 patients were included: age 65±11yrs (16-91). 61,9% HC strategy (94% priming reduction). No differences between groups in: comorbidities, preO SCr and eGFR. A U-shaped relationship between lowest Hct, AKI and RRT was observed, (lower rates in Hct 24-28%), in all eGFR spectrum. An excess (score) multivariable adjusted incidence of RRT was observed in the under 24% Hct group. AKI: 28 vs 31%, RRT: 1.9% vs 3.4%; PO Δ eGFR: 15.3 ml/min vs 17.9 ml/min, and OM (4.3% vs 5.8%) (*) were lower in the HC group, despite lower PreO Hct (36% vs 38%), but higher on-pump Hct (25,5% vs 23,9%) and lower HD (12,5% vs 14,6%) (*). The benefit of HC was observed in all the eGFR (*) spectrum, mainly in patients with preO Hct less than 34% (RRT: 2,7% vs 6%) (*). **Conclusion**: A direct relationship between lowest on-pump Hct and AKI was observed, with a 2-fold reduction of RRT incidence with the HC strategy, resulting in a lower 30-day mortality. Few other preventive strategies described in the literature demonstrated such impact on PO AKI after CS.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO042

**Impact of Statin Use on Incidence of Acute Kidney Injury in Critically Ill Patients with Sepsis** Ravish Shah,[OSUMC] Anil K. Agarwal.[OSUMC] *[1]Division of Nephrology, The Ohio State University Medical Center, Columbus, OH; [2]Division of Nephrology, The Ohio State University Medical Center, Columbus, OH.*

**Background:** Sepsis is the leading cause of death in critically ill patients. Development of sepsis related acute kidney injury (AKI) significantly increases the mortality. Cellular mechanisms for AKI include generation of oxygen free radicals and inflammatory cytokine production. Hydroxy-3 methylglutaryl co-A reductase inhibitors (statins) directly influences these inflammatory mechanisms. The aim of the study was to determine the impact of statin use on incidence of AKI, need for renal replacement therapy (RRT) and mortality in patients with sepsis.

**Methods:** This retrospective study was performed at a university affiliated tertiary care center and approved by the institutional review board. Patients admitted to ICU with a diagnosis of sepsis were included. They were then divided into two groups based on statin use. Demographics, co-morbid conditions and laboratory data were obtained. Statistical analyses were performed after adjusting for all the co-variables.

**Results:** A total of 302 patients met the inclusion criteria. Of those, 74 patients were in statin group and 228 were in no-statin group. Patients in statin group were older and had higher incidence of diabetes mellitus, hypertension, dyslipidemia, coronary artery disease (P < 0.01). Those with chronic kidney disease and systolic heart failure were more likely to develop sepsis related AKI (P< 0.01). Statin group had significantly less mortality (61%) compared to no-statin group (90%) (P<0.01). No statistically significant differences were noted in incidence of AKI or the need for RRT between both the groups.

**Conclusion:** Despite of higher incidence of co-morbid conditions, the statin group had significantly lower mortality. Statin use had no impact on incidence of sepsis related AKI or need for RRT. The reason for disparities in the outcome related to mortality and AKI may be related to differences in the disease severity, which could not be assessed given the retrospective nature. Prospective randomized controlled trials are warranted to investigate the potential benefit of statin in sepsis related AKI.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO043

**Performance of Three Generations of Severity Scoring Systems Sequentially Evaluated in Acute Kidney Injury (AKI) Critically Ill Patients** Veronica T. Costa e Silva,[1] Isac Castro,[1] Fernando Liano,[2] Luis Yu.[1] *[1]Nephrology Service, Unversity of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Nephrology Service, University of Sao Paulo School of Medicine, Sao Paulo, Brazil.*

Validated scoring systems are necessary to guarantee adequate randomization in clinical trials. The third generation of illness severity models (SAPS 3, APACHE IV and MPM III) have never been assessed in AKI non-dialysis patients. The aim of this study was to compare the performance of three AKI specific and nine general intensive care unit (ICU) scoring systems, including the new third generation models, for AKI patients in the ICU. A prospective study was done in six ICUs in a brazilian academic tertiary care center.

AKI was defined as an increase ≥ 50% in baseline serum creatinine. Scores discrimination and calibration were expressed by the area under the receiver operating characteristic curve (AUCROC) and Hosmer-Lemeshow (HL) goodness-of-fit tests, respectively. The observed-to-predicted mortality ratio (SMR) was calculated whenever possible. Scores were applied on AKI diagnosis day, on the day when AKI specific criteria were met and on the day of nephrology consultation (NC). Three hundred sixty six patients were analyzed. SAPS II and SHARF scores presented good performance at all evaluated time points (AUCROC curves over 0.80 on the third day and sustained-HL test ($p > 0.20$) over time). Customized SAPS 3 had good discrimination (AUCROC curve: 0.81 on third day), the best SMR (range over time: 1.09 – 1.13), but showed unsatisfactory HL test results on the third day (p: 0.025). APACHE IV presented similar discrimination and satisfactory HL tests over time but underestimated mortality (SMR range over time: 1.69 – 1.72). All scores presented progressive performance improvement over time. On the third day of analysis, most scores presented AUROC values over 0.70, and six models demonstrated AUCROC around 0.80. In conclusion, a sequential evaluation in early AKI improved the models' performance, demonstrating that severity scoring systems could perform well in critically ill AKI patients. The third generation models presented good discrimination but calibration difficulties were observed.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO044

**Validation of RIFLE Criteria across Different Categories of Preoperative Estimated Glomerular Filtration Rate (eGFR) in Cardiac Surgery Patients**

Raul Lombardi,[1] Alejandro Ferreiro.[2] *[1]Sanatorio IMPASA, Montevideo, Uruguay; [2]Instituto Nacional de Cirugia Cardiaca, Montevideo, Uruguay.*

Purpose: to validate serum creatinine-based RIFLE criteria across different stages of chronic kidney disease.

Methods: all adult patients submitted to cardiac surgery in INCC-IMPASA between 1/1/2000 and 12/31/2008 were enrolled. Patients with an eGFR less than 15 ml/min/m2 were excluded. The INCC prospectively-collected database with more than 490 variables was used for analysis. Demographics, comorbidities, intraoperative and postoperative (PO) variables, and 30-day mortality (OM) were registered. Acute kidney injury (AKI) was defined according to RIFLE criteria based on changes in serum creatinine. For estimation of GFR the modified MDRD formula was used. Patients were categorized into four categories of chronic kidney disease (CKD): 1) ≥ 90 ml/min/$m^2$; 2) 60 to 90 ml/min/$m^2$; 3) 45 to 60 ml/min/$m^2$; 4) 30 to 45 ml/min/$m^2$; 5) 15 to 30 ml/min/$m^2$. Incidence of AKI as well as mortality rate was established for each CKD category. The model was adjusted for preoperative risk (EuroSCORE) using logistic regression analysis.

Results: 6231 patients were included in the analysis. Mortality rate of AKI and non-AKI patientes are shown in Table 1.

Mortality rate across preoperative eGFR

| eGFR | AKI | Non AKI | |
|---|---|---|---|
| Cat 1 | 6.4 | 2.4 | 0.000 |
| Cat 2 | 8.9 | 2.5 | 0.000 |
| Cat 3 | 12.9 | 2.5 | 0.000 |
| Cat 4 | 22.0 | 4.3 | 0.000 |
| Cat 5 | 29.9 | 7.0 | 0.000 |

value are expresed in percentages

Relative risk for Eusroscore was (1.17); CKD cat 1: (1.47); CKD cat 2: (1.48); CKD cat 3: (2.40); CKD cat 4 (3.70); CKD cat 5 (11.9); whereas for RIFLE R (2.30); RIFLE I (4.61) and RIFLE F (14.1). As seen, AKI is associated to a higher mortality which rises as preoperative renal function falls, even when adjusted for preoperative risk.

Conclusions: the present study showed that AKI defined by RIFLE criteria is independently associated to mortality rate in all categories of preoperative renal funcion.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO045

**Reappraising the Urine Output Criteria of AKI Consensus Definitions**

Dinna N. Cruz, Francesco Garzotto, Pasquale Piccinni, Gramaticopolo Silvia, Claudio Ronco. *S Bortolo Hosp, Vicenza, Italy.*

The consensus definitions RIFLE and AKIN use two criteria: change in serum creatinine (sCr) from a baseline or reference value, and urine output (UO). Experts have noted that the UO criteria may not match well with respective sCr criteria. Furthermore, UO is affected by diuretic use. Our aim was to evaluate the concordance between sCr and UO criteria on a daily basis, in the presence or absence of diuretics.

**Methods**

We enrolled 601 ICU patients from 10 centers; we recorded daily the worst 6hr & 12h UO, total 24h UO, diuretic use and sCr during the entire ICU stay. The sCr was compared to baseline sCr values from old records whenever possible. These data were used to determine RIFLE class on a daily basis. Agreement between RIFLE class based on sCr criteria (RIFLE-Cr) or on UO criteria (RIFLE-UO) was evaluated with the Kappa test.

**Results**

We evaluated a total of 6101 patient-days, excluded 536 days when RRT was performed, leaving 5565 days for analysis. RIFLE class was: NonAKI in 4490, Risk in 700, Injury in 241 and Failure in 134 of 5565 days. Diuretics were used in 2923/5565 days.

On the whole, agreement between RIFLE-Cr and RIFLE-UO was very poor (k=0.07, p<0.001). It was slightly better on no-diuretic days (k=0.11, p<0.001), and worse on days with-diuretic (k=0.05, p<0.001).

RIFLE-UO criteria for Risk (<0.5 ml/kg/h x 6h) were fulfilled in only 21% of all RIFLE-Cr-Risk days (Fig 1); this proportion was slightly higher on no-diuretic days (24%) vs with-diuretic days (18%). Findings were qualitatively similar for 12h (not shown) & 24h UO (Fig 2, RIFLE-Cr-Failure, no-diuretic 37% vs with-diuretic 7%, p<0.001).

**Conclusion**

Agreement between RIFLE-Cr and RIFLE-UO is poor. Diuretic use, which is common in the ICU, further worsens this inconsistency. Revision of UO criteria may help to harmonize the two sets of criteria.

Fig 1:

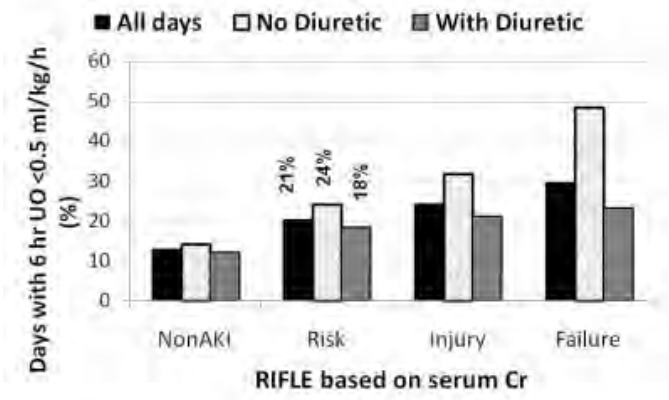
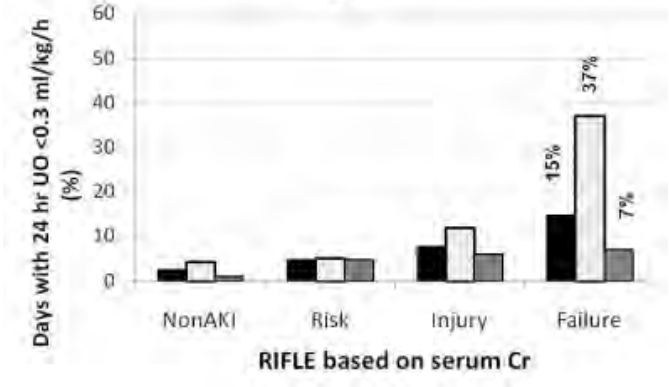



Fig 2:




Disclosure of Financial Relationships: Honoraria: Speaker Honoraria for Biosite/ Inverness Medical.

## TH-PO046

**Performance of a New Definition of Acute Kidney Injury Based on Creatinine Kinetics in Cardiac Surgery**

Fernando Liano,[1] Angel M. Candela-Toha,[2] Jose Del Rey,[3] Laura Garcia-Bermejo,[4] Jose Garrido.[5] *[1]Nephrology, Hospital Universitario Ramón y Cajal, Madrid, Spain; [2]Anesthesiology; [3]Biochemistry; [4]Pathology; [5]Cardiac Surgery, .*

A new definition of AKI based on creatinine kinetics (CK) models has been published recently (1). This definition and classification system uses absolute increases in sCr over clearly defined periods of time, instead of relative changes. The rationale under this proposal is that absolute increases should provide earlier diagnosis, specially in CKD patients.

Aim: Compare the CK definition of AKI with the RIFLE system in a cardiac surgery setting.

Methods: Retrospective analysis of a cardiac anesthesia database. We analyzed sCr data for the first postoperative week of 2103 patients operated of major cardiac surgery with CPB. Baseline sCr was considered the nearest value to the surgical date. Patients were assigned to the highest category of AKI by both definitions. Time to diagnosis was measured as the number of days elapsed till the first time a sCr value fullfilled criteria to be classified as AKI in any of the systems. We use weighted kappa coefficient to assess agreement across categories and nonparametric tests to compare time to diagnosis. Discrimination for outcomes (need for RRT and in-hospital mortality) was explored calculating and comparing AUC ROC.

Results: A significant greater proportion of patients were classified as AKI by the CK system as compared with RIFLE (28.5% vs 14.3%). Diagnosis of AKI by the CK scheme was earlier (1.8 d vs 2.5 d; p<0.001) for the 268 patients diagnosed of AKI by both systems. While need for RRT discrimination was excellent for both definitions (AUC 0.884 for CK vs 0.852 for RIFLE; p=n.s.) discrimination for in-hospital mortality was good (AUC 0.801 for CK vs 0.781 for RIFLE; p=n.s.)

Conclusion: The AKI definition and classification system based on CK models was more sensitive than RIFLE, detected AKI earlier and had a similar prognostic value in terms of need for RRT and in-hospital mortality.

Reference

1. JASN 2009;20:672-9

Disclosure of Financial Relationships: nothing to disclose

**TH-PO047**

**Definition of Iodinated Radiocontrast Agent Toxicity by Creatinine Elevation Alone Appears Questionable (BICAH Study: CRG010700102)**
Simon Desmeules, Mohsen Agharazii. *Medicine, CHUQ, Quebec, Qc, Canada.*

Introduction: Radiocontrast induced nephropathy (RCIN) is usually defined by an elevation of baseline creatinine (Cr) level by 0.5 mg/dL or 25%. Cystatin C (CyC) blood levels are well correlated with kidney function and can detect more rapidly subtle change of glomerular filtration rate (GFR). In theory, CyC should detect more RCIN than Cr alone. Our study evaluated RCIN incidence using Cr (0.5 or 25% elevation) and CyC (25% increase). Method: 170 patients with GFR<60 mL/min exposed to iodixanol were randomized to normal NaHCO3 (Bic), normal saline (NaCl) or NS+n-acetylcystein 600 mg BID x48h started the day before (NAC). Hydration protocols were identical in the 3 groups (3mL/kg x1H pre + 1mL/kg X6h post). Blood samples were drawn at T=0 and T=48H. Table shows baseline characteristics of the patients.

| | total | BIC | NAC | NaCl | p |
|---|---|---|---|---|---|
| n | 170 | 56 | 61 | 53 | |
| Age (y) | 63.7 | 65.1 | 63.1 | 63.1 | ns |
| Men (%) | 66.5 | 60.7 | 63.9 | 75.5 | ns |
| Weight (kg) | 75.5 | 77.3 | 72.3 | 76.9 | ns |
| Height (cm) | 168 | 169 | 168 | 169 | ns |
| BSA | 1.87 | 1.90 | 1.83 | 1.89 | ns |
| BMI | 26.4 | 26.9 | 25.6 | 26.9 | ns |
| DM (%) | 28.8 | 32.1 | 32.8 | 20.8 | ns |
| Transplant (%) | 31.6 | 26.8 | 37.7 | 30.2 | ns |
| Creat (µmol/L) | 158 | 163 | 150 | 161 | ns |
| MDRD (mL/min) | 41 | 41 | 42 | 42 | ns |
| Contrast vol (mL) | 144 | 143 | 137 | 153 | ns |
| Cont/bw (mL/kg) | 2.0 | 1.9 | 2.0 | 2.1 | ns |
| Hydratation (mL) | 668 | 658 | 653 | 695 | ns |
| Hydrat/bw (mL/kg) | 8.9 | 8.6 | 9.0 | 9.1 | ns |

Results: RCIN incidence was lowest in the NS group but no statistical difference was detected.

RCIN incidence (%)

| **RCIN def** | **Intervention** | | | |
|---|---|---|---|---|
| | Bic | NAC | NaCl | p |
| **Cr0.5** | 3.6 | 3.3 | 0 | ns |
| **Cr25** | 5.4 | 6.6 | 0 | ns |
| **CyC25** | 5.4 | 9.8 | 3.8 | ns |

No patient required dialysis. As expected, CyC diagnosed more cases of RCIN but concordance between the two methods was poor. 3/7 cases detected by Cr25% were also detected by CyC25% while Cr0.5 and CyC25% were in agreement on 2 of 4 cases. Overall, Cr and CyC were in agreement in less than 50% of cases. Conclusions: RCIN incidence in our study was low when defined with Cr elevation. Prevention with NS does not appear inferior to NAC or BIC and is less cumbersome. Finally, RCIN needs to be better defined in order to prevent clinically significant events.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO048**

**Episode Duration and Improvement Status Influence Outcomes from Acute Kidney Injury (AKI)**
Rakesh Malhotra,[1] Etienne Macedo,[1] Josee Bouchard,[2] Ravindra L. Mehta.[1] *[1]University of California, San Diego, San Diego, CA; [2]Université de Montréal, Montréal, QC, Canada.*

Background: AKI stage is well recognized as influencing outcomes. However,the duration of AKI episode and the completeness of renal functional improvement have not been evaluated as factors related to outcomes. We hypothesized that duration and completeness of improvement in renal function would correlate with clinical outcomes.

Methods: We studied 716 ICU patients screened for a prospective observational study on incidence of AKI from June 2006 to December 2008. AKI was defined by AKIN sCr criterion (≥0.3mg/dl absolute change within 48 hr). AKI patients were categorized based on the rate and completeness of change in sCR into three groups: "Transient Elevation" sCR return to reference within 72 hr; "AKI Slow Improvement": sCr improvement ≥ 0.3 mg/dl in > 72 hr without return to reference within 72 hr) and "Persistent AKI"(sCr change <0.3 mg/dl through ICU stay with no return to reference). ICU and hospital mortality, need for renal replacement therapy (RRT) and length of stay were compared among these groups stratified by AKIN stage.

Results: AKI patients had a significantly higher ICU and hospital mortality than No AKI subjects (19.8% vs. 2.1%; P <.0001 & 24.0% vs. 2.4%; P<.0001). Patients with persistent AKI had the worst outcomes followed by patients with slow improvement and transient elevation (Table). This effect was evident controlling for AKI stage (AKI Stage 1 mortality 11.8% vs. 15.8% vs. 19.7% in Transient, Slow and Persistent AKI).

Conclusion: Transient rise of sCr is associated with increased risk of mortality. Slow renal improvement or no improvement after an AKI episode contributes to worse outcomes. Future studies should focus on targeting early improvement in renal function to improve outcomes.

Clinical Outcome by AKI Groups

| | No AKI | AKIN AKI | | |
|---|---|---|---|---|
| | | Transient elevation | AKI slow Improvement | Persistent AKI |
| Total N | 374 | 42 | 28 | 122 |
| % ICU Mortality | 2.1 | 9.5 | 17.9 | 23.8 |
| % Hospital Mortality | 2.4 | 11.9 | 21.4 | 28.7 |
| % Need for RRT | 0.5 | 0 | 0 | 31.1 |
| Length of ICU stay (days) | 3 (2-4) | 5 (4-8) | 7 (5-13) | 4 (3-6) |
| Length of Hospital stay (days) | 5 (3-10) | 10 (6-18) | 11 (6-19) | 7 (4-15) |

Disclosure of Financial Relationships: nothing to disclose

**TH-PO049**

**Decline in Serum Creatinine: A Potential Criteria for Refining Diagnosis and Staging of Acute Kidney Injury (AKI)**
Rakesh Malhotra,[1] Etienne Macedo,[1] Josee Bouchard,[2] Ravindra L. Mehta.[1] *[1]University of California, San Diego, San Diego, CA; [2]Université de Montréal, Montréal, QC, Canada.*

Background: Diagnostic criteria for AKI (AKIN and RIFLE) rely on increments in serum creatinine(sCR) to define AKI. However, several patients present with an elevated sCr at hospital admission that subsequently declines during hospitalization. These patients cannot be classified as AKI based on current criteria and it is unclear if this decline in sCR represents an episode of AKI. We studied the prognostic significance of sCr decline in ICU patients who did not meet AKIN criteria during ICU stay.

Methods: We studied 716 patients admitted to ICU's who were screened for a prospective observational study on incidence of AKI from Jun 06 to Dec 08. Patients were stratified in three groups. "AKIN AKI": defined by AKIN sCr criterion (≥0.3mg/dl absolute change within 48 hours) ; "Presumed AKI": patients who did not meet AKIN criteria but had sCr decline ≥0.3 mg/dl during the ICU stay; "No-AKI": patients who did not meet either criteria. We compared ICU and hospital mortality, need for renal replacement therapy (RRT) and length of stay among these groups.

Results: 192 (26.8%) patients met the AKIN criteria. Presumed AKI patients had a trend towards higher ICU mortality (4.0% vs. 2.1%; P=0.37) compared with No AKI but lower ICU mortality (4.0% vs. 19.8%; P=<.0001) compared with AKIN AKI group (Table). Presumed AKI patients had significantly longer length of stays as compared to No AKI (P<.0001).

Conclusion: Patients with elevated sCr at ICU admission who have a decline during the course of ICU stay have an increased risk for mortality. Future studies are required to evaluate whether a decline in Scr should be a criterion to diagnose and stage AKI.

| | No AKI | Presumed AKI | AKIN AKI | P value |
|---|---|---|---|---|
| Total N | 374 | 150 | 192 | |
| % ICU Mortality | 2.1 | 4.0 | 19.8 | <.0001 |
| % Hospital Mortality | 2.4 | 6.0 | 24.0 | <.0001 |
| % Need for RRT | 0.5 | 0 | 19.8 | <.0001 |
| Length of ICU stay (days) | 3 (2-4) | 5 (3-6) | 5 (3-8) | <.0001* |
| Length of Hospital stay (days) | 5 (3-10) | 8 (5-15) | 8 (5-16) | <.0001* |

* P value No AKI vs Presumed AKI

Disclosure of Financial Relationships: nothing to disclose

**TH-PO050**

**Transient Changes in Renal Function and Outcomes in ICU Patients: A Prospective Cohort Study**
Etienne Macedo,[1] Rakesh Malhotra,[1] Josee Bouchard,[2] Ravindra L. Mehta.[1] *[1]University of California San Diego; [2]Université de Montréal.*

Prerenal failure is used to designate a transient increase in sCr, however, the time frame for reversibility as well as volume or hemodynamic improvement required to rule out the syndrome are not clearly designated. We assessed the influence of a transient decrease in urine volume or increase in sCr on outcomes of surgical ICU patients. We hypothesized that transient changes would be associated with better outcomes than prolonged azotemia and/or oliguria. Methods: We analyzed surgical ICU patients screened in study on incidence of AKI from Jun06 to Dec08. We characterized transient changes as an increase in sCr (at least 0.3 mg/dL or 50% from reference) that returned to reference within 72 h or a single episode of urine volume less than 3mL/kg for 6h. Patients were classified as sustained AKI by sCr if sCr did not returned to reference values within 72 h, or an episode of 12h with urine output < 6mL/kg; and AKI by both criteria when they met sustained UO and sCr criteria. Results: Of 315 patients, 168 (53%) were classified as AKI of which 43 (28%) met transient criteria, and 125 (82%) sustained AKI. Oliguric patients without increase in sCr were less often exposed to diuretic (20%) than patients that reach only sCr criteria (40%) or both criteria (70%). There was no difference in the use of vasopressor or diuretics between prerenal and sustained AKI patients. Length of ICU and hospital stay, as well as ICU and hospital mortality were different among the groups. Among survivors, AKI patients had a longer length of ICU stay compared to the prerenal group (p=0.05).

| | No AKI | Transient changes by sCr or UO | AKI by UO | AKI by sCr | AKI by UO and sCr |
|---|---|---|---|---|---|
| N | 147 | 43 | 55 | 36 | 34 |
| % ICU mortality | 2 | 4.6 | 2 | 11 | 24 |
| % Need for RRT | 1 | 0 | 0 | 8 | 12 |
| ICU stay (days) | 2(2-3) | 3(2-8) | 3(2-7) | 4(3-15) | 5(3-13) |
| Hospital stay (days) | 5(3-11) | 8(4-14) | 8(4-17) | 10(5-19) | 16(5-23) |

Conclusion: Patients with transient changes in urine flow and or sCr have worse outcomes than non AKI patients and may identify patients at risk for adverse events. Further studies are needed to assess whether transient alterations in renal function have a similar effect in non-surgical patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO051**

**Pattern of Serum Creatinine Decline Is Associated with Renal Recovery from Acute Kidney Injury in ICU Patients** Etienne Macedo,[1] Rakesh Malhotra,[1] Josee Bouchard,[2] Ravindra L. Mehta.[1] [1]*University of California San Diego;* [2]*Université de Montréal.*

Recovery of kidney function following an episode of AKI is influenced by several factors including the severity of renal functional change. The rate, magnitude and duration of change in sCr have been identified as potential factor associated with outcomes. We hypothesized that a rapid improvement in renal function characterized by early and persistent decline in sCr would be associated with higher level of renal recovery. **Methods:** We studied a cohort of 716 ICU patients screened from Jun06 to Dec08. AKI was defined by the AKIN sCr criterion (>= 0.3mg/dL within 48 hours). The slope of Scr change was computed from the last sCr value prior to its decline in non-dialyzed patients for 24, 48 and 72h time points from ICU admission. Percent recovery was calculated based on the last value at ICU discharge in comparison to the first sCr at ICU admission and time to recovery was computed as the time duration between the last peak sCr value and that at ICU discharge. Patients were stratified based on percentage change of recovery from; 25-49%, 50-99% and >=100%. We evaluated the relationship of the slope to level of and time to recovery. **Results:** Of 192 (26.8%) patients that met AKIN criteria, 38 were dialyzed and not included in this analysis. In 37%, sCr returned to reference during ICU stay, in half the last sCr was between 50 to 99% from the reference sCr %, and in 11% from 25 to 49%. The level and time to recovery were correlated (r=-.399; p=<.0001).

Serum creatinine slopes and renal recovery during ICU stay

| Recovery category | N | Slope 24 | Slope 48 | Slope 72 |
|---|---|---|---|---|
| 25-49% | 17 | .35(.3-.47) | .25(.2-) | .3(.2-) |
| 50-99% | 78 | .4(.15-.5) | .1(.1-.25) | .13(.06-.16) |
| >=100% | 57 | .4(.3-.6) | .25(.15-.35) | .2(.13-.23) |
| Correlation with final % of renal recovery - r (p) | 152 | .428(<0.001) | .449(0.001) | .323(0.051) |

*p for difference among groups

**Conclusion:** In this cohort, a rapid decline in Scr was associated with higher levels of renal recovery during the ICU stay. Future studies should target the time to improvement as a modifiable factor for improving renal functional recovery and other outcomes from AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO052**

**How Effective Is a Time-Independent Creatinine Change Criteria in Identifying Patients Developing Acute Kidney Injury as Defined by RIFLE/AKIN?** Andrew J. P. Lewington,[1] Ashley Garner,[2] Julian Barth.[2] [1]*Department of Renal Medicine, Leeds Teaching Hospitals, Leeds, West Yorkshire, United Kingdom;* [2]*Department of Clinical Biochemistry, Leeds Teaching Hospitals, Leeds, West Yorkshire, United Kingdom.*

Introduction

The NCEPOD AKI report identified a significant gap in the quality of care received by patients who died from AKI. Specifically there was an unacceptable delay in recognising patients who developed AKI post-admission. This clearly illustrates the need to improve the detection of AKI in hospitalised patients.

AKI can be detected by increases in serum creatinine using the RIFLE or AKIN definitions. However current UK hospital laboratory systems cannot apply these definitions to real-time changes in serum creatinine. An alternative criterion is to use a time independent laboratory Delta check to identify patients who experience a rise in creatinine.

Methods

The laboratory clinical biochemistry database was interrogated retrospectively for creatinine results for adult in-patients admitted to a teaching hospital (880 in-patient beds) during October 2008. Only in-patients were included, all out-patients were excluded. All creatinine results for these patients reported within 30 days following the day of admission were collected. The data was analysed using the RIFLE, AKIN and a Delta check criterion defined as an increase of >26 umol/L in two successive creatinine results over a period up to 30 days.

Results

A total of 10019 serum creatinine results were reported on 2822 adult in-patients. Of the 1315 patients who had multiple creatinine results 149 (73 males; 76 females) were identified as potentially suffering from AKI in accordance to either the RIFLE, AKIN or Delta check criteria. These 149 patients represented 5.3% of all patients with creatinine results and 11.3% of patients who had multiple creatinine results.

Conclusion

Utilisation of the Delta criterion detected most of the patients identified by both RIFLE and AKIN. This criterion could be easily incorporated into a routine Clinical Biochemistry alert system to identify patients developing AKI. Diagnosis of AKI would then be based on the application of the RIFLE/AKIN definitions.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO053**

**Hypertension, Cardiovascular Diseases and Multiple Organ System Failure: Flip Sides for an Acute Kidney Injury Outcome** Milan M. Radovic.[1,2] [1]*Clinic of Nephrology, Clinical Center of Serbia, Belgrade, Serbia;* [2]*University of Belgrade, School of Medicine, Belgrade, Serbia.*

BACKGROUND: Viewpoint on binomial acute kidney injury (AKI) outcome and factors that influence lack of complete recovery have not been elucidated.

AIM: The aim of the study was to compare impact of comorbidities and complications on fatal outcome and incomplete AKI stage 3 recovery.

METHODS: Retrospective, observational, single centre study was done in 825 AKI stage 3 patients (age 50.3 ± 15.8 years, 604 male) treated by hemodialysis, assigned to one out of 4 groups according to in-hospital outcome: 1 = no AKI recovery and death (N =369, 44.7%), 2 = AKI recovery but death (N=9, 1.1%), 3 = incomplete AKI recovery and survival (N=43, 5.2%), 4 = complete recovery and survival (N=404, 49%, p < 0.001). Individual severity score (ISS), age and frequencies of comorbidities and complications were compared between groups (ANOVA, logistic regression and Cox proportion hazard model).

RESULTS: Patients in group 1 were older than in group 4 and had greater ISS than other (p<0.001). Hypertension and cardiovascular diseases (p< 0.001) were more frequent in groups 1 and 3 than other groups. Sepsis (p = 0.038), ARDS and MOSF (p < 0.001) were more frequent in group 1 than group 4, and MOSF than in group 3 (p < 0.001). Patients with hypertension (OR 2.72, CI 1.2 - 6.2, p=0.018) and ischaemia (OR 6.49, CI 1.98 - 21.3, p=0.002), were likely not to recover AKI completely. Neoplasia (OR 1.62, CI 1.01 - 2.61, p = 0.045) and cardiovascular diseases (OR 2.43, CI 1.48 - 3.97, p< 0.001) were related with fatal outcome. Cox regression (p = 0.011) showed impact of hypertension (OR 4.66, CI 1.95 - 11.1, p=0.01) on incomplete AKI recovery. Patients with cardiovascular (OR 1.67, CI 1.2 - 2.3, p = 0.003) or hepatic (OR 1.52, CI 1.06 - 2.17, p = 0.024) comorbidities and ARDS as a complication (OR 2.23 , CI 1.37 - 3.67, p = 0.01) had greater hazard risk for fatal outcome.

CONCLUSIONS: Hypertension had the greatest impact on AKI stage 3 incomplete recovery. Cardiovascular diseases are the most important comorbidity and ARDS and MOSF complications related with fatal outcome.

Disclosure of Financial Relationships: Employer: Supported by research grant number 145043 - dj by Ministry of Science, Republic of SerbiaResearch Funding: Pharma Swiss, Belgrade, Serbia, Research grant 2009.; Other Relationship: Pharma Swiss, Belgrade, Serbia travel grant 2010.

**TH-PO054**

**Second Episode of AKI: An Infrequent but Deadly Outcome** Raul Lombardi. *On Behaf of the VENTILA Group, Getafe, Spain, IMPASA, Montevideo, Uruguay.*

Purpose: to establish the clinical profile and the outcomes of a second episode of acute kidney injury (AKI) in mechanically ventilated (MV) patients.

Methods: The VENTILA Group database was used for the study. Clinical and laboratory data were recorded daily until death, ICU discharge, or day 28, whichever came first. AKI was defined according to AKIN criteria. Regression of AKI (AKI-R) was considered when a sustained and progressive reduction of SCr ≥0.3 mg/dl was observed during more than 2 consecutive days. Persistence of AKI (AKI-P) was defined by the absence of regression. Second episode of AKI (AKI-2) was defined by a sustained rise in SCr≥0.3 mg/dl for two or more days in patients with AKI in regression. The remainder ptes were grouped as No-AKI. Study period: 10 days from start of MV. Primary outcome: in-hospital mortality; secondary outcome: days of MV, ICU-LOS and hospital-LOS.

Main results are in Table 1.

| | AKI-R | AKI-P | AKI-2 | No-AKI | TOTAL |
|---|---|---|---|---|---|
| Cases n, % | 330 (12.0) | 391 (14.1) | 49 (1.8) | 1991 (72.1) | 2761 |
| Age yrs (SD) | 60.0 (16.4) | 61.8 (17.1) | 55.9 (16.2) | 58.3 (17.4) | 59.0 (17.5) |
| Male % | 67.9 | 63.2 | 57.1 | 59.2 | 60.8 |
| SAPS II median,range | 48 (12-108) | 49 (5-109) | 41 (2-94) | 42 (2-109) | 44 (2-109) |
| Days of MV mean (SD) | 9.6 (7.1) | 6.5 (3.5) | 14.5 (16.2) | 8.1 (7.7) | 8.2 (7.8) |
| ICU-LOS mean (SD) | 16.1 (13.8) | 12.0 (11.6) | 25.1 (27.5) | 14.5 (13.7) | 14.6 (13.90 |
| Hospital-LOS mean (SD) | 30.1 (34.3) | 24.5 (26.7) | 36.1 (40.2) | 28.5 (29.7) | 28.3 (30.1) |
| Observed mortality % | 49.1 | 59.1 | 67.3 | 38.6 | 43.2 |
| Expected mortality % | 44.8 | 47.0 | 35.3 | 35.7 | 38.4 |
| Variation % (rate) | 8.8 (1.09) | 20.5 (1.25) | 47.3 (1.90) | 7.5 (1.08) | 11.1 (1.12) |

Kaplan-Meier analysis showed a drop in survival curve for AKI-2 ptes by day 6, in coincidence with the onset of AKI-2: 6.6 (1.9) d. $PaO_2$, plateau pressure, PEEP, MAP worsened 3 days before AKI-2 onset.

Conslusions: 1) a second episode of AKI almost doubled the expected mortality and prolonged MV time and LOS. 2) timing of onset of AKI-2 and drop in survival suggest a relationship between both events. 3) hemodynamic derangement and injurious MV seems to be risk factors. 4) protective ventilation and close hemodynamic support could prevent this deadly complication.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO055**

**Clinical Characteristics and Outcomes of Septic Acute Kidney Injury** Eunjung Cho, Sang-Kyung Jo, Won-Yong Cho, Hyoung-Kyu Kim. *Department of Internal Medicine, Division of Nephrology, Korea University Anam Hospital, Seoul, Korea.*

**Purpose**: This study aimed to determine the clinical characteristics and outcomes of critically ill patients with septic acute kidney injury (AKI) in comparison with non-septic AKI.

**Methods**: We retrospectively collected data of patients with AKI who were ≥18 years of age and admitted to the intensive care unit (ICU) for ≥24 hours between 1 April 2007 and 31 December 2009, and compared the clinical characteristics and outcomes of patients with and without sepsis. Outcome measures were in-hospital mortality, renal replacement therapy (RRT) requirements, duration of ICU and hospital stays, and renal recovery. We analyzed predictors of mortality using logistic regression.

**Results**: Of the 1075 patients, 333 (30.9%) had AKI, as defined by the RIFLE criteria, and 134 (40.2%) of them had AKI with sepsis. The predominant septic foci were thoracic (46.3%) and abdominal (29.9%). Septic AKI had significantly higher SAPS II and SOFA scores, and required more mechanical ventilation and vasoactive drugs than non-septic AKI. Furthermore, a significantly greater proportion of patients with septic AKI progressed to the failure category of the RIFLE classification compared to patients with non-septic AKI (62.7% versus 44.7%). Septic compared with non-septic AKI had a higher in-hospital mortality and required more RRT (63.2% versus 35.7%, p<0.001; 44.7% versus 23.2%, p<0.001, respectively). Amongst survivors, patients with septic AKI were more likely to recover renal function. Finally, a higher SAPS II score, and a greater requirement for vasoactive drugs and RRT were independently associated with increased in-hospital mortality in septic AKI after adjustment for other covariates.

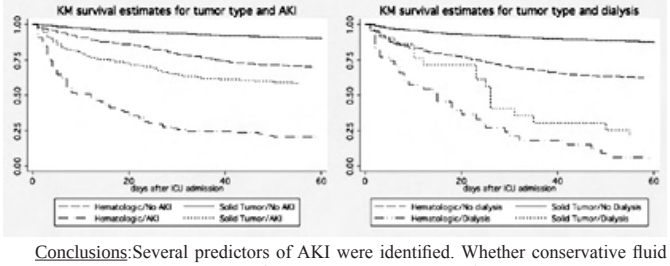
**Conclusion**: The incidence of AKI in ICU patients is high, for which sepsis is a leading contributing factor. Patients with septic AKI are sicker and have a higher burden of illness with an increased risk of death compared to patients with non-septic AKI, but renal function recovers better in survivors of septic AKI. Further studies are necessary to determine the clinical factors and actual pathophysiology related to outcomes and also to find valid treatment modalities in septic AKI.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO056

**Predictors, Costs, and Outcomes of Acute Kidney Injury in Critically Ill Patients with Cancer** Amit Lahoti, Abdulla K. Salahudeen. *General Internal Medicine AT/EC, Section of Nephrology, University of Texas M D Anderson Cancer Center, Houston, TX.*

Purpose:To identify predictors and the associated costs of AKI and dialysis in critically ill patients with cancer. Methods:Retrospective analysis of 2398 patients admitted to the ICU from Dec 2005 through Dec 2006 with a baseline serum creatinine (Scr)<=1.5 mg/dL. Primary endpoint was AKI defined as a minimum 50% increase in Scr from baseline at any point during the ICU admission. Regression analysis was used to determine predictors of AKI as well as the economic impact of AKI and dialysis on total hospital cost. Kaplan-Meier estimates of survival stratified by tumor type were determined. Results:Predictors of AKI included (Odds Ratio): vasopressors(2.7), mechanical ventilation(2.2), medical vs surgical service(2.0), IV diuretics(3.0), amphotericin(2.3), sepsis(1.9), and diabetes(1.4). There was a 2.1-fold increase in odds of developing AKI in patients receiving both mechanical ventilation and vasopressors (interaction). After adjusting for covariates, dialysis was associated with an increase of $76,911 in hospital cost. Independent of dialysis, each 1% increase in creatinine was associated with a $92 increase in hospital cost. Pts with hematologic malignancies who developed AKI had the lowest short-term survival. The need for dialysis in these patients was associated with a dismal 60-day prognosis (figure).



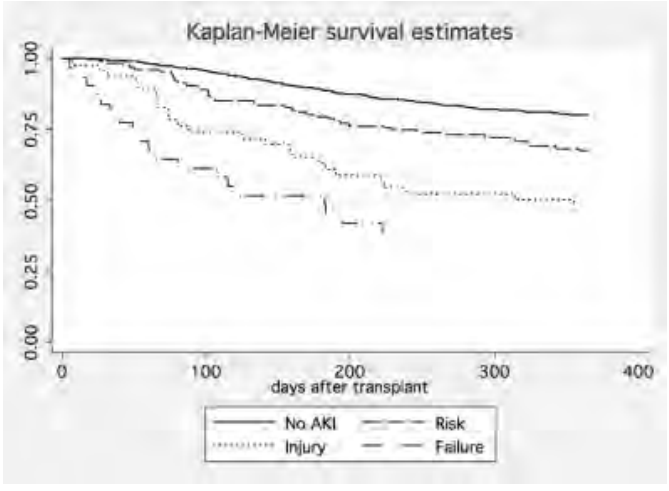

Conclusions:Several predictors of AKI were identified. Whether conservative fluid strategies decrease the incidence of AKI by reducing the need for mechanical ventilation and diuretics is of interest. Amphotericin should be reserved for patients who have a strong indication. Given the cost implications and poor survival, the utility of dialysis in critically ill patients with hematologic malignancies should be carefully examined. A limited trial of early goal directed therapy might be considered.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO057

**Acute Kidney Injury Occurs in a Significant Number of Patients Undergoing Stem Cell Transplant and Is Associated with Decreased Survival** Amit Lahoti,[1] Abdulla K. Salahudeen.[1] *[1]General Internal Medicine AT&EC, Section of Nephrology, University of Texas M D Anderson Cancer Center, Houston, TX; [2]Houston, TX; [3]Houston, TX.*

Purpose:To determine the incidence and outcome of acute kidney injury (AKI) in patients undergoing stem cell transplant. Methods:Retrospective analysis of 1048 patients who underwent stem cell transplant at M D Anderson Center from Sept 2004 to Sept 2006. AKI was defined by the RIFLE criteria using the maximum SCr within 60 days of transplant compared to baseline. Cox Proportional Hazards model was used to examine the effect of AKI on survival. Kaplan-Meier estimates of survival were determined by RIFLE category. Results: Demographics included average age 50 years, 60% male gender, and 76% Caucasian. 48% of patients underwent allogeneic transplant. 19% of patients had acute GVHD grade I-II, and 4.4% had acute GVHD grade III-IV. 12%, 4.4%, and 3% of patients were classified into the Risk, Injury, and Failure categories, respectively. AKI was significantly associated with mortality in an adjusted Cox proportional hazard model (hazard ratio): Risk (1.4), Injury (1.9), and Failure (2.7).




Conclusions: AKI develops in a significant number of patients undergoing stem cell transplant and is associated with decreased overall survival. Whether strategies that help to mitigate the incidence of AKI also translate to improved survival are of interest.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO058

**Acute Kidney Injury (AKI) in Cancer Patients: Incidence, Risk Factors and Outcomes** Abdulla K. Salahudeen, Amit Lahoti. *UT MD Anderson CC, Houston, TX.*

Purpose of Study: AKI appears to be on the rise in the cancer population, but its incidence, risk factors or influence on patient-outcomes is not known.

Methods: Electronic patient records were prospectively collected in 5491 patients hospitalized to UT MD Anderson Cancer Center for 3 months between May 1, 2006 and July 31, 2006. For each patient, demographic information and laboratory and pharmacy data were obtained. Descriptive, survival and logistic regression analyses were performed.

Results: The incidence of AKI defined as an absolute increase in serum creatinine ≥ 0.3 mg/dl was 28% among the 5013 patients. Preexisting AKI was noted in 14% patients admitted to hospital where as 14% patients acquired AKI in the hospital. The mean (±SD) age was 54±18 y, and men were 52%. In univariate analysis, frequency of AKI was significantly higher in patients on chemotherapeutic, anti-diabetic and antibiotic medications. Patients admitted to ICU were 50% more likely to develop AKI. AKI in cancer patients was found to be associated with 1) a high blood sugar of over 200 mg/dl (p<0.001), 2) admission through Emergency Center or admission to ICU (p<0.001), and 3) admission Service Group or Cancer types (p=0.001), for example, admission to Stem Cell Transplant Department or hematological malignancy. Patient with AKI had a mortality rate of 15% compared to patients without AKI who only had a mortality rate of 2.3 (p<0.001). In multivariate regression analysis, patient who had AKI had higher rates of hospital stay (OR 2.1, CI: 1.7-2.6; P<0.0001) and mortality (OR 6.0 , CI: 4.3-8.2; P<0.0001).

Conclusions: 1) the AKI incidence rate of 28% among patients admitted to cancer hospital is higher than the rate known for patients admitted to a community hospital, 2) the risk factors for AKI include diabetes, type of cancer and cancer therapy and 3) both mortality and hospital stay are adversely affected by the development of AKI in hospitalized cancer patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO059

**The Incidence and Clinical Course of Acute Renal Failure in Patients with Severe Acute Pancreatitis** Young Soo Kim, Hyun Gyung Kim. *Nephrology, Uijeongbu St. Mary's Hospital, The Catholic University of Korea, Uijeongbu-si, Kyunggi-do, Republic of Korea.*

**Purpose** : Although acute renal failure (ARF) commonly develops in patients with severe acute pancreatitis (SAP), the impact of ARF on disease severity is rarely reported in Korea. This study was performed to compare the clinical findings, morbidity and mortality between SAP patients with ARF and normal renal function (NRF). **Method** : We retrospectively evaluated the medical records of 102 patients with SAP between january 2001 and June 2008 in 3 Hospitals. We defined SAP by the 1992 International Symposium on Acute Pancreatitis in Atlanta. **Results** : Of the total 102 SAP patients, ARF was observed in 39 patients (38.2%). Eight of the 39 ARF patients (20.5%) received hemodialysis and ten patients (25.6%) died. When compared to NRF patiens, ARF patients (n=39) had higher incidence of loss of consciousness (17.9% vs 1.6%, p=0.003), and APACHE II scores more than 8 (92.3% vs 0%, p<0.001). The ARF group had also higher incidences of sepsis (35.9% vs 7.9%, p<0.001), multiorgan failure (15.4% vs 0%, p=0.001), respiratory failure (28.2% vs 4.7%, p=0.001) and mortality (25.6% vs 3.2%, p=0.001). In our study, thrombocytopenia (p=0.037), Hemoconcentration (p=0.023), high LDH (p=0.040) were significant predictors of ARF in patients with SAP.**Conclusion** : The incidence of ARF was high (38.2%) and ARF patients showed higher mortality, compared to NRF patients. We suggest that early management of ARF should be performed for reducing the mortality in SAP patients.

Multivariate analysis of risk factors for ARF in patients with severe acute pancreatitis

| | Regression coefficient | Regression Standard error | p value | Adjusted odds ratio | 95% C.I |
|---|---|---|---|---|---|
| Thrombocytopenia | 2.083 | 1.000 | 0.037 | 8.026 | 1.130-56.991 |
| Hemoconcentration | 1.526 | 0.673 | 0.023 | 4.602 | 1.229-17.226 |
| Hyponatremia | 0.741 | 0.643 | NS | 2.098 | 0.589-7.476 |
| Abnormal LFT | -0.301 | 0.674 | NS | 0.740 | 0.198-2.770 |
| High LDH | 1.646 | 0.801 | 0.040 | 5.188 | 1.080-24.913 |
| Sepsis | 1.188 | 0.792 | NS | 3.281 | 0.695-15.492 |
| Respiratory failure | 1.098 | 0.892 | NS | 2.997 | 0.521-17.228 |

Disclosure of Financial Relationships: nothing to disclose

## TH-PO060

**Predicting Kidney Recovery in Patients with Acute Kidney Injury Requiring Renal Replacement Therapy** Benjamin R. Bell,[1] Neill Adhikari,[1] Sean M. Bagshaw,[2] Jan O. Friedrich,[1] Michelle A. Hladunewich,[1] David Klein,[1] Stephen Lapinsky,[1] Ron Wald.[1] *[1]Department of Medicine, University of Toronto, Toronto, ON, Canada; [2]Critical Care Medicine, University of Alberta, Edmonton, AB, Canada.*

**Introduction:** A significant minority of patients with acute kidney injury (AKI) who require renal replacement therapy (RRT) go on to chronic dialysis. There is limited information on factors present at RRT initiation that may predict a favorable kidney prognosis in AKI.

**Methods:** This is a cohort study of consecutive patients admitted to critical care units at St. Michael's Hospital (Toronto, Canada) who required dialysis for AKI from April 2007-January 2010. We collected demographic (age, sex) and clinical variables (baseline and admission creatnine, diagnostic category, laboratory data, RRT modality, urine output, Charlson and Sequential Organ Failure Assessment scores) at RRT initiation and followed patients to 30 days. Factors associated with RRT independence at 30 days among survivors (analysis 1) and RRT-independent survival at 30 days (analysis 2) were determined using logistic regression/stepwise covariate selection.

**Results:** 256 critically ill patients with AKI required RRT, of whom 118 (46%) died within 30 days. Among the 138 survivors, 40 (29%) were dependent on RRT and 98 (71%) were RRT-free at 30 days. Factors that were independently associated with RRT independence among survivors (analysis 1) and RRT-free survival (analysis 2) are shown below.

| Analysis 1 | | Analysis 2 | |
|---|---|---|---|
| | OR (95% CI) | | OR (95% CI) |
| CCI | 0.80 (0.66-0.97) | CCI | 0.81 (0.70-0.95) |
| Nonoliguria | 2.55 (1.06-6.06) | Nonoliguria | 1.71 (0.91-3.20) |
| baseline eGFR (per 10 unit increase) | 1.14 (0.99-1.30) | SOFA score (per 1 unit increase) | 0.85 (0.78-0.92) |

CCI=Charlson comorbidity index (per 1 unit increase), Nonoliguria=>400cc urine in 24h period after RRT initiation. SOFA=sequential organ failure assessment

**Discussion:** Recovery of kidney function in AKI is associated with a lower preexisting comorbidity, greater urine output and premorbid kidney function. This information may inform clinical decision-making and discussions with patients and their families.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO061

**Prognosis of Acute Kidney Injury in Chronic Kidney Disease** Seung Yeup Han, Mi Hyun Jang, Choong-Hwan Kwak, Eun-Ah Hwang, Sung Bae Park, Hyun Chul Kim. *Internal Medicine, Keimyung University Dongsan Medical Center, Daegu, Korea.*

**Purpose** : In studies of acute kidney injury(AKI) in hospitalized patients, the prevalence of preexisting chronic kidney disease(CKD) has been estimated to be about 30%. This study performed to ascertain the incidence, causes and outcomes of AKI in CKD.

**Methods** : We reviewed medical records of 211 consecutive cases of AKI admitted in our hospital between Jan. 2003 and Dec. 2006.

**Results**: Altogether, 211 patients of AKI were identified. The mean age of the patients was 60.8±17.3 years, and there was a male predominance (1.6:1). The mean follow up duration was 18.9±24.1 months. During an average hospitalization of 15.2±16.4 days, 32 patients(15.2%) died. There were 48 patients (22.7%) with AKI in CKD. The causes of AKI were 77 infections including sepsis patients constituted the largest group(36.5%). The second common cause of the AKI was hypovolemia (39 patients, 18.5%), and then nephrotoxic drugs or contrast induced AKI (38 patients, 18.0%), cardiac problem (18 patients, 8.5%), malignancy (8 patients, 3.8%), post-operative (8, patients 3.8%). When the groups with AKI only (n=163) and AKI with preexisting CKD (n=48) were compared, the rates of dialysis requiring patients in hospitalization was significantly higher in those with underlying CKD than AKI only (22.1% Vs 58.3%, p=0.000). There were no statistically significant difference in age, blood pressure, hospital duration, cause of AKI between two groups. In AKI only, serum creatinine level was recovered known before the episode of AKI in 42.3%, compared with 8.3% for patients with CKD, and 4.9% of patients with AKI only progressed to end-stage renal disease(ESRD); 37.5% of patients with preexisting CKD progressed to ESRD (p=0.000). In hospital mortality rates were no difference between two groups, but renal survival rates with AKI only were 98% in 3month, 97% in 1year, 96% in 3years; AKI with preexisting CKD were 72.7%, 50.8%, 46.2% (p=0.000). **Conclusion**: AKI with preexisting CKD was an important part of AKI and at significantly increased risk for ESRD. So enough attention and prevention should be paid to this entity and causes such as infection or drugs.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO062

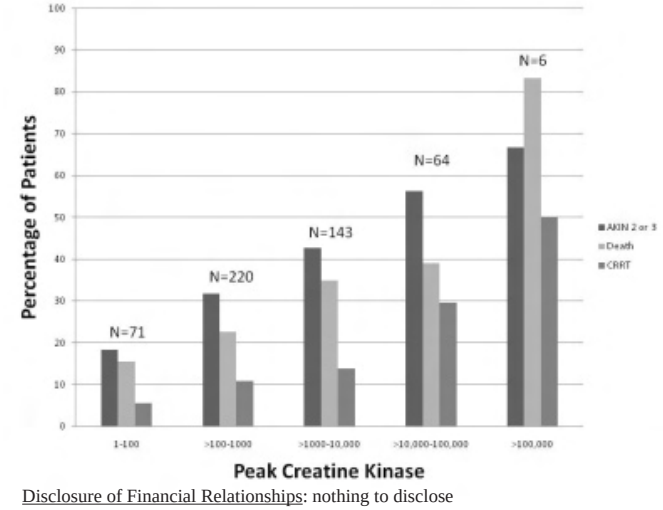
**Association of Rhabdomyolysis with Renal Outcomes and Mortality in Burn Patients** Ian J. Stewart,[1] Casey Cotant,[1] Molly A. Tilley,[1] Todd F. Huzar,[2] James K. Aden,[2] Jesse Sherratt,[1] Clinton K. Murray,[1] Kevin Chung.[2] *[1]San Antonio Military Medical Center; [2]United States Army Institute of Surgical Research.*

Purpose: The contribution of rhabdomyolysis to acute kidney injury (AKI) in the context of burn injury is poorly studied. We sought to determine the impact of rhabdomyolysis on AKI (defined by the AKIN classification), renal replacement therapy (RRT) and death.

Methods: Consecutive admissions to the burn intensive care unit at our institution from January 2003 to October 2008 were examined. Patients were included in the analysis if they had a serum creatine kinase (CK) measured anytime in the course of their hospitalization. Patients were excluded from the analysis if they had a diagnosis of myocardial infarction. Independent variables included gender, age, inhalation injury, electrical burn, percentage total body surface area burned (%TBSA), percentage of full thickness burns (%FT), injury severity score (ISS), log CK at admission and log peak CK. These variables were examined via multivariate logistic regression analysis against AKIN stage, RRT and death.

Results: Of 1,124 consecutive admissions, 306 met our eligibility criteria. Both log CK at admission (OR 1.5, 95% CI 1.1-2.2; p=0.02) and log peak CK (OR 2.0, 95% CI 1.5-2.6; p<0.0001) were found to be associated with AKIN stage 2 or greater. For RRT, log CK at admission (OR 1.9, 95% CI 1.2-3.0; p=0.007) and log peak CK (OR 1.7, 95% CI 1.2-2.3 p=0.003) achieved statistical significance. Finally, log peak CK (OR 1.8, 95% CI 1.3-2.6; p=0.003) was associated with an increased risk of mortality.

Conclusion: Each ten-fold increase in admission CK nearly doubles the probability of AKI and RRT while each ten-fold increase in peak CK nearly doubles the probability of AKI, RRT and death.




Disclosure of Financial Relationships: nothing to disclose

## TH-PO063

**Baseline Serum Markers Are Associated with Future Severe Rhabdomyolysis in Non-Whites** Lisa K. Prince, Kevin C. Abbott, Stephen W. Olson. *Internal Medicine, Nephrology Service, Walter Reed Army Medical Center, Washington, DC.*

Rhabdomyolysis can cause significant morbidity. Metabolic and electrolyte disorders may increase the risk of rhabdomyolysis. Known ethnic differences in baseline creatinine kinase (CK) levels suggest a potential difference in other serologic risk factors. No previous studies evaluate baseline serology prior to rhabdomyolysis. We hypothesize that non-white subjects with severe rhabdomyolysis have different baseline serology prior to diagnosis compared to matching controls.

**Methods**:Fourteen cases of severe rhabdomyolysis with acute kidney injury in non-whites were identified in the military database. The Department of Defense serum repository (DoDSR) sent serum samples for each study subject from *prior* to diagnosis and 3 age, sex, race, and age of serum matched controls to Quest Diagnostics© for measurement of CK, renal panel, lactate dehydrogenase (LDH), coenzyme Q10 (CoQ10) and aldolase levels.

**Results**:Study cases and matching healthy controls had a significantly different mean CK (341 vs. 158 U/L; p = 0.004), CoQ10 (0.52 vs. 0.66 U/L; p = 0.01), aldolase (9.35 vs. 8.11 U/L; p = 0.01), and phosphorus level (3.78 vs. 3.46 mg/Dl; p = 0.01). A greater number of study cases than matching controls met the combined thresholds of CoQ10<0.6 U/L & Aldolase>7.1 U/L (79% vs. 29%; p=0.002) and LDH>93 U/L & CoQ10<0.57 U/L (64% vs. 8%; p<0.0001). Combined thresholds of aldolase > 9.3 U/L & CoQ10 of < 0.6 U/L (43% vs. 0%; p=0.0001) was 100% specific for future severe rhabdomyolysis.

**Conclusions:**Lower baseline levels of coenzyme Q10, and higher levels of phosphorus, CK, and aldolase are associated with future severe rhabdomyolysis in non-whites. Combinations of serologic markers establish a more sensitive and specific evaluation for future severe rhabdomyolysis. These novel findings could provide a screening tool to identify those at risk for rhabdomyolysis in physically demanding or extreme environments. Targeted education of this population could provide early symptom recognition and therapeutic intervention to prevent further exacerbation of disease. The potential prophylactic benefit of CoQ10 replacement also warrants investigation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO064**

**Time Is Tubule. Consequences of Referral Delay in Acute Kidney Injury** Prasad Rajendran, Thangavelu Chandrasekar, Christopher Federick Wong, Kottarathil Abraham Abraham. *Department of Nephrology, University Hospital Aintree, Liverpool, United Kingdom.*

Aim: The purpose of our study was to determine in patients with Acute Kidney Injury(AKI), whether a delay in referral to a nephrologist, by more than 1 day was associated with worsening of the AKIN stage or poor outcome and to evaluate prognostic factors for AKI mortality.

Method: Hospitalized patients referred to our renal service from July 2007 to January 2008 were studied. Serial S.Creatinine values through the hospital stay at 48 hour intervals were used to estimate the AKIN stage prior to referral, at referral and post referral. Statistical analysis was performed on demography and outcome**.** Multivariate analysis was performed on various prognostic factors.

Results: Among the 135 patients referred with AKI, 60% were males. Pre-existing CKD was present in 73%.

| Referral Delay | AKI Stage Pre-referral | AKI Stage at Referral | AKI Stage post referral | In Hospital Outcome | Outcome at 2 years |
|---|---|---|---|---|---|
| < 1 day n=27 (20 %) | Stage 1 n=8(30%); Stage 2 n=5(18%); Stage 3 n= 14(52%) | Stage 1 n=8(30%); Stage 2 n=5(18%); Stage 3 n= 14(52%) | Stage 1 n=7(26%); Stage 2 n=5 (18%); Stage 3 n= 15 (56%) | Death 8(30%); Dialysis Dependant 0; Full Recovery 13(48%); Partial Renal Recovery 5(19%); No Renal Recovery 1(4%) | Dialysis Dependant 0; Death 16(59%) |
| >=1 day n=108 (80%) | Stage 1 n=59(55%); Stage 2 n=16(15%); Stage 3 n= 33(30%) | Stage 1 n=50(46%); Stage 2 n=15(14%); Stage 3 n= 43(40%) | Stage 1 n=42(39%); Stage 2 n=13(12%); Stage 3 n= 53(49%) | Death 25(23%); Dialysis Dependant 1(1%); Full Recovery 44(41%); Partial Renal Recovery 25(24%); No Renal Recovery 13(12%) | Dialysis Dependant 4(4%); Death 61(57%) |

**Presence of sepsis (OR 2.6, p<0.05) and being on diuretics at the onset of AKI (OR 2.8, p<0.05) were strong independent predictors of 1 year mortality.**

Conclusion: Early referral in less than 24 hours of AKI is associated with less patients progressing to higher AKI stages and having poor outcomes at discharge. Mortality outcomes at 2 years was similar but dialysis dependancy was minimised. Attention to the treatment of sepsis and avoidance of diuretics in those at risk can potentially minimize mortality in AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO065**

**Delayed Nephrology Consultation (NC) and the Outcome of Acute Kidney Injury (AKI) Patients in the Intensive Care Unit (ICU)** Veronica T. Costa e Silva,[1] Isac Castro,[1] Fernando Liano,[2] Luis Yu.[1] *[1]Nephrology Service, University of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Nephrology Service, Hospital Ramon y Cajal, Madrid, Spain.*

Delayed NC is supposed to affect the prognosis of AKI ICU patients. The aims of this study were to analyze: 1) factors related with early NC in critically ill AKI patients; 2) the impact of delayed NC on outcome of these patients. A prospective study was performed in 6 ICUs of University of São Paulo School of Medicine (Pneumology, Internal Medicine, Trauma, Surgery, Infectious Diseases and Emergency). Data were collected by an independent single observer, non-member of the ICU or nephrology staff, by daily visits looking for new AKI cases. NC was solicited by the ICU physician. AKI was defined as an increase of 50% in the basal serum creatinine (SCr). AKI was classified as Surgical or Clinical. Renal function recovery was defined as return to baseline SCr level. A total of 366 patients were included for analysis. AKI incidence was 18%. General mortality was 68%. NC was carried out in 196 (53%) pts and NC occurred 2.8 +/- 3.5 (mean +/- SD) days after diagnosis day (DD) as requested by the ICU physician. NC were done in the first 2 days after DD (66%, early- NC group). The remaining were considered as delayed-NC group which presented higher mortality (OR:4.04/CI:1.60-10.17) and reduced renal function recovery (OR:0.22/0.08-0.60). A PS for early-NC was created. The 6 variables retained on the model were: AKI of clinical origin (OR:2.62/CI:1.14-5.99), D (OR:0.99/CI:0.99-1.00), SCr (OR:2.04/CI:1.38-3.02), pH (OR:0.008/CI:0.001-0.20), Pneumology (OR:3.58/CI:1.06-12.06) and Internal Medicine (OR:5.95/CI:1.80-19.59) ICUs. After correction by the PS, Delayed NC was still associated with higher mortality (OR: 4.04/CI: 1.60 – 10.17) and reduced renal function recovery (OR: 3.61/CI: 1.14 – 11.40). In conclusion, factors influencing early NC were SCr, D, pH, ICU pt's origin and clinical AKI. Delayed NC was associated with higher mortality and worse renal function even after correction by confounders variables.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO066**

**Acute Kidney Injury (AKI) Management in the Intensive Care Unit (ICU): A Survey of Current Practice in the United Kingdom** Sarah L. Jones, Mark A. J. Devonald. *Nottingham Renal Unit, Nottingham University Hospitals NHS Trust, Nottingham.*

**Study Purpose**

To ascertain current UK ICU practice for investigating and managing AKI.

**Methods**

An online survey was distributed to all UK general adult ICUs between December 2009 and May 2010. We asked about details of renal replacement therapy (RRT) and investigation of AKI.

**Results**

188/233 ICUs (81%) started the survey and 167 (72%) completed it.

In over 40% of units a nephrologist is never or rarely consulted about AKI. 88% of responding units refer to nephrology if a primary renal disease is suspected. Only 19% of units routinely use the AKIN or RIFLE criteria. 57% of respondents always request a renal tract ultrasound scan but only 46% have 24 hour access to renal ultrasound scanning.

CVVH is the most frequently used form of RRT (56%) followed by CVVHDF (37%). Intermittent RRT is the main method in only 5% units. CRRT is managed exclusively by intensivists in 94% of units, whereas IHD is managed predominantly by nephrologists (57%).

The most frequently used criteria for initiating RRT are hyperkalaemia, fluid overload and pH (acidaemia). 66% of respondents have a lower threshold for commencing RRT in patients with severe sepsis. A satisfactory or improving urine output is the most commonly used indication for discontinuing RRT followed by normalisation of pH.

73% of units have a standard protocol for RRT with 35mls/Kg/hr as the most frequently prescribed dose (59%). In only 51% of units is delivered dose of RRT assessed.

**Conclusions**

There is considerable variation in the management of AKI in UK Intensive Care Units. This is presumably attributable to a lack of consensus on optimal practice.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO067**

**Can Trimetazidine Prevent Contrast Induced Nephropathy in Patients with Renal Impairment?** Muhammad Rafiqul Alam,[1] Abdul Wadud Chowdhury,[2] Kazi Shahnoor Alam,[3] Asia Khanam.[1] *[1]BSMMU; [2]DMCH; [3]NIKDU, Dhaka, Bangladesh.*

***Background:***

Contrast Induced Nephropathy (CIN) is an increasing cause of hospital acquired kidney injury and leads to a significant increase in mortality. With the increasing use of radiographic contrast media in diagnostic and interventional procedures, CIN has become an important cause of iatrogenic acute renal impairment. CIN is defined as a 25% increase in SCr from the baseline value or an absolute increase of at least 0.5 mg/dl (44.2 mmol/L), with 48 hours after the administration of radiographic contrast media that is not attributable to other causes.

**Objective:**

To estimate the prevalence of CIN in patients undergoing coronary procedure with pre-existing renal impairment.

To evaluate the efficacy of TMZ as an adjunct to saline infusion for prevention of CIN in patients with renal impairment.

**Method:**

This was a prospective randomized controlled trial comparing hydration therapy to additional TMZ for the prevention of CIN. A total of 90 patients with a SCr between 1.4 to 2.5 mg/dl undergoing elective coronary procedure in a specialized cardiac hospital were studied. Patients were randomly divided into 2 groups: TMZ (n=44) and control (n=46). Echocardiography was done in all patients. Hydration with isotonic normal solution (0.9%) at a rate of 1ml/kg/hr starting 12 hr before the coronary procedure and up to 12 hr there after was done in patients of both groups. TMZ was administered as a dose of 20 mg thrice day orally for 72 hr starting 48 hr before the procedure. Iopamiro-370 was used during all of the coronary procedure. Volumes of contrast agent were different depending upon the coronary procedure. SCr and urea concentration were measured 24 hr before, 48 hr and 7 days after the coronary procedure.

**Result:**

Out of 90 patients with pre-existing renal impairment 44 were in TMZ group and 46 in control group. There was no statistically significant difference in age, sex, blood pressure, baseline SCr, calculated creatinine clearance rate and presence of co-morbid condition between the two groups. Diabetes mellitus was found as predominant co-morbid condition.

***Conclusion:***

Trimetazidine cannot prevent CIN significantly in renal impaired patients.

Disclosure of Financial Relationships: nothing to disclose

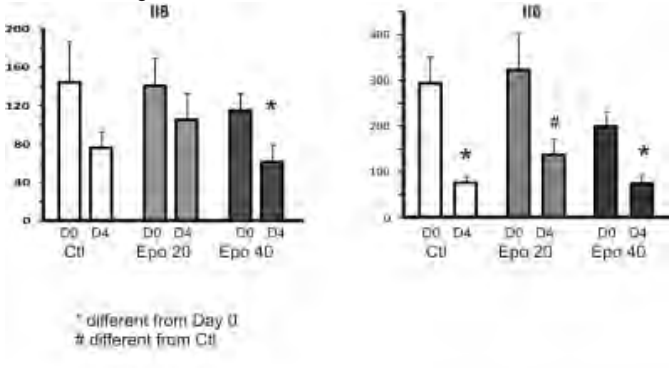
**TH-PO068**

**Epoetin Does Not Demonstrate Anti-Inflammatory Properties after Cardiac Surgery** Sophie M. De Seigneux, Belen Ponte, Jerome Pugin, Patrick Saudan, Pierre-Yves F. Martin. *Department of Nephrology, University Hospital of Geneva, Geneva, Switzerland.*

Introduction : The inflammatory state is deleterious for the mortality in the acute setting. Erythropoietin has been thought to have nephroprotective as well as antiinflammatory properties. We have previously demonstrated that a-epoetin (EPO) does not modify renal function after cardiac surgery (ASN 2009). In the same patients, we measured the cytokines blood levels and compared the levels between the groups.

Subjects and Methods: From June 08 to June 09, 80 patients having cardiac surgery were randomised to receive either 40 000 IU a-Epoetin (n=20) or 20000 IU a-Epoetin (n=20) or a normal saline injection after cardio-pulmonary bypass (CBP). To assess acute inflammatory response serum cytokines ( IL6, IL8, IL1beta, IL10, TNFalpha, IL12p70) were measured using a luminex technique at day 0 before Epoetin infusion and at day 4.

Results: Patient groups did not differ in terms of age, gender, comorbidities and baseline renal function. IL6, IL8, IL1beta, IL10, TNFalpha, IL12p70 were measured at day 0 and similar between groups. Only IL 6 and IL8 decreased at day 4 and this decrease was observed in all groups. However in the group treated with EPO 20000 units, the decrease of IL6 was significantly less important than in the control ( 77+/-12 vs 135+/-34pmol/ml, p<0.05). IL8 also appeared to decrease less with EPO treatment but this trend did not reach statistical significance.




Conclusions: A single administration of either 20000 or 40000 IU a-Epoetin after cardiac surgery does not alleviate the inflammation observed in the post operative state and rather appeared to impede the spontaneous decrease in pro inflammatory cytokine post-operatively, mostly IL6. These results, associated with the negative results on renal function do not support the use of EPO in acutely ill patients for nephroprotection.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO069**

**AKI: Are We Biased Against Peritoneal Dialysis?** Sergio Gaiao,[1,2] Massimo De Cal,[1] Fredric O. Finkelstein,[3] Claudio Ronco,[1] Dinna N. Cruz.[1] *[1]S Bortolo Hosp, Vicenza, Italy; [2]Sao Joao Hosp, Porto, Portugal; [3]St Raphael Hosp, New Haven, CT.*

**Introduction:**

Peritoneal Dialysis (PD) is employed for Acute Kidney Injury (AKI) management in developing countries. However, in developed countries, this modality is rarely used in this context.

This is a relevant issue in the AKI field, in part related to the cost and infrastructure needs for hemodialysis. In disasters, PD is also the simplest method for RRT.

Our aim was to evaluate actual practice as well as practitioner opinion regarding PD use in AKI.

**Methods**

A questionnaire was distributed to selected attendees of 3 PD meetings held in 2009 (International Course on PD in Vicenza, ISPD in Hong Kong and North American Chapter of ISPD in Vancouver).

**Results**

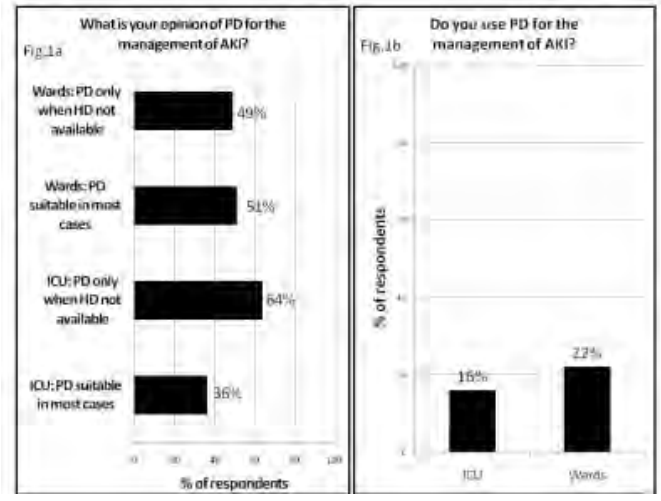
We analyzed 484 completed questionnaires. Responders were from Europe (74%), followed by Asia Pacific (14%) and North America (11%). Most work in nephrology departments (88%).

Thirty-six percent think that PD is a suitable therapeutic option in most cases of AKI in ICU, while 51% think that PD is suitable for AKI in the wards (Fig 1a). In contrast, only 16% actually use PD for AKI management in the ICU, while 22% use it in the wards (Fig 1b). The most common modality used is Acute Intermittent PD, 39% in the ICU and 36% in the wards, followed by Tidal PD (24% in ICU and 30% in wards).

Overall 66% responders were not certain of the most appropriate PD dose for AKI in the ICU, while 61% were uncertain in the wards. However, among actual users of PD, a smaller number (34-50%) were uncertain about appropriate dose.

**Conclusions**

Although 36-51% of respondents feel that PD is suitable for AKI, only 16-22% actually use it. This is similar to the disparity between opinion and practice regarding PD in chronic kidney disease. There is much uncertainty about the appropriate PD dose for AKI. Further studies on barriers to implementation of acute PD are needed.




Disclosure of Financial Relationships: nothing to disclose

**TH-PO070**

**Impact of Acute Renal Failure on Nosocomial Infections Prevalence in Critically-Ill Patients** Jean-Sebastien Rachoin, Daniel Fabius, Christa Schorr, Jean-Pierre El-Khoury, David R. Gerber, Lawrence S. Weisberg. *Medicine, UMDNJ-RWJ Medical School, Camden, NJ.*

Critically-ill patients with dialysis-dependent ARF have a high mortality rate which may be due, in part, to acute infection. We hypothesized that ARF itself, and not just renal failure, increases the risk for nosocomial infection. We studied nosocomial infection and mortality in critically-ill patients on dialysis for either ARF or end-stage renal disease (ESRD). We performed a retrospective observational study of all patients admitted to a medical/surgical ICU from 2003 through 2009. We defined ARF as that requiring RRT (A-RRT). We recorded the following nosocomial infections: pneumonia, bacteremia and UTI. Patients with onset of nosocomial infection before RRT were classified as not having ARF. 9441 patients were initially considered for inclusion, of whom 7202 had complete data available. 256 patients (3.6%) had A-RRT, of whom 27 had nosocomial infection before RRT, leaving 229 evaluable patients with A-RRT. 403 patients (5.2%) were receiving RRT for ESRD (E-RRT). 446 of 7202 patients (6.2%) had one or more nosocomial infection. 34 patients with A-RRT (15%) had at least one nosocomial infection, compared with 41 patients (10%) with E-RRT and 368 of 6570 patients (5.6%) receiving no RRT. After adjusting for comorbid conditions and severity of illness, patients with A-RRT were significantly more likely to develop nosocomial infection than patients with E-RRT (OR 2 [1.3-3.0],p=0.001). In patients with A- RRT, nosocomial infection was associated with a trend towards increased mortality (OR 2 [0.9-4.5], p=0.087). Thus, critically-ill patients with dialysis-dependent ARF are at higher risk of nosocomial infections than patients with dialysis-dependent chronic kidney disease, even after adjustment for severity of illness. The effect of modality of RRT remains to be determined.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO071**

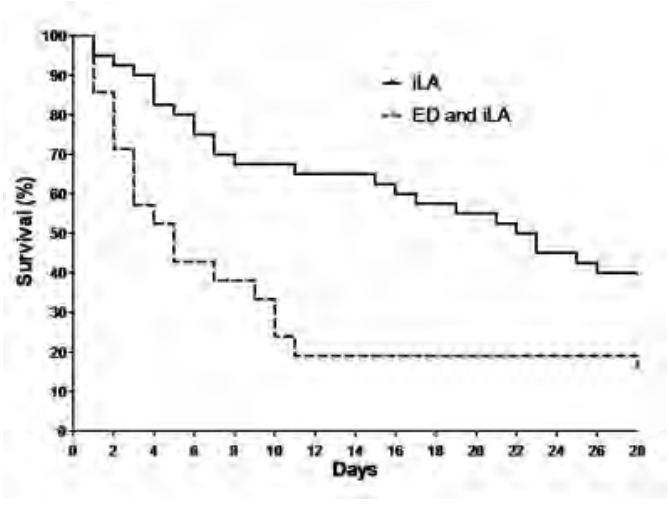
**Effect of Acute Kidney Injury Requiring Extended Dialysis on Survival of Patients Undergoing Interventional Lung Assist Membrane Ventilator Treatment** Jan T. Kielstein,[1] Sören Tolk,[1] Olaf Wiesner,[2] Hermann G. Haller,[1] Carsten Hafer,[1] Marius M. Hoeper.[2] *[1]Department of Nephrology and Hypertension, Medical School Hannover, Hannover, Germany; [2]Department of Pulmonary Medicine, Medical School Hannover, Hannover, Germany.*

**Background:** Extracorporeal lung assist devices are increasingly used in the intensive care unit setting to improve extracorporeal gas exchange mainly in patients with acute respiratory distress syndrome (ARDS). In the setting of multi-organ dysfunction syndrome ARDS is frequently accompanied by acute kidney injury. It is unknown how the combination of these two conditions affects survival of critically ill patients.

**Patients and Methods:** At a tertiary care centre we retrospectively evaluated all patients undergoing interventional lung assist (iLA membrane ventilator) treatment between January 1st 2005 and December 31st 2009. 61 patients (31F/30M), median age 40 (28 to 52) years were followed up to one year.

**Results:** Of the 61 patients undergoing iLA membrane ventilator treatment 21 patients had acute kidney injury network (AKIN) stage 3 treated by extended dialysis. The mean 28 day survival of all patients was 33 %. While patients without extended dialysis showed a mean 28 day survival of 40%, the survival of patients with extended dialysis was only 19%. Patients on extended dialysis were not different in respect to age, weight, Horowitz index and underlying disease.

**Discussion:** The concomitant necessity for extended dialysis (ED) in patients undergoing iLA membrane ventilator treatment is associated with increased mortality in ICU patients.




Patients in whom iLA was placed as a bridge to lung transplantation and that were successfully transplanted showed the best outcome. Future studies have to clarify whether it is possible to identify patients that benefit from the combination of these two extracorporeal treatment methods.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO072**

**Prognosis of Patients on Extracorporeal Membrane Oxygenation: The Impact of Acute Kidney Injury on Mortality** Yung-Chang Chen,[1] Feng-Chun Tsai,[2] Ya-Chung Tian,[1] Ming-Yang Chang,[1] Chang-Chyi Jenq,[1] Chih-Wei Yang.[1] *[1]Kidney Research Center, Department of Nephrology, Chang Gung Memorial Hospital, Taipei, Taiwan; [2]Division of Cardiovascular Surgery, Chang Gung Memorial Hospital, Taipei, Taiwan.*

**Purpose:** Extracorporeal membrane oxygenation (ECMO) has been utilized for patients in critical condition such as those with life-threatening respiratory failure or post-cardiotomy cardiogenic shock. This study analyzed the outcomes of patients treated with ECMO and identified the relationship between prognosis and the Acute Kidney Injury Network (AKIN) obtained at post-ECMO support 48 h (AKIN 48-hour). Additionally, AKIN 48-hour is compared with other acute kidney injury scoring systems.

**Methods:** This study reviewed the medical records of 82 critically ill patients on ECMO support at a specialized intensive care unit (CVSICU) at a tertiary care university hospital between March 2002 and February 2006. Demographic, clinical and laboratory variables were retrospectively collected as survival predicators.

**Results:** Overall mortality rate was 59.8%. The most common condition requiring ECMO support was cardiogenic shock. Goodness-of-fit was good for AKIN 48-hour criteria. The Risk of renal failure, Injury to the kidney, Failure of kidney function, Loss of kidney function, and End-stage renal failure (RIFLE) 48-hour and AKIN 48-hour scoring systems had excellent areas under the receiver operating characteristic curve (0.845+/-0.048 and 0.881+/-0.041, respectively). Furthermore, AKIN 48-hour correlated strongly with RIFLE 48-hour scores for individual patients ($r^2$=0.608, $p$<0.001). Finally, cumulative survival rates at 6-month follow-up after hospital discharge differed significantly ($p$<0.05) for AKIN 48-hour stage 0 *vs.* AKIN 48-hour stage 1,2 and 3, and AKIN 48-hour stage 1 and 2 *vs.* AKIN 48-hour stage 3.

**Conclusions:** During ECMO support, the AKIN 48-hour scoring system proved to be a reproducible evaluation tool with excellent prognostic abilities for these patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO073**

**The Acute Flank Pain Syndrome: A Common Presentation of Acute Renal Failure in Young Adults in Iceland** Margret Arnadottir,[1] Helga Margret Skuladottir,[1] Sverrir Hardarson,[2] Margret B. Andresdottir.[1] *[1]Department of Nephrology, Landspitali University Hospital, Reykjavik, Iceland; [2]Department of Pathology, Landspitali University Hospital, Reykjavik, Iceland.*

**Purpose**: The acute flank pain syndrome (AFPS) was a common side effect of the NSAID suprofen which was withdrawn in the eighties. Since then, only a few reports of AFPS have been published, in association with intake of other NSAIDs and/or binge drinking. The goal of the present study was to calculate the incidence and describe the cases of AFPS in Iceland during a 10 year period.

**Methods**: AFPS was defined as severe flank and/or abdominal pain in combination with acute renal failure, both features unexplained except for the possible consumption of ethanol and/or an NSAID. The hospital records of the patients that fulfilled the following criteria were reviewed with regard to AFPS: age 18-41 years, acute renal failure, and admission during the period January 1998 – December 2007. Information about the sales figures of ibuprofen and diclofenac were also collected.

**Results**: One-hundred and six patients received the diagnosis acute renal failure, of which 21 (20%) had AFPS. The incidence of AFPS increased during the study period but the average countrywide incidence was 20.2/million/year. There was a history of recent consumption of ethanol in 15 patients, an NSAID in 15 patients, either in 20 patients and both in 9 patients. Eighteen of 21 patients were males. The maximal serum creatinine concentration was 251 (137-529) micromol/L. In all cases, spontaneous regression of pain and renal failure was observed. In 2007, 29.8 DDD of ibuprofen and 20.7 DDD of diclofenac were sold. The increase in the incidence of AFPS was parallel to that of the over-the-counter sales of ibuprofen.

**Conclusions**: The study revealed a high incidence of AFPS and the largest case series of AFPS that has been reported since the suprofen experience. As in previous reports, almost all the patients had a history of recent consumption of ethanol and/or an NSAID. The incidence of AFPS in Iceland may be explained by heavy consumption of NSAIDS in combination with binge drinking pattern. AFPS may be underreported in other countries.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO074**

**Retrospective Evaluation of the Renal Effect of Phosphate Soda Enema in Hospitalized Patients** Bruce S. Spinowitz, Prince Mohan, Gabriel EL-Kass, Wing Fun Leo-To, Bessie Vardianos, Chaim Charytan. *Nephrology & Pharmacy, NY Hospital Queens, Flushing, NY.*

Acute phosphate nephropathy has been reported in patients who have received oral sodium phosphate products. Patients who have received phosphate soda via enema have been reported to experience severe hyperphosphatemia, hypocalcaemia, seizures and coma. Acute kidney injury(AKI) has not been reported in adults. Our objective was to explore a relationship between sodium phosphate enema administration in the hospital setting and subsequent rise in serum creatinine, which might suggest the possibility of AKI consequent to this laxative use.

Methods: Data was collected from our hospital pharmacy patient profiling system from September 2008 through September 2009. 1347 patients had an order for phosphate enema. 510 patients were excluded (pediatric patients, ER patients, patients with fewer than three days of post-dose data). Of the remaining 837 patients, 364 received a phosphate enema. 473 who did not receive an enema served as the control group. AKI was defined as an increase of 25% from baseline serum creatinine or an absolute serum creatinine increase of 0.5 mg/dl. Serial creatinine and eGFR were analyzed post enema administration in all patients. Concomitant medications and selected co-morbidities were analyzed.

Results: 32 of the 364 patients (8.8%), who received sodium phosphate enema, were found to have AKI. 17 out of 473 patients (3.6 %) who never received enema, had AKI(OR=2.58,p<0.002). 26 of 32 patients with AKI in enema group returned to baseline renal function in an average of 6.3 days. In the control group 12 of 17 patients returned to baseline renal function in an average of 2 days. The two groups were comparable with respect to age and baseline eGFR.

Conclusions: The temporally related increase in serum creatinine associated with phosphate soda enema administration suggests, but does not prove, a causal relationship. This data, plus the association of oral phosphate use and acute renal failure, warrant caution when prescribing phosphate enemas as a purgative. To further evaluate this relationship, studies are needed with serum phosphate levels measured pre/post enema administration.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO075**

**Incidence and Predictors of Acute Kidney Injury in Hospitalized Clostridium Difficile Infected Patients (Pts)** Venkata A. Suda, Lalathaksha Murthy Kumbar, Paul A. Fein, Ashwini M. Shadakshari, Sara Asadi, Parampreet S. Ghuman, Jyotiprakas Chattopadhyay, Morrell M. Avram. *Avram Division of Nephrology, Long Island College Hospital, Brooklyn, NY.*

Clostridium difficile infection (CDI) is recently becoming more frequent, severe and difficult to treat. Medical literature about the relationship between CDI and development of acute kidney injury (AKI) is minimal. The objective of this study was to examine the incidence and predictors of AKI (serum creatinine >0.3 mg/dL above baseline) in hospitalized pts with CDI. One hundred forty two pts who were diagnosed with CDI between November 2008 and September 2009 were enrolled in this study. Demographics, clinical, and biochemical data were recorded. Sixty one percent of the pts (n=87) developed AKI. Pts with AKI were older than those without AKI (67 vs. 59 years, p=0.02). Pts with hypertension (p=0.004), diabetes (p=0.002), cardiovascular disease (p=0.008), chronic kidney disease (p=0.03), and those who received pressors (p<0.0001) were significantly higher in AKI group compared to those without AKI. At the time of admission, the patients who developed AKI had higher serum creatinine (p<0.0001), urine specific gravity (p=0.013) proteinuria (p=0.002), hematuria (p=0.07), low serum albumin (p=0.016) and serum bicarbonate (p=0.002). By univariate logistic regression analysis, the above factors were significantly associated with AKI. The administration of intravenous contrast was lower in AKI group compared to non AKI (16 vs.31 p=0.037). Using multivariate logistic regression analysis, controlling for other confounding variables, admission creatinine (odds ratio: 26.2, p=0.001), CVD (odds ratio: 5.63, p=0.059), diabetes (odds ratio: 9.66, p=0.009) and proteinuria (odds ratio: 6.36, p=0.03) were significant predictors of AKI in these pts. Twenty seven percent (n=24) of pts with AKI expired during the study period compared to 2% in pts without AKI. In conclusion, AKI is highly prevalent in pts with CDI and carries a high mortality. Further studies are needed to assess these risk factors and interventions aimed at decreasing the high mortality.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO076**

**Risk Factors of Colistin Induced Nephrotoxicity** Sung Chang Bae, Sung Jin Moon, Sang Hun Lee, Hyeong Cheon Park, Sung-Kyu Ha. *Internal Medicine, Gangnam Severance Hospital, Seoul, Korea.*

In recent years, colistin was increasingly reused into clinical practice despite its nephrotoxicity, depending on the multidrug-resistant (MDR) gram-negative bacteria is becoming more prevalent. But only limited data are available to prevent its nephrotoxicity. In this study, the incidence, clinical characteristics and risk factors of colistin-induced nephrotoxicity was investigated. The data was obtained retrospectively from 57 cases receiving colistin sodium methanesulfonate for treatment of MDR gram negative bacterial infections between March 2009 and May 2010 at Gangnam Severance Hospital, 900-bed tertiary care facility located in Seoul, South Korea. The study excluded the patients who used the colistin for less than two days or who was creatinine concentration more than 4.0 mg/dL. Thirty five (61.4%) of the 57 cases developed nephrotoxicity and 9 (15.8%) of whom underwent renal replacement therapy. By Acute Kidney Injury Network, AKI stage 1 (R), stage 2 (L) and stage 3 (F) were 13 (22.8%), 14 (24.6%) and 8 (14%), respectively. In the univariate analysis, old age, hypoalbuminemia, septic shock, malignancy and concominanat use of prepenem or vancomycin were more frequent in the patients with AKI. However, multivariate logistic regression analysis showed that the malignanacy (OR=67.8; 95% CI 1.34 – 3426.13; P = 0.035) and concominant use of vancomycin (OR = 20.7; 95% CI 1.09–391.95; P = 0.043) were independently associated with a colisin induced nephrotoxicity. In conclusion, maliganancy and concomitant use of vancomycin were risk factors of colistin induced nephrotoxicity. Colistin may have to be used with caution in these patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO077**

**Outcomes of Critically Ill Patients with Acetaminophen Induced Acute Liver and Kidney Injury Requiring Renal Replacement Therapy** Andrew J. P. Lewington,[1] Simon Lines,[1] Ashley Wood,[1] Mark Bellamy.[2] *[1]Renal Department, Leeds Teaching Hospitals, Leeds, West Yorkshire, United Kingdom; [2]Department of Intensive Care Medicine, Leeds Teaching Hospitals, Leeds, West Yorkshire, United Kingdom.*

Introduction:

We report here on the outcomes of patients with acetaminophen (paracetamol) induced liver and kidney injury necessitating renal repacement therapy (RRT) admitted to the intensive care unit (ICU) of a tertiary referral liver unit. We analysed which admission variables predicted mortality.

Methods:

We performed a retrospective review of all patients admitted to the ICU over a 6 year period. A subgroup of these with acetaminophen induced liver injury and requiring RRT were identified for further analysis.

Results

Of the 5582 admissions during this period, 73 patients met study criteria. Mean age was 37 years (s.d. ± 13.9 yrs) with a 60% female preponderance; overall hospital mortality was 58%. The ten patients who underwent a liver transplant had a lower mean age of 28 years ($p<0.05$); 8 of the 10 survived. Using logistic regression modeling, low pH and higher MELD score were predictive of mortality ($p<0.05$); age, gender, albumin, serum transaminase and sodium, hemoglobin, platelet count and APACHE2 score were not. Admission pH was the best discriminator of survival; all patients with an admission of pH of ≤ 7 died (n=8) and those with a pH of > 7.3 (n=14) fared best with a mortality of 35%.

Conclusion

These data add to the small body of evidence on acetaminophen-induced acute kidney injury in critically ill patients. A low admission pH, a likely surrogate for liver dysfunction and global disease severity, is the most useful parameter measured on admission for predicting outcome. Traditional ICU prognostic scores, such as APACHE2, seem to perform less well in this subset of patients. These prognostic criteria are similar to the King's College (UK) prognostic criteria for acute acetaminophen poisoning.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO078**

**Acute Kidney Injury (AKI) in Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD) and Pneumonia** Qi Qian,[1] Carlos R. Franco-Palacios,[1] Robert Hartman,[2] Ladan Zand,[3] Xiangling Wang,[1] Guangxi Li,[2] Rodrigo Cartin-Ceba.[2] *[1]Division of Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [2]Department of Radiology, Mayo Clinic, Rochester, MN; [3]Division of Internal Medicine, Mayo Clinic, Rochester, MN; [4]Pulmonary & Critical Care Medicine, Mayo Clinic, Rochester, MN.*

**Background:** ADPKD is a common inherited disease, leading to kidney failure in >50% affected individuals. AKI occurs in patients with pneumonia, leading to an increase in one-year mortality. We compared the occurrence and severity of AKI in pneumonia patients with and without ADPKD.

**Methods:** Mayo Clinic Rochester in-patient database from 1998 to 2008 were screened. A total of 45 pneumonia episodes (by chest x-ray and clinical presentations) in ADPKD patients and 112 consecutive non-ADPKD patients with pneumonia and with a comparable eGFR were identified. Patients with end stage renal failure, with organ transplantation or on chronic immunosuppressants were excluded. The remaining pneumonia episodes, 26 in ADPKD and 82 in non-ADPKD, were analyzed.

**Results:** ADPKD patients were younger than non-ADPKD patients, mean age of 61.9 and 84.3 years, respectively, $P < 0.01$. Seventeen of the 26 (65.5%) pneumonia episodes in ADPKD and 26 of the 82 (31.7%) episodes in non-ADPKD were associated with the development of AKI ($P < 0.01$). In both groups, those who developed AKI had a lower baseline eGFR (mil/min/BSA) than those who did not (49.2 vs. 60 in ADPKD and 41.1 vs. 54.4 in non-ADPKD). One year mortality rate in ADPKD with or without AKI was 41.1% (7 of 17) vs. 11.0% (1 of 9), odds ratio 5.6 (95% confidence interval: 0.6-55.4). The one year mortality rates in non-ADPKD with and without AKI were 42.3% vs. 30.4%, odds ratio 1.7 (95% confidence interval: 0.6-4.4). In both ADPKD and non-ADPKD groups, pneumonia patients with AKI had more frequent ICU admission and mechanical ventilation and a longer hospital stay than those without AKI.

**Conclusion:** ADPKD patients with pneumonia show a higher occurrence of AKI than those without ADPKD (with comparable eGFR), suggesting that ADPKD patients are more susceptible to kidney injury in the context of infection.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO079**

**Clinical Manifestations of Renal Dysfunction Are Associated with Low Individual Awareness of Chronic Kidney Disease** Delphine S. Tuot,[1] Laura C. Plantinga,[1] Chi-Yuan Hsu,[1] Regina Jordan,[2] Nilka Rios Burrows,[2] Elizabeth Hedgeman,[3] Jerry Yee,[4] Neil R. Powe.[1] *[1]University of California, San Francisco, San Francisco, CA; [2]Centers for Disease Control and Prevention, Atlanta, GA; [3]University of Michigan, Ann Arbor, MI; [4]Henry Ford Hospital, Detroit, MI.*

Background: Awareness of chronic kidney disease (CKD) among primary care providers and persons with CKD is low. We examined whether well-known complications of kidney disease that should trigger CKD recognition among providers are associated with high individual CKD awareness.

Methods: CKD awareness was assessed in 2323 adults with CKD stages 1-4 using 1999-2006 National Health and Nutrition Examination Survey data. CKD awareness was a "yes" answer to "Have you ever been told you have weak or failing kidneys?" A clinical manifestations score (CMIS) ranging from 0-7 and consisting of equally weighted indicators of abnormal values of serum potassium, serum bicarbonate, serum phosphate, blood urea nitrogen, anemia, proteinuria and uncontrolled hypertension, was created. Multivariable logistic regression was used to estimate odds and percentages of awareness by CMIS, adjusted for demographic characteristics and diabetes, weighted to the US population.

Results: Among individuals with CKD, those with proteinuria or hyperkalemia had a 3-fold greater odds of CKD awareness ($p<.01$ & $p=.03$) than those without. Patients with a CMIS of 2, 3-4 and >5, had higher odds of being aware of their CKD status than those without measurable complications of renal dysfunction, independent of eGFR (adjusted odds ratio 2.1 $p=.01$; 3.9 $p<.001$; 5.0 $p=.02$). There was a linear trend of greater CKD awareness with an increasing CMIS ($p =.003$). Nonetheless, 86.5% of individuals with a CMIS score >5 were unaware of their disease.

Conclusions: CKD awareness is low, even among individuals who manifest many complications of renal dysfunction. A better understanding of mechanisms of awareness, such as physician recognition of clinical manifestations and provider-patient communication, is required to intervene and improve risk factor modification, facilitate earlier detection of CKD to slow its progression, and minimize associated complications.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO080**

**An Easily Administered Self-Report Depression Scale Can Predict Poor Outcomes in Patients with CKD** Susan Hedayati,[1,2] Masoud Afshar,[2] Abu T. M. Minhajuddin,[2] Robert D. Toto,[2] Madhukar Trivedi,[2] Augustus John Rush.[3] *[1]Nephrology, Dallas VA Medical Center; [2]Nephrology, Clinical Sciences, and Psychiatry, University of Texas Southwestern Medical Center at Dallas; [3]Duke-NUS, Singapore.*

We previously demonstrated that a major depressive episode (MDE) as assessed by a structured psychiatric interview is associated with excessive morbidity and mortality in patients with CKD (*JAMA* 2010; 303: 1946). To investigate whether an easily administered patient self-report scale of depression is associated with poor outcomes, we administered the 16-item Quick Inventory of Depression Symptomatology Self-Report Scale (QIDS-SR) to 260 consecutive VA outpatients with CKD. Scores on the QIDS-SR range from 0 to 27, with higher scores indicating a greater severity of depressive symptoms. A cutoff of ≥10 was validated to indicate a MDE in CKD patients. Patients were followed prospectively for 12 months for a composite outcome of progression to ESRD, hospitalization or death. Cox proportional hazards models were used to explore the associations of both the QIDS-SR score and the QIDS-SR cutoff of ≥10 with the composite outcome. Models were adjusted for age, race, eGFR, serum albumin, hemoglobin, calcium-phosphorus product, medical comorbidities and drug and alcohol abuse.

Mean age was 64.5 ±12 years. Thirty-seven percent were African American and 55% had diabetes mellitus. Six percent had stage 2, 38% stage 3, 41% stage 4 and 15% predialysis stage 5 CKD. Mean QIDS-SR score was 7.1 ±4.9. There were 126 composite events. The risk for ESRD, hospitalization or death was 6% higher for each one point increase in QIDS-SR score, HR 1.06 (95% CI 1.02-1.09). This association remained statistically significant in adjusted models, HR 1.05 (1.01-1.09). QIDS-SR score ≥10 was also significantly associated with the composite outcome, HR 1.52 (1.05-2.21) in unadjusted and 1.55 (1.05-2.28) in adjusted models.

The score on a short and easily administered depression questionnaire was independently associated with an increased risk of CKD progression, death or hospitalization. The QIDS-SR may be used to screen patients with CKD for depression in the outpatient setting and predict clinical outcomes.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO081**

**Ethnic Differences in ESRD and Death in the Diabetes Mellitus Treatment for Renal Insufficiency Consortium (DIAMETRIC) Study** Tahira P. Alves,[1] David K. Packham,[2] Jamie P. Dwyer,[3] Dick De Zeeuw,[4] Julia Lewis,[3] Robert C. Atkins,[5] Hiddo Jan Lambers Heerspink.[4] *[1]Medicine, Nephrology Division, University of Texas Health Science Center at San Antonio, San Antonio, TX; [2]Melbourne Renal Research Group and Royal Melbourne Hospital, Melbourne, Australia; [3]Medicine, Nephrology Division, Vanderbilt University Medical Center, Nashville, TN; [4]Clinical Pharmacology, University of Groningen, Groningen, Netherlands; [5]Medicine, Nephrology Division, Vanderbilt University Medical Center, Nashville, TN; [6]Monash University, Melbourne, Australia; [7]Clinical Pharmacology, University of Groningen, Groningen, Netherlands.*

In recent studies, black patients with non-diabetic chronic kidney disease appear to be more likely to reach ESRD than die from any cause. The purpose of this study was to compare the risks for ESRD, CVD death and all cause mortality among different ethnic groups with established type 2 diabetic nephropathy and CKD in the DIAMETRIC database. Data on 3228 adult patients with type 2 diabetic nephropathy from the IDNT and RENAAL trials were combined to establish the DIAbetes MEllitus Treatment for Renal Insufficiency Consortium (DIAMETRIC) database established in 2009. Mean follow-up for the trials was 2.8 years. There were 628 ESRD events and 576 deaths ( 304 CV related and 272 non-CV related) during the follow-up period. The rates for ESRD, CV death and all cause mortality were significantly higher ($p<0.05$) for Hispanics (11.7/100 pt yr, 2.2/100 pt yr, and 4.8/100 pt yr, respectively) followed by Asians (8.8/100 pt yr, 1.7/100 pt yr, and 3.3/100 pt yr, respectively) and blacks (7.6/100 pt yr, 1.4/100 pt yr, and 2.2/100 pt yr, respectively), when compared to white patients (5.8/100 pt yr, 1.3/100 pt yr, and 2.1/100 pt yr, respectively). The incidence rate ratio (IRR) of ESRD to mortality was significantly higher ($p<0.05$) for blacks (IRR 3.5) and whites (IRR 2.8) closely followed by Asians (IRR 2.7) and Hispanics (IRR 2.4). In conclusion, all ethnic groups with type 2 diabetic nephropathy were significantly more likely to reach ESRD than die from any cause.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO082**

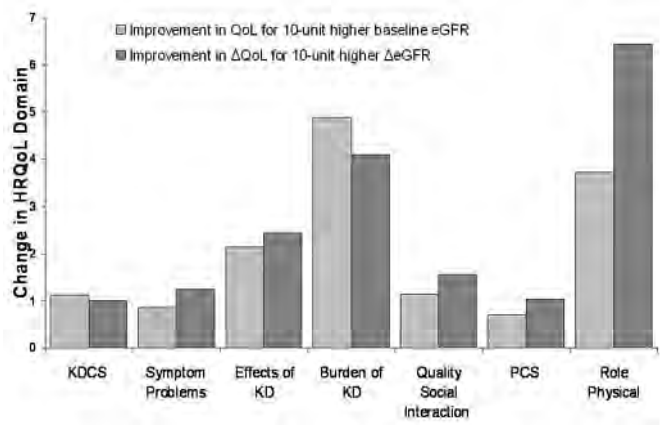
**Change in Health Related Quality of Life (QoL) with Progression of Chronic Kidney Disease (CKD): The Study of Treatment for Renal Insufficiency: Data and Evaluation (STRIDE)** Anca Tilea,[1] Brenda W. Gillespie,[1] Fredric O. Finkelstein,[2] George Eisele,[3] Margaret A. Kiser,[4] Rajiv Saran,[1] Peter Kotanko.[5] *[1]U. of Michigan, Ann Arbor, MI; [2]Hospital St Raphael Yale University, New Haven, CT; [3]Medical College of Albany, Albany, NY; [4]University of North Carolina, Chapel Hill, NC; [5]RRI, New York, NY.*

Declining renal function may result in deterioration in QoL.We examined the association between change (Δ) in QoL domain score and progression of CKD in a multicenter CKD cohort.

STRIDE is a national prospective cohort of 2,162 CKD patients (median follow-up 3 years).The Kidney Disease QoL Short Form-36 was administered at baseline and follow-up visits. ΔQoL and ΔeGFR were calculated as the difference between the baseline visit and the last visit with both QoL and eGFR(n=991).Pearson correlation and linear regression were used to assess the associations between each QoL domain and baseline eGFR,and between ΔQoL domains and ΔeGFR.Models were adjusted for age, gender, race, diabetes and hypertension.

The average age was 63±14 yr, mean eGFR was 25±11 ml/min/1.73m$^2$, with 52% males, 23% black, 38% diabetics and 54% hypertensives.The average ΔeGFR was -0.6±8.7 ml/min/1.73m$^2$/year.Results showed significant associations ($p<0.05$) between ΔeGFR and ΔKidney Disease Component Summary score, Δ(symptom problems), Δ(effects of kidney disease, Δ(burden of KD), Δ(quality of social interaction), Δ(Physical Component Summary score), Δ(role-physical) and marginal association with Δ(energy/fatigue) ($p=0.06$).The figure shows the significant associations of baseline eGFR predicting baseline QoL, and ΔeGFR predicting ΔQoL domains.

Declining eGFR is associated with a decline in a variety of QoL domains.Understanding mechanisms of these associations and implications for patient management require further investigation.




Disclosure of Financial Relationships: nothing to disclose

**TH-PO083**

**Racial Disparity in Mortality by Identified CKD Stages in Elderly Medicare Patients** Suying Li,[1] Jiannong Liu,[1] David T. Gilbertson,[1] Robert N. Foley,[1,2] Allan J. Collins.[1,2] *[1]U. S. Renal Data System Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, U of MN, Minneapolis, MN.*

The United States Renal Data System has shown that mortality rates for African Americans (AAs) are lower than those for Whites in dialysis patients. A study using the Third National Health and Nutrition Examination Survey data showed that in patients with early CKD stages (1-5) AAs had significantly higher risk for death than Whites among persons who were < 65 yrs old, but this was not seen among those who were >= 65 yrs old.

Beginning October 2005, new ICD-9 codes were introduced to identify CKD by stage in the Medicare population. This study includes point prevalent Medicare patients on January 1, 2008, aged > 65 yrs old. CKD stages and comorbidities were identified from 2007 claims. Patients were excluded if they were enrolled an HMO, Medicare as secondary payor, or diagnosed with ESRD in 2007. Patients were followed from January 1 to December 31, 2008 censored at ESRD date or the end of Medicare entitlement. Adjusted mortality rates by CKD stages were based on Cox regression and adjusted for age, gender, and comorbidities with the all CKD cohort as reference. The relative risks (RRs) were adjusted for age, gender, comorbidities, and CKD stages. These comorbidities included anemia, cancer, GI bleeding, liver disease, hypertension, and all cardiovascular diseases.

There were total 37,320 patients with specific CKD stages codes and identified as Whites or AAs included in this study. Overall, AAs had higher risk of death than Whites (RR=1.10; p=0.0486). More results by CKD stages are reported in the following table.

| CKD stages | Race | N | Percent of death | Adjusted mortality (per 1000 pat-yrs) | Adjusted RR |
|---|---|---|---|---|---|
| 1-2 | Whites | 5694 | 10.5 | 124 | 1.00 |
| | AAs | 68 | 7.9 | 115 | 0.95 (0.74-1.23) |
| 3-5 | Whites | 27683 | 13.5 | 151 | 1.00 |
| | AAs | 3875 | 12.2 | 172 | 1.12 (1.02-1.23) |

In Medicare 5% random sample, we have found that AAs with CKD had significantly higher risk of death, especially with stages 3-5. We conclude that the improved AA survival on dialysis may reflect a survival bias compared to Whites.

Disclosure of Financial Relationships: Research Funding: Amgen.

**TH-PO084**

**Predicting Loss of eGFR in Patients with Diabetes Mellitus – What Is the Impact of Ethnicity?** Gavin Dreyer,[1] Rohini Mathur,[2] Sally Hull,[2] Alistair Chesser,[1] Magdi Yaqoob.[1] *[1]Renal Unit, Royal London Hospital, United Kingdom; [2]Centre for Health Sciences, Queen Mary University, United Kingdom.*

Introduction

In patients with diabetes mellitus (DM), South Asian ethnicity is associated with a greater prevalence of severe CKD (stages 4-5) compared to Afro-Caribbean or Caucasian ethnicity. The effect of ethnicity on the progression of CKD in patients with DM has not been studied. We conducted a prospective study of 2,305 patients to examine the impact of ethnicity on the yearly change in eGFR of patients with DM.

Methods

Computer databases in 138 primary care facilities in London were searched for patients with a diagnostic code for DM and concomitant CKD (eGFR <60ml/min/m$^2$, 4 variable MDRD equation adjusted for ethnicity). Patients aged 30-75 years with a recording of ethnicity at study entry in April 2006 were included. The mean follow up was 4.4 years. Progression of CKD was measured as change in eGFR per year.

Results

At study entry, 1,970 patients had CKD stage 3 compared to stage 4 (n=110) and stage 5 (n=45). In a logistic regression analysis for patients with CKD stage 3 (adjusted for age, gender, clustering, mean arterial pressure, cholesterol, HbA1c, use of ACE/ARB), South Asian and Afro-Caribbean patients had a faster rate of decline in eGFR compared to the Caucasian population. In stage 4 CKD, only South Asian patients had a significant reduction in eGFR.

Mean change in eGFR by ethnic group per year

| | | Change in eGFR (ml/min/year) | 95% confidence interval |
|---|---|---|---|
| | White | -0.55* | -0.89, -0.21 |
| Stage 3 | South Asian | -0.91* | -1.29, -0.13 |
| | Afro-Caribbean | -0.91* | -1.43, -0.39 |
| | | | |
| | White | 0.38 | -0.84, 1.62 |
| Stage 4 | South Asian | -1.51* | -2.61, -0.57 |
| | Afro-Caribbean | -1.01 | -2.40, 0.38 |

*=p<0.001

Conclusions

Ethnicity significantly affects the change in eGFR in patients with DM. South Asian and Afro-Caribbean patients progress faster than Caucasian patients which may in part explain higher numbers of non-Caucasian patients receiving dialysis or transplants in the UK. Further studies are required to determine the optimal treatment strategy for each ethnicity to minimise loss of kidney function and reduce health disparities between ethnic groups.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO085

**Socioeconomic Factors and Low Albumin Levels in Rural CKD Patients** Rebecca J. Schmidt, Bethany S. Pellegrino. *Nephrology, West Virginia University, Morgantown, WV.*

The prognostic value of serum albumin for dialysis patients is well known and low levels are due to both the 'chronic inflammatory state' and nutritional factors. Albumin levels of patients starting dialysis in the US have changed little since 1996 (3.1-3.2 g/dl), suggesting the problem prior to the start of dialysis. Nutrition may be equally as important as the 'chronic inflammatory state,' particularly in patients whose poor nutritional habits are longstanding.

Laboratory values from 4623 patients with Stages 3-5 chronic kidney disease (CKD ) seen between January 1, 2001 and May 31, 2010 were analyzed by county of residence. Values at first presentation to the CKD clinic were utilized. Census statistics for the percent of residents living below the federal poverty level (%<FLP) ($10,827 and $18,346 for an individual and family of 3, respectively), were ranked for each county.

The majority (4299 or 93%) of patients represented 18 of West Virginia's 55 counties. Mean albumin levels were similar for all CKD patients of both genders (mean±SE: 3.58±0.023 g/dl for females vs. 3.54±0.025 g/dl for males) and did not correlate with age or year of visit. Almost half (45%) of all CKD patients presented with albumin levels ≤3.5 g/dl and for Stages 3-5, 34%, 42% and 70% of patients presented with albumins ≤ 3.5 g/dl. Albumin levels (mean±SD) ranged from 3.66±1.00 for Stage 3 to 3.14±0.79 for Stage 5.

Albumin levels (mean±SE) ranged from 3.38±0.08 to 4.23±0.13 with 17 of 18 counties having a mean albumin level below 3.92±0.09 and a single county having a mean albumin level exceeding 4 g/dl (p<0.0001). Mean albumin in this county (4.23±0.13) was higher than the mean albumin for the 17 other counties, though statistically different from only 11 (p<0.0001). The %<FLP ranged from 15.5 to 25.2% in these 17 counties, the county with the highest albumin having the lowest %<FLP (12.9%).

Among our rural CKD patients, mean albumin levels were highest in the county with the lowest %<FLP. Additional study of the impact of socioeconomic status on nutritional forces throughout the continuum of CKD may shed light on the stagnant and low albumin levels seen in patients starting dialysis.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO086

**Racial and Ethnic Differences in the Onset of CKD as Defined by Proteinuria** Stephen F. Derose,[1] Jean Q. Wang,[1] Peter W. Crooks,[2] Jiaxiao Shi,[1] Mark P. Rutkowski.[3] *[1]Research & Evaluation, Kaiser Permanente Southern California, Pasadena, CA; [2]Renal Program, Kaiser Permanente Southern California, Pasadena, CA; [3]Baldwin Park Medical Center, Kaiser Permanente Southern California, Baldwin Park, CA.*

Racial disparities in the onset of CKD as defined by proteinuria are not well characterized. We conducted a retrospective cohort study to determine the risk of developing proteinuria among a diverse population with an initially normal urine protein test and eGFR >60 mL/min/1.73m$^2$.

Subjects were members of Kaiser Permanente Southern California from 1st Jan 1998 to 31st Dec 2006 and had equivalent health insurance coverage. Proportional hazards regression was used to estimate risk associated with race after adjustment for age and sex. Study entry was defined by a negative urine test. The outpatient serum creatinine test closest in time and prior to the entry urine test was used to estimate eGFR based on the CKD-EPI equation. The primary endpoint was proteinuria as defined by two quantitative urine tests >6 days apart without concurrent evidence of UTI by ICD-9 codes or positive leucocyte esterase or nitrites. Censoring events were death, disenrollment, or the end of the study period. Members with unknown race or multiple race were excluded (26% of those who were otherwise eligible).

Subjects were 42.5% male, median age 49.2 years, with 42.4% White, 34.7% Hispanic, 13.1% Black, 8.1% Asian and Pacific Islander, 0.12% Native American, and 1.62% for all other race and ethnicity. Compared to Whites, the hazard ratios and 95% CI's for the 769,915 subjects were: Black 1.32 (1.28-1.36); Hispanic 1.57 (1.53-1.61); Asian and Pacific Islander 1.59 (1.54-1.65); and Native American 1.77 (1.32-2.37).

Compared to Whites, the other major racial and ethnic groups were at higher risk of developing proteinuria after a normal test and when eGFR was in the normal to mildly decreased range. Blacks, who are often observed to be at higher risk for ESRD, were at slightly lower risk than other non-White groups.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO087

**Deprivation but Not Rurality Is Associated with Increased Incidence of End-Stage Renal Disease: A Spatial Analysis** Cécile Couchoud,[1] Chantal Guihenneuc-Jouyaux,[2] Florian Bayer,[1] Benedicte Stengel.[3] *[1]REIN Registry, Biomedicine Agency, Saint Denis La Plaine, France; [2]MAP5 CNRS UMR 8145, Paris 5 University, Inserm U780-IFR69, Villejuif, France; [3]Paris-Sud University, Inserm U780-IFR69, Villejuif, France.*

Objective. To quantify the extent to which deprivation and rurality may explain the spatial pattern of ESRD incidence, after adjustment for several confounders and application of geographically appropriate methods.

Methods. The association between ESRD incidence in France in 2006-2007 and 6 district-level indicators of socioeconomic environment (percentage of people aged 20-59 years receiving minimum guaranteed income allowances, percentage unemployed, percentage receiving free health care, percentage receiving minimum guaranteed income allowances for the elderly, gross domestic product (GPD) per capita, and educational level) and 2 indicators of rurality was explored at the district level. Confounders included diabetes prevalence, cardiovascular mortality (competitive risk), and indicators of health-care supply and clinical practices.

Results. Crude ESRD incidence by district ranged from 80.4 to 238.6 per million inhabitants. Each 1.3% increase in those receiving minimum guaranteed income allowances was associated with a 14% increase in ESRD incidence before and an 11% increase after adjustment for 9 potential confounders. Similar increase was found with % unemployed and % receiving free health care, but not with other socioeconomic or rurality indicators. The association between rurality and ESRD incidence seemed to be explained largely by the deprived environment, and much less by other confounders.

Conclusion. Living in deprived areas may explain some between-area variation in ESRD incidence rates. Better understanding of the local geography of these rates might help public officials to adjust supply and demand at the district level. It may also prove useful for devising and monitoring strategies for prevention, especially among subgroups with low socioeconomic status.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO088

**Racial and Ethnic Differences in Mortality among Individuals with Chronic Kidney Disease (CKD): Results from the Kidney Early Evaluation Program (KEEP)** Stacey Jolly,[1] Nilka Rios Burrows,[2] Shu-Cheng Chen,[3] Suying Li,[3] Claudine T. Jurkovitz,[4] Keith C. Norris,[5] Michael Shlipak.[6] *[1]Cleveland Clinic, Cleveland, OH; [2]Centers for Disease Control, Atlanta, GA; [3]Chronic Disease Research Group, Minneapolis, MN; [4]Christiana Care Health System, Newark, DE; [5]Charles R. Drew University, Los Angeles, CA; [6]University of California San Francisco, San Francisco, CA.*

*Objective:* To estimate associations of race/ethnicity with all-cause mortality among those with early and late stage CKD.

*Methods:* Cross-sectional analysis; KEEP participants from 2000-2008 with CKD, not on dialysis, nor with prior kidney transplant who self-reported race/ethnicity. Early stage CKD defined as urinary albumin to creatinine ratio ≥ 30 mg/g among those with eGFR ≥ 60 mL/min/1.73 m$^2$. Late stage CKD defined as eGFR < 60. Covariates: age, sex, obesity, DM, HTN, smoking, family history, education, health insurance, geographic region, year screened.

*Results:* 19,205 participants with prevalent CKD, 55% ($n$ = 10,560) were white, 27% ($n$ = 5237) African American, 9% ($n$ = 1638) Hispanic, 5% ($n$ = 951) Asian, and 4% ($n$ = 813) American Indian/Alaska Native (AIAN). There were 1043 deaths (5%) among those with CKD; 214 (3.5%) among those with early stage CKD; 829 (6%) among those with late stage CKD.

Adjusted Hazards Ratios of Risk of Death for KEEP Participants with CKD by Race/Ethnicity, 2000-2009

| | CKD | Albuminuria (eGFR >=60) | eGFR <60 |
|---|---|---|---|
| | N= 19,205 | N = 6068 | N = 13,137 |
| | HR (95% CI) | HR (95% CI) | HR (95% CI) |
| White | 1.00 | 1.00 | 1.00 |
| African American | 1.01 (0.87 – 1.18) | 1.03 (0.75-1.42) | 0.99 (0.83-1.17) |
| Hispanic | 0.65 (0.48 – 0.88)* | 0.62 (0.33-1.14) | 0.64 (0.45-0.91)* |
| Asian | 0.72 (0.50 – 1.04) | 0.44 (0.20-0.95)* | 0.84 (0.56-1.27) |
| AIAN | 1.41 (1.10 – 1.18)* | 1.32 (0.80-2.18) | 1.39 (1.04-1.84)* |

*p-value <0.05

*Conclusions:* Hispanics and Asians had better survival prognosis compared with Whites; AIAN had the worst prognosis. Further research is needed to understand these disparities and create novel interventions to address them among those with CKD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO089**

**Health Related Quality of Life (HRQoL) Predicts Mortality and ESRD in Chronic Kidney Disease (CKD): The Study of Treatment for Renal Insufficiency: Data and Evaluation (STRIDE)** Anca Tilea,[1] Brenda W. Gillespie,[1] Fredric O. Finkelstein,[2] Margaret A. Kiser,[3] George Eisele,[4] Peter Kotanko,[5] Rajiv Saran.[1] *[1]U. of Mich., Ann Arbor, MI; [2]Hosp. of St. Raphael Yale U., New Haven, CT; [3]U. of N. Carolina, Chapel Hill, NC; [4]Med. College of Albany, Albany, NY; [5]RRI, New York, NY.*

Poor patient outcomes are a major concern in CKD. However, previous studies have mainly investigated these outcomes in relation to HRQoL in the End Stage Renal Disease (ESRD) population only. We examined the association between HRQoL and patient outcomes (death, dialysis and transplantation) in a multi-center CKD cohort.

The STRIDE is a prospective cohort study of 2,162 CKD patients (median follow-up 3-years) at 79 US renal clinics. The Kidney Disease and Quality of Life Short Form (KDQoL SF-36) was administered at enrollment and follow-up visits. The Kidney Disease Component Summary (KDCS), Mental Component Summary (MCS), and Physical Component Summary (PCS) scores were calculated. Cox regression was employed to assess the associations between mortality and ESRD with each of the baseline HRQoL domain score, adjusted for age, gender, race, diabetes, hypertension, hemoglobin, serum albumin and eGFR.

The average age was 63±15 years. The average eGFR (MDRD) was 24±10ml/min/1.73m$^2$. Fifty-four percent were males, 23% were black, 39% with diabetes and 53% with hypertension.There were 85 deaths and 278 ESRD events. In general, higher HRQoL scores were significantly associated with lower risk of mortality,and ESRD. In particular, the PCS score and KDCS score showed the strongest association. Our results show significant associations between low HRQoL and poor patient outcomes. Systematic monitoring of HRQoL could become a useful tool in risk stratifying CKD patients and thus might improve their management.

| | Death | | ESRD | |
|---|---|---|---|---|
| HRQoL-SF36 Domains** | HR | P | HR | P |
| KDCS | 0.83 | 0.01* | 0.99 | 0.78 |
| Symptom Problems | 0.89 | 0.02* | 0.94 | 0.01* |
| Effects of KD | 0.95 | 0.28 | 0.97 | 0.16 |
| Burden of KD | 0.94 | 0.04* | 0.98 | 0.12 |
| Cognitive Function | 0.96 | 0.08 | 1.01 | 0.21 |
| Quality Social Interaction | 1.04 | 0.42 | 1.05 | 0.01* |
| PCS | 0.80 | <0.0001* | 0.94 | 0.07 |
| Physical Functioning | 0.94 | 0.01* | 0.98 | 0.09 |
| Role Physical | 0.92 | 0.02* | 0.98 | 0.23 |
| Pain | 0.96 | 0.11 | 0.98 | 0.22 |
| General Health | 0.88 | 0.02* | 0.92 | <0.0001* |
| MCS | 0.93 | 0.37 | 0.94 | 0.12 |
| Emotional Well-Being | 0.92 | 0.09 | 0.96 | 0.07 |
| Role Emotional | 0.99 | 0.73 | 0.99 | 0.40 |
| Social Function | 0.96 | 0.11 | 0.98 | 0.16 |
| Energy/Fatigue | 0.91 | 0.01* | 0.97 | 0.04* |

* significant at p<0.05

**HRQoL domains are on a 5-unit change. HRQoL domains were scaled from 0 to 100, where higher scores correspond to better QoL. Adjusted for age, sex, race, diabetes, hypertension, baseline hemoglobin, serum albumin and eGFR.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO090**

**Depressed Mood, Usual Activity Level, and Continued Employment on Dialysis** Nancy G. Kutner,[1] Rebecca H. Zhang,[1] Yijian Huang,[1] Kirsten L. Johansen.[1,2] *[1]USRDS Rehabilitation/QoL Special Studies Ctr, Emory University, Atlanta, GA; [2]San Francisco VA Medical Ctr, UCSF, San Francisco, CA.*

**Background**. Employment is valued in American society, and individuals' ability to engage in productive activity provides a marker of the effectiveness of care. Many chronic kidney disease (CKD) patients leave the labor market when they start dialysis, however. **Methods**. We investigated the association of depressed mood and usual activity level with patients' continued employment after starting dialysis, independent of disability income and patient characteristics. 1643 incident dialysis patients aged ≥18 from 296 randomly selected clinics were surveyed in the USRDS Comprehensive Dialysis Study (CDS). Participants provided information about current and prior employment, disability income receipt, education, depressive symptoms (Patient Health Questionnaire-2, i.e. PHQ-2), and usual activity level/energy expenditure (Human Activity Profile, i.e. HAP). Age, gender, race, medical insurance, diabetes, inability to ambulate or transfer, COPD, cardiovascular comorbidity, and hemoglobin and serum albumin values at treatment start were obtained from USRDS files. Dialysis modality was defined at time of interview, i.e. approximately 4 months post treatment start. **Results**. 32.6% of patients who worked in the year before dialysis (191/585) continued working. Patients who stopped working were almost three times more likely to report probable or possible depression (PHQ-2 ≥3; P < 0.0001). Mean (sd) HAP scores were 46.1 (18.6) among patients who stopped working vs. 60.2 (14.8) among those who continued working; P < 0.0001. Lower PHQ-2 scores (P = 0.06) and higher HAP scores (P < 0.0001) remained associated with increased likelihood of continued employment in logistic regression analyses adjusted for disability income, socioeconomic and clinical characteristics, and patient clustering in facilities. **Conclusion**. Screening and management of depressive symptoms and encouragement of increased activity in CKD patients are mutually reinforcing interventions that could facilitate individuals' continued employment in addition to generally improving overall quality of life on dialysis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO091**

**Genetic Ancestry, Poverty and Albuminuria among African Americans: The Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) Study** Deidra C. Crews,[1] Edgar R. Miller,[1] Neil R. Powe,[2] Mike Nalls,[3] Andrew Singleton,[3] Alan B. Zonderman,[3] Michele Kim Evans,[3] Wen Hong Linda Kao,[1] Carmen A. Peralta.[2] *[1]Johns Hopkins University; [2]University of California, San Francisco; [3]National Institute on Aging, National Institutes of Health.*

A susceptibility gene for non-diabetic proteinuric ESRD was recently identified and found to be more common among African Americans. However, whether genetic ancestry, environmental factors (i.e. poverty), or their interaction account for most of the risk for albuminuria at early stages of kidney disease is unknown. We examined the relation of genetic African ancestry and poverty with albuminuria among African Americans in a community-based study of adults aged 30-64 years living in Baltimore, MD, and selected to have socioeconomic diversity. Percent African ancestry was estimated with STRUCTURE using 2000 ancestry informative markers. Poverty (annual household income <125% of 2004 Poverty guideline) and 3 income categories were examined. Urinary albumin/creatinine ratio (ACR) was log transformed, then back transformed to a relative difference (RD) in multivariable linear regression models. Among 474 participants, 53% were women, 31% lived in poverty. African ancestry ranged from 46% to 99%. ACR ranged from 0.10 to 279 mg/g. Overall, the correlation between ACR and percent African ancestry was -0.07 (P= 0.15), and each 10% increase in African ancestry was not associated with higher ACR, RD -2.6 % (95% CI, -8.7% to 4.0%). In contrast, poverty (compared to non-poverty) was independently associated with higher ACR [RD 16.6% (95% CI, 2.8% to 32%)] after adjustment for African ancestry, age, sex, diabetes, hypertension, obesity, total cholesterol, and estimated GFR; and there was no significant interaction between poverty and African ancestry. Additionally, with adjustment, there was a graded, independent association between greater income and lower ACR [RD -20.4% (95% CI, -33.3% to -4.9%), when the highest annual income (>$40,000) was compared to the lowest income (<$17,500) category]. Poverty, an environmental factor, is associated more strongly than genetic ancestry with early kidney damage among African Americans.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO092**

**Racial and Ethnic Differences in the Onset of CKD as Defined by eGFR Loss** Stephen F. Derose,[1] Mark P. Rutkowski,[3] Jean Q. Wang,[1] Jiaxiao Shi,[1] Peter W. Crooks.[2] *[1]Research & Evaluation, Kaiser Permanente Southern California, Pasadena, CA; [2]Renal Program, Kaiser Permanente Southern California, Pasadena, CA; [3]Baldwin Park Medical Center, Kaiser Permanente Southern California, Baldwin Park, CA.*

Racial disparities in the onset of kidney disease as defined by GFR loss are not well characterized. We conducted a retrospective cohort study to determine the risk of losing half of initial kidney function among a diverse population with eGFR >90 or >60 mL/min/1.73m$^2$.

All subjects were members of Kaiser Permanente Southern California and had equivalent health insurance. Two or more outpatient serum creatinine tests >90 days apart from 1st Jan 1998 to 31st Dec 2006 were used to estimate eGFR based on the CKD-EPI equation. Proportional hazards, including competing risks, regressions were used to determine risk asociated with race after adjustment for age and sex. Study entry was defined by the first available eGFR. The primary endpoint was the eGFR after which all subsequent eGFR's were less than half the entry value. Censoring occurred for death (the competing event), disenrollment, or the end of the study period. Members with unknown race or multiple race were excluded (30% of those who were otherwise eligible).

Subjects were 41% male, median age 51 years, with 47.9% White, 31.3% Hispanic, 12.6% Black, 7.8% Asian and Pacific Islander, 0.1% Native American, and 0.4% for all other race and ethnicity. Compared to Whites, the hazard ratios (HR) and 95% CI's for 1,054,696 subjects with entry GFR >60 were: Black 3.4 (3.1-3.8); Hispanic 2.9 (2.6-3.2); Asian and Pacific Islander 1.9 (1.6-2.2); and Native American 4.4 (1.8-10.6). For the 742,260 subjects with entry eGFR >90, the HRs were: Black 2.5 (2.1-3.0); Hispanic 2.9 (2.6-3.2); Asian and Pacific Islander 1.4 (1.1-1.8); and Native American 4.4 (1.8-10.6).

Compared to whites, the other major racial and ethnic groups had a greater risk of losing half their entry eGFR from a starting point of normal (>90), or normal to mildly decreased (>60), kidney function. Blacks, Hispanics and Native Americans were at highest risk.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO093**

**Is Awareness of Chronic Kidney Disease Associated with Greater Receipt of Evidence-Based Medical Therapies?** Delphine S. Tuot, Laura C. Plantinga, Chi-Yuan Hsu, Neil R. Powe. *University of California, San Francisco, San Francisco, CA.*

Background: Awareness of chronic kidney disease (CKD) is low. Efforts are underway to increase recognition of CKD among providers and patients, assuming that they will lead to better outcomes in CKD patients through wider application of, and adherence to, proven therapies. However, few studies have tested this assumption.

Methods: Blood pressure (BP) control, use of Angiotensin Converting Enzyme Inhibitors (ACEI) or Angiotensin Receptor Blockers (ARB) and glycemic control were assessed in 3200 adults with CKD stages 1-4 using 1999-2006 National Health and Nutrition Examination Survey data. BP control was defined as an average BP <140/90 in subjects without proteinuria and <130/80 in the presence of proteinuria; ACEI/ARB use was self-reported; glycemic control was a hemoglobin A1C <7mg/dl. CKD awareness was a "yes" answer to "Have you ever been told by a healthcare provider you have weak or failing kidneys?". All estimates were weighted to the US population.

Results: Fewer than 50% of individuals with kidney dysfunction attained BP control and only 18% were treated with an ACEI/ARB, regardless of CKD awareness. Among diabetics with CKD, glycemic control was achieved in 30% of those aware of their CKD and 50% of those unaware. Odds of BP control and ACE/ARB use according to evidence-based guidelines were not different among subjects aware and unaware of their CKD (p=.08 and p=.77), regardless of proteinuria (p=.54 and p=.3), CKD stage (p=.58 and p=.51) or diabetes (p=.38 and p=.73). Those aware of their CKD status had lower odds of glycemic control compared to those who were unaware, independent of eGFR and severity of diabetes (adjusted odds ratio =.23; p=.01).

Conclusions: Contrary to expectation, awareness of CKD was not associated with greater odds of BP control or ACEI/ARB use and was actually associated with decreased odds of glycemic control. Inference regarding causality is limited by the cross-sectional nature of the analysis but our results suggest that future policy in this area should be guided by a more sophisticated understanding of physician and patient behavior.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO094

**Sources of Drug Coverage among Medicare Beneficiaries with End-Stage Renal Disease** Wendy L. St. Peter,[1,2] Diane L. Frankenfield,[3] Christopher Powers,[3] Eric D. Weinhandl,[1] James P. Ebben,[1] Benjamin L. Howell.[3] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Pharmacy, U of MN; [3]ORDI, CMS, Baltimore, MD.*

**Background:** Drug coverage sources in end-stage renal disease (ESRD) patients since Medicare Part D inception are unknown. Objectives were to describe 1) distribution of drug coverage in the ESRD patient population, and 2) which types of stand-alone Part D plans ESRD patients voluntarily enrolled in during 2007. **Methods:** Data from the Chronic Condition Warehouse linked with administrative data from the United States Renal Data System were used. A 20% random sample of Medicare beneficiaries who were alive and had ESRD in December 2007 were identified. Patients were classified as having no known coverage, Part D coverage (with or without the low-income subsidy [LIS]), coverage from a former employer, or coverage from other creditable sources. To describe the stand-alone Part D plans in which ESRD patients voluntarily enrolled, we considered only patients who self-enrolled into plans and excluded patients eligible for auto-enrollment via LIS. Plan characteristics considered included deductibles (Y/N), gap coverage (Y/N), and premiums (by quartile). **Results:** Among ESRD patients, 16.9% had no known source of drug coverage, 17.9% had Part D without LIS, 45.7% had Part D with LIS, 8.8% had coverage from former employer, and 9.5% had other creditable coverage (compared to 15.4%, 33.9%, 21.9%, 15.8%, and 12.9% in general Medicare population, respectively). ESRD patients who self-enrolled in Part D were more likely to choose more comprehensive plans, with 70.8%, 38% and 22.6% choosing plans with no deductible, with gap coverage, and with higher premium and superior benefits, respectively (compared to 68.9%, 33.7%, and 12.0% among all Part D program self-enrollees, respectively). **Conclusions:** In 2007, a large number of ESRD beneficiaries still lacked a known source of creditable drug coverage; reasons for this are unclear. Auto-enrollment into the Part D benefit via LIS appeared to be an important mechanism for many ESRD patients to obtain prescription drug coverage. ESRD beneficiaries who self-enrolled into Part D plans tended to prefer plans with more comprehensive benefits.

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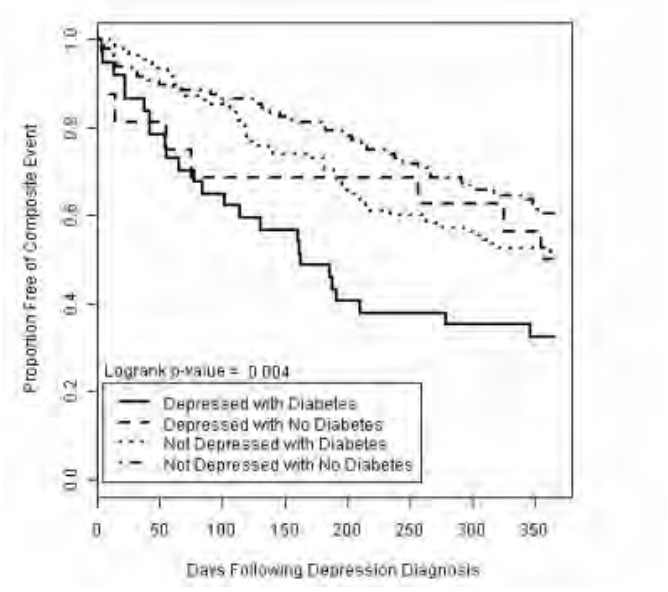
## TH-PO095

**Major Depressive Episode Increases Risk for Kidney Disease Progression, Hospitalization and Death in Type 2 Diabetics** Susan Hedayati,[1,2] Masoud Afshar,[2] Abu T. M. Minhajuddin,[2] Robert D. Toto,[2] Madhukar Trivedi,[2] Augustus John Rush.[3] *[1]Dallas VA Medical Center; [2]UT Southwestern Medical Center at Dallas; [3]Duke-NUS, Singapore.*

Depression is associated with treatment nonadherence and poor glycemic control in diabetics. To determine if patients with type 2 diabetes, CKD and a Major Depressive Episode (MDE) are at a higher risk for progression to ESRD, death or hospitalization, we prospectively studied 267 outpatients with stages 2-5 predialysis CKD recruited from a single VA Medical Center and followed for a year. The primary outcome was the composite of ESRD, death or hospitalization. Kaplan Meier survival curves were used to compare event-free survival times, and Cox proportional hazards to assess adjusted hazard ratios. Subjects were grouped by diabetes and MDE status: **Group A**: 39 had diabetes and MDE; **Group B**: 17 had MDE but no diabetes; **Group C**: 109 had diabetes but no MDE; **Group D** (referent group): 102 had neither MDE nor diabetes. Event-free survival based on the composite outcome was shortest in **Group A** (187 ±22 days), vs. 245 ±40 days in **Group B**; 261 ±12 days in **Group C**; and 280 ±12 days in **Group D**, Log-rank p =.004.




After adjusting for age, race, eGFR, serum albumin, hemoglobin, calcium-phosphorus product, cardiovascular disease, other comorbidities and drug and alcohol abuse, those with diabetes and depression (**Group A**) were twice as likely to progress to ESRD, die or become hospitalized than those without diabetes or depression (**Group D**), HR 2.1 (95% CI 1.2-3.6). Adjusted hazard ratios were statistically non-significant for **Group B** (1.9, 95% CI 0.9-4.3) and **Group C** (1.2, 95% CI 0.8-1.9).

Depression in patients with type 2 diabetes and CKD increases risk for kidney disease progression, hospitalization and death due to diabetic complications. Identification and treatment of depression in these patients may improve outcomes.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO096

**Racial Disparity in CKD Progression and Risk Factor Control: A Longitudinal Analysis of the Kidney Early Evaluation Program** Suying Li,[1] Shu-Cheng Chen,[1] Allan J. Collins,[1,2] Peter A. McCullough,[3] George L. Bakris,[4] Joseph A. Vassalotti.[5,6] *[1]Chronic Disease Research Group, MMRF, Minneapolis, MN; [2]Medicine, Univ. of Minnesota; [3]William Beaumont Hospital, Northville, MI; [4]Univ. of Chicago, Pritzker School of Medicine, Chicago, IL; [5]National Kidney Foundation, New York, NY; [6]Mt. Sinai School of Medicine, New York, NY.*

Starting in June, 2008, the Kidney Early Evaluation Program participants (KEEP) were asked to return annually. To examine differences in CKD progression and risk factor control between Whites (Ws) and African Americans (AAs), we studied KEEP participants who had a 2nd screening between 6/15/08 and 12/31/09. The overall returning rate was 9%.

This study included 2451 Ws with mean age of 63.8 and 1286 AAs with mean age of 58.3 years old. The mean and median screening interval in days between assessments was 642 and 408. The CKD prevalence at the 1st and 2nd screening was 30.0% and 35.6% for Ws and 17.7% and 21.5% for AAs. The MDRD-eGFR declined 4.8 mL/min/1.73 $m^2$ in Ws and 6.6 mL/min/1.73 $m^2$ in AAs between the two screenings. The blood pressure (BP) control (systolic BP<140 mm Hg and diastolic BP<90 mm Hg) at the 1st and 2nd screening was 42.4% and 43.6% for Ws and 37.9% and 42.6% for AAs. Among hypertensive participants who had not achieved BP control at the 2nd screening, AAs declined faster in eGFR than Whites.

As product of the KEEP program, BP control was improved on the 2nd screening. Despite this improvement, the rate of decline of eGFR was greater for AAs than Ws. These data suggest that more intensive intervention and care is needed with additional education on risk factor awareness and control.

| | Whites | AAs | p-value |
|---|---|---|---|
| **N** | 2451 | 1286 | |
| **Mean difference in eGFR between 2 screenings** | -4.8 | -6.6 | <0.0001 |
| **CKD prevalence at 1st screening, %** | 30.0 | 17.7 | <0.0001 |
| **CKD prevalence at 2nd screening, %** | 35.6 | 21.5 | <0.0001 |
| **BP control at 1st screening, %** | 42.4 | 37.9 | 0.0211 |
| **BP control at 2nd screening, %** | 43.6 | 42.6 | 0.6127 |
| **eGFR decline for those BP controlled at 2nd screening** | -5.8 | -6.5 | 0.4105 |
| **eGFR decline for those BP not controlled at 2nd screening** | -4.5 | -6.6 | 0.0053 |

Disclosure of Financial Relationships: Research Funding: Amgen.

## TH-PO097

**Ethnic- and Gender-Specific Cut-Offs for Urinary Albumin-Creatinine Ratio When Detecting Microalbuminuria in Han Chinese** Xiaohong Fan, Jianfang Cai, Lijun Mou, Bixia Gao, Xuejiao Liu, Xuemei Li, Xue-Wang Li. *Nephrology, Peking Union Medical College Hospital, Beijing, China.*

**Background** As Asian people tend to be smaller than Caucasians, establishing accurate cut-off values for simple screening tests of kidney injury, such as urinary albumin-creatinine ratio(ACR), is important.

**Methods** We analyzed a population based sample of 1056 people(50.2±12.1years, no known renal disease) from the epidemiological investigation of metabolic syndrome and chronic kidney disease in Pinggu district, Beijing. 8-hour overnight urinary albumin excretion(UAE) was regarded as the standard for defining the albuminuria. The ROC curve analysis was used to determine the ACR cut-off value for microalbuminuria, the 95$^{th}$ percentile method was then used to determine an ACR reference value in the healthy Han Chinese, both of which were compared to previously published data.

**Results** The discriminator value of ACR for microalbuminuria by ROC curve analysis was 1.95mg/mmol(sensitivity 97.6% and specitivity 88.6%)for men, 3.62mg/mmol(sensitivity 83.8% and specitivity 89.1%)for women and 2.78mg/mmol(sensitivity 88.7% and specitivity 85.9%)for overall. The upper boundary of ACR (the 95$^{th}$ percentile ) in healthy was 2.11mg/mmol for men,2.52 mg/mmol for women, 2.23 mg/mmol for overall. Compared with the ACR cut-off by KDOQI, the result of ROC curve analysis in this study had better agreement with the standard criteria(K value is 0.637 vs 0.568 of KDOQI), .

The kappa statistics and diagnostic test of calculated in this study and previously published ACR cut-off values for MA

| ACR cut-offs values for MA | Male | Female | K- value | Sensitivity (%) | Specitivity (%) | Positive likelihood ratio | Positive predictive value (%) |
|---|---|---|---|---|---|---|---|
| ROC curve analysis | 1.95 | 3.62 | 0.637 | 91.3 | 88.2 | 7.96 | 56.9 |
| 95th percentile | 2.11 | 2.52 | 0.536 | 90.7 | 83.3 | 5.44 | 47.4 |
| Beijing CKD STUDY | 1.58 | 2.26 | 0.479 | 94 | 78.6 | 4.39 | 42.1 |
| INTERMAP STUDY | 2.79 | 3.9 | 0.649 | 83.3 | 91.3 | 9.56 | 61.3 |
| KDOQI | 1.92 | 2.83 | 0.568 | 92.7 | 84.6 | 6 | 49.8 |

**Conclusions** Using a population sample, we report ethnic differences in cut-off values for detecting microalbuminuria using ACR. Ethnic difference must be considered when evaluating results using these methods.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO098

**Screening for CKD in Homeless Individuals in Mexico** Guillermo G. Garcia,[1] Jaime A. Gonzalez,[1] Alfonso Gutierrez,[1] Ma. Concepcion Ocegueda,[1] Salvador Plascencia,[1] Mario Marquez,[2] Marcello Tonelli.[3] *[1]Division of Nephrology, Hospital Civil de Guadalajara, Guadalajara, Jal, Mexico; [2]Secretaria de Salud, Jalisco, Mexico; [3]Nephrology Division, University of Alberta, Canada.*

CKD is a major cause of morbidity and mortality in Mexico. Data from other nations suggests that risk factors for CKD are disproportionally higher among the poor.

Since 1998, the Fundacion Hospitales Civiles de Guadalajara has used mobile units to provide health care for the poor in Jalisco, with emphasis on disease prevention and early detection. In partnership with IJAS, a state welfare agency, we screened homeless individuals in Guadalajara. We excluded individuals with known CKD and < 18 years of age. Trained personnel collected demographic and clinical data and collected blood samples for selected tests. In 02/2006, 260 individuals were screened. Findings were compared with the National Health Survey 2006 (ENSANUT), and with our own reported data on CKD in poor communities in Jalisco.

Results

| | Homeless n = 260 | ENSANUT n=33,624 | Homeless vs ENSANUT p | Jalisco n = 3742 | Homeless vs Jalisco p |
|---|---|---|---|---|---|
| Age (y) | 50.75 ±17.93 | NA | | 57.3±13.2 | 0.0001 |
| Male (%) | 193 (74.2) | 16,139 (49.7) | 0.0001 | 1097 (29.3) | 0.0001 |
| Known DM (%) | 15 (5.8) | 2,554(7.0) | 0.29 | 1625 (43.4) | 0.0001 |
| Blood Glucose>126 mg/dL (%) | 48 (18.9) | NA | | 1528 (41.6) | 0.0001 |
| Known HTN (%) | 9 (3.5) | 5,044 (15.0) | 0.0001 | 2217 (59.2) | 0.0001 |
| SBP≥140 or DBP≥90 (%) | 76 (31) | 10,423 (30.8) | 0.58 | 2128 (57.1) | 0.0001 |
| eGFR < 60 ml/min/ 1.73 m² (%) | 57 (22.4) | NA | | 593 (17.4) | 0.05 |
| BMI ≥ 30 (%) | 39 (17.6) | 10,444(31.1) | 0.0001 | 1580 (43.1) | 0.0001 |
| Current smoker (%) | 90 (34.6) | 2419 (21.5) | 0.0001 | 569 (15.2) | 0.0001 |

We conclude that 1) the prevalence of CKD and its risk factors are high among homeless individuals 2) Consideration should be given to target this high risk population in programs aimed to prevent CKD and its risk factors in Mexico.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO099

**Impact of Socioeconomic Factors to the Incidence of ESRD in DM Patients** Yafei Yang,[1] Fung-Chang Sung,[2] Chiu-Ching Huang.[1] *[1]Nephrology, China Medical University Hospital, Taichung, Taiwan; [2]School of Public Health, China Medical University, Taichung, Taiwan.*

**Objective** Diabetes mellitus (DM) is the major risk leading to the end stage renal disease (ESRD) in Taiwan. Studies investigating the relationship between socioeconomic status and incidence of ESRD in DM are limited. We investigated whether socio-demographic factors and comorbidity interact with DM in developing ESRD.

**Design and setting** Cohort study assembled from the National Health Insurance claims data of Taiwan and followed up to 2008.

**Study subjects** In 1997-2000, a cohort of 26,352 new diagnosed DM patients and a non-DM cohort of 131,694 controls were sampled from the claims data.

**Results** The incidence of ESRD was 29.9 per 10,000 person-years in the DM cohort, which was much higher than that in the non-DM cohort of 2.0 per 10,000 person-years. The incidence of ESRD increased from 8.0 per 10,000 person-years in the DM cohort for less than 20 years old to 29.5 per 10,000 person-years of 40-59 years old and 34.6 per 10,000 person-years of 60 years of age and older. There was a reversed dose-response relationship between the age-specific incidence rate ratio and age; the younger DM patients had a ratio of 47.2 while the oldest group had a ratio of 2.7. More DM patients lived in rural area. However, the adjusted hazard ratio of living in suburban and urban area to rural area were 1.18 and 1.08. All comorbidities contributed to develop ESRD.

Cox proportional hazard regression analysis for estimating risk of end stage renal disease by socio-demographic status and comorbidity

| | Crude Hazard ration (95% CI) | Adjusted HR (95% CI) |
|---|---|---|
| Population/km2 | | |
| Low | 1 | 1 |
| Moderate | 1.01(0.73-1.39) | 1.18(0.85-1.62) |
| High | 0.78(0.57-1.07) | 1.08(0.79-1.48) |
| Comorbidity | | |
| None | 1 | 1 |
| Hypertension | 9.94(7.68-12.9) | 2.67(2.01-3.54) |
| Kidney disease | 10.3(7.63-14.0) | 4.79(3.51-6.52) |
| Heart disease | 2.05(1.18-3.54) | 0.86(0.50-1.50) |
| Stroke | 2.86(1.51-5.43) | 1.32(0.69-2.52) |

**Conclusions** Patients with DM are at high risk of developing ESRD. Residential place and insured group didn't show significant interference to the development of ESRD in Taiwan.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO100

**Low Awareness of Chronic Kidney Disease in Japanese Health-Check Subjects** Yoshinari Yasuda,[1] Kinuko Komada,[1] Kiyoshi Shibata,[2] Shoichi Maruyama,[1] Enyu Imai,[1] Seiichi Matsuo.[1] *[1]Department of Nephrology/CKD Initiatives, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; [2]Kasugai City Health Care Center.*

*Background:* Chronic kidney disease (CKD) has been increasingly highlighted as a public health issue, worldwide. CKD prevalence in Japan is very high and estimated CKD patient number is 13.3 million in adult. However it is worried that the vast majority of CKD patients do not recognize their CKD, because CKD was the recently defined disease which usually lacks a subjective symptom.

Objective: To estimate awareness of CKD in Japan, past and present renal diseases were interviewed among health check subjects in relation to CKD and life-style related diseases.

Methods: The study subjects were 8,674 people (4,382 men and 4,292 females), who underwent health check in Kasugai City Medical Care Center, from 2006 to 2007. Medical interview was conducted by 10 nurses, and the awareness was defined by any past and present renal diseases. Estimated GFR (eGFR) was calculated by the Japanese eGFR equation, and cases with eGFR less than 60 (mL/min/1.73m²) or with proteinuria in urine dipstick were diagnosed as CKD. CKD awareness was compared in eGFR category, with/without proteinuria and treatment for hypertension, diabetes mellitus or hyperlipidemia.

Results: CKD awareness rate was 6.4% (555/8,674) in all and 10.0% (141/1,413) in CKD patients. The CKD awareness increases as CKD stage advances. CKD patients on treatment for lifestyle-related diseases recognize renal diseases at 9.2% in eGFR45-60, 22.4% in eGFR 30-45 and 60.0% in eGFR<30, which shows no statistical significance in comparison to CKD without treatment (7.7%, 22.4%, 100%, respectively). Even among CKD with proteinuria, treatment for lifestyle-related disease has no statistical impact for CKD awareness.

Conclusions: CKD awareness was low in Japanese CKD patients regardless of treatment for lifestyle-related disease which associate with CKD onset and/or progression.

Disclosure of Financial Relationships: Honoraria: Astellas, Banyu, Takeda, Novartis, Daiichi-Sankyo, Dainippon-Sumitomo, Kowa, Kirin, Mochida, Ohtsuka, Chugai, Phizer, Eisai, Tokyo-Tanabe-Mitsubishi,Fuji; Scientific Advisor: Astellas; Other Relationship: Astellas, Banyu, Chugai, Dainippon-Sumitomo, Phizer, Novartis.

**TH-PO101**

**High Normal Levels of Albuminuria and Risk of Hypertension in Indo-Asian Population** Saleem Jessani,[1] Andrew S. Levey,[2] Nish Chaturvedi,[3] Tazeen H. Jafar.[1,2,3] *[1]Aga Khan University, Karachi, Pakistan; [2]Tufts Medical Center, Boston; [3]Imperial College London, United Kingdom.*

Urine albumin excretion even in the high normal range (10-29 mg/g) as assessed by urine albumin to creatinine ratio (UACR) predicts hypertension in European-origin populations. Indo-Asians have lower muscle mass and reduced urine creatinine excretion, but also greater risk of elevated blood pressure (BP). However, the prognostic significance and clinical utility of UACR in the high normal range for incident hypertension is unclear in this population.

We conducted a nested cohort study within a cluster randomized controlled trial in Karachi, Pakistan on 1272 normotensive, non-diabetic adults aged ≥40 years with UACR <30 mg/g to examine the relationship of high normal levels of UACR and incident hypertension (defined as initiation of antihypertensive therapy during two years of follow-up or new onset of systolic blood pressure (SBP) ≥140 mm Hg or diastolic ≥90 mm Hg, confirmed at subsequent visit). BP was measured in the sitting position with a calibrated Omron 737 Intellisense device. Multivariable models were built. Logistic regression analysis was performed to determine the relationship of UACR with incident hypertension.

A total of 920 (72.3%) participants completed the 2-year final follow-up visit. During this time, 105 participants (8.3%) developed incident hypertension. In the multivariable model, participants with UACR values in the highest quartile (≥6.1 mg/g) had a 3-fold (adjusted OR: 2.72 (95% CI: 1.37 to 5.40), and those in the third quartile (3.8 to 6.0 mg/g) had 2-fold (adjusted OR: 2.11(1.06 to 4.22), greater risk of hypertension compared to those in the lowest quartile (<2.8 mg/g). The overall positive predictive value (PPV) of high normal UACR (≥6 mg/g) was (12.3% (9.1 to 16.3%) which was not different from PPV of 12.0% (9.8 to 14.5) of high normal baseline SBP (≥120 mm Hg) for incident hypertension.

Conclusion: Albuminuria as measured by UACR in the high normal range predicts hypertension in non-diabetic Indo Asians. Further research including economic evaluation is needed to assess the clinical application of these findings.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO102**

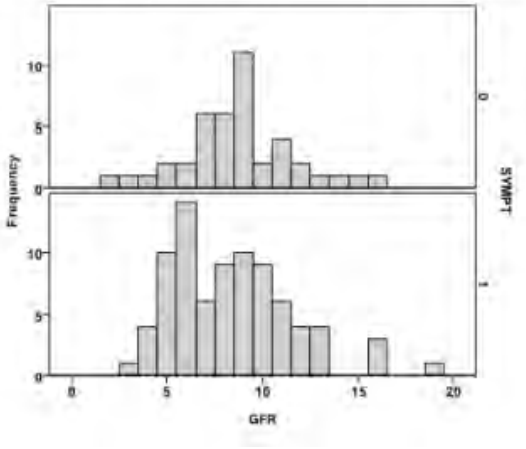
**Earlier Initiation of Dialysis in Males and Older Patients May Be Due to Poorer Tolerance of Uraemic Symptoms** Damien Ashby, Wendy Brown, Lynn Dahri, Elizabeth M. Dalby, Debra J. Gifford, Marie S. Henriksson, Samantha Ross, Claudia C. Schmalzhaf, Helen E. Watts, Peter Hill, Megan Griffith. *Imperial College Kidney & Transplant Institute, London, United Kingdom.*

There is wide variation in opinion and practice patterns regarding the timing of dialysis initiation. Age and sex influences have previously been noted, but the reasons are poorly understood.

To investigate this, data was prospectively collected on all patients starting dialysis from a group of low clearance clinics over a 2 year period to examine the effect of age and sex on GFR at dialysis initiation.

128 patients (aged 28 – 90, 66% male) commenced dialysis with a mean GFR +/-sd of 8.5+/-3.1ml/min. The primary reason for initiation was biochemical in 53.5%, fluid overload in 7% and uraemic symptoms in 39.5%, (although symptoms contributed to the decision in 67%). Mean GFR in symptomatic and asymptomatic patients was similar (8.4+/-3.1 vs 8.7+/-2.9ml/min p=0.3) but, as expected, there was a difference in the shape of the distributions with GFR in symptomatic patients being clearly skewed with greater variance and a long right sided tail.

Fig 1 Distribution of GFR at initiation of dialysis in asymptomatic and symptomatic patients




GFR was significantly higher at dialysis initiation in patients over 65 (9.0+/-3.1 vs 7.9+/-2.9ml/min p=0.041) and also in males (9.1+/-2.7 vs 7.4+/-3.3ml/min p=0.003) despite tending to be younger in this group. Analysing symptomatic and asymptomatic patients separately, both the age and gender effects were seen to be restricted to those patients with symptoms at dialysis initiation.

Males and older patients tend to start dialysis at a higher level of GFR when symptoms contribute to the decision but not when dialysis is initiated for biochemical reasons. These data suggest that gender, as well as age, influences the tolerance of uraemic symptoms. Whether this is the result of stoicism or differences in uraemic pathophysiology is unclear.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO103**

**Trend of Prevalence of Chronic Kidney Disease along with the Change in Pattern of Health-Related Behavior in the Korean Adult Population: Data from Korea National Health and Nutrition Examination Survey (KNHANES)** Seong-Woo Lee,[1] Sewon Oh,[2] Ho Seok Koo,[1] Ki Young Na,[1,2] Kwon Wook Joo,[1] Yon Su Kim,[1] Curie Ahn,[1] Dong Wan Chae,[1,2] Jin Suk Han,[1] Suhnggwon Kim,[1] Ho Jun Chin.[1,2] *[1]Internal Medicine, Division of Nephrology, Seoul National University College of Medicine, Seoul, Korea; [2]Internal Medicine, Seoul National Bundang Hospital, Seongnam, Korea.*

Background: Chronic kidney disease (CKD) is an increasing public-health problem. However, there have been limited data on the prevalence and trend of CKD in Korean adult population. Method: Data from 3rd and 4th KNHANES were used. A total of 14,938 participants with an age ≥20 years, with creatinine data, and with estimated glomerular filtration rate (eGFR) ≥15 ml/min/1.73m$^2$ were included for the analysis. Result: In the whole population, the proportion of CKD defined as eGFR < 60 ml/min/1.73m$^2$ was 7.5%. In 3rd KNHANES, the proportion of CKD was 8.8%, while 6.7% in 4th KNHANES (p <0.001). Mean age of participants in 3rd KNHANES was 47.2±15.3 years, while 49.3±16.3 years (p<0.001) in 4th KNHANES. In 3rd KNHANES, mean systolic and diastolic blood pressure were 119.3±17.9 mmHg and 77.3±10.8 mmHg, while 116.6±17.4 mmHg (p<0.001) and 75.1±10.7 mmHg (p<0.001) in 4th KNHANES, respectively. In 3rd KNHANES, 48.0% of participants walked over 60 minutes a day, while 57.5% did in 4th KNHANES (p<0.001). 14.1% of participants did strenuous exercise in 3rd KNHANES, while 15.4% did in 4th KNHANES (p=0.036). Total energy intake (1996.0±834.9 kcal/day vs. 1768.3±751.7 kcal/day; p<0.001) and sodium intake (5.7±3.3 g/day vs. 4.6±3.0 g/day; p<0.001) significantly decreased over the time. However, prevalence of hypertension (25.6% vs. 26.6%; p=0.200), diabetes (8.9% vs. 9.7%; p=0.101), obesity (32.6% vs. 31.9%; p=0.340) and proteinuria defined as dipstick ≥ 1+ (2.2% vs. 2.0%; p=0.479) were not changed over the survey period. Conclusion: In Korean adult population, proportion of CKD was deceased. This might be largely due to improved pattern of health-related behaviors, decreased blood pressure and stabilized prevalence of the other chronic diseases.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO104**

**Withholding Renal Replacement Therapy Does Not Explain the Low Incidence of ESRD in Iceland** Thorbjorg Karlsdottir,[1] Runolfur Palsson,[1,2] Olafur S. Indridason.[2] *[1]University of Iceland; [2]Division of Nephrology, Landspitali University Hospital, Reykjavik, Iceland.*

The incidence of treated ESRD in Iceland is lower than in most other Western countries. The aim of this study was to examine if this might be explained by withholding of renal replacement therapy (RRT) in a substantial proportion of patients with ESRD.

This was a retrospective study of all patients with ESRD in Iceland during the years 2000-2007, who did not receive RRT. We gained information from all health care institutions in Iceland on patients with serum creatinine levels >280 µmol/L (3.2 mg/dl) identified through electronic laboratory reports. Medical records of the patients were studied and death certificates examined. Primary care physicians and nephrologists were contacted for information on patients with advanced CKD. ESRD was defined as eGFR <15 ml/min/1.73 m$^2$, calculated by the MDRD study equation.

We identified 78 patients (43 males) who developed ESRD during the study period but did not receive RRT. In contrast, 164 patients initiated RRT yielding a total incidence of ESRD of 102.5/million/year. Thus, 32.2% of patients with ESRD did not receive RRT. Their mean age was 79.3 ±9.59 years, 73.1% were >75 years of age and they had on average 4 comorbid diseases. The last known eGFR averaged 9.6 ±3.44 ml/min/1.73 m$^2$ and 31 patients had eGFR between 10 and 15. A nephrologist was involved in the care of 76% of patients. In 36% of cases, the treating physician did not recommend RRT, 23% of the patients declined RRT and in 18% the decision not to use RRT was made jointly by the patient and the physician. Six patients (8%) were prepared for RRT but died before it could commence and in 10 patients (13%) with well compensated stage 5 CKD, no decision regarding RRT had been made as it was not considered necessary. Nine patients were alive at the end of the study. The most common causes of death were cardiovascular diseases (54%) and kidney failure (23%).

One third of patients who develop ESRD in Iceland do not receive RRT. However, the total incidence of ESRD remains low compared to other Western nations. Most of the untreated patients are elderly and burdened by comorbidity and, thus, may not benefit from RRT.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO105**

**Propensity Score Matched Evaluation of Epoetin Alfa and Darbepoetin Alfa Utilization in Patients with Chronic Kidney Disease Not on Dialysis**
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**Purpose:** To examine epoetin alfa (EPO) and darbepoetin alfa (DARB) treatment patterns and corresponding erythropoiesis stimulating agent (ESA) costs in patients with chronic kidney disease not on dialysis (CKD-NOD). Additionally, we compared the results observed in the total study population with a propensity score matched population to account for potential baseline difference that may affect ESA use.

**Methods:** A medical claims analysis was conducted from 1/1/2004-7/31/2009 using the HealthCore Integrated Research Database. Patients were age ≥18 years, newly initiated on EPO or DARB, received ≥2 doses, and had ≥1 claim for CKD prior to treatment. Patients with cancer, receiving chemotherapy or dialysis, or treated with both agents were excluded. Mean cumulative ESA dose and drug costs were evaluated in the total population and a propensity score matched population. Propensity score matching was used to identify similar populations in the EPO and DARB groups for comparison. January 2010 Wholesale Acquisition Cost was used to calculate drug cost.

**Results:** In the ESA-treated, CKD-NOD population, 1,660 EPO patients and 1,175 DARB patients were identified. Mean (SD) cumulative EPO dose was 300,596 (468,371) units for EPO and 1,205 (1,824) mcg for DARB. The mean drug cost was $4,554 for EPO and $6,279 for DARB (27% higher cost for DARB). The observed dose ratio (EPO units/ DARB mcg) was 244:1. The propensity score analysis identified 1,083 ESA treated CKD-NOD patients in each of the EPO and DARB groups that were not clinically different. Mean (SD) cumulative ESA dose was 299,194 (456,467) units for EPO and 1,185 (1,776) mcg for DARB. The mean drug cost was $4,533 for EPO and $6,174 for DARB (27% higher cost for DARB). The observed dose ratio (EPO units: DARB mcg) was 253:1.**Conclusion:** This study of ESA use in CKD-NOD observed higher drug costs for DARB compared with EPO. The observed cost difference and dose ratios were similar between the total study population and propensity score matched population.

Disclosure of Financial Relationships: Employer: Centocor Ortho Biotech Services, LLC.

**TH-PO106**

**Assessing Which Clinical Parameters Nephrologists Use When Predicting Rapid Progression of Chronic Kidney Disease**
Darren Green, Richard Hoefield, Aaron Poppleton, David I. New, Philip A. Kalra. *Dept. of Renal Medicine, Salford Royal Hospital, Salford, United Kingdom.*

For patients with chronic kidney disease (CKD) approaching dialysis, modality choice and timing of vascular access can affect survival and patient satisfaction. Accurate prediction of time to dialysis is therefore an important tool for the renal clinician.

The CRISIS prospective epidemiological investigation of CKD stages 3-5 has reported which clinical factors are associated with rapid progression of CKD. In this study, we analyzed which clinical factors most strongly influence physicians' assessment of when a patient will need dialysis. By comparing these data with data from CRISIS, factors which physicians over or undervalue when predicting time to dialysis can be evaluated.

Nephrologists seeing patients in a pre-dialysis clinic were asked to predict the time to dialysis for each patient, based on clinical information available during the consultation. The clinic was held at the hospital where CRISIS patients had been enrolled to give similar demographics. Multi-variate regression was used to define which factors were associated with the predictions of time to dialysis.

During the study, 6 clinicians assessed 124 patients. Baseline data compared to CRISIS were as follows: mean age = 64.8 years (vs. CRISIS = 65.1 years), gender = 62.9% male (vs. 63.7%), diabetes = 31.5% (vs. 32.0%). Factors associated with perceived likelihood of rapid progression to dialysis by clinician were MDRD calculated eGFR ($p<0.001$), and the presence of proteinuria ($p<0.001$), hypoalbuminemia ($p=0.002$), hyperphosphatemia ($p<0.001$) and anemia ($p=0.044$). Of these, multi-variate analysis of >1300 CRISIS patients has shown eGFR, anemia and proteinuria to be significant in predicting rapid progression of CKD. CRISIS also showed that younger age, male sex, current smoker, and hypertension were significant.

This study suggests that not all predictive parameters are used by clinicians when assessing time to dialysis but that only hyperphosphatemia and hypoalbuminemia are overvalued as predictors. Highlighting these discrepancies may improve physicians' judgement in the pre-dialysis setting and improve patient outcome and satisfaction.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO107**

**Hypertension Awareness, Treatment, and Control in the Korean Adult Population with and without Diabetes or Chronic Kidney Disease: The Fourth Korea National Health and Nutrition Examination Survey 2008**
Sang-Woong Han,[1,5] Ho-Jung Kim,[1] Joo-Hark Yi,[1] Ju-Hyun Lee,[1] Hyeong Cheon Park,[2] Sang Youb Han,[3] Bumsoon Choi,[4] Bo Youl Choi.[1,5] *[1]Hanyang University College of Medicine, Korea; [2]College of Medicine,Yonsei University, Korea; [3]Inje University, Ilsan-Paik Hospital, Korea; [4]The Catholic University of Korea, Korea; [5]Center for Healthy Aging and Longevity, Hanyang University Institute of Aging Society, Korea.*

Hypertension (HTN) is a well-established risk factor for cardiovascular disease and is common in diabetic or chronic kidney disease (CKD) patients. We aimed to analyze HTN awareness, treatment, and control in adults with and without diabetes or CKD, according to recent guidelines which recommend more stringent blood pressure (BP) control in high risk patients. A cross-sectional study of a representative sample of the Korean adult population (≥30 years old) was conducted using 5,923 participants in the 2008 KNHANES IV-2. HTN was defined as a BP of ≥140/90 mmHg for those without diabetes or CKD and as ≥130/80 mmHg for those with diabetes or CKD (an estimated GFR of <60 mL/ min/1.73 $m^2$ calculated by the MDRD equation). In 2008, the estimated prevalence of HTN, diabetes and CKD were 27.7%, 10%, and 5.9%, respectively. The estimated prevalence of HTN among the population with diabetes or CKD were 70% and 68.1%, respectively. Awareness, treatment, and control of HTN in the Korean adult population without diabetes or CKD were 59.2%, 51.6%, 36.8 % and 65 %, 59.9%, 22.6% in those with diabetes or CKD, respectively. Compared to the hypertensive population without diabetes or CKD (n=1,193), those with diabetes or CKD (n=625) were older (64±11.6 vs 59.4±13.3 yrs, $p<0.0001$), in lower education ($p=0.0018$) and in lower BP (systolic BP, 131.8±16.5 vs 134.3±16.5 mmHg, $p=0.0039$; diastolic BP, 79.6±10.8 vs 84.6±11.4 mmHg, $p<0.0001$) This study suggested that despite higher awareness and treatment of HTN, and lower level of BP in addition to higher prevalence, HTN remains poorly controlled in the Korean adult population with diabetes or CKD. Therefore, optimal management of HTN by health care personnel in this high risk population is strongly recommended.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO108**

**The Incidence of Pauciimmune Glomerulonephritis in Lancashire and South Cumbria (North-West of England)**
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Pauciimmune glomerulonephritis (PIGN) is the most common cause of rapidly progressive glomerulonephritis in adults. Most of these patients produce Anti Neutrophil Cytoplasmic Autoantibodies (ANCA). Incidence of PIGN varies according to geographical region and ethnicity. This study investigated the incidence of PIGN in North-West England over a 5-year period. The population of adults in this area in the 2001 census was 1.2million; 95.3% were Caucasian, 3.7% were Indo-Asian and 0.2% were Afro-Caribbean.

**Methods** Data from archived records was used to identify patients with histological evidence of PIGN between 1st January 1999 and 31st December 2003. The criteria for inclusion were cellular or fibrocellular crescents, tuft necrosis or vasculitis. Only patients with PIGN first detected within the study period and who were aged >17-year were collected.

**Results** Sixty-four cases were identified over 5-years with an overall annual incidence of 10.5 patients per million of the adult population. Thirty-five of these were under 65-years at diagnosis and 29 patients were at least 65-years old. Of the younger group, 20 were alive at 5-years compared to 12 in the older group (6 patients were lost to follow up). The male to female ratio was 37:27. Most patients (44) were Caucasian; 2 were Indo-Asian and 1 patient was Afro-Caribbean (ethnicity data unavailable for 17 patients). Forty-two patients were ANCA positive; 22 cANCA/PR3-ANCA. Fifteen patients were ANCA negative and data was not available for 7 patients.

**Conclusions** Our results are very similar to those found in a study in Greater Manchester over the same time period (ASN 2007 poster by Dhaygude et al). Estimates in the latter were of 9.8 per million of the adult population; this is in contrast to findings in South-West USA where the estimate was 3.1 per million population (13th International ANCA Workshop poster by Hogan et al). This supports the findings of other studies concerning regional variation and an increase in incidence with further distance north. Further studies are needed to determine the importance of environmental and genetic factors in affecting this distribution of cases.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO109**

**Canadian Pediatric IgAN/HSP: Baseline Patient Characteristics from the REDDCAPP Registry** Maury N. Pinsk,[1] Chantal Bernard,[5] Janusz Feber,[7] Ian W. Gibson,[2] Aviva M. Goldberg,[2] Christoph Licht,[3] Aicha Merouani,[6] Andrew W. Wade,[4] Tom D. Blydt-Hansen.[2] *[1]University of Alberta, Edmotnon, AB, Canada; [2]University of Manitoba, Winnipeg, MB, Canada; [3]University of Toronto, Toronto, ON, Canada; [4]University of Calgary, Calgary, AB, Canada; [5]McGill University, Montreal, QC, Canada; [6]Universite de Montreal, Montreal, QC, Canada; [7]University of Ottawa, Ottawa, ON, Canada.*

The Renal Disease Database in the Canadian Pediatric Population (REDDCAPP) is a multicentre effort to track the incidence and characteristics of IgA nephropathy (IgAN) and Henoch Schoenlein purpura nephropathy (HSPN) in Canadian children. Methods: Pediatric patients with biopsy-proven IgAN/HSPN were enrolled from four Pediatric Nephrology centres across Canada from 2008-2010. Results: 30 patients (47% were males) with complete data sets were included in the analysis. 53% were self-declared Aboriginal/ First Nations, 27% were Caucasians, and the remainder were from South Asian/East Asian or Latin American Countries. The mean age at biopsy was 10.8 ± 3.8 years. 36.7% of the patients had a BMI>26 kg/m$^2$, and 37% of patients presented with hypertension (7% >90th percentile, 30% >95th percentile). Presentation as HSPN occurred in 47%. Presenting signs were macrohematuria (63%) and microhematuria (33%). One patient without hematuria presented with nephrotic syndrome. Urine protein creatinine ratio (UPCR)was < 20 mg/mmol in 6%, 20-200 mg/mmol in 33% and >200 mg/mmol in 61% . The average UPCR was 631 mg/mmol (range 11-2641) compared to normal value of <20 mg/mmol. Renal function was abnormal in 20% with 7% CKD II, 10% CKD III and 3% CKD IV. No patients presented with a GFR < 15 ml/min/1.73m$^2$. No patient presented with low serum C3, although 38% had C4 levels below 0.16 g/L. Conclusion: A significant proportion of Canadian children who were biopsied with IgAN/HSPN suffer from hypertension (37%), proteinuria (94%) and decreased renal function (20%).

Disclosure of Financial Relationships: nothing to disclose

**TH-PO110**

**Low Serum Albumin Associated with Death in Patients with Chronic Kidney Disease** Rebecca J. Schmidt, Bethany S. Pellegrino. *Nephrology, West Virginia University, Morgantown, WV.*

Serum albumin is known to correlate with survival in patients on dialysis. Albumin levels of patients starting dialysis in the United States have changed little since 1996 (3.1-3.2) g/dl) and in our rural West Virginia dialysis patients low albumin levels are the norm.

Albumin values from 5406 patients with Stages 3-5 chronic kidney disease (CKD) seen between January 1, 2001 and May 31, 2010 were analyzed. Values at first presentation to the CKD clinic were utilized. Age of the patient population (mean±SD ) was 65.7±16.67 and 57% of the group was older than 65. Less than 3% of the group was African American, the predominant race being white (93.41%).

Mean albumin levels were similar for gender (mean±SE: 3.58±0.023 for females vs. 3.54±0.025 for males) and did not correlate with age. Almost half (44%) of Stage 3-4 patients presented with albumin levels ≤3.5 g/dl and for Stages 3-5, 34%, 42% and 70% of patients presented with albumin levels ≤3.5. Mean±SD for WV CKD patients ranged from 3.37±0.75 for Stage 3 to 3.16±0.74 for Stage 5.

Albumin levels for patients who ultimately died prior to starting dialysis were significantly less than for those who did not (3.21±0.05 vs. 3.61±0.02 (mean±SE); P< 0.0001.

The prognostic value of low albumin in non-dialysis-dependent CKD patients has been suggested to portend similar adverse outcomes, though prevalence of albumin levels ≤ 3.5 among those with higher levels of renal function is not widely reported.

In our patient population, a low serum albumin at first presentation was associated with a higher likelihood of death and perhaps represents an adjunctive marker of prognosis in CKD patients moving toward dialysis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO111**

**Low Serum Albumin Common in Rural Patients with Chronic Kidney Disease** Rebecca J. Schmidt, Bethany S. Pellegrino. *Nephrology, West Virginia University, Morgantown, WV.*

Serum albumin is known to correlate with survival in patients with on dialysis and low levels are an Achilles heel for many caregivers of dialysis patients. Albumin levels of patients starting dialysis in the United States have changed little since 1996 (3.1-3.2) g/dl) and in our rural West Virginia dialysis patients, low albumin levels are the norm.

Albumin values from 5406 patients with chronic kidney disease (CKD 3-5) seen between January 1, 2001 and May 31, 2010 were analyzed. Values at first presentation to the CKD clinic were utilized. Age of the patient population (mean±SD ) was 65.7±16.67; 57% were older than 65. Less than 3% were African American, the predominant race being white (93.41%).

Mean albumin levels were similar for all CKD patients of both genders (mean±SE: 3.58±0.023 for females vs. 3.54±0.025 for males) and did not correlate with age or year of visit. Mean levels ≤ 3.5 g/dl were highly prevalent (45%) in all stages of CKD among our rural patients and also among those < 65 years of age. Albumin levels for WV CKD patients are shown with NHANES data for the US general population by stage below, along with percents of WV CKD patients with albumin levels ≤ 3.5 g/dl compared with data from a large integrated health system (Go 2004) below:

CKD Albumins

| | WV CKD Patients | US General Population | WV CKD with Albumin ≤ 3.5 (%) | Large Health Care System with Albumin ≤ 3.5 (%) |
|---|---|---|---|---|
| Stage 3-45-59 | 3.66±1.00 | 4.2±0.01 | 10.6% | 2.8% |
| Stage 3-30-44 | 3.63±0.58 | 4.1±0.03 | 21.5% | 5.8% |
| Stage 4-15-29 | 3.54±0.67 | 4±0.04 | 41.8% | 13.7% |
| Stage 5 <15 | 3.14±0.79 | 4.1±0.02 | 70.4% | 24% |

p < 0.0001 for WV CKD vs. NHANES IIII population and large health care system

Compared to the NHANES III population and members of a large health care system, WV CKD patients had significantly greater prevalence of hypoalbuminemia at varying levels of renal function. Low albumin levels present earlier in the continuum of CKD likely contribute to the persistently low albumin levels seen in patients starting dialysis and the particularly high prevalence of hypoalbuminemia in WV dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO112**

**Effect of Statin on Muscle Sympathetic Nerve Activity in Patients with Chronic Kidney Disease** Laima Siddiqi,[1] Liam P. Oey,[2] Peter J. Blankestijn.[1] *[1]Nephrology and Hypertension, University Medical Center Utecht, Utrecht, Netherlands; [2]Neurophysiology, University Medical Center Utecht, Netherlands.*

Background: Hypertensive chronic kidney disease (CKD) patients often have sympathetic hyperactivity which appears to contribute to the pathogenesis of hypertension and cardiovascular organ damage. Experimental studies and some clinical studies have shown that statin therapy can reduce central sympathetic activity by different mechanisms. The aim of this study was to evaluate the effect of 6 weeks treatment with statins (Lipitor 20mg/day) added renin inhibitors on sympathetic activity in hypertensive stage 2-4 CKD patients.

Methods: In ten CKD patients (8 males, aged 44 ± 11 years, eGFR 59 ± 22 ml/min per 1.73 m$^2$) blood pressure and sympathetic activity (quantified by assessment of muscle sympathetic nerve activity, MSNA) were assessed, while lipitor 20mg/day was added to renin inhibitor (aliskiren 300mg/day), and during 6 weeks only aliskiren 300 mg/day. Total cholesterol and low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride levels were measured before and after treatment with Lipitor.

Results: Mean arterial blood pressure remained stable throughout the study (93 ± 5 with Lipitor vs 94 ± 5 without Lipitor 20mg/day). MSNA was reduced from 32 ± 8 to 25 ± 6 bursts/minute (P = 0.01) while heart rate remained stable during the study (62 ± 2 with Lipitor vs 61 ± 3 without Lipitor). Total cholesterol and LDL, HDL and triglyceride levels were elevated when patients were off medication however, the difference was not significant. Conclusion: Addition of statin on renine inhibitors has a substantial sympatholytics effect in hypertensive CKD patients, while blood pressure and heart rate remain unchanged.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO113**

**Clinical and Pathological Phenotype of Leukocyte Cell-Derived Chemotaxin-2 Amyloidosis (ALECT2)** Julian D. Gillmore,[1] Jennifer H. Pinney,[1,2] Janet Gilbertson,[1] Simon Gibbs,[1] Prayman Sattianayagam,[1] Ashutosh D. Wechalekar,[1] Helen J. Lachmann,[1] Ahmet Dogan,[3] Philip Hawkins.[1] *[1]National Amyloidosis Centre, Division of Medicine, UCL Medical School, London, United Kingdom; [2]Centre for Nephrology, Division of Medicine, UCL Medical School, London, United Kingdom; [3]Departments of Laboratory Medicine and Pathology and Haematology, Mayo Clinic, Rochester, MN.*

LECT2 is a chemokine and growth factor that was recently identified as a novel amyloid fibril protein from renal biopsy specimens. In this study, we report the clinical and pathological features in 7 patients with LECT2-associated amyloidosis (ALECT2).

Diagnosis was confirmed in all patients by histology and mass spectrometry based proteomic analysis of amyloid deposits. Deposition of LECT2 amyloid within kidney biopsy specimens was mainly interstitial and vascular with relative sparing of the glomeruli. The LECT2 gene and promoter region was sequenced in all cases. Although no novel mutations were identified within the coding region, all patients were homozygous for a described non-synonymous SNP.

Median age at presentation was 62 (52-70) years. Five patients were Punjabi and 2 were North African. Six of 7 patients presented with low level proteinuria (median 0.5 g/24hr) and/or renal impairment (median eGFR 32 ml/min). In the remaining patient, LECT2 amyloid deposits were incidentally discovered in a liver biopsy for unrelated liver disease. No patient had amyloid involvement of the heart or nerves, and none had a family history. Amyloid was imaged in the spleen and kidneys by SAP scintigraphy in all 7 patients, and the liver and adrenal glands were also involved in 4 and 6 cases respectively.

Median follow up was 24 (6-49) months during which no patient developed extra-renal amyloidotic organ dysfunction. GFR was stable or declined slowly in all patients (range 0-10 ml/min/yr).

ALECT2 is characterized by renal, splenic, hepatic and adrenal amyloid deposition and a clinical picture of slowly progressive chronic kidney disease with low level proteinuria. The pathogenesis of ALECT2 remain unclear. Accurate recognition of ALECT2 amyloidosis is essential to avoid inappropriate treatment for other types of amyloidosis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO114**

**High Prevalence of CKD May Be Socioeconomically Driven** Rebecca J. Schmidt, Bethany S. Pellegrino. *Nephrology, West Virginia University, Morgantown, WV.*

Chronic kidney disease (CKD) has reached epidemic proportions nationwide, and in 2007, West Virginia led the nation in numbers of patients starting dialysis (434.3 vs 353.8 per million). The state's high prevalence of diabetes (12 vs 7% nationally), along with its older population (15.7% > 65 years) and the geographic distance to specialty care likely contribute to the high prevalence of CKD.

A predominantly rural state of 1.8 million people, West Virginia has a high prevalence of non-Hispanic whites (93.5% vs 65.6% for US) with an African American prevalence of 3.6% (vs 12.8% for US). Compared to national figures, the percent of West Virginians living below the federal poverty level (%<FPL) in 2008 was 17.4% compared to 13.2% nationally. Median household income is $37,528 compared to $52,029 for the US.

Of 5406 patients with CKD Stages 3-5 seen from 54 counties in West Virginia between January 1, 2001 and May 31, 2010, African Americans comprised 3.0% and the prevalence of non-Hispanic whites was 93.4%. These estimates differ significantly ($p<0.0001$) from national CKD data, which report a prevalence of 7.3% for African American and 87.3% for non-Hispanic whites (UDRDS).

Of the 29 counties represented by 4532 patients total, two had a lower %<FPL than the national average and only one county had a median income exceeding the national average of $52,029. Median household income ($35,896) and % <FPL (17.6) for the CKD group was similar to the state overall. No African American patient came from a county where the %<FPL exceeded 16.4%. Only two counties were represented by African American patients; these counties had median household incomes exceeding those of 21 other counties.

No state started more patients on dialysis in 2007 than West Virginia, reflecting the high prevalence of CKD. The proportion of African Americans in our CKD population is lower than that of the nation. More West Virginians than the national average live at levels below the FPL and have lower median household incomes. Socioeconomic factors, irrespective of race, may impact the prevalence of CKD and additional study is warranted to ascertain the strength and independence of this potential risk.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO115**

**A Ten-Year Review of Percutaneous Kidney Biopsies in an Adult Afro-Caribbean Population. Specific Place of Lupus Nephritis** Jean-Marc Roger Dueymes-Laporte,[1] Raluca Ples,[2] Christophe Deligny,[4] Alex Ranlin,[1] Maryvonne R. Dueymes,[3] Hawa Traore.[1] *[1]Nephrology Department, Centre Hospitalier, Lamentin, Martinique, Martinique; [2]Anatomo-Pathology Laboratory, Centre Hospitalier Universitaire, Fort de France, Martinique; [3]Immunology Laboratory, Centre Hospitalier Universitaire, Fort de France, Martinique; [4]Internal Medicine Department, Centre Hospitalier Universitaire, Fort de France, Martinique.*

Objectives

A retrospective ten-year study (2000-09) study of histological findings obtained by kidney biopsy (KB) was made in the only Nephrology Dpt of a French West Indies Island (400000 inhabitants).The aim of the study was to review histological aspects of primary and secondary glomerulonephritis (GN), with special attention to Lupus Nephritis(LN).

Methods

Clinical and histological reports were reviewed for KB performed in the Nephrology Department from 2000 to 09. Different Data were collected from the Histology requisition form.

Results

243 biopsies(147female, 96male) were performed. Creatininemia was under 120micromol/l in 51%, over 300micromol/l in 20%, proteinuria over 3gr/24h in 171patients. Post biopsy,10pts had a transient gross hematuria or peri-renal hematoma. The biopsy was usable for histology examination in 93%. Membranous glomerulonephritis (MGN)was observed in 61 pts(27%),Focal Segmental GN (FSGN) in 37pts(16%), MPGN in 33pts, Minimal Change in 27pts. Vascular lesions were observed in 58% and tubulo-interstitial lesions in 77%.GN was primitive in 85pts(38%), secondary to systemic disease in 158pts. 75/158 were LES with Lupus Nephritis LN(47.4%). Histology of LN according to the ISN/RPS 2003 classification showed classIV in 32pts, classV in 23pts, classIII in 12pts,classII in 7pts.LN patients tested positive for antiRNP(58.7 %),antiSSA(47.2 %),antiSm(37.1%). Epidemiological parameters of LN pts were found to be similar to those described in a previous ten-year period in the same population.

Conclusion

In this Afro-Caribbean population, the most common histological findings following KB for Primary GN were MGN, FSGS, MPGN.For LN, diffuse proliferative and Membranous GN were the main types (73.3%).

Disclosure of Financial Relationships: nothing to disclose

**TH-PO116**

**Long Term Follow-Up of Overweight and Obese Living Kidney Donors** H. Tent,[1] Mieneke Rook,[1] Hendrik Sijbrand Hofker,[2] Rutger J. Ploeg,[2] Jaap Homan vd Heide,[1] Gerjan Navis.[1] *[1]Internal Medicine Division of Nephrology, University Medical Center Groningen, Netherlands; [2]Surgery, University Medical Center Groningen, Netherlands.*

Due to donor organ shortage, living kidney donor selection has become more liberal with acceptation of overweight and obese donors. We found that early after donation overweight donors have a higher risk for impaired GFR and lower renal reserve. Whether this results in a worse long term outcome is unknown.

We evaluated 5–year outcome in 64 donors who donated at our center. All had GFR ($^{125}$I-iothalamate) and ERPF ($^{131}$I-hippuran) measured 4 months prior, and 2 months and 5.2±1.1 year after donation. Delta GFR from 'single kidney status' was calculated as [GFR long term – (pre-donation GFR/2)].

Results are shown in the table, with a break-up for pre-donation BMI. On regression analysis ΔGFR was associated negatively with pre-donation age ($R^2$ 0.13, $p<0.05$), but not with BMI. Long term blood pressure was positively related to age and BMI ($R^2$ 0.18 and 0.08, $p<0.05$). No proteinuria occurred pre- and post-donation.

In conclusion, in this small population overweight donors have lower ERPF prior and higher blood pressure prior and post-donation. However, long term course of renal function is equal to lean donors and not determined by BMI. We want to emphasize that although these overweight donors perform well, close long term monitoring and adequate blood pressure treatment remains necessary.

| BMI pre-Unx | BMI<25 | BMI 25-28 | BMI≥28 |
|---|---|---|---|
| N (%male) | 28 (40) | 20 (45) | 16 (38) |
| Age at Unx (years) | 44±12 | 48±8 | 53±9* |
| Duration of follow-up (years) | 5.3±1.3 | 5.3±0.8 | 4.9±1.1 |
| BMI pre-Unx | 23±2 | 27±1 | 32±5 |
| BMI early post-Unx | 23±2 | 27±1* | 31±5*$ |
| BMI long term post-Unx | 24±2 | 27±2* | 33±6*$ |
| GFR pre-Unx | 113±13 | 118±19 | 117±24 |
| GFR early post-Unx | 74±11 | 74±10 | 76±12 |
| GFR long term post-Unx | 81±14 | 84±12 | 84±12 |
| ΔGFR to long term | 26±10 | 24±10 | 25±8 |
| ERPF pre-Unx | 435±84 | 449±56 | 397±64$ |
| ERPF early post-Unx | 295±57 | 295±37 | 280±48 |
| ERPF long term post Unx | 300±62 | 292±36 | 290±56 |
| MAP pre-Unx | 87±8 | 93±9* | 97±8* |
| MAP early post-Unx | 92±9 | 93±7 | 98±6*$ |
| MAP long term post-Unx | 92±8 | 92±9 | 99±9*$ |

Unx: donation. *$p<0.05$ vs BMI<25; $p<0.05$ vs BMI 25-28. (Δ)GFR and ERPF in mL/min, MAP in mmHG.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO117**

**A Randomized Controlled Trial of Enzyme Replacement Therapy in Fabry Disease: The Canadian Fabry Disease Initiative at Year Three** Michael L. West,[1] Daniel G. Bichet,[2] Sandra Sirrs,[3] Christiane Auray-Blais.[5] *[1]Medicine, Dalhousie University, Halifax, NS, Canada; [2]Medicine, Universite de Montreal, Montreal, NS, Canada; [3]Medicine, University of British Columbia, Vancouver, BC, Canada; [4]Pediatrics, Universite de Sherbrooke, Sherbrooke, QC, Canada.*

Fabry disease is a rare X-linked lysosomal storage disorder which causes cardiomyopathy, renal failure, strokes and death by 60 years if untreated. Enzyme replacement therapy (ERT) with recombinant agalsidase is not reimbursed in Canada by government due to high cost and lack of clinical effectiveness. The Canadian Fabry Disease Initiative (CFDI) study provides ERT (agalsidase alfa 0.2 mg/kg or agalsidase beta 1.0 mg/kg I.V. every 2 wks) to Fabry patients and collects outcomes data (Clinical Trial Registration protocol # NCT 00455104). Fabry patients ages 5 - 85 years in Canada are eligible with cohort 1a those on prior ERT; cohort 1b ERT naïve patients who meet Canadian ERT guidelines; cohort 1c those refusing or not meeting ERT criteria. Patients are randomized In cohort 1b to agalsidase alfa or beta. Data are collected prospectively as to clinical outcomes (eGFR, proteinuria, LVMI, unstable angina, MI, PTCA, ICD, pacemaker, CHF, arrhythmia, hospitalization, stroke, TIA, acute deafness, death), MSSI, quality of life, and pain. Over 36 mos 277 patients were enrolled with data available on 266: cohort 1a 30.2%, cohort 1b 21.5% and cohort 1c 48.9%. Mean duration of follow up was 23.0 mos. In cohort 1b, females predominated (34, 63.0%) over males (20, 37.0%) but were balanced between the agalsidase treatment groups. Clinical features at baseline did not differ in the two treatment arms. Anti-agalsidase antibodies were more prevalent and of higher titre in the agalsidase beta group. Urine globotriaosylceramide was lower with ERT and in females. Outcomes data in cohorts 1a and 1b up to 30 months showed no differences between agalsidase-alfa and agalsidase-beta. Similar results were seen with a composite clinical outcome. In the short term no significant differences in clinical outcomes can be shown between agalsidase alfa and beta for Fabry disease.

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Sumitomo Pharma
Amicus Therapeutics; Scientific Advisor: Shire Human Genetic Therapies.

**TH-PO118**

**Adverse Renal Outcomes after Nephrectomy for Renal Tumours: A Population-Based Analysis** Scott Klarenbach,[1] Ron Moore,[2] Dave Chapman,[1,2] James Dong,[1] Branko Braam.[1] *[1]Medicine/Nephrology, Univ. of Alberta, Edmonton, Canada; [2]Surgery/Urology, Univ. of Alberta.*

Oncologic outcomes are excellent for early stage renal cell carcinoma (RCC). However, patients may be at risk for chronic kidney disease (CKD) given the shared risk factors of HTN, smoking, and age. We sought to describe adverse renal outcomes in a population-based cohort of subjects undergoing partial (PN) or radical nephrectomy (RN) for renal tumors, and identify baseline risk factors associated with poor renal outcomes.

All subjects undergoing nephrectomy for renal tumors between 2002 and 2009 were identified from inpatient encounters using ICD9/ICD-10 codes. Baseline comorbid conditions (including DM, HTN, CVD, CHF, and Charleson Comorbidity), proteinuria status, and eGFR was determined. Descriptive statistics and Cox proportional hazards were used, and the composite outcome of CKD (eGFR < ml/min/1.73m$^2$), rapid CKD progression (eGFR <60 and delta eGFR ≥4 ml/min/1.73m$^2$/year), or acute or chronic renal replacement therapy was considered.

Over follow-up of 1,151 patients, 23 (2.0%) developed ESRD or required acute dialysis, 114 (9.9%) developed CKD, and 24 (2.1%) developed rapidly progressive CKD. The 132 (11.5%) subjects that developed an adverse renal outcome were older, had lower baseline eGFR, and had more concomitant comorbidity. In adjusted analysis, partial nephrectomy was associated with a HR of 0.56 (95% CI 0.33-0.96) of adverse renal outcomes. In addition to lower baseline eGFR, greater Charlson Comorbity score and presence of proteinuria were also associated with adverse renal outcomes, with HR of 1.12 (1.02-1.23) and 2.44 (1.54-3.87) respectively.

In this large population based cohort of patients undergoing nephrectomy for renal masses, baseline renal function and proteinuria were important predictors of adverse renal outcomes. Renal outcomes were negatively affected by RN versus PN. The study supports routine assessment of eGFR, proteinuria and comorbidity prior to surgery to assist in the decision for RN or PN, and identify high risk patients for follow-up.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO119**

**Cost-Effectiveness of Paricalcitol in Type 2 Diabetes, Nephropathy, and Residual Albuminuria** Hiddo Jan Lambers Heerspink,[1] Mark Nuijten,[2] Steven E. Marx,[3] Raimund K. Sterz,[3] Dick De Zeeuw.[1] *[1]University Medical Center, Groningen, Netherlands; [2]Ars Accessus Medica, Erasmus University, Rotterdam, Netherlands; [3]Abbott, Abbott Park, IL.*

**Introduction:** The Selective Vitamin D Receptor Activator for Albuminuria Lowering (VITAL) Study showed that paricalcitol at doses of 2 mcg per day significantly reduced residual albuminuria over 6 months in addition to ACEI and/or ARB therapy. The objective of this analysis is to assess the cost-effectiveness of paricalcitol with ACEI and/or ARB therapy in type 2 diabetic patients with nephropathy.

**Methods:** A Markov model was used to simulate the progression from microalbuminuria to end stage renal disease. We use data from the VITAL study and published literature (Delea JASN 2008) to estimate probabilities of progression of renal disease, utilities and costs. The comparator was ACEI and/or ARB therapy compared to ACEI and/or ARB therapy plus paricalcitol 2 mcg per day. The data was analyzed from a US healthcare payer perspective, using 2007 costs and discounted at 3%. The efficacy outcome, reduction in urinary albumin creatinine ratio, was extrapolated to lifetime life-years gained (LYS) and quality adjusted life-years gained (QALY).

**Results:** Compared to ACEI and/or ARB therapy, the addition of paricalcitol 2 mcg per day leads to additional lifetime healthcare costs of $9,622 (USD). The health benefits of paricalcitol lead to an increase in LYG of 0.411 and a gain in QALYs of 0.498. The use of paricalcitol results in an incremental cost-effectiveness ratio of $19,333/QALY and $23,387/LYS. These values are in a range which is accepted as cost-effective (<$50,000/QALY).

**Conclusion:** This model showed increase in life expectancy and quality-adjusted life years saved. The additional lifetime costs for paricalcitol were offset by the savings associated with delayed in chronic kidney disease progression. The addition of paricalcitol 2mcg per day to ACEI and/or ARB therapy in patients with type 2 diabetes and nephropathy is cost-effective from a US healthcare perspective.

Disclosure of Financial Relationships: Research Funding: Abbott Research Grant. Payments directed to institution.

**TH-PO120**

**Temporal Hemoglobin Trends in Erythropoiesis-Stimulating Agent Treated Chronic Kidney Disease Patients Not on Dialysis** Robert A. Bailey,[1] Marie-Helene Lafeuille,[2] Isabelle Raymond,[2] Mekre Senbetta,[1] Patrick Lefebvre.[2] *[1]Health Economics and Outcomes Research, Centocor Ortho Biotech Services, LLC, Horsham, PA; [2]Group d'Analyse, Ltee, Montreal, QC, Canada.*

**Purpose:** To evaluate hemoglobin (Hb) values over time in patients with chronic kidney disease (CKD) not on dialysis treated with erythropoiesis-stimulating agents (ESAs).

**Methods:** Data on CKD patients receiving ESAs in nephrology clinics in the United States collected between November 2001 and December 2009 were analyzed. Visits associated with ESA treatment and a Hb value were selected for analysis. Patients receiving renal dialysis were excluded. Hemoglobin values were reported as either baseline or on ESA treatment and were categorized in this manner for analysis. Mean baseline and on-treatment Hb values were evaluated by semester (S1: Jan-Jun; S2: Jul-Dec) and for two distinct periods: Pre-Kidney Disease Outcomes Quality Initiative (KDOQI) period (visits prior to 04/01/2007), and Post-KDOQI period (visits on 04/01/2007 or after).

**Results:** A total of 10,053 (baseline: 1,109, on-treatment: 8,944) Hb values associated with ESA treatment were analyzed. Mean hemoglobin (SD) was 10.4 (1.3) g/dL at baseline and 11.2 (1.3) g/dL for on-treatment. Semi-annual mean baseline and on-treatment Hb were relatively stable over the study period (across semesters, g/dL: Baseline: mean (SD): 10.4 (0.2), median: 10.3, 25$^{th}$ and 75$^{th}$ percentiles: 10.2 and 10.6; On-treatment: mean (SD): 11.2 (0.3), median: 11.2, 25$^{th}$ and 75$^{th}$ percentiles: 11.0 and 11.5). The proportion of Hb values ≥12 g/dL decreased from 12.0% in the Pre-KDOQI period to 2.9% in the Post-KDOQI period for baseline (*P*<.0001) and from 28.5% to 15.2% for on-treatment visits (*P*<.0001).

**Conclusions:** This study observed a stable trend in Hb values and a reduction in the proportion of hemoglobin readings greater than 12 g/dL over the period from November 2001 to December 2009 in CKD patients not on dialysis receiving ESAs in nephrology clinics.

Disclosure of Financial Relationships: Employer: Centocor Ortho Biotech Services, LLC.

**TH-PO121**

**Comparative Effectiveness of Incident Oral Antidiabetic Drugs (OADs) on Kidney Function in Veterans with Type 2 Diabetes** Adriana Hung,[1,2] Christianne Roumie,[1,2] Robert Greevy,[1,2] Xulei Liu,[1,2] Carlos Grijalva,[1,2] Harvey J. Murff,[1,2] M. M. Huizinga,[3] Marie Griffin.[1,2] *[1]Medicine, Vanderbilt University, TN; [2]GRECC, VA Tennessee Valley Healthcare System, TN; [3]John Hopkins University, MD.*

OADs are the mainstay of therapy for patients with type 2 diabetes; however, data comparing their effect on kidney function are sparse. Of 18123 veterans who filled an incident OAD prescription at the VA-VISN9 between 1/1/2000-12/31/2007, we identified 10418 who met our inclusion criteria: 1 or more baseline creatinine measures, a GFR> 60 ml/min, and complete covariates. Incident users of sulfonylurea (SU) or combination metformin + sulfonylurea (MET-SU) were compared to metformin (MET) users (reference group), using a time to event analysis. The primary outcome was: a persistent (confirmatory value within 3-12 months) eGFR decline of 30 ml/min (GFR event), confirmed end stage renal disease (ESRD), or death, and the secondary outcome was: GFR event or ESRD, censoring at death. This analysis was performed in all individuals to reflect real clinical practice settings (n=10418) and on the subgroup with available urine protein test during baseline (n=7110). The analysis was performed in patients while on their incident OAD regimen. Exposure ended with: a gap in medication > 90 days, addition or changed to a different OAD or insulin, leave the VA, or end of the study. Covariates included: age, sex, race, blood pressure, history of cardiovascular disease, body mass index, glycosylated hemoglobin, specific medications, marital status, year, healthcare utilization, and, in the n=7110 subset, proteinuria.

For the primary outcome, the SU vs MET group had an adjusted hazard ratio (aHR) of 1.23 (95% CI 1.04, 1.45) for all patients and 1.32 (95% CI 1.07, 1.62) for the proteinuria subgroup and for the secondary outcome an aHR 1.17 (0.97, 1.41) and 1.33 (95%CI 1.07, 1.67) respectively. There was no difference observed between the combination group and metformin in either analysis.

Our findings suggest that patients on sulfonylureas have a higher risk of a clinically significant decline in their kidney function or death, independent of glycemic control.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO122**

**Long-Term Renal Survival Analysis of Patients with IgA Nephropathy: Results from a Cohort of 590 Cases in a Chinese Population** Wei-Bo Le, Kang-Ping Deng, Yang-Lin Hu, Cai-Hong Zeng, Zhi-Hong Liu. *Research Insitute of Nephrology, Jinling Hospital, Nanjing University of Medcine, Nanjing, Jiangsu, China.*

Although numerous long-term renal survival analysis and related risk factors of patients with IgA nephropathy (IgAN) have been published, data from a large cohort of Chinese patients are sparse. We performed a retrospective study of patients with biopsy-proven primary IgAN in the NanJing Glomerulonephritis Registry from January in 1989 to December in 2004. Patients with estimated glomerular filtration rate (eGFR, MDRD formula) less than 30ml/min per 1.73m$^2$, or follow-up less than 12 month, were excluded. Clinical features at the time of renal biopsy were reviewed, and all the follow-up data was updated to May, 2010. There were 590 patients enrolled. Among those patients, 69 (12%) developed ESRD during the follow-up. The 5, 10, 15, and 20-year cumulative renal survival rate were 96%, 84%, 74% and 65% respectively. Univariate analysis indicated that lower initial eGFR, hypertension at presentation, no history of recurrent macroscopic hematuria, higher level of proteinuria, lower serum albumin, and hyperuricaemia, were the risk factors of ESRD. Gender, age, body mass index and microscopic hematuria were irrelevant factors.. Multivariate Cox regression confirmed that eGFR<60 ml/min per 1.73m$^2$ at biopsy(HR 4.0, *P<0.001*), the square root of proteinuria at biopsy (HR, 3.3, *P<0.001*), hypertension at presentation (HR 2.5, *P<0.001*), were the independent risk factors of long-term renal survival, and patients with recurrent macroscopic hematuria (HR 0.23, *P=0.047*) were strongly predictive of a favorable outcome. While the follow-up data were concerned, the median of proteinuria > 1.0g/24h(HR 8.9, P<001), the mean of mean artertial pressure(HR 1.02, *P=0.02*), and the initial eGFR<60 ml/min per 1.73m2 (HR 3.2, *P<0.001*), were the most important risk factors of a unfavorable outcome. In Conclusion, IgAN is a progressive disease. Our data shows that the renal survival was 84% within 10

years, and 65% within 20 years. The major independent risk factors were impaired eGFR, hypertension, and proteinuria, while the recurrent macroscopic hematuria was associated with a favorable outcome.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO123

**Renal and Patient Death of Idiopathic Membranous Nephropathy in Japan** Shinji Kitajima,[1] Yasuyuki Shinozaki,[1] Tadashi Toyama,[1] Akinori Hara,[1] Kiyoki Kitagawa,[1] Kengo Furuichi,[1] Hitoshi Yokoyama,[2] Takashi Wada.[1] *[1]Department of Nephrology, Kanazawa University Hospital, Kanazawa, Japan; [2]Department of Nephrology, Kanazawa Medical University Hospital, Kanazawa, Japan.*

BACKGROUND: The 20-year renal survival of Idiopathic membranous nephropathy (IMN) in Japanese adults with nephrotic syndrome was reported around 60%. On the other hand, the long-term patient survival of IMN remains unclear so far. In this study, we evaluated the predisposing clinicopathological factors of IMN patients with renal or patient death.

METHODS: One hundred forty three patients (84 males and 59 females; mean age 46.2 years) with biopsy proven IMN from 1965 to 2006 in Kanazawa Universitiy Hospital were evaluated in this study. Clinicopathological features were evaluated for renal and patient death.

RESULTS: Renal death was observed 14 out of 143 patients (9.8%). The percentage of nephrotic syndrome was higher in renal death group than that in non renal death group (92.9% vs. 69.0%). Based on the electron microscopic findings, the patients were assigned to two distinct groups, homogeneous type and heterogeneous type (synchronous electron dense deposits or various phases of dense deposits in basement membrane, respectively) as we previously published in Kidney International in 2004. The heterogeneous group showed higher renal death rate than that in homogeneous group (25.0% vs. 1.4%). Moreover, the patient group with massive proteinuria (> 3.0g/day) under any treatment had the higher rate of renal death. Patients death was observed 24 four out of 143 patients (16.8%). The major three causes of patients death were cardiovascular disorder (9 patient, 38%), malignant tumors (7 patients, 29%), and infection (5 patient, 21%). Patient death due to infection was observed in early clinical period of IMN. Older age and higher rate of nephrotic syndrome at onset were significant predictive factors for patient death due to infection.

CONCLUSION: Onset with nephrotic syndrome, heterogeneous type, and no response to treatment were predictors of progression to renal death. Infection was one of major cause of death in early clinical period of IMN, and older patients with nephrotic syndrome needs careful treatment.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO124

**Influence of Anti-Agalsidase Antibody on Clinical Outcomes in the Canadian Fabry Disease Initiative Study** Michael L. West,[1] Daniel G. Bichet,[2] Sandra Sirrs,[3] Christiane Auray-Blais.[4] *[1]Medicine, Dalhousie University, Halifax, NS, Canada; [2]Medicine, Universite de Montreal, Montreal, NS, Canada; [3]Medicine, University of British Columbia, Vancouver, BC, Canada; [4]Pediatrics, Universite de Sherbrooke, Sherbrooke, QC, Canada.*

Fabry disease is a rare X-linked lysosomal storage disorder which causes renal failure, cardiomyopathy and premature strokes. Enzyme replacement therapy (ERT) with recombinant agalsidase stabilizes renal function with a sustained fall in urine globotriaosylceramide (Gb3), the glycosphingolipid enzyme substrate that accumulates in the kidney in Fabry disease. Antibodies (Ab) to agalsidase develop in many patients with Fabry disease but the clinical significance of this is unknown. We report the anti-agalsidase antibody status of patients in the Canadian Fabry Disease Initiative study (CFDI) (Clinical Trial Registration protocol # NCT 00455104) receiving ERT (agalsidase alfa 0.2 mg/kg, agalsidase beta 1.0 mg/kg) IV every 2 wks. No difference in clinical outcomes has been seen between the 2 forms of agalsidase so far. Anti-agalsidase antibody ELISA assays done by the drug manufacturers vary in method and in sensitivity. Of the 288 patients in the CFDI study at 42 mos, 149 received ERT: antibody results were done in 121: 89 (73.6%) negative and 32 (26.4%) positive, median titer 1/800, range 1/50-1/25,600. Mean age, time on ERT and clinical parameters did not differ between the two groups at baseline. Ab + was more likely in males (40.8%) and in those on agalsidase beta (81.2%). Urine Gb3 levels fell less at 24 mos in Ab + patients (26.1%) vs Ab – patients (65.1%). A greater number of clinical events (renal, cardiac, stroke, death) occurred in Ab + (25%) vs. Ab – patients (20.2%). Intermediate/high Ab titer (≥ 1/800) was associated with no fall in urine Gb3 but no difference in clinical outcomes vs. low Ab titer. The presence of anti-agalsidase Ab of any titer is associated with lower urine Gb3 and worse clinical outcomes. While this suggests that Ab interferes with ERT action in the kidney in some way, an effect of male gender with more severe disease cannot be excluded in the Ab + group.

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Shire Human Genetic Therapies
Sumitomo Pharma
Amicus Therapeutics; Scientific Advisor: Shire Human Genetic Therapies.

### TH-PO125

**Altered Regulations of Matrix Metalloproteinase (MMP)-2 Are Associated with a Rat Model of CKD-MBD** Neal X. Chen,[1] Kalisha O'Neill,[1] Xianming Chen,[1] Kraiwiporn Kiattisunthorn,[2] Vincent H. Gattone,[1] Sharon M. Moe.[1,3] *[1]Indiana University School of Medicine, Indianapolis, IN; [2]Faculty of Siriraj Medical School, Mahidol University, Bangkok, Thailand; [3]Roudebush Veterans Medical Center, Indianapolis, IN.*

MMPs are responsible for extracellular matrix degradation which is critical for bone and arterial remodeling and therefore may play a role in the pathogenesis of CKD-MBD. We have previously demonstrated that the Cy/+ rat is a progressive model of CKD-MBD, with spontaneous hyperphosphatemia, hyperparathyroidism and vascular calcification. The objective of the current study was to determine if altered regulation of MMP-2 may predispose to extracellular matrix degradation, facilitating arterial calcification. Sera were collected from Normal or Cy/+ rats at 20, 29, 34 or 38 weeks and the MMP-2 level and activity determined by ELISA or zymography. The results demonstrated that at each time point, serum total MMP-2 levels are increased in CKD rats compared to that in normal rats, as were levels of tissue inhibitor of metalloproteinases (TIMP)-1. Importantly, MMP-2 activity by zymography was progressively increased in the Cy/+ rats with the development of CKD-MBD (20 week=28507±1101 Odu vs. 38 week 45199±5055 Odu, overall p over time<0.001). MMP-2 expression by real time RT-PCR in aorta tissue from 34 week old animals showed increased expression in Cy/+ rats compared to Normal rats (5.7±1.1 vs. 2.6±0.4, p<0.02). To further investigate the mechanism by which MMPs might be involved, we used the aorta ring culture model which calcifies in the presence of sodium phosphate and alkaline phosphatase. Immunohistochemistry demonstrated an increased expression of MMP-2 in areas of calcification compared to non calcified section. Furthermore, MMP inhibitors, GM6001 and doxycycline, significantly reduced calcification of the aorta rings (95.6±16.6 μmol/g, control; 58.1±11.7 μmol/g, GM6001; 34.3±8.6 μmol/g, doxycycline, p<0.001). In conclusion, MMP-2 activity increases with progressive CKD-MBD in vivo and ex vivo, and blockade of MMP acitivity ex vivo can inhibit arterial calcification. Thus, matrix remodeling may be important contributor to CKD-MBD.

Disclosure of Financial Relationships: Research Funding: Amgen, Inc; Patent: Licensing agreement with AMGEN.

### TH-PO126

**Development of Klotho-FGFR1/3 Dependent Resistance to FGF-23 in Human Aortic Smooth Muscle Cells Exposed to Calcifying Stress** Kenneth Lim,[1,2] Tzong-Shi Lu,[1] Daniel Zehnder,[2] Li-Li Hsiao.[1] *[1]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Clinical Sciences Research Institute, Warwick Medical School, Coventry, United Kingdom.*

Vascular calcification (VC) is a significant contributor to cardiovascular mortality in chronic kidney disease (CKD). Osteo/chondrocytic transformation and simultaneous dedifferentiation of smooth muscle cells (SMC) are important in the pathogenesis of VC. FGF-23 is a bone-derived circulating hormone that binds to FGFR1/3-Klotho receptor complex at the kidney to counteract hyperphosphatemia. FGF-23 levels rise in CKD, however there are currently conflicting data on the role of FGF-23 in the inhibition of VC. Our results show for the first time that Klotho and FGFR1/3 are expressed in healthy human arteries, in vivo and normal HA-SMCs, in vitro. Co-expression of Klotho with FGFR1/3 was observed in normal HA-SMCs on immunostaining. We found that Klotho, FGFR1/3 expression were downregulated in HA-SMCs grown in borderline high (2.0mM CaCl2 and 2.7mM β-Glycerolphosphate(GP)) and high (5mM CaCl2 and 5mM β-GP calcification medium, in short-term (3 and 6 hours) and long-term (21 day) cultures, in vitro. These cells exhibited concomitant core binding factor alpha-1-dependent osteo/chondrocytic transformation and loss of serum response factor (SRF)-dependent SMC contractile phenotype. Downregulation of klotho expression was also observed following treatment of HA-SMCs with the SRF inhibitor, CCG-1423 (10μM), suggesting SRF-dependent regulation of Klotho. We next showed that FGF-23 treatment (1ng/ml) of HA-SMCs induced upregulation of P-ERK and P-AKT expression, in vitro. However, FGF-23 failed to induce P-ERK and P-AKT expression in HA-SMCs exhibiting loss of klotho expression. In summary, we show for the first time that Klotho and FGFR1/3 are co-expressed in HA-SMCs; loss of Klotho and FGFR1/3 was associated with the development of VC; regulation of Klotho expression is SRF-dependent; and FGF-23 failed to initiate intracellular signaling in the absence of Klotho. We therefore conclude that downregulation of Klotho-FGFR1/3 expression in arteries exposed to calcifying stress may mediate vascular resistance to FGF-23.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO127

**Antioxidant Ameliorates the Progression of Vascular Calcification in Uremic Rats** Shunsuke Yamada,[1] Masatomo Taniguchi,[1] Masanori Tokumoto,[3] Kazuhiko Tsuruya.[2] *[1]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [3]Department of Medicine, Fukuoka Dental College, Fukuoka, Japan.*

(Background) Oxidative stress has been reported to be involved in the pathogenesis of vascular calcification (VC). This study was aimed to elucidate the role of oxidative stress in VC progression by assessing the effect of apocynin, an antioxidant and a specific inhibitor of NADPH oxidase.

(Methods) Ten-week-old SD rats were divided into the three groups (n=12 for each group); 1) control rats (fed normal diet and water)(CNT), 2) CKD rats (fed adenine diet and normal water)(CKD), 3) CKD rats treated with apocynin (fed adenine diet and 1.5 mM apocynin water) (CKD+APO). All rats were killed after 7 weeks. Oxidative stress of the aorta was assessed using immunohistochemistry (IHC) for 8-hydroxydeoxyguanosine(8-OHdG), and urinary 8-OHdG content was assessed as systemic oxidative stress. The calcified area was confirmed by von Kossa staining, and the features of calcified area were assessed by IHC. The aortic calcification was quantitatively determined by Ca content in the aorta.

(Results) CKD and CKD+APO developed progressive renal failure over 7 weeks in contrast with CNT. Von Kossa staining revealed arterial medial calcification in CKD and CKD+TMP. Twenty-four-hour urinary 8-OHdG content and Ca content in the aorta in CKD significantly ($p<0.05$) increased compared to CNT. The urinary 8-OHdG content showed significant ($p<0.05$) positive correlation with Ca content in the aorta. The calcified area of aorta in CKD was positive for Runx2, osteocalcin and 8-OHdG as assessed by IHC, suggestive of the involvement of osteoblast-like cells. The aortic Ca content and the urinary8-OHdG content in CKD+APO were significantly ($p<0.05$) lower than those in CKD.

(Conclusion) Apocynin prevented the progression of VC in uremic rats, signifying that oxidative stress plays pivotal roles in the progression of VC in uremia.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO128

**PA21, a New Iron-Based Phosphate Binder Prevents Arterial Calcification in Chronic Renal Failure Rats?** Olivier Phan,[1] Marc P. Maillard,[1] Christine Perregaux,[1] David Mordasini,[1] Stehle J. C Jean-Christophe,[3] Felix W. Funk,[2] Michel Burnier.[1] *[1]Nephrology, CHUV, Lausanne, Switzerland; [2]Vifor (International) Inc, St Gallen, Switzerland; [3]MPF UNIL, CHUV, Switzerland.*

BACKGROUND: The use of calcium-based phosphate binders to control hyperphosphatemia can induce hypercalcemia and has been associated with progression of vascular calcifications. The purpose of the present study was to evaluate the effects of PA21, a new iron-based non calcium phosphate binder, on the development of vascular calcifications in uremic Wistar rats.

METHODS: Chronic renal failure (CRF) was induced by feeding rats with an adenine-enriched diet for 4 weeks. Then CRF rats were randomized to receive either PA21 5% or calcium carbonate ($CaCO_3$) 3% in the diet or a placebo for another 4 week period. Drugs were added to the food and control non-CRF rats received the same diet (n=6-11/group). Vascular calcifications were assessed blinded on random sections of several vessels (aorta, carotid and femoral arteries). The degree of Von Kossa positivity was scored semi-quantitatively with scores ranging from 0 to 3 depending on the surface of Von Kossa positivity.

RESULTS: At randomization, no statistical difference was observed for serum calcium, phosphate, creatinine and body weight between the CRF groups. At sacrifice, serum phosphate was similarly decreased by $CaCO_3$ 3% and PA21 5% (to 2.02 and 2.21 mmol/l respectively) compared with CRF control rats (2.86 mmol/l, $p <0.005$). Serum calcium, creatinine and weight were similar between the CRF groups. Preliminary results show that despite comparable effects on serum phosphate and calcium, PA21 5% was associated with a significantly lower score of vascular calcifications ($p= 0.043$, chi-square test) when compared with $CaCO_3$ 3%-treated CRF rats and controls.

CONCLUSION: These preliminary results suggest that the new iron-based phosphate binder PA21 reduces the development of vascular calcifications in rats with chronic renal failure beyond the control of phosphatemia and hypercalcemia.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO129

**Serum Glycoprotein Fetuin-A Is Degraded in Renal Proximal Tubular Epithelial Cells** Kazunori Inoue,[#1] Isao Matsui,[#1] Chikako Nakano,[#1] Hiroki Omori,[#1] Jun-Ya Kaimori,[#1] Takayuki Hamano,[#2] Toshiki Moriyama,[#1] Hiromi Rakugi,[#1] Yoshitaka Isaka.[#1] *[1]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, School of Medicine, Philadelphia, PA.*

Serum glycoprotein fetuin-A is synthesized in the liver, secreted into blood and thereby exerts potent inhibition of calcification. Although it has been revealed that hepatic and serum fetuin-A levels are repressed by inflammatory changes or malnutrition, the degrading pathway of fetuin-A is poorly understood. To assess the fetuin-A-degrading pathway, we performed immunohistochemical analysis using normal Wistar rat tissues. In addition to fetuin-A staining in the liver, we found dotted pattern staining of fetuin-A in renal proximal tubular epithelial cells. Because normal rat kidney dose not express mRNA for fetuin-A, this result suggested an uptake of fetuin-A in these cells. For the inhibition of proximal tubular reabsorption, a basic amino acid L-lysine or histidine-tagged soluble receptor-associated protein (His-RAP) was administrated. After the treatment with L-lysine, dotted pattern staining of fetuin-A completely disappeared concomitantly with the excretion of fetuin-A into the urine. Glycine administrated rats, which served as control in L-lysine administrated experiments, did not show such changes. Megalin, a key player of proximal tubular reabsorption, was localized at the base of brush border in glycine administrated rats, whereas L-lysine administration was associated with an intense brush border labeling for megalin to the tip of the microvilli. Similar to the results in L-lysine administrated rats, His-RAP injected rats showed a decreased staining of fetuin-A in the proximal tubular cells. We further analyzed whether lysosomal blockade changes fetuin-A distribution in proximal tubular cells. Immunohistochemistry revealed that intraperitoneal injection of leupeptin accumulated fetuin-A in the proximal tubular cells. Western blot analysis confirmed the lysosomal distribution of fetuin-A in the leupeptin injected rats. Kidney plays a role in fetuin-A metabolism.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO130

**Breast Arterial Calcification: A Window into the Prevalence, Pathogenesis, and Significance of Medial Vascular Calcification in Chronic Kidney Disease** W. Charles O'Neill,[1] Valerie S. Duhn,[1] Amy Adams,[2] Ellen M. D'Orsi,[3] Carl J. D'orsi.[3] *[1]Renal Division, Emory University, Atlanta, GA; [2]Department of Pathology, Emory University, Atlanta, GA; [3]Department of Radiology, Emory University, Atlanta, GA.*

Renal failure predisposes to medial vascular calcification but its prevalence and significance are unknown because imaging is not routinely performed, has unknown sensitivity, and cannot distinguish it from intimal calcification. Because of the frequency of mammography and breast excision, breast arterial calcification (BAC) may provide a useful window into medial vascular calcification in CKD. Medial calcification but not intimal calcification was present in all 18 specimens (visible only by von Kossa staining in 5) of breast tissue from patients with ESRD (n=8) or CKD (creatinine 1.0-3.9). Coarse calcifications were present in 5, while 13 cases showed granular staining. Prominent linear staining of the internal elastic lamina (IEL) was noted in 8 cases. There was no staining for osteocalcin, a marker of osteogenesis. Mammograms in 71 patients with ESRD and 66 controls without renal disease (serum creatinine < 1.0 mg/dl) matched for age and diabetes were retrospectively reviewed. The mean age of the patients was $60.4 \pm 2.1$ (range: 35-85), mean ESRD duration was $5.7 \pm 0.9$ yr, and 55 % had diabetes. The prevalence of BAC was 63 % in ESRD patients compared to 15 % in controls ($p<0.001$). Of 16 patients with subsequent mammograms, 8 showed progression of BAC. Peripheral vascular disease (OR = 3.0; $p = 0.028$) but not coronary artery disease (OR = 1.05) was more prevalent in those with BAC. BAC was present in 36 % of mammograms performed prior to ESRD ($5.5 \pm 0.7$ yrs). We conclude that BAC is limited to the media and the internal elastic lamina and does not occur in the intima, and that early medial calcification may be universal in CKD. Our data do not support osteogenic differentiation of smooth muscle as an initial event. The prevalence of BAC on mammography is markedly increased in ESRD and CKD and may correlate with peripheral vascular disease. Breast arterial calcification will be a useful tool to study medial vascular calcification in CKD.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO131

**Thiosulfate Inhibits Vascular Calcification In Vitro Independent of Interactions with Calcium or Hydroxyapatite** W. Charles O'Neill,[1] Kenneth Hardcastle,[2] George R. Dubyak.[3] *[1]Renal Division, Emory University, Atlanta, GA; [2]Department of Chemistry, Emory University, Atlanta, GA; [3]Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH.*

Thiosulfate has been shown to inhibit vascular calcification in rats and may inhibit calcification in humans but whether this is due to a systemic or local action is unknown. The underlying mechanism is also unclear but complexation of calcium ions has been proposed. In vitro assays were used to determine the effect of thiosulfate on vascular calcification and hydroxyapatite formation. Thiosulfate ($EC_{50}$: 1-2 mM) prevented calcification of cultured aortas induced by injury but not calcification induced by alkaline phosphatase. Similar results were obtained with sulfate, which was more potent. There was no effect on reversal of calcification. Thiosulfate had no effect on the calcification of purified elastin or on the formation of hydroxyapatite from seed crystals. There was no effect on pyrophophosphate metabolism or on the inhibition of hydroxyapatite formation by pyrophosphate. Measurements with an ion-sensitive electrode (corrected for changes in ionic strength) revealed a very weak interaction between thiosulfate and calcium ($K_a = 3.5 \times 10^{-5}$ L/mol). Adjustment of the calcium concentration to account for this did not prevent the inhibition of aortic calcification by thiosulfate.

It is concluded that thiosulfate can directly inhibit vascular calcification but at millimolar concentrations. This effect is not specific for thiosulfate since sulfate has similar properties. Inhibition is dependent on the cause of calcification and cannot be explained by interactions with elastin, pyrophosphate, hydroxyapatite, or calcium. The mechanism by which thiosulfate inhibits vascular calcification remains to be elucidated.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO132

**The Effect of Sodium Thiosulfate (STS) on Measures of MBD** Tracy Jack Mayne, Tasos Constantin, Carey Colson, Mahesh Krishnan. *DaVita Inc., Denver, CO.*

STS has been used to treat calciphylaxis in ESRD. Though the MOA involves mobilization of calcium, the longitudinal effect on markers of MBD has not been explored in a large setting. A retrospective analysis of 203 dialysis patients treated with STS from 01/01/09 to 12/31/09 was completed at a US dialysis organization. Changes in pre/post initiation Ca, P, PTH and IV paracalcitrol dose (IV D) were examined using repeated measures general linear models. Change in % of patients treated with cinacalcet was assessed using Cochran's Q. We examined patients treated for 1 (n=203), 2 (N=68) and 3 (N=42) consecutive months.

Results:

| STS Treatment | Mo. -2 | Mo. -1 | Mo. 0 | Mo. 1 | Mo. 2 | Mo. 3 | p-value |
|---|---|---|---|---|---|---|---|
| **1 Mo.** | | | | | | | |
| | | | | | | | |
| Ca | 8.9±0.9 | 8.8±0.8 | 8.9±0.9 | 8.9±1.0 | | | 0.81 |
| P | 6.2±2.1 | 6.0±2.1 | 5.5±1.8 | 5.5±2.0 | | | <0.01 |
| PTH | 549.5±714.4 | 505.4±585.2 | 422.4±459.5 | 424.0±587.1 | | | <0.01 |
| IV D | 5.7±4.1 | 5.7±4.0 | 5.2±3.6 | 4.7±3.4 | | | 0.55 |
| Cinacalcet | 30.5% | 34.5% | 34.0% | 25.6% | | | 0.05 |
| **2 M.** | | | | | | | |
| | | | | | | | |
| Ca | 9.0±0.7 | 8.9±0.6 | 8.9±0.8 | 8.9±0.8 | 9.0±0.7 | | 0.64 |
| P | 5.7±1.7 | 5.4±1.8 | 5.2±1.8 | 5.1±1.7 | 5.4±2.1 | | 0.11 |
| PTH | 578.0±1035.9 | 485.0±737.1 | 457.9±563.6 | 403.6±672.3 | 575.3±1158.7 | | 0.38 |
| IV D | 5.5±4.6 | 4.9±4.2 | 4.8±3.8 | 4.1±3.1 | 4.1±3.2 | | 0.97 |
| Cinacalcet | 32.4 | 35.3 | 41.2 | 35.3 | 32.3 | | 0.21 |
| **3 M.** | | | | | | | |
| Ca | 8.9±0.7 | 8.9±0.6 | 8.9±0.7 | 8.9±0.8 | 8.9±0.8 | 9.0±0.7 | 0.95 |
| P | 5.7±1.7 | 5.4±1.9 | 5.4±1.8 | 5.2±1.9 | 5.6±2.1 | 5.6±1.7 | 0.21 |
| PTH | 423.4±679.2 | 343.6±353.5 | 302.9±382.2 | 415.0±860.7 | 414.5±619.7 | 478.7± 706.0 | 0.04 |
| IV.D | 5.5±4.8 | 5.3±4.6 | 4.8±4.0 | 3.9±2.7 | 4.3±3.2 | 4.4±3.1 | 0.40 |
| Cinacalcet | 40.5 | 38.1 | 42.9 | 33.5 | 35.7 | 38.5 | 0.50 |

This may be the largest case series of patients receiving STS treatment for clinically diagnosed calciphylaxis. STS treatment was associated with reductions in P, PTH and cinacalcet in the first 2 mo following initial treatment. Serum Ca and IV paricalcitrol dose showed no change. Results suggest STS transiently improves MBD outcomes, but improvements aren't maintained over time. A RCT is needed to determine the benefit of STS on intermediate and terminal outcomes.

Disclosure of Financial Relationships: Employer: DaVita, Inc.; Honoraria: Sanofi-Aventis.

## TH-PO133

### Sodium Thiosulfate (STS) Dosing Strategies for Treatment of Calciphylaxis

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**Background:** Calciphylaxis is a poorly understood but life threatening disorder with limited therapeutic options. STS ($Na_2S_2O_3$) has reported efficacy, thought due to it dissolving calcium deposits by producing the calcium salt ($S_2O_3Ca$), which is extremely soluble and cleared by hemodialysis (HD). The exposure to the STS (area under curve, AUC) is thought to be proportional to its ability to dissolve calcifications.

**Aim:** Given that there are successes using an empiric 25 gm IV 3x/wk given after HD, we performed simulations to determine dosing strategies for alternative, more or less intense dialysis regimens so as to produce equivalent AUC drug exposure.

**Methods:** Since a 2 compartment model has described pharmacokinetics of STS in healthy humans, we used it to simulate exposure for HD. The modeled prescriptions varied HD time from 12-40 hrs/week over 3-6 sessions, QB 200-400 ml/min, QD 500 or 800 ml/min, or CVVHD at low flow rates, using F160 high-flux filters, and also accounting for residual renal function. The simulations to calculate the AUC exposure were performed using nonlinear mixed-effect modeling software (NONMEM).

**Results:** Simulations showed a marked variation in STS doses depending upon HD frequency and duration. Blood and dialysate flows have a less prominent effect. Assuming no residual renal function, HD prescription permutations caused the dose to vary from 75-168 gm/week, with examples in the table below.

| Dialyses per week | 3 | 4 | 5 | | 6 | CVVHD |
|---|---|---|---|---|---|---|
| QB (ml/min) | 400 | 400 | 400 | 400 | 400 | 200 |
| QD (ml/min) | 800 | 800 | 800 | 800 | 800 | 33 |
| HD Time (hr) | 4 | 3 | 2.5 | 8 | 2 | continuous |
| Dose post-HD (g) | 25 | 22 | 18 | 24 | 16 | 24, daily |
| Weekly Dose (g) | 75 | 88 | 90 | 120 | 96 | 168 |

**Conclusion:** Based on 1) the success reported for one STS dosing regimen and 2) assuming AUC exposure of STS is proportional to its effect, pharmacokinetic simulations can be used to calculate the dose for alternative, higher or lower intensity dialysis regimens. These strategies are imperative to assure adequate treatment for this mortal disease, as well as to avoid toxicity from excess dosing.

Disclosure of Financial Relationships: Other Relationship: Consultancy and honoraria from Genzyme and Shire Corps.

## TH-PO134

### Response of Calcific Uraemic Arteriopathy to Sodium Thiosulphate; Ten Case Reports

Nicholas J. New, Dougal Stirling Carlisle, Dwarakanathan Ranganathan, Sree Krishna Venuthurupalli. *Nephrology Department, Royal Brisbane Women's Hospital, Brisbane, QLD, Australia.*

**INTRODUCTION:** Calcific uraemic arteriopathy (CUS) is an uncommon but increasingly recognized condition, associated with high morbidity and mortality. Pathogenetic mechanisms of calciphylaxis are incompletely understood, however calcium and phosphate homeostasis are integrally involved. Current multimodality treatment involves aggressive serum calcium and phosphate control with dialysis, non-calcium phosphate binders, cinacalcet and parathyroidectomy, wound care including surgical debridement and infection control. Sodium Thiosulphate and hyperbaric oxygen are emerging as promising agents for the treatment of CUS.

**AIM:** To assess Sodium Thiosulphate therapy for CUS at a tertiary care hospital.

**METHODS:** A retrospective chart review of all patients over the previous 5 years, treated for CUS using Sodium Thiosulphate. Treatment modalities were identified and success of treatment, as determined by survival, reduction in pain and/or size of skin lesions, was examined.

**RESULTS:** Ten patients received sodium thiosulphate for treatment of CUS over the previous 5 years, 9 of whom had End Stage Renal disease (ESRD) on dialysis. Complete resolution of lesions occurred in 1 patient who in addition underwent parathyroidectomy and received hyperbaric oxygen therapy. A further 4 patients demonstrated a reduction in both pain and size of skin lesions, 3 of whom also received hyperbaric oxygen. Only one death was directly attributable to CUS.

**CONCLUSIONS:** The small population size of our study does not permit definitive conclusions. Literature supporting the use of sodium thiosulphate remains limited to case studies. Further prospective research is required in order to fully determine the potential benefits of sodium thiosulphate in the treatment of CUS in ESRD.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO135

### Effect of Sodium Thiosulfate on Urinary Lithogenicity in Normal Adults

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**Purpose**: Sodium thiosulfate (STS) has been shown to reduce calcium stone formation in humans (Yatzidis H; Clin Nephrol 1985) and hypercalciuric rats (Asplin et al; J Am Soc Nephrol 2009). We studied the effects of STS on urine chemistry in normal adults.

**Methods**: 5 healthy people with a mean age of 34 years completed the study. Two baseline 24-hour urine collections were done and then repeated after oral intake of STS 10 mmol BID for 7 days. Participants kept a diet diary during baseline urine collections and replicated the diet during the STS urine collections. Results were compared by non parametric Wilcoxon signed rank test.

**Results**:

Effect of STS on 24 Hour Urine Chemistry

| 24 hour excretion: | Calcium (mg) | Oxalate (mg) | Phos (mg) | pH | NH4 (meq) | Citrate (mg) | SO4 (meq) |
|---|---|---|---|---|---|---|---|
| Mean baseline | 111.5 | 37.8 | 805 | 6.67 | 24.8 | 740.6 | 44 |
| SD | 24.9 | 15.1 | 410.2 | 0.39 | 5.9 | 504.7 | 18.8 |
| Mean post STS | 147 | 36.3 | 830 | 6.08 | 43.4 | 713.9 | 85.8 |
| SD | 43.9 | 17.3 | 496.5 | 0.40 | 17.3 | 548.6 | 29.3 |
| p value | 0.08 | 0.9 | 0.5 | <0.05 | <0.05 | 0.5 | 0.08 |

SD: standard deviation

Urine pH decreased while urinary $NH_4$ excretion increased without any significant change in urinary citrate. Urinary Ca excretion rose; $PO_4$ and oxalate excretion did not change. Urinary $SO_4$ excretion increased but urinary thiosulfate concentrations were negligible post STS. Urine volume, creatinine, urea, sodium and other measured analytes remained unchanged, confirming replication of diets during the collections.

**Conclusions**: Increased urinary $Ca^{2+}$ excretion would be counterproductive for prevention of stones. Also, STS may be causing metabolic acidosis, suggested by a fall in urinary pH and increased urinary $NH_4$. Interestingly, a rise in urine $SO_4$ with negligible urine thiosulfate suggests a conversion of thiosulfate to sulfate, hence challenging the previously proposed theory of $Ca^{2+}$ chelation by STS as its mechanism of action. Whether STS reduces stone formation in hypercalciuric stone formers has not been tested in a controlled study.

Disclosure of Financial Relationships: Consultancy: Takeda, Watson; Ownership: Ravine GroupResearch Funding: Amgen, Kibow; Honoraria: Genzyme.

## TH-PO136

### Urinary Response to an Oral Oxalate Load after Roux-en-Y Gastric Bypass

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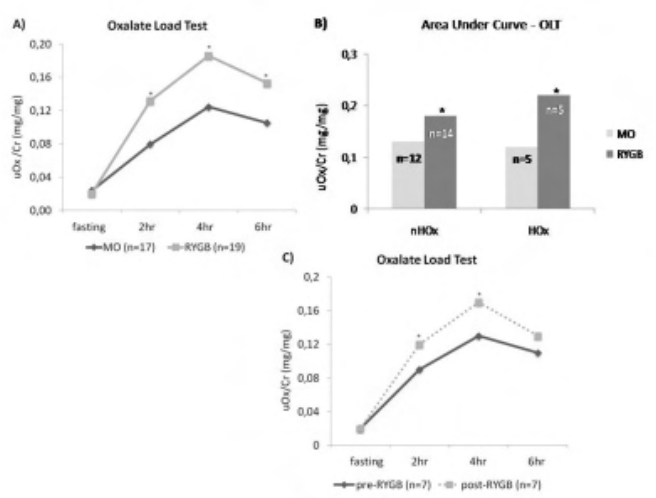
Several studies have reported increased oxalate excretion and risk for stone formation following bariatric surgery but the underlying mechanisms for hyperoxaluria are not fully elucidated yet. We aimed to evaluate the urinary response to an oral oxalate load by patients who had undergone Roux-en-Y gastric bypass (RYGB) for at least 6 months. Methods: A total of 19 RYGB patients and 17 morbidly obese (MO) subjects (controls) collected a 24-hour urine specimen and were classified as Hyperoxalurics (HOx) or non-Hyperoxalurics (nHOx). There have been 5 HOx and 3 stone formers in each group. All patients were further submitted to an oral oxalate loading test (OLT) consisting of two-hour urine samples obtained after an overnight fast, 2, 4 and 6 hours after a 375mg oxalate load (spinach). In addition, a prospective longitudinal analysis of 7 patients who underwent the OLT both before (pre-RYGB) and after 6 months of the procedure (post-RYGB) was performed. Results: Calcium excretion did not differ between the periods of the test. The mean urinary oxalate-to-creatinine (uOx/Cr) ratio in RYGB was significantly higher vs. MO at all periods after the load (figure 1A) and the mean area under the curve (AUC) of RYGB was higher vs. MO ($0.20 \pm 0.05$ vs.$0.13 \pm 0.04$, $p<0.001$). As shown in figure 1B, mean AUC of RYGB was significantly higher vs. MO both among nHOx and HOx but there was a trend for a higher mean AUC value achieved by the RYBG HOx group. Mean uOx/Cr of post-RYGB patients (figure 1C) was significantly higher 2 and 4 hours after the oxalate load vs. pre-RYGB and AUC value was also higher in the former ($0.18 \pm 0.05$ vs.$0.14 \pm 0.04$, $p = 0.02$).




Conclusions: RYGB patients exhibited an exaggerated urinary response to an oral oxalate load suggesting an increased intestinal absorption of dietary oxalate. These patients may benefit from oxalate restriction.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO137

**Increased Risk for Calcium Oxalate Nephrolithiasis after Roux-en-Y Gastric Bypass Surgery Starts in the Early Post-Operative Period** Varun Agrawal, Daniel L. Landry, Xiao Jie Liu, Ann Lagoy, Inta Discenza, Thomas Campfield, John Romanelli, J. Enrique Silva, Gregory Lee Braden. *Baystate Medical Center, Springfield MA.*

Hyperoxaluria and nephrolithiasis are known complications of Roux-en-Y gastric bypass surgery (RYGB), but little is known if this stone risk starts in the early post-operative period and whether it changes with time. We prospectively studied 13 morbidly obese adults who underwent RYGB and had urine collections 2 weeks before, 1, 2, 4 and 6 months after surgery. Relative saturation ratio (RSR) with regard to calcium oxalate was computed by EQUIL. Non-parametric analyses were performed and results expressed as median (25th-75th %ile). Eleven subjects were female, median age was 40(36-48)years and none had history of diabetes or nephrolithiasis. Body mass index decreased from 44.6 (41.6-47.6)kg/m² to 34.1 (30.4-36.8)kg/m² in 6 months, p<0.0001. We observed significant decreases in urine volume [2100 (1537-3290) to 1360 (1200-1700)ml, p<0.0001], urine sodium [207.3 (145.6-258.4) to 92.5 (57.8-203.8)mmol/24h, p=0.002], urine potassium [70.1 (39.7-111.4) to 32.1 (22.4-53.8)mmol/24h, p<0.0001], urine magnesium [8.7 (4.2-14.1) to 7.1 (3.3-11.4) mg/24h, p=0.016], urine phosphorus [1.0 (0.7-1.2) to 0.5 (0.4-0.9)g/24h, p=0.01] , urine uric acid [0.6 (0.4-0.8) to 0.4 (0.3-0.6)g/24h, p=0.015] and creatinine clearance [144.0 (117.5-193.1) to 108.5 (90.8-164.8)ml/min, p=0.046]. The increase in urine oxalate approached significance [28.6 (18.7-49.7) to 51.6 (25.2-85.0)mg/24hour, p=0.054]. No change was seen in urine pH, 24 hour urine creatinine or 24 hour urine calcium excretion. Calcium oxalate RSR increased after RYGB [1.7 (1.2-3.6), 4.5 (1.6-9.2), 5.73 (2.1-12.2), 5.27 (2.7-9.1), 6.3 (3.4-13.9) at baseline, 1, 2, 4 and 6 months respectively, p=0.020). One patient had critical supersaturation (RSR=23.7) at 1 month that peaked at 42.3 at 6 months after RYGB. We conclude that calcium oxalate RSR and risk for nephrolithiasis start early and persist in the first 6 months after RYGB due to decreased urine volume, magnesium and increased urine oxalate. Studies of increased fluid intake and oral agents to bind enteric oxalate to reduce this risk are needed.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO138

**Increased Intestinal Oxalate Absorption, Serum Oxalate Concentrations and Urinary Calcium Oxalate Supersaturation in Patients after RYGB Surgery** Rajiv Kumar, John C. Lieske, Maria L. Collazo-Clavell, Ellen Olson, Xujian Li, Eric J. Bergstralh, Zachary C. Ryan. *Mayo Clinic, Rochester, MN.*

Patients frequently develop hyperoxaluria after RYGB surgery for morbid obesity. The mechanism by which hyperoxaluria occurs is unknown. We hypothesized that hyperoxaluria and increased serum oxalate (Ox) concentrations occur as a result of the intestinal hyperabsorption of Ox. We tested 11 RYGB patients for the presence of intestinal Ox hyperabsorption before, 6, and 12 months (m) after surgery. Fasting serum and urine Ox, and urine Ox following oral administration of 120 mg of Ox were measured. Relevant urinary analytes and supersaturations for calcium (Ca) Ox, apatite, brushite, uric acid, and sodium urate were measured in 24-h urines. 72-h fecal fat was measured.

*Results:* The results (**Table)** demonstrate increases in serum Ox levels, urine CaOx supersaturation, and 72 h fecal fat excretion at 6 and 12 m after RYGB surgery. An increase in urine Ox levels following an oral dose of 120 mg of disodium Ox was observed at both 6 and 12 m.

Serum and Urine Values Before and After RYGB

| Serum or urine test | Baseline, 0 m, mean±SD | 6 m, mean±SD | 12 m, mean±SD | P-value, paired t-test, 0-6 m | P-value, paired t-test, 0-12 m |
|---|---|---|---|---|---|
| Serum Ox, mol/L | 1.2±0.36 | 2.2±1.26 | 1.9±0.84 | 0.018 | 0.016 |
| Urine Ox, mg/24h | 26.4±13.26 | 27.2±8.21 | 32.6±11.42 | 0.89 | 0.18 |
| Urine volume, mL/24h | 2091±768 | 1317±540 | 1596±568 | 0.02 | 0.11 |
| CaOx DG SS | 1.0±0.88 | 2.3±0.36 | 1.8±0.54 | 0.003 | 0.009 |
| Urine Ox following oral Ox load, mg/24h | 35.3±8.22 | 64.4±23.1 | 69.7±17.86 | 0.02 | <0.0001 |
| Fecal fat, g/72h | 4.1±3.48 | 9.3±6.93 | 7.7±4.31 | 0.026 | 0.055 |

*Conclusions:* An increase in the proportion of Ox absorbed in the intestine and excreted in the urine following a dose of oral oxalate accounts for the increase in serum oxalate concentrations and urinary CaOx supersaturation values at 6 and 12 m following RYGB surgery. Increases in fecal fat are likely to reduce the amount of free Ca available for binding to oxalate causing absorptive intestinal hyperoxaluria in RYGB patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO139

**Sex and Sib Prevalences of Upper Urinary Tract Stone Disease (UUTS): The Effects of Urinary Physicochemical and Inhibitory Activities** Suchai Sritippayawan. *Renal Division, Internal Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.*

We explored sex and sib prevalences of UUTS and study urinary physicochemical and inhibitory activities(IA) of stone formers(SF), nonstone former sibs(NSFS) and controls(C) in northeastern(NE) Thailand.

**Methods** Prevalence of UUTS in GP was studied by randomly choosing subjects>15 years(y) from the census records of 5 villages(n=344). Prevalence in sib study was hospital-based(n=430). Plain KUB film was done in all subjects. Fasting urine(U)Ca, oxalate(Ox), citrate(Cit), creatinine(Cr) and CaOx supersaturation(SS) were measured in 95 CaOxSF, 158 NSFS and 114 C. Urine was also dialyzed against distilled water using 80 kd cutoff membrane and 1 µg of urine protein was used for CaOx crystal aggregation assay by spectrophotometry. Bad inhibitor was the urine protein having IA less than the IA of 1 µg of control Tamm Horsfall protein.

**Results** UUTS was 3 times more prevalent in sib than in GP. Male(M):Female(F) ratio was 1.3-2.6:1 in subjects<70y, but the ratio reversed to 0.5:1 in subjects≥70y. SF had significantly(p<0.05) higher UCa/Cr(370vs257 mmol/mol), UCa/Cit(15.6vs5.9), UOx/Cr(30.5vs24.6 mmol/mol) and CaOxSS(6.4vs5.2) than C. NSFS had significantly higher CaOxSS(6.4vs5.2) and UCa/Cr(321vs257) than C but had significantly lower UCa/Cit(7.8vs15.6), UOx/Cr(24.6vs30.5) and higher UCit/Cr(1.2vs0.84 mmol/gCr) than SF. Bad inhibitor was significantly higher in SF than NSFS&C(37.5%vs10.9%&9.4% respectively). Males had significantly lower UOx/Cr and UCit/Cr than females in all 3 groups(C,NSFS,SF: UOx/Cr=20.5,21.4,23.5vs27.3:26.5vs35.3; UCit/Cr=0.67,0.85,0.55vs1.08,1.51,1.03 respectively). UCit/Cr and UCa/Cr had negative correlation with age. UCit/Cr was significantly lower in females≥70y than females<70y(0.66vs1.32) and closed to the UCit/Cr in males≥70y(0.54). UOx/Cr decreased in M≥70y but increased in females≥70y(<70vs≥70y, M:21.9vs18.4, F:28.7vs32.9).

**Conclusion** This study demonstrated UCit & genetic influence on the UUTS in NE Thailand. The increase prevalence of UUTS in elderly female may be from the decrease in UCit and increase in UOx. SF had worse urinary promoters and inhibitors than C while NSFS had better inhibitors and UOx than SF.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO140

**Gallstones and the Risk of Nephrolithiasis** Eric N. Taylor,[1,2] Gary C. Curhan.[2,3] *[1]Division of Nephrology and Transplantation, Maine Medical Center, Portland, ME; [2]Channing Laboratory, Brigham and Women's Hospital, Boston, MA; [3]Renal Division, Brigham and Women's Hospital, Boston, MA.*

**Background**. Existing data on the potential association between gallstones and nephrolithiasis are suggestive but limited.

**Methods**. To evaluate the relation between symptomatic radiographically confirmed gallstones and/or cholecystectomy (GS) and prevalent kidney stones (KS), we conducted a cross-sectional study of three large cohorts including over 240,000 participants: the Nurses' Health Study I (older women), the Nurses' Health Study II (younger women), and the Health Professionals Follow-up Study (men). We then prospectively studied the association between history of GS and incident symptomatic KS over a combined 56 years of follow-up. We also prospectively examined the relation between history of KS and incident GS. Multivariate regression models adjusted for age, body size, thiazide diuretic use, fluid intake, diet, and other factors.

**Results.** At baseline, the multivariate odds ratio of prevalent KS in individuals with GS compared to individuals without was 1.67 (95 % CI 1.48 to 1.89) in older women, 1.87 (95% CI 1.67 to 2.10) in younger women, and 1.62 (95% CI 1.41 to 1.85) in men. Prospectively, the multivariate relative risk of incident KS in participants with GS compared to participants without was 1.32 (95 % CI 1.16 to 1.51) in older women, 1.35 (95% CI 1.17 to 1.55) in younger women, and 1.34 (95% CI 1.10 to 1.64) in men. The multivariate relative risk of incident GS in participants with a history of KS compared to participants without was 1.18 (95 % CI 1.07 to 1.30) in older women, 1.29 (95% CI 1.17 to 1.42) in younger women, and 1.48 (95% CI 1.29 to 1.69) in men. Prospective "lag" analyses instituting a delay of 4 years between the diagnoses of GS and KS yielded similar results.

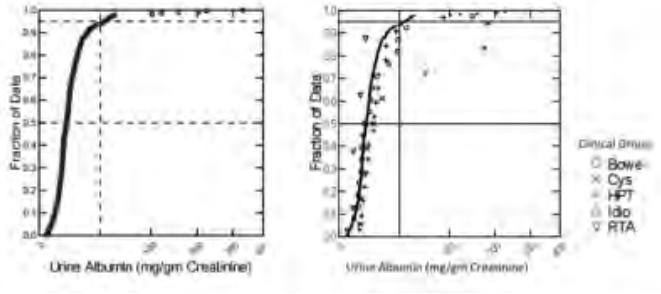
**Conclusion.** Gallstones are associated with kidney stones, independent of diet, body size, and other factors. Additional studies are needed to elucidate potential mechanisms underlying this association.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO141**

**Prevalence of Albuminuria Is Not Increased in Patients with Idiopathic Calcium Nephrolithiasis** Anna L. Zisman, Kristin J. Bergsland, Elaine M. Worcester, Fredric L. Coe. *Department of Medicine, Section of Nephrology, University of Chicago, Chicago, IL.*

Nephrolithiasis has been associated with decreased renal function in population studies, but those data are limited in that the stone forming population is poorly characterized. Microalbuminuria is associated with kidney injury. We hypothesized that stone formers (SF) would be more likely to show evidence of renal injury in the form of microalbuminuria. We evaluated the clinical and laboratory data for 549 SF who had submitted 24-hour urine collections in the past 2 years. We excluded urine samples with any evidence of hematuria (trace or above) by dipstick (n=134, 24.4%). After exclusion of patients with diabetes (n=46), proteinuria >1g/day (n=6), urine oxalate >100 mg/day (n=4), those with non-idiopathic calcium stones (n=51), and those with missing data (n=19), our sample included 289 SF (36% F) with a mean age of 45.5 ± 13.5 years. There was no relationship between albumin excretion and age, gender, weight, measured creatinine clearance, systolic blood pressure, number of ESWL, or urine pH (p=NS). Only 5% of patients demonstrated urinary albumin/creatinine ratio of >30 mg/g, which is similar to that documented for the general population in NHANES III (Figure, left panel). When we compared the albumin excretion of the idiopathic SF to that of the other stone forming populations (RTA, hyperparathyroidism, bowel disease, and cystinuria), only the cystine SF demonstrated higher albumin excretion (ANOVA, p<0.002). (Figure, right panel). **Conclusions:** Nearly one fourth of patients referred for kidney stone prevention present with at least microscopic hematuria. Idiopathic calcium SF without hematuria show no evidence of increased microalbuminuria. Cystine SF exhibit increased albumin excretion, potentially signifying the presence of greater renal injury.




Disclosure of Financial Relationships: nothing to disclose

**TH-PO142**

**Obesity in Adolescents Induces Insulin Resistance & Increased Risk for Uric Acid & Calcium Oxalate Kidney Stones** Gregory Lee Braden,[2] Rishita Tiwari,[1] Holley F. Allen,[1] Thomas Campfield,[1] Varun Agrawal,[2] Edward O. Reiter.[1] *[1]Dept of Pediatrics, Baystate Medical Ctr/Tufts University School of Medicine, Springfield & Boston, MA; [2]Dept of Medicine, Baystate Medical Ctr/ Tufts University School of Medicine, Springfield & Boston, MA.*

Obese adults are at risk for calcium oxalate(CaOx) & uric acid(UA) kidney stones(KS) from the metabolic syndrome & insulin resistance(IR) which inhibits renal ammoniagenesis leading to a low urine ph and insoluble UA. Whether these same risks occur in obese adolescents(ADs) are unknown. We studied 46 obese ADs, mean age 14.6 years, BMI 36 kg/m2 with no KS history. They formed 2 groups, 26 ADs with IR defined as a HOMA>4.4 (fasting glucose/fasting serum insulin) vs 20 obese AD controls ( HOMA < 4.4). Metabolic syndrome traits were measured: HOMA>4.4, HDL chol<40mg/dL, blood pressure>90th%, or triglycerides>150mg/dL. 24 hour urines were obtained for sodium(Na), magnesium, calcium(Ca), uric acid(UA), potassium, phosphorus, chloride, oxalate(Ox) & 24 hour ph. A fasting urine for all stone forming elements was obatined to calculate the relative CaOx saturation ratio(RSR)by the EQUIL program and urine ph by ion electrode. 24 hour osteopontin was measured by ELISA. There were no differences in mean daily excretion of Na,Ca,Ox,UA or osteopontin in the IR ADs vs Cs. However, the 24 hour urine ph correlated inversely with increasing HOMA value (r=-.31,p=.04). Fasting urine ph correlated inversely with increasing numbers of metabolic syndrome traits (r=-.34,p=.02). There was no relationship of HOMA to UA or Ox excretion but HOMA correlated positively to increasing calcium excretion(r=.34,p=.02) & positively to increasing CaOxRSR (r=.33,p=.03).We conclude: Similar to adults with metabolic syndrome, in obese ADs, IR correlates directly to increased urinary calcium excretion and increased CaOxRSR & lower urinary ph. Metabolic syndrome traits correlate directly to lower urinary ph. Obese ADs with IR & metabolic syndrome traits are at risk for UA & CaOx KS.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO143**

**A Comparison of Metabolic Variables among Obese and Non-Obese Kidney Stone Formers** Nikhil Johri, Stephen B. Walsh, William G. Robertson, Robert J. Unwin. *Centre for Nephrology, Royal Free Hospital NHS Trust and UCL Medical School, London, United Kingdom.*

Kidney stone disease is common worldwide and the incidence is growing. Increasing prevalence of obesity has been linked with this increase. Studies suggest that insulin resistant states may be associated with increased risk of uric acid stones. We studied metabolic differences between obese and non-obese stone formers.

We investigated the kidney stone database created between November 97 and November 09 (N=1654). Patients were divided into two categories – BMI ≤ 25.0 kg/m$^2$ and BMI ≥30.0 kg/m$^2$. Serum (S) and urinary (U) analytes were compared. We also compared the stone composition for these groups. Data were processed using 'GraphPad' software and statistical significance calculated using Mann-Whitney Test. Results are expressed as mean ± S.E .M.

Analysis showed 664 (40.1%) individuals had BMI ≤25 and 336 (20.3%) had BMI ≥30. In BMI≥30 group, S. Creatinine levels were significantly higher (95.6 ± 2.38 vs. 88.6 ± 1.56 umol/L p<0.001) as was S. Urate (371.0 ± 6.0 vs. 301.5 ± 4.0 umol/L p<0.001). S. Bicarbonate levels on the other hand were lower (26.5 ± 0.1 vs. 27.8 ± 0.2 mmol/L p<0.001). Higher levels of U.Oxalate (0.364 ± 0.0 vs. 0.348 ± 0.1 mmol/L p<0.001), U.Citrate (2.7 ± 0.1 vs. 2.4 ± 0.0 mmol/L p= 0.004), U.Urate (3.9 ± 0.07 vs. 3.19 ± 0.04 mmol/L p<0.001), U.Na (188 ± 4.0 vs. 150 ± 2.5 mmol/L p<0.001) and U.K (71 ± 1.3 vs. 68.4 ± 1.09 mmol/L p=0.037) were also seen in those with BMI ≥30. U.pH was significantly lower (5.974 ± 0.072 vs. 6.382 ± 0.023 p<0.001) in those with BMI ≥30. Differences in U.Volume, U.Mg and U.Ca were not significant. Stone composition data (N=468) showed 4% of stones to be composed of uric acid in those with BMI ≤25, whereas the proportion of uric acid stones was 27.8% in those with BMI ≥30.

Increasing BMI not only increases the overall risk of stone formation but also skews the risk factors in favor of uric acid stones. This greater risk is primarily due to raised U.Urate, U.Oxalate, U.Na and lower U.pH. U.Ca was not a significant additional risk factor. High BMI patients also have worse renal function which puts them at a higher risk of prospective renal dysfunction.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO144**

**Beta Thalassemia Major and Urolithiasis** Nikhil Johri, William G. Robertson, Robert J. Unwin, Stephen B. Walsh. *University College London Partners Renal Centre, London, United Kingdom.*

Patients with beta-thalassaemia major (BTM) are prone to urolithiasis, thought to be due to chronic hemolysis leading to uric acid (UA) stones. We interrogated our renal stone database in order to compare patients with BTM and kidney stones with the other stone forming patients.

13 BTM patients were compared with the other 432 stone forming (SF) patients. The probability of stone formation (PSF) was calculated. Significance was calculated using the unpaired t-test. Values expressed are means ± S.E.M.

BTM patients were younger than SF patients (31.4 ± 3 vs. 47.2 ± 1 years p=0.0001). All identified stones in the BTM group were calcium oxalate (CaOx) or mixed calcium oxalate/phosphate (CaOx/P).

Serum bicarbonate was higher in BTM patients (28.6 ± 0.5 vs. 26.4 ± 0.2 mmol/L p=0.01) but there was no difference in urine pH (6.18 ± 0.1 vs. 6.15 ± 0.1, ns).

Serum UA was lower in the BTM group (270 ± 34 vs. 340 ± 5 µmol/L, p=0.01), however the 24-hour urinary uric acid was higher in BTM patients (4.1 ± 0.4 vs. 3.3 ± 0.1 µmol/L, p=0.02).

24-hour urinary calcium was higher in BTM patients (9.5 ± 1.8 vs. 5.4 ± 0.2 mmol/L, p<0.0001), but they had a lower dietary calcium intake than SF patients (23.3 ± 2 vs. 24.6 ± 1 mmol/day, ns).

Serum phosphate was higher in the BTM group (1.2 ± 0.1 vs. 1.02 ± 0 mmol/L, p=0.001), as was 24-hour urinary citrate (3.4 ± 0.4 vs. 2.5 ± 0.1 mmol/L, p=0.03).

The PSF in BTM and SF patients was very low for UA stones (0.7 x $10^{-6}$ ± 0 vs. 0.04 ± 0, ns), and mixed UA and CaOx stones (0.5 x $10^{-4}$ ± 0 vs. 0.04 ± 0, ns).

The PSF was significantly higher in BTM patients for the formation of CaOx (0.57 ± 0.1 vs. 0.3 ± 0.01, p=0.005) and mixed CaOx/P stones (0.6 ± 0.1 vs. 0.3 ± 0.01, p=0.005).

Despite a higher concentration of urinary UA in the BTM group, these data do not support the common view that they are more likely to form UA stones. They are hypercalciuric and have a higher tendency to form CaOx and mixed CaOx/P stones than the other stone forming patients on the database. Resorptive bone disease is prevalent in BTM, which may be related to hypogonadism: we suspect that the consequent hypercalciuria is the cause of urolithiasis, rather than UA stone formation due to chronic haemolysis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO145**

**The Increasing Burden of Pediatric Urolithiasis in the United States** John D. Spencer, Halima S. Janjua, Rose M. Ayoob, John D. Mahan. *Division of Pediatrics, Department of Nephrology, Nationwide Children's Hospital, Columbus, OH.*

**Background**: Recent evidence suggests that the prevalence of urolithiasis is increasing in the USA. The epidemiology of pediatric urolithiasis is not well characterized. A recent NIH report identified pediatric urolithiasis as an insufficiently researched area.

**Objective**: To evaluate the demographics of pediatric urolithiasis admissions and identify trends in their utilization of healthcare resources.

**Design/Methods**: A retrospective analysis was performed on children ≤18 yrs of age, admitted with urolithiasis from 1997-2006, using the Healthcare Cost and Utilization Project Kids' Inpatient Database (KID).

**Results**: *Hospitalizations*: From 1997-2006, the KID captured 6.5 million annual discharges. During this period, 3,799 ± 152 children/yr were hospitalized with urolithiasis. Hospitalizations increased 30% (p<0.001); female admissions increased 36% and male admissions increased 27% (p<0.001). The average hospitalization was 2.2 +/- 0.1 days and did not vary by year, gender, or age group. *Patient Demographics*: Females were 1.5 times more likely to be admitted than males. Children ≤10 yrs of age were more likely to be hospitalized if they were male; patients ≥15 yrs of age were more likely to be hospitalized if they were female (p < 0.001). Children with ureteral calculi were 1.5 times more likely to be admitted than children with renal calculi. *Geographic Location*: Hospitalization rates in the South were 1.7 times more common than hospitalization rates in the Midwest and 2.5 times more common than in the Northeast or West. *Hospital Economics*: In 2000, estimated hospital costs for a urolithiasis admission were $3,384/hospitalization. In 2006, costs rose to $5,115/hospitalization. Mean hospital charges increased from $5,927 to $9,868 per hospitalization – representing a 66% increase.

**Conclusions:** Urolithiasis is common in the American pediatric population. Since 1997, hospitalization rates for urolithiasis rapidly increased. Hospitalization is more common in females, teens, and children from Southern states. Although lengths of hospitalization remain constant, hospital charges continue to rise.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO146

**Kidney Stones and the Risk of End-Stage Renal Disease (ESRD)** Ziad El-Zoghby,[1] John C. Lieske,[1] Robert N. Foley,[1] Eric J. Bergstralh,[2] Xujian Li,[2] Amy E. Krambeck,[3] Andrew D. Rule.[1] *[1]Nephrology and Hypertension, Mayo Clinic; [2]Biomedical Statistics and Informatics, Mayo Clinic; [3]Urology, Mayo Clinic, Rochester, MN.*

**Background:** Kidney stones are a risk factor for chronic kidney disease but it is unclear the extent they contribute to the development of end-stage renal disease (ESRD). To our knowledge, no cohort studies have assessed the ESRD risk among kidney stone formers in the general population.

**Methods:** The study cohort included all stone formers in Olmsted County, Minnesota first diagnosed between 1984 and 2003. Controls were matched 3:1 on age, sex, duration of medical record, and year of a clinic visit date. Incident ESRD cases were identified by querying the United States Renal Data System and Mayo Clinic dialysis and transplant databases validated by chart review through 12/31/2007. Co-morbidities (hypertension, diabetes, obesity, dyslipidemia, gout, alcohol dependence, tobacco use) were determined by diagnostic codes. Subjects were censored at ESRD or death (Minnesota death certificate).

**Results:** Mean follow up was 9.0 years in 4461 stone formers and 9.2 years in 10,638 controls. A total of 92 subjects developed ESRD after the index date. The incidence of ESRD was 1.02 (95% CI: 0.73 to 1.39) events per 1000 person years in stone formers and 0.52 (95% CI: 0.39 to 0.68) events per 1000 person years in controls. The risk of ESRD in stone formers increased with age-sex-adjustment (HR= 1.92, 95% CI: 1.27 to 2.90) and with adjustment for all comorbidities (HR=1.77, 95% CI: 1.16 to 2.68). The reported causes of ESRD among stone formers (n=41) were kidney stones in 3, diabetes in 10, glomerulonephritis/vasculitis in 5, hypertension in 4, multifactorial in 4, polycystic kidney disease in 3 and other/unknown in 12.

**Conclusion:** Kidney stone formers in the general population are at increased risk for ESRD, but kidney stones are usually not the primary cause attributed to the development of ESRD.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO147

**Temporal Trends in the Incidence of Kidney Stone Disease in Iceland: Results of a Nationwide Study** Vidar O. Edvardsson,[1,3] Olafur S. Indridason,[2] Runolfur Palsson.[2,3] *[1]Children´s Medical Center, Landspitali University Hospital, Reykjavik, Iceland; [2]Division of Nephrology, Landspitali University Hospital, Reykjavik, Iceland; [3]Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavik, Iceland.*

Recent reports have suggested a rising occurrence of kidney stone disease. The aim of the study was to examine the incidence and recent trends of kidney stone disease in the adult Icelandic population.

Computerized databases of all major hospitals and medical imaging centers in Iceland covering the years 1983-2008, were searched for ICD, radiology and surgical procedure codes indicative of kidney stones. Patients diagnosed with kidney stones before 1990 were excluded from the analysis due to incomplete electronic coding. Medical records were reviewed to confirm the diagnosis of kidney stone disease and to determine if the stones were associated with clinical symptoms. Time trends in the incidence of kidney stones were assessed by regression analysis.

During the years 1990 to 2008 the number of incident patients was 5026, of whom 3170 were men. Mean age at diagnosis was 52.8 ± 17.5 years for men and 48.9 ± 18.9 years for women. Symptomatic stones were present in 4202 patients (83.6%), 505 patients (10%) were asymptomatic and the presence of symptoms could not be determined in 319 patients (6.3%). The annual incidence of kidney stones ranged from 149 to 185 per 100,000 in men and remained unchanged over time (P=0.76). In women, the incidence was also similar throughout the study period, ranging from 72 to 123 per 100,000 (P=0.17). The annual incidence of asymptomatic stones increased over time from 10 to 24 per 100,000 in men (P <0.001) and from 6 to 21 per 100,000 in women (P=0.001). Asymptomatic patients were significantly older than symtomatic patients, 65.6 ± 15.0 vs. 49.4 ± 17.8 years, respectively (P <0.001).

The incidence of kidney stones in the Icelandic population has not increased over the past two decades. However, the diagnosis of asymptomatic stones has more than doubled during this period, possibly due to increased use of imaging studies in older patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO148

**Incidence of Kidney Stone Disease in Icelandic Children, 1990-2008: A Nationwide Study** Vidar O. Edvardsson,[1,3] Olafur S. Indridason,[2] Runolfur Palsson.[2,3] *[1]Children´s Medical Center, Landspitali University Hospital, Reykjavik, Iceland; [2]Division of Nephrology, Landspitali University Hospital, Reykjavik, Iceland; [3]Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavik, Iceland.*

The incidence of kidney stone disease in children has been rising according to recent studies. The aim of the study was to examine the incidence and temporal trends of confirmed kidney stone disease in Icelandic children.

Computerized databases of all major hospitals and medical imaging centers in Iceland covering the years 1983 to 2008, were searched for ICD, radiology and surgical procedure codes indicative of kidney stones in children and adolescents less than 18 years of age. Children diagnosed with kidney stones before 1990 were excluded from the analysis due to incomplete electronic coding. Medical records were reviewed to confirm the diagnosis of kidney stone disease and to determine if the stones were associated with clinical symptoms. Age-standardized incidence was calculated and trends in the incidence over time were assessed by regression analysis.

We identified 147 children who were diagnosed with their first kidney stone between 1990 and 2008, 66 males and 81 females. The medan (range) age for males was 13.7 (0.22-17.99) years and 15.1 (0.77-17.76) years for females. All but 6 patients were symptomatic at the time of diagnosis. The mean annual incidence was 9.9 ± 4.5 per 100,000 children. It was 8.7 ± 4.3 and 11.1 ± 7.2 per 100,000 for males and females, respectively (P=0.21). There was a borderline significant (P=0.058) linear increase in the annual incidence over time, that largely resulted from a relatively low annual incidence during the first 5 years of the study period.

The incidence of kidney stones in Icelandic children is high compared to other Western nations. Contrary to recent studies, the incidence of kidney stone disease in children has not increased significantly during the last two decades in Iceland.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO149

**Previous Shock Wave Lithotripsy Is Not Associated with Hypertension among Community Stone Formers at Long-Term Follow-Up** Amy E. Krambeck,[1] Andrew D. Rule,[2] Xujian Li,[3] Eric J. Bergstralh,[3] John C. Lieske.[1] *[1]Urology, Mayo Clinic, Rochester, MN; [2]Internal Medicine, Mayo Clinic, Rochester, MN; [3]Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN.*

Concern exists over the subsequent development of hypertension after use of shock wave lithotripsy (SWL) for the treatment of symptomatic urolithiasis. A referral bias and lack of long-term follow-up has been a limitation of prior studies.

We identified all Olmsted County, Minnesota residents with a diagnosis of urolithiasis from 1985 to 2008. The charts were electronically queried for hypertension and obesity by diagnostic codes and use of SWL by surgical codes. All patients first diagnosed with hypertension before or up to 90 days after their first documented kidney stone were considered to have prevalent hypertension. Cox proportional hazards models were used to assess the association of SWL with a subsequent diagnosis of hypertension.

6,077 incident urolithiasis patients were identified with greater than 90 days follow-up. We excluded 1,295 (21.3%) of the population for prevalent hypertension leaving 4,782 incident urolithiasis patients for analysis. During an average follow-up of 8.7 years, new-onset hypertension was diagnosed in 983 (20.6%) of the cohort at a mean of 6.0 years from index stone date. Only 400 (8.4%) of the cohort received SWL therapy. There was no significant association between SWL treatment and the development of hypertension in univariate (P=0.33) and multivariate modeling controlling for age, gender, and obesity (Hazard ratio [95% CI] =1.03[0.84, 1.27], P=0.77).

Therefore, in a large population-based cohort of kidney stone formers we failed to identify an association between SWL and the subsequent long-term risk of hypertension.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO150

**Renal Histology of Living Kidney Donors with and without Radiographic Stones and Past Symptomatic Stone Events** Merfake Semret,[1] John C. Lieske,[1] Lynn D. Cornell,[2] Elizabeth C. Lorenz,[1] Terri J. Vrtiska,[3] Andrew D. Rule.[1] *[1]Nephrology, Mayo Clinic, Rochester, MN; [2]Anatomic Pathology, Mayo Clinic, Rochester, MN; [3]Radiology, Mayo Clinic, Rochester, MN.*

Background: Certain risk factors for kidney stones (age, hypertension, obesity, hyperuricemia) associate with nephrosclerosis or increased nephron size on renal biopsy. Our objective was to determine if kidney stones were associated with changes in renal histology among living kidney donors.

Methods: Kidney donors at the Mayo Clinic from 2001 and 2009 were identified. During donor evaluation, radiographic kidney stones from computed tomography scans and past symptomatic kidney stones from clinic notes were identified. Renal biopsies at the time of transplant were reviewed for the presence of crystals, including using polarizing light microscopy for detection of Ca-Oxalate crystals. Nephrosclerosis was determined from 2 or more of the following: any glomerulosclerosis, any tubular atrophy, interstitial fibrosis >5%, any arteriosclerosis. Cortex per glomerulus (mm2), a measure of nephron size, was calculated [sectioned width x length x % cortex/number of glomeruli].

Results: There were 1143 kidney donors (58% female, mean age 44 years) of which 107 (9.4%) had radiographic stones and 21 (1.8%) had past symptomatic stones. Between persons with and without radiographic stones, there was no difference in the prevalence of crystals (3 vs 2%, p=0.54), nephrosclerosis (28% vs 28%, p=0.93), or nephron size (mean 0.48 vs 0.49 mm2, p=0.80). Between persons with and without past symptomatic stones, there was a difference in the prevalence of crystals (10 vs 2%, p=0.013), and possibly nephrosclerosis (43% vs 27%, p=0.12), but not nephron size (mean 0.48 vs 0.48, p=0.98). However, with age adjustment there is no association between past symptomatic stones and nephrosclerosis (p=0.52). Of the 23 donors with renal crystals, 21 had Ca-Phosphate, 3 had Ca-oxalate and 15 were in cortex.

Conclusion: There is no evidence that radiographic or past symptomatic kidney stones associate with nephrosclerosis or nephron size. Crystals in the cortex may contribute to kidney stones particularly in persons with past symptomatic stone passage.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO151

**Gender Differences in Urinary Osteopontin among Incident Stone Formers**

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Proteins have been identified in urine that inhibit crystal growth, and this activity appears reduced in certain stone formers. Osteopontin (OPN) is one urinary protein with crystal growth inhibition (CGI) activity.

Urine samples from a community-based cohort of recent first-time stone formers (n=81) and controls without a stone history (n=304) were collected. A seeded calcium oxalate crystal assay was used to measure total urinary protein CGI activity. For this study samples were selected from male and female stone formers and controls with CGI in the lower and upper quartile (n=10 each). OPN was quantitated by ELISA and creatinine (Cr) by enzymatic creatinase assay.

Stone formers and controls were matched by age at 43.3 ± 12.5 years and 41.6 ± 12.9 years respectively. OPN/Cr was higher in stone formers vs. controls, 246 ± 21 pg/mg and 141 ± 64 pg/mg respectively. When stratified by gender, OPN/Cr was higher in female stone formers (400 ± 119.6 pg/mg) compared to female controls (161 ± 34 pg/mg) and both male stone formers (134 ± 34 pg/mg) and controls (121 ± 25 pg/mg) (p=0.01). Furthermore, female stone formers with inhibition levels in the upper quartile had significantly higher OPN/Cr (599± 215 pg/mg, n=10) than stone forming females in the lower quartile (200 ±72 pg/mg, n=10) and all other groups (p<0.05). OPN/Cr positively correlated with urinary CGI in the female stone formers and controls but inversely correlated in the male stone formers and controls.

OPN appears to correlate with CGI in women but not men, and levels are higher in female stone formers than female controls, male stone formers, or male controls. These results suggests than OPN may increase in response to stone formation, perhaps in a compensatory manner, or be involved in stone generation. However, the role of OPN in stone pathogenesis may differ between men and women.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO152

**Timing Relative to Stone Events Determines Patient-Reported Outcomes (PRO) of Health-Related Quality of Life (HRQoL) Assessment in Kidney Stone Formers (SF)**

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Kidney stones are perceived as causing only transient effects on HRQoL. Studies assessing HRQoL of SF are few. We measured HRQoL in SF and compared it to normative values of the US population. We used an online version of SF-36 v2 administered to cystine and non-cystine stone formers. Patients were recruited through an active stone and urology practice and via online solicitation. We surveyed 295 SF. In a secondary data analysis, we determined the effect of the length of the interval between administering the HRQoL instrument and the last reported kidney stone event.

**Results:** For the complete sample, SF had worse HRQoL than the normative US data in all 8 domains composing the Physical Summary Score (PCS) and Mental Summary Score (MCS). In the secondary data analysis, we divided the complete sample into 3 subgroups based on the time since the last reported kidney stone event: within 30 days; > 1 to 6 months; 6 months – 1 year. Our results showed an improvement in all SF-36 domains with increasing time since the last kidney stone event (ANOVA P<0.05).

Effect of time since last stone episode on summary domain scores

| Summary Score | | N | Mean | SD |
|---|---|---|---|---|
| Physical | within 30 days | 91 | 40.53 | 9.34 |
| | 1 - 6 months | 60 | 47.93 | 8.80 |
| | 6 mo - 1 year | 99 | 51.26 | 9.18 |
| Mental | within 30 days | 91 | 35.81 | 13.44 |
| | 1 - 6 months | 60 | 47.53 | 10.70 |
| | 6 mo - 1 year | 99 | 48.03 | 12.25 |

**Conclusions:** SF have worse HRQoL than US normative population. Currently there is no disease specific HRQoL instrument available for kidney stones. When using a generic non-disease specific instrument like the SF-36, the appropriate timing of the assessment of HRQoL is important. The SF-36 is designed so that the majority of the questions elicit patient-reported HRQoL of the past 30 days (22 questions). While applying the SF-36, the time interval since the last stone episode has a major effect on HRQoL PRO.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO153

**Once Daily Extended Release Niacin (Niaspan®) Lowers Serum Phosphorus in Patients with the Metabolic Syndrome**

Susie L. Hu,[1] Gregory C. Shearer,[3] Michael Steffes,[2] Andrew G. Bostom.[1] *[1]Medicine, Division of Kidney Disease and Hypertension, Rhode Island Hospital, Warren Alpert School of Medicine at Brown University, Providence, RI; [2]Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN; [3]Sanford Health-USD Biomed Research, Sioux Falls, SD.*

**Introduction:** Hyperphosphatemia has been shown to be independently associated with cardiovascular disease and all-cause mortality among end-stage renal disease and chronic kidney disease patients. The hypophosphatemic effect of niacin compounds appears to be mediated via their direct inhibition of the small intestinal active transporter for phosphorus.

**Methods and Results:** We examined the phosphorous lowering effect of extended-release niacin (ERN, Niaspan®) among 59 subjects with metabolic syndrome during an 8-week randomized, placebo-controlled 2X2 factorial intervention trial assessing the effect of extended release niacin (ERN; Niaspan®) and Omega-3 Fatty acids (OMFA) on lipid and lipoprotein concentrations. Subjects were randomized into 8 weeks duration of dual placebo, OMFA (4 g/day), ER niacin (2 g/day), or both after a 6-week placebo run-in period. Baseline creatinine, and baseline as well as 8-week phosphorus, calcium, and albumin levels were determined. Estimated glomerular filtrations rates for the entire study group ranged from 58 to 122 ml/min/1.73 $m^2$. There was no evidence for ERN-OMFA treatment interaction (test for interaction by ANOVA, p=0.31) with regard to effects on serum phosphorus. After 8 weeks of treatment, ERN (n=29) lowered mean (± SD) baseline phosphorus levels (3.22 ± 0.51) by an average of -0.48 ± 0.48 mg/dl. Placebo ERN treatment (n=30) did not reduce serum mean phosphorus levels from baseline (3.01 ± 0.64 mg/dl; change= -0.03 ± 0.65; unpaired t-test comparison of between group changes, p=0.004). ERN effect on serum phosphorus persisted (at -0.40, SD ± 0.43 mg/dl, p=0.01) upon analysis of covariance adjustment for baseline concentrations of phosphorus.

**Conclusion**: Once daily ERN reduces phosphorus levels by ~12 % among subjects with metabolic syndrome free of advanced CKD.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO154

**PA21, a Novel Oral Iron Based Phosphate Binder (PB), Significantly Reduces Hyperphosphatemia in Hemodialysis (HD) Patients**

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**Introduction:** This study's purpose was to determine the ability of escalating doses of PA21 to effectively and safely lower serum phosphate (P) levels in HD patients.

**Methods:** This was a multicenter, randomized, open-label, active-controlled, parallel-group study. Major inclusion criteria were: ≥ 18 years old, on a P restricted diet, & on HD 3 times/wk for ≥ 3 months. After washout of the previous PB, subjects whose P levels increased to ≥ 5.5 mg/dL were randomized equally to 5 PA21 arms (1.25, 5.0, 7.5, 10.0, or 12.5g/day) or the control arm of sevelamer HCl (SEV) 4.8 g/day for 6 wks of treatment. The primary efficacy endpoint was the change in P from Baseline to End of Treatment. Secondary efficacy endpoints included time to reach the first controlled P level and the % of subjects achieving controlled P levels (≥3.5 to ≤5.5 mg/dL).

**Results:** Overall, 154 subjects were randomized. For the primary efficacy endpoint, the intent-to-treat group (n=147) showed a statistically significant (p≤ 0.006) decrease from baseline in all but the lowest PA21 dose (decrease of 1.3, 1.3, 1.6 and 1.8 mg/dL for the 4 higher PA21 doses respectively). SEV showed a decrease of 1.3 mg/dL. Median time to first controlled P level was 1 wk in the 4 higher PA21 groups and 2 wks in the SEV group. During treatment, the % of subjects with controlled P levels in the PA21 5.0 to 12.5g/day groups ranged from 68% to 91% and 83% in the SEV group. Apart from hypophosphatemia, adverse events reported with PA21 were not dose dependent, were mainly GI related & occurred with similar frequency to SEV group. There were no discontinuations due to GI events with PA21, but 2 with SEV (diarrhea).

**Conclusions:** PA21 exerted a significant dose dependent P lowering effect between 5.0 - 12.5g/day. The results for 5.0 and 7.5g/day appeared to be comparable to SEV 4.8g/day; 10.0 and 12.5 g/day achieved a greater lowering of P than the SEV dose. These results demonstrate that PA21 is an efficacious and well tolerated new PB.

Disclosure of Financial Relationships: Consultancy: Questcore PharmaceuticalsResearch Funding: Questcore Pharmaceuticals.

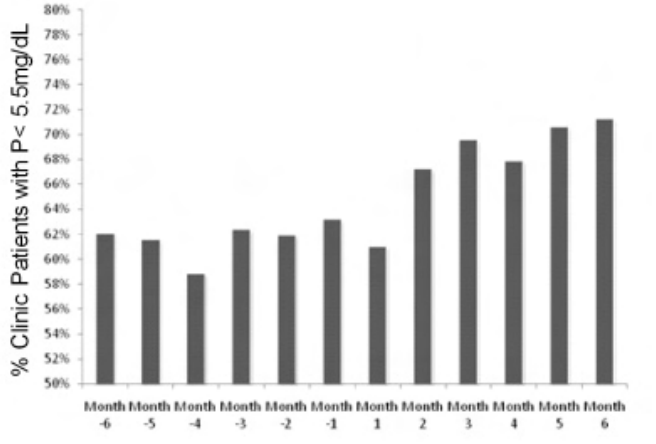
**TH-PO155**

**Focused Phosphorus Management Program Decreases Hyperphosphataemia in End Stage Renal Disease Patients** Tracy Jack Mayne,[1] Steven M. Wilson,[1] Carey Colson,[1] Kathy E. Ricketts.[2] *[1]DaVita Inc., Denver, CO; [2]Shire Pharmaceuticals, Chesterbrook, PA.*

Background End stage renal disease (ESRD) causes disregulation of bone and mineral metabolism, including an increase in serum phosphorus. KDOQI guidelines recommend maintaining phosphorus levels between 3.5 and 5.5 mg/dL. A focused phosphorus management program was designed to support patients with proper diet and phosphorus management through in-center phosphorus educational materials, as well as direct-to-patient adherence communications. It also includes education and access support specific to adherence to lanthanum carbonate.

Method This was an evaluation of 8 dialysis centers before and after initiation of the focused phosphorus management program; the level of analysis was the facility. Mean center size was 88+23 patients (range: 68-141). Centers were geographically diverse (CA, FL, GA, IL, NC, NJ, PA,TX). A repeated measures general linear model was performed.

Results There was a significant increase in the percent of center patients achieving KDOQI phosphorus goals (mean increase from month -6 to month 6=9.2%+6.5%; p<0.01). The percent of patients receiving lanthanum carbonate increased from 18.2% to 24.7% over 12 months. The correlation between change in percent of patients on lanthanum carbonate and change in percent achieving phosphorus < 5.5mg/dL from month -6 to month 6 was r=0.78 (p < 0.01).




Conclusion The focused phosphorus management program improved serum phosphorus goal attainment compared to baseline. Increased lanthanum carbonate use was associated with increased achievement of phosphorus target levels. A larger randomized controlled study is planned.

Disclosure of Financial Relationships: Employer: DaVita, Inc.; Honoraria: Sanofi-Aventis.

**TH-PO156**

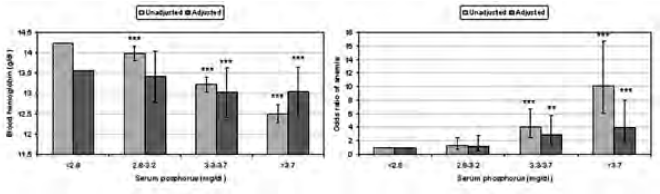
**Associations between Serum Phosphorus and Anemia in Kidney Transplant Recipients** Csaba P. Kovesdy,[1,2] Maria Eszter Czira,[3] Anna Rudas,[3] Akos Ujszaszi,[3] Laszlo Rosivall,[3] Myles S. Wolf,[4] Marta Novak,[3] Istvan Mucsi,[3] Miklos Z. Molnar.[3,5] *[1]Salem VA Medical Center, Salem, VA; [2]University of Virginia, Charlottesville, VA; [3]Semmelweis University, Budapest, Hungary; [4]University of Miami, Miami, FL; [5]Harbor-UCLA, Torrance, CA.*

Post-transplant anemia is a common complication in kidney transplant (KT) recipients and is associated with increase in mortality and morbidity. Abnormalities of mineral and bone metabolism have been associated with anemia in patients with CKD and ESRD, but such a link has not been examined in KT recipients.

We examined associations of serum phosphorus (PO4) with blood hemoglobin (Hgb) level and with the prevalence of anemia in 992 KT recipients. Associations were examined in regression models adjusted for age, sex, diabetes, smoking, transplant vintage, acute rejections, medication use, the malnutrition-inflammation score, estimated GFR and serum calcium, PTH, 25OH vitamin D, fibroblast growth factor-23, iron, erythropoetin, percent hypochromic reticulocytes, soluble transferrin receptor, ferritin, C-reactive protein and interleukin-6.

Higher PO4 was associated with significantly lower Hgb and higher risk of anemia (Figure). A 1 mg/dl higher PO4 was associated with 0.29 g/dl lower Hgb (95%CI: 0.42-0.16, p<0.001) and with an odds ratio of anemia of 2.1 (95%CI: 1.4-3.0, p<0.001) after adjustments. The results were consistent in subgroups of patients with different levels of kidney function and various markers of inflammation and mineral-bone disorder.

Higher serum PO4 is associated with anemia in KT recipients even within the normal range of serum phosphorus. This association is independent of kidney function, inflammation, PTH, vitamin D and FGF23. Future studies are needed to assess possible mechanisms of action (such as the effect of PO4 on uremic toxin metabolism), and to determine if lowering serum PO4 can improve Hgb levels.




Disclosure of Financial Relationships: Consultancy: GenzymeResearch Funding: Abbott, Genzyme, Shire; Honoraria: Genzyme, Novartis, Shire.

**TH-PO157**

**Sevelamer Therapy and Mortality in Pre-Dialysis Chronic Kidney Disease Patients** Ana Cabrita, Anabela Malho, Ana Pinho, Elsa Morgado, Ana Paula Silva, Pedro Neves. *Serviço de Nefrologia, Hospital de Faro, EPE, Faro, Portugal.*

Cardiovascular disease (CVD) is the main cause of morbidity and mortality in chronic kidney disease (CKD) patients. Vascular calcification was described as one of the risk factors of CVD, and recently, sevelamer hydrochloride (SH) therapy has been associated in some studies with a decreased calcification and mortality. The aim of this study was to evaluate the influence of SH on the survival of pre-dialysis patients.

We included 95 patients of our "low-clearance" outpatient clinic (f=41 m=54, mean age=69.4 years, mean eGFR = 16.1 ml/ min, mean follow-up=24.1 months). Our population was divided in 2 groups: G I (n = 76) – no phosphate binder therapy or calcium carbonate as the phosphate binder, G II (n = 19) – sevelamer as the phosphate binder. The groups were compared regarding the parameters analyzed and the survival at 24 months. We also looked for risk factors of mortality of our patients.

In the statistical analysis we performed descriptive statistics and used the student´s t-test and chi-square test and the Kaplan-Meier method and the Cox proportional hazard model.

We found at baseline, that G II were younger (59.0 vs 71.9 years, p= 0.001) and showed higher levels of phosphate (5.8 vs 4.5 mg/dl, p = 0.001), PTH (471 vs 304 pg/ml, p=0.036) and Ca x P (58 vs 44 $mg^2/dl^2$, p = 0.001). There were no differences regarding sex distribution, proportion of diabetes or presence of cardiovascular disease and eGFR, calcium, albumin and IL-6 levels. Using the Kaplan-Meier method the survival of G II at 2 years was significantly better (0.947 vs 0.690, logrank = 4.6, p = 0.032). Using the Cox proportional method we found that cardiovascular disease (p = 0.004) and IL-6 (p=0.049) were independent risk factors of death. The absence of phosphate binder therapy / use of calcium carbonate was associated with a higher trend (p=0.08) of increased mortality rate.

In conclusion, our observational study, with a mean 24 months of follow-up and a small number of patients, showed that sevelamer can have a beneficial effect on the survival of pre-dialysis patients. Further studies with more patients are needed to confirm our results

Disclosure of Financial Relationships: nothing to disclose

**TH-PO158**

**Non Calcium Containing Phosphate Binders May Excert Different Effects on Gastrointestinal Calcium Absorption** Geert J. Behets, Marc E. De Broe, Patrick C. D'Haese. *Laboratory of Pathophysiology, University of Antwerp, Antwerp, Belgium.*

Both Ca- and non Ca-containing phosphate binders (PBs) can increase gastrointestinal Ca absorption, which in subjects with chronic renal failure (CRF) may lead to increased serum Ca levels, low bone formation rate and increased risk for vascular calcifications, particularly in combination with vit D therapy. We observed in rats that administration of $La_2(CO_3)_3$ does not give rise to increased calciuria. In CRF patients, $La_2(CO_3)_3$ treatment is associated with a less pronounced decrease in serum PTH, compared to other PBs.

In the current study, Male Wistar rats (normal renal function (NRF) or CRF) received $La_2(CO_3)_3$, sevelamer, $CaCO_3$ or cellulose (2% in diet) for 8 days, followed by a 6-day washout.

Animals with NRF, treated with sevelamer showed increased serum iCa ($p < 0.05$), from 2 days after start, returning to normal within 1 day of washout. $CaCO_3$ treated animals showed a smaller increase. $La_2(CO_3)_3$treated animals did not show differences in serum iCa vs control.

In CRF animals, no significant differences in iCa were found between sevelamer, $CaCO_3$ or control groups. In the $La_2(CO_3)_3$ group, lower serum iCa ($p < 0.05$) were seen after 2 days of treatment which persisted during treatment. During washout, serum iCa returned to normal within 24h.

In a short-term experiment in which CRF animals received a single dose of any of the PBs under study showed that $CaCO_3$ induced a rapid increase in serum iCa already after 2h, whilst in the sevelamer group an increase was seen after 6 to 8 h. In contrast in the $La_2(CO_3)_3$ group a decrease in serum iCa was observed 2h after dosing. In all groups, serum iCa returned to normal within 24h.

In these experiments a concomitant inverse relationship between serum iCa and PTH was noted.

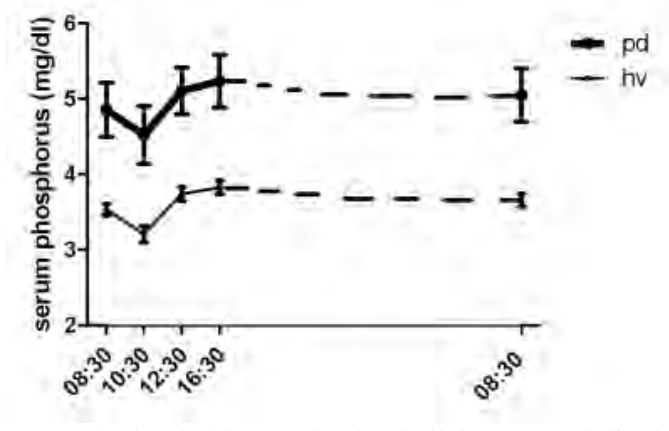
Our results show that Ca-containing as well as non Ca-containing PBs show a differential effect on serum iCa levels, likely due to the well-documented Ca-channel blocking of La at high concentrations. Our findings indicate that $La_2(CO_3)_3$ treatment may be of particular interest for CRF patients with increased risk of hypercalcemia and/or patients with low bone turnover and may facilitate the combined use of vit D.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO159

**Daytime Rhythm in Phosphorus Concentration in Healthy Volunteers and Peritoneal Dialysis Patients** Liesbeth Viaene,[1] Bjorn K. I. Meijers,[1] Bert Vermeiren,[1] Vanrenterghem Yves,[1] Dirk Vanderschueren,[2] Pieter Evenepoel.[1] *[1]Nephrology, University Hospital Leuven, Leuven, Belgium; [2]Endocrinology, University Hospital Leuven, Leuven, Belgium.*

Introduction: Serum phosphorus concentrations reflect a dynamic balance between dietary phosphorus absorption, urinary excretion and exchanges between intra- and extracellular stores. Methods: To elucidate the role of renal function, we compared the daytime rhythm in phosphorus concentration in 9 stable peritoneal dialysis (PD) patients and 10 healthy volunteers (HV). Patients were studied during a 24-hour period with standardized meals at 08:30 and 13:00 and blood sampling at 08:30, 09:30, 10:30, 12:30, 16:30 and 08:30 (day 2). Phosphate binder therapy was stopped two days in advance. All PD patients received the same dialysis regimen. Individual daytime nadir and absolute amplitude was determined. In addition, serum phosphorus levels were analyzed according to blood sample timing (07:30 to 09:30 vs 09:30 to 11:30 vs 11:30 to 13:00) in 152 unselected chronic kidney disease (CKD) patients stage 3 (eGFR 40.8±8.4 ml/min). Results: The daytime rhythm was similar in HV and PD patients: nadir: 09:42±00:40 vs 09:20±01:00; amplitude 1.14±0.33 vs 0.92±0.92 (Mean ± SD, p>0.05, all).




Serum phosphorus levels in CKD patients determined before 09:30 were significantly higher than those determined later in the morning (3.6±0.6 vs 3.1±0.6 + 3.2±0.6; p=0.02), independent of eGFR and fasted/non-fasted condition. Conclusion: The daytime rhythm in phosphorus concentration is similar in PD patients and healthy volunteers. Our data support the thesis that the daytime periodicity in serum phosphorus concentration reflects an endogenous rhythm, caused by a shift between the cellular and extracellular compartment. Blood sample timing should be accounted for when comparing serum phosphorus concentrations between groups.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO160

**Hormonal and Dietary Determinants of Urinary Calcium Excretion in Healthy Volunteers and Patients with Chronic Kidney Disease Stage 1-4** Liesbeth Viaene,[1] Bjorn K. I. Meijers,[1] Vanrenterghem Yves,[1] Dirk Vanderschueren,[2] Pieter Evenepoel.[1] *[1]Nephrology, University Hospital Gasthuisberg, Leuven, Belgium; [2]Endocrinology, University Hospital Gasthuisberg, Leuven, Belgium.*

Introduction: Calcium balance and homeostasis are disturbed in chronic kidney disease (CKD). In steady-state, 24-hour urinary calcium excretion (24h-$Ur_{Ca}$) corresponds to net daily gastrointestinal calcium absorption. We aimed to identify hormonal and dietary determinants of 24h-$Ur_{Ca}$ and 24 hour fractional ($FE_{ca}$) urinary calcium excretion. Methods: 20 healthy volunteers (HV) (8 male; age 34±10 year) and 170 stable CKD stage 1-4 patients (108 male; age 59±15 year) were studied. Fasting blood and 24-hour urine samples were analyzed for parameters of mineral metabolism including calcitriol and PTH. Dietary data were available in all HV and in 72 CKD patients. Results: Consistent with literature data, we observed decreasing calcitriol, increasing PTH and stable calcium levels along CKD progression. Dietary data and urinary indices are summarised in table1.

| | HV | >60 ml/min/m² | 30-60 ml/min/m² | 15-30 ml/min/m² | p-value |
|---|---|---|---|---|---|
| 24h-UrCa (mg/day) | 220 | 92 | 42 | 21 | <0.0001 |
| FEca (%) | 1.4 | 0.7 | 0.6 | 0.5 | 0.0003 |
| Calcium intake (mg/day) | 977 | 819 | 774 | 627 | 0.006 |

Median values are shown.

In univariate analysis high 24h-$Ur_{Ca}$ was significantly associated with male gender, younger age, high dietary calcium intake, high calcitriol, high eGFR and low PTH. In multivariate analysis calcitriol, dietary calcium intake and age were found to be independently associated with 24h-$Ur_{Ca}$ ($R^2$=0.32, p<0.0001). $FE_{ca}$ decreased along CKD progression. Remarkably, only 24h-$Ur_{Ca}$ was independently associated with $FE_{ca}$, explaining 78% of its variability (p<0.0001). Conclusions: Our data confirm and extend previous clinical and experimental observations. Urinary calcium retention seems to compensate for suppressed net intestinal calcium absorption along the progression of CKD. Low calcitriol levels contribute to this decreased absorption. Our data support the notion of a tenuous calcium balance in CKD patients stage1-4 and strengthen the need for formal calcium balance studies.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO161

**The Calcimimetic R568 Increases Serum Phosphate (P) Via Decreased Urinary P Excretion** Sean Morony, Jei-In Young, Edward Shatzen, Paul Harrington, Christopher Fotsch, Charles M. Henley III. *Amgen Inc.*

The calcimimetic (CaM) Cinacalcet lowers PTH, Ca, and P levels in dialysis patients; however in CKD 3-4 patients treatment induces hyperphosphatemia. This suggests a role for the kidney in the effect of CaM on serum P. We tested this hypothesis by treating 5/6Nx rats with research CaM that were either orally (R568) or not orally bioavailable (CpdA). Male SD rats (250g, 3wks post 5/6Nx, N=7) were dosed PO with R568 (30mg/kg, $t_{1/2}$=2.9h, F=6%), CpdA (30mg/kg, $t_{1/2}$=BQL, F=BQL), or vehicle. Urine and serum were collected 4 and 24h post-dose for PTH, Ca and P determination. Additionally, kidney and duodenum were analyzed for NaPi cotransporter mRNA expression.

| | 4h | | | 24th | | |
|---|---|---|---|---|---|---|
| Serum | Vehicle | R568 | CpdA | Vehicle | R568 | CpdA |
| Ca (mg/dL) | 11.6±0.1 | 9.2±0.2* | 11.6±0.1 | 11.3±0.1 | 10.5±0.2* | 11.1±0.1 |
| P (mg/dL) | 9.4±0.2 | 10.5±0.3* | 9.5±0.2 | 8.5±0.2 | 8.6±0.2 | 8.6±0.2 |
| PTH (pg/mL) | 1388±276 | 492±56* | 1318±174 | 999±126 | 1297±103 | 890±53 |
| Urine | | | | | | |
| FE Ca (%) | 1.7±0.2 | 5.1±0.5* | 2.8±0.7 | 0.4±0.1 | 1.7±0.3* | 0.7 ± 0.2 |
| FE P (%) | 15.1±3.0 | 2.8±0.9* | 13.2±1.4 | 22.0±1.6 | 23.6±2.3 | 20.1±2.2 |

Mean±SEM, N=7, *P<0.05 compared to vehicle

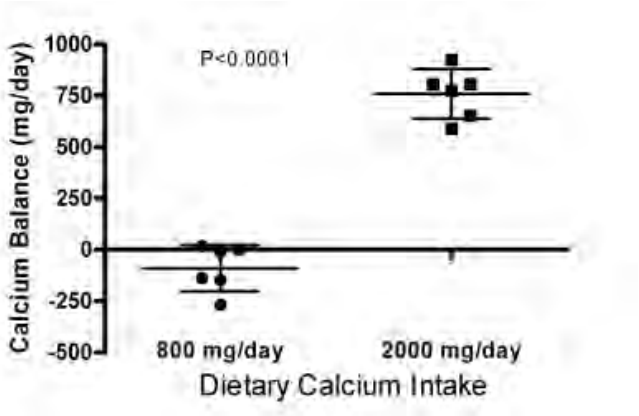
R568 decreased serum PTH and Ca while elevating P, compared to vehicle treated rats. Serum changes reflected urine Ca and P levels; as R568 increased $FE_{Ca}$ and decreased $FE_P$ compared to vehicle treated rats. In contrast, the non-orally bioavailable compound, CpdA, had no effect on serum PTH, Ca, P, or urine $FE_{Ca}$ and $FE_P$ compared to vehicle treated rats. Together these data suggest that decreased urinary P excretion mediates the elevated serum P noted in CaM treated animals with residual kidney function. To further evaluate the mechanism of CaM-induced hyperphosphatemia we determined NaPi cotransporter mRNA levels in kidney and duodenum and found no change in expression; however, post-translational modification of these transporters cannot be ruled out. Additionally, reduced kidney-mediated phosphaturic effects of PTH and FGF23 may explain the decreased urinary P excretion seen with R568 since CaM decrease both of these phosphaturic hormones.

Disclosure of Financial Relationships: Employer: Amgen Inc.; Ownership: Stock and options in Amgen Inc.

## TH-PO162

**Positive Calcium Balance in CKD** David M. Spiegel, Rebecca H. Moore. *Division of Renal Diseases and HTN, University of Colorado Denver, Denver, CO.*

Vascular calcification (VC) is common at dialysis initiation. Children starting dialysis have increased blood vessel calcium content. The pathophysiology of VC is complex but calcium loading has not been felt to play a role as it is assumed that non-dialysis CKD patients are in calcium balance. We investigated calcium balance in 6 late stage 3 or stage 4 CKD patients on 2 calcium diets (800 mg/day and 2000 mg/day). Both diets contained 1600 mg of phosphorus and all foods were prepared by the GCRC for each 9 day (7 outpt, 2 inpt) period. Following each 7 day outpatient diet period patients were admitted to the GCRC. Urine was collected over 48 hrs and blood was drawn each morning. Methylene blue was used to label the stool and all stool stained blue was collected during and after the GCRC stay. Calcium balance was calculated as (24 hour ca intake) - (24 hr stool ca) - (24 hr urine ca). Stool calcium was normalized to 24 hrs by assuming patients were in neutral phosphorus balance. The eGFR was 28.6±4.2 ml/min/1.73m². Serum ca and phos were not different on the 2 diets (low vs high: ca 8.8±0.4 vs 8.8±0.4, phos 4.2±0.5 vs 4.1±0.5). On the 800 mg/day ca diet patients were in slightly negative ca balance (mean± SD)(-91±113 mg/d). On the 2000 mg/day diet patients were in markedly positive ca balance (759±121 mg/d) p<0.0001 (non-paired t-test) (Fig). Furthermore, patients in late stage 3 and stage 4 CKD were unable to increase their urinary ca excretion despite increased net ca intake (low vs high ca diets; urinary ca: 82±48 vs 99±72 mg/d, p=0.65). This study suggests that positive calcium balance can occur in late stage 3 or stage 4 CKD if dietary calcium intake exceeds 1200 to 1300 mg/day and patients are in marked positive balance when intake is 2000 mg/day. This study suggests that calcium loading in late stage 3 and 4 CKD may contribute to tissue deposition including vascular calcification.

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Genentech
Ineos; Honoraria: Amgen, Inc
Genzyme; Scientific Advisor: Amgen, Inc.

### TH-PO163

**Ionized Calcium in Hemodialysis-Patients** Jean-Christophe Szelag, Myriam Pastural, Alejandra Lenz, Ignace Mpio, Nouredine Boumendjel, Carlos Cardozo, Elias Abdullah, Walid Arkouche. *AURAL Villon, AURAL, Lyon, France.*

**Background**: The last KDIGO guideline for the evaluation of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-BMD) do not recommend the use of ionized calcium measurement in haemodialysis-patients. However, ionized calcium is the physiologically active part of serum calcium and his measurement may be useful in case of major acid-base disorders or hypoalbuminemia.

**Methods**: We analyzed the bone and mineral metabolism of 164 HD-patients (68 women, 96 men, 61,8 yr) (total calcium, corrected calcium, phosphorus, PTH, 25-hydroxyvitamin D). Ionized calcium was simultaneously measured in optimal technical conditions.

**Results:** mean values were: total calcium 2,21+/-0,17 mmol/L, corrected calcium 2,23+/-0,19mmol/L, ionized calcium 1,14+/-0,11 mmol/L, phosphorus 1,55+/-0,49 mmol/L, PTH 385+/-319 ng/L, 25-hydroxyvitamin D 27,2+/-16,4 ng/mL. Hypocalcemia frequency was significantly higher by using total calcium (59%, threshold 2,2mmol/L, p<0,0001) or corrected calcium (53%, threshold 2,25mmol/L) that ionized calcium (34%, threshold 1,12mmol/L) (chi2 analysis). Low ionized calcium subjects have a lower 25-hydroxyvitamin D concentration (21,4 +/-9,4 *vs* 30,2 +/-18,4 ng/mL, p<0,01) and an higher phosphorus (1,68+/-0,51 *vs* 1,49+/-0,46 mmol/L, p<0,01, Mann Wythney analysis) than other patients.

In contrast, there was no significant difference on 25-OHvitamin D or phosphorus by using total or corrected calcium. 25OH-vitaminD under 20ng/mL was associated with a higher PTH (460+/-373 *vs* 328+/-261 ng/mL; p<0,05) and a lower ionized calcium (1,12+/-0,11 *vs* 1,16+/-0,10 mmol/L; p<0,05) without significant modification of total calcium. The rate of vitamin D was especially correlated to the ionized calcium (p<0,0005) and the PTH (p<0,005) (simple regression analysis).

**Conclusion:** Although the ionized calcium measurement cannot be routinely used because of technical difficulties and of its cost, it represents the most reliable parameter to analyze the BMD disturbances of low calcium HD-patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO164

**Differential Actions of Calcium Acetate (CaAc) in the Early CKD-MBD: Stage 2 Compared to Stage 3 CKD** Yifu Fang, Suresh Mathew, Keith A. Hruska. *Pediatric Nephrology, Washington University School of Medicine, St. Louis, MO.*

CKD induces the CKD-MBD associated with increased mortality by stage 2. We have shown the loss of bone anabolism and vascular smooth muscle cell phenotype before abnormalities of mineral metabolism are detected. The CKD-MBD in stage 2 CKD consists of an increase in vascular calcification (VC), a decrease in bone formation, and an increase in FGF23 levels. In stage 3 CKD, the onset of hyperphosphatemia increases VC, and Pi binders such as CaCO3 are protective against VC (Davies et al, JASN: 2005). However, calcium salt phosphate binders have been associated with calcium absorption and body burden contributing to VC. CaAc is commonly prescribed, and the CARE trial (Qunibi et al, Kid. Int., 2004) demonstrated that CaAc was more effective than others in controlling serum phosphorus and Ca X P product in ESKD. Therefore, we examined the role of CaAc in the treatment of early phase CKD-MBD. Ldlr deficient mice fed high fat diets were subjected to partial kidney ablation at 14 weeks of age to create an early CKD-MBD mouse model. At 22 weeks of age, treatment was begun with Vehicle, CaAc 1%, and CaAc 3% or 3% CaCO3 from 22 to 28 weeks. A relatively mild reduction in the GFR measured by inulin clearance was produced (76 % of normal, stage 2 CKD) at 22 weeks of age after renal cortical electrocautery and contralateral nephrectomy. Aortic Ca levels were increased at 28 weeks of age, while the serum levels of BUN, Ca, Pi and PTH were normal. Skeletal osteoblast surfaces and bone formation rates were diminished. FGF23 levels were elevated. Treatment with CaAc (both doses) increased the serum Ca from 8.8±1.4 to 9.88±2.2 or 9.65±2 respectively, decreased PTH levels and did not decrease the serum Pi. Neither CaAc nor CaCO3 decreased aortic Ca or FGF23 levels in the stage 2 CKD-MBD, but they reduced, aortic Ca, corrected hyperphosphatemia, and decreased serum FGF-23 levels, in stage 3 CKD (vehicle vs. treated CKD, P<0.05 for each parameter). In conclusion, CaAc controls aortic Ca levels and serum FGF-23 levels in hyperphosphatemic stage 3 CKD, but causes increased serum Ca but no effect on aortic Ca in stage 2 CKD-MBD.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO165

**Lowering Dietary Phosphate Intake Reduces Fibroblast Growth Factor 23 (FGF-23) in Chronic Kidney Disease Stages 3 and 4** Mhairi K. Sigrist, Monica C. Beaulieu, Adeera Levin. *Dept of Nephrology, University of British Columbia.*

FGF-23 is thought to play a critical role in the hormonal regulation of urinary phosphate. In the later stages of CKD both phosphate and FGF-23 are highly deranged and associated with both vascular calcification and mortality. This study describes the physiological response of altered dietary phosphate on circulating FGF-23 and associated biochemical parameters of mineral metabolism in early stages of CKD.

20 stage 3 and 4 CKD Subjects and 12 healthy controls with serum phosphate in the normal range were included in this study. Subjects followed 7-days of the following three diets; high phosphate (HP) (2500mg), low phosphate (LP) (750mg) and low phosphate plus an aluminum based phosphate binder (LP+B). Dietary compliance was encouraged with the provision of a daily eating plan including specified quantities and portion sizes and a grocery basket of appropriate foods. Fasting serum Calcium, phosphate ($PO_4$), intact PTH, intact FGF-23, 1,25 and 25 vitamin D and 24hr urinary excretion of Calcium and $PO_4$ were assessed at the end of each week. FGF-23 was assessed using the Kainos assay.

Baseline FGF-23 was 100pg/ml in the CKD group and 32 pg/ml in the control group (P<0.0001). Baseline phosphate was 3.3±0.5 mg/dl in the CKD group and 3.4±0.5 mg/dl in the control group (NS). In CKD FGF-23 was significantly higher with a HP diet (121± 7pg/ml) than with a LP+B diet (82±60 pg/ml) (P<0.01). Concurrently there were significant falls in urinary $PO_4$, PTH & 25 Vit D on the LP+B diet, although1,25 vit D rose while $PO_4$ stayed relatively stable. Results followed similar trends in the control group. FGF-23 significantly correlated with eGFR (r=-0.78, P<0.0001), intact PTH (r=0.51, P=0.002) and 1,25(OH)D (r=-0.68, P<0.0001).

Reductions in dietary phosphate result in reductions in FGF-23, even when serum phosphate levels are normal. These results demonstrate that a phosphate lowering diet could be beneficial for CKD patients well before their serum phosphate starts to rise late in the progression of CKD.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO166

**Results of a Targeted Phosphorus Management Quality Improvement Program ($PO_4$-QIP); Significant Benefits of the $PO_4$-QIP of ESRD Network of Texas (NW-14) during Year One** Donald A. Molony,[1] Glenda Harbert.[2] *[1]Internal Medicine / Renal Dis & Htn, University of Texas Houston Medical Sch, Houston, TX; [2]ESRD Network of Texas, Dallas, TX.*

Successful management of hyperphosphatemia amongst dialysis patients remains an important but incompletely achieved goal. The degree of successful management can vary widely amongst comparable dialysis facilities within a single region. In order to improve overall management of $PO_4$, NW-14 developed and implemented the $PO_4$-QIP to foster quality improvement on a facility level. All facilities in Texas were notified that $PO_4$ would be a special focus for 2009 and invited to participate in an educational activity. Hemodialysis (HD) and peritoneal-dialysis (PD) facilities reporting less than 40% of their patients within the KDOQI range for $PO_4$ of 3.5 to 5.5 mEq/L were enrolled in a focused intervention (focused facilities) whereby NW-14 provided technical assistance and education and additionally required reporting of facility quality improvement processes and outcomes. We report here the changes in successful $PO_4$ management amongst the focused facilities compared to all other facilities in Texas during year 1 of the QIP.

Improvements in Mineral Metabolism in Focused Facilities 12/08 to 12/09

| Facility Type | n | % of patients with PO4 >3.5 - <5.5, 12/08 | Change in absolute % (12/09) ± 95% CI | P | % facilities improved / deteriorated |
|---|---|---|---|---|---|
| HD, non-focused | n = 364 | 52.6 | 53.2 ± 0.9 | 0.211 | 52.7 / 45.9 |
| HD, focused | n = 38 | 36.1 | 49.3 ± 4.3 | 0.00001 | 94.7 / 5.3 |
| PD, non-focused | n = 79 | 58.8 | 57.4 ± 4.4 | 0.592 | 51.9 / 44.3 |
| PD, focused | n = 22 | 31.7 | 55.6 ± 6.8 | 0.00003 | 86.4 / 4.6 |

These findings from a large region, demonstrate that interventions from NW-14 as part of a QIP focused on improving quality on a facility level can result in significant sustained improvement in $PO_4$ management. Similar improvements did not occur with education alone. Such interventions may provide additional tools for achieving optimal patient-centered outcomes.

Disclosure of Financial Relationships: Honoraria: Genzyme Corp, Cambridge Ma
Amgen Corp, Thousand Oaks, CA
Novartis Corp, Basel Switzerland.

**TH-PO167**

**Sevelamer Based $PO_4$ Binders Result in Reduced Mortality Compared to Calcium-Based $PO_4$ Binders; a Meta-Analysis of Long-Term Randomized Controlled Trials** Donald A. Molony. *Internal Medicine / Renal Diseases and Htn, University of Texas Houston Medical Sch, Houston, TX.*

Animal and epidemiologic studies have supported the hypothesis that excess oral intake of calcium can result in vascular calcification and increased cardiovascular morbidity and mortality. Conversely, by reducing calcium and $PO_4$ burdens in ESRD patients, sevelamer-based binders may have the potential to improve survival. Demonstrating a by meta-analysis a survival advantage with sevelamer has been difficult, in part, because of the small sample size, short duration, or incomplete follow-up in studies conducted to date.

The purpose of the current study, was to re-examine the RCT literature in order to perform a meta-analysis (MA) which included only those studies in which some subjects were followed for a minimum of 1 year and to consider a subject as contributing to the estimate of risk for death from an exposure only as long as they were followed in the study. A systematic review of the literature was performed by searching PubMed, EmBase, the Cochrane trials registry and the gray literature through 5/31/10. Authors were contacted if they did not report deaths by group allocation. Seven RCTs reporting on unique populations were included in the final analysis. For these studies the combined odds ratio (OR) was 0.676, 95% CI 0.0438 – 0.998, heterogeneity p = 0.1623. The DCOR trial had the largest impact on the overall estimate of effect. The OR favoring a survival benefit with use of sevelamer was no-longer seen when RCTs of a duration of less than 1 year were included in the analysis. There was an insufficient number of RCTs to interrogate by MA whether older patients or incident dialysis patients might demonstrate a higher magnitude of risk reduction.

Thus, the meta-analysis reported here differs from others reported recently. The current meta-analysis shows a survival advantage with sevelamer. Meta-analysis is a particularly powerful tool when combining similar studies of inadequate power on populations of similar baseline risk but may soimetimes obscure potential benefits. Comparison of this MA with those published recently, illustrate the power and the limitation of this analytical framework.

Disclosure of Financial Relationships: Honoraria: Genzyme Corp, Cambridge Ma
Amgen Corp, Thousand Oaks, CA
Novartis Corp, Basel Switzerland.

**TH-PO168**

**Association between CCR7 and Vitamin D Deficiency in Patients with CKD Stages 3 & 4** Chun Khai Chong,[1] Kalisha O'Neill,[1] Braca Benizry,[1] Edward F. Srour,[1] Sharon M. Moe.[1,2] *[1]Indiana University School of Medicine, Indianapolis, IN; [2]Roudebush VAMC, Indianapolis, IN.*

**Background:** In vitro and in vivo animal studies have demonstrated that vitamin D modulates immune function. In patients with CKD, there is a high prevalence of vitamin D (25-hydroxyvitaminD (25D)) deficiency and high morbidity secondary to infection. We therefore tested the hypothesis that vitamin D insufficiency/deficiency (levels < 30 ng/ml) altered T-cell function.

**Methods:** We performed a cross-sectional study of 100 subjects with CKD 3 or 4. We measured blood levels of biochemistries, 25D, and intact PTH levels. Immune function was assessed by flow cytometry, analyzing CD4/CD8 lymphocyte ratio, and CD27 (TNF receptor), CD25 (Regulatory T-cell), CD197 (Chemokine Receptor 7 (CCR7)) and CD45RO (Memory T-cell) on both CD4 and CD8 subpopulations.

**Results:** One hundred subjects were recruited. The mean 25D level was 22±13 ng/ml, 75 subjects were 25D insufficient/deficient (DefD, levels < 30 ng/ml) and 19 subjects were 25D sufficient (SufD, levels ≥ 30 ng/ml). Levels of 25D correlated with iPTH (r=0.37, p<0.001). Mean flow cytometry values demonstrated 68% lymphocytes; 50% $CD4^+$, and 20% $CD8^+$ T-cells with a CD4/CD8 ratio of 3±1.5. However, there was no difference between the DefD and SufD groups. The comparison of specific receptors on CD4 or CD8 lymphocytes demonstrated that the $CD4^+CD27^+$ lymphocytes were 85±9 vs 92±5% in DefD vs SufD (p=0.001) and the $CD4^+CD197^+$ lymphocytes were 48±18 vs 63±10% in DefD vs SufD (p<0.001). There were no differences between both groups for any other markers. The 25D levels correlated with the percentage of $CD4^+CD27^+$ (r=0.35) and $CD4^+CD197^+$ (r=0.37) lymphocytes, both p<0.001.

**Conclusions:** Vitamin D insufficiency or deficiency in subjects with CKD 3 or 4 is associated with significantly lower percentage of $CD4^+CD197^+$ (CCR7) lymphocytes when compared to those who are vitamin D sufficient. CCR7 is critical for the generation of T-cell responses. Previous studies have shown that CCR7 deficiency leads to increased susceptibility of infections and therefore CCR7 might be the link between vitamin D deficiency and increased infection in CKD 3 or 4 patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO169**

**African-American Race Is an Independent Predictor of Increased PTH but Not Calcium and Phosphorus in Chronic Kidney Disease** Jennifer L. Ennis,[1] Susan E. Donahue,[1] John R. Asplin,[1] Elaine M. Worcester,[2] Fredric L. Coe.[2] *[1]Litholink Corporation, Chicago, IL; [2]Department of Medicine, Section of Nephrology, University of Chicago, Chicago, IL.*

**Purpose:** African-Americans (AA) comprise a significant and growing portion of the chronic kidney disease (CKD) population, but few studies have focused on bone and mineral abnormalities in this patient group. One prior study demonstrated higher rates of vitamin D (vit D) deficiency and increased serum calcium (Ca), phosphorus (P), and parathyroid hormone (PTH) levels in AAs compared to non-AAs with pre-dialysis CKD.[1] We sought to test whether these results would hold in a larger cohort of CKD patients from unselected US practices.

**Methods:** We performed a cross-sectional analysis of initial laboratory data collected on AA (n=817) and non-AA (n= 2162) stage 1-5 US CKD patients enrolled in the Litholink CKD program. Serum Ca, P, 25-hydroxy vitamin D (25 Vit D) and plasma PTH levels were compared between the two groups.

**Results:** Mean plasma PTH values were higher and mean 25 Vit D values were lower in AAs versus non-AAs across all CKD stages. Differences reached statistical significance in stages 2-5 for PTH and in stages 1-3 for 25 Vit D. Unlike Gutierrez et al, mean Ca and P did not differ between groups at any stage. In a general linear model with PTH as the dependent variable, and Ca, P, 25-Vit D, CKD stage, and AA race as the independent variables, AA race remained a significant predictor of PTH along with Ca, 25 vit D, and CKD stage (p<0.001). Serum P did not enter the model.

**Conclusions:** To our knowledge, this is the largest analysis of mineral metabolism in AA CKD patients. AAs have more severe vit D deficiency and elevations in PTH across all stages of CKD compared to other races. However, we could not confirm differences in serum Ca and P. We confirm that AA race is a novel and unexplained risk factor for elevated PTH in CKD patients independent of other established factors.

[1] Gutierrez O et al. *Kidney Int* 2008; 73: 956-62.

Disclosure of Financial Relationships: Employer: Litholink Corporation.

**TH-PO170**

**The Effect of Combining an ACE Inhibitor and a VDR Activator on Glomerulosclerosis, Proteinuria and Renal Oxidative Stress in Uremic Rats** Eduardo Slatopolsky,[1] Edu Suarez,[3] Kazim Husain,[2] Leon F. Ferder,[2] Michell Cruz,[3] Denis J. Glenn,[3] David Gardner,[3] Helen Liapis,[1] Jane L. Finch.[1] *[1]Washington University School of Medicine, St. Louis, MO; [2]Physiology and Pharmacology, Ponce School of Medicine, Ponce, PR; [3]Medicine, Diabetes Center, University of California, San Francisco, San Francisco, CA.*

ACE inhibitors can suppress the progression of renal disease. In combination with vitamin D receptor activators, they provide additional benefits. Previously, we showed suppression of renal TGFβ and Smad2 in uremic rats treated with a combination of enalapril and paricalcitol. In these studies, uremic (U) rats were treated as follows: U+vehicle (UC), U+enalapril (UE) (25mg/L in drinking water), U+paricalcitol (UP) (0.8ug/Kg, IP/3 x week), and U+Enalapril+ paricalcitol (UEP). Blood pressure (BP) was significantly elevated in the UC and UP rats versus normal animals (NC), while BP in enalapril-treated rats was well controlled. Despite hypertension in UP rats, proteinuria decreased by 32% versus UC rats. Addition of enalapril caused a further decrease (71%). Glomerulosclerosis increased from 5% (NC) to 22% in UC rats. Paricalcitol prevented this (7%). Interstitial infiltration increased from 4% (NC) to 42% in UC rats. Paricalcitol reduced it to 29% and co-treatment with enalapril to15%. Renal oxidative stress (OS) plays a critical role in inflammation and the progression of sclerosis. The antioxidant glutathione reductase was increased in all treatments groups (UE=44% and UP=67% compared to the UC group (p<0.001). In addition, expression of super oxide dismutase increased significantly in all treatment groups. NADPH oxidase and pro-inflammatory inducible nitric oxide synthase (iNOS) decreased in the treated groups. Also, peroxidase activity, a marker of OS, was increased in UC animals (550 vs. 410 nmol/min/ml (NC)). Paricalcitol alone prevented this increase which was further inhibited by addition of enalapril. In conclusion, paricalcitol can suppress progression of renal failure via TGFβ, Smad2, proteinuria, glomerulosclerosis, interstitial infiltration and OS reduction. These effects are amplified when an ACE inhibitor is added.

Disclosure of Financial Relationships: Employer: Washington University St. Louis, MOResearch Funding: Abbott and Genzyme; Honoraria: Abbott and Genzyme; Patent: University of Wisconsin.

**TH-PO171**

**Vitamin D Deficiency and Its Association with Physical Activity and Quality of Life: The Comprehensive Dialysis Study** Shuchi Anand,[1] George A. Kaysen,[2] Kirsten L. Johansen,[3] Glenn M. Chertow,[1] Barbara A. Grimes,[3] Manjula Kurella Tamura.[1] *[1]Nephrology, Stanford University School of Medicine, Palo Alto, CA; [2]Nephrology, University of California, Davis, Davis, CA; [3]Nephrology, University of California, San Francisco, San Francisco, CA.*

As research has identified a wide array of biological functions of vitamin D, the consequences of vitamin D deficiency in persons with and without CKD has attracted increased attention. The objective of this study was to determine the extent of 25-hydroxyvitamin D (25D) deficiency and its associations with self-reported physical activity and health-related quality of life (HRQoL) among participants of the Comprehensive Dialysis Study (CDS). The nutrition substudy of the CDS collected data on patients new to dialysis recruited from 68 units throughout the U.S. 25D concentration was measured using the Immunodiagnostic Systems Inc. Direct EIA assay. Physical activity was measured with the Human Activity Profile (HAP) Adjusted Activity Score (AAS) and Maximum Activity Score (MAS). HRQoL was measured with the SF-12 Physical Component Score (PCS) and Mental Component Scores (MCS). Mean age of the participants in the analytic cohort (n=192) was 62 years. There were 124 participants (65%) with 25D < 15ng/mL, indicating deficiency. After adjusting for age, sex, race/ethnicity, diabetes status, season and center, lower 25D levels were independently associated with lower scores on the HAP and a lower score on the MCS of the SF-12.

Association of 25OHD level with physical activity and quality of life.

| Dependent variable | Adjusted ß coefficient (p value) |
|---|---|
| HAP, AAS | 0.72 (0.03) |
| HAP, MAS | 0.55 (0.03) |
| SF-12, PCS | 0.25 (0.15) |
| SF-12, MCS | 0.41 (0.03) |

In a well-characterized cohort of incident dialysis patients, lower 25D concentrations are associated with lower self-reported physical activity and poorer self-reported mental health. Further research is required to determine whether hypovitaminosis D is causally linked to reduced activity, and whether supplementation can improve physical activity and HRQoL in the dialysis population.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO172

**Ethnic Differences in Response to Ergocalciferol Therapy in Chronic Kidney Disease** Iris Sanchez,[1] Roberto Mangoo-Karim,[1] Jason R. Stubbs,[2] George P. Yanev,[3] James B. Wetmore.[2] *[1]South Texas Kidney Specialists, PA, McAllen, TX; [2]Medicine, Division of Nephrology, University of Kansas Medical Center, Kansas City, KS; [3]Mathematics, University of Texas - Pan American, Edinburg, TX.*

Nutritional vitamin D deficiency is common in patients with chronic kidney disease (CKD). Previous investigations have suggested racial differences in prevalence of this disorder, but no studies have specifically examined differences between ethnic groups in response to vitamin D2 repletion.

We performed a retrospective analysis evaluating the effectiveness of D2 repletion in 261 Hispanic and Caucasian CKD patients from a solar-rich environment treated with the ergocalciferol replacement as recommended by KDOQI. We measured baseline and end-of-study 25(OH)D levels and compared responses to therapy. We stratified individuals by the presence of proteinuria to assess its effects on response to therapy.

Low 25(OH)D levels (<30 ng/mL) were found in 89.5% of Hispanics versus 53.1% of Caucasians with similar degrees of CKD. Treatment with ergocalciferol per KDOQI guidelines was clinically ineffective in restoring 25(OH)D levels to the normal range, with 86.1% of Hispanics and 50.0% of Caucasians remaining insufficient following therapy. Caucasians had a two-fold greater increase in 25(OH)D levels (2.2 +/- 0.5 ng/ml per 100,000 IU ergocalciferol) compared to Hispanics (0.9 +/- 0.2 ng/ml per 100,000 IU; between-group $p < 0.001$). Patients with proteinuria >300 mg/d demonstrated a poor response to therapy (-0.2 +/- 0.4 ng/ml per 100,000 IU) compared to individuals with lower levels of proteinuria (increase of 1.1 +/- 0.4 ng/ml per 100,000 IU; between-group $p = 0.03$).

25(OH)D deficiency is common in Hispanics, a growing population with high rates of CKD. Hispanics demonstrated less robust response to therapy than did non-Hispanics. The presence of proteinuria > 300 mg/d was associated with a poorer response to ergocalciferol therapy. Overall, use of the KDOQI protocol demonstrated suboptimal effectiveness. Future research should focus on establishing a more effective repletion strategy in patients with CKD, especially among Hispanics and in patients with proteinuria.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO173

**Seasonal Variation in 25-Hydroxyvitamin D in the Cardiovascular Health Study** Abigail B. Shoben, Bryan R. Kestenbaum, David Siscovick, Ian H. de Boer. *University of Washington, Seattle, WA.*

Low serum concentrations of 25-hydroxyvitamin D (25OHD) are associated with adverse health outcomes in diverse populations. However, serum 25OHD levels are expected to fluctuate seasonally with sun exposure, such that a single measurement may not accurately capture the vitamin D status of an individual. We investigated sinusoidal trends in population average levels of serum 25OHD among 2298 individuals enrolled in the Cardiovascular Health Study, a community-based study of adults aged 65 years and older. A sinusoidal model fit observed 25OHD levels significantly better than a mean model, which assumes no seasonal variation ($p<0.0001$). Peak 25OHD levels occurred in September with trough in March. From the sinusoidal model, the mean peak-trough difference in 25OHD levels was 9.6 ng/mL (95% CI: 9.5 to 9.7; see Figure). A greater peak-trough difference was associated with younger age, male sex, more northern study sites, and greater physical activity ($p<0.05$ for all). Neither overall mean 25OHD nor 25OHD peak-trough difference varied by estimated GFR. Serum levels of intact parathyroid hormone (PTH) and bone-specific alkaline phosphatase (BAP) also varied by season in a sinusoidal fashion ($p<0.0001$) reciprocal to 25OHD. Participants with estimated GFR < 60 mL/min/1.73m2 (N=406, 18%) had higher overall levels of PTH and BAP with no difference in seasonal variation. In conclusion, serum 25OHD varies in a sinusoidal manner throughout the year, with reciprocal changes in PTH and BAP. Single 25OHD measurements may not capture overall vitamin D exposure, and extent of misclassification may vary by key demographic and behavioral factors. Findings have implications for the interpretations of 25OHD levels clinically and in the research setting.

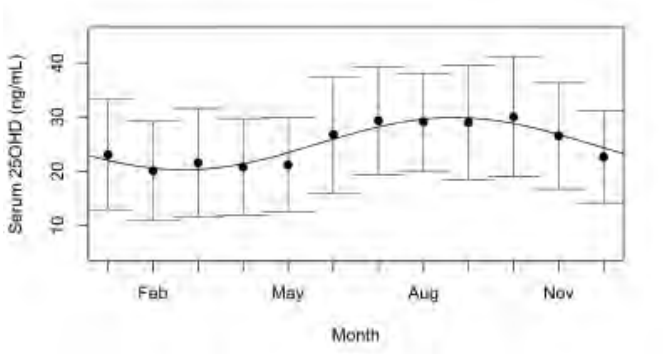



**Figure.** Sinusoidal model fit for observed 25OHD levels superimposed on plot of observed mean (+/- sd) 25OHD level by month.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO174

**Antiproteinuric Treatment Reduces Urinary Excretion of Vitamin D Binding Protein in Patients with Chronic Kidney Disease but Does Not Improve Serum Vitamin D Levels** Carolina R. C. Doorenbos,[1] Milton M. de Cuba,[1] Maartje C. J. Slagman,[1] Jacob Van den Born,[1] Reinold O. B. Gans,[2] Gerjan Navis,[1] Martin H. De Borst.[1] *[1]Internal Medicine, Nephrology, University Medical Center Groningen, Netherlands; [2]Internal Medicine, University Medical Center Groningen, Netherlands.*

Vitamin D deficiency is common in chronic kidney disease (CKD) and associated with its progression. Proteinuria (UP) is associated with urinary loss of vitamin D binding protein (VDBP), the main vitamin D transport protein. Urinary VDBP loss has been postulated to contribute to vitamin D deficiency in CKD.

Urine and plasma VDBP levels were measured in a randomized double-blind crossover study (n=13, clearance median 60(range 25-177) ml/min), at 3 timepoints: after a 4-wk washout period without treatment, or after 4-wk treatment with lisinopril 40 mg QD (LIS), or indomethacin 75 mg BID (IND), respectively. Age and sex matched individuals screened for donation (n=10) were included as controls. Patients with more marked UP (3.9(1.7-7.5) g/24h, n=10) treated with valsartan + lisinopril were studied separately.

UP decreased from 2.9(1.3-13.1) on placebo to 1.5(0.2-6.6) on LIS and 1.2(0.3-12.5) g/24h on IND. Urinary VDBP was increased during placebo (5413(155-211027) µg/24h) compared to controls (50(41-68) µg/24h, $p<0.001$). Treatment reduced VDBP excretion: LIS 4276(50-35800) IND 1653(97-129854) µg/24h ($p<0.05$). UP and urine VDBP were correlated. Plasma VDBP was normal and unaffected by intervention: controls 41(29-51), placebo 37(18-50), LIS 34(28-61), IND 34(22-49) mg/dl, just like plasma 25(OH) vitamin D: controls 43(16-80), placebo 80(33-110), LIS 48(21-78), IND 54(27-193) nmol/l. Fractional VDBP excretion was <1% in all samples. Patients with more marked UP (n=10) showed similar results.

Urinary VDBP loss is markedly increased in proteinuric CKD, and responds to antiproteinuric treatment in proportion to UP reduction irrespective of the mode of intervention. Urinary VDBP loss was not associated with plasma levels of VDBP or 25(OH) vitamin D. Fractional VDBP excretion remains low even in nephrotic range UP. These data suggest that urinary loss of VDBP does not contribute to vitamin D deficiency in proteinuric patients.

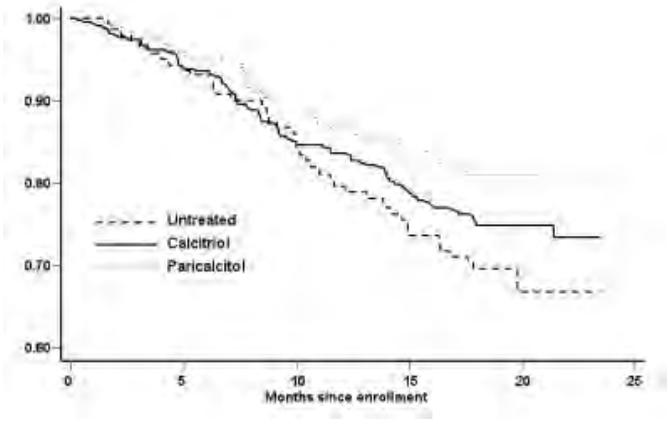
Disclosure of Financial Relationships: nothing to disclose

### TH-PO175

**VDRA Therapy Is Associated with Improved Survival in Dialysis Patients Even When Serum PTH Is Lower Than 150 pg/ml – Results of the Italian FARO Survey** Mario Cozzolino, Diego Brancaccio, Piergiorgio Messa, Giuseppe Cannella, Sandro Mazzaferro. *Renal Division, San Paolo Hospital, Milan, Italy.*

Chronic kidney disease (CKD) patients affected by MBD (Mineral Bone Disorders) have been shown to have higher rates for both all-cause and cardiovascular-related mortality. Surprisingly, most of hemodialysis (HD) patients have low serum PTH levels, but it has not been yet fully elucidated the reason why they have higher mortality compared to patients with serum PTH levels in the normal range. Using data from the FARO, a two year longitudinal study on 2378 Italian HD patients from 28 centers that collected data with standardized forms every 6 months, we have investigated the time-to-death (any cause) cumulative probability in patients with serum PTH ≤ 150 pg/ml.and in particular we investiagted the effect of vitamin D receptor activator (VDRA) therapy. Kaplan-Meier curves and proportional hazards regression models (including time-dependent covariates) were used, observing cumulative probability of time to death and adjusted hazard ratios for demographic, clinical, and/or CKD-MBD treatment characteristics. The cumulative probability of death for any cause was higher for patients with serum PTH levels ≤ 150 pg/ml., compared to HD with serum PTH > 150 pg(ml. In a model with time dependent covariates restricted to time-periods when patients had PTH ≤ 150 pg/ml, we observed a lower mortality in VDRA-treated compared to untreated patients ($P<0.001$). VDRA

treatment was associated with lower all-cause mortality hazard ratios regardless of all measured variables. Furthermore, patients who received intravenously (iv) paricalcitol, compared with either oral or iv calcitriol, showed a reduced mortality ($p<0.001$).




In conclusion, our results suggest that VDRA therapy was associated with improved survival in HD patients, even with lower serum PTH levels.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO176**

**Associations of 25 Hydroxyvitamin D Levels with Mortality Are Independent of Physical Activity and Physical Functioning in CKD: NHANES III** Vidya M. Raj Krishnamurthy,[1,2] Bradley C. Baird,[1] Guo Wei,[1] Tom H. Greene,[1,2] Srinivasan Beddhu.[1,2] *[1]Univ of Utah; [2]VA, SLC, UT.*

Vitamin D deficiency as well as poor physical functioning and lower physical activity are common in the CKD population. However, it is unclear whether the associations of low serum vitamin D levels with mortality are independent of the levels of physical activity and physical functioning. Therefore, we examined these in the National Health And Nutrition Examination Survey (NHANES III) data (n=1207). Physical activity was obtained based on the frequency and intensity of leisure time physical activity obtained by a questionnaire. Physical function was assessed by timed rising 5 times from a chair and timed walking on a 8 foot course. Mortality data were obtained by NCHS by linkage of NHANES III and National Death Index records through December 31, 2006.

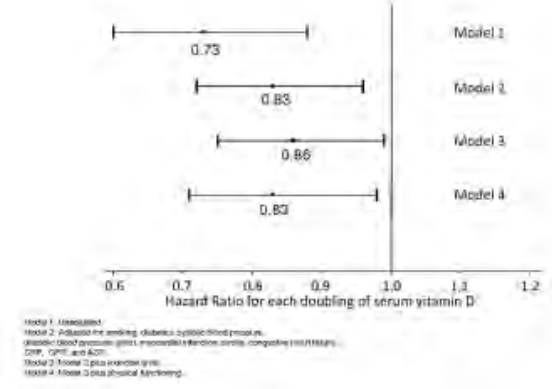
Baseline characteristics by serum vitamin D groups are summarized in Table 1.

Baseline Characteristics by Vitamin D Levels

| | < 15 ng/mL (10%) | 15-30ng/mL (52%) | > 30 ng/mL (38%) |
|---|---|---|---|
| Vitamin D (ng/mL) | 11.8±0.2 | 22.6±0.3 | 38.8±0.5 |
| Age (yrs) | 69±2 | 70±1 | 68±1 |
| Male (%)** | 24 | 30 | 48 |
| AA Race (%)** | 23 | 7 | 3 |
| MI (%) | 16 | 15 | 16 |
| CHF (%) | 13 | 13 | 12 |
| DM (%)** | 33 | 25 | 12 |
| Smoking (%) | 17 | 14 | 11 |
| Inactivity (%)* | 44 | 31 | 20 |
| Time to 5 stands (sec)** | 16.3±0.7 | 14.6±0.4 | 13.1±0.3 |
| Time to 8-foot Walk (sec)* | 5.3±0.5 | 4.2±0.1 | 3.8±0.1 |
| eGFR (ml/min/1.73m²)* | 45.5±1.3 | 49.1±0.5 | 50.4±0.5 |
| Waist (inch)* | 39.1±0.8 | 38.6±0.3 | 37.6±0.4 |

* $0.001<p\leq0.05$, ** $p\leq0.001$.

The associations of vitamin D with mortality are summarized in Figure 1.




We conclude that higher serum vitamin D levels are associated with better physical activity and functioning levels but the associations of higher vitamin D levels with lower hazard of death were independent of physical activity and functioning in CKD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO177**

**1,25-Dihydroxyvitamin D Inhibits Hyperglycemia-Induced Podocyte Apoptosis** Youli Wang, Dilip K. Deb, Yan Chun Li. *Medicine, The University of Chicago, Chicago, IL.*

Podocytes are a key component of the glomerular filtration barrier and podocyte loss is a major pathogenic factor in diabetic nephropathy. Our previous studies demonstrated that vitamin D inhibits diabetic nephropathy by reducing proteinuria and podocyte loss in diabetic mice, and the aim of this study is to explore the molecular mechanism whereby vitamin D blocks podocyte apoptosis. Chromatin condensation and DNA fragmentation assays demonstrated that exposure of podocyte cultures to high glucose (HG, 30 mM) caused apoptosis, and apoptosis was blocked by 1,25-dihydroxyvitamin D (1,25-VD) treatment. Podocytes exposed to HG showed up-regulation of angiotensinogen, renin and AT1 receptor, and the elevation of these proteins was attenuated by 1,25-VD. Treatment of podocytes cultured in low glucose (LG, 5 mM) condition with Ang II also induced apoptosis, which could be blocked by AT1 blocker losartan, but not by 1,25-VD. These observations suggest that activation of the local renin-angiotensin system (RAS) by HG plays a key role in podocyte apoptosis, and 1,25-VD blocks apoptosis by targeting the upstream components of Ang II, namely renin and angiotensinogen. HG stimulated Bak but had little effects on Bcl-2, Bcl-xL and Bax, and 1,25-VD stimulated Bcl-2 and down-regulated Bax and Bak expression. Moreover, 1,25-VD also blocked active caspase-3 in podocyte cultures. Studies are undergoing to assess whether 1,25-VD affects the increase in p38 phosphorylation and Erk induced by HG. Taken together, 1,25-VD appears to block HG-induced podocyte apoptosis by targeting the local RAS and the mitochondrial outer membrane permeabilization in hyperglycemia.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO178**

**Inhibition of Monocyte ADAM17 by Paricalcitol May Provide Renal and Cardiovascular Protection in Human Kidney Disease** Adriana S. Dusso,[1] M. Vittoria Arcidiacono,[2] Karla Giles,[1] Kathy Norwood,[1] Daniel W. Coyne,[1] Christine Pham.[3] *[1]Renal, Washington University, St. Louis, MO; [2]Experimental Nephrology, IRB Lleida, Lleida, Spain; [3]Rheumatology, Washington University, St. Louis, MO.*

Activation of the renin-angiotensin II system (RAS) and systemic inflammation are key determinants of renal and cardiovascular damage in chronic kidney disease (CKD). Whereas RAS activation-driven increases in renal ADAM17 cause renal lesions in mice and human CKD, elevated monocyte ADAM17 activation drives systemic inflammation and increases the risk of cardiovascular death in normal individuals. This work examined the role of ADAM17 inhibition in paricalcitol suppression of RAS activation and inflammation in 30 hemodialysis (HD) patients. Cell surface expression of ADAM17 on monocytes obtained from peripheral blood was assessed by flow cytometry and showed levels 15-fold higher in HD than in 18 age-matched normal individuals. ADAM 17 activity was measured by the release to the blood of the soluble forms of tumor necrosis factor α (TNFα), transforming growth factor α (TGFα), and vascular cell adhesion molecule 1 (VCAM-1). Serum levels of these markers of pro-inflammatory renal, endothelial, and cardiovascular lesions were 3.2-, 1.6- and 2.8-fold higher, respectively, in HD patients compared to normal controls ($p<0.05$). Furthermore, monocyte ADAM17 expression strongly correlated with serum VCAM-1, a known marker of endothelial dysfunction ($r=0.44$, $p<0.01$). More importantly, in a subpopulation of 16 HD patients receiving nutritional vitamin D and anti-RAS therapy, paricalcitol doses of 4 ug or higher reduced monocyte ADAM17 expression by 39.1% ($p<0.05$) and serum levels of intercellular cell adhesion molecule 1 (ICAM-1), a marker of pro-fibrotic lesions released by ADAM17 from epithelial cells, by 41.2% ($p<0.01$). These reductions were unrelated to changes in serum calcium, phosphate, or PTH. Thus, the active vitamin D analog, paricalcitol, synergizes with anti-RAS therapy in renal and cardiovascular protection through a pathway unrelated to PTH suppression that involves inhibition of ADAM17 to moderate renal and systemic inflammation.

Disclosure of Financial Relationships: Honoraria: I have received honoraria from Abbott Laboratories as a speaker and the research presented is funded by Abbott.

**TH-PO179**

**A Novel Organ Culture Model To Demonstrate That Direct Suppression of PTH by the Vitamin D Prohormones, Doxercalciferol and 25-Hydroxyvitamin $D_3$, Requires the Vitamin D Receptor** Cynthia S. Ritter, Alex J. Brown. *Renal Division, Washington University School of Medicine, St. Louis, MO.*

Vitamin D compounds regulate PTH at the transcriptional level, presumably via binding to the vitamin D receptor (VDR). We recently reported that the several vitamin D prohormones that have low VDR affinity suppressed PTH, even when their activation was inhibited. To test whether prohormone efficacy is associated with a VDR-dependent mechanism, we developed a novel organ culture that allowed the assessment of activities of the prohormones on PTH release from wild-type and VDR null thyroparathyroid explants. The cultures remained viable with respect to PTH release for at least two weeks. Full suppression of PTH by the native vitamin D hormone, 1,25-dihydroxyvitamin $D_3$,[$1,25(OH)_2D_3$] required two days, consistent with a transcriptional mechanism, and was reversible, indicating that reduced PTH was not attributable to cell death. Inhibition of PTH release by $1,25(OH)_2D_3$ and two prohormones, 25-hydroxyvitamin $D_3$ and 1α-hydroxyvitamin $D_2$ was observed in explants from wild-type mice but not in those from VDR null mice. These findings 1) are the first direct demonstration of the role of

the VDR in regulation of PTH by $1,25(OH)_2D_3$, 2) confirm that the suppressive actions of the vitamin D prohormones are mediated by the VDR, and 3) introduce a novel organ culture model that allows the *ex vivo* study of the function of parathyroid glands from transgenic animals.

Disclosure of Financial Relationships: Research Funding: Chugai Pharmaceuticals
Genzyme Corporation
Abbott Laboratories.

### TH-PO180

**A Vitamin D Analog Inhibits VSMC Mineralization Induced by TNF-α and Phosphate** Yumie Aoshima,[1] Masahide Mizobuchi,[1] Chiaki Kumata,[1] Fumiko Kondo,[1] Naoko Ono,[1] Ai Nakazawa,[1] Hiroaki Ogata,[2] Eriko Kinugasa,[2] Tadao Akizawa.[1] *[1]Division of Nephrology, Department of Medicine, Showa University School of Medicine; [2]Internal Medicine, Showa University Northern Yokohama Hospital.*

**Background:** Inflammatory cytokines such as tumor necrosis factor-α (TNF-α) have been shown to accelerate the highly regulated osteogenic process in vascular smooth muscle cells (VSMCs). On the other hand, vitamin D has been demonstrated to decrease the mortality in patients with CKD, which may be partly due to protective actions on the cardiovascular system. In the present study, we examined whether maxacalcitol (Maxa), a vitamin D analog, has a suppressive effect on VSMC mineralization induced by TNF-α and phosphate.

**Methods:** Human VSMCs were treated with either vehicle (ethanol) or Maxa ($10^{-9}$ M to $10^{-7}$ M) in 2.5 mM of phosphate media with TNF-α (1 ng/ml) for 9 days. VSMC mineralization was determined by von Kossa staining and quantitated by total calcium measurement. Expression of genes associated with the osteogenic process was examined by real-time RT-PCR. Gene expression of matrix metalloproteinase-2 (MMP-2) and MMP-2 concentration in media was also analyzed.

**Results:** Vehicle treated VSMCs showed massive mineralization (30.6±7.9 μg/mg protein) while Maxa inhibited the mineralization in a concentration-dependent manner (14.0±4.4 μg/mg protein at $10^{-7}$ M $p<0.05$, 26.7±3.1 μg/mg protein at $10^{-8}$ M, and 28.3±5.0 μg/mg protein at $10^{-9}$ M). While vehicle treated VSMCs increased mRNAs associated with the osteogenic process (Runx2, osteocalcin, and collagen type I) compared with VSMCs with a regular media without TNF-α (control), Maxa had a suppressive effect on this increase in mRNAs, suggesting that Maxa inhibits VSMC mineralization with the blockade of the osteogenic process. Furthermore, vehicle treated VSMCs increased both mRNA levels and concentrations in the media of MMP-2 while Maxa suppressed both levels.

**Conclusions:** Maxacalcitol, a vitamin D analog, has a potency to inhibit VSMC mineralization induced by TNF-α and phosphate with the blockade of the osteogenic process. Anti-inflammatory properties of maxacalcitol may contribute to this inhibitory effect.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO181

**Acute Hyperuricemia Reduces 1,25 Dihydroxy-Vitamin D in Rats** Diana I. Jalal,[1] Carlos Alberto Roncal-Jimenez,[1] Miguel A. Lanaspa,[1] Wei Chen,[1,2] Michel B. Chonchol,[1] Richard J. Johnson.[1,3] *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Department of Nephrology, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China; [3]Division of Nephrology, Hypertension, and Transplantation, University of Florida, Gainesville, FL.*

Patients with gout are reported to have lower 1,25 dihydroxy-vitamin D ($1,25(OH)_2D$) levels, that improve with treatment of hyperuricemia, suggesting that uric acid inhibits 1- α hydroxylase activity. We hypothesized that acute hyperuricemia would lead to lower $1,25(OH)_2D$ levels in rats. Sprague Dawley rats were randomized to 4 groups: control, the uricase inhibitor, allantoxanamide (150 mg/kg intraperitoneally), the xanthine oxidase inhibitor, febuxostat (30 mg/kg by gavage), or allantoxanamide and febuxostat. All rats were sacrificed after 24 hours, and serum and tissue samples were collected. $1,25(OH)_2D$ and 25 hydroxy- vitamin D (25(OH)D) were measured by Elisa assay, and the ratio of $1,25(OH)_2D$/ 25(OH)D was calculated for each animal as an indicator of 1- α hydroxylase activity. 1- α hydroxylase expression was evaluated by western blot of whole kidney lysates. Allantoxanamide administration resulted in significantly higher serum uric acid levels (3.3± 1.4 mg/dL) when compared to control (1.9± 0.1 mg/dL), and uric acid levels were lower (1.2± 0.3 mg/dL) in the rats treated with allantoxanamide and febuxostat (P value for trend 0.0002). When compared to the control group, the allantoxanamide group had significantly lower $1,25(OH)_2D$ levels while 25(OH)D levels did not differ significantly between groups. Consistently, the $1,25(OH)_2D$/25(OH)D ratio was significantly lower in the allantoxanamide group. Febuxostat prevented the allantoxanamide-induced drop in $1,25(OH)_2D$ levels and the reduction in the $1,25(OH)_2D$/25(OH)D ratio. In addition, 1- α hydroxylase protein expression in the kidney was reduced in the allantoxanamide group compared to the control animals and was restored in the group that received febuxostat and allantoxanamide. Our results suggest that uric acid suppresses 1- α hydroxylase activity and that treatment of hyperuricemia may improve vitamin D metabolism.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO182

**Vida-5: A Novel VDR Modulator with Expanded Therapeutic Index and Cardio-Renal Protective Effects** J. Ruth Wu-Wong, Megumi Kawai, Yung-Wu Chen, Masaki Nakane. *Vidasym, Chicago, IL.*

VDR modulators (VDRMs) such as calcitriol, paricalcitol and doxercalciferol are commonly used to manage hyperparathyroidism secondary to chronic kidney disease (CKD). Clinical observations demonstrate that VDRM therapy may provide cardio-renal protective and survival benefit for CKD patients. However, hypercalcemia remains a serious concern for current VDRMs due to narrow TI (therapeutic index: a ratio estimated from the minimum toxic hypercalcemic dose and minimum PTH suppressing dose) at 1-4-fold, which leads to the need for frequent dose titration and serum calcium monitoring. Significant clinical benefit can be derived from a VDRM with expanded TI and cardio-renal protective benefits. We have identified a series of novel VDRMs with significantly less hypercalcemic potential. Treatment (3x/week over a period of two weeks ) by Vida-5 (0.004-0.64 μg/kg, i.p.) of 5/6 nephrectomized (NX) male Sprague–Dawley rats with established uremia at Week 6 after surgery suppressed serum PTH in a dose-dependent manner without raising serum calcium or phosphate, demonstrating a >50-fold TI. Untreated NX rats exhibited compromised endothelial function and increased left ventricular (LV) weight. When the NX uremic rats with established LV hypertrophy were treated with Vida-5 for two weeks, Vida-5 at 0.004-0.16 μg/kg improved endothelium-dependent aortic relaxation and attenuated LV abnormalities in a dose-dependent manner without affecting serum calcium. Treatment with Vida-5 significantly reduced proteinuria in the NX rats. Similar results were obtained when Vida-5 was given to NX rats by oral gavage, once daily, for two weeks. Real-Time PCR and Western blotting results showed that Vida-5 induced CYP24A1 and CD14 expression in HL-60 cells with $EC_{50}$ values at 7 and 4 nM, respectively, and suppressed thrombospondin-1 expression in human coronary artery smooth muscle cells. These studies demonstrate that Vida-5 is a VDRM with greatly expanded TI and an overall therapeutic product profile that support expanded use in pre-dialysis CKD to realize the cardio-renal protective effects of VDR activation.

Disclosure of Financial Relationships: Ownership: Own stock options.

### TH-PO183

**Restoration of Physiological Concentrations of 25-OH-Vitamin D (25-OH-D) Enables Calcitriol (1,25-OH2-D) Production in Hemodialysis (HD) Patients: Evidence from a Multicentre Randomized Controlled Trial** Annick Massart,[1] Michel Dhaene,[3] Joelle L. Nortier,[1] Frederic D. Debelle.[3] *[1]Nephrology, Erasme Academic Hospital, Brussels, Belgium; [2]Nephrology, Universitair Ziekenhuis Brussel, Brussels, Belgium; [3]Nephrology, RHMS Baudour, Baudour, Belgium.*

AIM OF THE STUDY: To investigate the impact of correcting cholecalciferol deficiency on mineral and bone disorder (MBD) parameters in HD patients.

METHODS: Adult HD patients with 25-OH-D level <30 ng/ml from 2 Belgian centres were included. Participants were randomly allocated 1:1 in double-blinded manner to receive an oral dose of 25,000 IU 25-OH-D or placebo once a week for 13 weeks. Based on preliminary data, we estimated that 15 case subjects and 15 controls would provide >95% power to detect a standardized difference of 15 ng/ml in 25-OH-D levels, assuming a two-sided type 1 error rate of 5%.

RESULTS: 54 patients were randomized and data were analysed according to intention to treat. Baseline serum 25-OH-D and 1,25-OH2-D levels were similar in both groups (13.8 +/-5,51 vs 16.1 +/-7.96 ng/ml and 18 +/- 9.8 vs 16.1 +/-7.14 pg/ml, respectively p=NS). After 13 weeks, serum 25-OH-D, 1,25-OH2-D and calcium levels significantly increased in the treated group compared to the placebo group (31.3 +/- 12.6 vs 15.7 +/- 8.13 ng/ml, p <.0001; 26.1 +/- 12.2 vs 12.4 +/-5.36, p <.0001 and 8.80 +/- 0.57 vs 8.26 +/- 1.20 mg/dl, p=.029). No statistical difference was found for serum intact parathormone (376 +/-278 vs 463 +/- 288 pg/ml), phosphorus (4.56 +/-1.60 vs 5.02 +/-1.82), C-telopeptide (2257 +/-1261 vs 2393 +/-1085.4), and bone specific alkaline phosphatase (34.4 +/-25.3 vs 31.6 +/- 31.9) levels.

CONCLUSIONS: Weekly oral administration of 25.000 IU cholecalciferol for 3 months is safe and efficient to correct 25-0H-D as well as 1,25-OH2-D deficiencies in HD patients. Long term studies are required to hypothesize the impact of cholecalciferol therapy on MBD.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO184

**The Vitamin D Analog, Eldecalcitol [1α,25-Dihydroxy-2β-(3-Hydroxypropyloxy)Vitamin $D_3$], Is a Potent Regulator of Calcium and Phosphate Metabolism** Alex J. Brown, Jane L. Finch, Eduardo Slatopolsky, Cynthia S. Ritter. *Renal Division, Washington University School of Medicine, St. Louis, MO.*

The vitamin D analog eldecalcitol has been shown to increase bone mineral density in ovariectomized rats and in post-menopausal women. Here, we compared the effects of eldecalcitol and 1,25-dihydroxyvitamin $D_3$ [calcitriol] on calcium and phosphate metabolism in normal rats. In the 1st protocol, eldecalcitol and calcitriol (0. 7.5, 20 or 50 pmol) were given orally every other day for two wks. The highest dose of eldecalcitol elevated ionized Ca, enhanced intestinal Ca absorption and increased urinary Ca; calcitriol had no significant effects at these doses. The high dose eldecalcitol stimulated both intestinal Pi absorption and urinary Pi, but not serum Pi. The phosphaturia was attributable, in part, to an increase in serum FGF-23. In a 2nd protocol, the effects of the high dose eldecalcitol on Ca and

Pi absorption and urinary excretion, and the increased FGF-23, persisted for several days following cessation of treatment, likely due to its higher DBP affinity and slower clearance than calcitriol. Because vitamin D compounds can modulate mineral metabolism indirectly by altering PTH levels, a 3rd protocol examined the effects of eldecalcitol and calcitriol in PTX'd rats that were infused with PTH to normalize Ca levels. Eldecalcitol (50 pmol) had greater effects than calcitriol on serum Ca and urinary Ca. In this model, eldecalcitol and, to a lesser extent, calcitriol (50 pmol) increased intestinal Pi absorption, but decreased serum Pi. This was due to a greater increase in urinary P, secondary to elevations in FGF-23. These studies indicated that with chronic oral administration, eldecalcitol is more potent than calcitriol in stimulating intestinal Ca and Pi absorption and renal excretion of Ca and P. The higher urinary Pi with eldecalcitol is due to a greater stimulation of FGF-23. The greater effects of eldecalcitol on mineral metabolism are not due to VDR affinity, which is lower, but likely to its higher DBP affinity and resistance to metabolism. The role of DBP binding in the beneficial effects of eldecalcitol on bone is under investigation.

Disclosure of Financial Relationships: Research Funding: Chugai Pharmaceuticals
Genzyme Corporation
Abbott Laboratories.

## TH-PO185

**Pharmacodynamic and Pharmacokinetic Profiles of CTAP101 Capsules and Intravenous Calcifediol for Secondary Hyperparathyroidism in Stage 3 or 4 CKD** Joel Z. Melnick,[1] John R. Sedor,[2] Navindra J. Ramjit,[1] Samir P. Tabash,[1] Peg Pepping,[1] Nancy L. Samberg,[1] Susan C. Cronin,[1] Mojtaba Noursalehi,[1] Gerald Schulman.[3] *[1]Cytochroma Inc., Markham, ON, Canada; [2]Case Western Reserve University, Cleveland, OH; [3]Vanderbilt University Medical Center, Nashville, TN.*

Most CKD patients develop vitamin D insufficiency leading to secondary hyperparathyroidism (SHPT), bone and cardiovascular disease. Current therapies are poorly effective in normalizing serum total 25D and intact parathyroid hormone (iPTH). CTAP101 Capsules is a modified-release formulation of calcifediol being developed to treat SHPT associated with vitamin D insufficiency in Stages 3 and 4 CKD.

In this randomized open label single-dose study, 27 subjects with baseline serum total 25D between 16 and 29 ng/mL and serum iPTH above K/DOQI targets were dosed with CTAP101 (450 or 900 mcg) or IV calcifediol (450 mcg) to evaluate bioavailability, pharmacokinetics, pharmacodynamics, safety and tolerability. Serum calcium (Ca), phosphorus (P), iPTH, $25D_3$ and total 1,25D were monitored for 6 weeks. Mean (±SD) baseline $25D_3$ and iPTH were 20±10 ng/mL and 197±146 pg/mL, respectively. IV calcifediol rapidly raised mean $25D_3$ to 134±19 ng/mL. CTAP101 produced gradual increases in $25D_3$ to a mean of 25±10 (450 mcg) and 32±16 ng/mL (900 mcg). The bioavailability was ~10%. No confirmed hypercalcemia (>10.3 mg/dL) was observed in any treatment group. No SAEs were reported. Serum total 1,25D reached an increase of 13 pg/mL at 4 hrs after IV calcifediol, and of 3 and 7 pg/mL at 24 hrs after 450 and 900 mcg of CTAP101, respectively. IV calcifediol and 450 mcg of CTAP101 had no clinically meaningful effect on mean iPTH. CTAP101 dosed at 900 mcg reduced iPTH from baseline by a mean of 19% at 24 hrs. The rapid increase in serum total 1,25D after IV calcifediol may have triggered excessive expression of the vitamin D catabolic enzyme, CYP24, in the parathyroid glands, leading to local hormone resistance and limited iPTH suppression.

These findings demonstrate that CTAP101 Capsules gradually normalized $25D_3$ levels and suppressed elevated iPTH, and that the mechanism to lower iPTH may be more complex than simply boosting serum total 25D.

Disclosure of Financial Relationships: Employer: Cytochroma; Ownership: Cytochroma.

## TH-PO186

**Suppression of PTH by the Vitamin D Analog, Eldecalcitol [1α,25-Dihydroxy-2β-(3-Hydroxypropyloxy)Vitamin $D_3$], Is Modulated by Its High Affinity to the Serum Vitamin D Binding Protein and Resistance to Metabolism** Cynthia S. Ritter, Alex J. Brown. *Renal Division, Washington University School of Medicine, St. Louis, MO.*

The vitamin D analog, eldecalcitol [1α,25-dihydroxy-2β-(3-hydroxypropyloxy)vitamin $D_3$], has a greater efficacy than alfacalcidol (1α-hydroxyvitamin $D_3$) for the treatment of osteoporosis. The explanation for the greater stimulatory effects on bone formation and inhibitory actions on bone resorption is not clear. Eldecalcitol has been found to be less potent than 1,25-dihydroxyvitamin $D_3$ (calcitriol) in suppressing PTH in vivo. To define the mechanism for the latter observation, we compared the effects of eldecalcitol and calcitriol on PTH secretion by bovine parathyroid cells (bPTC). Dispersed bPTC were cultured in the presence of 15% calf serum. After 72 h, the cells were treated daily with eldecalcitol or calcitriol (0, 0.1, 1, 10 or 100 nM) for 3 d, and the rate of PTH release measured. Eldecalcitol was equipotent with calcitriol in regulating PTH under these conditions. Since eldecalcitol has a higher affinity for the serum vitamin D binding protein (DBP), we repeated the experiment in the absence of serum and found eldecalcitol to be ~100 times more potent than calcitriol in suppressing PTH release. Therefore, DBP appears to limit the uptake and activity of eldecalcitol in bPTC, providing an explanation for the lower PTH suppressing activity in vivo (100% serum). However, the 100-fold higher activity of eldecalcitol in the absence of serum was unexpected since the VDR affinity of eldecalcitol is half that of calcitriol. Preferential uptake was ruled out since $^3$H-eldecalcitol was taken up more slowly than $^3$H-calcitriol. The explanation for the potency of eldecalcitol was found to be its resistance to metabolism. While $^3$H-calcitriol was completely degraded within 24 h, $^3$H-eldecalcitol was not metabolized, despite the induction of the vitamin D catabolic enzyme, 24-hydroxylase (CYP24A). Thus, the unique properties of eldecalcitol in vivo can be attributed, in part, to its high DBP affinity which increases the half-life, but limits uptake, of eldecalcitol, and to its reduced metabolism, which prolongs the activity of this analog in target tissues.

Disclosure of Financial Relationships: Research Funding: Chugai Pharmaceuticals
Genzyme Corporation
Abbott Laboratories.

## TH-PO187

**Novel Synthetic Prohormonal Forms of Vitamin D for Use in the Treatment of Secondary Hyperparathyroidism Associated with CKD** Christian Helvig,[1] Christopher Hosfield,[1] Rina Glozman,[1] Dominic Cuerrier,[1] Gary H. Posner,[2] Aza Kharebov,[1] Martin P. Petkovich.[1] *[1]Research and Development, Cytochroma Inc., Markham, ON, Canada; [2]Department of Chemistry, The Johns Hopkins University, Baltimore, MD.*

The vitamin D prohormone, 25-hydroxyvitamin $D_3$ ($25D_3$), is hydroxylated at C-1 by the cytochrome P450, 1α-hydroxylase (CYP27B1), to form the active hormone, 1,25-dihydroxyvitamin $D_3$ ($1,25D_3$). In healthy individuals, CYP27B1 is most abundantly expressed in the kidney where its expression is regulated -positively by PTH and negatively by $1,25D_3$ and FGF23 - to tightly control blood levels of $1,25D_3$. CYP27B1 is also expressed at lower levels in essentially all other (extra-renal) tissues. While extra-renal CYP27B1 allows for local generation of $1,25D_3$ from circulating $25D_3$, total hormone production becomes insufficient to correct secondary hyperparathyroidism (SHPT) in late-stage CKD.

We have designed CTA192, a novel synthetic vitamin D prohormone which, when activated by CYP27B1, resists inactivation by CYP24, the cytochrome P450 24-hydroxylase specifically responsible for vitamin D catabolism. In HPK1a-*ras* cells expressing CYP27B1, CTA192 induced transcriptional activity approximately 5 -10% of that observed with the C-1 hydroxylated parent compound. Comparatively, the prohormone $25D_3$ exhibited approximately 10% of the activity of $1,25D_3$ in this cell line. Further *in vitro* studies demonstrated that CTA192 did not bind to the vitamin D receptor and was inactive when compared to its parent compound in U937 cells expressing low levels of CYP27B1. CTA192, when administered over a 2-week period (6 doses) to rats rendered uremic by exposure to adenine, was equally effective in lowering blood PTH levels as its parent compound at doses only 5-6 fold higher. In contrast to its parent, CTA192 did not raise serum calcium or FGF23 levels. These studies indicate that synthetic prohormones requiring CYP27B1 for activation, such as CTA192, may offer safety advantages over current hormone replacement therapies which frequently cause hypercalcemia.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO188

**Relationships of IV Vitamin D (IVVD) Use with Mortality and Hospitalization in Hemodialysis (HD) Patients Are Not Modified by Race/Ethnicity (R/E)** K. Servilla,[1] B. Horowitz,[1] W. Hunt,[1,2] P. Zager.[1,2] *[1]UNM, Abq, NM; [2]DCI, Abq, NM.*

R/E impacts outcomes of HD patients. Black & Hispanic HD patients have lower mortality than non-Hispanic whites (NHW). Wolf observed an interaction between R/E and IVVD use in FMC-NA HD patients (JASN 2008). Specifically in patients not receiving IVVD mortality was higher in blacks vs. NHW. The opposite was true in those receiving IVVD. We tested for effect modification by R/E on the association of IVVD with mortality and hospitalization in DCI HD patients. We studied an incident cohort (n=14,661; black, n=5,301; Hispanic n=633; NHW, n=8,727). Mean PTH (pg/ml) was highest in blacks (334), intermediate in Hispanics (253) & lowest in NHW (209). Blacks (80%) & Hispanics (67%) were more likely than whites (48%) to receive IVVD. IVVD doses were higher in blacks vs. Hispanics and NHW. We constructed proportional hazards (PHM) and marginal structural models (MSM) to test for associations of IVVD with mortality and hospitalization. Three-year mortality was lower in blacks & Hispanics vs. NHW, regardless of IVVD exposure (table).

HR for Mortality by R/E and IVVD (computed) in PHM

| | Black vs. NHW | Hispanic vs. NHW | Hispanic vs. Black |
|---|---|---|---|
| IVVD | 0.71 (0.65, 0.78) | 0.70 (0.56, 0.87) | 0.98 (0.79, 1.23) |
| No IVVD | 0.75 (0.67, 0.84) | 0.68 (0.52, 0.88) | 0.90 (0.68, 1.18) |
| All | 0.73 (0.68, 0.78) | 0.69 (0.58, 0.82) | 0.95 (0.80, 1.13) |

In aggregate, the HR (95% CI) for mortality with IVVD were 0.93 (0.87, 0.99) in PHM and 0.89 (0.80, 0.90) MSM. In an analysis stratified by R/E mortality tended to be lower in each group of IVVD treated patients, but these differences reached statistical significance only in the MSM for NHW (0.88 (0.78,0.99). There were no interactions between R/E & IVVD in either the PHM (p=0.72) or MSM (p=0.68). There was no significant relationship between IVVD & hospitalization. Summary: IVVD use was associated with decreased mortality. This relationship was not modified by R/E. Conclusion: The greater use of IVVD among blacks & Hispanics vs. NHW suggests that it may be prudent to add R/E as a variable in the expanded bundle.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO189**

**Safety of Rapid, High-Dose Vitamin D Repletion in ESRD Patients: A Pilot Study** M. S. Singapuri, Monnie Wasse. *Renal Division, Emory University School of Medicine, Atlanta, GA.*

Background: 25-hydroxyvitamin D (25-OHD) is an essential nutrient frequently deficient in up to 90% of ESRD patients due to impaired renal conversion of 25-OHD to 1,25-dihydroxyvitamin D, insufficient sunlight exposure, and low dietary intake. This pilot study evaluated the safety and efficacy of a novel vitamin D repletion regimen in ESRD patients.

Methods: We conducted a double-blind randomized controlled pilot study to evaluate the safety and efficacy of rapid, high-dose nutritional vitamin D repletion in ESRD patients deficient in 25-OHD. 22 ESRD patients were randomly assigned to receive placebo or Vitamin D3 (cholecalciferol), which were taken orally and were identical in shape and color. Subjects were directly observed taking vitamin D3 200,000 IU once weekly for 3 weeks (total 600,000 IU). Blood samples were collected at baseline and within two weeks following completion of medication dosing. Serum levels of parathyroid hormone, 25-OHD, phosphorus and calcium were compared before and after treatment with cholecalcifierol.

Results: In the overall cohort, mean 25-OHD concentration was 17.8 ng/dL, mean age was 54 years, and 95% of patients were Black. No differences in baseline characteristics were observed between placebo (n=12) vs. vitamin D3 (n=10) treated patients. Within the treatment arm, 25(OH) D levels increased from 15.8 ng/dL to 57.6 ng/dL (p=0.0002), and 1,25 (OH)D levels rose (10.5 ng/dL to 25 ng/dL {p=0.02}), while no significant change in 25-OHD or 1,25-OHD was observed within the placebo-treated arm. 100% of patients in the treatment arm achieved 25-OHD sufficiency without vitamin D toxicity. Changes in serum phosphorus (p=0.30), calcium (p=0.38) and PTH (p=0.28) were no different between treatment arms , although PTH trended downward in vitamin D-treated patients (488 pg/ml pre-treatment to 404 pg/ml post-treatment (p=0.26}.

Conclusion: Our regimen of 600,000 IU of cholecalcifierol given over 21 days achieved vitamin D sufficiency in 100% of cases, without vitamin D toxicity. Initial results of our rapid, high-dose vitamin D repletion regimen to correct vitamin D status in ESRD patients show that it is safe and effective in achieving vitamin D sufficiency.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO190**

**Heritability and Seasonal Variability of Vitamin D Levels in Male Twins** Cristina Karohl,[1] Shaoyong Su,[2] Meena Kumari,[3] Vin Tangpricha,[3] Emir Veledar,[4] Viola Vaccarino,[2] Paolo Raggi.[4] *[1]Department of Nephrology, Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil; [2]Department of Epidemiology, Rollins School of Public Health; [3]Department of Endocrinology; [4]Department of Cardiology, Emory University, Atlanta, GA.*

**Objective:** It is not clear to what extent genetic influences play a role in determining vitamin D status. We estimated the heritability of vitamin D levels and the impact of season on heritability estimates.

**Method:** We measured serum 25-hydroxyvitamin D (25(OH)D) in 510 middle-age male twins selected from the Vietnam Era Twin Registry [310 monozygotic (MZ) and 200 dizygotic (DZ) twins]. Generalized estimating equations were used to test the association between 25(OH)D and other study factors. Structural equation modeling was used to estimate the heritability of 25(OH)D. Winter was defined as November to March, and summer was defined as April to October.

**Results:** The mean age was 55±2.8 years. The mean 25(OH)D level was 38.4±23.3 ng/mL, with a substantial seasonal variation (6.1 ng/mL lower values during the winter compared to summer, P=0.003). About 70% of the variation in 25(OH)D levels during the winter was explained by genetic factors. However, in the summer, the levels of 25(OH)D did not show a heritable trait. During the summer, 53% of the variation in 25(OH)D levels was due to shared environmental factors and 47% to unique environmental factors.

Variance components in vitamin D explained by additive genetic factors (A), common (C) and unique (E) environmental factors during winter and summer seasons

| Season | A | C | E |
|---|---|---|---|
| Winter | 70 (31-80) | 0 (0-34) | 30 (20-46) |
| Summer | 0 (0-35) | 53 (21-64) | 47 (36-59) |

Data: parameter estimates (95% CI)

**Conclusion:** Serum 25(OH)D levels are highly heritable during the winter season. In the summer, environmental conditions (such as sun exposure) prevail over genetic background in determining serum 25(OH)D levels.

Disclosure of Financial Relationships: nothing to disclose

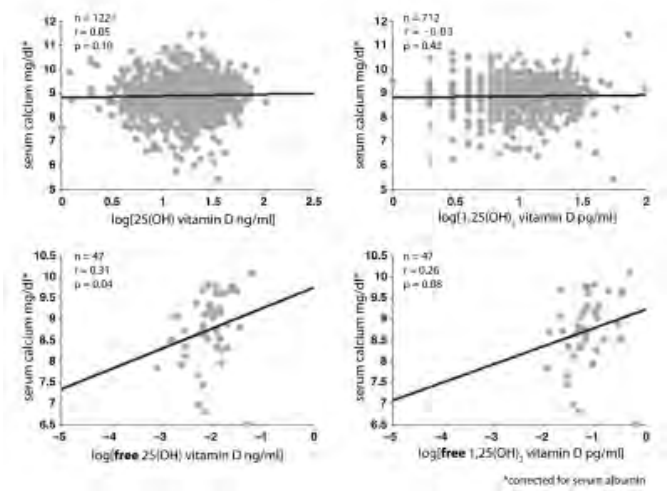
**TH-PO191**

**Vitamin D Binding Protein Modifies Relationship between Vitamin D and Calcium in Dialysis** Camille Elise Powe,[1] S. Ananth Karumanchi,[2] Julien L. Pham,[1] Jun Ye,[1] Catherine E. Ricciardi,[1] Elizabeth D. Ankers,[1] Ravi I. Thadhani,[1] Ishir Bhan.[1] *[1]Division of Nephrology, Massachusetts General Hospital, Boston, MA; [2]Division of Nephrology, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: The free hormone hypothesis stipulates that only unbound hormones are biologically active. Over 85% of circulating 25-hydroxyvitamin D 25(OH)D and 1,25-hydroxyvitamin D (1,25$(OH)_2$D) is bound to vitamin D binding protein (DBP). Few, if any, studies have examined free Vitamin D levels in subjects with end-stage renal disease (ESRD) and assessed their biological significance.

Methods: We measured levels of DBP, total 25(OH)D, total 1,25$(OH)_2$D, calcium, and albumin levels in incident dialysis patients from the Accelerated MOrtality on Renal Replacement (ArMORR) cohort. Using vitamin D, albumin, and DBP levels, we calculated free 25(OH)D and 1,25$(OH)_2$D levels using previously published formulae. We explored associations between vitamin D levels and levels of serum calcium, which are biologically linked to vitamin D action .

Results: Calcium levels (corrected for albumin) were correlated with free levels of 25(OH)D (r=0.31, p=0.04), but not total 25(OH)D (r=0.05, p=0.10). Corrected calcium levels appeared to be more strongly correlated with free 1,25$(OH)_2$D (r=0.26, p=0.08) than total 1,25$(OH)_2$D levels (r=-0.03, p=0.42). Figure 1 shows the relationship of corrected calcium with total and free vitamin D levels.




Conclusion: In ESRD, free levels of 25(OH)D and 1,25(OH)D are more strongly associated with serum calcium levels than total vitamin D levels. Future studies should explore the link between free vitamin D levels and outcomes in ESRD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO192**

**Vitamin D Supplementation in Chronic Kidney Disease: A Systematic Review and Meta-Analysis** Praveen Kandula,[1] Mirela A. Dobre,[2] Jesse D. Schold,[3] Rajnish Mehrotra,[4] Sankar D. Navaneethan.[3] *[1]Internal Medicine, Southern Illinois University School of Medicine, Springfield, IL; [2]Internal Medicine, Huron Hospital; [3]Nephrology, Cleveland Clinic, Cleveland, OH; [4]Nephrology, UCLA Medical Center, Torrence, CA.*

Vitamin D deficiency is highly prevalent among patients with CKD. We assessed the benefits and harms of vitamin D supplementation (ergocalciferol or cholecalciferol) in patients with CKD.

MEDLINE (1966-September 2009), SCOPUS (September 2009), and nephrology conference proceedings were searched for relevant observational and randomized controlled trials (RCTs). Treatment effects were summarized as relative risk (RR) or mean differences (MD) with 95% confidence intervals (CI) using a random effects model. Separate analyses were conducted for observational studies and RCTs.

Twenty-two studies (17 observational and 5 RCTs) were included. There was a significant improvement in 25-hydroxyvitamin D (25D)(24.1 ng/ml, 95% CI 19.6 to 28.6) as well as 1, 25 D levels and an associated decline in PTH levels (-41.7 pg/ml, 95% CI -55.8 to -27.7) among observational studies. PTH reduction was higher in dialysis patients. Among RCTs, there was a significant improvement in 25 D (14 ng/ml, 95% CI 5.6 to 22.4) and an associated decline in PTH levels (-31.5 pg/ml, 95% CI -57 to -6.1) but no significant change in 1, 25 D levels. A low incidence of hypercalcemia and hyperphosphatemia was reported with vitamin D supplementation. Effects of vitamin D supplementation on outcomes related to cardiovascular and bone disease have not been studied.

Available evidence from low to moderate quality observational studies and few RCTs suggests that vitamin D supplementation improves biochemical endpoints. However, whether such improvements translate into clinically significant outcomes is yet to be determined.

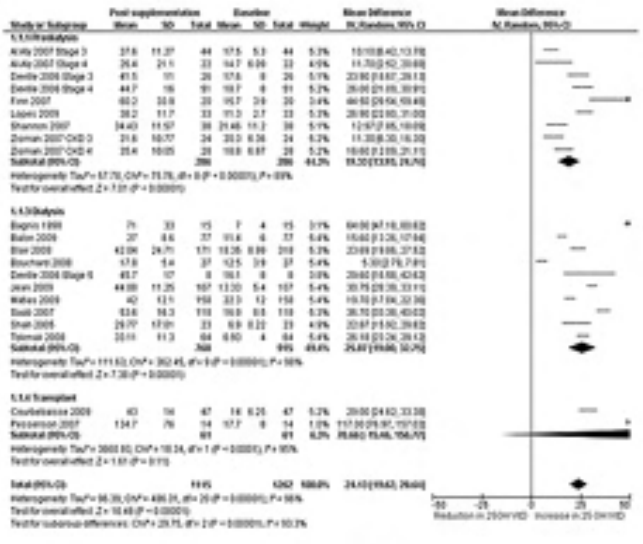


**Figure 1** Effect of vitamin D supplementation on the end of treatment 25(OH)D levels among observational studies in people with chronic kidney disease

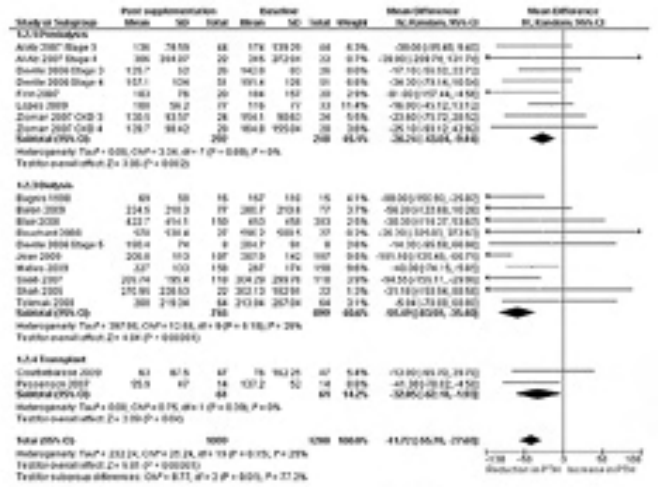


**Figure 2** Effect of vitamin D supplementation on the end of treatment PTH levels in observational studies in people with chronic kidney disease

Disclosure of Financial Relationships: nothing to disclose

## TH-PO193

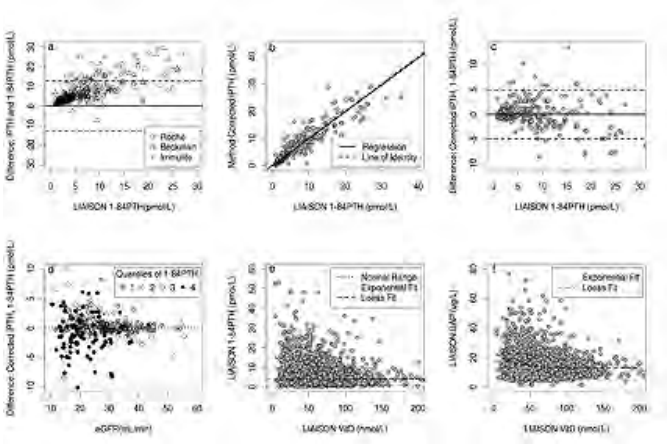
**Relationships between Diasorin LIAISON 1-84PTH, Intact PTH, Bone ALP, and 25(OH)Vitamin D in a CKD Cohort from the CanPREDDICT Study** Daniel T. Holmes, Ognjenka Djurdjev, Adeera Levin. *U. of British Columbia.*

We desired to investigate relationships between a new automated 1-84PTH assay, intact PTH (iPTH), and other bone markers in a CKD cohort. Specimens from 2374 pts from the CanPREDDICT study were analyzed for 1-84PTH, bone ALP (BAP),and 25(OH)VitaminD (VitD) on the Diasorin LIAISON analyzer. Recruiting sites performed iPTH,Ca,PO4,and Cre using local methods. Large biases exist between iPTH and 1-84PTH. After method-specific iPTH correction, correlations between iPTH, 1-84PTH and bone markers were similar. Difference plots show large scatter. Nonlinear regression shows little decrease in 1-84PTH or BAP for VitD>75 nM. VitD status is difficult to predict from PTH or BAP. 1-84PTH and iPTH assays are not directly comparable. Discrepancy can be modeled as a fn. of eGFR.

Descriptive Statistics and Correlations

| **Analyte** | **1-84PTH (pM)** | **BAP (ug/L)** | **VitD (nM)** | **Ca (mM)** | **PO4 (mM)** | **Cre (μM)** | **% in CKD Stages 3,4 & 5** |
|---|---|---|---|---|---|---|---|
| Median (IQR) | 4.7 (2.8-8.0) | 12.8 (9.2-17.4) | 60 (41-83) | 2.3 (2.2-2.4) | 1.17 (1.0-1.3) | 199 (160-253) | 36, 59 ,5 |
| N. Range | 0.70-3.90 | see below | >75 | 2.18-2.58 | 0.80-1.60 | 60-100 M, 50-90 F | - |
| **Correlations [CI]** | 1-84PTH | BAP | VitD | Ca | PO4 | Cre | eGFR |
| 1-84PTH | 1 | 0.49 [0.40,0.57] | -0.34 [-0.44,-0.24] | -0.35 [-0.44,-0.24] | 0.21* [0.05-0.35] | 0.39 [0.29-0.48] | -0.43 [-0.51,-0.34] |
| iPTH (All - corrected) | 0.92 [0.90-0.94] | 0.48 [0.39,0.56] | -0.33 [-0.43,-0.23] | -0.34 [-0.43,-0.24] | 0.23 [0.08-0.37] | 0.40 [0.31-0.49] | -0.43 [-0.52,-0.34] |

R for PTH vs VitD and eGFR are log/log. All p <0.01 for R's except *, where p=0.01. For correlations, n=301-321



Disclosure of Financial Relationships: nothing to disclose

## TH-PO194

**Effective Management of Hyperparathyroidism, Vitamin D and Mineral Metabolism Following Roux-en-Y Gastric Bypass Surgery** Rajiv Kumar, John C. Lieske, Ellen Olson, Xujian Li, Zachary C. Ryan, Eric J. Bergstralh, Maria L. Collazo-Clavell. *Nephrology Research, Mayo Clinic, Rochester, MN.*

Patients with morbid obesity frequently undergo RYGB surgery for weight loss. Patients often develop secondary hyperparathyroidism and elevated alkaline phosphatase concentrations, placing them at risk for bone loss. We examined the effects of aggressive vitamin $D_3$ and calcium (Ca) citrate supplementation on parameters of mineral metabolism in 11 patients at 6 and 12 months (m) post RYGB surgery. The patients received an average of 5000 IU of vitamin $D_3$/per day and 1600 mg of elemental calcium/day immediately following surgery.

*Results:* The results immediately prior to surgery, and at 6 m and 12 m following surgery (**Table)**, demonstrate stable serum Ca, phosphorus, total and bone alkaline phosphatase, and intact PTH concentrations. Total 25(OH)D increased to 38.5 ng/mL at 12 months from a baseline level of 26.5 ng/mL. Serum $1,25(OH)_2D$ and urine Ca did not change.

Serum and Urine Values Prior To and Following RYGB Surgery

| **Serum or urine test** | **Baseline, 0 m, mean±SD** | **6 m, mean±SD** | **12 m, mean±SD** | **P-value, paired t-test, 0-6 m** | **P-value, paired t-test, 0-12 m** |
|---|---|---|---|---|---|
| Serum Ca, mg/dL | 9.4±0.35 | 9.5±0.33 | 9.3±0.27 | 1.0 | 0.38 |
| Ionized serum Ca, mg/dL | 4.9±0.13 | 5.0±0.17 | 5.0±0.15 | 0.18 | 0.1 |
| Serum phosphorus, mg/dL | 3.8±0.65 | 3.8±0.43 | 4.2±0.39 | 0.77 | 0.051 |
| Serum PTH, pg/mL | 55.5±12.7 | 48.1±14.48 | 48.9±20.0 | 0.22 | 0.27 |
| 25(OH)D, ng/mL | 26.5±8.4 | 36.8±10.4 | 38.5±12.3 | 0.036 | 0.017 |
| $1,25(OH)_2D$, pg/mL | 41.1±7.22 | 51.4±20.57 | 52.1±20.0 | 0.11 | 0.07 |
| Total serum alkaline phosphatase, U/L | 86.3±20.4 | 100.8±32.6 | 92.0±24.8 | 0.20 | 0.53 |
| Bone serum alkaline phosphatase, U/L | 27.1±9.9 | 33.3±10.66 | 30.6±11.5 | 0.82 | 0.64 |
| Urinary Ca, mg/24h | 120.1±76.6 | 141.1±60.85 | 111.4±52.1 | 0.82 | 0.58 |

*Conclusions:* Following RYGB surgery secondary hyperparathyroidism and reductions in 25(OH)D can be effectively prevented by a regimen that contains 5000 IU vitamin $D_3$ and 1600 mg of elemental Ca/day in the form of Ca citrate.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO195

**Impact of Hypovitaminosis D and Alfacalcidol Therapy on Survival of Haemodialysis Patients: Results from the French ARNOS Study** Guillaume Jean,[1] X. Moreau-Gaudry,[3] Denis Fouque.[2] *[1]Hemodialyse, Centre de Rein Artificiel, Tassin, France; [2]Néphrologie Hemodialyse, Hopital Edouard Herriot, Lyon, France; [3]Hemodialyse, AGDUC, Montelimar, France.*

**Background:** In chronic kidney disease (CKD) and dialysis patients, vitamin D deficiency is associated with mortality. In some observational studies, calcitriol analogues therapy was associated with a better survival rate in haemodialysis (HD) patients. The aim of this study was to determine the relationship between serum 25-hydroxyvitamin D (25-OHD) levels and alfacalcidol therapy with the HD patients' outcomes.

**Methods**: We measured baseline 25-OHD levels using a cross-sectional analysis in 648 HD prevalent patients from the regional ARNOS French cohort. A 42-month survival analysis was applied according to serum 25-OHD level and CA therapy.

**Results**: The prevalence of 25-OHD insufficiency < 30 ng/ml was high (73%) with only 22% taking native vitamin D supplementation. A baseline 25-OHD level above the median value (18 ng/ml) was associated with lower all-cause mortality (hazard ratio [HR], 0.73[0.5–0.96]; $P$ = 0.02) after adjustment for age, gender, dialysis vintage, calcaemia, phosphataemia, cardiovascular disease, and diabetes. Only in monovariate analysis, low doses oral alfacalcidol therapy, was associated with a better survival rate in patients with and without 25-OHD deficiency (HR, 0.7 [0.5–0.92]; $P$ = 0.05).

**Conclusions:** Our study shows that, among prevalent HD patients, low 25-OHD levels affects mortality. Alfacalcidol therapy, especially in small doses, may provide compensation, but this needs to be further confirmed using prospective controlled studies comparing native and active vitamin D compounds.

Disclosure of Financial Relationships: Employer: my wife works for Merk laboratory; Consultancy: fresenius medical care, genzyme; Ownership: noneResearch Funding: Amgen; Honoraria: Shire, Amgen, Fresenius, Genzyme; Patent: none; Scientific Advisor: Fresenius medical care, Amgen, Genzyme; Other Relationship: none.

## TH-PO196

**Vitamin D Deficiency (Vit D Def) Is Highly Prevalent in Pediatric (ped) Patients (pts) Receiving Chronic Dialysis** Poyyapakkam Srivaths,[1] Eileen D. Brewer,[1] Stuart Goldstein,[3] Douglas M. Silverstein.[2] *[1]Pediatrics, Renal Section, Baylor College of Medicine, Houston, TX; [2]Pediatric Nephrology, Children's National Medical Center, Washington, DC; [3]Pediatric Nephrology, Cincinnati Children's Hospital, Cincinnati, OH.*

**Background**: Vit D def is widespread in adults receiving chronic dialysis. Prevalence and risk factors associated with vit D def are not well known in ped pts.

**Aim**: Assess prevalence and potential demographic risk factors for vit D def in ped pts receiving chronic dialysis.

**Method**: Cross-sectional study of 25-hydroxy vit D (25OHD) levels in 101 ped pts (median age 15y; range 0.4-26y; 42 Black,39 Hispanic,16 Caucasian,4 other); 53 hemodialysis (HD)/48 peritoneal dialysis (PD) pts at 2 centers (Houston TX & Washington DC). 66 pts also had 1,25-dihydroxy vit D (1,25D) levels assessed; among those 56/66 pts (85%) were taking calcitriol.

**Results**: 94/101 pts (93%) had vit D def (25OHD<30ng/ml); 29% had severe deficiency (25OHD<7ng/ml). Black & Hispanic pts had lower 25OHD (Black 13.3±7.9, Hispanic 13.1±7.7 vs Caucasian 21.6±9.7, p=0.01). 1,25D levels were 31.3±21.6pg/mL (range 6-86; normal 15-75). Vit D def was negatively correlated with age and positively correlated with 1,25D levels, even after controlling for calcitriol therapy and anephric state (10/66), but was not correlated with serum Ca, P or PTH levels (Table 1).

25OHD Correlation with Variables

| | Age | 1,25D | Ca | P | PTH |
|---|---|---|---|---|---|
| Pearson R | -0.43 | 0.39 | -0.11 | -0.09 | -0.14 |
| p value | 0.0001 | 0.001 | 0.23 | 0.32 | 0.16 |

25OHD levels did not differ by modality (HD vs. PD) or center (Houston vs DC).

**Conclusions:** Our data show 1) vit D def is highly prevalent in ped dialysis pts, 2) race, ethnicity and older age are significant risk factors, 3) low 25OHD levels are associated with low 1,25D levels, even in the presence of calcitriol therapy, and 4) residing in more southern latitude does not seem to offer protection from vit D def. We conclude that since vit D def is highly prevalent, calciferol supplementation may be desirable for overall health and improvement of 1,25D levels in ped pts receiving chronic dialysis.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO197

**Urinary and Dialysate Losses of Vitamin D Binding Protein May Contribute to Vitamin D Deficiency in Children with CKD and on Dialysis** Agnieszka Prytula,[1 and 2] David R. Wells,[1] Timothy I. Mclean,[3] Filipa Rola Balona,[1] Ambrose M. Gullett,[1] Kimberly Rita Hassen,[1] Sarah Ledermann,[1] Lesley Rees,[1] Rukshana C. Shroff.[1] *[1]Renal Unit, Great Ormond Street Hospital for Children, NHS Trust, London, United Kingdom; [2]Department of Pediatric Nephrology, Erasmus Medical Center- Sophia Children's Hospital, Rotterdam, Netherlands; [3]North West London Hospitals NHS Trust, Harrow, United Kingdom.*

Vitamin D deficiency is widely prevalent in CKD and dialysis patients. We hypothesise that low circulating levels of vitamin D metabolites 25-hydroxyvitamin D (25OHD) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) are due to a loss of vitamin D binding protein (VDBP) in urine and dialysate.

We studied serum, urine and dialysate VDBP levels and compared these with circulating 25OHD and $1,25(OH)_2D$ levels in 16 children on automated PD, 14 on HD and 10 in pre-dialysis CKD stage 4-5. The mean age of the study population was 9.7± 5.6 years and the median time on dialysis 8.5 (1-33) months.

Serum VDBP and 25OHD levels were significantly lower in children on HD than on PD or pre-dialysis patients ($p = 0.03$ and $p = 0.023$ respectively). There were no significant differences in $1,25(OH)_2D$ serum levels, but all except 3 children received daily oral 1-alfahydroxycholecalciferol (alfacalcidol). In the pre-dialysis group urine VDBP losses strongly correlated with urinary albumin losses ($p = 0.0008$, $r = 0.91$). Serum VDBP levels decreased with increasing time on dialysis ($p = 0.0004$, $r = -0.77$) in PD children, but not in HD. In PD children there was a correlation between serum VDBP levels and total dialysate and urine losses ($p = 0.03$, $r = -0.53$). VDBP losses in the long daytime dwell were higher than in the overnight drain ($p = 0.04$), but did not correlate with the type of dialysis fluid used. Patients with high VDBP losses in dialysate also had higher dialysate and urinary albumin losses.

61% of children were 25OHD deficient and 42.5% $1,25(OH)_2D$ deficient despite alfacalcidol supplementation, possibly as a result of high VDBP losses. VDBP losses are higher in patients with high albumin losses and increase with peritoneal membrane changes over time.

Disclosure of Financial Relationships: nothing to disclose

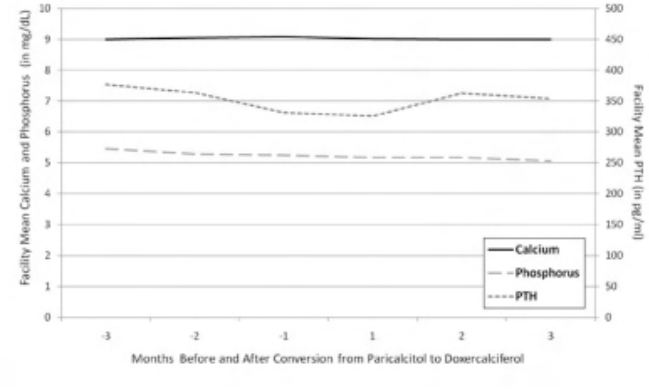
## TH-PO198

**Conversion from Paricalcitol to Doxercalciferol Does Not Affect Bone and Mineral Outcomes** Tracy Jack Mayne,[1] Heather Ashbaugh,[2] Gilbert Marlowe,[1] Mahesh Krishnan.[1] *[1]DaVita Clinical Research, Minneapolis, MN; [2]DaVita Inc., Seattle, WA.*

Background: The issue of comparative effectiveness of drugs has become a national healthcare priority, but with the impending Medicare payment changes, its importance in dialysis is immediate. While a variety of research can be conducted, prospective studies are recognized to be of a higher evidentiary standard. In order to assess the comparative effectiveness of intravenous vitamin D agents used in dialysis, we conducted a prospective conversion from paricalcitol to doxercalciferol and monitored bone and mineral outcomes at the clinic level before and after conversion.

Methods: We conducted a single arm, prospective study of 7 dialysis centers in which patients were initially exclusively prescribed paricalcitol. Patients were converted over a 2-month period to doxercalciferol. Calcium, phosphorus and parathyroid hormone (PTH) were monitored monthly. Clinic level means were computed for each outcome. General Linear Models were used to test for changes in outcomes.

Results: All 7 clinics were located in the Southwest (AZ and NV). Clinics ranged in size from 36 to 247 patients (mean=129±52.4). Utilization at the clinics went from 100% paricalcitol three months before conversion to 100% doxercalciferol three months after. Average paricalcitol clinic dose pre-conversion was 10.7±1.8 mcg. Average doxercalciferol clinic dose post-conversion was 5.7±1.2 mcg. As shown, though there was a numerical trend towards better phosphorus and PTH measures post-conversion, the effects were not statistically significant.




Conclusions: In this small prospective study, clinics successfully converted from paricalcitol to doxercalciferol with non-significant improvements in bone and mineral measures. In this time period, dose conversion was 1.9 mcg paricalcitol to 1.0 mcg doxercalciferol. There was no evidence of the superiority of either agent.

Disclosure of Financial Relationships: Employer: DaVita, Inc.; Honoraria: Sanofi-Aventis.

## TH-PO199

**Active Vitamin D Therapy Is Associated with Lower Mortality, Especially in Low 25(OH)D Levels** Masatomo Taniguchi,[1] Shunsuke Yamada,[1] Masanori Tokumoto,[3] Kazuhiko Tsuruya,[2] Hideki N. Hirakata.[4] *[1]Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University; [2]Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University; [3]Medicine, Fukuoka Dental College; [4]Fukuoka Red Cross Hospital.*

**Background.** Vitamin D therapy contributes to mortality or development of cardiovascular disease (CVD) in hemodialysis patients. Low 25(OH)D levels have recently been shown to be associated with all-cause mortality. The present study described the 25(OH)D levels in hemodialysis patients, and the influence of vitamin D treatment on morality or CVD.

**Methods.** In December 2007, we measured serum 25(OH)D levels in a prospective cohort of 2,854 hemodialysis patients, and investigated two-year mortality, onset of CVD, infection- or cancer-related death. Analysis was mainly done using multivariate Cox regression models, adjusted for patient demographics, serum albumin, corrected calcium, phosphate, intact PTH, CRP, urea nitrogen, Kt/V, nPCR, history of CVD and dialysate calcium.

**Results.** Of these patients, 78% were considered 25(OH)D insufficient (<25ng/ml) with 39% considered severely deficient (<15ng/ml). In Cox analysis, the correlation between lower 25(OH)D levels and mortality was not significant. While, injective or oral vitamin D therapy was closely correlated with lower risk of mortality [Hazard ratio (HR) 0.54, 95%CI: 0.51-0.71]. Patients with vitamin D therapy had a significant lower risk of CVD [HR 0.75 (0.58-0.98)], but not that of infection- or cancer-related death. In 25(OH)D insufficient state (<25ng/ml), vitamin D treatment was associated with lower risk of mortality [HR 0.55 (0.40-0.74)], compared with untreated patients. This association was not significant in 25(OH)D levels with more than 25ng/ml [HR 0.53 (0.25-1.12)].

**Conclusions.** The present study suggests that vitamin D therapy contributes to a lower risk of death, especially in 25(OH)D insufficient state.

Disclosure of Financial Relationships: nothing to disclose

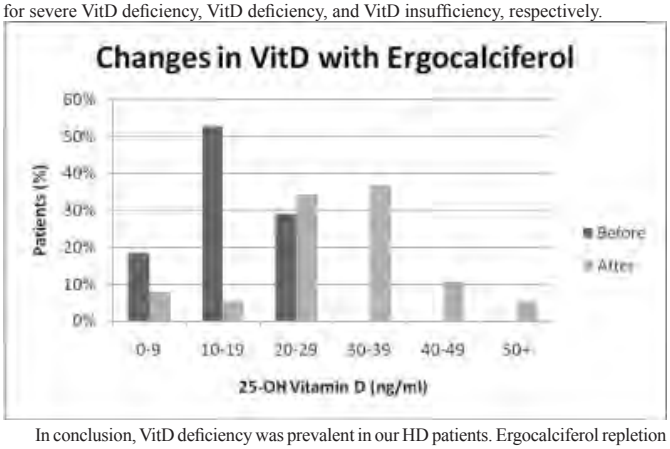
### TH-PO200

**Prevalence of 25-OH Vitamin D Deficiency and Efficacy of Ergocalciferol Supplementation on Hemodialysis Patients** Anna C. Porter,[1] Cheryl L. Gilmartin,[2] Sanjeev Akkina.[1] *[1]Medicine, U. IL, Chicago, IL; [2]Pharmacy, U. IL, Chicago, IL.*

Studies suggest a high prevalence of 25-OH vitamin D (VitD) deficiency among hemodialysis (HD) patients, but the optimal replacement regimen is unknown. This study aimed to investigate the prevalence of VitD deficiency in HD patients in Chicago, IL (latitude 41), and to determine the efficacy of an oral ergocalciferol repletion regimen.

Patients received ergocalciferol replacement according to KDOQI guidelines for CKD stages III-IV based on initial VitD levels (ng/ml) for 6 months. For serum levels <5, 50,000 units (U) weekly were given for 12 weeks, then monthly; for levels 5-15, 50,000 U weekly were given for 4 weeks, then monthly; and for levels 16-30, 50,000 U were given monthly. VitD levels were rechecked after 6 months.

There were 111 HD patients in the study. African-Americans comprised 68% of patients, 29% were Hispanic, and 3% were Caucasian. At baseline, 8% had normal VitD levels (>30 ng/ml), 41% had VitD insufficiency (16-30), and 51% had VitD deficiency (<15). Age, race, history of renal transplant, dialysis vintage, activated vitamin D use, and binder use/type were not associated with baseline VitD levels. Seventy-three patients received repletion according to protocol. Of these, 27% had normal VitD levels after 6 months. Thirty-eight of the 73 patients were treatment-compliant (by pharmacy records) and the results after 6 months are shown in Figure 1. The 3 treatment regimens were 50%, 44%, and 44% effective for severe VitD deficiency, VitD deficiency, and VitD insufficiency, respectively.




In conclusion, VitD deficiency was prevalent in our HD patients. Ergocalciferol repletion resulted in improved VitD levels in compliant patients. However, these regimens were less than 50% effective in normalizing VitD levels in 6 months. The optimal ergocalciferol replacement regimen in HD patients must be determined with further studies.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO201

**Relationship of 25(OH)D Testing on MBD Outcomes and Medication Utilization in Hemodialysis (HD) Patients** Joe Weldon, Mary B. Burgess, Deborah A. Benner, Karen M. Spach, Mahesh Krishnan. *DaVita Inc., Denver, CO.*

**Background:** KDIGO recommends evaluation of 25-hydroxyvitamin D [25(OH)D] in patients with stage 5D CKD and repletion with nutritional vitamin D given the low serum 25(OH)D levels in this population. Small studies have shown nutritional vitamin D use may improve mineral and bone disease (MBD) outcomes and decrease active vitamin D and erythropoietin stimulating agents (ESAs) requirements. We evaluated 25(OH)D testing at a dialysis facility level and correlated the percent of patients being tested with MBD outcomes. **Methods:** We assessed the 25(OH)D testing patterns of 1393 HD facilities. All patients treated more than 13 times in 2009 and at a single facility throughout 2009 were included. Of those, the 730 that tested for 25(OH)D deficiency in their patients were grouped by percent of patients in the facility tested in 2009 at any one point in time. This was correlated with MBD outcomes (phosphorus ≤ 5.5 mg/dL; corrected calcium < 9.5 mg/dL and PTH 150-300 ng/ml) and medication utilization for December 2009. **Results:** Percent of patients meeting the MBD outcome parameters between facilities stratified by percent of patients being tested did not differ. Utilization patterns of medication use are displayed below.

| % of Patients in Facility with 25(OH)D Tested | Facilities | Mean Paricalcitol per tx (mcg) | Mean ESA per tx (U) |
|---|---|---|---|
| None | 663 | Reference | Reference |
| 1-24% | 518 | -0.1 | -128 |
| 25-49% | 44 | -0.5 | -753 |
| 50-74% | 48 | -0.5 | -560 |
| 75-100% | 120 | -0.6 | -472 |

**Conclusions:** Patients in facilities that selectively test 25(OH)D show lower paricalcitol and ESA doses than those that do not test, despite comparable MBD outcomes. If testing is a surrogate for detection and treatment of Vitamin D deficiency with cholecalciferol or ergocalciferol, then our findings are consistent with those of smaller clinical trials regarding the potential benefits of testing and treatment of 25(OH)D deficiency in ESRD patients.

Disclosure of Financial Relationships: Employer: DaVita Inc.

### TH-PO202

**A Phase IV, Randomized, Blinded Single-Center Study of the Effects of Calcitriol and Paricalcitol on Vascular Calcification in Chronic Kidney Disease: Vitamin D and Coronary Calcification Study (VCOR)** Sylvia E. Rosas, Philip T. Cochetti. *Medicine, University of Pennsylvania, Philadelphia, PA.*

Coronary artery calcification (CAC) has been found to predict cardiovascular events and mortality. In animal models, calcitriol significantly increased the serum calcium-phosphate product and aortic calcium content, while paricalcitol had no effect. The objective of this phase IV, randomized, blinded single-center study is to determine the differential effect of calcitriol and paracalcitol on vascular calcification in chronic kidney disease (CKD)patients . The primary endpoint will be percent change in coronary artery (CAC) score at week 48. Inclusion criteria included CKD stage 3-4 and secondary hyperparathyroidism (SHPT) with any CAC. The recruitment goal is 50 participants. Aorto-femoral pulse wave velocity (PWV) and pulse wave analysis were performed to assess aortic stiffness.We present clinical and physiologic parameters on the first 19 participants and compare them with individuals with SHPT that had a baseline research visit but were not eligible to participate due to lack of CAC. There were no differences in measures of mineral metabolism or measures of central pressures (not shown). However, PWV was significantly elevated in those with CAC compared to those without CAC.

| Variable | | No CAC (n=11) | CAC (n=19) | p-value |
|---|---|---|---|---|
| Age (years) | | 57.6 (3.9) | 63.5 (4.3) | 0.11 |
| Gender | Male | 5 (38.5%) | 8 (61.5%) | |
| | Female | 6 (35.3%) | 11 (64.7%) | 0.58 |
| Race | AA | 8 (33.3%) | 16 (66.7%) | |
| | Non-AA | 3 (50.0%) | 3 (50.0%) | 0.38 |
| Diabetes | | 4 (44.4%) | 7 (58.3%) | 0.51 |
| eGFR | | 28.5 (4.9) | 27.2 (2.2) | 0.78 |
| cCalcium* (mg/dL) | | 9.34 (0.08) | 9.47 (0.09) | 0.36 |
| Phosphate (mg/dL) | | 3.85 (0.20) | 4.20 (0.16) | 0.20 |
| iPTH (pmol/L) | | 18.7 (3.5) | 19.2 (3.3) | 0.91 |
| 25(OH)D (ng/mL) | | 20.8 (3.6) | 20.4 (2.1) | 0.91 |
| CAC | | 0 | 261.1 (94.6) | <0.01 |
| PWV | | 9.40 (.73) | 12.00 (.55) | 0.02 |

*cCalc = corrected calcium for serum albumin

Conclusion: Aorto-femoral PWV is significantly higher in patients with CKD and SHPT with CAC compared to those without CAC. CKD patients with SHPT but no CAC have normal PWV.

Disclosure of Financial Relationships: Research Funding: Abbott Laboratories; Scientific Advisor: American Heart Association.

### TH-PO203

**Role of Statin on Vitamin D Usage among Maintenance Hemodialysis Patients** Osama W. Amro,[1] Malik Ladha,[1] Gita Verma,[1] Mahboob Rahman,[2] Saima Iqbal,[1] Salman Rasheed Mallick,[1] Charity Kankam.[1] *[1]Internal Medicine, Saint Vincent Charity Medical Centre, Cleveland, OH; [2]Division of Nephrology, University Hospitals of Cleveland, Case Western Reserve University, Cleveland, OH.*

Statin has been recently proposed to be a vitamin D analogue. Our aim is to test the potential clinical benefit of statin treatment on mineral metabolism in patients with end stage renal disease, favorable outcome will support of the postulated theory that statin indeed might be a vitamin D analogue and its use will be of particular importance in patient with ESRD. Study design was retrospective in fifteen centers in north east Ohio, patients who were on paricalcitol treatment were followed for up to two years. The outcome measure was weekly usage of paricalcitol and intact PTH (iPTH) between patients who were on statin therapy versus those who were not. Final sample had 1685 patients, where 735 were in statin group and 950 were in non-statin group, iPTH was 397.5 ± 333.7 versus 475.9 ± 465.7 (p=<0.001) , weekly dose of paricalcitol was 10.82 ± 6.01 versus 11.46 ± 7.13 (p=0.05), serum calcium was 9.07 ± 0.52 versus 9.0 ± 0.56 (p=0.01), calcium phosphate product was 47.1 +- 8.57 versus 48.64 ± 9.1 (p=0.001), alkaline phosphatase was 112.9 ± 8.6 versus 48.6 ± 9.1 (p=0.01) in the statin group versus non-statin group respectively. When patients were divided in four groups on basis of weekly paricalcitol usage group 1- up to 10 microgram per week, group 2 -10 to 20 microgram per week, group 3- 20 to 30 microgram per week and group - 4 more than 30 microgram per week. Patients percentage on further sub-grouping on basis of statin use versus no statin use , group1; 51.5% versus 48.7%, group 2; 42% versus 41%, group3; 5.2% versus 7.9%, group4; 1.4% versus 2.4% respectively. This data suggest that there may beneficial effects of statin use on iPTH and other markers. Further stronger prospective controlled trials would be needed to test the hypothesis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO204**

**Prevalence and Densitometric Characteristics of Incomplete Renal Tubular Acidosis in Men with Recurrent Nephrolithiasis** Spyridon Arampatzis, Barbara Rieben, Kurt Lippuner. *Osteoporosis Policlinic, University of Bern, Bern, Switzerland.*

Background: The prevalence and skeletal effects of incomplete distal renal tubular acidosis (iRTA) in recurrent nephrolithiasis patients are insufficiently documented.

Methods: 150 male patients with idiopathic nephrolithiasis were evaluated at a tertiary care hospital for iRTA. The diagnosis of iRTA (urinary pH > 5.32, measured from 2 h fasting urine) was confirmed by an additional acid-loading test. Serum and urinary biochemistry were obtained from 10 iRTA and 50 BMI- and age-matched non iRTA recurrent stone formers (nonRTA). Bone mineral density (BMD) of lumbar spine (LS) and femoral neck (FN) was measured by DXA in both groups.

Results: Prevalence of primary iRTA was 6.7% (10/150) in recurrent stone formers. Patients with iRTA had significantly higher urinary pH levels (mean: 5.8 ± 0.3 SD vs 5.1 ± 0.3, p=<0.001) and urinary calcium excretion (8 ± 4 vs 6 ± 2 mmol/day, p=0.013) but significantly lower urinary citrate levels (2 ± 1 vs 3 ± 1 mmol/day, p=0.021) compared to nonRTA patients. No significant difference in skeletal status was found between iRTA and nonRTA patients at the lumbar spine (LS-BMD: 1.046 ± 0.245 vs. 1.004 ± 0.117 g/cm2, LS-Z-score: -0.4 ± 1.8 SD vs. -0.7 ± 0.8 SD, LS-T-score: 0.3 ± 1.8 SD vs. -0.1 ± 0.9 SD) and femoral neck (FN-BMD: 0.829 ± 0.135 vs. 0.852 ± 0.127 g/cm2, FN-Z-score: -0.9 ± 1.1 SD vs.- 0.7 ± 1.0 SD, FN-T-score: 0.3 ± 1.2 SD vs. 0.2 ± 1.5 SD).

Conclusion: This study suggests that iRTA is a prevalent condition in male patients with recurrent urolithiasis. Moderate metabolic acidosis of iRTA did not significantly affect bone mineral density of lumbar spine and femoral neck.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO205**

**Maxacalcitol Ameliorates Kidney Injury in Dahl Salt-Sensitive Rats in a Renin-Independent Manner** Kazunori Inoue,[1] Isao Matsui,[1] Chikako Nakano,[1] Tomonori Kimura,[1] Yoshitsugu Takabatake,[1] Takayuki Hamano,[2] Masaru Horio,[1] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, PA.*

Recent studies have revealed vitamin D receptor activators (active vitamin D) exert renoprotective roles by repressing renin mRNA transcription. It remains uncertain whether vitamin D protects kidney in a renin-independent manner.

Male Dahl salt-sensitive (DS) rats were randomly divided into four groups; 0.3% NaCl + vehicle or maxacalcitol (LS+V, LS+M), 8% NaCl + vehicle or maxacalcitol (HS+V, HS+M) at age 4 weeks. Vehicle or 0.2 μg/kgBW of maxacalcitol was intraperitoneally injected every day for 6 weeks. To investigate the mechanisms for renoprotection by maxacalcitol, in vitro experiments were performed using normal rat kidney epithelial (NRK52E) cells.

Maxacalcitol therapy did not affect the development of hypertension in HS rats. HS+V group rats showed a marked elevation in urinary protein excretion and serum creatinine levels at age 10 weeks compared to rats fed with LS. Maxacalcitol significantly attenuated proteinuria and renal interstitial fibrosis, and hence blunted serum creatinine increase in HS rats. Renal renin mRNA expression levels in two HS groups were significantly lower than those of LS groups. Maxacalcitol did not affect renal renin mRNA expression levels in HS groups. In NRK52E cell, maxacalcitol inhibited TGFβ1-induced epithelial mesenchymal transition along with the decrease in collagen and fibronectin mRNA expression levels.

Renin-angiotensin-aldosterone system (RAAS) plays an important role in the progression of chronic kidney disease (CKD). Despite rigorous therapeutic interventions for RAAS, CKD remains a major public-health problem. Our findings indicated that maxacalcitol protects kidney independent of its renin repressive effect. Maxacalcitol may serve as a new additional therapy for CKD even concomitantly with RAAS inhibitors including direct renin inhibitor.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO206**

**Low Vitamin 25(OH)D Levels in Peritoneal Dialysis and Hemodialysis: Comparative Analysis and Risk Factors** Gianpaolo Amici, Adriana Caberlotto, Piergianni Calzavara, Carmelo Cascone. *Nephrology and Dialysis, Regional Hospital, Treviso, Italy.*

KDIGO guidelines on CKD-MBD suggest vitamin 25(OH)D monitoring to identify and treat deficiency status. Many papers report vitamin 25(OH)D deficiency in peritoneal dialysis (PD) due to chronic loss of vitamin D binding protein in PD fluids, but this issue is not considered in guidelines. Serum vitamin 25(OH)D has been assayed by LIAISON DiaSorin method in 29 PD patients (APD 18, CAPD 11) and 40 hemodialysis (HD) patients (standard 28, hemodialfiltration 12), matching was controlled for body weight (PD 70.8±12.2 vs HD 67.2±13.2 kg, p=ns), age (PD 65.9±16.6 vs HD 65.1±13.9 years, p=ns), sex (M/F PD 20/9 vs HD 23/17, p=ns) and diabetes (PD 7/22 vs HD 15/25, p=ns). Statistical analysis was performed by JMP SAS software. PD and HD groups showed differences for: serum 25(OH)D PD 16.0±8.6 vs HD 51.8±31.3 nmol/L (p<0.001), APD 15.8±8.9, CAPD 16.4±8.4, HD 54.5±31.5, HDF 45.6±31.5, (p<0.001), median time on dialysis PD 2.6 (0.2-8.0) vs HD 4.3 (0.3-50.6) years (p=0.0023), vitamin D therapy, 59 treated: PD 28/1 vs HD 31/9 (p=0.0369), albumin PD 3.7±0.4 vs HD 3.9±0.4 g/dL (p=0.0409). There were no differences for antihypertensive medication calculated as median Defined Daily Dose (ATC-WHO) overall 1.75 (0-14): PD 1.75 vs HD 1.75 (p=ns), patients were treated with paricalcitol: PD 2/27 vs HD 6/34 (p=ns), phosphate binding therapy (sevelamer, lantanium, aluminum, calcium) PD vs HD p=ns, cinacalcet treatment: PD 4/25 vs HD 1/39 (p=ns), serum Ca PD 9.2±0.8 vs HD 9.2±0.7 mg/dL (p=ns), serum Pi PD 5.1±1.4 vs HD 5.1±1.1 mg/dL (p=ns), serum ALP PD 85.5±36.9 vs HD 86.6±40.6 U/L (p=ns), iPTH PD 240±183 vs HD 251±213 ng/mL (p=ns). Applying a multiple linear regression model, 25(OH)D serum level (Rsq=0.652) results dependent to: dialysis type (F=38.4, p<0.001), time on dialysis (F=7.5, p=0.008), diabetes (F=7.1, p=0.010), non significant variables in the model: sex, age and vitamin D therapy. PD and HD showed a markedly different circulating pool of vitamin 25(OH)D and this finding, together with other reports as corroborative evidence, should be considered in treatment; other risk factors of vitamin D deficiency can be time on dialysis and diabetes.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO207**

**Phosphate Management with Reduced Calcium Load Increases Administration of Vitamin D** Naomi Sasaki, Nobuo Hashimoto. *HN Medic, Sapporo, Japan.*

**Background** Phosphate management is particularly important for chronic kidney disease mineral and bone disorder (CKD-MBD).Even at the same apparent serum phosphorus level, VD administration sometimes varies depending on which phosphate binder is used. Specifically, when calcium carbonate (CC) or calcium acetate is used as a phosphate binder, it becomes a calcium load and may prevent the administration of VD. **Methods** The subjects were 116 chronic dialysis patients at our facility who were being orally administered CC. We discontinued or drastically decreased the dosage of CC.We compared the effect of VD administration and the levels of serum albumin-corrected calcium (Ca (Alb)), phosphorus (P), and intact parathyroid hormone (iPTH) before, immediately after, and 1 year after the change in CC dosage.**Results** The number of patients receiving CC was reduced to 30, and the mean dosage was reduced from 1.82±0.72 to1.53±0.37 g/day(p<0.05). Before the change, the serum Ca (Alb), P, and iPTH had been 9.21±0.48mg/dl, 5.12±0.73mg/dl, and 127±81pg/ml respectively. Immediately after the change, the serum Ca (Alb) decreased to 8.96±0.53(p<0.001). The serum P and iPTH increased to 5.48±0.67, and 177±87(p<0.001). One year after the change, the serum Ca (Alb) and P were 9.17±0.63 and 5.19±0.61; both levels were no longer significantly different from the levels before the change. The serum iPTH decreased to149±70. We compared the effect of VD administration before and one year after the change.

VD administration

| | Before(%) | One year after(%) |
|---|---|---|
| non VD | 11.2 | 1.0 |
| 1α(OH)$D_3$ ,oral / mean dosage(μg/day) | 51.8 / 0.12±0.11 | 25.2 / 0.24±0.06* |
| 1α25(OH)$_2$$D_3$,oral/ mean dosage(μg/day) | 37.0 / 0.12±0.14 | 63.5 / 0.36±0.13* |
| 22-oxacalcitol | – | 10.3 |

*p<0.001 vs before

The percentage of patients who were not administered VD decreased dramatically from 11.2% to 1.0%, which was only one patient. The mean dosage of VD increased significantly. **Conclusion** It was clear that when CC was administered, VD administration decreased because of increase in serum calcium and inhibition of PTH secretion. Phosphate management with reduced calcium load increased the administration and dosage of VD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO208**

**Dose Comparison Study of CTAP201 Injection and Doxercalciferol (Hectorol®) Injection in Hemodialysis Patients with Secondary Hyperparathyroidism** Joel Z. Melnick,[1] Navindra J. Ramjit,[1] Donna Radjenovich,[1] Nancy L. Samberg,[1] Susan C. Cronin,[1] Samir P. Tabash,[1] Mojtaba Noursalehi,[1] Kotagal Shashi Kant,[2] Mariliza Bernardo.[3] *[1]Cytochroma Inc., Markham, ON, Canada; [2]University of Cincinnati, Cincinnati, OH; [3]Southwest Houston Research, Houston, TX.*

Secondary hyperparathyroidism (SHPT) is a serious condition developing in chronic kidney disease (CKD) as vitamin D levels decline. Left untreated, SHPT causes bone and cardiovascular disease. CTAP201 (1,25-$(OH)_2D_2$) Injection is being developed to treat SHPT in hemodialysis (HD) patients.

In this open label pharmacokinetic (PK) cross-over study, CTAP201 Injection was compared to doxercalciferol (DOX) injection in 24 HD patients. Subjects were enrolled sequentially into 2 dose groups and were randomized to receive single doses of CTAP201 and DOX, separated by a 2-week wash-out. One dose group received 1 mcg of CTAP201 and 4 mcg of DOX while the other received 3 mcg of CTAP201 and 6 mcg of DOX. PK, serum calcium (Ca), phosphorus (P) and intact parathyroid hormone (iPTH) were assessed over 68 hrs. Ca, P and iPTH were compared across dose group, sequence, period, time and treatment using mixed effect model.

Subject demographics, baseline clinical labs, and medical history were comparable between dose groups and treatment sequences. Mean total exposures ($AUC_{0-t}$ and $AUC_{0-last}$) to 1,25-$(OH)_2D_2$ from CTAP201 at the 3 mcg dose were comparable to those from the 6 mcg dose of DOX, while exposures at the 1 mcg dose were approximately 50% lower than those from the 4 mcg dose of DOX. Despite comparable % changes in iPTH after CTAP201 and DOX, significantly lower Ca (8.93±0.15 vs. 9.12±0.15) and a trend toward lower P (4.08±0.17 vs. 4.28±0.17) were observed with CTAP201.

CTAP201 Injection was safe and well tolerated in HD patients. Treatment with CTAP201 Injection may result in lower serum Ca and P than with DOX when similar control of iPTH is achieved.

Disclosure of Financial Relationships: Employer: Cytochroma; Ownership: Cytochroma.

## TH-PO209

**Development of Vitamin D, iPTH, Calcium, Phosphate and BAP in Hemodialysis Patients over 8 Years** Frans A. Zantvoort. *Renal Transplant Center, Central Hospital, Bremen, Germany.*

We analyzed, retrospectively the development of 25-OH-D3 levels over the years in combination with other related parameters, per year in the timeperiod 2002 - 2009. In our renal transplant center, all recipients have blood taken immediately before transplantation before any drugs have been given. This reflects the status in patients on renal replacement in the north-western part of Germany. For this abstract we took serum Ca (normal range 2.20-2.70 mmol/l), serum Ph (0.81-1.62 mmol/l), iPTH (1.6-6.9 pmol/l), 1,25-diOH-D3 (20-65 pg/l) and bone specific alkaline phosphatase (BAP). For 25-OH-D3 there are no normal values, as 'normal' values tend to be sub-optimal. Values < 20 nmol/l are regarded as a sign of severe deficiency, values > 70 nmol/l are regarded as sufficient. Results are shown in table 1:

Labvalues over the years

| Year | N | Ca (mmol/l) | Ph (mmol/l | 25-D (nmol/l) | 1,25-D (pg/ml) | iPTH (pmol/l) | BAP (µg/l |
|---|---|---|---|---|---|---|---|
| 2002 | 42 | 2.58 ± 0.21 | 1.87 ± 0.59 | 14.0 ± 17.3 | 27.1 ± 20.6 | 18.8 ± 23.0 | 15.4 ± 2.8 |
| 2003 | 36 | 2.49 ± 0.28 | 1.78 ± 0.53 | 11.9 ± 8.1 | 24.5 ± 16.8 | 27.9 ± 28.0 | 12.5 ± 7.2 |
| 2004 | 43 | 2.46 ± 0.24 | 1.69 ± 0.49 | 12.1 ± 8.9 | 22.3 ± 13.8 | 26.5 ± 26.3 | 12.7 ± 9.4 |
| 2005 | 34 | 2.43 ± 0.26 | 1.80 ± 0.61 | 13.0 ± 14.0 | 30.6 ± 18.1 | 43.1 ± 70.4 | 16.4 ± 17.4 |
| 2006 | 50 | 2.37 ± 0.29 | 1.71 ± 0.46 | 37.8 ± 28.8 | 19.3 ± 12.0 | 33.1 ± 37.9 | 18.6 ± 21.3 |
| 2007 | 42 | 2.44 ± 0.21 | 1.68 ± 0.41 | 61.7 ± 38.8 | 17.3 ± 10.5 | 24.9 ± 24.4 | 15.7 ± 10.2 |
| 2008 | 33 | 2.40 ± 0.29 | 1.76 ± 0.55 | 52.3 ± 36.5 | 25.6 ± 18.9 | 23.8 ± 30.9 | 14.1 ± 11.7 |
| 2009 | 31 | 2.39 ± 0.21 | 1.74 ± 0.55 | 75.6 ± 51.0 | 24.5 ± 15.9 | 34.0 ± 33.6 | 18.4 ± 10.7 |

In the period < 2006 123 P. (79.4%) had a severe 25-OH-D3 deficiency, only 2 patients (1.3%) had a level regarded as sufficient. After 2006 the numbers were respectively 10 (9.4%) and 35 (33%). None of the other parameters given changed over time.

In conclusion, nephrologists in the north-western part of Germany are apparently well informed and react rapidly to new insights and information. For 25-OH-D3 this change happened in 2006.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO210

**Use and Outcomes for Oral Vitamin D Preparations Used in Peritoneal Dialysis Patients** Carey Colson, Joe Weldon, Mahesh Krishnan. *DaVita, Denver, CO.*

**Background:** Oral vitamin D agents used for patients on peritoneal dialysis (PD) varies by prescriber preference. However, little is known about the relative differences between achieved outcomes for those patients between agents. We utilized an electronic health record to understand the comparative effectiveness between products in a PD population.

**Methods:** Patients were included in the analysis if they were on continuous ambulatory PD (CAPD), continuously cycling PD (CCPD), or intermittent PD in March of 2010 with the following criteria: > age 18 as of the end of the month, at least one treatment in the month, on dialysis for ≥ 90 days, ≥ 1 of the lab tests below within the last 90 days, and an open order for one (and only one) oral vitamin D preparation during the month. Oral medication records were reviewed and the predominantly prescribed oral vitamin D was used to categorize patients into calcitriol, doxercalciferol, and paricalcitol groupings. Demographic characteristics of each group were generated. Propensity score matching was then performed for the three different agents. Relevant bone and mineral outcomes for March 2010 for each oral agent were generated for each group and compared.

**Results:** Demographic characteristics for patients using the various preparations were not significantly different. 1,372 patients were included in each group following the propensity score analysis. In those patients, lab outcomes for each oral agent are listed below.

| Vitamin D preparations (oral) | Albumin ≥ 3.5 g/dL | PTH 150-600 pg/ml | Ca 8.5-10.2 mg/dL | Ca >10.2 mg/dL | Phos ≤5.5 mg/dL |
|---|---|---|---|---|---|
| Calcitriol | 68.1% | 70.0% | 80.9% | 3.5% | 66.4% |
| Doercalciferol | 67.4% | 70.3% | 80.7% | 3.6% | 66.5% |
| Paricalcitol | 70.0% | 71.8% | 83.4% | 3.9% | 64.5% |
| p-value | NS | NS | NS | NS | NS |

**Discussion:** Baseline demographic characteristics did not differ between the groups. MBD-related outcomes, including albumin, PTH, calcium and phosphate levels showed no between-group differences.

**Conclusion:** In propensity-matched groups of PD patients, use of either of three predominant oral vitamin D preparations produces no clinically significant differences in key MBD outcomes.

Disclosure of Financial Relationships: Employer: DaVita Inc.

## TH-PO211

**Has the Control of Mineral Metabolism Disorders Improved in the Past Three Years? Data Analysis of the OSERCE I and OSERCE II Studies** Jose L. Gorriz,[2] Celestino Pinera,[3] Alberto M. Martinez-Castelao,[4] Javier Nieto,[5] Guillermina Barril.[6] *[1]Servicio de Nefrologia, Fundacio Puigvert, Barcelona, Spain; [2]Servicio de Nefrologia, Hospital Univ Dr Peset, Valencia, Spain; [3]Servicio de Nefrologia, Hospital M Valdecilla, Santander, Spain; [4]Servicio de Nefrologia, Hospital Bellvitge, Barcelona, Spain; [5]Servicio de Nefrologia, Hosp Ciudad Real, Spain; [6]Servicio de Nefrologia, Hosp La Princesa, Madrid, Spain.*

**Objective:** To analyze the degree of compliance with the K/DOQI guidelines in chronic kidney disease (CKD) patients with GFR < 60 mL/min/1.73m2 (stages 3, 4, and 5 not on dialysis) by comparing the results of the OSERCE I and OSERCE II studies.

**Patients and methods**: The OSERCE I study is a multicenter, cross-sectional study that collected consecutive data in April and May 2006 in 634 patients from the outpatient clinics of 32 sites. The OSERCE II study also intends to analyze the degree of compliance in mineral and bone disorders in CKD patients, in addition to other primary objectives including 742 patients from 39 sites, with the same inclusion criteria (April and May 2009).

The degree of compliance with the K/DOQI guidelines parameters is showed in table 1.

Compliance with K/DOQI guidelines

| Compliance with K/DOQI guidelines | inadequate (%) | higher (%) | lower (%) |
|---|---|---|---|
| i-PTH (pg/mL) OSERCE I | 68.4 | 50.7 | 17.7 |
| i-PTH (pg/mL) OSERCE II | 70 | 50.2 | 19.8 |
| Corrected calcium (mg/dL) OSERCE I | 44 | 40 | 4 |
| Corrected Ca (mg/dL) OSERCE II | 64.4 | 61 | 4.4 |
| Phosphorus (mg/dL) OSERCE I | 25.1 | 18.8 | 6.3 |
| Phosphorus (mg/dL) OSERCE II | 23.5 | 5.7 | 17.7 |
| Ca x P (mg2/dL2) OSERCE I | 3 | 3 | - |
| Ca x P (mg2/dL2) OSERCE II | 1 | 1 | - |
| 25-(OH)-Vit D (ng/mL) OSERCE I | 81.5 | - | 81.5 |
| 25-(OH)-Vit D (ng/mL) OSERCE II | 84 | | 84 |

i-PTH was measured with Immulite 2000 (chemiluminescece) .

**Conclusions**: It is still very difficult to achieve the objectives established by the K/DOQI guidelines for mineral and bone metabolism disorders in stage 3, 4, and 5 CKD patients not on dialysis; no improvement was seen in the past three years. The main compliance difficulty is with the PTH-i goals. The levels of 25-(OH)-Vit D are inadequate in a high percentage of patients in both studies.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO212

**Effect of Ergocalciferol Supplements on Bone Metabolism Parameters and ESA Use in Hemodialysis Patients** Anna C. Porter,[1] Cheryl L. Gilmartin,[2] Sanjeev Akkina.[1] *[1]Medicine, U. Illinois, Chicago, IL; [2]Pharmacy, U. IL, Chicago, IL.*

There has been growing interest in the role of 25-OH vitamin D (VitD) supplements in patients with ESRD. Previous studies suggested that VitD repletion results in improved bone metabolism parameters, reduced erythropoiesis-stimulating agent (ESA) doses, and improved albumin levels. This retrospective study evaluated the effects of oral VitD supplements on these parameters in a group of hemodialysis (HD) patients in Chicago.

Baseline VitD, PTH, hemoglobin, phosphorus, and calcium levels were obtained. Based on initial VitD levels, participants received ergocalciferol repletion according to KDOQI guidelines for CKD. These parameters were reassessed after 6 months of treatment.

We examined data for 111 HD patients with VitD levels in Oct. 2009. At baseline, 8% had normal VitD levels (>30 ng/ml), 41% had insufficiency (16-30), and 51% had deficiency (≤15). Seventy-three patients received ergocalciferol, 38 of whom were compliant with treatment, the remainder noncompliant. Only phosphorus levels improved in the compliant group while sevelamer dose increased in both groups.

| | Compliant (change) | p | Non-compliant (change) | p |
|---|---|---|---|---|
| VitD (ng/ml) | 13.7 | <0.001 | -1.1 | 0.21 |
| PTH (pg/ml) | -13.6 | 0.79 | -108 | 0.28 |
| Calcium (mg/dl) | 0.07 | 0.45 | -.03 | 0.81 |
| Albumin (g/dl) | 0.08 | 0.14 | 0 | 0.97 |
| Corrected Ca (mg/dl) | 0.02 | 0.83 | -0.03 | 0.82 |
| Phos. (mg/dl) | -0.98 | 0.0002 | -0.2 | 0.56 |
| Doxercalciferol dose (mcg/treatment) | -0.4 | 0.23 | -0.04 | 0.93 |
| Sevelamer dose (caps/day) | 0.97 | 0.009 | 0.8 | 0.02 |
| Cinacalcet dose (mg/day) | 0.8 | 0.74 | -6.9 | 0.25 |
| Hgb (g/dl) | -0.1 | 0.65 | -0.2 | 0.53 |
| Darbepoetin dose (mcg/week) | +1.6 | 0.75 | -6.5 | 0.35 |

Ergocalciferol replacement resulted in improved phosphorus levels in compliant patients, though sevelamer use also increased. In contrast to findings of previous studies, other bone parameters and reduced ESA dose did not occur. Further studies should determine the effects of ergocalciferol repletion on other clinical outcomes in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO213**

**Fibroblast Growth Factor 23 in Long-Term Kidney Transplantation** Sinee Disthabanchong, Supinda Sirilak, Vasant Sumethkul. *Division of Nephrology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Phayathai, Bangkok, Thailand.*

Fibroblast growth factor 23 (FGF-23) is synthesized by bone cells whose function is regulation of calcium-phosphate metabolism. In CKD, as GFR declines, FGF-23 increases in concert with PTH resulting in phosphaturia alleviating phosphate retention. Recent evidence added the role of FGF-23 in direct suppression of PTH and FGF-23 may be released in response to high PTH. In early post-transplantation, despite significant phosphate wasting, FGF-23 accumulates accentuating hypophosphatemia. The relationship between FGF-23 and phosphate as well as allograft function in long-term kidney transplantation is largely unexplored. Phosphate retention is less of a problem in these patients and the role of FGF-23 may be different than that in CKD. The present study investigates FGF-23 in 229 kidney allograft recipients at least 1-year post transplantation and its relationship to other mineral parameters. A comparison was made to CKD with equivalent GFR. The average GFR was 56±19 mL/min/1.73m$^2$. Serum phosphate was normal in 93%, low in 6% and high in 1%. Inverse correlations were observed between phosphate, FGF-23 and PTH with GFR ($p<0.001$). Serum phosphate correlated modestly with FGF-23 ($p=0.045$). Urinary phosphate excretion increased as GFR declined and correlated well to the increase in PTH ($p<0.001$) and FGF-23 ($p=0.006$). In multiple regression analysis, the only independent predictors for FGF-23 levels were increased PTH and decreased GFR. The relationships between FGF-23 with serum and urinary phosphate were lost after adjustment suggesting closer relationship between FGF-23 and PTH than phosphate. Comparison to CKD patients with equivalent GFR, PTH and serum calcium were higher and serum phosphate was lower in the transplant group ($p<0.001$) confirming the existence of persistent hyperparathyroidism that may promote FGF-23 secretion. Using ROC analysis, AUC of FGF-23 for predicting PTH>100 pg/mL was the highest among other parameters (AUC= 0.653). In conclusion, the increase in FGF-23 appeared to associate with the severity of hyperparathyroidism rather than phosphate retention in long-term kidney transplantation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO214**

**Functional Analysis of a Novel Klotho Mutation Identified in a Patient with End-Stage Renal Disease** David De Brauwere,[1] Pablo A. Urena,[1,3] Caroline Kannengiesser,[4] Christine Leroy,[1,2] Bernard Grandchamp,[4] Dominique Prie,[1,2] Laurent Beck,[1,2] Gerard Friedlander.[1,2] *[1]INSERM U845, Paris, France; [2]Universite Paris Descartes, Paris, France; [3]Clinique du Landy, Saint-Ouen, France; [4]Hopital Bichat, Paris, France.*

Klotho is implicated in the regulation of phosphate (Pi) homeostasis by modulating the phosphaturic action of fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH). In a previous clinical study, comparing normophosphatemic to hyperphosphatemic hemodialysis patients, we found that, in a significant proportion of patients, the hyperphosphatemia was independent from serum levels of PTH, vitamin D, circulating bone markers, the effectiveness of dialysis, dietary Pi intake and Pi lowering therapies, suggesting that a genetic background could predispose to hyperphosphatemia. We hypothesized that alteration in the klotho gene could be responsible for the hyperphosphatemia. We identified a novel klotho mutation (c.1041delT) in a patient belonging to the hyperphosphatemic group. The aim of this study was to elucidate whether this mutation could account for the observed phenotype. We first asked whether soluble klotho (1-980 aa) through its beta-glucuronidase activity could have a direct effect on intestinal Pi transport. To test this hypothesis, Pi uptake was assessed in intestinal CaCo-2 cells transfected with NPT2b following treatment by beta-glucuronidase, klotho and/or FGF23. We found that Pi uptake was not modified by beta-glucuronidase nor by recombinant Klotho (Kl) or conditioned media from Kl-transfected HEK cells. Similarly, the addition of FGF23 to Kl or Kl-conditioned media did not affect Pi uptake. We then asked whether soluble klotho could modulate systemic Pi uptake in fibroblastic NIH-3T3 cultured cells. We found that recombinant Klotho (Kl) or Kl-conditioned media only in combination with FGF23 increased PiT-2 mRNA expression and Pi uptake by 2 fold, while mutated klotho did not have any effect. In conclusion, for the first time, we demonstrate that klotho plus FGF23 up-regulated PiT-2 mRNA expression and increased Pi uptake in NIH-3T3 cells and that a novel klotho mutation completely lacks this effect, which might explain the patient phenotype

Disclosure of Financial Relationships: nothing to disclose

**TH-PO215**

**Purification of Intestinal Phosphatonin from Porcine Intestine** Zachary C. Ryan, Rajiv Kumar. *Dept of Medicine, Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

The proximal intestine releases a factor(s) that inhibits renal phosphate (P) transport upon exposure to a high P meal. Previous experiments have demonstrated that the factor is present in the intestinal mucosa and in the liver. Further experiments have shown that rat intestinal cells maintained in culture also express and secrete a factor that inhibits sodium-dependent P transport in opossum kidney (OK) cells. To identify a readily available and abundant source of this factor, we isolated porcine intestinal mucosa and tested purified fractions for the presence of an inhibitor of renal P transport.

*Results:* Porcine intestinal mucosa was scraped and homogenized in isotonic saline at 4 °C. The homogenate was treated with trichloroacetic acid (to a final concentration of 3%). The acidified homogenate was centrifuged at 27,000 x g in an SS34 rotor at 4 °C for 60 minutes. The pellet was discarded and the supernatant was neutralized with 1M NaOH and dialyzed extensively against water at 4 °C. The dialyzed material was lyophilized and resuspended in 25mM HEPES, 100mM NaCl, pH 7.2 (Buffer A). This material was chromatographed on a 26/60 Superdex 75 column in Buffer A at a flow rate of 0.5 mL/min. Fractions were collected, dialyzed against water, lyophilized, resuspended in P-free DMEM, and tested for P uptake inhibitory activity in OK cells. The **Table** demonstrates the presence of P uptake inhibitory activity in different fractions.

OK Cell P Uptake Inhibitory Activity in Fractions of Porcine Intestine

| *Purified fractions from porcine intestine* | *Percent inhibition (mean ± SEM) of sodium dependent phosphate uptake in OK cells* |
|---|---|
| Original homogenate | 12.58±2.2 |
| Supernatant following TCA treatment | 11.8±2.1 |
| ***Fractions from Superdex column*** | |
| • >67 kDa | 21.8±1.8 |
| • 67-16.7 kDa | 39.6±1.3 |
| • 16.7-5.7 kDa | 4.4±2.2 |
| • 5.7-0.2kDa | 10.2±3.6 |
| • <0.2kDa | 3.9±2.9 |

*Conclusions:* Porcine intestinal mucosa contains a factor that inhibits renal phosphate transport. Purification of the inhibitor by trichloroacetic acid precipitation, and size exclusion chromatography effects a substantial purification of the inhibitor. Further characterization of the inhibitor should be possible using porcine intestine as the source of material.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO216**

**Intracellular Angiotensin II Induces the Expression of $Na^+/HCO_3^-$ Co-Transporter in Mouse Proximal Tubule Cells Via MAPK- and NF-κB-Dependent Signaling** Xiao C. Li,[1] Julia Cook,[2] Ulrich Hopfer,[3] Jia L. Zhuo.[1] *[1]Division of Hypertension and Vascular Research, Department of Internal Medicine, Henry Ford Hospital, Detroit, MI; [2]Department of Medical Genetics, Ochsner Clinic Foundation, New Orleans, LA; [3]Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH.*

Extracellular angiotensin II (ANG II) stimulates sodium and bicarbonate reabsorption in proximal tubules (PT) via activation of cell surface ANG II receptor-induced expression/activity of $Na^+/HCO_3^-$ co-transporters. However, whether intracellular ANG II plays a physiological role is unknown. The present study tested the hypothesis that intracellular ANG II stimulates $Na^+/HCO_3^-$ cotransporter expression in mouse PT cells via MAPK- and NF-κB-dependent signaling. Expression of a cyan fluorescent fusion of ANG II chimera (ECFP/ANG II) in PT cells induced time-dependent increases in intracellular ANG II levels with a peak response at 48 h after transfection (control: 191.9 ± 17.6 *vs.* ECFP/ANG II: 484.0 ± 31.8 pg/mg protein, $p<0.01$). However, ECFP/ANG II was not released into the medium. ECFP/ANG II increased $Na^+/HCO_3^-$ cotransporter expression by >2-fold (control: 0.18 ± 0.03 *vs.* ECFP/ANG II: 0.46 ± 0.06 $Na^+/HCO_3^-$/actin ratio, $p<0.01$), which was associated with 2.7-fold increases in MAP kinases p-ERK1/2 (control: 0.18 ± 0.04 *vs.* ECFP/ANG II: 0.49 ± 0.14 p-ERK1/2/t-ERK1/2 ratio, $p<0.01$) and >10-fold increases in the p65 subunit of NF-κB proteins (control: 0.16 ± 0.02 *vs.* ECFP/ANG II: 1.96 ± 0.31 NF-κB/actin ratio, $p<0.01$). ECFP/ANG II-induced activation of p-ERK1/2 and NF-κB/p65 and expression of $Na^+/HCO_3^-$ cotransporter were blocked by the $AT_1$ receptor antagonist losartan (10 μM; $p<0.01$), the MEK1 & MEK2 inhibitor UO126 (10 μM; $p<0.01$), or by the NF-κB inhibitor, Ro 106-9920 (10 μM; $p<0.01$). By contrast, ECFP/ANG II had no specific effects on p-ERK1/2, NF-κB/p65 or $Na^+/HCO_3^-$ cotransporter protein expression in PT cells of $AT_{1a}$-KO mice. These results suggest that intracellular ANG II induces $Na^+/HCO_3^-$ cotransporter expression in PT cells via $AT_{1a}$ receptor-mediated activation of MAP kinases and NF-κB signaling mechanisms.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO217**

**Angiotensin II Stimulates Heterogenous Nuclear Ribonucleoprotein F and K Expression in Renal Proximal Tubular Cells Via Reactive Oxygen Species Generation** Chao-Sheng Lo,[1] Shiao-Ying Chang,[1] Isabelle Chenier,[1] Shao-Ling Zhang,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] John S. D. Chan.[1] *[1]Res. Ctr., CHUM-Hotel Dieu Hosp, Montreal, QC, Canada; [2]Res. Ctr., Maisonneuve-Rosemont Hosp, Montreal, QC, Canada; [3]Pediatr Nephrol Unit, Mass Gen Hosp, Boston, MA.*

Heterogeneous nuclear ribonucleoprotein F (hnRNP F) and hnRNP K (hnRNP F/K) bind to the rat angiotensinogen (Agt) gene promoter and inhibit Agt gene transcription in vitro (JBC, 2006). The present study investigated whether angiotensin II (Ang II) stimulates hnRNP F/K expression, thereby exerting 'negative feedback' on Agt gene expression in renal proximal tubular cells (RPTCs) and subsequently attenuates the Ang II-effect on RPTC injury. Adult male transgenic (Tg) mice specifically overexpressing rat Agt in their RPTCs (10 weeks of age) were given ± RAS blockers (losartan (30 mg/kg/day) plus perindopril (4 mg/kg/day)) and euthanized at week 20 while untreated non-Tg littermates served as controls. Kidneys were processed for histology and immunostaining for hnRNP F/K expression. Renal proximal tubular hnRNP F/K mRNA and protein expression were quantified by respective real time qPCR assay and Western blotting. Rat RPTCs stably transfected with the plasmid containing Agt cDNA were cultured in 5 mM D-glucose DMEM ± losartan or DPI (an inhibitor of NADPH oxidase). RPTC reactive oxygen species (ROS) generation was determined by lucigenin assay. HnRNP F/K expression were significantly increased in RPTCs of Agt-Tg mice as compared to non-Tg littermates and normalized by RAS blockers in vivo. Furthermore, Tg mice overexpressing both Agt and catalase in their RPTCs normalized hnRNP F/K expression. Finally, hnRNP F/K mRNA expression and ROS generation were significantly increased (2-fold increase) in Agt-stable transformants,

and these parameters were reversed by losartan and DPI in vitro. These data demonstrate that Ang II stimulates both hnRNP F/K expression via Ang II type 1 receptor and ROS generation in RPTCs. HnRNP F/K expression might play an important 'negative feedback' role in attenuating Agt gene expression and subsequently attenuating hypertension and kidney injury induced by intrarenal RAS activation in vivo.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO218**

**Epithelial Sodium Channel Regulation by Cell Surface Sgk1** Sheela V. Thomas, Madhumitha Rajagopal, Carol Charlton, Alan C. Pao. *Medicine/ Nephrology, Stanford University, Stanford, CA.*

Serum and glucocorticoid regulated kinase 1 (Sgk1) participates in diverse biologic processes including cell growth, osmoregulation, and sodium homeostasis. In the kidney, Sgk1 mediates aldosterone-regulated sodium transport in the distal nephron by stimulating surface expression of the epithelial sodium channel (ENaC). Recent studies have suggested that subcellular localization of Sgk1 may represent a mechanism for modulating Sgk1 function. We used a mammalian kidney cell ($mpkCCD_{c14}$) line to examine the subcellular localization of the aldosterone-regulated isoform of Sgk1. By cell fractionation studies, we identified aldosterone-stimulated Sgk1 to be mostly localized to the membrane and the cytosol rather than to the nuclear fraction. To determine if membrane-associated Sgk1 is at the plasma membrane, we used surface biotinylation assays and immunocytochemistry to confirm that the aldosterone-regulated isoform of Sgk1 is present at the apical surface of kidney collecting duct cells. Cell surface Sgk1 expression in $mpkCCD_{c14}$ cells was attenuated with pre-treatment with methyl-b-cyclodextrin (MβCD), a compound that depletes membrane cholesterol, suggesting that Sgk1 requires intact lipid raft formation to associate with the cell surface. Furthermore, in HEK293T cells, Sgk1 associated with the cell surface only when ENaC was co-transfected, suggesting that Sgk1 associates with ENaC at the cell surface. This Sgk1-ENaC association was also confirmed by co-immunoprecipitation assays. To evaluate the functional significance of cell surface Sgk1, we treated $mpkCCD_{c14}$ cells with MβCD (or vehicle) and used an Ussing chamber to measure aldosterone-stimulated short-circuit currents that are sensitive to the Sgk1 inhibitor GSK650394. MβCD treatment attenuated baseline amiloride-sensitive currents modestly but it significantly diminished aldosterone-stimulated amiloride-sensitive currents and aldosterone-stimulated GSK650394-inhibitable currents. We propose that Sgk1 associates with ENaC and lipid raft microdomains at the cell surface, where an assembly platform may exist for the regulation of ENaC by Sgk1.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO219**

**Uninephrectomy (UNX) Reduces 11beta-Hydroxysteroid Dehydrogenase Type 1 (11beta-HSD1) and Type 2 (11-beta-HSD2) in Rats** Meret Lauterburg, Brigitte Frey, Bernhard Dick, Geneviève Escher, Felix J. Frey. *Department of Nephrology and Hypertension, University Hospital of Bern, Switzerland.*

RATIONALE. Renal allograft donors are at risk to develop hypertension and a metabolic syndrome by an unknown mechanism. We hypothesized that this risk is at least in part explained by an enhanced intracellular availability of glucocorticoids due to an increased 11beta-HSD1, an intracellular prereceptor activator of corticosteroids in glucocorticoid target tissues (Escher G, J Exp Med.1997:189-98) and/or a diminished 11beta-HSD2, an inactivator of corticosteroids in mineralocorticoid target tissues (Frey FJ, Curr Opin Nephrol Hypertens. 2004 :451-8).

METHODS. Uninephrectomized or sham operated rats (n=7 per group) were investigated four weeks after UNX.

The mRNA of 11beta-HSD1 and 11beta-HSD2 were assessed by RT-PCR in liver and kidney tissue, respectively and the enzyme activities were directly quantified in the corresponding tissues by determining the ratios of (THB+5alpha-THB)/THA ((tetrahydrocorticosterone+5alpha-tetrahydrocorticosterone)/ tetrahydrodehydrocorticosterone) and the B/A (corticosterone/dehydrocorticosterone) by gas chromatography-mass spectrometry. The apparent total body activities of 11beta-HSD1 and 11beta-HSD2 were estimated by the urinary ratios of (THB+5alpha-THB)/THA and B/A, respectively.

RESULTS. The hepatic mRNA content and the hepatic and urinary ratios of (THB+5alpha-THB)/THA were decreased after UNX, indicating diminished access of glucocorticoids to glucocorticoid receptors. In renal tissue 11beta-HSD2 mRNA and measures of activity were diminished and, as a corollary, the renal and urinary ratios of B/A were increased, indicating enhanced access of glucocorticoids to mineralocorticoid receptors ($p < 0.01$ for all parameters analyzed).

CONCLUSION. Both 11beta-HSD1 and 11beta-HSD2 are down-regulated after UNX in rats, a constellation known to induce salt-sensitive hypertension but not insulin-resistance.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO220**

**Angiotensin II Type 2 Receptor Binding and Signaling in Mesothelial Cells** Michael E. Ullian,[1,2] Thomas Morinelli.[1,2] *[1]Medicine/Nephrology, Medical University of South Carolina, Charleston, SC; [2]Medicine, Ralph H. Johnson VA Medical Center, Charleston, SC.*

The process of thickening and inflammation of the peritoneal mesothelium is a major problem in patients undergoing chronic peritoneal dialysis. We have been exploring the role of angiotensin II (AngII) receptor subtypes (AT1 and AT2) in this phenomenon, using MET5A mesothelial cells as a model system. Monolayers of these cells display typical mesothelial cell cobblestone appearance. Radioligand binding competition studies with 125I-AngII or 125I-CGP42112 (a specific AT2 receptor agonist) and 100-fold excesses of unlabeled AngII or unlabeled losartan (a specific AT1 receptor antagonist) or unlabeled PD123319 (a specific AT2 receptor antagonist) in crude membrane suspensions revealed specific binding to AT2 receptors but not AT1 receptors; 125I-AngII was displaced by 19% by PD123319 but only by 7% by losartan, and 125I-CGP42112 was displaced by 32% by PD123319 and only 4% by losartan ($N = 5$, both $p < 0.05$). In intact cells, the absence of AngII-stimulated (1 nM - 100 nM) increases in intracellular calcium concentration, an AT1 receptor-coupled signaling event, as measured with the fluorescent probe Fluo-3 and a fluorescence imaging plate reader, confirmed the absence of AT1 receptors ($N = 1$). Next, we investigated signaling [phosphorylation of extracellular-regulated kinase (p42/44 ERK) by immunoblotting] through AT2 receptors in intact cells. Uniform protein loading was verified by immunoblotting of total p42/44 ERK. AngII-stimulated (100 nM for 5 minutes) increases in p42/44 ERK phosphorylation were decreased by 27% by a 100-fold excess of PD123319 ($p < 0.05$) but not by losartan ($N = 3$). These preliminary results suggest that AngII might contribute to mesothelial membrane thickening and inflammation through AT2 receptors during the course of peritoneal dialysis. Studies of the effects of AngII, losartan, and PD123319 on p42/44 ERK phosphorylation in primary cultures of rat peritoneal mesothelial cells and on peritoneal membrane thickening and inflammation in rats models of peritoneal dialysis are ongoing.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO221**

**Angiotensin II (AII)-Induced AT1A Receptor (AT1AR) Internalization and Subsequent Increased Cyclooxygenase 2 (COX-2) Protein Expression in Rat Aortic Vascular Smooth Muscle Cells (RASMC) Are Mediated by NF-κB Activation** Thomas Morinelli,[1,2] Michael E. Ullian.[1,2] *[1]Medicine Division of Nephrology, Medical University of South Carolina, Charleston, SC; [2]Research Services Ralph H. Johnson VA Medical Center, Department of Veteran's Affairs, Charleston, SC.*

Previously, we showed that AII activation of COX-2 transcription and protein expression is dependent upon AII AT1AR internalization and nuclear localization (Morinelli, et al, 2007; 2008; 2009). Others have shown that the transcription factor NF-κB mediates AII-induced COX-2 expression (Hu et al 2002;Ohnaka et al, 200). Activation of the NF-κB protein complex is initiated by phosphorylation and degradation of its IκB component and nuclear localization and DNA binding of the p65 component. The present study examines the involvement of NF-κB complex activation in AII AT1AR internalization and subsequent COX-2 protein expression in RASMC. The NF-κB inhibitor Ro-1069920 blocked AII-induced COX-2 expression in a concentration-dependent manner as determined by COX-2 immunoblots ($5.7 \pm 2.2$ fold increase for AII, 100 nM, 3 hrs. vs. $0.75 \pm 0.6$ fold with 10 μM Ro-1069920 pre-treatment, $n=3$, $p<0.05$). AII-induced NF-κB nuclear localization (immunofluorescence) and AII-induced AT1AR internalization as measured by radioligand binding assays ($57.3\% \pm 11\%$ internalization after 5' for Control vs. $28.2 \pm 6\%$ for Ro-1069920 pre-treatment, $n=4$; $p < 0.05$) were also inhibited. Additional inhibitors of the NF-κB pathway were tested. Both parthenolide, like Ro-1069920 inhibits degradation of IκB, and curcumin an inhibitor of NF-κB/DNA complex formation and subsequent transcription also blocked AII-induced COX-2 expression (80% and 76% inhibition, respectively, n=3). In contrast, betulinic acid, an inhibitor of NF-κB phosphorylation, had no effect on COX-2 protein expression induced by AII. These data suggest that inhibition of IκB degradation inhibits AII-induced AT1AR internalization and subsequent COX-2 protein expression, possibly linking internalization of the AT1AR with activation of the NF-κB pathway. Supported by Dialysis Clinic Incorporated and the U.S. Department of Veteran's Affairs.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO222**

**AGTR1 and AGTR2 Modulation by Ang II and Cytokines in Human Monocytes, Granulocytes and Lymphocytes** Branko Braam, Wenqing Zhuang. *Medicine/Nephrology, Univ. of Alberta, Edmonton, AB, Canada.*

**Introduction**: Angiotensin II (Ang II) is a key player in hypertension, inflammation and oxidative stress, all associated with atherosclerosis. Although several circulating cells reportedly express AT1 receptors (AGTR1), its regulation has not been studied. Angiotensin AT2 receptor (AGTR2) expression in circulating cells is largely unknown. We measured cell surface expression of AGTR1 and AGTR2 in human leukocytes and modulation by Ang II itself and the cytokines IFNg, IL6 and TNFa.

**Methods and results**Monocytes (MONO), granulocytes (GRAN) and B-cells (B), but not T-cells, expressed AGTR1 and AGTR2 assessed by FACS on fresh whole blood (n=5 healthy humans). MONO showed the strongest expression. Western blotting revealed AGTR1 protein expression in all cell types including T-cells. Next, whole blood was stimulated with Ang II (100 nM), a combination of IL6 (20 IU/ml), IFNγ (4 ng/ml) and

TNFa (20 ng/ml) (CytMix), or CytMix+Ang II for 4h ex vivo at 37C under gentle automated movement. Ang II by itself tended to depress MONO AGTR1 (81±10%) and AGTR2 (87±14%) expression assessed by FACS on whole blood. GRAN and B AGTR1 and -2 were not affected by Ang II. CytMix and CytMix+Ang II increased MONO AGTR1 (to 133±34 and 126±27%, resp.) and AGTR2 (to 137±26 and 126±15%,resp.) to levels exceeding Ang II alone (both $P<0.05$). When tested in U937 human MONO cell line, these results were reproduced for AGTR1. IFNγ seemed responsible for AGTR1 induction by CytMix.

**Conclusion**These data demonstrate that AGTR1 and -2 are present on cell surface of freshly isolated MONO, GRAN and B cells. Ang II by itself did not significantly modulate AGTR1 or AGTR2 expression. However, cytokines (presumably IFNg) cell-specifically stimulate AGTR1 and -2 expression in MONO. This absence of ligand repression of AGTR1 and -2 would indicate continued receptor stimulation in the presence of Ang II, which could induce a vicious circle leading to more inflammation and oxidative stress. These observations prompt exploration of aberrant AGTR expression in pro-atherosclerotic disease states such as CKD and HTN.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO223

**Roles of NO/sGC/cGMP/PKG Pathway in the Regulation of NBCe1 Activity by Angiotensin II** Ayumi Shirai, Osamu Yamazaki, Motonobu Nakamura, Hideomi Yamada, Masashi Suzuki, Shoko Horita, George Seki, Toshiro Fujita. *Internal Medicine, Tokyo University, Tokyo, Japan.*

Although nitric oxide (NO) has been known to affect renal proximal tubule transport, whether NO signaling is stimulatory or inhibitory in this segment has been controversial. Moreover, the roles of NO in the regulation of proximal transport by angiotensin (Ang) II have not been clarified. In the present study we investigated the roles of NO in the regulation of renal Na-$HCO_3$ cotransporter (NBCe1) activity by Ang II. Cell pH in isolated mouse renal proximal tubules was monitored by the pH sensitive dye BCECF, and the NBCe1 activity was determined by the rates of cell pH reduction in response to bath bicarbonate concentrations. As previously reported, Ang II had a biphasic effect on NBCe1 activity via AT1 receptor: 26% stimulation by $10^{-10}$ M AngII, no effect by $10^{-8}$ M Ang II, and 31% inhibition by $10^{-6}$ M Ang II. In the presence of nitric oxide synthetase (NOS) inhibitor L-NAME, however, all the concentrations of Ang II stimulated the NBCe1 activity by approximately 30%. In the presence of soluble guanylyl cyclase (sGC) inhibitor ODQ, $10^{-6}$ M Ang II stimulated the NBCe1 activity by 32%. In the presence of protein kinase G (PKG) inhibitor KT5823, $10^{-6}$ M Ang II also stimulated the NBCe1 activity by 42%. On the other hand, the addition of NO donor sodium nitroprusside dose-dependently inhibited the NBCe1 activity: 17%, 32%, and 40% inhibition by $10^{-5}$ M, $10^{-4}$ M, and $10^{-3}$ M SNP, respectively. Moreover, the addition of membrane permeable cGMP analog 8Br-cGMP (2 x $10^{-5}$ M) significantly reduced the NBCe1 activity by 24%. SNP also reduced the NBCe1 activity in the presence of cytosolic phospholipase A2 (cPLA2) inhibitor AACOCF3. By contrast, the addition of arachidonic acid significantly inhibited the NBCe1 activity, but failed to inhibit the NBCe1 activity in the presence of L-NAME. These results indicate that NOS/NO/sGC/cGMP/PKG pathway mediates the inhibitory action of Ang II in this segment. Furthermore, this pathway may lie downstream of cPLA2/arachidonic acid pathway, which was previously identified as another mediator of Ang II inhibitory action.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO224

**Role of Rho-Kinase (ROCK) and Epidermal Growth Factor Receptor (EGFR) Activation in Glomerular Injuries Induced by Angiotensin II (AngII)-Dependent Hypertension** Hiroyuki Suzuki,[1] Tatsuo Yamamoto,[2] Yoshihide Fujigaki,[1] Akira Hishida.[1] *[1]First Department of Medicine, Hamamatsu University School of Medicine, Japan; [2]Department of Health and Nutritional Sciences, Hamamatsu University, Japan.*

Among many kinases that are activated by AngII, role of ROCK and EGFR in the glomerular injuries are not well known. Therefore, we investigated the effect of fasudil (ROCK inhibitor) and gefitinib (EGFR inhibitor) on the progression of glomerular injuries using AngII-dependent hypertension model of rats.

Male Wistar rats were infused with AngII at a rate of 400ng/kg/min for 14 days. Fasudil (20mg/kg) and gefitinib (3mg/kg) were administered once every day intraperitoneally and orally, respectively.

We found that staining intensity of phosphorylation of myosin light chain at Ser19, downstream target of ROCK, and phosphorylation of EGFR at Tyr1068 were increased in kidneys at day 14, suggesting ROCK and EGFR in the kidney were activated by AngII. AngII infusion increased blood pressure (BP) (220.3±18.7mmHg: $p<0.05$) as well as urinary protein excretion (118.0±19.0mg/day: $p<0.05$) and the number of proliferating cells in glomeruli judged by Ki67 and PCNA staining by day 14. Also, p27 expressions in the glomeruli were reduced, suggesting AngII-induced cell proliferation is partly dependent on p27. AngII infusion induced dissociation of immunostaining patterns of nephrin and podocin, suggesting that proteinuria in this model might be related with slit diaphragm dysfunction. Fasudil significantly reduced proteinuria (57.2±17.5mg/day: $p<0.05$), completely inhibited cell proliferation and reduction of p27. On the other hand, gefitinib also completely inhibited glomerular cell proliferation and reduction of p27 in glomeruli. However, proteinuria was not reduced by treatment with gefitinib (133.3±30.9mg/day) at all. In agreement with the result of reduced proteinuria, dissociation of expression patterns of podocin and nephrin was ameliorated by fasudil but not by gefitinib.

In conclusions, AngII activates ROCK and mediates both proteinuria and glomerular cell proliferation. Although AngII also activates EGFR, it may induce glomerular cell proliferation but not proteinuria.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO225

**Adiponectin Attenuates Angiotensin II-Induced NADPH Oxidase Activity in Human Kidney Cells** Fei Fang, George Chu Liu, Xiaohua Zhou, James W. Scholey. *Medicine, University of Toronto, Toronto, ON, Canada.*

Although the mechanism(s) responsible for the progression of chronic kidney disease (CKD) to end-stage renal disease have not been fully elucidated, two important determinants of CKD are activation of the renin-angiotensin system (RAS) and obesity. The generation of angiotensin II (AngII) contributes to kidney injury, at least in part, by leading to NADPH oxidase activation and superoxide generation. Obesity is associated with reduced levels of adiponectin, and adiponectin attenuates high glucose-induced superoxide generation in endothelial cells. However the effect of adiponectin on Ang II-induced NADPH oxidase activation has not been studied. Accordingly, we sought to determine if there was an interaction between adiponectin and AngII in cultured human renal epithelial (HRE) cells. Primary cultured HRE cells expressed the adiponectin receptors, AdipoR1 and AdipoR2, and exhibited a robust increase in NADPH oxidase activity and superoxide production after incubation with AngII (0.1μM). Pre-treatment with adiponectin decreased the AngII-induced NADPH oxidase in a dose dependent manner. The effect of adiponectin on AngII-induced NADPH oxidase activity was mimicked by treatment with stable cAMP analogues (pCTP cAMP, 20μM; db cAMP, 25μM,) and was blocked by both the adenylyl cyclase inhibitor (SQ22536, 0.1mM) and the protein kinase A (PKA) inhibitor (H89, 1μM). In addition, incubation with an AMP activated kinase (AMPK) agonist, AICAR (1mM), normalized AngII-induced superoxide generation, and incubation with an AMPK antagonist, compound C (1μM), reversed the effect of adiponectin on AngII-induced NADPH oxidase activity. In conclusion, adiponectin attenuates AngII-induced superoxide production in human kidney cells, and this effect is mediated by both cAMP-PKA and AMPK signal transduction pathways. This finding suggests that declining levels of adiponectin may contribute to the deleterious effect of obesity on progression of CKD.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO226

**Local Balance of Angiopoietins (Ang) Is Essential To Protect the Glomerulus in Diabetes** Marie Jeansson,[1] Chengjin Li,[1] Susan E. Quaggin.[2] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [2]Division of Nephrology, St Michael's Hospital/University of Toronto, Toronto, ON, Canada.*

Endothelial dysfunction is central to complications of diabetes, sepsis, and malaria. An elevated ratio of Ang2:Ang1 has been associated with increased morbidity and mortality in these conditions. Ang1 and Ang2 are ligands for the tyrosine kinase receptor, Tie2, found on endothelial cells. Ang1, produced by endothelial support cells (pericytes, podocytes), is an agonist and promotes endothelial cell stabilization and quiescence. Ang2, produced by endothelial cells, is an antagonist and causes endothelial activation, destabilization, and inflammation. We hypothesize that imbalance between Ang1 and Ang2 is a critical factor in pathogenesis of endothelial dysfunction, particularly in diabetes.

Standard KO of Ang1 is embryonic lethal so to test this hypothesis, we generated a conditional allele for Ang1. Timed whole body KO of Ang1 was accomplished using a doxycycline sensitive ROSArtTA/tetOcre system. Deletion of Ang1 at E10.5 resulted in lethality at E17.5, with abnormal vascular remodeling. In the kidney, glomeruli had dilated Bowman's capsules and markedly enlarged capillary loop structures. Interestingly, when Ang1 was deleted at E16.5 or later the mice appear NORMAL.

Mice lacking Ang1 from E16.5 or later were then challenged with diabetes (STZ). Diabetic Ang1 KO mice had decreased survival (80%) and significantly higher urinary albumin/creatinine ratios. Histology showed increased glomerulosclerosis and mesangial matrix expansion. To study local Ang2 regulation, glomerular endothelial cells were isolated from diabetic mice and found to have an increased expression of Ang2. Also, cultured glomerular endothelial cells demonstrate marked increase in release of Ang2 when exposed to D-glucose, AngII or TGFb, known mediators of progression of diabetic nephropathy.

Taken together, the data shows that Ang1 is not required in the fully developed quiescent glomerulus, but is essential to prevent glomerular injury in the presence of enhanced Ang2 release, and demonstrates for the first time that local balance of Ang1:Ang2 plays a pathogenic role in diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO227

**Parathyroid Hormone Induces ICAM-1 Protein Expression and Oxidative Stress in Human Aortic Endothelial Cells** Wei Chen,[1,2] Miguel A. Lanaspa,[1] Diana I. Jalal.[1] *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Department of Nephrology, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China.*

Parathyroid hormone (PTH), inflammation, and oxidative stress are all associated with the development of cardiovascular disease in patients with chronic kidney disease (CKD). Although PTH has been shown to induce proatherosclerotic mediators such as interleukin 6 in endothelial cells, the effect of PTH on other inflammatory mediators such as intracellular adhesion molecule-1 (ICAM-1) or on oxidative stress has not been explored. We hypothesized that PTH induces ICAM-1 and oxidative stress in human aortic endothelial cells (HAECs). HAECs were purchased from Lonza, and grown at 37°C with 5% $CO_2$, then treated with PTH at various dosages (1 $X10^{-12}$, 1 $X10^{-11}$, $1X10^{-10}$ nmol/mL ). ICAM-1 protein expression was determined in whole cell lysates by western blot. Western blots were normalized by detection of GAPDH expression. Reactive oxygen species (ROS) were measured by Image-iT™ LIVE Green Reactive Oxygen Species Detection Kit and

imaged by confocal fluorescence microscopy. In addition, time course experiments were conducted at the following time points: 0, 1, 3, 6, and 24 hours, and PTH- mediated activation of Nuclear Factor κ-B (NFκ-B) was evaluated by western blot for NFκ-B-p65 expression in nuclear extracts. PTH significantly increased ICAM-1 protein expression compared to the negative control in a dose- and time- dependent manner. ROS increased progressively in response to increasing PTH dosages peaking at a dose of $1X10^{-10}$ nmol/mL. NFκB -p65 expression increased in the nuclear extracts in response to PTH treatment in a time dependent manner. In conclusion, the present study demonstrates that PTH induces ICAM-1 and oxidative stress in HAECs. These findings support a direct role for PTH in inflammation and oxidative stress in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO228**

**VEGF-Induced Glomerular Endothelial Cells Hyperpermeability Via PI3K/Akt-Mediated Rac1 Activation** Zengchun Ye, Hui Peng, Cheng Wang, Canming Li, Tan-Qi Lou. *Division of Nephrology, Department of Nephrology, The Third Affiliated Hospital of Sun Yet-Sun University, Guangzhou, Guangdong, China.*

Purpose: To investigate the roles of PI3K/Akt and Rac1 in vascular endothelial growth factor (VEGF) -induced hyperpermeability in glomerular endothelial cells (GEnCs). Methods: The GEnCs permeability was detected by measuring the transendothelial electrical resistance and flux of FITC-BSA across the GEnCs monolayers. Rac1 activity was detected by pull-down assay, and Akt phospholylation was measured by western blotting. ZO-1 and Occludin were detected by immunofluorescence. Membranous and cytoplasmic proteins were separated and western blotting was performed to analyze the re-distribution of ZO-1 and Occludin. Results: Rac1 is activated within 15 min of VEGF treatment, and this activation was reduced by the PI3K antagonist, suggesting Rac1 activation required PI3K activity. Additionally, treatment with VEGF caused Akt phospholylation in wild type Rac1 GEnCs, dominant negative Rac1 mutant GEnCs and constitutively active Rac1 mutant GEnCs within 15 min, while PI3K inhibitor, wortmannin, can completely block these effects, suggesting that Akt is the upstream of Rac1. VEGF-stimulated induction of ZO-1 and occludin disassembly was diminished by inhibiting PI3K activity in wild type GEnCs and was increased by a constitutively active Rac1 mutant, which cannot be inhibited by wortmannin. Whereas dominant negative Rac1 mutant cells dramatically attenuated the effect of VEGF on ZO-1 and occludin. Furthermore, VEGF increases paracellular permeability of wild type Rac1 GEnCs and constitutively active Rac1 mutant GEnCs. The PI3K antagonist can inhibit the effect of VEGF on wild type Rac1 GEnCs permeability and have no effect on constitutively active Rac1 mutant GEnCs. Conversely, VEGF has no effect on dominant negative Rac1 GEnCs permeability. Conclusion: Collectively, these data suggest that VEGF leads to a PI3K/Akt-mediated generation of Rac1, which induce disassembly of ZO-1 and occludin in GEnCs, ultimately regulating tight junction integrity and endothelial cells permeability.

* Zengchun Ye and Hui Peng contributed equally to this work.
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Disclosure of Financial Relationships: nothing to disclose

**TH-PO229**

**Chronic Celebrex Promotes Early Glomerular Changes of Diabetic Nephropathy in OVE26 Mice** Rania Nasrallah,[1] Susan J. Robertson,[2] Richard L. Hebert.[1] *[1]Cellular and Molecular Medicine, Kidney Research Centre, University of Ottawa, Ottawa, ON, Canada; [2]Pathology, Ottawa Hospital, Ottawa, ON, Canada.*

Cyclooxygenases (COX-1 and -2) contribute to renal prostaglandin synthesis, and are implicated in the regulation of many kidney processes. Given the controversies concerning the role of COX-2 in the development of renal pathologies, we compared the effect of COX inhibition by non-steroidal anti-inflammatory drugs (NSAIDs; non-selective: Ibuprofen, COX-2 selective: Celebrex) on the onset of diabetic change in type I diabetic OVE26 mice. FVB (control) and OVE26 (diabetic) mice received 15 or 50 mg/kg/day of NSAIDs in standard chow from 8 to 20 weeks of age. All OVE26 mice developed sustained hyperglycaemia (>600mg/dL) prior to 5 wks of age. Systolic blood pressures were monitored bi-weekly by tail-cuff from 8wks, and levels were comparable between OVE26 and FVB mice up to 20 wks (125-140 mmHg), but increased gradually in response to Celebrex in diabetics (155 mmHg at 20 wks). By 20 wks, OVE26 mice had increased kidney/body weights (2-fold) and urinary albumin/creatinine was increased 5-fold, but these were unaltered by NSAIDs. Enzyme immunoassays of urinary prostaglandins showed elevated levels of $PGE_2$ (3-fold), PGEM (4.2-fold), $TXB_2$ (3.2-fold), and 6-keto-PGF1α (3.3-fold) in diabetics, but $PGE_2$ and 6-keto-PGF1α were reduced 50% and 30% respectively by high dose Celebrex. Cortical fibronectin levels as assessed by Western blotting were increased 2.3-fold in diabetics but unaltered by NSAIDs. Glomerular structure was examined by electron microscopy. Glomerular diameters were increased by 17% but were unaltered by NSAIDs. Although no difference was noted between untreated OVE26 and FVB mice, diabetics receiving Celebrex had diminished glomerular basement membrane thickness (22%) and slit pore diameters (17%). Foot process densities were reduced by 27% in OVE26 mice compared to FVB, and further reduced by 15% in Celebrex treated diabetic mice. In summary, our study indicates that chronic Celebrex may promote the development of early glomerular changes characteristic of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO230**

**Effects of Paricalcitol on Endothelial Cells Stimulated with Advanced Glycation End Products** Eliezer Golan,[1,2,3] Sydney Benchetrit,[1,2,3] Jacques Bernheim,[1,2,3] Janice Green,[1] Tali Zitman-Gal.[1] *[1]Renal Laboratory, Meir Medical Center, Kfar Saba, Israel; [2]Nephrology & Hypertension, Meir Medical Center, Kfar-Saba, Israel; [3]Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, Israel.*

**Background**: Increased formation of advanced glycation end products (AGEs) play a role in the development of vascular complications in kidney failure, aging, and diabetes. Paricalcitol, a vitamin D receptor activator, is a synthetic analogue of calcitriol. It has already been demonstrated that calcitriol may inhibit endothelial proliferation, blunt angiogenesis, and be a cardioprotective agent.

**Objectives**: To investigate the possible impact of various concentrations of paricalcitol on gene and protein expression in human umbilical vein cord endothelial cells (HUVEC) stimulated with AGEs.

**Methods:** Cultured HUVEC were treated with paricalcitol at $10^{-9}$, $10^{-10}$, and $10^{-11}$ mol/L, and human serum albumin (HSA) or AGE-HSA for 6 and 24 hours. Pro-inflammatory markers such as the receptor of AGEs (RAGE), interleukin-6 (IL6), interleukin-8 (IL8), NFKB-p65, and endothelial nitric oxide synthase (eNOS) were tested at the mRNA and/or protein levels.

**Results:** Our results demonstrate that after 6 hours of stimulation with AGE-HSA, paricalcitol significantly decreased RAGE mRNA and protein expression and significantly increased eNOS mRNA, while p65, IL6, and IL8 mRNA levels were not changed. However, after 24 hours of stimulation p65, IL6, and IL8 mRNA levels were significantly down regulated. At the level of protein expression, after 24 hours, paricalcitol significantly decreased RAGE protein expression, while eNOS showed no significant change.

**Discussion**: Paricalcitol seems to have a beneficial effect on the expression of genes that are involved in the inflammatory processes that take place following the stimulation of HUVEC with AGEs. We observed that RAGE and eNOS, which are located upstream in the inflammatory signal pathway started to change at 6 hours, while changes in genes that are downstream in the inflammatory pathway such as p65, IL6 , IL8 were modified only after 24 hours. These findings indicate that paricalcitol may act as a vascular protective agent starting as early as 6 hours after induction.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO231**

**The Effect of Silibinin on the Expression of Vascular Endothelial Growth Factor (VEGF) and the Type IV Collagen in Human Podocytes Induced by High Glucose** Changying Xing, Jia Liu. *Dept of Nephrolgy, First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China.*

To explore the effects of silibinin on the expression of vascular endothelial growth factor (VEGF) and the secretion of type IV collagen in normal immortalized human podocytes induced by high glucose (HG). The normal immortalized human podocytes were cultured at 33° and allowed to differentiate at 37° for 2 weeks. The differentiated podocytes were incubated with medium (1%FBS and 5.5mmol/L D-glucose) for 24 hours to synchronize cell growth. Podocytes were divided into several groups: 1. High glucose (30mmol/L D-glucose) was given on different time course (0h, 24h, 48h, and 72h). 2. The podocytes were divided into seven groups of normal medium (NG, 5.5mmol/L D-glucose), mannitol (MN, 24.5mmol/L mannitol plus 5.5mmol/L D-glucose), high glucose (HG, 30mmol/L D-glucose), HG plus low concentration of silibinin (HG+LS, silibinin5μg/ml), HG plus medium concentration of silibinin(HG+MS, silibinin 10μg/ml),HG plus high concentration of silibinin(HG+HS, silibinin 20μg/ml), and HG plus DMSO (HG+D, DMSO1mg/ml). These groups were incubated for 48h. The expressions of VEGF mRNA and protein in podocytes were detected by semiquantitative reverse transcription-PCR and Western blot analysis respectively. The concentrations of collagen IV in culture supernatant secreted by the podocytes were determined by ELISA. Results: 1. Compared with 0h group, high concentration of glucose increased the expressions of VEGF and collagen IV in podocytes in a time-dependent manner ($P<0.05$ or $P<0.01$). 2. Compared with NG group, the levels of VEGF and collagen IV expression were increased markedly after HG treatment for 48 hours ($P<0.01$). Compared with HG group, the different concentrations of 5, 10, 20μg/ml silibinin effectively suppressed HG-induced expressions of VEGF mRNA, VEGF protein and the secreted collagen IV in a dose-dependent manner ($P<0.05$).

High concentration of glucose increases the expressions of VEGF and collagen IV by podocyte. Silibinin may exert a protective effect on podocyte and the effects of prevention and treatment on diabetic nephropathy via reducing the over-expressions of VEGF and collagen IV induced by HG.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO232**

**Effects of Apelin on Hemodynamic Function in the Rat Kidney** Annette Hus-Citharel,[1] Nadine Bouby,[2] Laurence Bodineau,[1] Alain Frugière,[1] Jean-Marie Gasc,[3] Catherine Llorens-Cortes.[1] *[1]INSERM U691, Paris, France; [2]INSERM U872, Paris, France; [3]INSERM U833, Paris, France.*

Apelin, a recently discovered peptide, and its receptor have a wide tissue distribution not only in the brain but also in the periphery, especially in the kidney. Central injection of apelin inhibits the electrical activity of vasopressin neurons, reduces plasma vasopressin levels and increases aqueous diuresis, thus showing a central role in the control of body fluid homeostasis. We recently reported that apelin receptor mRNA is expressed in the four renal zones with the highest density in the inner stripe of the outer medulla. Along the nephron,

the highest apelin receptor mRNA expression is found in glomeruli and to a lesser extent in the nephron segments, including outer and inner medullary collecting ducts that express $V_{1a}$ and $V_2$ vasopressin receptors. Apelin receptor mRNA is also found in endothelial cells and vascular smooth muscle cells of glomerular arterioles. These observations led us to investigate the functional role of apelin in the kidney. Apelin 17 induced vasorelaxation of AngII-preconstricted juxtamedullary afferent (AA) and efferent (EA) arterioles as shown by an increase in arteriolar diameter (from 18.6±0.5 to 21.8±0.7 μm, *p<0.01*). This vasorelaxation observed in AA and EA was NO-dependent and directly linked to the inhibition by apelin 17 of AngII-elicited $[Ca^{2+}]_i$ increase (43±5 and 44±7%, respectively). In glomeruli, apelin 17 significantly inhibited forskolin-induced cAMP production by 46% (*p<0.05*). This effect may increase glomerular filtration. Consistent with the distribution of apelin receptors in collecting ducts, intravenous injection of increasing doses of apelin 17 in lactating female rats caused a dose-dependent increase in diuresis. Taken together, these data showed that apelin exerts a complex and fine regulation on renal hemodynamic functions through actions on glomeruli and pre- and post-glomerular microvasculature. Moreover, in addition to its central action, apelin may directly counteract antidiuretic effects of vasopressin at the tubular level.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO233

**Hematide™/Peginesatide Binds to the Human Erythropoietin Receptor with High Affinity and Favorable Thermodynamics** Jennifer M. Green,[1] Yijun Pan,[1] Rishi Arora,[2] Robert K. Suto,[2] Peter R. Young,[1] Christopher P. Holmes.[1] *[1]Affymax, Inc., Palo Alto, CA; [2]Xtal BioStructures, Inc., Natick, MA.*

Peginesatide is a PEGylated, investigational, peptide-based erythropoiesis-stimulating agent that was designed and engineered to stimulate specifically the erythropoietin receptor (EPOR) dimer that governs erythropoiesis. Peginesatide is currently being developed for the treatment of anemia of chronic kidney disease. Peginesatide stimulates erythropoiesis by binding to the EPOR at a site cross-competitive with EPO. Methods: Isothermal titration calorimetry (ITC) was used to compare peginesatide with EPO by assessing the thermodynamic parameters that are associated with binding to the extracellular domain of the EPOR. ITC measures the binding equilibrium directly by determining the heat gained or lost when 2 molecules interact in solution. The thermodynamics of binding are characterized by free energy (ΔG), enthalpy (ΔH), entropy (ΔS), heat capacity of binding (ΔCp), and the stoichiometry of the interaction. Results: Calorimetric titrations at 25 °C of both peginesatide and EPO into a solution containing the soluble extracellular domain of the EPOR show strong, high affinity binding events characterized by equilibrium dissociation constants (Kd) of 631 and 172 pM, respectively. The large negative overall free energy associated with peginesatide binding (ΔG = -12.6 kcal/mol), comprised of favorable enthalpic (ΔH = -24.4 kcal/mol) and unfavorable entropic (ΔS = -11.9 kcal/mol/K) components, was greater in magnitude than it was for EPO binding (ΔG = -9.22 kcal/mol). The large negative heat capacities calculated for both peginesatide and EPO binding (ΔCp = -1.047 and -1.230 kcal/mol/K, respectively) suggest that a large amount of the water-accessible surface area on the EPOR is occupied and buried upon ligand binding. As expected, 1 peginesatide molecule was found to bind to 2 EPOR chains, similar to the known 1:2 interaction between EPO and the EPOR. Conclusions: The thermodynamic parameters of binding indicate a favorable and high affinity interaction between peginesatide and the EPOR that may contribute to the prolonged in vivo activity of peginesatide.

Disclosure of Financial Relationships: Employer: Affymax, Inc.; Ownership: Affymax, Inc.

## TH-PO234

**Prostaglandin Transporter PGT Regulates PGE2 Signaling** Yuling Chi, Victor L. Schuster. *Medicine, Albert Einstein College of Medicine, Bronx, NY.*

Prostaglandin E2 (PGE2) triggers cellular events by binding to EP1, EP2, EP3, or EP4 receptors. The EPs 1-3 reside stably on the cell surface, whereas EP4 undergoes ligand-induced internalization. Net signaling has typically been considered to be a function of the number of cell-surface receptors and cell-surface [PGE2]. However, plasma membrane PGT (Km for PGE2 = 50 nM) could remove cell-surface PGE2 and thus locally regulate PGE2 access to its receptors. To test this hypothesis directly, we established two experimental systems: 1) an "EP1 system" consisting of wild type HEK 293 cells ± EP1 ± PGT; and 2) an "EP4 system" consisting of MDCK cells ± EP4 ± PGT. In the EP1-HEK system, exogenous PGE2 (0.1 nM – 10 μM) induced a dose-dependent Ca2+ spike. When EP1-HEK also expressed plasma membrane PGT, the PGE2 dose-response curve was right-shifted. A potent PGT inhibitor, T26A, reversed this shift. Moreover, when bradykinin was used to induce endogenous PGE2 release and autocrine EP1 signaling, expression of PGT also induced a (T26A-inhibitable) reduction in the Ca2+ spike. In the EP4-MDCK system, bradykinin induced autocrine signaling as judged by acutely raised cellular cAMP levels. Expression of plasma membrane PGT induced a (T26A-inhibitable) reduction in this acute cAMP accumulation. Finally, we assayed the ability of pharmacological exogenous PGE2 to induce EP4 internalization, as judged by cell-surface ligand binding, immunocytochemical EP4 receptor localization, and cAMP accumulation. PGT reduced PGE2-induced EP4 receptor internalization in a T26A-inhibitable fashion. Summary: as judged by EP1 signaling (Ca2+), acute EP4 signaling (early cAMP), and longer-term EP4 internalization (binding, labeling, delayed cAMP), PGT at the plasma membrane reduces PGE2's ability to interact with its receptors. Conclusion: Plasma membrane PGT regulates EP signaling by internalizing cell-surface PGE2, making it unavailable for EP receptor activation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO235

**Novel Transcription Regulation of Endothelin-1 in the Collecting Duct** Donald E. Kohan, Kevin A. Strait, Peter K. Stricklett. *Division of Nephrology, University of Utah, Salt Lake City, UT.*

Collecting duct (CD) endothelin-1 (ET-1) is an important autocrine inhibitor of CD Na reabsorption. Salt loading increases CD ET-1 production, however the cellular mechanisms involved are poorly understood. Herein, we studied factors modulating ET-1 gene transcription in cultured rat inner medullary CD (IMCD). Transiently transfected rat ET-1 promoter-reporter constructs revealed enhancer activity in the 500 bp region between 1.2-1.7 kb 5' to the transcription start site. This 500 bp region is sufficient to drive reporter expression from a heterologous promoter. Activity of the proximal 1.7 kb ET-1 or the 500 bp heterologous promoter was almost abolished by calmodulin inhibition. Sequence analysis revealed two NFAT consensus binding sites in the 1.2-1.7 kb region which could potentially be regulated by calmodulin. Mutation of either NFAT site (at -1563 and -1263) reduced activity of the 1.7 kb ET-1 promoter by over 80%. Electrophoretic mobility shift confirmed nuclear protein binding to these NFAT sites. Supershift analysis showed NFATc3 binding to the -1563 site and NFATc1 binding to the -1263 site. INCA-6, an NFAT inhibitor, markedly reduced ET-1 secretion and promoter activity. Several AP-1 sites were also identified in the 1.7 kb ET-1 promoter. PKC inhibition (calphostin C) reduced (35-40%) ET-1 secretion, mRNA and 1.7 kb promoter activity. PMA rapidly increased ET-1 mRNA and promoter activity, but was inhibitory after 1 hr of exposure. PKC inhibition reduced ET-1 promoter activity by about 35% in all constructs from -366 to -3048 bp. Mutation of the AP-1 consensus binding site at -186, but not at -1219 or -1394, inhibited .36 kb and 1.7 kb ET-1 promoter activity by over 60%. In summary, Ca/calmodulin-sensitive NFAT isoforms and PKC stimulate IMCD ET-1 promoter activity through interaction with unique cis-acting domains. This suggests that changes in intracellular calcium (as can occur with altered tubule fluid flow), via NFAT and PKC, may regulate IMCD ET-1 production, thereby providing a novel mechanism coupling salt intake to tubule Na reabsorption.

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## TH-PO236

**Endogenous PPAR-gamma Ligand,15-Deoxy-(Delta)12,14-Prostagalndin $J_2$ (15d-$PGJ_2$) Has a Protective Effect on $H_2O_2$ Induced Cell Damage in MDCK Cells** Tomoya Makihara, Masaru Horio. *Osaka University, Suita, Japan.*

**Background:** Oxidative stress in renal tubular cell is a possible factor inducing the cell damage and the tubulointerstitial fibrosis. 15-d-$PGJ_2$ is a prostaglandin and an endogenous ligand of PPAR-gamma. 15-d-$PGJ_2$ was reported to increase cellular glutathione (GSH) content in some cultured cells. We examined the effect of 15-d-$PGJ_2$ on GSH content and protective effect on $H_2O_2$-induced cell damage in MDCK cells.

**Methods:** Cell damage was assessed by MTT assay and LDH activity in culture medium. GSH content, activity of glutamate-cysteine ligase (GCL), the rate-limiting enzyme of GSH synthesis, and activity of NADPH oxidase, one of the primary sources of superoxide, were measured.

**Results:** $H_2O_2$ induced cell damage in MDCK cells in dose dependent manner. Pre-incubation and simultaneous addition of 0.5, 1 and 2μM 15-d-$PGJ_2$ protected the $H_2O_2$-induced cell damage assessed by LDH activity in culture medium. 15-d-$PGJ_2$ significantly increased GSH content up to 2 fold the value of control cells after 8h. But, higher concentration (5 and 10 μM) of 15-d-$PGJ_2$ did not show further increase of GSH, but induced cell death after 24h. Simultaneously addition of 10μM GW9662, an inhibitor of PPAR-gamma, did not inhibit the induction of the GSH content by 1μM 15-d-$PGJ_2$. Ciglitazone (1, 2 and 10μM), a PPAR-gamma agonist, did not change GSH content significantly, indicating that the increase of GSH by 15-d-$PGJ_2$ was PPAR-gamma independent mechanism. GCL activity were not increased but significantly decreased after the addition of 15d-$PGJ_2$. GW9662 did not have significant effect on the decrease of GCL. Ciglitazone did not have any significant effect on GCL activities. Change of GCL activity by 15-d-$PGJ_2$ seems to be the result of negative feedback of the induction of GSH contents. NADPH activity was not changed significantly after addition of 15d-PGJ2. The mechanism of inducing GSH content by 15-d-PGJ2 in MDCK cells needs further investigation. **Conclusion:** 15d-$PGJ_2$ has a protective effect on $H_2O_2$ induced cell damage in MDCK cells, suggesting the possibility of a participation against renal cell damage by oxidative stress.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO237

**Retinoic Acid Activity in Collecting Duct: Potential Target Gene and Anti-Fibrotic Potential** Yuen Fei Wong,[1] Jeffrey B. Kopp,[2] Bruce M. Hendry,[1] Qihe Xu.[1] *[1]Renal Medicine, King's College London, London, United Kingdom; [2]Kidney Disease Section, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.*

Retinoic acid (RA), the primary bioactive derivative of vitamin A, plays an essential role in nephrogenesis by promoting ureteric bud branching via retinoic acid receptor (RAR) activation. We hypothesized that RA-RAR response system remained active in kidneys after birth, and may serve as an inbuilt defence mechanism against kidney injury or enhance wound repair by regulating the expression of certain genes. By employing a retinoic acid response element (RARE)-*hsp68-lacZ* transgenic mouse model, we found that RA activity is present specifically in the renal collecting ducts of neonatal, young and adult mice. A well-established mouse inner medullary collecting duct cell line (mIMCD-3) was used as an *in vitro* model to identify potential target genes of RA. Transient transfection

of RARE-luciferase reporter plasmid to mIMCD-3 revealed presence of endogenous RA; exogenous RA treatment increases luciferase activity in a dose-dependent manner, which was blocked by AGN193109, a pan-antagonist of RAR, suggesting an intact RA-RAR-RARE response system. Wnt7b, Pax2 and Bmp7 genes were selected as these genes are expressed in collecting duct and are reported to mediate repair mechanisms during renal injury. Effects of RA on mRNA expression of these genes were examined with quantitative PCR. Of these candidate genes, basal expression of Bmp7 was blocked by AGN193109. Furthermore, Bmp7 expression was induced by RA in a dose dependent manner and blocked by AGN193109. As Bmp7 is best known for its anti-fibrotic property, fibrogenesis was induced in mIMCD-3 by transforming growth factor-β1 (TGF-β1) to examine the role of RA in this context. RA did not prevent TGF-β1-induced loss of epithelial marker (E-cadherin) and *de novo* gain of mesenchymal marker (α-smooth muscle actin) but suppressed the induction of fibronectin, a main fibrogenic matrix protein. The mechanism of Bmp7 induction by RA and its role in regulating fibrogenesis in mIMCD-3 are currently being investigated.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO238

**Hormonal Status Influences mTOR Signaling Pathway in Adaptive and Maladaptive Myocardial Hypertrophy** Angelika Kusch, Maria Schmidt, Rusan Catar, Bjoern Hegner, Duska Dragun. *Department of Nephrology and Intensive Care Medicine/Center for Cardiovascular Research, Charité, Berlin, Germany.*

Background

Activation of PI3K/Akt/mTOR pathway is a hallmark of both, adaptive and maladaptive myocardial hypertrophy (MH). Hormonally induced differences in regulation of this pathway might influence clinical outcome in a sex-specific manner. Aim of our study was to investigate how beta-estradiol (E2) modulates PI3K/Akt signaling in response to hypertrophic stimuli and determine consequences of mTOR inhibition in the context of female cardiomyocyte.

Methods

Female HL-1 cardiomyocytes were treated with physiologic (IGF-1) and pathologic (ET-1) stimuli in the presence or absence of estradiol or mTOR inhibitor rapamycin. Cell size was determined by immunocytochemistry and FACS-analysis. Signal transduction was assessed by immunoprecipitation with anti-mTOR polyclonal antibodies and westernblotting using polyclonal antibodies against raptor/ rictor, phospho-specific antibodies against Erk, Akt to monitor TORC2-activity, p70S6K to monitor TORC1-activity, polyclonal antibodies against respective non-phosphorylated forms of proteins and SERCA2A. Taqman was applied to investigate genomic changes.

Results

E2, IGF-1 and ET-1 induced phosphorylation of Akt and p70S6K in a time-dependent manner. E2 reversed the increase in p70S6K-phosphorylation upon ET-1-stimulation, whereas co-treatment of E2 with IGF-1 increased p70S6K-phosphorylation. Estradiol increased both, mTOR-complex formation with raptor and rictor. SERCA2A protein expression was upregulated by E2. ANP-mRNA-expression increased significantly with E2-cotreatment and most markedly with additional ET-1. Rapamycin inhibited cardiomyocyte hypertrophy and p70S6K-phosphorylation by IGF-1 and ET-1 irrespective of E2-cotreatment, whereas positive feedback loop towards Akt-phosphorylation was differentially regulated by rapamycin dependent on the presence or absence of E2.

Conclusions

mTOR inhibition effectively inhibits female cardiomyocyte hypertrophy irrespective of the presence or absence of E2. However, E2 differentially modulates TORC1 and TORC2 activities dependent on the nature of hypertrophic stimulus and rapamycin treatment.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO239

**Cardiac Fibrosis Is Modulated by Endogenous PAI-1** Jianyong Zhong,[1] Haichun Yang,[2] Agnes B. Fogo,[2] Valentina Kon,[1] Iekuni Ichikawa,[1] Ji Ma.[1] *[1]Pediatrics, Vanderbilt University, Nashville, TN; [2]Pathology, Vanderbilt University, Nashville, TN.*

The profibrotic effects of plasminogen activator inhibitor-1 (PAI-1) are postulated to reflect decreased extracellular matrix degradation due to decreased plasmin activity and increased inflammation due to PAI-1 binding to vitronectin (Vn). However, recent findings suggest that, depending on the tissue, increased plasmin activity can also promote fibrosis. This study was designed to determine the importance of plasmin *vs.* vitronectin pathways in angiotensin II (Ang II)-induced cardiac fibrosis.

Uninephrectomized (UNx) mice were fed a high salt diet and infused with Ang II for 8 weeks. Different human stable PAI-1 variants, including PAI-1AK (retaining protease inhibitory effects of native PAI-1 but not binding to Vn), or PAI-1RR (competitive blocker of Vn), or CPAI (control PAI-1, retaining all known functions of native PAI-1), or PBS was injected daily.

Increased systolic blood pressure and albuminuria were found in all mice with Ang II infusion. In the heart, PAI-1AK-injected mice showed significantly more extensive myocardial and interstitial fibrosis compared with the other Ang II-infused mice (PAI-1AK 1.79±0.26%, PAI-1RR 0.91±0.18%, CAPI 0.81±0.12%, PBS 1.15±0.26% and UNx/salt 0.24±0.04%; n= 9 for PAI-1AK, 11 for PAI-1RR, 11 for CAPI, 7 for PBS and 4 for UNx/salt). Active plasmin was increased in kidneys of PAI-1AK-injected mice, but it was not changed by Ang II and was not different among the groups treated with PAI-1 variants. However, although Ang II increased the level of endogenous total mouse PAI-1 in heart tissue, this was further elevated by PAI-1AK (1.40±0.23 *vs.* 0.93±0.09 in CPAI, $P<0.05$).

Our study suggests that the severity of cardiac fibrosis induced by high circulating Ang II is dependent on endogenous tissue PAI-1, which is influenced by the balance between the two main PAI-1 pathways.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO240

**Fusion of Activated Omentum with Kidney Attenuates Progression of Chronic Kidney Disease** Nishit Pancholi,[2] Jilpa Patel,[2] Krishnamurthy P. Gudehithlu,[1,2] George Dunea,[1,2,3] Jose A. L. Arruda,[3,1,2] Ashok K. Singh.[1,2] *[1]Nephrology, John H. Stroger Hospital of Cook County, Chicago, IL; [2]Nephrology, Hektoen Institute of Medicine, Chicago, IL; [3]Nephrology, University of Illinois at Chicago, Chicago, IL.*

In previous work we showed that fusion of activated omentum, a tissue rich in stem cells and growth factors, to the injured liver facilitated repair and regeneration of the liver. Here we tested whether fusion of activated omentum to the kidney could ameliorate chronic kidney disease (CKD) in rats. CKD was induced in rats by renal mass reduction (5/6 nephrectomy by removal of right kidney and excision of the two poles of the left kidney). After inducing the injury, the rats were divided into two groups. Group 1 rats (N = 22; treated) received one intraperitoneal injection of polydextran gel particles to activate the omentum and promote its fusion to the wounded edges of the kidney, while Group 2 rats (N = 22; control) underwent total omentectomy. Following surgery, both groups showed a gradual rise in plasma creatinine and urea over baseline (baseline plasma creatinine 0.8 ± 0.02 mg/dL, plasma urea 56 ± 1.9 mg/dL, creatinine clearance 0.31 ± 0.017 mL/min/100g). However at weeks 6 and 12, as compared to control rats, the treated rats had significantly lower plasma creatinine and urea levels and higher creatinine clearance.

Table 1.

| | Week 6 | | Week 12 | |
|---|---|---|---|---|
| | TREATED | CONTROL | TREATED | CONTROL |
| **Creatinine (mg/dL)** | 1.5 ± 0.00* | 1.9 ± 0.02 | 1.6 ± 0.02* | 2.2 ± 0.03 |
| **Urea (mg/dL)** | 103 ± 0.5* | 155 ± 3.6 | 105 ± 2.0* | 197 ± 8.5 |
| **Creatinine Clearance (mL/min/100g)** | 0.22 ± 0.007* | 0.16 ± 0.011 | 0.18 ± 0.012* | 0.10 ± 0.012 |

*(p <0.05 versus respective control)

Weight gain in treated rats was higher than in controls. Histologically, the treated rats had a lower kidney injury score, with less glomerulosclerosis, tubular dilatation, and interstitial fibrosis, as well as lower immunoreactivity for α-SMA in the tubulo-interstitial areas. These results show that fusion of activated omentum to the kidney slows the progression of CKD in rats.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO241

**Smad Anchor for Receptor Activation (SARA) Regulates High Glucose Induced EMT Via Modulation of Smad2 and Smad3 Activities in Renal Tubular Epithelial Cells** Guanghui Ling,[1] Wenbin Tang,[1] Yuncheng Xia,[1] Lin Sun,[1,2] Yinghong Liu,[1] Youming Peng,[1] Yashpal S. Kanwar,[2] Fu-You Liu.[1] *[1]Department of Nephrology, Second Xiangya Hospital, Center of Kidney Disease and Dialysis in Hunan Province, Central South University, Changsha, Hunan, China; [2]Department of Pathology and Medicine, Northwestern University Medical School, Chicago, lL.*

Recent studies demonstrated that SARA is an essential adaptor protein in TGF-β1 signaling, and it is also involved in the process of epithelial-mesenchymal transition (EMT) and fibrosis. We investigated the effect of SARA on high glucose (HG) induced EMT and extracellular matrix (ECM) synthesis in renal tubular epithelial cells, HK-2 cells. The cells were transfected with the following plasmids: wild-type SARA (SARA-WT), SARA mutant (SARA with Smad binding domain deletion, SARA-dSBD) and SARA-WT + SARA-dSBD, and then subjected to high glucose ambience (HG, 30mM). The expression levels were assessed by QPCR, Western-blot analysis, and Immunofluorescence and Confocal microscopy. The HG induced EMT phenotype with increased expression of ECM genes in HK-2 cells. This was associated with the decreased expression of SARA and Smad2. In comparison with HG group, over-expression of SARA in HK-2 cells, a relatively high up-regulation of mRNA and protein expression of ZO-1 was seen; while that of vimentin, fibronectin and collagen I was decreased. No change in the expression of EMT profile was observed in cells doubly transfected with SARA-WT and SARA-dSBD. The Smad2 protein expression was increased in HK-2 cells after transfection with SARA WT plasmid. Interestingly, the over-expression of SARA prolonged the activity period of Smad2 and shortened that of Smad3, which seemed consistent with the change of EMT phenotype and ECM changes in HK-2 cell induced by HG. In conclusion, SARA regulates HG induced EMT and ECM accumulation and modulation via the activation of Smad2 and Smad3 in renal tubular epithelial cells. In view of these findings it is conceivable that SARA may serve as a potential novel target in pre-EMT states for the amelioration renal fibrosis seen in chronic kidney diseases.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO242**

**Dysfunctional Regulation of PGC-1α in Skeletal Muscle during CKD and Diabetes: A Potential Mechanism for Sustaining Atrophy** Russ Price, Haiyan Li, Bin Zheng, Myra Woodworth-Hobbs. *Renal Division, Emory University, Atlanta, GA.*

PGC-1α is a transcription coactivator protein that plays an integrative role in energy and protein metabolism in skeletal muscle. It also regulates muscle fiber-type specification and is less abundant in MHC II fibers than MHC I fibers. In CKD or diabetes (DM), MHC II fibers preferentially undergo atrophy and fiber-type switching from MHC I to MHC II has been noted; PGC-1α expression is also reduced. To elucidate the mechanism for the reduction in PGC-1α, we studied the signaling process that controls its transcription. The cAMP signaling pathway controls fiber type by regulating PGC-1α gene expression via CREB. Paradoxically, phosphorylated CREB (i.e, activated) was >7-fold higher in muscle of DM rats or dexamethasone-treated L6 muscle cells vs controls (P<0.03 in each model). Under the same conditions, PGC-1α was suppressed >50% (P<0.05 in each model). This led us to hypothesize that dysfunctional cAMP/CREB signaling contributes to the reduction in PGC-1α during muscle atrophy. To study the regulation of PGC-1α transcription by CREB, we transfected L6 muscle cells with a PGC-1α-luciferase (Luc) plasmid or with PGC-1αΔCRE-Luc which contains PGC-1α-Luc without the CRE binding site. PGC-1αΔCRE-Luc activity was reduced 72% vs PGC-1α-Luc (P<0.01). Treatment of transfected cells with forskolin (FSK) to raise cAMP did not increase luciferase activity from either PGC-1α reporter plasmid whereas FSK increased luciferase activity >9-fold (P<0.001) in cells transfected with a generic CRE-Luc reporter gene. These results suggested that CREB regulates PGC-1α transcription but that cAMP alone is insufficient to drive PGC-1α expression. To explore potential defects in cAMP/CREB signaling, we examined TORC1, a CREB coactivator that participates in PGC-1α transcription, in DM and control rat muscle. TORC1 protein was reduced 31% in DM muscle (P<0.005). Thus, defective cAMP/CREB signaling contributes to the abnormal transcriptional regulation of PGC-1α during skeletal muscle atrophy. Reduced PGC-1α and the concurrent fiber-type switching could serve as a mechanism to sustain atrophy in CKD and DM.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO243**

**Determination of Optimal Time Point of Conversion from Cyclosporine to Sirolimus Using Experimental Model of Chronic Cyclosporine** Jungyeon Ghee,[1] Ji-Hyun Song,[1] ShangGuo Piao,[1] Sol Kim,[2] Chul Woo Yang.[1] *[1]Department of Internal medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea; [2]Department of Bioengineering, University of Pennsylvania, Philadelphia.*

**BACKGROUND:** Sirolimus (SRL) is a promising drug for replacing calcineurin inhibitors, but the conversion point is still undetermined. We performed this study to determine optimal time point of conversion of SRL in cyclosporine (CsA)-induced nephrotoxicity.

**METHODS:** Three separate studies were performed in rats. First, CsA was treated with or without SRL for 28 days. Second, CsA (15mg/kg per day) was switched to SRL (0.3mg/kg per day) at day 7 (early conversion). Third, CsA was switched to SRL or withdrawn at day 28 and followed for 4 weeks (late conversion). The conversion effect from CsA to SRL was evaluated by renal function, histopathology (interstitial inflammation and fibrosis), inflammatory and fibrotic markers (osteopontin and beta-igh3), and apoptotic cell death (TUNEL-positive cells and caspase 3).

**RESULTS:** Combined treatment of CsA and SRL further deteriorated renal function, increased interstitial fibrosis and beta-igh3 and apoptotic cell death. CsA treatment for one week did not decrease renal function and increase interstitial fibrosis compared with the control group, and early conversion to SRL did not affect these parameters. CsA treatment for four weeks significantly deteriorated renal function and increased interstitial inflammation and fibrosis. But, withdrawal of CsA after four weeks CsA treatment improved renal function and decreased interstitial fibrosis and inflammation. Late conversion to SRL aggravated both parameters compared with the CsA withdrawal group.

**CONCLUSION:** Early conversion of from CSA to SRL is effective in preventing CsA-induced renal injury, but later conversion aggravates CsA-induced renal injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO244**

**Functional Research of CD2-Associated Protein Gene Mutation in Podocytes** Nan Chen, Xiaoxia Pan, Caixia Zhao, Xiaobei Feng, Weiming Wang, Zhaohui Wang, Qi Long. *Ruijin Hospital.*

**Objective:** To investigate the influence of CD2-associated protein (CD2AP) gene mutation on other podocyte associated proteins and cytoskeletal structures in mouse podocytes.

**Methods:** CD2AP heterozygous mutation V54I in exon 2, which was found in one Chinese patients with sporadic focal segmental glomerular sclerosis (FSGS) in our department, was selected. Wild-type and V54I mutant CD2AP plasmid were constructed and transfected into mouse podocytes using lipofectamin 2000. The mRNA level and protein expression of CD2AP and other podocyte associated proteins such as nephrin, podocin and synaptopodin were detected by realtime PCR and flow cytometry respectively. The distribution of nephrin, podocin and cytoskeletal structures in podocytes were observed by immunofluorescent technique.

**Results:** Mutant CD2AP had no obvious effects on the protein expression of nephrin, podocin and synaptopodin in podocytes. There was no significant difference of mRNA level of nephrin and podocin between mutant and wild-type CD2AP expression group. However the mRNA level of synaptopodin in mutant group significantly decreased, comparing to the wild-type group. No significant differences on the distribution of nephrin and podocin were observed between the wild-type and mutant groups. In interphase of cell division, stress fibers destructed in cytoplasm, and appeared thick, short dot-like structures mainly around the nuclei in podocytes expressing wild-type CD2AP, while in podocytes expressing mutant CD2AP, stress fibers mainly expressed along the cytoplasm membrane. In the metaphase of cell division, F-actin filaments aggregated encircling podocyte in wild-type group, however the F-actin in the mutant group did not show similar changes, but simply an increase in cytoplasm with some regional aggregation.**Conclusion:** The V54I mutant CD2AP has no obvious effects on genetic transcription and protein translation of podocin and nephrin. However the mRNA level of synaptopodin significantly decreased in mutant podocytes, comparing to the wildtype podocytes. It might affect podocyte skeletal construction of F-actin.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO245**

**Cigarette Smoking Affects Unfavorably the Renal Function of Hypercholesterolemic Rats** Barbara Costa,[1] Gloria E. Mendes,[1] Monique Martinez,[1] Leandro Pires,[1] Marcos Luz,[1] Emmanuel A. Burdmann.[1,2] *[1]Sao Jose do Rio Preto Medical School; [2]University of Sao Paulo Medical School, Brazil.*

The aim of this study was to assess the effects of the association of cigarette smoking (CS) and hypercholesterolemia on the renal function of rats. Male adult rats were exposed to CS (smoking chamber) or sham procedure (smoking chamber without CS) for 10 min, twice a day for 18 weeks (w). From the 14 to the 18th w rats received low salt diet (0.06%) and half of them received diet supplemented with 2% of cholesterol (CO) and 0.5% of cholic acid. Final groups (5 to 8 rats/group) were: normal CO+No-CS, normal CO+CS, high CO+No-CS, and high CO+CS. On day 126, GFR (inulin clearance, ml/min/100g), RBF (Doppler ultrasound, ml/min), RVR (mmHg/ml/min), CO serum level (mg/dl), urinary osmolality (UO, mOsm/Kg), hematocrit (Ht, %) and tubulointerstitial fibrosis (TIF, score 0 to 4) were assessed. Data (mean±SE) were analyzed by one-way ANOVA with post-test.

| | Normal cholesterol | | High cholesterol | |
|---|---|---|---|---|
| | No-CS | CS | No-CS | CS |
| GFR | 0.72±0.05 | 0.69±0.03 | 0.40±0.06 * | 0.20±0.03 **, ♦ |
| RBF | 4.0±0.4 | 3.5±0.2 | 4.0±0.5 | 2.2±0.3 **, ♦ |
| RVR | 33±6 | 30±2 | 24±2 | 43±5 **, ♦ |
| UOsm | 382±45 | 235±13 ♣ | 199±20 * | 169±7 |
| TC | 63±2 | 60±1 | 85±5 * | 83±2 ** |
| Ht | 55±2 | 53±1 | 52±2 | 49±1 |
| TIF | 0.03±0.005 | 0.05±0.007 | 0.13±0.02 * | 0.17±0.02 ** |

*p<0.05 vs. NLCO No-CS; **p<0.05 vs. NLCO CS; ♦ p<0.05 vs. HCO No-CS; ♣ p<0.05 vs NLCO NoCS

The obtained results demonstrated the efficacy of the supplemented cholesterol diet in promoting hypercholesterolemia. High cholesterol diet induced GFR decrease, impaired UOsm and TIF. Cigarette smoking associated to hypercholesterolemia worsened GFR decreased and caused RBF decrease and RVR increase.

In conclusion, these data suggest that cigarette smoking can enhance the negative impact of hypercholesterolemia on renal function.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO246**

**Mechano-Growth Factor Regulation by Diabetes and GLUT1 in Glomeruli** Minghui Xiang,[1] Kathleen O. Heilig,[1] N. Stanley Nahman,[2,1] Charles W. Heilig.[1] *[1]Medicine, University of Florida COM Jacksonville, Jacksonville, FL; [2]Medicine, Charlie Norwood VA Medical Center & Medical College of Georgia, Augusta, GA.*

Mechano-growth factor (MGF) is a stretch- and hypoxia-induced splice variant of IGF1. It is involved in tissue repair, has anti-apoptotic properties, induces cell hypertrophy, and in skeletal muscle recruits satellite cells to proliferate in response to cell stretch. It is expressed in many cell types, including vascular smooth muscle. However, it has not been investigated in the kidney where its expression potentially could modulate diabetic glomerular disease. We hypothesized it would be expressed in glomeruli where it could be induced by stretch or hyperglycemic stress with diabetes. **Methods**: All mice had the C57BL6 background. Diabetic (db/db, glucose > 250 mg/dl) and non diabetic (db/m) mice, transgenic mice with overexpression of GLUT1 (GT1S), and diabetic mice deficient in mesangial GLUT1 (db/db/antisense-GLUT1) were sacrificed at 8 or 26 weeks of age. Kidneys were harvested, fixed and embedded in paraffin for immunolabeling with antibodies against MGF. Whole kidney and glomeruli were assessed and scored by 2 observers. Primary cultured mouse mesangial cells (MC) were also labeled with the MGF antibodies. **Results**: Glomeruli and some cortical tubular segments exhibited MGF expression. Glomerular MGF was found in the mesangium and capillaries. In the diabetic and GT1S kidneys, glomerular MGF was particularly high. Specifically, glomerular MGF increased 144% (p< 0.0001) in response to diabetes and increased 33% (p< 0.001) in GT1S mice without diabetes. In contrast, GT1AS mice with 50% reduced MC GLUT1 demonstrated protection against diabetes-induced glomerular MGF expression, with 55% (p<0.0001) reduction in MGF. Primary cultured MC's from normal mice expressed MGF in both the nuclei and cytoplasm. **Conclusions**: MGF is expressed in the kidney, particularly in glomeruli where it was detected in the

mesangium and capillaries. Glomerular MGF increased in response to both diabetes and GLUT1-overexpression. This suggests that GLUT1 may contribute to the MGF response in diabetes, where glomerular GLUT1also is increased.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO247

**Resistance to Leucine Stimulated Anabolic Signaling in Work-Overloaded Skeletal Muscle of CKD Rats** Yu Chen,[1,2] Sumita Sood,[1,2] Kevin L. Mcintire,[1,2] Ralph Rabkin.[1,2] *[1]Nephrology, Stanford University School of Medicine, Palo Alto, CA; [2]Nephrology, Veterans Affairs Palo Alto Healthcare System, Palo Alto, CA.*

Muscle wasting is a common and significant complication of advanced CKD caused partly by insensitivity to insulin/IGF-1 induced signal transduction and low serum and tissue levels of branched chain amino acids (BCAA). In an earlier study of CKD rats we reported that resistance exercise fully activates the depressed IRS1/PI3kinase/AKT pathway and induces an increase in muscle mass (Kidney Int.2006). In humans undergoing resistance exercise, ingestion of the BCAA leucine (LEU) enhances exercise induced signaling through the downstream mTOR pathway. This pathway involves phosphorylation of p70 ribosomal S6 kinase-1 ($p70^{S6K}$) and S6 ribosomal protein (rpS6) and increases exercise induced protein synthesis. To test whether in the setting of uremia, administration of LEU can enhance exercise induced mTOR anabolic signaling, we studied the response to LEU in a model of unilateral plantaris work overload (WO) in CKD and control (Con) rats. WO was created by partially ablating the gastrocnemius muscle and tendon which causes significant plantaris muscle hypertrophy. After 7 days of WO the animals were gavaged with LEU or saline (S) and sacrificed 30 mins later. Western blot analysis showed that as anticipated, LEU loading did not affect AKT phosphorylation since it acts downstream of AKT. Nonetheless LEU did activate and normalize the depressed mTOR phosphorylation in CKD muscle. Also phosphorylation of $p70^{S6K}$ increased 3 fold in response to LEU in CKD ($p<0.05$), but this response was significantly less than occurred in Con muscle (5 fold increase). Furthermore LEU failed to stimulate the phosphorylation of the downstream protein rpS6 in CKD while significantly increasing rpS6 phosphorylation in Con muscle. Thus it appears that the uremic state induces resistance to LEU induced anabolic signaling in skeletal muscle undergoing hypertrophy due to sustained work overload. This differs from our findings in unexercised uremic muscle which is sensitive to leucine loading.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO248

**Transforming Growth Factor-β3 Expression and Obesity-Linked Renal Disease** David A. Maddox,[1,2,3] Karen A. Munger.[1,2,3] *[1]VA Medical Center, Sioux Falls, SD; [2]Avera Research Institute, Sioux Falls, SD; [3]Sanford School of Medicine of the University of South Dakota, Sioux Falls, SD.*

**Background:** Obesity closely associated with hypertension, Type II diabetes mellitus, and hypercholesterolemia, which are among the leading causes of end-stage kidney disease. Obese Zucker rats develop these symptoms and die of kidney disease. This is prevented by food limitation to lean animal levels. In this study we examined gene expression of TGF-β2 and TGF-β3 to gain insight into the matrix synthesis cascade in obesity-linked renal disease.

**Methods:** Obese Zucker rats six weeks of age were fed either *ad libitum* or restricted in food intake to that consumed by lean Zucker, then tested at 6, 12, and 18 weeks for each condition and compared to lean animal values. Kidneys were perfusion-fixed with RNA*Later*™, tissue RNA was extracted from kidney cortex, and single stranded cDNA prepared for the study of the two genes of interest, TGF-β2 and TGF-β3. Housekeeping genes (RpL19 and Cyclophilin A) served as reference genes.

**Results:** TGF- β3 gene expression was significantly increased in the 6, 12, and 18 week-old obese ad lib animals compared to lean. Limiting food intake at 6 weeks of age in the obese animals to that consumed by lean rats lowered TGF-β3 expression to lean animal levels by 12 weeks of age and maintained these levels at 18 weeks. Proteinuria did not increase until 12 weeks of age in the obese rats fed ad libitum and food restriction in obese animals beginning at 6 weeks of age completely prevented the development of proteinuria. The TGF- β2 isoform had no elevated expression levels in any of the animals.

**Discussion:** Gene expression of TGF- β3 in the obese rat was already elevated at 6 weeks of age, prior to the development of proteinuria. This is earlier than we previously observed for TGF-β1. Data suggest that elevations in the expression of TGF-β3 is one of the initiating events leading to kidney damage in these animals and that one of the primary benficial effects of food limitation at 6 weeks of age that leads to the prevention of kidney disease is to restore TGF-β3 expression to normal.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO249

**Systemic Depletion of $CD4^+$ T Cells Does Not Alter Susceptibility or Resistance to Development of Chronic Kidney Disease (CKD) in Mice after Reversible Unilateral Ureteral Obstruction (rUUO)** Mohammed I. Shakaib, Richard J. Quigg, Tipu S. Puri. *Medicine/Nephrology, The University of Chicago, Chicago, IL.*

Using a rUUO model, we have determined that C57BL/6 mice are susceptible to development of CKD after obstruction-mediated kidney injury while BALB/c mice are resistant. Histological studies demonstrated a significant difference between strains in the extent and resolution of tubulointerstitial inflammation during obstruction and after release of obstruction. The C57BL/6 and BALB/c strains are known to favor different subtypes of helper T-lymphocytes ($T_h$) in their immunologic responses. C57BL/6 mice favor the $T_h1$ subtype which promotes cell-mediated immune responses whereas BALB/c mice favor the $T_h2$ subtype which promotes humoral immune responses. To investigate whether differential extent or type of $T_h$ cell responses might contribute to the differences in susceptibility to development of CKD after rUUO, we used an anti-CD4 antibody (GK1.5) to systemically deplete $CD4^+$ T cells prior to and throughout the rUUO protocol. Flow cytometry confirmed complete depletion of CD4+ T lymphocytes from peripheral blood in both C57BL/6 and BALB/c mice 24 hours after intraperitoneal administration of the GK1.5 antibody that was maintained by re-administration of the antibody every 4 days. Blood urea nitrogen (BUN) levels at 14 days after release of a 6 day obstruction in C57BL/6 mice treated with the GK1.5 antibody were 78.9 ± 5.0 mg/dl as compared to 67.8 ± 6.0 mg/dl in vehicle treated mice. BALB/c mice treated with either the GK1.5 antibody or vehicle remained resistant to development of CKD with BUN levels similar to baseline pre-rUUO levels in both groups. Quantitative assays of the $T_h1/T_h2$ relevant cytokines IL-4, -5, -10, -12, and IFN-g from whole kidney protein preparations also showed no significant differences in these cytokines between strains. Cytokine analysis supported that the immunologic response to obstruction-mediated injury does not involve a $T_h1$ response in C57BL6 mice or a $T_h2$ response in BALB/c mice. Together, our results confirm that differences in $T_h$ lymphocyte responses do not contribute to susceptibility or resistance to development of CKD after rUUO.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO250

**TNF-alpha and TGF-beta 1 Exert Synergistic Effect on the Induction of EMT Via Blocking VDR Expression** Xiaoyue Tan,[1] Youhua Liu.[2] *[1]Pathology, Medical School of Nankai University, Tianjin, China; [2]Pathology, Medical Centre, University of Pittsburgh, Pittsburgh, PA.*

Our previous experiments have showed that vitamin D can abrogate both inflammation and EMT, thus alleviate the renal fibrosis. Herein, we performed the study to further explore the mechanism underlying the renal beneficial effect of vitamin D.

In vivo, Immunohistological stain and western blot were used to reveal the expression of VDR. The results showed VDR expression was largely inhibited after UUO, even from the very early stage (1st day after operation. Administration of vitamin D can protect VDR expression compared with control. Immunohistological stain revealed that VDR expression was down-regulated in the patients with chronic renal diseases; moreover, the level of inflammation seemed negative related with VDR expression.

In vitro, we treated cultured HK-2 cells with different dosage of TNF-a (1, 2, 5 and 10 ng/ml). The results suggested TNF-a can inhibit the expression of VDR. Then, we treated cultured HK-2 cells by 5ng/ml TNF-a, relative low dose of TGF-b1 (0.1ng/ml, 0.2ng/ml and 0.5ng/ml) or their combination separately. We used western blot to access the expression of VDR and EMT related protein (E-cadherin, a-SMA, Fibronetin and PAI). The results suggested that TNF-a and TGF-b has synergistic effect on the induction of EMT. Further, VDR RNAi and VDR over express plasmid were used to knock down and high regulate the expression of VDR. The results showed that knocking down VDR gene expression through siRNA could mimic the affect of TNF-a while over expression of VDR through VDR plasmid could abolish it. parallel experiments with different dosage ($10^{-9}$, $10^{-8}$, $10^{-7}$, $10^{-6}$M) of vitamin D showed [/bold]Vitamin D can rescue the VDR expression inhibited by TNF-a and also the effect of VDR knock-down on EMT.

These data demonstrate that inflammation factor TNF-a has synergistic effect on EMT induced by TGF-b1 and this effect might be mediated by blocking the expression of VDR. Our study sheds new light on the mechanism underlying the renal beneficial role of vitamin D.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO251

**The Effect of Low Protein Diet with Keto-Acid Supplement on Local RAS Activation in Rats with Subtotal Renal Ablation** Chuan-Ming Hao. *Huashan Hospital, Fudan university.*

**Objective** To investigate whether low protein plus keto-acid diet ameliorates progression of CKD by influence local RAS.**Methods** 30 male SD rats received subtotal renal ablation(Nx) and were placed on 18% normal protein diet (NPD), 6% low protein diet (LPD) or 5% low protein plus 1%keto-acid diet (LK) for 12 weeks (n=10 in each). 10 male SD sham-operated rats fed with NPD were used as control. Mesangial cells(MsC) were cultured in plasmas (10%) collected from animals with or without losartan ($10^{-7}$M) for 48hs. **Results** Nutritional indices were comparable among the four groups. The urinary protein excretion in Nx-rats markedly increased vs. control. Reducing dietary protein intake was associated with less proteinuria. Serum creatinine in Nx-rats increased significantly vs. sham group, but did not show statistical difference among Nx rats. Plasma and kidney AngII levels in NPD rats were 4.89 and 3.37 times higher vs. control. Renal AT1 mRNA and protein levels also markedly increased in NPD rats vs. sham group, but no changes of renin expression were observed. LPD diet greatly reduced renal cortical AngII, $AT_1$ and renin protein expression, accounting for only 56%, 73.7% and 61.8% of those in NPD group respectively. LK diet further decreased these parameters. In cultured MsC, NPD plasma induced higher levels of FN, LN, TGF-β and AngII in the supernatant vs. those in control group. The mRNA expression of these parameters as well as renin and AT1 receptor in cells were increased to 2.5, 1.72, 5.22, 1.57, 5.45 and 2.64 folds vs. control respectively. LPD plasma significantly decreased the production of ECM and RAS expression in MsC, while LK plasma showed stronger inhibitory effects. Application losartan to block the activity of RAS further reduced the expression of FN, LN and TGF-β in different plasma treated group, all of them were less than 1/5 vs. corresponding losartan negative groups.**Conclusion** Low

protein diet and keto-acid supplement was associated with reduced local RAS activation, which may contribute to its beneficial effect on the kidney in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO252

**TWEAK in Renal Fibrosis** Alvaro C. Ucero,[1] Alberto Benito Martin,[1] Isabel Fuentes-Calvo,[2] Beatriz Santamaria Pérez,[1] Carlos Martinez-Salgado,[2] Jose M. Lopez-Novoa,[2] Marta Ruiz-Ortega,[1] Jesus Egido,[1] Alberto Ortiz.[1] *[1]Experimental Nephrology Laboratory, Instituto de Investigación Sanitaria - FJD, Madrid, Spain; [2]Unidad de Fisiopatología Renal y Vascular, Universidad de Salamanca, Salamanca, Castilla y Leon, Spain.*

Renal fibroblasts are key players in renal fibrosis. TNF-related weak inducer of apoptosis (TWEAK) is a TNF superfamily member involved in different processes like angiogenesis, proliferation, differentiation and apoptosis. Other members of this family, TNF-α and Fas, induce apoptosis in murine renal interstitial fibroblasts. However, there is no information on the role of TWEAK in renal fibrosis.

In cultured murine renal interstitial fibroblasts addition of TWEAK increased viability at 24, 48 and 72 h. Flow cytometry of DNA content showed that this was the result of both decreased apoptosis and increased proliferation, which were dose-dependent. TWEAK also increased cyclin D1 protein expression which is necessary for the cell cycle G1/S phase transition. TWEAK promoted an early activation of extracellular-regulated kinase (ERK) pathway and pretreatment with an ERK inhibitor (PD98059) reversed both proliferation and cyclin D1 overexpression induced by TWEAK. We also studied the effect of TWEAK in mice embryonary fibroblasts (MEF) observing the same effects, but cell viability of MEFs lacking N- and H-Ras isoforms was not increased after TWEAK treatment. In addition, TWEAK also activated ERK in MEFs in a N- and H-Ras-dependent manner, suggesting the involvement of Ras/ERK pathway.

Renal interstitial fibroblasts play an active role in the recruitment of inflammatory cells into the interstitium. In TFBs TWEAK upregulates mRNA expression of inflammatory cells chemoattractants (MCP-1 and RANTES), adhesion molecules (ICAM-1) and inflammatory cytokines (IL-6 and CXCL16) in an NFκB-dependent way. This effect was inhibited by parthenolide. Immunofluorescence and EMSA showed that NFκB translocated to the nucleus and bound a DNA NFκB consensus sequence after TWEAK treatment. In conclusion, TWEAK increases fibroblast number and promotes a pro-inflammatory phenotype through the ERK and NFκB pathways. These results suggest that TWEAK may contribute to renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO253

**HSP47 Mediate ECM Accumulation Induced by TGF-beta1 in Proximal Tubular Cells** Hong-Bo Xiao, Li Xiao, Lin Sun, Yuncheng Xia, Guanghui Ling, Fu-You Liu, Rui-Hong Liu. *Department of Nephrology, The 2nd XiangYa Hospital, Kidney Institute of Central South University, ChangSha, Hunan, China.*

To demonstrate the role of Heat shock protein (HSP)47 in TGF-beta1 induced ECM accumulation in proximal tubular cells and the possible mechanism in it. HSP47 expression and ECM related protein collagen (Col) IV and fibronectin (FN) were detected by immunohistochemistry method in UUO rat models. In vitro study, HSP47, Col IV, FN and PAI-1 expressions were evaluated with the treatment of TGF-beta1 in HK-2 cells (a proximal tubular cell line). The results showed that there was obvious up-regulation expression of HSP47 both in UUO rat model and HK-2 cells with the stimulation of TGF-beta1, which was correlated with ECM protein and PAI-1 expression. However, these effects were dramaticaly reversed with pre-treatment of HSP47-siRNA. In addition, the pathway involved in HSP47 induced by TGF-beta1 was explored, HSP47 expression was also observed in HK-2 cells pretreated with ERK1/2 inhibitor(PD98059) or JNK inhibitor(SP600125) under TGF-beta1 condition. Intertestingly, the effect of TGF-beta1 on HSP47 expression was obviously inhibited with ERK1/2 or JNK inhibitor. These data suggests that HSP47 plays an important role in ECM accumulation induced by TGF-beta1 in HK-2 cells, both in ECM production and degradation. ERK1/2 and JNK pathway maybe involved in the regulation of TGF-beta1 on HSP47 production. HSP47 will be served as a vital target to prevent tubular-interstitial fibrosis.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO254

**Predictive Factors for Chronic Kidney Disease Following AKI Post Cardiac Surgery** Henrique Palomba,[1] Isac Castro,[3] Luis Yu.[2] *[1]Nephrology, University of São Paulo Medical School, São Paulo, São Paulo, Brazil; [2]Nephrology, University of São Paulo Medical School, São Paulo, São Paulo, Brazil; [3]Nephrology, University of São Paulo Medical School, São Paulo, São Paulo, Brazil.*

Introduction: Acute Kidney Injury (AKI) following cardiac surgery is strongly associated with perioperative morbidity and mortality, but its impact on long term development of renal dysfunction is uncertain.

Patients and Methods: A total of 350 patients (pts) submitted to cardiac surgery between July/2005 and July/2006 were evaluated for AKI, defined according to AKIN Stage I (serum creatinine (SCr) > 0,3 mg/dL over baseline value). Univariate and multivariate analysis were utilized for evaluation of pre, intra and post-operative parameters associated with occurrence of chronic kidney disease (CKD), defined as creatinine clearance < 60 mL/min, after 12 months of follow-up.

Results: AKI incidence was 41% (n=102). The incidence of CKD after 12 mo. of follow-up was 37% (n=130) and it was greater in patients with AKI and previous CKD (88%, n=38), compared with AKI patients without previous CKD (25%, n=15). CKD incidence was lower in non-AKI patients without previous CKD (9%, n=14). In the multivariate logistic regression model, adjusted by previous CKD, the following factors were determined as predictors of long-term renal dysfunction development: AKI (OR 4.74, 95% CI 1.75-12.79), intra-aortic balloon pump use (OR 8.91, 95% CI 1.07-81.99), age > 68 y/o (OR 4.07, 95% CI 1.52-10.91), serum bicarbonate < 24 mEq/L at ICU admission (OR 11.29, 95% CI 1.8-71.06) and pre-operative serum albumin < 3.5 mg/dL (OR 3.49, 95% CI 1.26-9.70).

Conclusions: AKI is frequent following cardiac surgery and presents an important impact on the long-term renal function outcome.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO255

**Sunitinib (SU), a VEGFR Blocker, Aggravates Glomerular but Not Interstitial Injury without Reducing Capillary Density in the 5/6 Renal Ablation (Nx) Model** Flavia G. Machado, Patricia Semedo Kuriki, Clarice K. Fujihara, Camilla Fanelli, Simone R. Costa, Claudia R. Sena, Denise M. Malheiros, Marcos Dall'oglio, Niels Olsen Saraiva Camara, Roberto Zatz. *Univ of Sao Paulo, Brazil.*

The role of angiogenesis (ANG) in CKD is unclear. New capillaries may be needed to oppose hypoxia and mitigate capillary rarefaction (CR). Conversely, ANG may worsen injury by favoring influx of inflammatory cells. VEGF, a major growth factor in ANG, has also autocrine/paracrine effects on podocytes and endothelial cells. We investigated the effect of SU on CKD. One day after ablation, Nx rats were assigned to Groups Nx+V (vehicle) or Nx+SU (SU, 4 mg/Kg/d). One week later, serum creatinine ($S_{Cr}$), glomerulosclerosis index (GSI), % cortical interstitium (%INT), % glomerular endothelial area (%GE), interstitial capillary density (ICD) and glomerular volume ($V_G$) were measured in 7 NX+V and 7 Nx+SU rats. The remaining 19 rats were followed until 45 days after Nx. Twelve sham-operated rats given vehicle (S+V) or SU (S+SU) were also studied at this time.

| | $S_{Cr}$ | GSI | %INT | %EG | ICD | $V_G$ | Hct |
|---|---|---|---|---|---|---|---|
| $Nx+V_{7d}$ | 1.2±0.1[b] | 0.2±0.2 | 0.3±0.1[b] | 88±1 | 621±17 | 0.7±0.1 | 47±1 |
| $Nx+SU_{7d}$ | 1.2±0.1[b] | 1.5±0.6[ab] | 0.2±0.1 | 87±1 | 592±21 | 0.6±0.1[b] | 48±1 |
| $Nx+V_{45d}$ | 1.1±0.1[b] | 19.9±5.9 | 2.3±0.4[bc] | 77±3[c] | 359±40[bc] | 1.3±0.1[bc] | 45±1 |
| $Nx+SU_{45d}$ | 1.3±0.1[b] | 57.7±13.2[abc] | 2.2±0.4[bc] | 72±4[c] | 288±33[bc] | 1.1±0.1[abc] | 41±1[abc] |
| $S+V_{45d}$ | 0.6±0.1 | 0.1±0.1 | 0.1±0.1 | 87±1 | 595±49 | 0.7±0.1 | 49±1 |
| $S+SU_{45d}$ | 0.6±0.1 | 0.1±0.1 | 0.1±0.1 | 82±2 | 531±78 | 0.8±0.1 | 49±1 |

Mean±SE, [a]p<0.05 vs. respective untreated, [b]p<0.05 vs. S receiving same treatment, [c]p<0.05 vs. 7 days.

SU had no effect on %EG, ICD or any other parameter in S. At day 7, no CR was seen in Nx. SU had no effect on %EG or ICD in Nx, but worsened GSI. On day 45, CR was evident in Nx. SU caused anemia, a numerical decrease in ICD and a marked increase in GSI vs. Nx+V. Renal injury seems unrelated to impaired ANG. Rather, these preliminary data suggest that VEGF may be necessary for glomerular cell viability in the context of tuft hypertrophy such as observed in Nx.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO256

**Renin Angiotensin Aldosterone Blockade (RAS) Reverses Experimental Cyclosporine Nephropathy** Fernanda Cristina Mazali,[1] Jose B. Lopes de Faria,[1] Richard J. Johnson,[2] Marilda Mazzali.[1] *[1]Division of Nephrology, State University of Campinas, Campinas, SP, Brazil; [2]Division of Renal Diseases and Hypertension, University of Colorado, Denver, CO.*

Chronic cyclosporine nephropathy (CsAN) is associated with renin-angiotensin system activation that aggravates arteriolar vasoconstriction and progressive fibrosis. Aim: Analyze the effect of RAS blockade in the established CsAN model. Methods: Male SD rats, 200 to 250g, received daily injections of cyclosporine (15 mg/kg SC) in presence of low salt diet. After 5 weeks, control group [CsA5] was sacrificed. Remaining animals were divided in 5 groups: CsA withdrawal [CsAWT], CSA for 9 weeks [CsALOS]with losartan 12,5mg/dL[CsA9LOS], enalapril 10 mg/dL[CsA9ENL] or espironolactone 20 mg/dL[CsA9ESP] in drinking water. At sacrifice, renal function and morphology were analyzed. Results: Control animals [CsA5] developed CsAN, with tubular atrophy and stripped interstitial fibrosis, comparable to CSAWT (p=ns). In CsA9 group, CsAN was progressive and more severe (p<0.05 vs CsA5).In treatment groups [CsA9LOS, CsA9ENL and CsAE9SP],despite CsA therapy, RAS blockade was associated with recovery of renal function and reduction of arteriolar and interstitial lesions, with lower interstitial inflammation and fibrosis, independently of oxidative stress regulation.

| | CsA5 | CsAWT | CsA9 | CsAENL | CsALOS | CsAESP |
|---|---|---|---|---|---|---|
| creatinine (mg/dl) | 0.6±0.1 | 0.6±0.1 b | 1.1±0.2 a | 0.6 ±0.2b | 0.6±0.1 a,b | 0.5 ±0.05 b |
| arteriolar hyalinosis % | 74.3±1.3 | 72.1±1.0 b | 77.7±2.7a | 54.9±3.5a,b,c | 48.2±2.7a,b,c | 45.7±3.7 a,b,c |
| interstitial fibrosis % | 9.2±0.7 | 9.2±0.7 b | 11.2±0.5a | 6.0±1.5b,c | 5.2±0.4a,b,c | 5.1±0.3 a,b,c |
| Macrophages (cel/mm3) | 18.6±2.2 | 17.4±1.4 b | 24.4±2.1 a | 18.3±2.5 b | 17.2±1.3a,b | 16.45±2.0b |
| 8-OHdG (cel/mm3) | 96.3±13.2 | 55.2±13 | 130.3±22.5 a,c | 89.78±8 b,c | 100.0±6.7 b,c | 89.7±9.3 b,c |

a-p<0,05 vs CSA 5, b-p<0,05 vs CSA9, c p<0.05 vs.CSAWT

Conclusion: RAS blockade improved renal function and reduced interstitial and vascular lesions in the established CsAN model, suggesting that angiotensin II and aldosterone have a negative impact in the progression of chronic CsAN.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO257**

**Correction Factor for e-GFR Calculated with Schwartz Formula To Match with MDRD and CDK – EPE in Adolescents** Licia Peruzzi, Roberta Camilla, Caterina Grillo, Giovanni Conti, Giovanni Montini, Teresa Papalia, A. Amore, Guglielmo Bracco, Rosanna Coppo. *For the Study Group Adult and Child of the Italian Society of Nephrology.*

Estimated glomerular filtration rate (e-GFR) in children and adults is calculated with Schwartz, MDRD and CKD-EPI formulas with frequent discrepancies. Enzymatic creatinine (isotope dilution traceable international standard (IDMS) calibration) does not solve the problem. We aimed at comparing e-GFR obtained by Schwartz (k of 0.413), MDRD and CKD-EPI in young Caucasian subjects, aged 15-19 y. We analyzed 644 subjects 354 males (M) and 290 females (F); e-GFR was calculated with the 3 formulas.

The 3 formulas correlate (r2= 0.77; p<0.0001): MDRD and CKD-EPI give higher results than Schwartz (M +31±18% and +37±16% respectively; F: +13%±10 and +21±11%). To overcome discrepancies we calculated with a linear mathematic model new K for Schwartz in subjects aged 15-19 to match with e-GFR obtained with MDRD and CKD-EPI.

| | a) e-GFR-Schwartz(k=0.413) | b) e-GFR-MDRD | c) e-GFR-CKD-EPI | Wilcoxon test a vs b vs c vs a |
|---|---|---|---|---|
| M Median (IQR) | 75.9 (53.6-90.1) | 104.5 (74.7-131.0) | 117.0 (81.9-140.6) | P<0.0001 |
| F Median (IQR) | 81.4 (68.8-98.0) | 94.0 (76.5-115.7) | 108.2 (87.4-129.6) | P<0.0001 |
| | | | | |
| | | | | |
| K*L/Scr | k to match with MDRD | k to match with CKD-EPI | Mean k to match either with MDRD or CKD-EPI | |
| M Bland Altman | 0.58 ± 0.08 | 0.62 ± 0.09 | 0.60 ± 0.08 | |
| F Bland Altman | 0.47 ± 0.04 | 0.52 ± 0.09 | 0.50 ± 0.05 | |

In M the conversion factor to compare e-GFR-Schwartz$_{(k=0.413)}$ to MDRD was 1.415 and to CKD-EPI was 1.506; in F 1.146 to MDRD and 1.268 to CKD-EPI. Mean conversion factors of 1.46 for M and 1.207 for F can be adopted to convert e-GFR-Schwartz$_{(k=0.413)}$ to both formulas, with analogous accuracy.

We showed a significant difference in e-GFR obtained by different formulas: to allow comparison of data in adolescents from 15 y we propose the adoption of a new k for Schwartz formula of 0.6 in M and 0.5 in F and the conversion factor of 1,506 in M and 1.207 in F for e-GFR-Schwartz to be matched to MDRD or CKD-EPI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO258**

**Changes in the TRPC6 Expression in Podocytes and Effect of Calcinurin-Inhibitor on the Glomerular Damages in 5/6 Nephrectomized Rats** Hajime Hasegawa, Takatsugu Iwashita, Kaori Takayanagi, Taisuke Shimizu, Yosuke Tayama, Juko Asakura. *Nephrol & Hypertens, Saitama Med Center, Saitama Med Univ, Kawagoe, Saitama, Japan.*

Glomerular hyperfiltration (GHF) is a major factor leading to podocyte injury and subsequent glomerular sclerosis. Recent works reveal that TRPC6 may function as a mechanosensor for intra-glomerular pressure load, and calcinurin-NFAT cascade may involve in the TRPC6 signaling. In this work, changes in the TRPC6 expression in the glomerular hyperfiltration and the possible effect of calcinurin-inhibitor on the TRPC6-related glomerular damages.

5/6 nephrectomy (Nx) or sham operation (S) was applied to male SD rats who were sacrificed 2 weeks after the operation. Tacrolimus (Tac; 0.08 mg/kg, s.c.) was administered to some rats in Nx group until sacrifice (Nx+Tac). Gene expressions were studied by real-time PCR in the isolated glomerulus by magnetic beads method. Glomerular damages and expressions were studied by histology and immunohistochemistry. Existence of TRPC6 in podocytes was confirmed by double immunostaining with WT-1 or synaptopodin.

In results, Nx showed an increase in the glomerular volume, urine protein excretion and desmin expression score which were partially recovered by Tac (glomerular vol: 1449±30 in S, 2257±55 in Nx, 1966±45 in Nx+Tac, urine protein: 215.4±67.3 in S, 619.4±412.4 in Nx, 106.1±17.6 mg/mgCr in Nx+Tac, desmin score: 0.98±0.05 in S, 1.92±0.06 in Nx, 1.75±0.07 in Nx+Tac). Gene expression of TRPC6 was up-regulated in Nx group and partially recovered by Tac (108.5±30.7 in S, 146.3±22.6 in Nx, 134.7±23.3% in Nx+Tac), although expression of NFATc3 was not significantly changed. Immunostaining studies demonstrated the increased TRPC6 expression in podocytes in Nx group, which was partially recovered by Tac.

Present study might suggest that the TRPC6 was up-regulated by glomerular pressure load, indicating that the TRPC6 might be involved in the development of hyperfiltration-related glomerular damages. Inhibition of calcinurin might provide clinical advantages in the hyperfiltration-associated glomerular damages.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO259**

**Treatment with an Antioxidant Inflammation Modulator (AIM) Increases Glomerular Filtration Rate Monitored by Inulin Clearance in Rats** Christian Wigley,[1] Ron Bumeister,[1] Rhesa D. Stidham,[1] Yanfeng Ding,[2] Marc L. Sprouse,[2] Colin Meyer,[1] Deborah A. Ferguson,[1] Rong Ma.[2] *[1]Reata Pharmaceuticals, Inc., Irving, TX; [2]UNT Health Sciences Center, Ft. Worth, TX.*

Chronic hyperglycemia and activation of the renin-angiotensin system result in production of reactive oxygen species, which stimulate pro-inflammatory pathways and lead to the development of chronic kidney disease (CKD). Activation of the Keap1-Nrf2 pathway suppresses oxidative stress and inhibits inflammatory signaling in various tissues. Bardoxolone methyl (BARD), the lead AIM, potently induces the activity of Nrf2, a transcription factor that regulates expression of many antioxidant and detoxification genes. AIMs exhibit significant protective effects in models of renal injury and, in a Phase 2 clinical study in CKD patients, BARD significantly improved several parameters of renal function including estimated glomerular filtration rate (eGFR). To test whether the increased eGFR observed in patients upon treatment with BARD reflects a *bona fide* increase in GFR, inulin clearance was monitored to directly measure GFR in rats treated with RTA 405, a BARD analog. To explore the underlying mechanism, the effect of AIMs on Angiotensin II (Ang II)-induced contraction of cultured mesangial cells and isolated rat glomeruli and on Nrf2 and NF-κB activity in cultured mesangial cells were evaluated. Consistent with the clinical trial results, RTA 405 inhibited Ang II-induced decrease in GFR and significantly increased the renal filtration fraction relative to controls. BARD induced expression of various Nrf2 target genes and substantially inhibited NF-κB signaling. A statistically significant inhibitory effect on Ang II-induced mesangial cell contraction was also observed. In addition, the Ang II-induced reduction in total glomerular volume was significantly attenuated. Results from this study demonstrate that AIMs inhibit reduction in GFR and also provide support for the evaluation of BARD in CKD, in which RAS activation, oxidative stress, and inflammation contribute to the pathology. A 12-month Phase 2b pivotal study in CKD patients is currently underway.

Disclosure of Financial Relationships: Employer: Reata Pharmaceuticals, Inc.; Ownership: Reata Pharmaceuticals, Inc.

**TH-PO260**

**Indoxyl Sulfate Stimulates Reactive Oxygen Species Leakage from Rat Kidney Mitochondria and AST-120 Inhibits This Process in Experimental Acute Kidney Injury** Shigeru Owada,[#1] Sumie Goto,[#2] Shunsuke Ito,[#2] Hideyuki Yamato,[#2] Fuyuhiko Nishijima,[#2] Aki Hirayama.[#3] *[1]Dialysis Center, Asao Clinic, Kawasaki, Kanagawa, Japan; [2]Biomedical Research Laboratories, Kureha Co., Ltd., Tokyo, Japan; [3]Internal Medicine, Tsukuba University of Technology, Tsukuba, Ibaragi, Japan.*

Aim: Free radicals both initiate and contribute to the progress of AKI but there is no direct evidence of free radical production in the injured cells. We have succeeded to identifying free radical leakage (O2- and OH radicals; ROS) from mitochondria (Mito) directly using an EPR spin trapping technique. To clarify the role of Mito stress on development of AKI, we have investigated leakage of ROS from Mito and immunostaining of survivin in the kidney using AKI model rats.

Methods: Ischemic AKI was induced in 29 SD male rats and divided into the following two groups; AKI control ( C ) and AST-120 ( A ). Sham operated rats were used as a normal group (N; n=28). 1-2 days after induction, kidneys were isolated and Mito fractions were prepared by fractional centrifugation. Serum and intra cellular IS levels were measured. Free radicals were detected by the EPR spin trapping method using CYPMPO as a spin trapping agent. ROS adducts were recorded every 1 min for 20 min. Peak height (PH) of ROS were accumulated and expressed as percentage compared to values of sham rats. SOD activities (U/mg protein) in the Mito and survivin expression in the kidney were measured. Results: Levels of serum Cr and IS were significantly lower in the A group compared to the C group. ROS leakage rates (%) were significantly higher in the C group and lower in the A group (N=100±13, C=112±18, A=95±16). Mito SOD activities were lower in the C group (N=9.7±2.4, C=3.1±0.6, A=6.0±28). Intracellular IS levels (n mol/mg protein) and survivin area (%) were higher in the C group (IS;N= 16.2±2.0, C=36.8±6.8,A=12.0±9.3, Survivin; N=5.45±1.17, C=6.44±1.48, A=5.03±0.49). There were significant correlations found between ROS leakage, intracellular IS levels and survivin.

Conclusions: AST-120 protected renal function in Ischemic AKI. It is speculated that one mechanisms of this effect of AST-120 was based on inhibition of Mito ROS leakage and apoptosis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO261**

**Distinct Roles of mPGES-1 in Mediating Renal Injury and Stimulating Renal Erythropoietin Expression in the 5/6 Nephrectomy Mouse Model** Zhanjun Jia,[1,2] Haiping Wang,[1,2] Tianxin Yang.[1,2] *[1]Internal Medicine, University of Utah, Salt Lake City, UT; [2]Veteran Affairs Medical Center, Salt Lake City, UT.*

COX-2 activity has been shown to contribute to the pathogenesis of chronic kidney disease with a poorly characterized mechanism. In present study, we investigated the role of microsomal prostaglandin E synthase-1 (mPGES-1), in the progression of chronic renal failure in a mouse model of 5/6 nephrectomy (5/6NX). After 4 weeks of 5/6NX, wild-type mice exhibited increases in plasma BUN, Cr, and blood phosphorus, all of which were attenuated in mPGES-1 KO mice (BUN: 41.86 ± 2.8 vs. 55.2 ± 4.8 mg/dl;

Cr: 0.485±0.017 vs. 0.61±0.02 mg/dl; blood phosphorus: 7.27 ± 0.4 vs. 9.2 ± 0.88 mg/dl, p<0.05, respectively). 5/6NX induced a 2.6-fold increase in urinary albumin excretion in WT mice, which was completely abolished by mPGES-1 deletion. Urine concentrating defect was less in 5/6NX KO mice than in 5/6NX WT mice (urine volume: 1.9 ± 0.23 vs. 2.8 ± 0.4 ml/24 h, p<0.05; water intake: 5.26 ± 0.41 vs. 7.01 ± 0.83 ml/24 h, p<0.05; urine osmolality: 1289 ± 76.2 vs. 1021.1 ± 51.6 mOsm/kg $H_2O$, p<0.05). In the remnant kidney, COX-2 and mPGES-1 mRNAs elevated 2.76-fold and 2.73-fold, respectively, contrasting to unchanged mRNA expression of COX-1. Despite the significant reduction of kidney mass, 24-h urinary PGE2 excretion in 5/6NX WT mice was comparable to that in sham animals (940.95 ± 146.5 vs. 855.5 ± 317.9 pg/24 h, p>0.05), evidence of increased PGE2 production in the remnant kidney. Paradoxically, 5/6NX induced more severe anemia in mPGES-1 KO mice than WT controls as assessed by hematocrit and splenomegaly (Hct: 40.1% vs. 46.3%, p<0.05; spleen weight: 159.3 ± 30.8 vs. 103.1 ± 8.4 mg, p<0.05). Erythropoietin (EPO) mRNA in the remnant kidney of WT mice elevated 19-fold, which was reduced by 50% the KO group. Remnant kidney TNF-α protein increase by 85% in the 5/6NX WT mice, which was entirely abolished in KO mice. We conclude that: (1) mPGES-1 deletion ameliorates chronic renal failure in the mouse model of 5/6 nephrectomy, and (2) mPGES-1 deletion paradoxically exacerbates anemia in this model likely via suppression of EPO.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO262**

**Bone Morphogenetic Protein 2 Gene Polymorphisms Contribute to the Development of Childhood IgA Nephropathy (IgAN)** Jin-Soon Suh, Won-Ho Hahn, Byoung-Soo Cho. *Department of Pediatrics, Kyung Hee University Hospital, Seoul, Korea.*

**Background :** Bone morphogenetic proteins (BMPs) are multi-functional growth factors belonging to the TGF-ß superfamily and have been shown to be important in both preservation of kidney function and resistance to injury. BMP2 is highly regulated in the kidney and high affinity binding sites for BMP2 has been identified in kidney epithelial cells. And it has been demonstrated to play various roles in the pathogenesis of renal diseases. However, the role of BMP2 gene in glomerulonephritis is not investigated yet. We aimed to evaluate the association of BMP2 polymorphisms with immunoglobulin A nephropathy (IgAN) in Korean children.

**Methods :** We evaluated 187 pediatric patients with biopsy-proven IgAN and 262 healthy controls. Two single nucleotide polymorphisms in the coding region of BMP2 gene (rs235768 [missense, Arg190Ser] and rs1049007 [synonymous, Ser87Ser]) were selected and genotyped by direct sequencing methods.

**Results :** The genotypes of rs235768 ($p$= 0.0023, OR (95% CI) = 6.39 (1.46-28.03) in the recessive model) and rs1049007 ($p$= 0.0002, OR (95% CI) = 13.67 (1.81-103.42) in the recessive model) were associated with childhood IgAN. And in haplotype analysis, haplotype TG ($p$= 0.011, OR (95% CI) = 6.76 (1.55-29.50) in the dominant model) and haplotype AA ($p$= 0.0137, OR (95% CI)= 0.08 (0.01-0.59) in the recessive model) showed association with IgAN.

**Conclusion :** Polymorphisms in BMP2 gene may contribute the susceptibility to IgAN in Korean children.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO263**

**Aldosterone Receptor Blocker Attenuates Glomerulosclerosis but Not Proteinuria** Nobuaki Takagi, Ji Ma, Valentina Kon, Iekuni Ichikawa. *Division of Pediatric Nephrology, Vanderbilt University Medical Center, Nashville, TN.*

We previously showed that inhibition of angiotensin type 1 receptor (AT1) attenuates podocyte damage and glomerulosclerosis in the NEP25 transgenic mouse model of podocyte-specific injury-induced glomerulosclerosis. However, our studies in NEP25 mice genetically deficient in podocyte AT1 revealed that the protective effect of AT1 blocker (ARB) is not through the podocyte AT1. Therefore, we now test the possibility that the protective effect of ARB on podocytes is systemic, and involves mineralocorticoid inhibition. For this purpose, we compared the ability of ARB, mineralocorticoid receptor blocker (MRB) or both to protect against podocyte injury and subsequent glomerulosclerosis.

NEP25 mice treated with the MRB, spironolactone (25 mg/kg/day, N=10), the ARB, losartan (250 mg/kg/day, N=11), combination of the two (Comb, N=8) or vehicle (Veh, N=9) in drinking water starting from day -7 until sacrifice at day 9. LMB2 was injected to induce podocyte injury at day 0. Proteinuria and systolic blood pressure were followed, and glomerular sclerosis and podocyte population were assessed at sacrifice.

Although MRB treatment did not reduce systolic blood pressure or proteinuria, it significantly decreased collagen type IV deposition and preserved WT-1-positive podocytes compared with glomeruli of untreated controls (Collagen IV: MRB 29.9±1.7% *vs.* Veh 38.9±1.2%; WT-1: MRB 10.2±0.4 *vs.* Veh 8.4±0.3 cells per glomerulus; both $P$<0.05). These benefits were comparable to those observed in ARB-treated mice (33.1±1.6% for Collagen IV, and 10.7±0.4 cells per glomerulus for WT-1). Further, addition of non-BP lowering MRB to the already large dose of ARB significantly attenuated sclerosis (glomerular sclerosis index: Comb 1.67±0.19 *vs.* ARB 2.35±0.19, $P$<0.05) and deposition of collagen type IV (Comb 23.7±1.4% *vs.* ARB 33.1±1.6%, $P$<0.05).

Taken together these data suggest that, mineralocorticoid receptor participates in the later stages of glomerulosclerosis but not in the early events of podocyte injury manifest as proteinuria, an effect that is independent of systemic blood pressure.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO264**

**Renal Arginase (A) Activity in Kidney Cortex (KC) of Rats with Chronic Kidney Disease (CKD)** Sergey Zharikov,[1] Gin-Fu Chen,[1] Natasha C. Moningka,[1] Sidney M. Morris, Jr.,[3] Christine Baylis.[1,2] *[1]Physiol., University of Florida; [2]Med., University of Florida, Gainesville, FL; [3]Biochem., University of Pittsburgh, Pittsburgh, PA.*

Kidney is the major site of synthesis of circulating arginine (Arg) but also contains A (A-II), which consumes some Arg. Moradi et al (2006) report increased urea inhibits A (in liver), which may limit the importance of A as a competitor for Arg, in CKD. In this study we investigated the impact of CKD on KC urea content, A abundance and activity using puromycin aminonucleoside (PAN; Pcreat = 1.09±0.24, sham = 0.22±0.01mg/dl) and 5/6 ablation/infarction of renal mass (5/6AI; Pcreat =0.62±0.07) CKD models. KC urea content was > 50% above sham control values after both 11 weeks PAN and 4-5 weeks 5/6AI. In PAN vs sham there was no difference in abundance of A-II but A activity (using physiologic Arg = 1.8mM and measuring urea accumulation) was low, ~30% of sham. In contrast, in 5/6AI, A-II abundance was low, ~ 40% of sham, but A activity was ~25% higher. We investigated the effect of different concentrations of exogenous urea on arginase activity in normal KC homogenates (1.8mM Arg; measuring ornithine production). Baseline KC urea concentration was 37±3mM and A activity was 2.50±0.01 (µmoles of ornithine/mg/h); with addition of exogenous urea inhibition of A activity occurred to 2.35±0.03 + 10mM, 2.30±0.08 + 25 mM, 2.20±0.04 +50 mM and 2.10±0.02 + 100mM. Of note, the inhibitory effect of urea was mild and declined with increasing concentration. Kinetic analysis of A activity in control KC (± 50mM urea) showed that urea-induced inhibition is due to a decrease in A affinity for Arg. Although baseline A activity was much lower in PAN rats (baseline urea 58±6mM; A activity 0.73±0.01), addition of urea led to increased A activity, to 0.74±0.03 + 10mM, 0.83±0.02 + 25 mM, 0.91±0.00 +50 mM and 0.97±0.08 + 100mM. Thus, the A activity in KC is not predictably related to KC urea content in CKD but varies with injury/model. Further, there are variable post-translational modifications of A having profound effects on activity in different models. Therefore, A activity may contribute to local Arg (and hence NO) deficiency in some types/stages of CKD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO265**

**Deletion of Scavenger Receptor A I/II Protects Kidney from a High Fat Diet-Induced Superoxide Production, Macrophage Infiltration and Tubulointerstitial Fibrosis** Zhaoyong Hu,[1] Wenjian Wang,[2] William E. Mitch.[1] *[1]Internal Medicine, Baylor College of Medicine, Houston, TX; [2]Internal Medicine, Guangdong General Hospital, Guangzhou, Guangdong, China.*

Tubulointerstitial fibrosis is common in kidney diseases, the degree of fibrosis pathologically is associated with the severity of progressive renal insufficiency. In unilaterally nephrectomized mice fed a high fat died (HFD) for 4 months, we found there was extensive deposition of lipids in tubular cells and cells of the renal interstitium compare to unilateral nephrectomy fed with normal chow. The deposition of lipids in tubular cells was accompanied by progressive tubulointerstitial fibrosis, including increased deposition of fibronectin plus collagens I & III. Because the scavenger receptor AI/II (SRAI/II) is known to mediate lipid uptake into tubular cells, we hypothesized that these receptors are involved in the production of tubulointerstital fibrosis. To address this possibility, we studied mice with global KO of the SRAI/II. Despite the stimulus of hight fat diet, there was a substantial decrease in lipids accumulation in kidney cells. SRAI/II KO also inhibited fibrosis and the deposition of fibronectin and collagens I & III. Using DHE staining and measurements of kidney MDA production, we found that SRAI/II KO caused a dramatic decrease in superoxide production. Moreover, both resident (CD68+) and bone marroe derived (CD68+/CD11c+) macrophages were decreased in kidney of SRAI/II KO mice fed the HFD. In the cultured HK-2 cells, silencing SRAI/II (siRNA interference technique) causes an inhibition of ox-LDL uptake and reduced TGF-beta1 expression and the phosporylation of Smad2/3, providing a mechanism for the suppression of fibrosis. The mechanism underlying the decrease in macrophage infiltration into the tubulointerstitium involved suppression of MCP-1 expression. We conclude that tubulointerstial fibrosis evolves from HFD to uptake ox-LDL by tubular cells leading to stimulation of superoxide formation. This results in increased production of MCP-1, macrophages infiltraion and stimulation of TGF-beta1/Smad signaling. These responses are blocked by inactivating the SRAI/II in tubular cells.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO266**

**Simvastatin Ameliorates Renal Ephithelial-Mesenchymal Transition by Modulating Uterine Sensitization-Associated Gene-1 (USAG-1)** Yoshifumi Hamasaki, Kent Doi, Koji Okamoto, Eisei Noiri, Toshiro Fujita. *Department of Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan.*

[Background & Purpose] BMP-7 suppresses renal fibrosis by counteracting with TGF-β-induced epithelial-mesenchymal transition (EMT). Uterine sensitization-associated gene-1 (USAG-1), the dominant BMP-7 antagonist expressing in distal tubular epithelial cells, promotes renal fibrosis. Although a number of clinical studies have reported the protective effect of statins on chronic kidney disease progression, the mechanism is not fully revealed. In this study, we investigated whether simvastatin (SIM) attenuates renal fibrosis by modulating USAG-1 mediated pathway.

[Method & Results] C57/BL6 mice fed by adenine-containing diet for 4 weeks showed significant increase of BUN accompanied with severe tubulointerstitial fibrosis. SIM treatment (50mg/kg/day) for two weeks significantly reduced BUN compared with

the control group. In pathological analysis, the area of fibrosis evaluated by Masson's trichrome staining and the expression of α-smooth muscle actin were significantly attenuated by SIM. On quantitative PCR, USGA-1 expression was significantly reduced in the SIM group without affecting BMP-7 expression. Immunoblotting analysis showed that SIM increased pSmad1/5/8 expression.

In vitro experiments, MDCK cells incubated with TGF-β (5ng/ml) for 72 hours showed increased expression of USAG-1, but SIM significantly reduced USAG-1 expression without affecting BMP-7 expression.

It has been reported that USAG-1 gene expression is regulated by a transcriptional factor HOXA13 in organ development. In MDCK, suppression of USAG-1 by SIM was not observed when HOXA13 gene was knocked down by RNA interference.

[Conclusion] Our data showed that SIM ameliorated EMT by suppressing USAG-1 expression, not up-regulating BMP-7 expression. Decreased USAG-1 levels may enhance the effect of BMP-7 relatively. We also demonstrated that a new mechanism of action of statins that regulates USAG-1 by modulating HOXA13.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO267

**Clinical Causes of Acute Kidney Injury and Outcome of ICU Patients** Helmut Schiffl. *Medizinische Klinik Innenstadt, University of Munich, Munich, Germany.*

Background: Acute kidney injury (AKI) secondary to acute tubular necrosis (ATN) is common in critically ill patients. The underlying pathophysiology of ICU AKI can be divided into pure ischemic, pure nephrotoxic, and mixed causes. This posthoc analysis of a prospective single-cohort 7 year study aimed to test the hypothesis whether the cause (pure versus mixed) of renal insults resulting in AKI affects the outcome of survivors of AKI.

Methods: A total of 425 critically ill patients with AKI secondary to clinically diagnosed ATN were divided in three groups according to the cause of ATN. Of these patients, 215 had mixed ATN, 203 had pure ischemic ATN, and 7 had pure nephrotoxic ATN. All patients had one episode of AKI. No patient had pre-insult chronic kidney disease. Patients were followed throughout their hospital stay (mortality rate, recovery of renal function at discharge) and up to 7 years after initiation of RRT.

Results: The three patient groups differed in their demographic and renal characteristics at iniation of RRT. The in-hospital mortality rate( 47 % for the cohort ) was 55% for the mixed ATN group, 39% for the pure ischemic group, and 29% for the pure nephrotoxic group. Complete renal recovery at discharge was documented in all 5 surviving patients with pure nephrotoxic ATN and in 92 out of 124 survivors with pure ischemic ATN, but only in 29 out of 97 surviving patients with mixed ATN. All patients discharged alive from the hospital completed the 7-year follow-up. At the end of the observation period, 60% of the survivors of pure ATN, compared with 22% of the survivors of mixed ATN, were alive. At 7 years, 6% of the living patients with pure ATN had early stage chronic kidney disease, but 38% of the mixed group patients had severely decreased eGFR or end-stage renal disease.

Conclusions: The cause of ATN has an impact on short- as well as long-term outcomes. The challenge for intensivists is to prevent further insults to the acutely injured kidneys.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO268

**Periostin: Novel Tissue and Urinary Biomarker of Progressive Renal Injury Induces a Coordinated Mesenchymal Phenotype in Tubular Cells** Bancha Satirapoj,[1,4] Ying Wang,[1] Cynthia C. Nast,[1,2] Mina Patel-Chamberlin,[1] Janine A. La Page,[1] Hans-Henrik Parving, Tiane Dai,[1] Xiwei Wu,[3] Rama Natarajan,[3] Sharon G. Adler,[1] Denis Ouimet.[5] *[1]Harbor-UCLA Los Angeles Biomedical Research Institute; [2]Cedars-Sinai Medical Center; [3]City of Hope National Medical Center, CA; [4]Phramongkutklao Hospital, Bangkok, Thailand; [5]Service de Nephrologie, Hopital Maisonneuve-Rosemont, Montreal, QC, Canada.*

Periostin is involved in cell survival, differentiation and migration, but data concerning periostin expression in kidney injury is scanty. Periostin was identified by microarray and confirmed by real-time PCR in renal tissue after 5/6 nephrectomy (5/6Nx), and streptozotocin-induced diabetes demonstrating generalizability of the periostin increment in renal injury. Periostin was expressed predominantly in distal nephron tubular cells and in tubule cells shed into the lumen. In affected distal nephron tubule cells after 5/6Nx, periostin expression appeared de novo, the epithelial cell adhesion molecule E-cadherin became undetectable, and tubule cells displayed the mesenchymal marker proteins fibroblast specific protein-1 (FSP1) and matrix metalloproteinase-9 (MMP9). To assess whether periostin plays a direct role in mediating the loss of the tubular differentiation marker E-cadherin and the gain of mesenchymal markers, we overexpressed periostin in cultured distal convoluted tubule cells. Tubules overexpressing periostin dramatically increased MMP9 and FSP1 protein, and decreased E-cadherin protein expression. Urine periostin excretion increased over time after 5/6Nx, and it was also excreted in the urine of CKD patients. Urine periostin ELISA at a cutoff value of 32.66 pg/mg creatinine demonstrated sensitivity and specificity for distinguishing patients with proteinuric and non-proteinuric CKD from healthy people (92.3%, and 95.0%, respectively). These values were similar to urine NGAL ELISA by ROC analysis. These data demonstrate that: 1) Periostin is a mediator and marker of mesenchymal phenotype; 2) Tubule cells that are shed into the tubular lumen express periostin-associated mesenchymal phenotype; and 3) Periostin is a promising tissue and urine biomarker for kidney injury in experimental models and in clinical renal disease.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO269

**The Balance of Beneficial and Deleterious Effects of Hypoxia-Inducible Factor Activation by Hydroxylase Inhibitor in Rat Remnant Kidney Depends on the Timing of the Stimulus** Xiaofang Yu, Xiaoqiang Ding, Yi Fang, Jiaming Zhu, Xialian Xu, Suhua Jiang. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

**Background.** The purpose of the present study was to characterize HIF expression during the course CKD development, and to investigate the effects of HIF activation on CKD by using prolyl hydroxylase (PHD) inhibitor L-mimosine (L-Min) at different stages of CKD. **Methods.** Rats with remnant kidneys (RK) were sacrificed at wk 1, 2, 4, 6, 8, 12 after subtotal nephrectomy. Then additional groups of RK rats were treated with L-mimosine at different stages of RK basing on the expression pattern of HIF-a . **Results.** The nuclear expression of HIF-1α and -2α, as well as typical HIF target genes VEGF, HO-1, GLUT-1 and EPO, were all up-regulated in the early stage of RK when renal function was stable, and returned to the basal level later, accompanied by impaired renal function and interstitial fibrosis. RK rats were then divided into early treatment group (L-Min administration at week2-12), advanced treatment group (L-Min administration at week4-12) and end stage treatment group (L-Min administration at week8-12). Early L-Min treatment in RK aggravated renal function, tubulointerstitial fibrosis and peritubular capillary loss. Advanced L-Min treatment improved renal function, decreased renal fibrosis and protect peritubular capillary networks. End stage L-Min treatment had no influence on these parameters. Furthermore, compared with the control group, nuclear accumulation of HIF-1a as well as CTGF in the early treatment group was increased soon after the administration and kept increasing during the treatment, while expression of HIF-2a and VEGF were only increased a little in the last 4 weeks; in the advanced administration group, only HIF-2a and VEGF expression kept increasing during the continued L-Min treatment.

**Conclusions.** There was a transient HIF-a activation in the remnant kidney of rats at the early stage following subtotal nephrectomy. HIF-a activation by PHD inhibitor L-Min has dual roles in the development of RK, depending on the timing of administration and, differential activation of HIF-a isoforms and their target genes might be the course.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO270

**The Anti-Oxidant Effect of Paricalcitol Improves Chronic Cyclosporine A Nephropathy** ShangGuo Piao,[1] Joonchang Song,[1] Ji-Hyun Song,[1] Jungyeon Ghee,[1] Sol Kim,[2] Chul Woo Yang.[1] *[1]Department of Internal Medicine, The Catholic University of Korea, Seoul, Republic of Korea; [2]Department of Bioengineering, University of Pennsylvania, Philadelphia.*

Oxidative stress is one of main mechanisms in cyclosporine(CsA)-induced renal injury. Vitamin D has anti-oxidative effect on chronic kidney disease patients. We evaluated whether paricalcitol has renoprotective effect on CsA-induced renal injury using experimental model of chronic CsA nephropathy.

Male SD rats were divided 4 groups; vehicle, CsA, CsA+paricalcitol 50 ng/kg (P50) and CsA+paricalcitol 200 ng/kg (P200), respectivley. CsA (15 mg/kg/day) was injected daily for 28 days and paricalcitol was injected as the concentration of 50 ng/kg/day(low) and 200 ng/kg/day (high) for 28 days. The responses of paricalcitol were evaluated by measuring renal functional parameters and the degree of the fibrosis by Masson's trichrome stain and the number of ED-1 positive cells. We also assessed the mechanism of the effect on renal pathology by analyzing the expression of βig-h3, caspase-3 immunoblotting and 24-h urinary and tissue expression of 8-hydroxy-2'-deoxyguanosine (8-OHdG).

Increased oxidative stress in the CsA group was significantly decreased compared to the P50 or P200 groups. The number of ED-1 positive cells was also lower in the P200 group than CsA group ($36.8 \pm 1.6/0.5mm^2$, vs. $52.3 \pm 2.0/0.5mm^2$, $P < 0.05$). At molecular basis, increased βig-h3 expression in the CsA group was significantly decreased in the P200 compared to CsA group ($9750 \pm 101\%$ vs $6897 \pm 465\%$, $P < 0.05$). Increased caspase-3 expression in the CsA group protein level for caspase-3 was decreased in paricalcitol groups. But lower paricalcitol dosage group (P50) did not show the significant difference with CsA group. Four weeks treatment of CsA developed typical chronic CsA nephropathy, characterized by interstitial inflammatory cells infiltration and tubulointerstitial fibrosis(TIF). Quantification for TIF revealed lower TIF score in the P200 group than CsA group ($15.5 \pm 2.3\%$ vs. $23.5 \pm 0.9\%$, $P < 0.05$).

Paricalcitol attenuates CsA-induced interstitial fibrosis and inflammation by decreasing oxidative stress, and this effect was dose-dependent.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO271

**Sympathetic Blockade Prevents the Decrease in Cardiac VEGF Expression and Capillary Supply in Experimental Renal Failure** Kerstin U. Amann,[1] Kerstin Benz,[2] Roland Veelken.[3] *[1]Pathology, University of Erlangen-Nürnberg, Erlangen, Germany; [2]Pediatrics, University of Erlangen-Nürnberg, Erlangen, Germany; [3]Hypertensiology and Nephrology, University of Erlangen-Nürnberg, Erlangen, Germany.*

**Background:** Uremic cardiomyopathy of men and rodents is characterized by lower myocardial capillary supply which in rats could be prevented by central and peripheral blockade of the sympathetic nervous system. The underlying pathomechanisms remain largely unknown. We investigated whether alterations of cardiac VEGF gene and protein expression were involved.

**Methods:** 1. Long-term experiment: We investigated whether VEGF gene and protein expression was altered in the heart of male Sprague-Dawley rats with either sham operation (sham, n=10) or subtotal nephrectomy (SNX, n=10). 2. Short term experiment (17 sham, 24 SNX):, The effect of a putative downregulation of sympathetic nervous activity by surgical renal denervation (interruption of renal afferent pathways) on cardiac gene expression of VEGF, flt-1 and flk-1 and on myocardial capillary supply was analysed.

**Results:** In the long-term study cardiac capillary supply (3484±532 vs 4258±829 mm/mm$^3$ and VEGF gene and protein expression were significantly lower in SNX than in sham. In the short-term experiment cardiac capillary supply (244.7±83.9 in SNX vs. 323.5±95.3 per mm$^2$ of myocardium in sham, $p<0.05$) and VEGF mRNA expression was significantly lower in untreated SNX (4258±2078 units) than in both sham groups (11709±4169 and 8998±4823 units); the decrease in capillarisation and VEGF expression was significantly prevented by renal denervation (8190±3889, $p<0.05$).

**Conclusion:** Cardiac VEGF gene and protein expression is reduced in experimental CRF and this may be considered as one potential reason for impaired myocardial adaptation under the situation of cardiac hypertrophy. The beneficial effect of sympathetic downregulation on cardiac structure and function in CRF may be at least in part explained by increased cardiac VEGF gene expression.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO272

### Molecular Targets for FGF23 in a Mouse Model of Chronic Kidney Disease

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The Col4a3$^{-/-}$ mouse is an animal model of Alport's disease caused by intrinsic defects in basement membrane collagens. 12-week old mice display loss of renal function and show features of CKD-MBD, including increased FGF23 levels. Progression of CKD in Col4a3$^{-/-}$ might result from the primary defect or extrinsic factors, such as circulating hormones, that modulate renal damage. Indeed, the kidney is the principal target for FGF23, which regulates phosphate transport, vitamin D metabolism and independently predicts progression of CKD. To determine the intrinsic and FGF-23 dependent expression of renal genes in progressive CKD, we performed microarray expression profiling on kidneys derived from 12-week-old Col4a3$^{-/-}$ and WT mice and compared these data with previously published data from Hyp and Fgf23 transgenic mice. Based on a minimum 2-fold change in expression of significantly modified genes, Col4a3$^{-/-}$ mice showed a marked up-regulation of 904 genes and downregulation of 148 transcripts. Up-regulated transcripts included lipocalin2 (+53.8 fold), Timp1 (+23.7), amiloride binding protein 1 (+16.7) and Col1a1 (+13.5). Several protein families were up-regulated including tumor necrosis factor (38 transcripts) and metalloproteinases (20 transcripts for MMPs and ADAMs). Downregulated transcripts included Dnase1 (-8.4 fold), Hbb-b2 (-7.2), Hbb-b1 (-4.6) and Klotho (-2.3). Also, 55 proteins belonging to the solute carrier transporters and 10 members of the cytochrome P450 superfamily were downregulated in Col4a3$^{-/-}$ mice. We identified 30 FGF23-depedent genes in Col4a3$^{-/-}$ mouse kidneys (10 upregulated, including Vcam1 and Ramp2, and 20 downregulated genes, including Dnase1, Klotho and Ace2). Pathway analysis of FGF23-dependent genes identified networks enriched for "Cell Death, Connective Tissue Development" related functions and disease categories such as "Immunological Disease" and "Renal Nephritis", suggesting that FGF23 may be directly involved in the renal disease progression. Although additional analysis of Col4a3$^{-/-}$ mice with an FGF23 endocrine clamp is needed to verify the FGF23-reponsive genes, these data set the stage for identifying specific FGF23-dependent pathways and investigating their role in the development of CKD-MBD.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO273

### The Expression of Glia Maturation Factor-β Promoted Aging Process of the Kidney by the Induction of an Aberrant Form of Lamin A

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Glia Maturation Factor-β (GMF) is induced by proteinurea in the kidney (*KI* 2002) and its expression causes apoptosis in cultured renal proximal cells because of vulnerability to oxidative stress (*J. Biol. Chem.*, 2003). To get insights into this process, transgenic mice expressing GMF (GMF-TG) was constructed, indicating early aging phenotype, similar to those of laminopathies, the premature-aging diseases such as Hutchinson -Gilford progeria syndrome. As a mechanism for this, we identified an excess amount of prelaminA in the kidney, which might be caused through a decrease of cleaving enzyme (Face-1) for this aberrant protein.

C57BL/6 (WT) was used to construct transgenic mice (GMF-TG). After genotyping, we found the followings;. 1) Kaplan-Meier representation of the survival curves demonstrated that GMF-TG died earlier than WT mice. 2) The hair on GMF-TG appeared to be sparser, like alopecia than that of WT. Hair growth declines linearly as aging in mice, which was assessed by hair growth assay (*Nature*, 2002). We measured the amount of hair growth 14-21 days after removal of hair from a dorsal area. Hair regrowth of GMF-TG was decreased significantly at 60 weeks of age compared with that of WT. 3) We also found induction of senescence associated genes, e.g. TGF-β and CTGF, in GMF-TG kidney. 4) Western blot analysis revealed that abnormal form of the nuclear protein lamin A (prelamin A) was expressed in the kidney of GMF-TG, but not in WT. The accumulation of this aberrant form is known to cause laminopathy and this accumulation is also detected in physiological aging (*Science* 2006). 5) The gene expression of Face-1, a zinc-metalloprotease that cleaved prelamin A to lamin A, decreased significantly in the kidney of GMF-TG at 60 weeks comparing to control.

Conclusion: The expression of GMF induced an aberrant form of lamin A, resulting in the promotion of premature aging process of the kidney.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO274

### Basic Fibroblast Growth Factor Reduces Renal Damage and Induces Repairing Nephrogenes and Angiogenes in Experimental Chronic Kidney Disease

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Chronic renal failure (CRF) is a clinical syndrome characterized for a progressive and irreversible structural damage of the nephron. Several therapies have been employed to retard the loss of renal function and reduce proteinuria, but have not been successful in limiting the progression of the disease. With the hypotheses that basic Fibroblast Growth Factor (bFGF) is involved in renal tissue repair, our objective was to study the evolution of CRF in animals treated with the repairing factor bFGF, an inductor of nephrogens, thus we expected that the damage induced in CRF could be decreased.

Male Sprague Dowley rats were submitted to nephrectomy 5/6 and were divided in two groups: injected with bFGF 30 mg/Kg or injected with saline that was used as control (Sham rats) (n = 7 per group). After 5 weeks, animals were sacrificed and the kidney was removed. The procedures were performed according with institutional and international standards for care and use of laboratory animals (PHS, NIH). The renal function was assessed by the serum creatinine levels; the morphological damage was evaluated by HE stain and the presence of damage markers ED-1 (macrophages) and interstitial α-SMA. Nephrogenes, angiogenes and transcriptional factors were analyzed by immunohistochemistry, Western blotting and RT-PCR.

Animals injected with bFGF had a significant reduction in serum creatinine levels, as well a reduction in the damage markers ED-1 and α-SMA compared to sham rats ($p<0.05$). Further, the animals injected with bFGF showed an important re-expression of nephrogenes BMP-7, LIM, bFGF, NOGGIN, and WNT-4. Also angiogenic proteins VEFG, TIE-2 and transcriptional factors HIF-1α, SMAD and p-SMAD were induced by bFGF ($p<0.05$).

These results indicate the potential therapeutic effect of bFGF on renal function in CRF by improving renal damage through the re-expression of nephrogenes and angiogenes (Fondecyt 11075029, Fondecyt 1080590, PFB 12-2007).

Disclosure of Financial Relationships: nothing to disclose

## TH-PO275

### Cysteamine Ameliorates Progressive Interstitial Fibrosis by Modulating Oxidative Stress and Attenuating Extracellular Matrix Synthesis during Chronic Kidney Injury

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Few therapeutic options exist to slow or halt the relentless expansion of interstitial extracellular matrix (ECM) leading to nephron loss and progressive decline of kidney function. Cysteamine treatment has had a tremendous impact on the severity and progression of nephropathic cystinosis, however, the primary renoprotective mechanisms remain poorly understood. The effect of cysteamine on the degree of renal fibrosis was investigated in a model of unilateral ureteral obstruction (UUO) using two doses, 400 and 600 mg/kg/day, and compared to mice that received vehicle alone (n = 8/timepoint: days 3, 7, and 14). Both doses were well-tolerated and measured serum levels of cysteamine were appropriate. Total kidney collagen content was significantly reduced by 21% in both the 400 and 600mg/kg doses in cysteamine-treated mice at day 14. ECM gene transcription levels were significantly down-regulated in UUO kidneys of cysteamine-treated mice: procollagen I mRNA levels were 56% lower in the mice treated with 600 mg/kg at day 14; and at day 7, despite no difference in total collagen, there was a nearly 40% reduction in kidney fibronectin and procollagen III mRNA levels in mice treated with 400mg/kg and a nearly 60% reduction in fibronectin, procollagen I and procollagen III at higher doses of cysteamine (600mg/kg). There was a significant reduction in alpha-SMA positive myofibroblasts by nearly 30% in both doses at day 14 in cysteamine-treated mice. There was a significant reduction in interstitial macrophage infiltration by 34% in mice treated with 600mg/kg. Total kidney thiol content, a measure of antioxidant status, was significantly increased by 36% in high dose cysteamine-treated mice (600mg/kg) compared to controls. Taken together, these data suggest that cysteamine may affect both myofibroblast accumulation and interstitial macrophage infiltration by modulating oxidative stress within the intersitium during chronic kidney injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO276**

**Leucine Stimulates mTOR Anabolic Signal Transduction in Muscle of CKD Rats** Sumita Sood,[1,2] Yu Chen,[1,2] Kevin L. Mcintire,[1,2] Ralph Rabkin.[1,2] *[1]Nephrology, Stanford University School of Medicine, Palo Alto, CA; [2]Nephrology, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA.*

There is recent evidence that the branched chain amino acid (BCAA) leucine (LEU), stimulates muscle protein synthesis in part by directly activating the mTOR signaling pathway, with phosphorylation of p70 ribosomal S6 kinase-1 ($p70^{S6K}$), S6 ribosomal protein (rpS6) and eukaryotic factor 4E-binding protein1 (4E-BP1). Since muscle wasting in CKD arises in part due to resistance to insulin/IGF-1 activation of the downstream PI3-kinase/Akt pathway and also low serum and tissue levels of BCAA, we tested whether LEU can effectively activate the mTOR anabolic signaling pathway in uremia. CKD and control (Con) rats were gavaged with LEU (L) or saline (S) and sacrificed 30 mins later. Western blot analysis showed that LEU loading did not affect AKT phosphorylation as anticipated, but did effectively stimulate down-stream phosphorylation of mTOR, $P70^{S6K}$ and rpS6 in both Con and CKD rats (fig 1). Of note, basal levels of phospho-rpS6 were depressed in CKD (saline treated) rats and following LEU gavage increased similarly in CKD and Con rats.

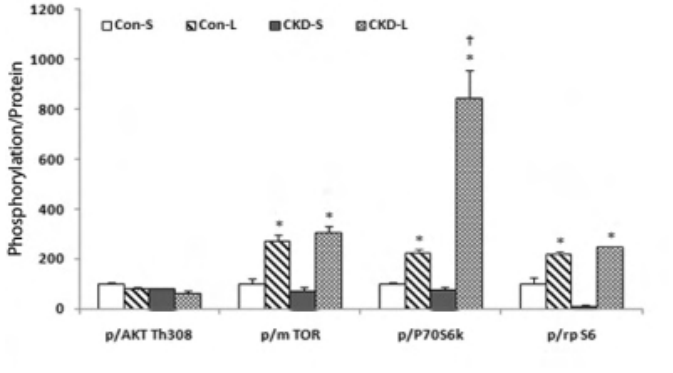


Fig 1. LEU (L) stimulated m TOR anabolic signal transduction down-stream of Akt in skeletal muscle of CKD and control rats. * - p<0.01 vs saline (S); † - p<0.0001 vs Con-L

Thus uremia appears to inhibit distal mTOR activated anabolic signaling, a significant defect that can be overcome by oral administration of LEU. Taken together these findings suggests that LEU supplements maybe of particular value in managing uremic muscle wasting, especially as serum LEU levels are commonly reduced in advanced CKD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO277**

**Fenofibrate Attenuates Proteinuria but Is Not Effective on Metabolic Parameters with Excess Dietary Salt Intake in SHR/NDmcr-cp** Junichi Yatabe,[1,2] Midori Sasaki Yatabe,[2,3] Pedro A. Jose,[4] Hironobu Sanada,[1] Tsuyoshi Watanabe.[2] *[1]Div. of Health Science Research, Fukushima Welfare Federation of Agricultural Cooperatives, Fukushima, Japan; [2]Dept. of Nephrology, Hypertension, Diabetology, Endocrinology and Metabolism, Fukushima Med. Univ., Fukushima, Japan; [3]Dept. of Pharmacology, Fukushima Med. Univ., Fukushima, Japan; [4]Center for Molecular Physiology Research, Children's National Medical Center, Washington, DC.*

While fenofibrate is used widely to manage dyslipidemia, whether its effects are influenced by salt intake has not been elucidated. This study aimed to determine the metabolic and renal effects of fenofibrate in metabolic syndrome model rats, SHR/NDmcr-cp (SHR-cp), on high-salt diet.

SHR-cp at 12 weeks of age (n=5 /group) were fed 0.3 % normal- or 4% high-NaCl chow and given oral fenofibrate (30 mg/kg/day) or vehicle for 4 weeks.

On normal-salt diet, fenofibrate significantly reduced fasting plasma glucose (control vs. fenofibrate; 124±0.4 vs 91±1.0 mg/dl, p<0.05), total cholesterol (131±6.7 vs 91±7.3 mg/dl, p<0.01), and proteinuria (27.3±3.2 vs 13.3±1.9 mg/g.crea, p<0.01) without changing blood pressure. However, with high-salt diet, fenofibrate rather elevated fasting plasma glucose (88.6±2.8 vs 101±4.9 mg/dl, p<0.05), postprandial glucose (176±16 vs 267±9 mg/dl, p<0.05) and plasma insulin (3.4±0.9 vs 5.7±1.9 IU/L, p<0.05). Total cholesterol, triglyceride, LDL cholesterol, free-fatty acid, BNP concentrations, systolic blood pressure and urinary sodium excretion were similar between fenofibrate and control groups on high-salt diet. However, body weight was less in the fenofibrate group (474±6 vs 424±5 g, p<0.01) with similar food intake. Moreover, the fenofibrate group showed significantly less urinary protein excretion (20.9±1.7 vs 15.0±2.2 mg/g.crea, p<0.05), and reduced renal cortical fibronectin protein (100±16.5 vs 35.4±10.8 %, p<0.05).

In conclusion, in SHR-cp on high-salt diet, fenofibrate decreased proteinuria and renal fibronectin expression without improving either glucose or lipid metabolism. The results of this study suggest that fenofibrate may be renoprotective while excess dietary NaCl may abrogate the metabolic benefits of fenofibrate.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO278**

**Investigation of Prevalence of Chronic Kidney Disease in 1031 Consecutive Cases with Coronary Angiography** Bi-Cheng Liu, Hong Liu, Kun Ling Ma. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China.*

**Objective** To investigate the prevalence of CKD in patients who had undergone coronary angiography with suspected coronary heart disease (CHD).

**Methods** Coronary angiography was performed in 1031 patients with suspected CHD in ZhongDa Hospital from December 2008 to November 2009. The definition of CKD was based on the estimated Glomercular filtration rate (eGFR) , which was estimated with the Modified Diet in Renal Disease (MDRD) equationand or proteinuria. Luminal narrowing at least one lesion ≥50% in the main branches of coronary artery was considered as CHD.

**Results** The mean age of patients were 64 ± 11 years. There were 543 males and 488 females, including 551 patients with CHD and 134 patients with CKD (13%). Patients with CHD had a significantly higher prevalence of CKD compared with patients without CHD (18.33% vs 6.88%, p < 0.001). With the increasing number of stenosed coronary vessels (n=0, 1, 2, 3), eGFR was declined significantly (84.25±19.00, 81.61±23.92, 75.16±20.99, 73.92±20.66ml/min per 1.73 $m^2$, p<0.001) significantly, and the percentage of proteinuria increased (0.42%, 0.82%, 1.96%, 3.25%; P=0.006), the prevalence of CKD increased accordingly (6.88%, 13.11%, 21.57%, 23.38%; P<0.001). Within the group of CHD patients, logistic regression analysis indicated that age (odds ratio, 1.106; 95% confidence interval, 1.075 to 1.139), hypertension (odds ratio, 2.248; 95% confidence interval, 1.156 to 4.372), systolic insufficiency (odds ratio, 4.541; 95% confidence interval, 2.452 to 8.412) and increasing numbers of stenosed coronary vessels (odds ratio=1.423; 95% confidence interval, 1.100 to 1.840) were the risk factors for the development of CKD.

**Conclusion** This study suggested a higher prevalence of CKD in patients with CHD diagnosed by coronary angiography, and it is closely related with the increasing number of stenosed coronary vessels. Aging, hypertension, low LVEF and elevated numbers of stenosed coronary vessels were important risk factors for angiographic CHD patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO279**

**Effect of Nephrotic Syndrome (NS) on Homocysteine (Hcy) Metabolism** Mohammad A. Aminzadeh, Pavan Gollapudi, Nosratola D. Vaziri. *Medicine/ Nephrology, University of California, Irvine, Irvine, CA.*

Proteinuria and hyperhomocysteinemia are independently associated with increased risk of atherosclerosis and cardiovascular disease. The available data on plasma Hcy level in patients with NS are contradictory with increased, decreased and unchanged values reported by different investigators. The reason for the disparity among the reported studies is unknown and could be due to differences in severity of hypoalbuminemia, concomitant renal insufficiency or underlying systemic diseases among others. In this study we explored the effect of NS on plasma Hcy concentration, urinary Hcy excretion and hepatic expression of methylenetetrahydrofolate reductase (MTHFR) and cystathionine-β-synthase (CBS), the key enzymes in trans-methylation and trans-sulfuration of Hcy respectively. Sprague-Dawley rats were rendered nephrotic by IP injection of puromycin aminonucleoside. Compared to placebo-injected controls the nephrotic rats showed heavy proteinuria (691±70 vs. 56±6 mg/24hr, P=0.003), hypoalbuminemia (1.8±0.1 vs. 3.3±0.1 g/dl, P<0.001), hypercholesterolemia (496±28 vs. 95±8 mg/dl, P<0.001), normal plasma creatinine (0.74±0.08 vs. 0.75±0.04 mg/dl) and creatinine clearance (1.36±0.2 vs. 1.4±0.02 ml/min), reduced plasma Hcy (3.0±0.3 vs. 5.6±0.8 µmol/L, P<0.03), increased urinary Hcy (3.4±0.2 vs. 9.6±0.2 µmol/24 hr, P<0.03 ) and down-regulation of CBS but not MTHFR expression. Plasma Hcy correlated directly with plasma albumin (r =0.77, P=0.009) and inversely with proteinuria (r =-0.78, P=0.02) and urinary Hcy correlated directly with proteinuria (r =0.98, P<0.001). Thus heavy proteinuria results in significant reduction in plasma total Hcy concentration which is due to diminished protein-bound fraction as opposed to its biologically-active free fraction. Increased urinary Hcy is, in part, due to the loss of albumin which is a major Hcy-binding protein and increased Hcy pool occasioned by its reduced CBS-catalyzed trans-sulfuration.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO280**

**Renin-Angiotensin-Dependent and -Independent Antihypertensive Therapies Exhibit Similar Degrees of Cardio- and Renoprotection in 5/6-Nephectomized Ren-2 Transgenic Rats** Ludek Cervenka. *Department of Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.*

Hypertension plays a critical role in the progression of chronic kidney disease (CKD) to end-stage renal disease (ESRD) and it has been postulated that antihypertensive drugs which block the renin-angiotensin system (RAS) exhibit class-specific renoprotective actions beyond their blood pressure (BP)-lowering effects. Since this notion has been recently questioned, the present study was performed to compare the effects of a RAS-dependent antihypertensive therapy, a combination of the angiotensin-converting enzyme inhibitor (ACEI) trandolapril and the angiotensin II (ANG II) receptor subtype 1A receptor antagonist losartan with a RAS-dependent antihypertensive therapy, a combination of the alfa- and beta-adrenoreceptor antagonist labetalol with the diuretics hydrochlorothiazide and furosemide, on the progression of CKD after 5/6 renal ablation (5/6 NX) in Ren-2 renin transgenic rats (TGR), a model of ANG II-dependent hypertension. Normotensive transgene-negative Hannover Sprague-Dawley (HanSD) rats with 5/6 NX served as controls.

The RAS-dependent and -independent antihypertensive therapies normalized BP and survival rate and prevented the development of cardiac hypertrophy and glomerulosclerosis to the same degree in 5/6 NX HanSD rats as in 5/6 NX TGR. Our findings indicate that the renoprotection, at least in rats after 5/6 NX animals, is predominantly BP-dependent. When equal BP-lowering effects leading to normotension were achieved the cardio- and renoprotective effects were equivalent independently of the type of antihypertensive therapy. This information derived from our present study should be considered in attempts for developing new therapeutical approaches and strategies to prevent the progression of CKD and lower the incidence of ESRD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO281**

**New Biomarkers of CKD Progression Discovered with a Multiplexed Aptamer Proteomic Technology** Roxana M. Bologa,[1] Daniel Levine,[1] Thomas Parker,[1] Trudi Foreman,[2] Nebojsa Janjic,[2] Robert E. Mehler,[2] Alex Stewart,[2] Jeffrey J. Walker.[2] *[1]The Rogosin Institute and Weil Cornell Medical College, New York, NY; [2]SomaLogic, Boulder, CO.*

The progressive loss of renal clearance that occurs in chronic kidney disease (CKD) is expected to be accompanied with an overall increase in plasma concentration of small proteins. We have tested this hypothesis using a highly multiplexed, aptamer-based proteomics array technology that simultaneously measures large numbers of proteins ranging from very low (0.1pg/mL) to high (50mg/mL) abundance in plasma. Plasma, obtained from 11 subjects with early- (stages 1-2) and 31 with late-stage CKD (stages 3-5), was used to measure 614 human proteins simultaneously in a 15uL volume. We identified 60 proteins that varied significantly between the two groups, using the Mann-Whitney test statistic, with a q-value (FDR-corrected p-value) of 4.2 x 10-4. Cystatin C and β2-microglobulin, which are important known biomarkers of CKD, were found, but many of the remaining 58 proteins are newly discovered biomarkers of declining renal function. Nine of the 11 proteins with the most significant variation (q-values <3.5 x 10-7) are relatively small proteins (MW <25 kDa) with an inverse correlation between abundance and renal clearance: β2-Microglobulin (11.7kDa), Folistatin-like-3 (FSTL3, 25.0kDa), Pleotrophin (15.3kDa), TNF sR-I (extracellular domain, 21.2kDa), Complement Factor D (24.4kDa), IL-15 Rα (extracellular domain, 18.4 kDa), Matrilysin (19.1kDa), Cystatin C (13.3kDa), C-C motif chemokine 14 (HCC-1 small isoform, 8.7kDa), Coiled-coil domain containing 80 (Ccdc80 or URB small isoform 10.3kDa).

Accumulation in plasma of small proteins appears to be a major change in the plasma proteome in CKD. However, the fact that the biomarkers are not simply ranked according to their molecular weights shows that renal tissue injury and secondary systemic effects resulting from reduced renal function clearly also affect the proteome. This example demonstrates that we can recover known biomarkers and discover new biomarkers that can be used to build diagnostic signatures for measuring CKD progression.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO282**

**A Comparison of Comorbidity in CKD Patients Based on MDRD vs. CKD-EPI Equations** David T. Gilbertson,[1] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

Estimation of GFR is most commonly done using the MDRD equation published in 2006. Inaccuracies using this equation, particularly in pts with mild CKD, led to the search for better equations such as the CKD-EPI equation published in 2009. Estimation using this equation, which was developed on pts across wide ranges of GFR, better estimates actual GFR in patients with GFR > 60 mL/min/1.73 m2.

Using i3 Ingenix data on individuals with coverage in 2008 with ≥ 1 serum creatinine, we computed eGFR using both equations and compared comorbidity burdens across CKD stages, particularly for pts classified differentially. For pts with > 1 creatinine, we used the average. Since ACR data was mostly unavailable, we compared the following categories/ CKD stages: >=90, 60-90 (mL/min/1.73 m2), stage 3, 4 or 5.

We identified 786,348 persons with continuous insurance coverage and ≥ 1 serum creatinine during 2008. 49.7%, 45.4%, and 4.6% were identified as >=90, 60-90, and stage 3, respectively, by CKD-EPI. 28.3%, 63.2%, and 8.1% were similarly classified by MDRD. The table shows the comorbidity burden for those classified the same by both equations compared to those differentially classified by the 2 equations. For pts classified as lower eGFR by MDRD than CKD-EPI (lower half of table), the comorbidity burden was generally similar to the comorbidity burden of the higher eGFR group (top half of table). These results are consistent with other studies showing more accurate estimation of actual GFR with the CKD-EPI equation for pts with less severe CKD.

| | | **Comorbidity Burden of Patients Classified the Same by Both Equations** | | | | |
|---|---|---|---|---|---|---|
| **MDRD & CKD-EPI Agree** | | ASHD | Anemia | CHF | Cancer | DM |
| >= 90 | | 2.7% | 5.5% | 0.7% | 3.2% | 12.3% |
| 60 - 90 | | 5.7% | 4.6% | 1.3% | 4.8% | 13.1% |
| Stage 3 | | 13.4% | 11.6% | 5.7% | 8.5% | 28.9% |
| Stage 4 | | 21.4% | 48.5% | 17.9% | 9.3% | 49.9% |
| | | | | | | |
| **MDRD & CKD-EPI Disagree** | | **Comorbidity Burden of Patients Classified with Higher eGFR by CKD-EPI** | | | | |
| MDRD | CKD-EPI | ASHD | Anemia | CHF | Cancer | DM |
| 60 - 90 | >= 90 | 2.8% | 4.6% | 0.6% | 3.2% | 10.2% |
| Stage 3 | 60 - 90 | 5.5% | 5.9% | 1.8% | 5.1% | 14.6% |
| Stage 4 | Stage 3 | 14.0% | 36.6% | 9.1% | 8.3% | 44.2% |
| Stage 5 | Stage 4 | 4.0% | 64.0% | 8.0% | 12.0% | 56.0% |

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**TH-PO283**

**Implications of the CKD-EPI GFR Estimation Equation in a Health Care System** Sankar D. Navaneethan,[1] Jesse D. Schold,[2] Stacey Jolly,[3] Susana Arrigain,[2] Welf Saupe,[4] John W. Sharp,[2] Emilio D. Poggio,[1] James F. Simon,[1] Martin J. Schreiber,[1] Anil K. Jain,[3,4] Joseph V. Nally.[1] *[1]Nephrology & Hypertension, Cleveland Clinic; [2]Quantitative Health Sciences, Cleveland Clinic; [3]Medicine Institute, Cleveland Clinic; [4]eCleveland Clinic eResearch, Cleveland Clinic.*

***Purpose:*** Chronic kidney disease (CKD) is a significant public health problem whose diagnosis and staging relies upon equations to estimate glomerular filtration rate (GFR), including the new CKD-EPI equation. CKD-EPI demonstrated superior performance compared to the existing MDRD equation during development and validation, but has not been applied to a population within a healthcare system.

***Methods:*** We identified 53,759 CKD patients based on either the MDRD or CKD-EPI equations using two eGFR measures <60 mL/min/1.73 m² greater than 90 days apart from an outpatient setting. We compared the prevalence, patient characteristics and time to inclusion using the two equations.

***Results:*** Prevalence of CKD decreased 10% based on the CKD-EPI equation. This change varied substantially by patient characteristics with a 35% decrease among patients <60 years and 10% increase among patients over 90 years. Females and non-African Americans had a greater decline in CKD identification.

| Patient Characteristics (n=53,759) | Method of CKD Classification | | | % Change in CKD patients based on the CKD-EPI Equation |
|---|---|---|---|---|
| | Both MDRD and CKD-EPI equations | MDRD equation only | CKD-EPI equation only | |
| Age 18-59 (n=33413) | 65% | 35% | 0% | -35% |
| Age 60-69 (n=12301) | 89% | 11% | 0% | -11% |
| Age 70-79 (n=5986) | 96% | 4% | 1% | -3% |
| Age 80-89 (n=2033) | 90% | 0% | 9% | 9% |
| Age 90+ (n=1726) | 91% | 0% | 9% | 10% |
| African American (n=6251) | 93% | 2% | 5% | 2% |
| Non-African American (n=47508) | 87% | 12% | 1% | -11% |
| Diabetes present (n=11004) | 91% | 7% | 1% | -6% |
| Diabetes absent (n=42755) | 86% | 12% | 2% | -11% |
| Male gender (n=23779) | 89% | 9% | 2% | -7% |
| Female gender (n=29980) | 86% | 13% | 1% | -12% |
| BMI < 20 kg/m² (n=1813) | 88% | 9% | 3% | -6% |
| BMI 20-24 kg/m² (n=13052) | 87% | 10% | 2% | -8% |
| BMI 25-29 kg/m² (n=17719) | 88% | 10% | 2% | -8% |
| BMI 30-34 kg/m²(n=10858) | 87% | 12% | 1% | -11% |
| BMI 35+ kg/m² (n=8133) | 85% | 13% | 1% | -14% |
| Overall – n (%) | 46985 (87%) | 3951 (13%) | 823 (2%) | 10% |

Adjusted for other characteristics, patients aged >70 had over two-fold hazard for time to inclusion in the registry with CKD-EPI relative to patients <60 (AHR=2.17, 95% C.I.2.11-2.23) as compared to a reduced hazard with MDRD (AHR=0.90, 95% C.I.0.88-0.93).

***Conclusions:*** Application of CKD-EPI equation resulted in a substantial decline in equation-based inclusion of CKD patients in our health care system. Further research is needed to determine whether widespread use of CKD-EPI based on current CKD classification guidelines could lead to delayed needed care among younger patients or excessive referrals among older patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO284**

**Predicting ESRD and CVD Risk Using GFR Estimated by the CKD-EPI and MDRD Equations: Strong Heart Study (SHS)** Nawar M. Shara,[1,2] Hong Wang,[1] Elizabeth A. Carter,[1] Mihriye Mete,[1] Yaman Rai Balha,[1,3] Barbara V. Howard,[1,2] Jason G. Umans.[1,2] *[1]Biostatistics, MedStar Health Research Institute, Hyattsville, MD; [2]Georgetown University, Washington, DC; [3]School of Medicine, Damascus University, Syrian Arab Republic.*

**Background:** Estimated glomerular filtration rate (eGFR) is the best available kidney function measure. The CKD-EPI collaboration has published a new equation to improve GFR estimates. Whether the new equation is better than the MDRD equation in predicting ESRD and CVD in populations with diabetes and obesity has not been studied. **Methods:** We analyzed data from the SHS, a longitudinal study of CVD risk in 4549 American Indians ages 45-74y. We excluded those with missing serum creatinine (n=173); with ESRD (n=21) or with CVD (n=331), leaving 4024participants (40% male) for this analysis. ESRD was defined as eGFR<15 or incident kidney transplant or dialysis. Of the 4024 participants, 156 had incident ESRD during subsequent SHS phases, while 1249 had incident CVD during a 15-y median follow-up. Logistic regression models and Cox proportional hazards models, adjusted for age, gender, LDL-C, HDL-C, hypertension (yes/no), smoking (never, past, or current smoker), and albuminuria, were used to assess the association of baseline $eGFR_{MDRD}$ or $eGFR_{CKD-EPI}$ with subsequent ESRD or CVD seperately. We categorized eGFR measures into groups as follows: [120-90), [90-60), [60-30], and < 30 mL/min/1.73 m². **Results:** Risk, assessed by either eGFR measure was similar in models predicting ESRD

and CVD separately. Adjusted ORs for ESRD were identical using either eGFR measure for models with or without adjustment for albuminuria. HRs for CVD were also identical using either eGFR measure in models with or without adjustment for albuminuria. Harrell's C were identical for models including either eGFR measure. No substantial reclassification to different eGFR strata (NRI=0.01, p=0.2) was observed. **Conclusions:** In an American Indian population with a high prevalence of diabetes, $eGFR_{MDRD}$ or $eGFR_{CKD\text{-}EPI}$ was equally predictive of ESRD or CVD and provided similar discriminatory power, suggesting that eGFR as calculated by either equation may be used in risk stratification.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO285

**Modification of the CKD-EPI Equation for Japanese: Accuracy and Use for Population Estimates** Masaru Horio,[1] Enyu Imai,[2] Yoshinari Yasuda,[2] Tsuyoshi Watanabe,[3] Seiichi Matsuo.[2] *[1]Functional Diagnostic Science, Osaka University Graduate School of Medicine, Osaka, Japan; [2]Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [3]Third Department of Medicine, Fukushima Medical University, Fukushima, Japan.*

**Background:** We previously reported a Japanese-GFR equation and a coefficient-modified MDRD Study equation for use in Japan. Recently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) developed a new equation, the CKD-EPI equation, that is more accurate than the MDRD Study equation and yields a lower prevalence of CKD in the US than the MDRD Study equation. We modified the CKD-EPI equation and evaluated its accuracy. We compared the prevalence of CKD in Japan using the equations. **Subjects**: We used same data set from which the coefficient of MDRD Study equation was obtained. Total 763 Japanese patients (413 for development and 350 for validation) were included. GFR were measured by inulin renal clearance. Prevalence estimates were based on 574,024 participants from the annual health check program. **Results:** The Japanese coefficient of CKD-EPI equation was 0.813 (95% CI: 0.794 - 0.833). In validation dataset, bias (measured minus estimated GFR, SD) of the coefficient-modified CKD-EPI equation, the coefficient-modified MDRD Study equation and the Japanese-GFR equation was 0.4±17.8, 1.3 ±19.8 and 2.1±19.0 ml/min/1.73 m², respectively. The coefficient-modified CKD-EPI equation performed significantly better than the coefficient-modified MDRD Study equation overall and for subjects with mGFR≥60 ml/min/1.73 m². The modified CKD-EPI equation also performed significantly better than the Japanese GFR equation for subjects with mGFR≥60 ml/min/1.73 m², although the Japanese GFR equation was better for subjects with mGFR< 60 ml/min/1.73 m². Modified CKD-EPI equation yields a lower estimated prevalence of CKD than the MDRD Study equation (7.9% vs. 10.0%), primarily because of a lower estimated prevalence of stage 3 (5.2% vs. 7.5%). **Conclusion:** The coefficient modified CKD-EPI equation is more accurate than the coefficient modified MDRD Study equation for Japanese and leads to a lower estimated prevalence of CKD in Japan.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO286

**Does Use of eGFR in Donor Screening Unnecessary Reduce the Donor Pool? Long Term Follow-Up of Donors with Pre-Donation eGFR<80** H. Tent, Mieneke Rook, Jaap Homan vd Heide, Gerjan Navis. *Internal Medicine Division of Nephrology, University Medical Center Groningen, Netherlands.*

Due to low costs and easy use, many centers rely on estimated GFR (eGFR) for initial living donor screening, with a cut-off of <80 mL/min/1.73m². Since eGFR tends to be imprecise and underestimates true measured GFR (mGFR) this may lead to unnecessary rejection of potential donors. Here we evaluate follow-up of donors with pre-donation eGFR<80 mL/min/1.73m².

Evaluated were 81 donors (38% male, mean age at follow-up 52±11) who donated at our center. Screening and follow-up were performed by mGFR ($^{125}$I-Iothalamate), 4 months prior and 2 months and 5.1±1.3 years post-donation. MDRD and CKD-EPI were determined from creatinine samples from the same days. Furthermore, a cohort of 291 living kidney donors (44% male, mean age 49±11) of whom only short term follow-up was available yet was analyzed.

Pre-donation mGFR, MDRD and CKD-EPI were 104±15, 83±18 and 88±14 mL/min/1.73m². 43 donors had pre-donation MDRD<80, 26 CKD-EPI<80 mL/min/1.73m². Renal function data is shown in the table. Long term mGFR of donors with pre-donation MDRD and CKD-EPI>80 was 77±11 and 75±11 mL/min/1.73m² respectively (both p<0.01 vs pre-donation value<80). In the larger group eGFR underestimated in similar extent: 170 donors had pre-donation MDRD<80, 96 CKD-EPI<80 mL/min/1.73m². In both groups, donors with eGFR<80 were older than those with eGFR>80 mL/min/1.73m².

Thus, both MDRD and CKD-EPI underestimate mGFR at screening which could potentially lead to rejection of a large proportion of potential donors (56% for MDRD and 33% for CKD-EPI). On follow-up, donors with pre-donation MDRD and CKD-EPI<80 had lower mGFR than donors with pre-donation value>80 mL/min/1.73m², mGFR was however stable and sufficient. To optimize the donor pool, we argue the use of more reliable methods for screening along with long term follow-up to ensure donor safety.

| | MDRD<80 | CKD-EPI<80 |
|---|---|---|
| Pre-Unx mGFR | 99±14 | 97±15 |
| Early post-Unx mGFR | 62±9 | 61±10 |
| Long term post-Unx mGFR | 68±9 | 67±9 |
| Pre-Unx eGFR | 70±6 | 74±5 |
| Early post-Unx eGFR | 46± | 48±6 |
| Long term post-Unx eGFR | 49±7 | 52±7 |

Unx: donation; mGFR and eGFR in mL/min/1.73m²

Disclosure of Financial Relationships: nothing to disclose

### TH-PO287

**Internal Standardization of Cystatin C Measurements in the Chronic Renal Insufficiency Cohort (CRIC) Study** Amanda Hyre Anderson, Dawei Xie, Kelvin Tao, J. Richard Landis, Daniel J. Rader, Harold I. Feldman. *University of Pennsylvania School of Medicine.*

One major limitation of widespread use of cystatin C to estimate GFR is the lack of standardization of laboratory procedures and assays. Owing to this fact, the International Federation of Clinical Chemistry and Laboratory Medicine working group for the standardization of cystatin C is working to disseminate secondary reference preparations of cystatin C. The NIDDK-sponsored Chronic Renal Insufficiency Cohort (CRIC) Study will track changes in kidney function over time among 3,939 participants, in part, by measuring cystatin C using the Siemens Diagnostics nephelometric assay at all study visits. All assays will be performed at the study's central biochemistry laboratory located at the University of Pennsylvania. To monitor for any drift in the Siemens cystatin C assay across testing batches in CRIC, a set of control specimens have been run repeatedly. In 2009, quality control checks identified a downward drift in cystatin C values of 19% over 1.5 years in these control samples (Table). This drift was verified at the central biochemistry laboratory for several large NHLBI studies. Potential sources of the assay drift were investigated across a range of cystatin C values from approximately 0.5-6.0 mg/L, and CRIC Investigators observed varied performance of the assay across reagent and calibrator lots. Cystatin C values were corrected to a specific Siemens reagent and calibrator lot combination using data from a subset of retested specimens and linear regression models to ensure longitudinal measures would not be confounded by downward assay drift. With the future achievement of international standardization of cystatin C, these corrected values will be able to be calibrated to gold standard materials ensuring the comparability of CRIC Study results to external groups.

Table. Mean uncorrected and corrected cystatin C values in N=30 control samples in the CRIC Study

| Calibrator lot | Reagent lot | Uncorrected mean cystatin C | Corrected mean cystatin C |
|---|---|---|---|
| 44 | 34 | 0.77 | 0.68 |
| 45 | 35 | 0.70 | 0.70 |
| 48 | 39 | 0.65 | 0.72 |
| 49 | 40 | 0.62 | 0.68 |
| 50 | 41 | 0.70 | 0.70 |

Disclosure of Financial Relationships: nothing to disclose

### TH-PO288

**Cystatin C Assay Calibration: An Important Determinant of Cystatin C Concentrations and GFR Estimation** Christine A. White,[1] Andrew D. Rule,[2] Ayub Akbari,[3] John C. Lieske,[2] Greg A. Knoll.[3] *[1]Dept. of Internal Medicine, Queen's University, Kingston, ON, Canada; [2]Division of Nephrology, Mayo Clinic, Rochester, NY; [3]Division of Nephrology, University of Ottawa, Ottawa, ON, Canada.*

Cystatin C has emerged as a promising new marker of glomerular filtration rate (GFR) and several prediction equations have been derived to estimate GFR from its serum concentration. Marked heterogeneity in the performance of these novel equations in similar populations exist even when the same methodology and reagents from the same manufacturer are utilized. This study was designed to examine differences in cystatin C assay calibration and its effect on GFR estimation using the common PENIA assay (Siemens). Cystatin C was measured in 97 split samples of frozen serum in the laboratories of the Mayo Clinic and of the Children's Hospital of Eastern Ontario (CHEO). GFR was estimated by the Mayo equation using cystatin C measured in both laboratories. The analysis was repeated after stratifying patients by cystatin C values greater than (n=45) and lesser than or equal to (n=52) the median Ottawa cystatin C value of 1.33 mg/L. For the whole cohort, the mean Ottawa cystatin C was 0.19 ± 0.32 mg/dL lower than the mean Mayo cystatin C (p<0.0001). For those with a cystatin C >1.33 mg/dL and cystatin C ≤ 1.33 mg/dL, the mean CHEO cystatin C value was 0.22 ± 0.38 mg/dL and 0.16 ± 0.23 mg/dL lower than the mean Mayo cystatin C value respectively (p<0.0001 for both). The results of the estimated GFRs using the 2 cystatin C assays are shown below.

| | eGFR (Mayo) ± SD (ml/min/1.73m²) | eGFR (CHEO) ± SD (ml/min/1.73m²) | Mean difference eGFR (CHEO) - eGFR (Mayo) ± SD (ml/min/1.73m²) |
|---|---|---|---|
| whole cohort (n=97) | 52.1 ± 22.1 | 60.5 ± 26.5 | * 8.4 ± 14.9* |
| Cystatin C ≤ 1,33 mg/L (n=52) | 67.2 ± 18.5 | 78.9 ± 22.3 | *11.7 ± 18.8 |
| Cystatin C > 1.33 mg/L (n=45) | 34.6 ± 9.1 | 39.3 ± 9.8 | *4.6 ± 6.7 |

*p<<0.0001

Significant differences in GFR estimation result from differences in assay calibration and this is more pronounced at higher GFRs. Cystatin C assay standardization should be a priority as this novel analyte becomes increasingly utilized.

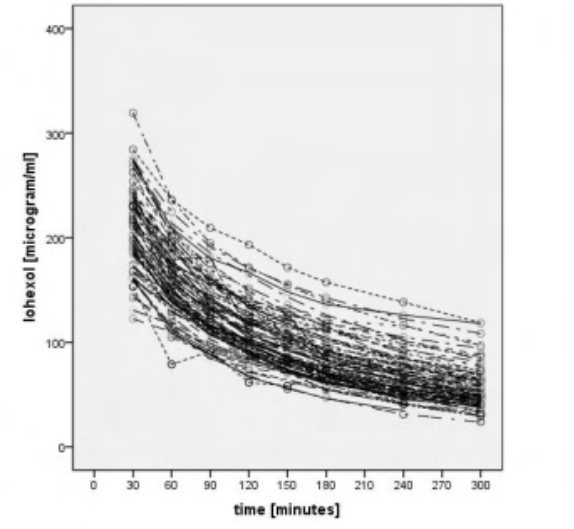
Disclosure of Financial Relationships: nothing to disclose

**TH-PO289**

**Iohexol Clearance Measurement in the Elderly – What Is the Better Protocol?** Natalie R. Ebert,[1] Peter Martus,[1] Jens Gaedeke,[1] Martin K. Kuhlmann,[4] Markus van der Giet,[1] Elke Schaeffner.[1] *[1]Nephrology, Charité, Berlin, Germany; [2]Nephrology, Klinikum im Friedrichshain, Berlin, Germany.*

Above the age of 70 no GFR formula has been validated. To construct a future estimation equation for the elderly we measured as goldstandard plasma clearance of iohexol which is known to be a safe and precise method. To find the ideal sampling protocol we compared several measurement points within the pilot of the *Berlin Initiative Study*.

Iohexol was measured every 30 min. with 8 measurements (30-300 min.). The analysis compares 4 (60, 120, 180, 240) vs. 8 measurement points (add. 30, 90, 150, 300). A half logarithmic linear model was applied in each of these analyses to the last three observations; slopes and correlation coefficients were compared and correlated to age, weight, height, BMI, and serum creatinine.




From 71 subjects one had to be excluded due to low correlations. 46 male (median 77ys, range 70-96) and 24 female (77 ys, 71-88) subjects entered the analysis. Creatinine (1.06±0.36 vs. 0.81±0.29, p = 0.004) was significantly different between males and females. The regression slope was –0.0042±0.0010 for 4 measurement points and –0.0036±0.0010 for 8 measurement points ($p<0.001$) with a non-significant steeper slope for females for each method of analysis ($p<0.15$). The slopes were highly correlated (r=0.825) and the linear fit was better for 8 (r=-0.995) compared to 4 measurements (r=-0.992, p =0.063 Wilcoxon signed rank test). Correlations of regression slope with creatinine and weight were higher for 4 (r=0.73, r=0.30) vs. 8 measurements (r=0.63, r = 0.23). Correlation to age and height was higher for 8 (r = 0.44, r= 0.25) vs. 4 measurement points r=0.35, r=0.18). BMI was not correlated with the slope parameters.

Measurements with 8 data points are feasible in the elderly. The later measurement points seem to be more adequate to determine a constant filtration rate.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO290**

**Accurate Prediction of True Glomerular Filtration Rate (GFR) by Both Immediate Post-Angiography Iodixanol Clearance, and Pre-Angiography Estimated GFR** Susie L. Hu,[1] Fatemeh Akhlaghi,[2] Shripad D. Chitnis,[2] Ritche C. Chiu,[3] Subil C. Go,[4] Preeti Rout,[5] Michael Steffes,[6] J. Dawn Abbott,[7] Lance D. Dworkin,[1] Andrew G. Bostom.[1] *[1]Div of Kidney Dis & HTN, Brown University, Providence, RI; [2]Biomed & Pharmaceutical Sciences, University of Rhode Island, Kingston, RI; [3]Nephrology Assoc of SW Ohio Inc, Cincinnati, OH; [4]N Valley Nephrology, Yuba City, CA; [5]Div of Nephrology, BI Deac Medical Center, Boston, MA; [6]Laboratory Medicine & Pathology, University of MN, Minneapolis, MN; [7]Div of Cardiology, Brown University, Providence, RI.*

**Introduction:** Creatinine-based GFR formulas may be inaccurate due to wide variability of creatinine among patients with relatively preserved GFR. GFR measurement by iodinated contrast media elimination at routine angiography (angiography GFR) has been internally validated. We evaluated whether the angiography GFR more accurately reflects true GFR compared to estimation equations on a day-to-day basis.

**Methods and Results:** Eighteen subjects had determination of **1)** GFR by iodixanol clearance at routine coronary angiography, and **2)** estimated GFR (eGFR) using pre-angiography creatinine. They were validated against an outpatient standard GFR measurement (with 5-ml intravenous iodixanol bolus) performed at a 2-week interval. Patients were selected from 4 GFR categories (30 to >75 ml/min/1.73m$^2$). Subjects with acute kidney injury were excluded. Precision of the measured angiography GFR with respect to the standard measurement of GFR was high with $R^2$ of 0.78 (R=0.88). $R^2$ of eGFR by the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation using pre-catheterization creatinine levels was also 0.78. Precision by mean coefficient of variation was 13 and 12% respectively. Both methods of GFR determination had good accuracy with 83% of values ranging within 30% of the standard GFR, and 94% within 50% of the standard GFR.

**Conclusions:** GFR measurements using iodixanol elimination at routine coronary angiography and estimation equations using pre-catheterization creatinine are precise and accurate on a day-to-day basis. GFR measurement during routine angiography does not appear to be better than GFR determination with estimation equations.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO291**

**Estimation of GFR from Cystatin C and Creatinine in the General Population – Results from the Renal Iohexol-Clearance Survey in Tromsø 6 (RENIS-T6)** Bjorn Odvar Eriksen,[1,2] Ulla Dorte Mathisen,[1,2] Toralf Melsom,[1,2] Marit D. Solbu,[2] Trond G. Jenssen.[1,3] *[1]Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway; [2]Section of Nephrology, Clinic of Internal Medicine, University Hospital of North Norway, Tromsø, Norway; [3]Section of Nephrology, Department of Internal Medicine, Oslo University Hospital, Oslo, Norway.*

**Background** Although GFR estimated from cystatin C alone ($eGFR_{CYS}$) or combined with creatinine ($eGFR_{CYS\&CRE}$) predict cardiovascular mortality better than estimates from creatinine ($eGFR_{CRE}$), there is no evidence that existing cystatin C-based equations estimate GFR better than $eGFR_{CRE}$ in the general population. However, these equations were developed in patients with kidney disease. We studied the fit of regression models in the middle-aged general population to explore whether cystatin C can improve estimation of GFR.

**Method** A representative sample of 1593 persons (50 to 62 years) from the general population without diabetes, cardiovascular or kidney disease was studied with iohexol-clearance, enzymatically measured creatinine, and a turbidimetric cystatin C immunoassay. Non-linear methods were used to regress GFR on age, sex, height, weight, cystatin C and/or creatinine. Fit was measured as $R^2$ and root mean square error (RMSE) on the log-scale. The CKD-EPI equation is included for comparison.

**Results**

**Fit of models**

| Dependent variable | Independent variables | RMSE(95% CI) | | $R^2$(95% CI) | |
|---|---|---|---|---|---|
| Log(GFR) | Sex, log(age), log(creatinine) | 0.112 | 0.108, 0.116 | 0.387 | 0.349, 0.424 |
| Log(GFR) | Sex, log(age), log(cystatin C) | 0.114 | 0.110, 0.118 | 0.369 | 0.332, 0.406 |
| Log(GFR) | Sex, log(age), log(cystatin C), log(creatinine) | 0.103 | 0.099, 0.107 | 0.481 | 0.446, 0.516 |
| GFR* | Sex, height, weight, age, log(creatinine), cystatin C | 0.102 | 0.098, 0.106 | 0.494 | 0.458, 0.527 |
| CKD-EPI* | | 0.122 | 0.118, 0.126 | 0.296 | 0.259, 0.332 |

*fit measured on log-scale

**Conclusion** The fit of $eGFR_{CYS\&CRE}$ was superior to both $eGFR_{CYS}$ and $eGFR_{CRE}$. In the general population, equations combining both cystatin C and creatinine may improve GFR estimation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO292**

**The Discrepancy between Two Reference GFR Measurements: Plasma Clearance of $^{99m}$Tc-DTPA Used in China and a Modified Renal Clearance of Insulin Used in Japan** Li Zuo, Shanshan Dai. *Renal Division, Peking University First Hospital, China.*

**Background and objective:**Racial coefficient added to the original Modification of Diet in Renal Disease (MDRD) equation by Chinese and Japanese study was 1.233 and 0.81, respectively. Our hypothesis is that different reference GFR methods may contribute to such a big racial coefficients difference. In this study,dual plasma sampling $^{99m}$Tc-DTPA plasma clearance (CL_DTPA)used in Chinese study and modified inulin renal clearance(CL_IN) used in Japan were compared.

**Methods:**Plasma clearance of $^{99m}$Tc-DTPA and a modified renal clearance of inulin were performed simultaneously on each subject according to the original Chinese and Japanese study protocol,a single dose of 37MBq $^{99m}$Tc-DTPA was bolus injected in the forearm and inulin solution was continuously administrated intravenously after oral hydration.Blood drawing,urine collection and GFR calculation were performed according to the original studies.Paired t test, correlation analyze and Bland-Altman plot were used to compare the discrepancy of two methods.

**Results:**Thirty-nine CKD patients and 2 healthy volunteers were enrolled (15 females),the average age was 42.59±11.88 years.There was a significant difference between CL_DTPA and CL_IN by paired t-test ($p < 0.01$).The mean CL_DTPA was 53.18±29.98ml/min/1.73m$^2$,CL_IN was 40.17±25.50ml/min/1.73m$^2$.All CL_DTPA correlated well with CL_IN, and spearman correlate coefficient r was 0.976 ($p < 0.01$).The discrepancy between two reference GFR methods was shown in Figure 1,mean difference between CL_DTPA and CL_IN was 13.00±7.63ml/min/1.73m$^2$,CL_DTPA exceed CL_IN by 10.60 to 15.41ml/min/1.73m$^2$ ($p<0.01$).

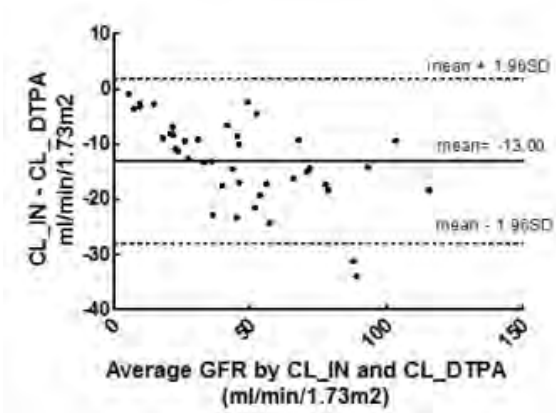



Figure 1 Bland-Altman plot of the CL-DTPA and CL-IN

**Conclusions:** Plasma clearance of $^{99m}$Tc-DTPA used in Chinese study was systemically higher than the modified inulin renal clearance used in Japanese study.This could partly explain why Chinese study got a higher racial coefficient.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO293

**Variation in the $^{125}$-I Iothalamate Glomerular Filtration Rate (GFR) Procedure in the Chronic Renal Insufficiency Cohort (CRIC) Study** Amanda Hyre Anderson, Wei Yang, J. Richard Landis, Dawei Xie, Valerie L. Teal, Harold I. Feldman. *University of Pennsylvania School of Medicine.*

In an effort to develop a more accurate GFR estimating equation for use in the NIDDK-sponsored Chronic Renal Insufficiency Cohort (CRIC) Study, approximately one-third of the cohort (i.e., the subcohort) was selected to have GFR measured using $^{125}$-I iothalamate clearance testing (iGFR). As part of the test, plasma and urine were collected during four 30-minute clearance periods after an initial equilibration period of 60 to 90 minutes. Period-specific iGFR was monitored to detect any systematic or large differences across testing periods as a quality control procedure given the stability of true GFR over the brief testing interval of 3 to 3.5 hours. Among subcohort members with four testing periods (N=1288; Table), iGFR was higher during the first period compared to the time-weighted average of periods 2 through 4 (delta = 3.97 mL/min/1.73m²; p-value = <0.001). Comparisons of time-weighted average iGFRs from all periods and from periods 2 through 4 revealed a mean (SD) difference of 1.15 (4.43) mL/min/1.73m². After developing two CRIC GFR estimating equations using; 1) data from all periods, and 2) data from all but the first testing period, a similar difference (SD) in estimated GFR of 1.11 (1.09) mL/min/1.73m² was observed. Using linear regression methods, several technical aspects of the iGFR test including time between saturated solution of potassium iodine administration and Glofil injection, time between Glofil injection and initiation of Period 1, and the discard urine volume were significant predictors of differences between average iGFRs before and after the exclusion of the first period. Exclusion of the first iGFR testing period provides an estimate of GFR that appears to more closely reflect true GFR within CRIC subcohort members.

Table. Description of iGFR testing periods for CRIC subcohort members (N=1288)

| | Period 1 | Period 2 | Period 3 | Period 4 | Overall time-weighted mean iGFR (Periods 1-4) | Time-weighted mean iGFR (Periods 2-4) |
|---|---|---|---|---|---|---|
| Mean (SD) iGFR, mL/min/1.73m² | 52.01 (25.66) | 49.11 (21.90) | 47.73 (20.80) | 47.59 (21.41) | 49.19 (20.91) | 48.04 (19.83) |

Disclosure of Financial Relationships: nothing to disclose

### TH-PO294

**New Adjusted Equations for Estimated GFR from Serum Creatinine in Korean** Beom Seok Kim,[1] Hoon Young Choi,[1] Ho Yung Lee,[1] Yong Kyu Lee,[1] Sung-Kyu Ha,[1] Sug Kyun Shin,[2] Seung-Ok Choi,[3] Ho-Jung Kim,[4] Kang Wook Lee,[5] Yang Wook Kim,[6] Yong-Lim Kim,[7] Yoshinari Yasuda,[8] Enyu Imai,[9] Masaru Horio,[9] Hirofumi Makino,[10] Seiichi Matsuo.[8] *[1]Yonsei Univ; [2]Ilsan Hospital; [3]Wonju Christian Hospital; [4]Hanyang Univ.; [5]Chungnam National Univ.; [6]Inje Univ.; [7]Kyungpook National Univ.; [8]Nagoya Univ.; [9]Osaka Univ.; [10]Okayama Univ.*

Introduction: Modification of Diet in Renal Disease (MDRD) Study equation is a standard method to estimate glomerular filtration rate (GFR). However, it's accuracy in Asians is often unsatisfactory. In this sub-study of the Asian Collaborative Study for Creation of GFR Estimation Equation (ACOS-CG-FREE) which is designed to develop the equation of estimated GFR (eGFR) for Asians, we focused on developing the modified eGFR for Korean population.

Methods: Patients with non-dialysis chronic kidney disease were enrolled. Measured GFR (mGFR) was computed from renal clearance of inulin (Cin). All the laboratory tests were performed at the central laboratory in Japan. The Korean coefficient was statistically determined by minimizing the sum of squared errors between eGFR and mGFR.

Results: Among 202 patients enrolled, 200 cases were analyzed as the development data set currently. Male to Female ratio was 56:44%. Mean age of the patients was 46.5 ± 16.7. The percentage of patients with diabetes mellitus and hypertension were 24.7% and 55.7%, respectively. From out data, modified eGFR equation in Korean was GFR (ml/min/1.73 m2) = 194 × Cr-1.094 × Age-0.287 (female × 0.739). With this equation, 30% accuracy was 57% (R = 0.907).

Conclusion: We showed that the eGFR equation obtained by this study is different from the standard MDRD study equation. Further study validating the accuracy of new eGFR equation should be considered to clinically apply this equation.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO295

**Equations To Estimate Creatinine Excretion Rate: The CKD Epidemiology Collaboration** Joachim H. Ix,[1,2] Christina Wassel,[2] Lesley A. Stevens,[3] Gerald J. Beck,[4] Marc C. Froissart,[5] Gerjan Navis,[6] Roger A. Rodby,[7] Vicente E. Torres,[9] Yaping Lucy Zhang,[3] Tom H. Greene,[9] Andrew S. Levey.[3] *[1]UC San Diego; [2]UC San Diego; [3]Tufts; [4]Cleveland Clinic; [5]Paris Descartes Univ.; [6]Univ. Med Center Groningen; [7]Rush; [8]Mayo Clinic; [9]Univ. of Utah.*

**Background:** Creatinine excretion rate (CER) can be used evaluate collection accuracy of timed urine specimens, but extremes of normal ranges vary by up to 100%, so collections may be deemed inaccurate only if grossly over -or under-collected. We aimed to develop and externally validate new CER equations using commonly available variables that mark muscle mass.

**Methods:** Individuals from 3 kidney disease studies (n=2466) were divided into development (random 2/3rd) and internal validation (1/3rd) datasets. Linear regression was used to develop new equations. Equation estimated CER was compared to measured 24-hr urine CER among 987 participants from 3 additional studies for external validation.

**Results:** Mean GFR was 60 ± 40 ml/min/1.73m², and measured CER (± SD) was 1400 ± 453 mg/day. Age, sex, race, weight, and serum phosphorus levels improved new equation fit in the development dataset. Because phosphorus is not always available in clinical practice we carried forward 2 equations (with or without serum phos). In external validation, the new CER equations showed little bias and moderate precision.

Performance of CER Estimation Equations in the External Validation Dataset (n=987)

| | % Difference (Measured-Estimated CER) | | Accuracy | | |
|---|---|---|---|---|---|
| | Median (95% CI) | IQR | $P_{15}$ (95% CI) | $P_{30}$ (95% CI) | RMSE (95% CI) |
| Equation without Phosphorus* | -0.7% (-2.6%, 1.0%) | 32% | 51% (47%, 54%) | 79% (76%, 81%) | 35.7 (33.1, 38.7) |
| Equation with Phosphorus** | 0.3% (-2.6%, 3.1%) | 27% | 54% (50%, 59%) | 81% (77%, 85%) | 33.9 (29.5, 37.8) |

* eCER = 880 + 12.5*weight - 6.2*age (+ 34.5 if black) (-379 if female)
** eCER = 1115 + 12*weight - 5.8*age - 60*phos (+ 53 if black) (- 368 if female)

Abbreviations: eCER=estimated CER; IQR=interquartile range; CI=confidence interval; P15=percent of estimated CERs within 15% of measured CERs; P30=percent of estimated CERs within 30% of measured CERs; RMSE=Root mean squared error

**Conclusions:** Common clinical variables can estimate expected CER, to which measured CER can be compared. The new equations may facilitate evaluation of accuracy of timed urine specimens.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO296

**Performance of Equations To Estimate 24-Hour Creatinine Excretion in Healthy Subjects and Renal Transplant Recipients** Steef Jasper Sinkeler,[1] Stephan J. L. Bakker,[1] Joachim H. Ix,[2] Gerjan Navis.[1] *[1]Nephrology, University Medical Center Groningen, Groningen, Netherlands; [2]Medicine, Division of Nephrology, University of California San Diego, San Diego, CA.*

24h urine allows assessment of renal function, proteinuria and dietary factors but is fraught with collection errors. As 24h creatinine excretion (CER) derives from muscle mass the appropriate 24h CER can be predicted from anthropometric data and thus serve to identify collection errors. Several formulas derived from CKD cohorts were proposed for CER estimation. We tested performance in renal transplant recipients (RTR) and healthy subjects.

595 RTR (mean age 52, 42% female, all Caucasian, mean GFR (iothalamate) 43 mL/min/1.73 m², mean time after transplantation 7 yr) and 263 healthy subjects (mean age 53, 52% females, all Caucasian, mean GFR 100 mL/min/1.73m²) were studied. The formula by Cockroft & Gault (eCERCG) (28 – 0.2*Age) *Body Weight (*0.85 if female) and 2 new equations by Ix et al. were used to estimate CER (eCER): eCERsimple=879.89+12.51*Weight–6.19*Age(-379.42 if female) and, when serum phosphorus was available, eCERphos = 1115.89+11.97*Weight–5.83*Age–(60.18*[Serum Phosphorus]) (-368.75 if female) .

In RTR median CER was 1267 [inter-quartile range (IQR) 532], eCERsimple 1434 [528], eCERphos1465 [519] and eCERCG 1266 [534] mg/day. R² for both eCERsimple and eCERphos was 0.43, and for eCERCG 0.41. Median bias was 7.0% [IQR 30.0%] for eCERsimple, 9.5% [31.1%] for eCERphos and 1% [31.2%] for eCERCG, and 15%-accuracy was 51, 48 and 49%; the 30%-accuracy 78, 75 and 83%, respectively.

In healthy subjects median CER was 1458 [628], eCERsimple 1375 [542], eCERphos 1391 [538] and eCERCG 1261 [470]. R² for both eCERsimple and eCERphos was 0.52 and for eCERCG 0.43. Median bias was -9.3% [19.4%] for eCERsimple and -8.3% [23.3%] for eCERphos, as compared to -14.7% [34.3%] for eCERCG, with 15%-accuracy of 51, 52 and 37%; the 30%-accuracy was 87, 87and 76%, respectively.

Thus, empirical equations estimate CER reasonably well in RTR and healthy subjects with precision corresponding to data in CKD. Their use can improve the suitability of 24h urine for monitoring of renal function and dietary factors.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO297

**Detection of Early Impairment of Glomerular Filtration Rate: Serum Creatinine Versus Low-Molecular Weight Proteins** Carlo Donadio,[1] Danika Tognotti,[1] Angeliki Kanaki,[1] Elena Donadio.[2] *[1]Internal Medicine, University, Pisa, Italy; [2]Biochemistry, University, Pisa, Italy.*

Serum creatinine is an efficient marker of GFR impairment only at CKD stages ≥3b (GFR<45 mL/min/1.73 m$^2$), while serum concentrations of low molecular weight proteins (LMWP) have been proposed as a more sensitive marker of GFR impairment.

Aim of this study was to assess the accuracy of serum levels LMWP (cystatin C, Cys; beta2-microglobulin, β2M; retinol-binding protein, RBP; beta-trace protein, BTP) as indicators of impairment of GFR, in comparison with serum creatinine (Creat) and urinary excretion of albumin (U-Alb).

Two hundred and ninety-five adult CKD patients (F 137), affected by different kidney diseases with various impairment of renal function (S-Creat 0.40-12.1 mg/dL), participated to this study. GFR was measured as the renal clearance of $^{99m}$Tc-DTPA. Creat, Cys, β2M, RBP, BTP and U-Alb were measured with standard laboratory methods.

Serum concentration of all markers increased with the reduction of GFR. The slight increases found at CKD stage 2 resulted already statistically significant versus the values found in the group of CKD at stage 1. Statistical significance was higher for S-Creat, S-β2M and S-BTP than for S-Cys and S-RBP. Some differences among the different markers were found according to the gender of patients. In any case, a very high correlation was found between GFR, and serum Creat (r=0.9254), Cys (r=0.9361), β2M (r=0.9381), and BTP (r=0.9251). The lowest correlation was that of RBP (r=0.6917). In general, the criterion values to screen the same GFR impairment were higher for male than for female patients. Furthermore, to screen patients with mild impairment in GFR, one must use criterion values lower than those necessary to screen patients with a more advanced impairment in GFR. All serum markers showed a similar accuracy as indicators of different degrees of GFR impairment, except RBP, which resulted significantly less accurate.U-Alb was inadequate as indicator of any level of GFR impairment.

In conclusion, serum levels of LMWP are probably not more sensitive or accurate than serum creatinine as an early indicator of GFR impairment. U-Alb gives no information on GFR impairment.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO298

**Cystatin C, Mortality Risk, and Clinical Triage in US Adults: Threshold Values and Hierarchical Importance** Robert N. Foley,[1,2] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

It has been suggested that cystatin C may be a superior measure of estimated glomerular filtration rate (eGFR) than creatinine-based methods. The utility of cystatin C for clinical triage in community-based settings, however, is unknown. Hence, we identified cystatin C thresholds that maximize sensitivity and specificity ($Max_{Sn+Sp}$) for predicting death, and subsequently applied classification tree methodology considering serum creatinine, creatinine-based eGFR, urinary albumin-creatinine ratio, and conventional modifiable risk factors to define subgroups, interactions, and hierarchical ranks in fasting US adults (National Health and Nutrition Examination Survey 1988-1994, followed through 2006). A threshold cystatin C value of 0.94 mg/L exhibited the best maximum combined value of sensitivity and specificity for predicting death ($Max_{Sn+Sp}$, Sn 0.64/Sp 0.78). When all variables were considered jointly in a classification tree, cystatin C and albumin-creatinine ratio were the primary mortality discriminators in subgroups that added up to 41% and 14% of the study population, respectively; serum creatinine and creatinine-based eGFR were non-discriminatory. Cystatin C may be useful for risk-based clinical triage in public health settings.

Disclosure of Financial Relationships: Consultancy: Affymax,Amgen,Ortho,Luitpold, Merck, Novartis.

### TH-PO299

**UK Prevalence of Chronic Kidney Disease (CKD) and Associated Cardiovascular (CV) Co-Morbidities: The Baseline Data from the QI-CKD Study** Hugh Gallagher,[1] Charles Tomson,[2] Kevin P. G. Harris,[3] Simon de Lusignan.[4] *[1]St Helier Hospital; [2]Southmead Hospital; [3]Leicester General Hospital; [4]St George's University of London.*

**Background:**

The high quality of computerised medical records in UK family practice provides a rich source of data for use in quality improvement and research. Here we report the prevalence of CKD in the UK from a very large primary care dataset, using stringent criteria for case definition. We also compare the abilities of the four-variable MDRD and CKD-EPI formulae to identify those at higher risk of CV disease.

**Method:**

Analysis of routine data collected from 930,997 patients registered with 139 general practices across England. Data were extracted to enable the calculation of eGFR using the MDRD and CKD-EPI formulae. We took into account whether local creatinine assays were traceable to the reference IDMS method. CV co-morbidities were documented.

**Results:**

The UK prevalence of stage 3 to 5 CKD (defined using two measurements of eGFR <60 ml/min/1.73m$^2$ at least three months apart with no interim values >60 ml/min/1.73m$^2$) is 5.41% (7.34% in females and 3.48% in males) using MDRD. Assuming white ethnicity in all and ignoring interim values gives prevalence estimates of 5.49% and 5.55% respectively. Just using the latest eGFR (as in NHANES and NEOERICA) inflates the figure to 6.4%. Using CKD-EPI and the full case definition, the prevalence is lower at 4.76% (6.14% in women and 3.39% in men).

Ischaemic heart disease is recorded amongst 15.3% of patients with CKD3a (compared with 6.5% in those with eGFR>90 ml/min/1.73m$^2$), 26.9% in CKD3b, 29.2% in CKD4, and 28.5% in CKD5. A major increase in prevalence between CKD3a and 3b is also seen for other CV co-morbidities.

The prevalence of CV co-morbidities in stages 3a, 3b, and 4 is approximately 10% higher using CKD-EPI compared with MDRD.

**Conclusions:**

The application of strict case definition criteria to high quality routinely collected data results in a lower estimate of prevalence of stage 3 to 5 CKD than that previously reported in the UK, whether the MDRD or CKD-EPI is used. CV co-morbidities are far more prevalent in CKD3b than 3a. CKD-EPI identifies a smaller, higher-risk population as CKD3a.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO300

**Changes in Estimated Glomerular Filtration Rate (eGFR) in Apparently Healthy Adults with an Elevated High-Sensitivity C-Reactive Protein (hsCRP) Level in the JUPITER (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin) Trial** Michael D. Cressman,[1] Donald G. Vidt,[2] John Monyak.[1] *[1]AstraZeneca; [2]Cleveland Clinic Foundation.*

**Purpose:** The aim of this analysis was to assess changes in eGFR among a population of apparently healthy adults across a range of baseline eGFR levels. **Methods:** JUPITER subjects were apparently healthy men ≥50 or women ≥60 years of age with an hsCRP ≥2.0 and an LDL-C <130 mg/dL. Changes in eGFR were assessed using the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations among 16 279 evaluable participants in JUPITER. Changes from baseline in eGFR were determined in all subjects in the placebo and rosuvastatin groups, and in subsets with baseline eGFR ≥90, 60–89, or <60 mL/min/1.73 m$^2$. **Results:** Mean (SD) serum creatinine increased 0.10 (0.16) mg/dL and 0.09 (0.18) mg/dL in the placebo and rosuvastatin groups, respectively, after a mean follow-up of 2.3 yr (p=0.0045). Reductions in eGFR (mL/min/1.73 m$^2$) were greater with placebo vs rosuvastatin based on both the MDRD (–8.4 [13.3] vs –7.9 [13.5], p=0.0007) and the CKD-EPI equation (–7.4 [12.1] vs –6.8 [12.2], p=0.0004). Reductions in eGFR (placebo and rosuvastatin groups) were apparent in study participants with a baseline eGFR ≥90 [MDRD: –22.6 (14.3) and –22.3 (14.5); CKD-EPI: –19.3 (11.2) and –18.7 (11.1)] or in those with a baseline eGFR 60–89 [MDRD: –7.0 (10.9) and –6.3 (11.3); CKD-EPI: –7.5 (11.2) and –6.7 (11.5)] but not in subjects with baseline eGFR <60 [MDRD: 0.2 (8.4) and 0.3 (8.5); CKD-EPI: 0.2 (8.4) and 0.3 (8.6)]. **Conclusions:** In JUPITER, reductions in eGFR based on the MDRD or CKD-EPI equation were greater than expected among individuals with baseline eGFR levels over 60 mL/min/1.73 m$^2$. Results of longitudinal studies that include individuals with higher eGFR levels may be misleading when eGFR is used to assess change in renal function.

Disclosure of Financial Relationships: Employer: AstraZeneca; Ownership: AstraZeneca.

### TH-PO301

**eGFR Variability in Primary Care: Normal Variation or Portending Increased Risk? Work in Progress** Christopher K. T. Farmer,[1] Jean Irving,[1] Edmund J. Lamb,[2] Adeera Levin,[3] Paul E. Stevens.[1] *[1]Kent Kidney Care Centre, East Kent Hospitals, Canterbury, Kent, United Kingdom; [2]Clinical Chemistry, East Kent Hospitals, Canterbury, Kent, United Kingdom; [3]Division of Nephrology, St. Paul's Hospital, University of British Columbia, Vancouver, BC, Canada.*

Introduction

Biological and analytical variability of serum creatinine (SCr) is ~ 5%, but in the real world may be greater as sampling is not standardised, leading to variation in reported eGFR and uncertainty in clinical decision making.

Aim

To describe the variability in a large primary care cohort with stable renal function over time.

Methods

Patients with ≥4 SCr tests and a rate of decline of eGFR <2 mL/min/1.73m$^2$/y over at least 2 y were identified from a primary care population of 279,000. Demographic, co-morbidity and prescription data were recorded. Patients were stratified by baseline eGFR. The number of excursions of SCr >26.4 μmol/L from baseline was the outcome of interest.

Results

47,191 of 128,370 pts with SCr tests fulfilled the entry criteria, 20% had DM and 68% hypertension, total number of SCr tests was 465,346 (mean 10/pt). 3111 (6.6%) of pts had significant excursions during follow up. DM, hypertension and medication were predictors of excursions (Table).

| GFR | Patients | Mean age | SCr excursions >26.4 umol/L | OR for excursions ( 95%CI) | | |
|---|---|---|---|---|---|---|
| (mL/ min/1.73m²) | (n) | (y) | Patients (number ) | Diabetes | Hypertension | Drugs* |
| All | 47971 | 59 | 3111 (6652) | 1.37 (1.29-1.45) | 1.15 (1.08-1.22) | 1.21 (1.14-1.30) |
| >90 | 9963 | 51 | 354 (534) | 1.08 (0.91-1.28) | 1.14 (0.97-1.33) | 1.13 (0.95-1.33) |
| 60-89 | 28276 | 58 | 1194 (2031) | 1.61 (1.47-1.77) | 1.14 (1.03-1.25) | 1.21 (1.10-1.34). |
| 45-59 | 6787 | 68 | 806 (1736) | 1.44 (1.27-1.62) | 1.02 (0.90-1.15) | 1.13 (0.98-1.30) |
| 30-44 | 1827 | 73 | 582 (1621) | 1.20 (1.01-1.41) | 1.03 0.86-1.22) | 1.07 (0.89-1.29) |
| <30 | 338 | 72 | 175 (730) | 1.02 (0.72-1.45) | 0.97 (0.70-1.33) | 1.02 (0.73-1.40) |

*Patients on one or more of ACE/ARB, Diuretic, NSAID

Conclusion

Significant eGFR variability in patients with stable renal function over time is uncommon and predicted by comorbidity and prescription data.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO302

**Pre-CKD: A Novel Risk Factor for CKD in Older Adults** C. Barrett Bowling,[1,2] Ruth C. Campbell,[2] Marjan U. Mujib,[2] Arnold B. Alper,[4] George L. Bakris,[3] Richard M. Allman,[2,1] Wilbert S. Aronow,[5] L. Lee Hamm,[4] Paul W. Sanders,[2,1] Ali Ahmed.[2,1] *[1]Birmingham VAMC; [2]UAB; [3]UChicago; [4]Tulane; [5]NY Medical College.*

**Purpose**: To determine the prevalence and effect of pre-CKD, defined as eGFR 60–89 ml/min/1.73m² on incident CKD.

**Methods**: Of the 5125 community-dwelling older adults ≥65 yrs in the original Cardiovascular Health Study, 2878 had data on eGFR (CKD-EPI) at baseline (BL) and year 9 (Y9). Of these, 2270 had eGFR ≥60 ml/min/1.73m², of which 1804 had pre-CKD. Propensity scores for pre-CKD were used to assemble a cohort of 428 pairs with (w) and without (w/o) pre-CKD who were balanced on 62 BL characteristics. Logistic regression models were used to estimate the association of BL pre-CKD with incident CKD at Y9.

**Results**: Participants (n=856) had a mean age of 70 (±3) yrs and 79% women and 4% Afr. Amer. Incident CKD occurred in 34% and 8% of matched pts w and w/o pre-CKD respectively (OR, 6.28; 95%CI, 4.16–9.48; p<0.001; **Table**). Pre-CKD also predicted incident eGFR <45 (OR, 6.58; 95%CI, 2.27–19.06; p=0.001) which occurred in 6% and 1% of those w and w/o BL pre-CKD respectively. Pre-match associations and the dose-response association between pre-CKD and new-CKD are displayed in the **Table**.

**Conclusions**: Among older adults w/o CKD, pre-CKD (eGFR 60–89) is common and associated with increased risk of incident CKD.

Association of pre-CKD with incident CKD

| | Matched OR (95% CI) | Unadjusted OR (95% CI)* | MV-adjusted OR (95% CI)*† |
|---|---|---|---|
| No pre-CKD (eGFR ≥90 ml/ min/1.73 m²) | 1 | 1 | 1 |
| Pre-CKD (eGFR 60–89 ml/ min/1.73 m²) | 6.28 (4.16–9.48); p<0.001 | 6.05 (2.45–14.90); p<0.001 | 7.52 (5.13–11.02); p<0.001 |
| Dose-response | | | |
| eGFR 60–74 ml/min/1.73 m² | 14.01 (8.62–22.78); p<0.001 | 16.51 (11.37–23.95); p<0.001 | 20.06 (13.25–30.37); p<0.001 |
| eGFR 75–89 ml/min/1.73 m² | 3.84 (2.44–6.04); p<0.001 | 3.79 (2.61–5.51); p<0.001 | 4.25 (2.86–6.33); p<0.001 |

* Based on 2270 pre-match cohort, † Adjusted for all 62 baseline covariates used in the propensity model

Disclosure of Financial Relationships: nothing to disclose

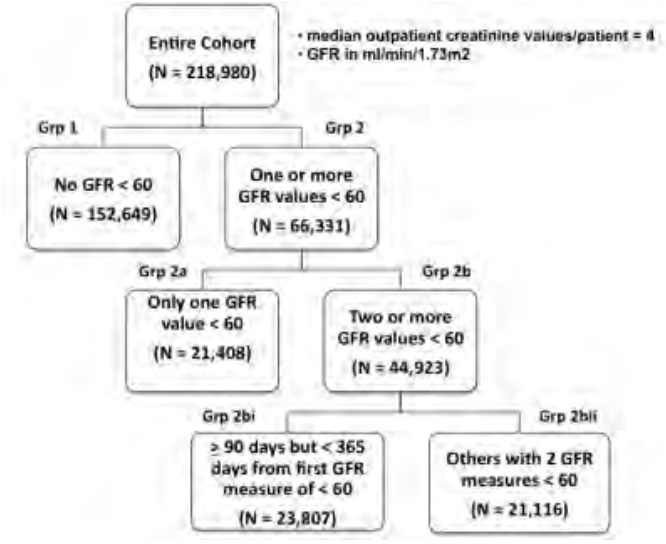
### TH-PO303

**Validity of Definition and Classification of Chronic Kidney Disease (CKD) in Observational Databases** Charuhas V. Thakar,[1,2] Christine Edie,[2] Kristen Schmitt,[2] Loretta Simbartl.[2] *[1]University of Cincinnati, Cincinnati, OH; [2]Cincinnati VA, Cincinnati, OH; [3].*

K/DOQI guidelines define CKD as estimated glomerular filtration rate (GFR) < 60 ml/min/1.73m2 measured > 90 days but < 365 days apart. Criteria to define CKD in a retrospective observational cohort are unclear.

We examined validity of classification of CKD in a multi-center cohort of 218,980 veterans seeking care across 5 Veterans Affairs healthcare systems in Ohio between 1/1/06 and 4/1/10. All outpatient creatinine values during the study were extracted, along with demographic information (age at first creatinine, gender, race). Primary outcome was all cause mortality. Estimated GFR was expressed in ml/min/1.73m2. Patients were classified into 5 groups based on number of GFR measures < 60, and compared to those with no GFR measures < 60 (Figure). Logistic regression models tested the association of GFR groups with mortality after adjusting for age, gender and race; results were expressed as odds ratios (OR) and 95% confidence intervals (CI).

The sample was 93% male, median age was 62 yrs. Prevalence of CKD was 30% defined as only one GFR measure < 60, 20.5% defined as any two GFRs < 60, and 11% when defined as two consecutive GFRs of < 60, > 90 but < 365 days apart.




Mortality was 4.4% in Grp 1 vs 13% in Grp 2 (Chi-square p < 0.0001). In multivariable models, the OR of mortality associated with different groups, with Grp 1 as a reference, were: Grp 2: 1.76 (95% CI, 1.70, 1.82); Grp 2a: 1.89 (95% CI, 1.80, 1.99); Grp 2b: 1.67 (95% CI, 1.61, 1.74); Grp 2bi: 1.44 (95% CI, 1.37, 1.51), and Grp 2bii: 1.88 (95% CI, 1.79, 1.97).

In an observational cohort, even a single measure of GFR < 60 is associated with increased risk of mortality; magnitude of mortality risk is comparable in patients with 2 GFR values < 60, regardless of timing of measurement.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO304

**Prevalence of Chronic Kidney Disease in the Chinese, South Asian and White Populations of Alberta, Canada** Joslyn D. Conley,[1] Sony Brar,[1] Hude Quan,[1] Braden J. Manns,[1] Marcello Tonelli,[2] Brenda Hemmelgarn.[1] *[1]University of Calgary, Alberta Kidney Disease Network; [2]University of Alberta.*

The reported prevalence of chronic kidney disease (CKD), eGFR < 60mL/min/1.73m², is highly variable among ethnic groups. The purpose of this study was to determine the prevalence of CKD for three ethnic groups in Alberta.

Using the Alberta Kidney Disease Network province-wide laboratory database we identified a cohort (n=777,354) of people with at least one out-patient measurement from January – December 2005 in Alberta, Canada. Chinese (4.09%) and South Asian (3.98%) populations were established using validated surname identification algorithms. eGFR was calculated using the MDRD equation, with the Alberta Health and Wellness registry file (n=2,397,981) used to determine the denominator.

The age and sex adjusted prevalence of CKD was highest in the white population (5.46%), followed by South Asians (3.65%) and Chinese (2.94%).

Table: Age and sex adjusted prevalence of CKD by eGFR stage and ethnicity

| eGFR | Total | White | South Asian | Chinese | P* |
|---|---|---|---|---|---|
| >60 mL/min | 94.74 | 94.53 | 96.34 | 97.06 | <0.01 |
| 46-60 mL/min | 3.81 | 3.97 | 2.62 | 2.05 | <0.01 |
| 30-45 mL/min | 1.11 | 1.15 | 0.77 | 0.62 | <0.01 |
| <30 mL/min | 0.34 | 0.35 | 0.26 | 0.27 | <0.01 |

* chi-square test of ethnic groups

The prevalence of CKD in South Asian and Chinese ethnic groups is lower than the white population, and similar to those reported from their countries of origin.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO305

**CKD among Disadvantaged Populations in Mexico** Guillermo G. Garcia,[1] Hector R. Perez,[1] Alfonso Gutierrez,[1] Martha Mendoza,[1] Librado De la Torre-Campos,[1] Karina Renoirte,[1] Marcello Tonelli.[2] *[1]Nephrology, Hospital Civil de Guadalajara, Guadalajara, Mexico; [2]Nephrology, University of Alberta, Edmonton, Canada.*

CKD is a major cause of morbidity and mortality in Mexico. In other nations, risk factors for CKD are disproportionally higher among individuals with low educational levels. Since lower education is itself a potentially reversible risk factor, this warrants further study.

Since September 2006, the Fundacion Hospitales Civiles de Guadalajara has used mobile units to screen for CKD in residents of poor communities in Jalisco, Mexico. Potential participants were informed of risk factors for CKD. We excluded individuals with known CKD and < 18 years of age. Trained personnel collected demographic and clinical data and collected blood and urine samples for selected tests. Between 09/2006 and 12/2009 9,619 were screened. The majority of participants (56%) had less than a primary school education. Findings were compared according to the level of education.

Results

| | ≤primary school or illiterate n= 5260 | ≥junior High School n= 4069 | p |
|---|---|---|---|
| Age (y) | 58.5 ±12.7 | 50.9 ±13.6 | 0.0001 |
| Male (%) | 1279 (24.3) | 1452 (35.7) | 0.0001 |
| Known DM (%) | 2409 (45.9) | 1510 (37.1) | 0.0001 |
| Blood glucose (mg/dL) | 148.2 ± 75.8 | 138.1 ± 71.2 | 0.0001 |
| >126 mg/dL (%) | 2249 (43.3) | 1432 (35.8) | 0.0001 |
| Known HTN (%) | 2866 (54.5) | 1921 (47.2) | 0.0001 |
| SBP≥140 or DBP≥90 (%) | 2894 (55.2) | 1888 (46.6) | 0.0001 |
| BMI (Kg/m²) | 29.5±5.3 | 29.3 ±5.4 | 0.039 |
| +dipstick proteinuria (%) | 969 (19.3) | 757 (20.8) | 0.081 |
| Current smoker (%) | 894 (17.0) | 802 (19.7) | 0.001 |
| eGFR (ml/min/1.73 m²) | 77.1 ±27.8 | 80.5 ±22.4 | 0.0001 |
| eGFR<60 ml/min/1.73 m²) (%) | 901 (17.1) | 487 (12.0) | 0.0001 |

We conclude that 1) the prevalence of CKD and its risk factors are higher among individuals with low level or no education. 2) Consideration should be given to target this high risk population in programs aimed to prevent CKD and its risk factors in Mexico.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO306

**A Nationwide Study of Mass Urine Screening Tests on Korean School Children and Implications for Chronic Kidney Disease Management**

Byoung-Soo Cho,[1] Won-Ho Hahn,[1] Jin-Soon Suh,[1] Hae Il Cheong,[2] Inseok Lim,[3] Cheol Woo Ko,[4] Tae-Sun Ha,[5] Su-Young Kim,[6] Dae-Yeol Lee.[7] *[1]Department of Pediatrics, Kyung Hee University Hospital; [2]Seoul National University Children's Hospital; [3]Yongsan Hospital, Chung-Ang University College of Medicine, Seoul; [4]Kyungpook National University Hospital, Daegu; [5]Chungbuk National University Hospital, Cheongju; [6]Pusan National University Hospital, Gyeongnam; [7]Chonbuk National University Hospital, Jeonju, Korea.*

We report the findings of a school urinary screening program that analyzed pediatric patients with proteinuria and/or hematuria. Between 1999 and 2008, 5,114 children were referred to pediatric nephrologists at 7 hospitals. These children had isolated hematuria (IH) (72.82%), isolated proteinuria (IP) (10.79%) or combined hematuria and proteinuria (CHP) (16.3%). Renal biopsies were performed on 1478 children (28.79% of total subjects; 26.77% in IH, 9.09% in IP and 51.19% of CHP) who showed abnormal renal function, persistent hematuria and/or proteinuria for more than 6 months, nephrotic-range proteinuria, or those with underlying systemic diseases. The most common findings in renal biopsies are IgA nephropathy in 38.97%, mesangial proliferative glomerulonephritis in 24.29%, and thin basement membrane nephropathy in 13.13%, respectively. Chronic kidney disease (CKD) was detected in 25.17% of all visiting subjects. Compared with the relative frequency of renal diseases associated with urinary abnormalities, the CHP group (46.9%) and nephrotic-range proteinuria group (69.96%) had more frequent CKD than the others. Abnormal findings on the renal ultrasound with or without Doppler scan were noted in 462 cases (suspected nutcracker phenomenon, 159; increased parenchymal echogenicity, 92; hydronephrosis, 75; simple cyst, 47). In conclusion, mass urine screening tests could detect asymptomatic CKD in its early stages. Initial aggressive diagnosis and treatment for CHP and nephrotic-range groups may prove helpful as interventions that delay CKD progression. These findings may inform the development of diagnostic and management guidelines for relatively mild urinary abnormalities, such as IH or low-grade IP.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO307

**Measured *vs.* Estimated GFR in the Detection of Early GFR Decline in Type 1 Diabetes Patients**

Aleksandra Kukla, Maria Luiza A. Caramori, John H. Eckfeldt, Michael Mauer. *University of Minnesota.*

Early GFR decline is difficult to detect. Serum creatinine changes over time are commonly used, however, this may not be sufficiently sensitive. We assessed, whether creatinine and cystatin C based GFR estimates (eGFR) could detect early measured GFR changes in 244 normoalbuminuric (NA) normotensive type 1 diabetic patients (pts) in the Renin Angiotensin System Study (RASS).

**Methods**: Pts underwent iohexol GFR measurements (iGFR) at baseline and at 5 yrs. MDRD-IDMS [eGFR(MDRD)], Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) and cystatin C [eGFR(cysC)] eGFRs were also calculated at these times.

**Results**: eGFR(CKD-Epi) and eGFR(MDRD) underestimated while eGFR(cysC) overestimated (perhaps partially explained by methodological changes) iGFR (p<0.001 for each). Correlations with iGFR were: eCKD-Epi, r=0.49; eGFR(MDRD), r=0.49; eGFR(cysC), r=0.35 (p<0.001 for each). GFR declined by all 4 methods (p<0.001 for each; see table; *in ml/min/1.73m*).

| N=244 | iGFR | eGFR(CKD-Epi) | eGFR(MDRD) | eGFR(cysC) |
|---|---|---|---|---|
| Baseline | 128±19 | 119±15 | 116±27 | 139±22 |
| 5 years | 120±21 | 115±14 | 110±25 | 136±20 |
| baseline-5 years | -7.6±15.9 | -3.6±9.6 | -5.8±20 | -3.0±18 |

The decline, numerically greatest by iGFR, was not statistically different from that for eGFR(MDRD) but it was less for eGFR(CKD-Epi) and eGFR(cysC) *vs*. iGFR (p=<0.001 for both) and for eGFR(CKD-Epi) (p=0.008) and eGFR(cysC) (p=0.02) *vs*. eGFR(MDRD). The decline by iGFR was weakly correlated with those by eGFR(MDRD) (r=0.24, p<0.001), eGFR(CKD-Epi) (r=0.28, p<0.001) and eGFR(cysC) (r=0.39, p<0.001). A 2 arm clinical trial powered for an 80% probability to detect a 50% reduction in the observed GFR decline would require a total of 552 pts for iGFR, 896 for eGFR(CKD-Epi), 1,496 for eGFR(MDRD), and 4,524 for eGFR(cysC).

**Conclusions:**

All 4 methods detected GFR decline over 5 yrs in NA T1DM subjects. For detecting subtle GFR changes in individual patients eGFR(CysC) was most closely correlated with iGFR changes, but if iGFR is considered the gold standard, no eGFR method accurately predicted these early changes. For clinical trials aiming to slow early GFR loss, iGFR is far superior. ** for the RASS Group.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO308

**Quantile Regression Better Estimates Effect of Diabetes on eGFR**

Mihriye Mete,[1] Nawar M. Shara,[1,2] Barbara V. Howard,[1,2] Jason G. Umans.[1,2] *[1]Medstar Health Research Institute, Hyattsville, MD; [2]Georgetown University, Washington, DC.*

**Background**: Estimated glomerular filtration rate (eGFR) is commonly associated with diabetes mellitus (DM) and other covariates using multiple linear regression models (MLRM). However, MLRM may obscure important associations when the covariates exert differential effects across the distribution of the outcome. Such differentiation would be expected for DM, which causes hyperfiltration at higher levels of GFR and accelerated chronic kidney disease (CKD) at lower levels. We quantified the impact of DM on eGFR (MDRD) using quantile regression (QR) and compared these results to those of an MLRM.

**Methods**:Data from 3,137 American Indians in the Strong Heart Family Study were used to examine relationships between DM and eGFR. MLRM provide a single set of coefficients estimating the mean response of eGFR to DM. In contrast, QR estimates the response in different locations across the eGFR curve, allowing varying covariate effects.

**Results**: Results of the MLR and QR models of eGFR on DM, adjusted for age, sex, hypertension, and protein intake, are shown (Table). MLRM estimate that eGFR is, on average, 14 mL/min/1.73m2 higher for participants with DM (p<0.001) than for those without DM. QR results show that the effect of DM varies across eGFR percentiles, revealing the expected negative effect of DM on eGFR (-30 to -0.5) at lower percentiles and suggesting diabetic hyperfiltration at higher percentiles. Indeed, at eGFR values ≥ the 25th percentile (eGFR≥ 83 in our sample), DM increased eGFR by 9-25. Both these positive and negative effects of DM were masked in the MLRM.

**Conclusions**: MLRM fail to capture the varying effects of DM on kidney function by masking differential impacts at the low and high ends of the eGFR distribution. QR reveals both hyperfiltration and accelerated CKD due to DM in our population and may improve mechanistic and genetic inferences from observational studies.

Effect of DM on eGFR estimated by MLRM vs. QR

| MLRM (95% CI) | QR estimates at different percentiles (95% CI) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1st | 5th | 10th | 25th | 50th | 75th | 95th |
| 14(11,16) | -30(-50,-9) | -8(-23,7) | -0.5(-6,5) | 9(5,12) | 14(11,17) | 18(14,21) | 25(22,28) |

Disclosure of Financial Relationships: nothing to disclose

## TH-PO309

**Repeat Testing Significantly Reduces the Estimated Prevalence of Chronic Kidney Disease and Identifies a Population with Increased Cardiovascular Risk**

Matthew O. Brook,[1] Matthew James Bottomley,[1] Alena Svistunova,[3] Aleh Kalachik,[3] Paul N. Harden.[1] *[1]Oxford Kidney Unit, Churchill Hospital, Oxford, United Kingdom; [2]Department of Biochemistry, John Radcliffe Hospital, Oxford, United Kingdom; [3]National Centre for Nephrology, Minsk, Belarus.*

**Purpose**

To investigate the impact of repeat testing of renal function on estimated prevalence of chronic kidney disease (CKD) and cardiovascular risk.

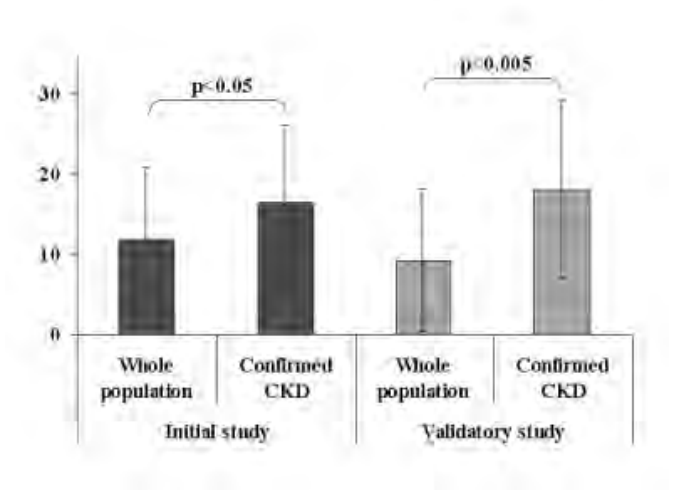
**Methods**

We carried out 2 observational studies in Minsk, Belarus. Blood and urine samples were taken with repeat testing 3 months later to confirm the diagnosis of CKD. 10-year general cardiovascular disease risk was calculated using criteria determined by the Framingham Study.

An initial study recruited 512 participants. Of these, 142 with abnormal or near abnormal results consented to repeat samples. A second validatory study recruited 528 individuals. 214 provided follow up samples 3 months later.

**Results**

Repeat testing reduced the estimated prevalence of CKD from 8.2% (initial study) and 10.8% (validatory study) based on single point testing to 4.1% and 4.2% respectively. Figure 1 demonstrates elevation of mean 10-year general cardiovascular risk in those with CKD confirmed by repeat testing over that of the whole population.




Importantly, cardiovascular risk in those individuals with only a single result indicating CKD was the same as the general population.

Finally, we identified a significant elevation in systolic hypertension in those with confirmed CKD in both studies (140 vs 148, p<0.05 and 133 vs 150, p<0.05).

**Conclusion**

We demonstrate that repeat testing reduces estimated prevalence of CKD and reveals a population with greatly increased cardiovascular risk likely related to systolic hypertension.

We conclude that studies based on a single test are likely to signficantly overestimate CKD prevalence and underestimate cardiovascular risk.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO310**

**High Prevalence of Undiagnosed Kidney Disease in Those Presenting with Troponin Positive Acute Coronary Syndrome** Christopher W. M. Horner, Sumith C. Abeygunasekara, Galil Rahman Ali. *Dept of Nephrology, Division of Medicine, Broomfield Hospital, Chelmsford, United Kingdom.*

*Aim*

The aim was to assess the prevalence of CKD in those presenting to a District General Hospital (DGH) with troponin positive acute coronary syndrome (ACS) as compared to a sample of the general population and determine the proportion of undocumented CKD in this patient group.

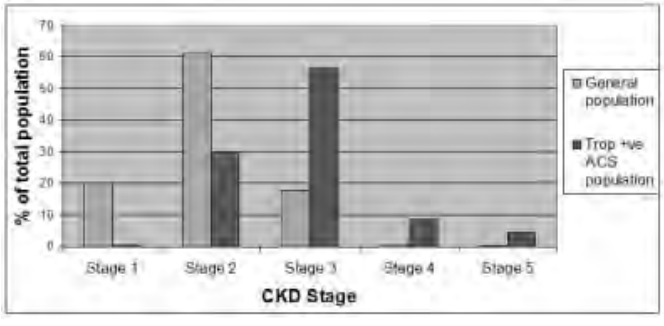
*Methods*

A retrospective observational study. Data was collected from proformas completed during the 18 month period from 01/11/07 to 30/04/09 for patients admitted to a large DGH for Troponin positive ACS. Demographic data, initial creatinine/eGFR and whether patient was documented to have CKD prior to admission were recorded.

Stage of CKD was calculated and the data was compared to that from de Lusignan *et al*. (2005). The t-test statistic was used to compare means. The proportion of undocumented CKD at presentation was also calculated.

*Results*

936 patients (600 men, 336 women) presented with troponin positive ACS, their mean CKD stage = 2.874 +/- 0.024. This was significantly different from the mean stage of CKD = 1.999 +/- 0.004 found in the general practice population (of similar demographics) sampled by de Lusignan *et al*. (2005) (p<0.001).




35.5% of patients with CKD stage 5, 65.7% of patients with CKD stage 4 and 92.7% of patients with CKD stage 3 had no knowledge or documentation of their renal impairment.

*Conclusions*

The management of patients with significant renal impairment should be optimised through the input of a nephrologist. Our data shows that the population of patients presenting to hospital with troponin positive acute coronary syndrome contains many patients with severe renal impairment who are unaware of their diagnosis, thus providing a useful starting point for the screening for renal disease and specialist nephrology input. Joint renal and cardiac clinics may offer better care for this group of patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO311**

**Association between Kidney Function and Coronary Artery Plaque on Computed Tomographic Angiography in Patients with Newly Diagnosed Coronary Artery Disease** Sejoong Kim, Jiyoon Sung, Jae Hyun Chang, Sun Young Na, Ji Yong Jung, Hyun Hee Lee, Wookyung Chung. *Internal Medicine, Gachon University Gil Hospital, Incheon, Gyeonggi, Korea.*

Cardiovascular disease (CVD) is the most frequent cause of morbidity and mortality in patients with chronic kidney disease (CKD). However, the association of kidney dysfunction with coronary atherosclerosis has not yet been fully elucidated. The purpose of this study was to evaluate the association of CKD and coronary artery plaque composition in a cohort of subjects without a previous diagnosis of coronary artery disease. A total of 1192 plaques from 470 patients (2.54 plaque/patient) with any coronary artery plaques on multislice computed tomographic angiography (CTA) were analyzed. Calcified plaque (CP), noncalcified plaque (NCP), and mixed plaque (MP) were identified on the CTA. The mean of eGFR was 80.6 ± 15.9 mL/min/1.73m$^2$, and the prevalence of CKD (defined as eGFR < 60 ml/min/1.73m$^2$) was 5.6%. Multivariate linear regression analysis was used to determine the association of clinical factors including CP, MP, NCP, and total plaque counts. CKD was associated with MP counts and total plaque counts ($r$=0.223, $P$<0.001; $r$=0.248, $P$<0.001, respectively). After the adjustment of covariables such as age, gender, diabetes mellitus, hypertension, smoking, anemia, albumin, calcium-phosphorus product, and lipid profiles, CKD was independently associated with MP counts and total plaque counts ($r$=0.132, $P$=0.01; $r$=0.15, $P$=0.002, respectively). The association was not significant between CKD and the CP or NCP counts. CKD was positively associated with coronary artery plaque counts, especially MP counts. These findings suggest that kidney function may play a role in the formation of coronary arterial plaques.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO312**

**The Presence of Ischaemic Heart Disease Magnifies the Risk of CKD in Patients with Essential Hypertension** Gavin Dreyer,[1] Sally Hull,[2] Ellena Badrick,[2] Alistair Chesser,[1] Magdi Yaqoob.[1] *[1]Nephrology, Royal London Hospital, London, United Kingdom; [2]Centre for Health Sciences, Queen Mary University, London, United Kingdom.*

Introduction:

Early identification of CKD reduces cardiovascular co-morbidity and delays progression to end stage kidney disease. Identifying high risk groups for developing CKD can direct screening services to identify and treat those most at risk for CKD. We studied a cohort of patients with essential hypertension (EH) to determine if a concomitant diagnosis of ischaemic heart disease (IHD) predicts an increase risk for CKD.

Methods:

Computer databases in 148 east London primary care practices were searched using Morbidity Information Query and Export Syntax software. 75,103 adults with a computerised diagnostic code for EH were identified. Patients with an eGFR measurement (MDRD 4 variable, n=29,749) during the study period (1/1/07 and 31/3/08) were included for analysis. Patients with diabetes mellitus were excluded from the analysis allowing us to evaluate the effect of EH in isolation.

Results:

The crude prevalence of EH was 9.5% and for CKD 3-5 was 22%. A diagnosis of IHD was present in 3,779 (12.7%) patients. The crude prevalence of CKD 3-5 in patients with EH and IHD is 30% compared to 19% in those without IHD (Chi$^2$ p<0.001). In a logistic regression analysis, the odds ratio for CKD 3-5 in patients with IHD compared to those without is 1.33, 95%CI 1.22-1.47 (adjusted for age, sex, systolic BP, smoking, cholesterol, ethnicity and clustered by practice). Severe CKD (stages 4-5) is more prevalent in patients with EH and IHD than those with EH alone (OR 1.37, 95%CI 1.03-1.82, adjusted as previously). Mean BP is lower in patients with CKD 3-5 and IHD than those without IHD (133/73 mmHg vs 137/76 mmHg, p<0.001). This may reflect greater use of anti-hypertensive medications in patients with CKD and IHD (2.36 vs 2.13 medications, p<0.001).

Conclusions:

CKD and particularly more severe CKD are significantly more prevalent in patients with EH and concomitant IHD despite a lower mean BP in this group. The public health implications of these findings should encourage clinicians to adopt an aggressive screening policy for CKD where EH and IHD co-exist.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO313**

**History of Nephrolithiasis Is Associated with Chronic Kidney Disease in Chinese General Population** Jianfang Cai, Xiaohong Fan, Hang Li, Xuemei Li, Xue-Wang Li. *Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China.*

**AIM** To explore the the association between history of nephrolithiasis and chrionic kidney disease (CKD) in Chinese general population.

**DESIGN AND METHODS:** In this cross-sectional study, a random sample of 6856 Chinese rural adults aged 30 to 75 years were selected in Beijing during 2008 to 2009. History of nephrolithiasis was assessed by asking:"Did a doctor ever say you had kidney stones?" Reduced eGFR was defined as eGFR <60ml/min/1.73m², albuminuria as albumin-creatinine ratio (ACR) ≥3.39 mg/μmol of first-void urine samples and CKD as the presence of reduced eGFR and/or albuminuria. The estimated marginal means (EMMs) of eGFR and the logarithm of urine ACR by history of nephrolithiasis were calculated and compared with generalized linear modelsThe odds ratios (ORs) of reduced eGFR, albuminuria and CKD associated with nephrolithiasis were obtained with logistic regression. Estimates of EMMs and ORs were adjusted for age, gender and other confounding factors (e.g. hypertension, diabetes, dyslipidemia, obesity, smoking).

**RESULTS:**History of nephrolithiasis had an overall prevalence of 7.41% and was more frequent in men than in women (9.12% vs. 5.71%, p<0.0001). There was significantly higher prevalence of reduced eGFR, albuminuria or CKD between persons with and those without nephrolithiasis (3.9% vs. 1.4%, p<0.0001; 14.4% vs. 10.4%, p=0.003; 15.7% vs 11.2%, p=0.002; respectively). The multivariate-adjusted EMM of eGFR was lower (90.09±0.89 vs. 91.26±0.74 ml/min/1.73m2, p<0.0001) and that of logarithm of ACR (0.316±0.026 vs. 0.267±0.022, p=0.002) was higher in the persons with than in those without history of nephrolithiasis. The multivariate-adjusted ORs of reduced eGFR, albuminuria, microalbuminuria and CKD associated with history of nephrolithiasis were 2.69 (95%CI 1.59-4.55, P<0.0001), 1.44 (95%CI 1.10-1.89, P=0.008), 1.51 (95%CI 1.15-2.00, p=0.003) and 1.44 (95%CI 1.11-1.87, P=0.006) respectively.

**CONCLUSIONS:** History of nephrolithiasis has a high prevalence and significant associations with reduced renal function, albuminuria and CKD in Chinese general population.

Disclosure of Financial Relationships: nothing to disclose

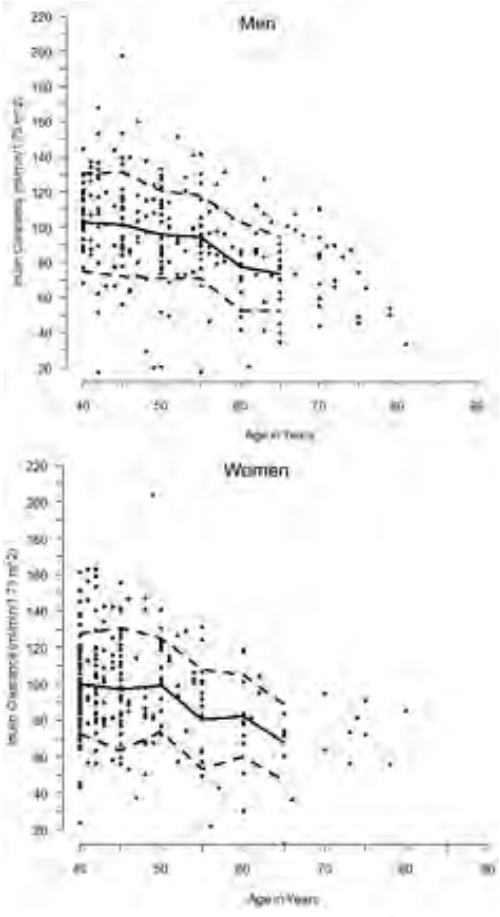
**TH-PO314**

**Kidney Function in South Asians-Measured Glomerular Filtration Rate in the General Population** Tazeen H. Jafar,[1,3] Muhammad Islam,[1] Saleem Jessani,[1] Lesley A. Stevens,[3] Christopher R. Mariat,[2] Andrew S. Levey.[3] *[1]Nephrology, Medicine and Community Health Sciences, Aga Khan University, Karachi, Pakistan; [2]CHU de Saint-Etienne, Université Jean Monnet, Saint-Etienne, France; [3]Nephrology, Tufts Medical Center, Boston, MA.*

Background: South Asian population is at high risk of chronic kidney disease (CKD) due to increased exposure to conventional risk factors, social determinants and low birth weight. Some reports on potential South Asian kidney donors indicate low glomerular filtration rate (GFR) as their normal level, and lowering of GFR thresholds for defining CKD has been suggested for this population. However, the level of kidney function in general adult population of South Asian origin has not been studied.

Methods: We measured GFR using the gold standard of inulin clearance on 530 subjects aged 40 years and above. Participants were randomly selected from communities in Karachi, Pakistan, using multi-stage cluster sampling.

Results: The mean age of participants was 46.9 (9.5) years, 50% were men. About 34% had hypertension (blood pressure >=140/90 mm Hg on two separate days or on antihypertensive medications) and 31% had diabetes (fasting plasma glucose >= 126 mg/dl or on hypoglycemic drugs). The mean inulin clearance (SD) adjusted for BSA was 94.1 (28.6) ml/min/1.73m2: 93.3 (28.1) in men vs 94.9 (29.1) in women (p=0.50). The age specific inulin clearance in men and women are shown in figures below, respectively.




Conclusions: The measured GFR in adults from the general population is not dramatically lower than levels in European-origin counterparts, as suggested by previous reports. Our findings do not support lowering GFR thresholds for defining CKD in Pakistan. Further studies are needed to assess GFR levels across a wider age range, and any regional variation in GFR levels in South Asia.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO315**

**Determinants of Measured Glomerular Filtration Rate in Adults from General Population in Pakistan** Tazeen H. Jafar,[1] Saleem Jessani,[1] Christopher R. Mariat,[2] Andrew S. Levey.[3] *[1]Nephrology, Medicine and Community Health Sciences, Aga Khan University, Karachi, Pakistan; [2]CHU de Saint-Etienne, Université Jean Monnet, Saint-Etienne, France; [3]Nephrology, Tufts Medical Center, Boston.*

Background: Nutritional status and diet influence glomerular filtration rate (GFR) in patients with chronic kidney disease (CKD). South Asians are at high risk of CKD and have poor nutritional indicators. However, the determinants of GFR in unselected general population of South Asian origin have not been studied.

Methods: We assessed the sociodemographic, nutritional, and clinical factors associated with measured GFR (mGFR) in 530 subjects aged 40 years and above. GFR was measured using the gold standard of inulin clearance. Participants were randomly selected from communities in Karachi, Pakistan, using multi-stage cluster sampling. Multivariable models were built using linear regression analysis.

Results: The mean age of participants was 46.9 (9.5) years, 50% were men, 34% had hypertension, 31% had diabetes. The mean body mass index was 25.8 (5.1) kg/m2 and serum albumin was 3.7 (0.3) g/dl. The mean (SD) mGFR adjusted for BSA was 94.1 (28.6) ml/min/1.73m2: 93.3 (28.1) in men vs 94.9 (29.1) in women (p=0.50). In the multivariable model the factors (beta coefficient (SE) ml/min/1.73m2, p value) independently associated with mGFR were: increasing age (-1.90 (0.25) for each 10 year increase, P<0.001), fasting plasma glucose (0.90 (0.17), for each 10 mg/dl increase, p <0.001), serum albumin (5.03 (1.88), for each 0.5 g/dl increase, p=0.008), high vs medium to low dietary meat consumption (5.46 (2.6), (11 or more vs 5 or less servings per week), p=0.04), and urine urea nitrogen (2.03 (0.49), each 1 g/d increase, p<0.001)). Sex was not significant (0.30 (2.47) for men vs women), and no interaction was detected between age and sex on mGFR.

Conclusions: Higher dietary protein intake and better nutritional status are associated with higher mGFR levels in the South Asians from the general population in Pakistan. Further research is needed to determine whether these factors account for regional and ethnic variations in GFR levels among general populations.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO316**

**Inappropriate Use of Metformin in Nursing Home Diabetic Population with Renal Failure** Leah Balsam, Jignesh Shah, Ankita Patel. *Medicine/Nephrology, Nassau University Medical Center, East Meadow, NY.*

**Purpose:** Metformin use has been contraindicated in renal impairment or congestive heart failure and advanced age unless measurement of creatinine clearance shows that renal function is not reduced. Concern remains over the side effect of lactic acidosis, a condition with mortality of up to 50%. Our study was undertaken in a group of nursing home diabetic residents to assess if appropriate considerations with regard to renal function were made before prescribing metformin.

**Methods:** This was a retrospective observational study. It included 64 residents of an Extended Care Facility with history of Diabetes Mellitus type 2 on Metformin. Data regarding age, sex, race, height and creatinine was obtained. Creatinine clearance was calculated using the Cockcroft-Gault equation modified for ideal body weight based on height and gender. Based on prior studies a creatinine clearance more than or equal to 60 mL/min was used as a cutoff for appropriate use of Metformin.

**Results:** In our study 48.5 % (31) of residents were females and 51.5 % (33) were males. The group comprised 40.6 % (26) Caucasians, 37.5 %( 24) African Americans, 17.2 % (11) Asians and 3.1 % (2) Hispanics. The average age was 65.5 years. The average age of females was 71.1 years and that of males was 61.1 years. 17.2 % (11) of the patients had a creatinine of greater than 1.5 mg/dl. 56.3 % (36) patients had a calculated creatinine clearance of less than 60 ml/min.

**Conclusion:** Our study demonstrates that more than half of elderly diabetic nursing home residents were prescribed metformin without adequate consideration to creatinine clearance. Although only 17.2 % had a serum creatinine that would be considered out of the normal range, 56.3 % had significantly decreased renal function. Use of estimated creatinine clearance should be advocated instead of serum creatinine when prescribing metformin.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO317**

**Evaluation of MDRD and Cockroft-Gault Equations for Sitagliptin Dosing** Joanna Q. Hudson,[1] M. Shawn Mcfarland,[2] Brandon M. Markley,[2] Utpal P. Patel.[3] *[1]Dept of Clinical Pharmacy, University of TN, Memphis, TN; [2]Dept of Veterans Affairs, TN Valley Healthcare System; [3]Murfeesboro Medical Center, Murfeesboro, TN.*

The MDRD equation is now advocated along with the Cockcroft-Gault (CG) equation for drug dosing. Currently most dose recommendations by manufacturers are based on estimated creatinine clearance (eCrCl) determined by CG. Few studies have evaluated differences in dosing using MDRD and CG. Sitagliptin is a dipeptidyl peptidase IV inhibitor used for type 2 diabetes mellitus (T2DM) with dose adjustments based on eCrCl. We assessed discordance rates in initial sitagliptin doses recommended using MDRD and CG.

Adult patients with T2DM prescribed sitagliptin in the outpatient clinic Oct 2006-June 2009 were included. Estimated GFR (eGFR) and eCrCl were calculated by the 4-variable MDRD and CG equations, respectively. Sitagliptin dose based on manufacturer's labeling was determined. Discordance in doses recommended using MDRD and CG estimates were compared overall and by subgroup based on eCrCl category (eCrCl >50, 30-50, and <30 mL/min). Discordance in drug dosing between methods was compared using the $x^2$ test; alpha was set at 0.05 a priori with 80% power.

A total of 121 patients were included; 52% male; 90% Caucasian; mean age 61±12 yrs; weight 93±19 kg; ideal body weight 62±10 kg; BSA 2.0±0.22 $m^2$. Mean eGFR was 70±15 mL/min and eCrCl was 67±17 mL/min. Discordance in sitagliptin dose was observed in 17 patients (14%) with MDRD compared to CG ($p<0.001$). Discordance by subgroup was as follows: n=1/2 (50%) for eCrCl < 30, n=8/10 (80%) for eCrCl 30-50, and 8/109 (7%) for eCrCl > 50 mL/min. All patients with eCrCl ≤50 would have received a higher sitagliptin dose using MDRD while patients with eCrCl > 50 would have received a lower dose.

Overall there was agreement in initial sitagliptin dose using MDRD and CG. Discrepancies resulted in underestimation of dose at eClCr above 50 mL/min and overestimation of dose at lower eClCr. Clinical implications are the potential for excessive initial dosing for individuals with kidney dysfunction. Since many agents have similar dosing stratification by eCrCl, this pattern is likely for other renally eliminated agents.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO318**

**Defining "Normal" Kidney Function in Phase I Clinical Trials: An Emerging Issue** Suzanne K. Swan,[1] William B. Smith,[2] Thomas C. Marbury,[3] Harry Alcorn,[1] Mahesh Krishnan,[1] T. Christopher Bond.[1] *[1]DaVita Inc, Denver, CO; [2]New Orleans Center for Clinical Research, New Orleans, LA; [3]Orlando Clinical Research Center, Orlando, FL.*

Background: Though it is generally accepted that renal function declines with age, equations that estimate glomerular filtration rate (GFR) or creatinine clearance do not have defined age-adjusted normal ranges. This leads to problems identifying an appropriate "normal" kidney function, age-matched groups for phase I/II pharmacokinetic trials involving investigational pharmaceutical compounds, particularly in light of a new draft FDA guidance defining "normal" kidney function or eGFR as > 90 ml/min.

Methods: We reviewed pharmacokinetic trial data from 3 phase I clinical research sites (Orlando, New Orleans, and Minneapolis) within the U.S. Renal Network, which performs a large percentage of pharmacokinetic trials in renal impairment subjects annually in the U.S. "Normal" eGFR lab values from 478 age-matched subjects over the past 7 years were available. Simultaneous results for Cockcroft-Gault (CG), Modified Diet in Renal Disease Study (MDRD) equation, or 24 urine collections for creatinine clearance (Ccr) were compared.

Results: The average eGFR of trial participants (as measured by MDRD) was less than 80 ml/min among all men and women aged 40 to 80. In subjects between 50 and 79 years of age, mean eGFR value ranged between 69 and 76 ml/min. If "normal" kidney function is redefined as eGFR > 90 ml/min by the MDRD equation, few, if any, age-matched subjects with "normal" kidney function would be identified for renal impairment subjects. As expected, CG estimates of Ccr were higher on average, with the discrepancy between the methods declining with age of subjects.

Conclusions: A proposed revision of the current FDA guidance on conducting pharmacokinetic drug studies in renal impairment subjects defines normal kidney function > 90 ml/min. However, this standard, if adopted, would complicate the conduct of early phase trials thus delaying drug approval. A lower, or age-adjusted "normal" eGFR is warranted.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO319**

**Validation of Estimating Equations for Lean Body Mass in Morbidly Obese Females** Ion D. Bucaloiu,[1] G. Craig Wood,[2] Evan Norfolk,[1] James E. Hartle,[1] Christopher D. Still,[1] Robert M. Perkins.[1,2] *[1]Geisinger Medical Center, Danville, PA; [2]Center for Health Research, Danville, PA.*

Background: Accurate estimation of lean body mass (LBM) is essential for appropriate determination of creatinine clearance and drug dosing in morbidly obese (MO) individuals (BMI≥40 kg/m²). The validity of existing LBM estimating equations is unknown in this population. Methods: Six previously reported LBM estimating equations were compared with LBM determined by dual energy absorbtiometry body composition (DEXA) in female, MO, Caucasian patients. All were enrolled in a comprehensive weight management program at a tertiary medical center. Correlation and bias against measured LBM were assessed. Results: 70 female patients [mean (SD) age 43.0 (11.0), weight 128.1 (13.8) kg, body mass index (BMI) 48.3 (4.8) kg/m²] underwent DEXA.

Performance of LBM estimating equations among females with morbid obesity

| | | BMI Category, kg/m² | | |
|---|---|---|---|---|
| **Reference** | **LBM Estimating Formula** | **40-44.9** | **45-49.9** | **≥50** |
| | | n=20 | n=27 | n=23 |
| Measured LBM (DEXA), mean (SD), (kg) | NA | 59.3 | 62.4 | 68.0 (5.7) |
| | | r (bias) | | |
| Garrrow (1985) | 0.287 x TBW + 9.74 x Ht² | 0.868 (-0.1) | 0.782 (-0.7) | 0.865 (-1.4) |
| Dufful (2004) | 1.75 x TBW – 0.0242 x BMI x TBW – 12.6 | 0.765 (9.7) | 0.587 (0.6) | -0.013 (-8.0) |
| Janmahasatian (2005) | [9270 x TBW]/[8780 + 244 x BMI] | 0.865 (-3.8) | 0.774 (-5.3) | 0.808 (-7.9) |
| Boddi (1972) | 1.07 x TBW - 0.0148 x BMI x TBW | 0.765 (-9.4) | 0.586 (-6.2) | -0.014 (-9.7) |
| Benezet (1997) | (TBW – IBW)/2 | 0.867 (26.1) | 0.785 (27.5) | 0.871 (30.4) |
| Amato (1995) | IBW + 0.32 x (TBW - IBW) | 0.863 (15.2) | 0.777 (14.8) | 0.836 (14.5) |

IBW - ideal body weight; TBW - total body weight; LBM - Lean Body Mass; BMI - body mass Index; r - Pearson's correlation coefficient.

Conclusions: In this female population with BMI > 40 kg/m², not all previously reported LBM estimating equations performed well. The estimating equation of Garrow et al. best correlated with DEXA LBM and minimized bias.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO320**

**Morbid Obesity: A Common *Noxa* to Kidney and Liver** Sara Goncalves,[1] Mariana Machado,[2] Adília Costa,[3] Fátima Carepa,[4] João Coutinho,[4] Helena Cortez-Pinto.[2] *[1]Nephrology, HSM - CHLN, Lisbon, Portugal; [2]Gastroenterology, HSM - CHLN, Lisbon, Portugal; [3]Histopathology, HSM - CHLN, Lisbon, Portugal; [4]Surgery, HSM - CHLN, Lisbon, Portugal.*

**Introduction and aims**: Obesity is a risk factor for chronic kidney disease (CKD) and nonalcoholic fatty liver disease (NAFLD), suggesting common pathways of lesion. We sought to evaluate early changes in renal function in morbid obese patients with NAFLD.

**Methods**: Morbid obese patients submitted to bariatric surgery, with liver biopsy classified by NAFLD Activity Score. Insulin resistance (IR) was defined as HOMA ≥3. Evaluation of renal function with CKD-EPI (CKD-Epidemiology Collaboration) estimated glomerular filtration rate (eGFR). Determination of plasmatic adiponectin, ghrelin and leptin, by ELISA.

**Results**: 111 patients, 94 women, age 42±11 years old, body mass index (BMI) 46±7 kg/m², IR in 53% and metabolic syndrome in 45%, eGFR 105±17 mL/min/1.73 $m^2$ (<90 mL/min/1.73 $m^2$ in 19%). Liver biopsies were classified as: nonalcoholic steatohepatitis (NASH)/ significant lobular inflammation (≥2), in 23% of patients, and simple steatosis, in 77%. There were no associations between eGFR and lipid profile, presence of metabolic syndrome or IR. eGFR correlated negatively with AST and bilirubin (-0.193, p=0.043 and -0.374, p<0.001, respectively), and was lower in patients with arterial hypertension (99±16 *vs* 110±17 mL/min/1.73m²; p=0.001), in patients with NASH/ significant lobular inflammation as compared to simple steatosis (98±21 *vs* 107±16 mL/min/1.73m²; p=0.034) and in patients with any fibrosis (104±18 *vs* 113±8 mL/min/1.73m²; p=0.0018), with no relation with steatosis severity. eGFR <90 mL/min/1.73m² was associated with NASH/ significant inflammation (52% vs 17%, p=0.001), any lobular inflammation (71% vs 39%, p=0.007) and hepatocyte ballonization (19% *vs* 4%, p=0.04). Neither eGFR nor liver histology was associated with adiponectin, ghrelin or leptin levels.

**Conclusions**: In morbidly obese patients, NASH, and particularly lobular inflammation, is associated with early decreases in eGFR, suggesting a common inflammatory link between liver and renal lesion. Adipokines role seems to be blunted in this high metabolic risk group.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO321

**The Prevalence of Renal Disease and Risk Factors for Renal Disease in Australian HIV-Infected Patients in the Primary Care Setting** David M. Gracey,[1] Mark T. Coulson,[2] Derek Chan.[3] *[1]Renal Unit, Royal Prince Alfred Hospital, Sydney, NSW, Australia; [2]Gilead Sciences, East Melbourne, Victoria, Australia; [3]Albion Street Centre, Surry Hills, NSW, Australia.*

**Aim:** To assess the prevalence of renal disease in the Australian HIV-infected cohort and to evaluate current practices in the management of renal disease and risk factors for renal disease by HIV physicians.

**Methods:** Internet-based data collection undertaken as part of a program to assess the care of common co-morbidities in HIV-infected patients. Primary care physicians (n=51) reported information relating to the renal health of their HIV-infected cohort (n=512).

**Results:** Patients demonstrated a low proportion thoroughly assessed for renal disease. This is despite a high prevalence of kidney disease and a high burden of risk factors. In this study population 93% were male, 39% were smokers, 20% had known hypertension, 5% had diabetes, 5% were morbidly obese and 32% had dyslipidaemia. 83% were on HAART and 6% were coinfected with hepatits C. Of those tested for renal function (n=484), 4% demonstrated an eGFR <60 (n=19); 91% of these patients were proteinuric. 3 patients (0.5%) had an eGFR <15; none were on dialysis. Less than half of all patients had been screened for proteinuria (n=252); of those tested 11.5% had > +1 on urine dipstix (n=29). The majority of patients with proteinuria were not on an ACE inhibitor or ARB (76%). Despite a history of hypertension (n=103), 21% of hypertensive patients were not medicated (n=22); this group had an average blood pressure of 145/92mmHg. Those on antihypertensives (n=66) demonstrated average blood pressure readings of 137/83mmHg. Use of potentially nephrotoxic medications was reported in 16% (n=85). NSAIDs and antiretorvirals were the most commonly reported nephrotoxins. Despite the burden of risk for renal disease and its high prevalence in this population, none of these patients had ever been referred to a Renal Physician for review.

**Conclusions:** This study demonstrates a high burden of renal disease in the HIV-infected population. The current screening and management practices fall short of suggested guidelines. Specialist input from Renal Physicians appears underutilised.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO322

**Chronic Kidney Disease in Patients Alive More Than 10 Years after Allogeneic Hematopoietic Stem Cell Transplantation** Tatsunori Shimoi,[3] Minoru Ando,[1] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [2]Internal Med IV, Tokyo Women's Medical University, Tokyo, Japan; [3]Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan.*

**Introduction:** Chronic kidney disease (CKD) is common in survivors of hematopoietic stem cell transplantation (SCT). However, evolution over time of kidney dysfunction and its association with peri-SCT acute kidney injury (AKI) are unclear.

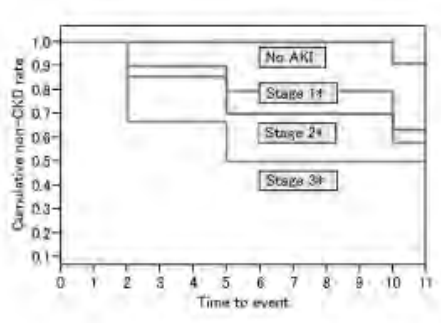
**Methods:** A retrospective cohort study was performed in 100 myeloablative allogeneic SCT patients who lived without relapse over 10 years after SCT. CKD was defined as a sustained decrease in estimated GFR (less than 60 ml/min/1.73 m$^2$) for a period more than 3 months. Prevalence of CKD was studied at 2, 5 and 10 years after SCT. Peri-SCT AKI was classified into three stages according to the AKIN criteria within 100 days after SCT. Cumulative CKD proportion was evaluated by the Kaplan-Meier analysis. The factors associated with 'CKD at 10 years' and 'mortality after 10 years' were examined, using Cox regression analysis.

**Results:** Prevalence of CKD after SCT is shown in **Table 1**. The cumulative CKD rate increased according to increasing AKI stages with significant difference between stages ≥1 and no AKI (**Figure 1**). Cox regression showed that each AKIN stage was a significant predictor of 'CKD at 10 years' [HR (95% CI): stage3, 19 (3.4-154); stage 2, 8.9 (2.0-64); stage 1, 7.8 (2.0-53)] and that CKD at 2 years was significantly associated with 'mortality after 10 years' [HR (95% CI): 11.6 (1.6-103)].

**Conclusions:** Prevalence of CKD increased during the 10-year follow-up period. Peri-SCT AKI, regardless of the AKIN stages, is significantly involved in presence of CKD. Early onset of CKD is potential risk for late mortality in patients alive without relapse.

Prevalence of post-SCT CKD

| Time after SCT | 2 years | 5 years | 10 years |
|---|---|---|---|
| Prevalence | 13% | 23% | 34% |




Disclosure of Financial Relationships: nothing to disclose

### TH-PO323

**Chronic Kidney Disease in Cancer Patients** Shin-Young Ahn,[1] Sewon Oh,[2] Dong Ki Kim,[1] Kook-Hwan Oh,[1] Ki Young Na,[2] Kwon Wook Joo,[1] Chun-Soo Lim,[1] Yon Su Kim,[1] Dong Wan Chae,[2] Curie Ahn,[1] Jin Suk Han,[1] Suhnggwon Kim,[1] Ho Jun Chin.[2] *[1]Department of Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea; [2]Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul, Republic of Korea.*

Chronic kidney disease (CKD) may be a risk factor for cancer and also cancer can cause CKD. As well, CKD in cancer patients can influence both clinical eligibility for treatment and mortality. Therefore, we investigate the prevalence of CKD, elucidate the risk factors of CKD in cancer patients and assess the impact of CKD on cancer patient survival.

This retrospective study reviewed 3769 patients who were classified according to the diagnostic code, as C-code in ICD-10, and visited the Seoul National University Bundang Hospital at least once in 2004. Patients aged 18 years or more were included. Glomerular filtration rate (GFR) was calculated based on serum creatinine (sCr) using the MDRD formula. CKD was defined as an estimated GFR (eGFR) of less than 60 ml/min/1.73m$^2$.

The study sample consisted of 3716 patients (2078 males; mean age, 59.2 ± 14.1 yrs). Among the 3716 patients, 82.1% had sCr at the time of diagnosis. The prevalence of CKD in cancer patients was 12.6%. Patients with diabetes, dyslipidemia, acute coronary syndrome (ACS), and cerebrovascular accident (CVA) had significantly higher prevalence of CKD than those without such comorbidities. Age ≥60yrs, male gender, diabetes and ACS remained an independent risk factors for CKD in cancer patients after multivariate analysis. During the observational period, all cause mortality rate was 36% and mean follow up duration was 50.6 month. Patients with CKD showed poorer mortality rate than those without CKD (33.6% vs 46.7%, p<0.001). In multivariate analysis adjusted by age, gender, diabetes and cancer types, CKD remained an independent risk factor for all cause mortality (OR 1.562, 95% CI 1.244-1.962).

The prevalence of CKD in cancer patients is higher than general population (12.6% vs 5.1%). Age ≥60yrs, male gender, diabetes and ACS are risk factors for CKD in cancer patients. CKD is one of the major independent risk factors for survival outcome in cancer patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO324

**Proteinuria and Microalbuminuria as Predictors of Renal Dysfunction in a Cohort of Marfan Syndrome Patients** Enrique Morales,[1] Eduardo Gutierrez,[1] Alberto Forteza,[2] Raquel Bellot,[2] Violeta Sanchez,[3] Maria Paz Sanz,[1] Jose Cortina,[2] Manuel Praga.[1] *[1]Nephrology, Hospital 12 de Octubre, Madrid, Spain; [2]Cardiac Surgery; [3]Cardiology, .*

Proteinuria and microalbuminuria are considered predictors of renal dysfunction but also as cardiovascular risk indicators. There is no information in the literature on renal involvement in patients with Marfan syndrome (MS)

Objectives: To assess the prevalence of proteinuria and microalbuminuria as predictors of renal dysfunction in Marfan syndrome.

Methods: Forty-eight patients with MS according Ghent criteria have been evaluated to assess renal function. Mean age was 32,3 ± 8,7 and 54% were male. Urine samples of 24 hours were analyzed in each patient to determine proteinuria and microalbuminuria. Additional measures like serum creatinine, creatinine clearance, glomerular filtration, hematuria, presence of ANAs or immunoglobulin A levels were also considered.

Results: Significant proteinuria was found in 25% of patients (0,23 ± 0,03 g/24 hour) and two more patients (4%) presented isolated microalbuminuria. Seven patients had hypertension but only one had proteinuria. Seventeen patients were with drugs blocking renin-angiotensin system. Immunoglobulin A levels were increased in 8 patients (16,7%). The serum creatinine was 0,83 ± 0,19 mg/dL (0.5-1.3). Glomerular filtration rate was 106.2 ± 24.3 ml/min/m2 (66.4-177.2). None patient had GFR below 60 ml/min/m2.

Conclusions: Prevalence of proteinuria and microalbuminuria in MS patients are higher than in general population. Although renal function was not affected it could be an early renal dysfunction predictor. Immunoglobulin A levels were higher than expected, but its relevance is unknown.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO325**

**Fibroblast Growth Factor 23 Is Associated with Proteinuria** Marc G. Vervloet,[1] Arjan D. Van Zuilen,[2] Peter J. Blankestijn,[2] Pieter M. Ter Wee,[1] Jack F. Wetzels.[3] *[1]Nephrology, VU University Medical Center, Amsterdam, Netherlands; [2]Nephrology, University Medical Center Utrecht, Utrecht, Netherlands; [3]Nephrology, Radboud University Medical Cener, Nijmegen, Netherlands.*

Introduction:

Fibroblast Growth Factor 23 (FGF23) may be a risk factor for cardiovascular disease (CVD) in CKD-stages 3 and 4. However, its relation to other risk factors for CVD is ill-defined.

Methods:

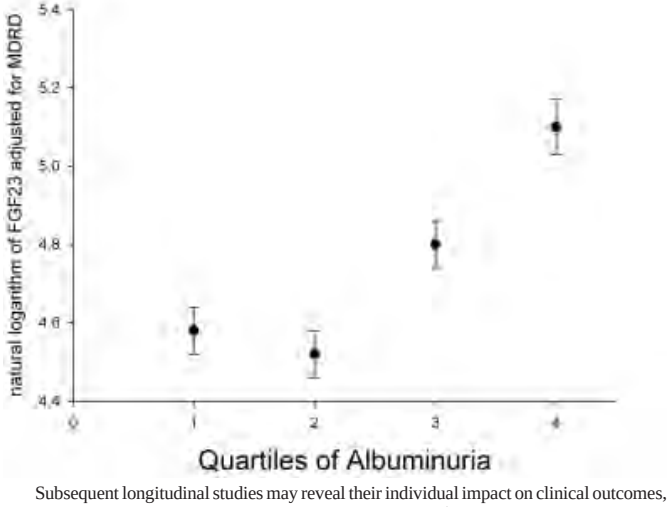
In a large cross-sectional cohort of patients with CKD stage 3 and 4 (The Masterplan Cohort) we assessed the correlation between c-terminal FGF23 levels and creatinine, eGFR, age, gender, race, history of cardiovascular disease, presence of diabetes, blood pressure, 24 hour proteinuria, presence of left ventricular hypertrophia (LVH), socioeconomic background, smoking habits, PTH and phosphate levels. Medications used were recorded. Several multivariate models were used, FGF23 was analyzed after log-transformation.

Results:

We evaluated 701 subjects, with an eGFR of 37 ± 14 ml/min; age 59 ± 13 years; 68% males; 92% Caucasians; 24% diabetics; 24% smokers; 29% with a history of CVD; and 14% with LVH. Blood pressure was 136/78 ± 21/11 mmHg; phosphate 1.09 ± 0.24 mmol/l and PTH 9 ± 9 nmol/l. Proteinuria median was 300 (100-700 IQR)mg/24 hrs. In univariate analysis females, diabetes, smoking, history of CVD, systolic blood pressure, phosphate, PTH, use of phosphate binders, proteinuria and active vitamin D usage were all significantly correlated with FGF23. Using multivariate modelling eGFR ($p<0.001$), PTH ($p<0.001$), phosphate ($p<0.006$), and proteinuria ($p<0.001$) were associated with FGF23.

Conclusion:

Both eGFR and proteinuria are highly significantly and independently associated with FGF23 in stage 3 and 4 CKD.




Subsequent longitudinal studies may reveal their individual impact on clinical outcomes, and may strengthen the hypothesis that FGF23 is a target of therapy.

Disclosure of Financial Relationships: Consultancy: Consultancy fees by Amgen and Abbott

Research funded by Genzyme and Abbott.

**TH-PO326**

**A Comparison of Prediction Equations for Estimating Glomerular Filtration Rate in Preeclampsia** Mahfuzur Rahman, Arnold B. Alper. *Nephrology & Hypertension, Tulane University Health Science Center, New Orleans, LA.*

**Background:**

Accurate estimation of the Glomerular Filtration Rate (GFR) in patients with preeclampsia is often difficult or impossible to accomplish. Measurement of the GFR either with Cockroft-Gault (CG) or the MDRD formula has not been validated in this setting.

**Objective:**

In a previous study, a newly created formula, the Preeclampsia GFR (PGFR) formula was proposed to accurately estimate GFR in preeclamptic patients. This study compared the utility of the CG, MDRD, and PGFR equations to predict the GFR in preeclampsia in a larger, more ethnically diverse population.

**Methods:**

This was a retrospective chart review study that compared the estimated GFR calculated from the above formulas with the creatinine clearance values (gold standard) obtained from a 24-hour urine collection in 515 preeclampsia patients from 5 large hospitals.

**Results:**

The Cockcroft-Gault formula provided the highest mean GFR (160 ml/min) and the MDRD formula provided the lowest (107ml/min). The PGFR formula provided an estimated GFR (131ml/min) that was very similar to that provided by the 24 hour Creatinine clearence (132 ml/min). The CG formula had the highest relative bias (19.5%) significantly overestimating GFR in all subjects, while the MDRD formula had smaller but significant relative bias (-12.1%) underestimating the GFR. The PGFR formula had the smallest relative bias (2.25%) and performed significantly better in predicting a GFR that was within 10% or 30% of the gold standard.

**Conclusion:**

Current GFR estimation equations based on serum creatinine values in non-pregnant patients are not reliable measures of renal function in patients with preeclampsia. The use of proposed PGFR formula is recommended.

Disclosure of Financial Relationships: nothing to disclose

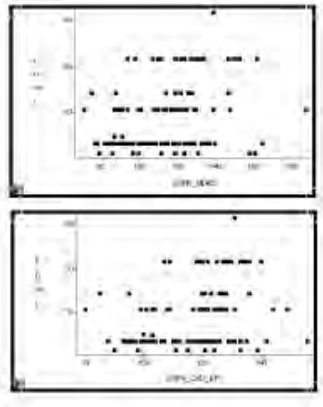
**TH-PO327**

**Retrospective Review of Serum Creatinine and Creatinine-Based Measures of Estimated Glomerular Filtration Rate in an Amputee Population** Casey Cotant,[1] Molly A. Tilley,[1] Ian J. Stewart,[1] Ellen E. Im,[3] Kelly D. Heegard,[3] Kevin Chung.[2] *[1]Nephrology, Wilford Hall Medical Center, Lackland Air Force Base, TX; [2]Institute of Surgical Research, Brooke Army Medical Center, Ft Sam Houston, TX; [3]Internal Medicine, SAUSHEC, Lackland Air Force Base, TX.*

**Background:** The MDRD and CKD-EPI equations may be over estimating the renal function of the wounded warrior amputee population potentially leading to erroneous medication dosing and inadequate disease surveillance.

**Methods:** Serum creatinine levels obtained from chart review of 206 patients with a history of traumatic amputation at the Center for the Intrepid were analyzed and averaged. Patients with acute kidney injury (AKI), as defined by Acute Kidney Injury Network (AKIN) 1 criteria or greater, were excluded. Estimated percentage of body weight lost (EBWL) was calculated for each patient using a formula derived by Osterkamp that takes into account the location and type of amputation. Estimated GFR (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation as well as the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. The data was recorded as a scatter plot demonstrating the relationship between EBWL and eGFR by MDRD and CKD-EPI equations. Spearman correlation coefficients were calculated.

**Results:** Both MDRD and CKD-EPI were significantly positively correlated with EBWL, with r values of 0.32 and 0.27 and p values of 0.0001 and 0.0010, respectively.



**Conclusions:** In patients with decreased muscle mass secondary to amputations, both the MDRD and CKD-EPI equations over estimate GFR, as evidenced by weak but statistically significant increases in eGFR with greater percentages of lost muscle mass. Further research comparing estimating equations to gold standard methods of GFR measurement is needed in the amputee population.

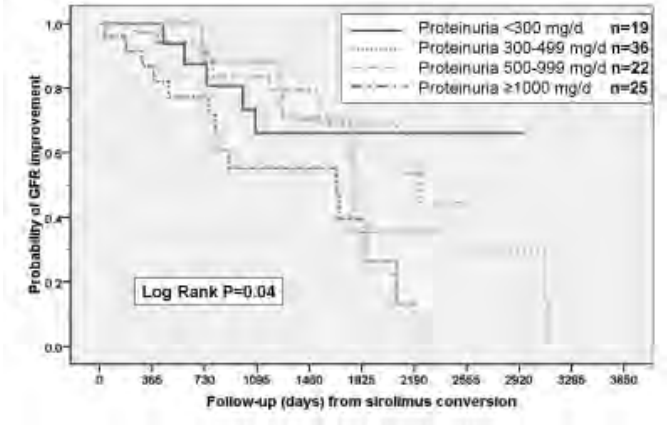
Disclosure of Financial Relationships: nothing to disclose

**TH-PO328**

**Sirolimus Induced Proteinuria Is Associated with Deterioration in Kidney Function in Liver Transplant Recipients with Calcineurin Inhibitor Nephrotoxicity** Hani Wadei, Ziad S. Zaky, Barry Rosser, Andrew Keaveny, Thomas A. Gonwa. *Transplantation, Mayo Clinic, Jacksonville, FL.*

**Background:** Proteinuria develops following sirolimus conversion in liver transplant (LT) recipients with calcineurin inhibitor (CNI) nephrotoxicity but its effect on post-conversion improvement of kidney function is unknown. **Methods:** Data of 102 consecutive LT recipients with CNI nephrotoxicity who were converted from cyclosporine (n=3) or tacrolimus (n=99) to sirolimus and remained on sirolimus monotherapy for ≥4 months were retrospectively reviewed. Kidney function was assessed using serum creatinine (Cr) and MDRD eGFR at time of conversion and serially thereafter. 24-hr urine for protein excretion was collected annually or when clinically indicated. **Results:** The follow-up period from sirolimus initiation to death, sirolimus discontinuation or last Cr was 2.9 ± 2.0 years. Stabilization or improvement in kidney function was defined as ΔeGFR (eGFR at last followup-eGFR at conversion) of ≥0 ml/min and developed in 65 (64%) patients. Post-sirolimus proteinuria ≥300mg/d developed in 83 (81%) and had a graded negative impact on eGFR improvement post-conversion with the highest probability of eGFR deterioration in those with proteinuria ≥ 1gr/day. **Figure 1.**

**Figure 1:** Kaplan-Meier plot showing the relationship between proteinuria and probability of GFR improvement in LT recipients with CNI nephrotoxicity converted to sirolimus




Sirolimus was discontinued in 11 patients for proteinuria which subsequently improved in 9 (82%). eGFR changed from 35.8±7.7 to 44.2±9.0 ml/min (P=0.06) in 8 patients following sirolimus discontinuation while 3 remained dialysis dependent. **Conclusions:** 1) Proteinuria was common following sirolimus conversion in LT patients with CNI nephrotoxicty 2) Post-sirolimus proteinuria showed a graded negative impact on the probability of post-conversion kidney function improvement 3) Proteinuria and kidney function improved following sirolimus discontinuation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO329

**Vitamin D Deficiency and Cardio-Renal Risk Factors in Children: National Health and Nutrition Survey (NHANES) 1999-2004** Peter Chau,[1] Sheena Cecille Marie Go,[2] Dulcie Kermah,[4] Keith C. Norris,[1,4] Kamyar Kalantar-Zadeh,[1,2,3] Gangadarshni Chandramohan.[1,2,3] *[1]University of California, David Geffen School of Medicine, Los Angeles, CA; [2]Pediatrics, Harbor-UCLA Medical Center, Torrance, CA; [3]Los Angeles Bio-Medical Research Institute, Torrance, CA; [4]RCMI, Charles Drew University, Los Angeles, CA.*

Vitamin D deficiency is associated with poor cardiac and renal outcomes in adults. In children, prevalence of vitamin D (VitD) deficiency has been increasing. We analyzed the risk VitD deficiency based on gender, ethnicity and other risk factors in children. **Methods**: VitD deficiency (<15ng/ml), insufficiency (15-29ng/ml) and combined (vitDdef). High waist circumference (WC): >75th percentiles. Obesity: BMI >95$^{th}$ percentile. Dyslipidemia (Dyslip): abnormal lipid profile. Abnormal fasting blood sugar (abFS): > 100mg/dl. High blood pressure (HBP): >90th percentile. Metabolic syndrome (MetS): 3 or 4 components of MetS. **Results**: Analyzed 2202 children, 6 -17 yrs; 51% males, 66% White, 15% Black, 19% Hispanic, 11% obese, 27% with high WC, HBP 9%, Dyslip 52%, abFS 7% and MetS 3%. VitD deficiency found in 78% and was significantly high in females, Blacks, Hispanics, obese, those with high WC, Dyslip and MetS. Odd ratio for vitDdef was only high in females, Blacks, Hispanics, obese and with Dyslip.

Odds Ratio for Vitamin D Deficiency Associated with Various Risk Factors

| Variables | Odds Ratio | 95% Confidence Interval |
|---|---|---|
| Gender (Female=1) | | |
| Male | 0.6 | .39 - .82 |
| Races (White=1) | | |
| Black | 58.1 | 15 - 224 |
| Hipanic | 6.2 | 3.3 - 11.7 |
| Waist circumference (Normal=1) | | |
| *High | 1.6 | 1.0 - 2.5 |
| **Obesity (No=1) | | |
| Yes | 1.8 | 1.1 - 3.1 |
| Blood Pressure (Normal=1) | | |
| ^High | 0.6 | 0.3 - 1.0 |
| Dyslipidemia (No=1) | | |
| Yes | 1.5 | 1.0 - 2.3 |
| Fasting blood sugar (Normal=1) | | |
| Abnormal | 1.5 | 0.7 - 3.4 |
| Metabolic syndrome (No=1) | | |
| Yes | 1.4 | 0.3 - 7.0 |

*High waist circumference >75th percentile, **Obesity=BMI >95th percentile, ^Blood pressure - high=systolic and/or diastolic >90th percentile

**Conclusion**: Risk of Vitamin D deficiency was high in female, Black, Hispanic, obese and Dyslip children. Dietary factors perhaps the major culprit. Further studies needed to identify the mechanisms of development of vitDdef in children.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO330

**Cocaine Use and Chronic Kidney Disease in the United States** Ruchir Patel,[1] Ankit Rathod,[1] Apurva Badheka,[1] Zeenat Yousuf Bhat.[2] *[1]Internal Medicine, Wayne State University/Detroit Medical Center, Detroit, MI; [2]Division of Nephrology, Wayne State University/Detroit Medical Center, Detroit, MI.*

**Background:**

Epidemiologic data regarding cocaine associated chronic kidney disease (CKD) is lacking.

**Methods:**

All adults (>18years) in the publicly available dataset of the National Health and Nutrition Examination III (NHANES III) survey between 1988-1994 (n=11990) were analyzed. History and frequency of cocaine use was self reported. The two groups were analyzed on basis of lifetime cocaine use as present or absent. We excluded subjects with data missing regarding cocaine use, serum creatinine and BMI (n= 5090). Creatinine clearance (ml/min) was calculated using Cockcroft-Gault equation and GFR was calculated using MDRD equation. CKD was graded per established criteria. Other baseline variables included in the model were age, sex, race, lipid profile, diabetes, hypertension, smoking, BMI, stroke, myocardial infarction, peripheral arterial disease and homocysteine level. Beta parameter estimate and odds ratio were calculated using linear and logistic regression and considered significant for p<0.05. All analysis were performed using SAS 9.2.

**Results:**

Our cohort consisted of 6900 adults with 49% males, 5.1% MI, 3.9% stroke, 26% hypertensives, 7.8% diabetics and 26% smokers. Mean age, BMI and eGFR were 48 years, 25.9 kg/m$^2$ and 86 ml/min respectively. Cocaine use was not associated with decreasing eGFR or creatinine clearance (beta 0.14, p = 0.91) on multivariate analysis. Cocaine use was also not associated with worsening CKD stage as an ordinal variable(OR = 1.05, 95%C.I 0.86-1.24,p =0.70). Even in patients with >100 lifetime exposures to cocaine this relationship did not change.

**Conclusion:**

Cocaine use was not associated with CKD in this large cross sectional survey representative of the adult US population after controlling for race, hypertension and cardiovascular risk factors. This is in contrast to prior data from small case series or single center studies. It is unknown whether cocaine per se is causes pathologic changes and CKD. Our results indicate that CKD among cocaine users is secondary to cocaine associated cardiovascular co-morbidities.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO331

**Role of Lean Body Mass in Estimating GFR in Alzheimer Disease** James B. Wetmore,[1] Jeffrey M. Burns.[2] *[1]Medicine, Division of Nephrology and Hypertension, University of Kansas Medical Center, Kansas City, KS; [2]Neurology, University of Kansas Medical Center, Kansas City, KS.*

**Introduction**

The association between estimated glomerular filtration rate (eGFR) and progression of Alzheimer Disease (AD), as measured by cognitive decline and brain atrophy, has not been systematically studied. Since AD is characterized by sarcopenia and other changes in body composition, determining the applicability of various eGFR equations, particularly those that account for lean mass (LM), is important.

**Methods**

Participants were drawn from a prospective longitudinal study of brain aging and AD in community-dwelling individuals. Control ($n$ = 60)and AD ($n$ = 61) participants had baseline and 2-year assessments of both cognitive function and brain volume. Estimated GFR was calculated using the 4-variable MDRD and Lean Mass (LM) GFR (eGFR = (2.4 * LM) - (0.75 * LM * creatinine)) equations. Association of eGFR and the outcomes was examined.

**Results**

Individuals with AD demonstrated a paradoxical finding in which lower baseline MDRD eGFR was associated with lower rates of both cognitive decline ($p$ =0.04) and brain atrophy ($p$ = 0.02), a phenomenon not observed in non-AD controls. This finding was abolished in the AD individuals when the LM GFR equation was used. While significant group-by-eGFR interactions were present for cognitive decline ($p$ = 0.006) and brain atrophy ($p$ = 0.001) when MDRD was used, no group-by-eGFR interactions were present when LM GFR was used ($p$ = 0.92 and $p$ = 0.52 for cognitive decline and brain atrophy, respectively).

**Conclusions**

Accounting for LM in GFR estimation appears to significantly mitigate counterintuitive relationships between measures of AD progression and eGFR as calculated by more traditional measures of renal function. This suggests that consideration of lean mass in eGFR calculations may be important in the AD population.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO332**

**Recognition of Chronic Kidney Disease: Prevalence and Predictors** Rajyalakshmi Gadi,[1] Lakshmi Venkitachalam,[2] John Spertus,[2] John Powers,[3] Paul S. Chan.[2] *[1]Nephrology, Univ of Kansas Med Ctr, KS; [2]CV Research, Saint Lukes' Mid America Heart Inst., MO; [3]Kaiser Permanente Inst. of Research, CO.*

**Background:** Because chronic kidney disease (CKD) is associated with significant cardiovascular (CV) morbidity and mortality, early recognition of incident cases would facilitate appropriate care. However, it is unknown whether the introduction of the new ICD-9-CM codes by CKD stages in early 2006 has increased physician recognition rates of this disease entity.

**Methods:** Within the Kaiser Permanente Colorado hypertension registry of 186,195 patients enrolled from 2000-2007, we identified 16,833 patients with incident CKD, defined as 2 consecutive estimated glomerular filtration rate's (eGFR) below 60ml/min/1.73m$^2$ and which were 3-12 months apart. The primary outcome, clinician recognition of incident CKD was defined as documentation of CKD with an ICD-9 code within 1 year of the second eGFR. Using multivariable logistic regression, we compared CKD recognition rates before and after 2006.

**Results:** Overall, CKD was recognized by physicians in 1446 (8.6%) patients, in 7.5% (1218/16122) with moderate CKD (eGFR 30-59) and 32.0% (228/711) with severe CKD (eGFR 15-29). The CKD recognition rate increased from 6.9% (969/13847) before 2006 to 15.9% (477/2986) after 2006. After multivariable adjustment, the likelihood of CKD recognition increased by more than 3-fold after 2006 (Adjusted odds ratio of 3.30, P<.0001).

Independent Predictors of CKD Recognition

| Covariate | Adjusted OR | P value |
|---|---|---|
| Age | 0.99 | <0.0001 |
| Male sex | 3.10 | <0.0001 |
| Black race | 2.56 | <0.0001 |
| Diabetes | 1.50 | <0.0001 |
| CHF | 2.05 | <0.0001 |
| Albuminuria | 1.10 | 0.75 |
| CKD stages 4-5 | 7.99 | <0.0001 |
| Introduction of new CKD ICD codes[a] | 3.30 | <0.0001 |

[a] After 2006 vs. Before 2006

**Conclusion:** While CKD recognition rates remain low, they have increased 3-fold since the new CKD ICD coding system has heightened awareness about CKD. Future studies need to examine whether improved CKD recognition resulted in more intensive CV risk factor modification and avoidance of nephrotoxic medications.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO333**

**BMP7 Maintains Nephron Progenitor Population and Determines Nephron Number** Mayumi Tomita,[1] Itsuro Kazama,[2] Nariaki Asada,[1] Atsuko Y. Higashi,[1] Shuichiro Endo,[1] Toru Kita,[3] Atsushi Fukatsu,[1] Aris N. Economides,[4] Elizabeth Robertson,[5] Jordan A. Kreidberg,[6] Motoko Yanagita.[1] *[1]Graduate School of Medicine, Kyoto University, Japan; [2]Tohoku University, Sendai, Japan; [3]Kobe City Medical Center, Hyogo, Japan; [4]Regeneron Pharmaceuticals, Inc., NY; [5]University of Oxford, United Kingdom; [6]Division of Nephrology, Children's Hospital Boston, MA.*

The number of nephrons varies between individuals, and low nephron number associates with the risk of hypertension and progression of renal insufficiency. However, the molecular mechanisms determining nephron number has not been clarified. Bone morphogenic protein 7 (BMP7) knockout mice demonstrate aplastic kidneys with few nephrons, however, the precise mechanism has not been clarified, because the nephrogenesis is arrested at the early stage of kidney development. Systemic knockdown of BMP7 after the initiation of kidney development enabled us to observe the prominent apoptosis and accelerated epithlialization and maturation of metanephric mesenchyme, which is known to include nephron progenitor population. Knockdown of BMP7 in kidney explants resulted in the accelerated epithelialization of metanephric mesenchyme. Inhibition of Smad1/5/8 in wild-type kidney explants similarly demonstrated the accelerated epithelialization. We also examined the inhibitory effect of BMP7 on epithelialization in colony-forming assay, in which metanephric mesenchymal cells epithelialize and form sheet-like colonies. Administration of BMP7 to this culture system dose-dependently prevented colony formation. Taken together, BMP7 inhibits apoptosis and differentiation of nephron progenitors through Smad signaling. In conclusion, BMP7 seems to maintain nephron progenitors and regulate nephron number at birth.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO334**

**Inhibitory Effect of Mesenchymal Stem Cells on the Progression of Experimental Peritoneal Fibrosis in a Rat Model** Toshinori Ueno,[1,2] Ayumu Nakashima,[2] Yoshihiko Taniguchi,[4] Takeshi Kawamoto,[3] Yukio Kato,[3] Noriaki Yorioka.[2] *[1]Department of Molecular and Internal Medicine; [2]Department of Advanced Nephrology; [3]Department of Dental and Medical Biochemistry, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan; [4]Department of Pharmaceutical Science, Hiroshima International University, Kure, Japan.*

**Background:** Recent studies have suggested that mesenchymal stem cells (MSCs) can be employed for various types of tissue damage because of their regenerative capacity and immunoregulatory properties, but the involvement of MSCs in peritoneal fibrosis is not clear.

**Methods:** Using male SD rats or Fisher 344 rats, a peritoneal fibrosis model was created by intraperitoneal injection of 0.1% chlorhexidine gluconate in 15% ethanol dissolved in saline, while 15% ethanol in saline was injected as a control. MSCs were obtained from the bone marrow of GFP transgenic rats and cultured. Then MSCs ($1\times10^7$ cells) suspended in 1 mL of PBS or vehicle were injected intraperitoneally into the peritoneal fibrosis model rats and control rats. At 7 and 14 days after injection of MSCs, the peritoneal tissues were taken for immunohistochemical analysis. In a separate in vitro investigation, we cultured human peritoneal mesothelial cells (HPMCs) with a high concentration of D-glucose (4%) and co-cultured HPMCs with human MSCs by using a trans-well system. To clarify the effect of MSCs, TGF-β mRNA expression by HPMCs was evaluated with RT-PCR.

**Results:** We found GFP-positive MSCs on the peritoneal surface in MSC-treated rats with fibrosis, but GFP-positive MSCs were not seen in MSC-treated control rats. Injection of MSCs suppressed myofibroblast accumulation (α-SMA), macrophage infiltration (ED-1), and the expression of TGF-β and MCP-1 in the peritoneum. TGF-β mRNA expression by HPMCs was increased 4.3-fold after exposure to a high concentration of D-glucose compared with the control (P<0.01), while there was 0.65-fold suppression by co-culture with human MSCs (p<0.05).

**Conclusions:** Our findings suggest that MSCs were mobilized to sites of peritoneal damage and that these cells may have anti-inflammatory and anti-fibrotic effects against peritoneal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO335**

**Effect of hMSCs Microvesicles on Cystine Content of *CTNS* Mutant Human Fibroblasts** Reyhan El-Kares,[1] Francesco Emma,[2] Nicoletta Eliopoulos,[3] Paul R. Goodyer.[1] *[1]Pediatrics, McGill University, Montreal, QC, Canada; [2]Nephrology and Urology, Bambino Gesù Children's Hospital, Rome, Italy; [3]Experimental Medicine, McGill University, Montreal, QC, Canada.*

Cystinosis is caused by mutations in the *CTNS* gene, encoding a cystine transporter in the lysosomal membrane. In affected children, cystine accumulates within lysosomes, causing progressive multi-organ dysfunction and a need for renal replacement therapy in the second decade of life. Recently, Syres *et al* reported that an infusion of heterologous wildtype bone marrow stem cells dramatically reduced pathologic cystine accumulation and reversed renal dysfunction in *Ctns* knockout mice. However, there was little evidence of replacement of the mutant tissue by transdifferentiation of wildtype stem cells. We hypothesized, therefore, that human mesenchymal stem cells (hMSCs) might correct the primary defect by shedding microvesicles (MVs) that transfer wildtype cystinosin to the mutant tissue.

Cystinotic fibroblasts derived from a patient with homozygous deletion (57kb) of the *CTNS* gene were co-cultured with hMSCs (in a 4:1 ratio) for 96 hours. Cystine level of the mixed cell population was 50% of the baseline level in mutant fibroblasts alone.

To test our hypothesis, we isolated MVs by ultracentrifugation from hMSC-conditioned medium and added them to human cystinotic fibroblasts. After 24 hours, we noted a MVs dose-dependent reduction of cystine content in the mutant fibroblasts. Exposure to 1700μg/ml of MVs reduced cystine content to the level in control non-cystinotic fibroblasts.

These observations indicate that the powerful effect of stem cells to reverse the pathologic cystine accumulation in *CTNS*$^{(-/-)}$ tissue is conferred by microvesicle transfer and cannot be explained by other postulated paracrine effects of stem cells such as stem cell transdifferentiation, suppression of inflammation or stimulation of endogenous cell proliferation. We speculate that microvesicle transfer of wildtype protein and/or mRNA may be relevant to stem cell therapy for cystinosis and other inherited renal diseases.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO336**

**Mesonephric Development and Regeneration in Transgenic Zebrafish** Weibin Zhou,[1] Rudrick Casey Boucher,[1] Christoph Englert,[3] Friedhelm Hildebrandt.[1,2] *[1]Pediatrics and Human Genetics, University of Michigan, Ann Arbor, MI; [2]Howard Hughes Medical Institute; [3]Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, Germany.*

Zebrafish represents a valuable vertebrate model for kidney research. The majority of previous studies were focusing on the pronephros of zebrafish, which consists of only two nephrons. It is structurally and functionally simpler than the mesonephros of adult fish and the metanephros of mammals. To evaluate the zebrafish system for studies of kidney development and regeneration, we investigated the development and post-injury regeneration of mesonephros in adult zebrafish.

Utilizing two transgenic zebrafish lines (wt1b::GFP and Pod::NTR-mCherry), we characterized the developmental stages of individual mesonephric nephron and the temporal-spatial pattern of mesonephrogenesis. We found the mesonephrogenesis continues throughout the life of zebrafish, with a rapid growth phase during the juvenile period and a slower phase in adulthood. Interestingly, the total nephron number of juvenile and adult fish linearly correlates with the body mass.

Following gentamicin-induced renal injury, the zebrafish mesonephros can undergo *de novo* regeneration of mesonephric nephrons, known as neo-nephrogenesis. We here used transgenic zebrafish to investigate the initialization of neo-nephrogenesis. As early as 48 hours post injury *wt1b* expression was induced in individually dispersed cells in the mesonephric mesenchyme. This was followed by aggregation of *wt1b*-expressing cells and nephron formation, suggesting that *wt1b* may serve as an early marker of fated renal progenitor cells. The gentamicin-induced injury causes overly synchronized mesonephrogenesis, useful for developmental studies of nephrons.

With the inducible podocyte injury model we have generated, we characterized the repair process initiated after podocyte injury in mesonephros and found that *wt1b* expression expands into Bowman's capsule. This indicates a podocyte-specific repair mechanism in zebrafish mesonephros, a process also known to occur in metanephros following podocyte injury. Thus zebrafish mesonephros may serve as a suitable animal model for studies of glomerular repair.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO337

**Clonal Analysis, Sphere-Formation and Cell Sorting Identify Human Adult Kidney Cells Capable of Enhanced Tubular Regeneration *In Vivo*** Ella Buzhor,[1] Orit Harari-Steinberg,[1] Dorit Omer,[1] Michal Mark-Danieli,[1] Tzahi Noiman,[2] Ronald S. Goldstein,[2] Benjamin Dekel.[1] *[1]Pediatric Stem Cell Research Institute, Sheba Medical Center, Tel Hashomer, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [2]Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel.*

Human adult kidney (hAK) stem/progenitor cells are ideal targets for gene therapy, cell transplantation and tissue engineering. However, their identity remains elusive. Cells from dissociated human tissues that express tissue-specific stem cell markers are clonogenic and form spheres *in vitro* and capable of tissue regeneration *in vivo* often represent progenitor cells. Based on these assays we have now analyzed expanded heterogeneous cultures of kidney epithelial cells retrieved from nephrectomy samples and sorted cell subpopulations for progenitor potential.

Clonal analysis of individual cells in cultures from 5 hAK samples revealed clone frequency of 1-4% and clonogenic expansion, indicative of self-renewal, mostly up to passage 3-5, with a single clone expanded to P10. The addition of human fetal kidney culture conditioned media (hFKCM) to hAK cells augmented clonogenic frequency and self-renewal. When switched to low-attachment conditions 'nephrospheres' developed in hAK cultures resulting in up-regulation of renal progenitor markers. Although, human nephrospheres were not entirely clonally-derived, sphere cells grafted in the chick embryo displayed enhanced regenerative capacity of the entire tubular spectrum. Finally, fractionation of hAK cells according to *NCAM1* expression, an embryonic renal progenitor surface antigen that re-appeared in culture, selected a cell subpopulation that overexpressed renal 'stemness' genes (*Six2/Sall1/Pax2/Wt1*) and proximal tubule markers (*aquaporin1, aminopeptidaseA*), exclusively formed well-defined 'nephrospheres' and was highly clonogenic when seeded on matrigel with FKCM. When grafted into the chick embryo NCAM1+ cells showed preferential reconstruction of proximal tubules.

Thus, we have identified hAK growth conditions and specific cell subsets that harbor progenitor potential and promote renal regeneration.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO338

**Expression of Receptor-Type Tyrosine Phosphatases VE-PTP and PTPμ in Developing and Adult Renal Endothelium** Keiko Takahashi,[1] Colette R. Hunt,[1] Rebecca S. Weller,[1] Hiroki Fujita,[1] Dietmar Vestweber,[2] Nicholas W. Gale,[3] Takamune Takahashi.[1] *[1]Nephrology, Vanderbilt University Medical Center, Nashville, TN; [2]Max Planck Institute for Molecular Biomedicine, Muenster, Germany; [3]Regeneron Pharmaceuticals, Tarrytown, NY.*

Renal vascular development is a coordinated process which requires ordered endothelial cell proliferation, migration, intercellular adhesion, and morphogenesis. In recent decades, extensive efforts have defined the pivotal role of endothelial receptor tyrosine kinases (RPTKs) in the development of renal vasculature. However, the role of receptor tyrosine phosphatases (RPTPs), counter enzymes of RPTKs, remains unknown. Therefore, here we evaluated the expression of endothelial RPTPs, VE-PTP and PTPμ, in developing (from E12.5 to P7) and adult renal vasculature using the heterozygous LacZ knock-in mice as well as the specific antibodies. In adult kidney, both RPTPs are abundantly expressed in arterial and glomerular endothelial cells. They are also expressed in segments of vasa recta, whereas their expression is limited or absent in peritubular capillaries (PTCs) and venous circulations. In developing kidneys, VE-PTP is expressed in branching renal arteries, developing glomerular endothelium (as early as S-shaped stage), and medullary vessels (from E15.5 stage). Its expression becomes prominent as the vasculatures mature. Interestingly, substantial VE-PTP expression was observed in the PTCs of P3 and P7 kidneys, whereas it is down-regulated in adult PTCs; the finding indicates that VE-PTP expression is strictly controlled during the development of PTCs. Compared with VE-PTP, PTPμ protein expression is highly limited in embryonic and neonatal renal vasculature, although the promoter activity is observed in developing renal arteries and glomerulus. Its protein expression in renal vasculature is remarkably increased from P3 stage. Thus, compared to RPTKs, VE-PTP and PTPμ are expressed at later stage of renal vascular development and the differences in their expression timing suggest distinct role of these RPTPs in maturation and maintenance of the renal endothelium.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO339

**Angiotensin (Ang) II-Dependent Ureteric Bud (UB) Branching Morphogenesis Is Mediated by UB Cell Movements** Renfang Song, Graeme James Preston, Ihor V. Yosypiv. *Pediatrics, Tulane University School of Medicine, New Orleans, LA.*

Directional UB cell migration represents an important cellular mechanism of UB branching morphogenesis. Here, we tested the hypothesis that Ang II-induced UB cell migration is essential for UB branching morphogenesis. Quiescent UB cells were grown overnight on top of transwell filters (8-μm-pore size, Corning, Acton, MA) the bottom side of which was precoated with 2 μg/ml fibronectin at 370 C and 5% CO2. Ang II ($10^{-5}$ M) or media (control) were added to the bottom of the well. The cells on the upper surface of the filter were removed with a cotton swab, the membrane was fixed, stained with hematoxylin-eosin, and mounted on a slide. The mean number of cells that migrated through each membrane was determined by counting the number of cells in 5 high-power fields (x400)/well (n=4 wells/treatment group). Ang II increased the number of cells that migrated through the filter compared with control (336±5.1 vs. 301±4.4, $p<0.05$). To determine whether Ang II can directly stimulate UB morphogenesis, E11.5 HoxB7-GFP+ intact UBs were suspended in Matrigel and grown at 37° C for 5 days on transwell filters located on top of DMEM/F12 medium containing GDNF (120 ng/ml) and FGF (100 ng/ml) (n=6) or GDNF/FGF and Ang II (10-5 M, n=6). Ang II increased the number of UB tips on day 5 compared to control (20.0±2.1 vs. 12.1±0.7; $p<0.01$). To identify UB cell-autonomous genes expression of which is altered by Ang II signaling, we examined the effect of Ang II (10-5 M) or media (control) on global gene expression in intact UBs after overnight culture using Agilent mouse GE4X44K microarray. 19 transcripts were upregulated by Ang II by four-fold or more and 68 by two-fold. Ang II downregulated 14 transcripts by four-fold or more and 52 by two-fold. Detailed analysis is underway to identify gene and signal transduction networks downstream of Ang II. In summary, Ang II stimulates UB cell migration and directly induces morphogenetic response in intact UBs. We hypothesize that: 1) Ang II-dependent cell movements play an important role in UB branching morphogenesis, and 2) Gene networks downstream of Ang II mediate the effects of Ang II on UB branching.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO340

**Use of the E2F1 Transgenic Suicide-Inducible Mice Permit Regeneration of Completely Human Kidneys** Kei Matsumoto,[#1] Takashi Yokoo,[#1] Shinya Yokote,[#1] Tetsuya Kawamura,[#1] Toya Ohashi,[#1] Tatsuo Hosoya,[#1] Osahiko Tsuji,[#2] Hirotaka James Okano,[#2] Hideyuki Okano,[#2] Eiji Kobayashi.[#3] *[1]The Jikei University School of Medicine, Tokyo, Japan; [2]Keio University School of Medicine, Tokyo, Japan; [3]Jichi Medical University, Tochigi, Japan.*

Introduction: We have grown a chimeric kidney using human mesenchymal stem cells (MSCs) that produces urine and erythropoietin (EPO) but is derived from both the human MSCs and the host animal cells. To remove the animal component from the chimera we have established a novel system, in which the xeno-tissue component can be eliminated during development.

Methods:

We have established the transgenic ER-E2F1 suicide-inducible mice, which express estrogen receptor-E2F1 fusion protein. E2F1 is a transcription factor which regulate cell proliferation and its ectopic expression induces apoptosis in differentiated cells, therefore cells fate from ER-E2F1 mouse can be eliminated on demand by tamoxifen administration. Metanephroi from ER-E2F1 mice (E2F1 group) and C57BL/6 mice (control group) were cultured with and without tamoxifen for 14 days. Cell fate was observed by fluorescence microscopy. Metanephroi were transplanted to Splague-Dawley rats (N=10 in both groups), and FK506 and tamoxifen were administered daily for two weeks. The average weight of the grown transplants was recorded and any changes were observed histologically. The expression of EPO was assayed by q-PCR using species-specific primers.

Results: In vitro tamoxifen treatment of metanephros cells derived from ER-E2F1 mice successfully eliminated these cells whereas wild-type cells remained viable. The percentage of the grown transplants in vivo, was 32% in the E2F1 group versus 56% in control group ($P=0.045$). The final weight of transplants was 2.4±0.8 mg in the E2F1 group versus 8.7±1.3 mg in the control group ($P=0.035$). Histological analysis showed that kidney tissue is replaced by fibrous tissue in the E2F1 group. qPCR revealed that rat EPO was produced from grown metanephros in both groups and that there was no significant difference between the two groups.

Conclusion: Metanephroi derived from ER-E2F1 mice are a powerful tool to regenerate completely human-derived kidney tissue.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO341**

**The Epigenetic Signature of the Developing Nephron** Nathaniel J. D. Mclaughlin, Fenglin Wang, Zubaida R. Saifudeen, Samir S. El-Dahr. *Pediatrics, Tulane University School of Medicine, New Orleans, LA.*

Kidney development is orchestrated by intricate regulatory networks of pioneer transcription factors. However, how distinct gene expression domains and cell fate decisions are propagated and maintained faithfully throughout generations of cell divisions is not completely understood. We postulate that epigenetic inheritance of combinatorial histone modifications (signature) play a key role in "imprinting" and maintenance of cell fate decisions during nephrogenesis. Cell lineages and fates were co-immunolocalized with histone marks and corresponding enzymes and examined by confocal microscopy. At E11.0, the Wolffian duct, ureteric bud (UB) and uninduced metanephric mesenchyme (MM) are decorated with a broad epigenetic landscape composed of both activating (H3-K4me3, K36me3, R17me2; H4-K16ac) and repressive marks (H3-K9me3, K27me3; H4-R3me2, K20me3). Quantitative analysis further indicated that certain repressive marks, e.g., H3K27me3 and H4R3me2, are domain-enriched in MM and UB, respectively. On E15.5, Six2/Pax2$^+$ MM cap cells are highly enriched with repressive marks/enzymes (H3K9me3/G9a, K27me3/EzH2) but also express activating marks/enzymes (H3K4me3/Ash2l and R17me2/Carm1), suggesting that the genome remains poised in nephron progenitors. The progeny of Six2$^+$ cells give rise to Lhx1$^+$ renal vesicles, which are now decorated predominantly with activating marks. Proximal K-cadherin$^+$ tubules are rich in H3K4me3, whereas distal E-cadherin$^+$ segments are enriched in H3K79me3 and its enzyme, Dot1l. Unlike other marks, H3K79me3/Dot1l increase in abundance with maturation. WT1$^+$ podocyte progenitors possess a repressive signature characteristic of terminally differentiated cells and are also enriched with H3K79me3. In summary, differentiation along the nephric lineage is characterized by dynamic changes in the epigenome. Renal progenitors possess a "bivalent" epigenetic signature, allowing for plasticity of gene expression in their progeny during nephrogenesis and differentiation. Our data support the hypothesis that epigenetic inheritance plays an important role in the generation and maintenance of cell fate decisions during kidney development.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO342**

**Distinct Epigenomic Landscapes of Renal Developmental Regulators** Nathaniel J. D. Mclaughlin, Jiao Liu, Zubaida R. Saifudeen, Samir S. El-Dahr. *Pediatrics, Tulane University School of Medicine, New Orleans, LA.*

According to the histone code hypothesis, combinatorial modifications of histone tails create an epigenetic landscape, recognized by specific effector protein complexes facilitating either activation or repression of gene transcription. In the embryo, pluripotent progenitor cells have a high number of "bivalent" or "poised" promoters marking important developmental regulators compared to differentiated cells, as indicated by the repressive H3K27me3 and the active chromatin mark H3K4me3. To explore whether this model applies to cellular differentiation during organogenesis, we compared the epigenetic landscapes of three renal developmental regulators, Six2, Pax2 and Lhx1, in two lines of immortalized mouse E11.5 metanephric mesenchyme cells, utilizing ChIP-PCR and ChIP-Seq. mK3 cells express Six2 but lack Pax2 and Lhx1; in contrast, mK4 cells express Pax2 and Lhx1 but lack Six2. The results demonstrate that in mK4 cells, the Pax2 promoter is enriched with H3K4me3 and phosphorylated form of RNA PolII; in contrast, occupancy of H3K27me3 is much higher in mK3 cells. Other repressive marks, H3K9me3 and H3R2me2, are not different. The Lhx1 promoter harbors two active marks, H3K4me3 and H3K79me3, in mK4 cells, but is enriched in two repressive marks, H3K9me3 and H3K27me3 in mK3 cells. The Six2 promoter harbors the active mark, H3K4me3, in mK3 cells, and two repressive marks H3R2me2s and H3K27me3 in mK4 cells. Overexpression of the H3K4 histone methyltransferase, Ash2l, in mK4 cells, stimulated H3K4me3 accumulation and induced endogenous *Six2* mRNA; in contrast, expression of the H3K27 methyltransferase, EzH2, stimulated H3K27me3 accumulation and further suppressed *Six2* gene expression. Ash2l and EzH2 had no effect on *GDNF* expression. Our data demonstrates that renal developmental regulators possess distinct epigenetic landscapes, which correlate strongly with gene expression patterns. While the three developmental genes share H3K4me3 as a common activation mark, they diverge in their repressive marks, lending support to the idea that distinct epigenetic signatures play a role in differential gene regulation during cellular differentiation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO343**

**Reduced Six2 Expression Compromises Nephron Development** Suwit Jack Somponpun, Nora Matutino, Thomas Hynd, Scott Lozanoff. *Anatomy, Uni of Hawaii, Honolulu, HI.*

Suboptimal kidney development resulting in an in-born deficit in nephron number is associated with cardio-renal disease in later life. We identified a heritable mutation affecting embryonic expression of sine oculis 2 (six2) in Brachyrrhine (Br) mice who display renal hypoplasia, indicating critical role of six2 in nephrogenesis. In this study, we determine whether six2 is involved in establishing nephron number during development. E12.5 kidney explants from wild-type, Br/+, and Br/Br were cultured in DMEM for 72h and stained for six2 and calbindin. In subsequent experiment siRNA was used to inhibit six2 transcription in developing cultures and its effect on ureteric bud (UB) branching examined. Explants were incubated with 3μM of six2-siRNA and after 72h, stained for six2 and calbindin or processed for qPCR for six2 and other factors' mRNA. Six2-ir and number of UB tips were determined. Cultured kidney rudiments from Br/+ and Br/Br mice were substantially smaller and exhibited fewer UB tips, suggesting disruption of kidney growth in the absence of six2. In wild-type tissue, six2 was expressed in metanephric mesenchymes, typical of developing kidneys. Expression of six2 corresponds with branching pattern and number of UB, indicating successful kidney development in our preparation. Conversely, six2 was markedly reduced in Br/+ explants and absent from Br/Br. Correspondingly, number of UB tips decreases in Br/+ and are least amongst Br/Br kidneys. Explants exposed to six2-siRNA exhibit a substantial reduction in six2+ cells and a marked depletion of UB tips over 72h. qPCR demonstrated decreases in six2, PAX2, and Cited1 mRNA, whereas WT1 was elevated in response to six2-siRNA. Based on the degree and pattern of six2 and the number of UB tips among wild-type, Br/+, and Br/Br kidneys, results indicate that reduced six2 expression is associated with reduced UB branching and deficient nephron number in developing kidneys. This is further supported by a knockdown of six2 with siRNA, leading to a reduction in six2-expressing cells and UB branching morphogenesis. Our results implicate the role of six2 in early nephrogenesis and suggest that six2 may constitute the genetic basis for renal nephron endowment.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO344**

**Epigenetic Reprogramming of Mouse Hematopoietic Stem and Progenitor Cells for Treatment of Acute Kidney Injury** Ling Li, Fangming Lin. *Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX.*

The objective of this study is to develop stem cell-based therapy for treatment of acute kidney injury. Hematopoietic stem and progenitor cells were isolated from mice with renal epithelial-specific expression of EYFP (KspCre;R26R-EYPF mice) and treated with protein factors in culture. After one week of treatment, a large number of reprogrammed cells were generated with 6.3% expressing EYFP. The cells expressed a panel of renal developmental genes and proteins, and exhibited an epithelial-like phenotype. The cells did not express embryonic stem cell marker Oct-4 or revert to a pluripotent state. Our results indicate the activation of a renal program in treated cells. An increased level of histone acetylation was detected on the promoters for cadherin 6 and 16. Further treatment of the cells with a histone deacetylase inhibitor trichostatin A resulted in a 6-fold increase in EYFP-expressing cells and in renal gene expression. These results suggest the importance of histone acetylation in reprogramming. Reprogrammed cells integrated into developing tubules of the embryonic kidney organ cultures whereas mouse embryonic fibroblasts failed to integrate. To test the therapeutic effect of reprogrammed cells, we transplanted the cells into mice with renal ischemic injury and detected improved renal function, increased proximal tubular proliferation, and decreased tubular injury. Subcapsular injection of the cells did not form teratomas, supporting the safety of cell treatment. However, only a small number of transplanted cells were founded in the regenerating tubules, indicating that cell replacement by the transplanted cells is not the main mechanism of renal protection. Instead, reprogrammed cells may offer beneficial effects in a paracrine and/or endocrine fashion. This possibility was confirmed by renal functional improvement seen after the injection of conditioned medium and the detection of renotrophic factors from reprogrammed cells. In conclusion, our studies demonstrate that hematopoietic stem and progenitor cells can be reprogrammed to generate a large number of therapeutically valuable cells in a short period of time to treat acute kidney injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO345**

**Validation of a New Developmental Cre Driver Strain for Kidney Development** Yoshiro Maezawa,[1] Matthew Binnie,[2] Chengjin Li,[1] C. C. Hui,[4] Susan E. Quaggin.[1,2,3] *[1]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [2]Department of Medicine and Division of Nephrology, St Michael's Hospital, Toronto, ON, Canada; [3]Institute of Medical Science, University of Toronto, Toronto, ON, Canada; [4]Endocrine Research, Hospital for Sick Children, Toronto, ON, Canada.*

Cell type-/tissue-specific gene targeting strategies have provided enormous insights for the development of various organs. The Cre-loxP system is most widely used and requires introduction of loxP sites around an essential region of the gene of interest followed by cell type-specific Cre recombinase-mediated gene excision. To study renal development, a number of Cre-driver mouselines have been created, including Six2-Cre, HoxB7-Cre, and Pax2-Cre. Here, we report a new Cre driver mouse line, which, under control of the endogenous Pod1(Tcf21)-promoter, directs Cre recombinase expression in metanephric mesenchyme and its derivatives, including interstitial cells and all the epithelial components of the nephron from podocytes down to distal tubules of the developing kidney. This Pod1-Cre line also expresses Cre throughout the embryonic mesenchyme, including lung, heart, gastrointestinal tract and pancreas. We tested the utility of the Pod1-Cre line using four floxed lines. Pod1-Cre line bred with floxed VEGF-A mice resulted in striking loss of mesangial cells. When crossed to the beta-catenin floxed line, this mouseline generated hydroureter, hydronephrosis and hypoplastic kidneys. A conditional gain of function model of beta-catenin resulted in fused kidneys as well as a striking poorly differentiated sarcoma that is spread throughout the mesenchyme of multiple organs of the embryo. Finally, a conditional knockout of the gene encoding Patched-1, a receptor for sonic hedgehog signaling, resulted in cyst formation and lack of medullary space in the kidney. Surprisingly, these Pod1-Cre/ Patched-1 mice also developed an aggressive embryonic sarcoma, which is virtually identical to that seen in the conditional gain of function model of beta-catenin. These results demonstrate that the Pod1-Cre mouse is a powerful tool to study the development and function of the kidney in transgenic mice.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO346**

**High Salt Intake in Pregnancy Alters Maturation of Glomeruli in the Rat Offspring** Nadezda Koleganova,[2] Grzegorz Piecha,[2] Eberhard Ritz,[1] Marie-Luise Gross.[2] *[1]Dept. of Internal Medicine, University of Heidelberg, Heidelberg, Germany; [2]Institute of Pathology, University of Heidelberg, Heidelberg, Germany.*

Faulty fetal programming leads to alterations in kidney morphology in the offspring. A low number of glomeruli is known to cause high blood pressure later in life. It was the purpose of the present study to clarify whether high salt intake in pregnancy alters kidney development in the offspring.

Sprague-Dawley rats were fed normal (0.15%), medium (1.3%), or high (8.0%) salt diet during pregnancy and weaning. The number of glomeruli (mature, immature, and S-shape bodies) was assessed at 1 week postnatally. The expression of proteins involved in kidney development was assessed (by western blotting) at term and at 1 week postnatally.

At age 1 week the number of S-shaped bodies was significantly lower (405±308) and the number of mature glomeruli (818±405) and layers of developing glomeruli (7.1±0.6) was higher in the offspring of mothers on high-salt compared to the other groups (1044±490, 460±304, and 5.9±0.9 respectively). As a net result total number of glomeruli was significantly lower in the offspring of mothers on high-salt (9476±1264) compared to the other groups (11175±1920). At 1 week of age in the offspring of mothers on high salt the glomeruli were bigger compared to lower salt intake. The expression of Pax-2 (54±23% vs. 100±28%) and FGF-2 (72±33% vs. 100±30%) was significantly lower in the offspring of mothers on high-salt consistent with their causative role. There was no difference between the groups with respect to litter size, birth weight, and placenta size

We conclude that high maternal salt intake during pregnancy accelerates maturation of glomeruli in the offspring, but reduces the final number of glomeruli.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO347**

**Postnatal Overnutrition Brings Lifelong Obesity and Renal Impairment in Adult Male Rats** Hyung Eun Yim, Kissu Ha, In Sun Bae, Young Sook Hong, Joo Won Lee, Kee Hwan Yoo. *Pediatrics, Korea University Guro Hospital, Seoul, Korea.*

Accelerated growth in early infancy has been associated with later cardiovascular and metabolic diseases. We recently showed that postnatal overnutrition led to hyperleptinemia, obesity and the acquired reset of key intrarenal hormone systems in the juvenile male rats. In this study, we investigated the influence of overnutrition during neonatal periods on the development of lifelong renal pathophysiological changes in adult offspring rats. Three or 10 male pups per mother from rat pup litters were assigned to either the overnutrition or control groups during the first 21 days of life. The effects of early postnatal nutrition excess on body weight, blood pressure, blood glucose and potential renal changes related to obesity were measured by 3 and 6 months of age, respectively. Smaller litter male pups persistently weighed heavier than controls between 7 days and 6 months of age ($P < 0.05$). By 3 and 6 months of age, there were no differences of kidney weight per body weight ratio, blood glucose and plasma leptin levels between the two groups. However, increased systolic, diastolic and mean blood pressure levels were observed in obese 6 month-old rats ($P < 0.05$). Serum creatinine was increased in each 3 and 6 month-old obese rats ($P < 0.05$). ED-1 positive macrophages and glomerulosclerosis were also increased by overnutrition at 3 and 6 months of ages ($P < 0.05$). Apoptotic cortical renal cells increased in neonatally overfed 6 month-old rats ($P < 0.05$). In immunoblots and immnnohistochemistry, matrix metalloproteinase (MMP)-9 protein expressions decreased and tissue inhibitor of MMP-1, tumor necrosis factor-α, osteopontin and adiponectin expressions increased in obese 3 month-old rats ($P < 0.05$). In contrast, increased MMP-9 and osteopontin expressions were found in obese offsprings of 6 months old ($P < 0.05$). Renin, angiotensin II type (AT) 1 and AT2 receptor expression was not changed. Our data demonstrates that postnatal overfeeding leads to lifelong obesity and renal dysfunction. Obesity induced by early postnatal overnutrition may have a detrimental impact on kidney structure, function and intrarenal inflammatory cytokines.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO348**

**Genome-Wide Identification of Target Genes of the Distal Nephron Transcription Factor CP2-Like 1 (Tcfcp2l1)** Kai M. Schmidt-Ott,[1,2] Melanie Viltard,[3] Jonathan M. Barasch.[3] *[1]Max-Delbrueck Center for Molecular Medicine, Berlin, Germany; [2]Experimental and Clinical Research Center, Charité-Universitätsmedizin Berlin, Berlin, Germany; [3]Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY.*

Transcription factors of the CP2 family control aspects of epithelial differentiation across species from Drosophila to man. Two transcription factors of this family are specifically expressed in the ureteric bud and in the distal nephron of the developing and adult kidney, grainyhead-like 2 (Grhl2) and transcription factor CP2-like 1 (Tcfcp2l1). We have set out to understand genomic target gene regulation by these factors in the kidney. We conducted chromatin immunoprecipitation (ChIP) coupled with massively parallel sequencing (ChIP-seq) in postnatal day 2 kidneys using a specific Tcfcp2l1 antibody and compared to rabbit IgG as a control. Sequence alignment to the mouse genome produced 3,171,121 aligned matches and the top 5000 clusters (representing genomic windows covered by at least 6 reads) were selected for further characterization. 50 of these Tcfcp2l1-binding peaks were randomly selected and 41 of these were confirmed independently by ChIP-PCR experiments corresponding to a validation rate of 82%. We combined data on the genomic localization of Tcfcp2l1 binding sites with respect to genes with expression profiles of the ureteric bud we had previously obtained. We identified genes that contained at least one Tcfcp2l1 binding site within 2000 bp of the transcriptional start site and found a highly significant overrepresentation of ureteric bud genes ($p<0.0001$). Tcfcp2l1 target genes constituted several key constituents of distal nephron epithelia, including cytokeratin 7 (Krt7), aquaporin 3 (Aqp3), and cadherin 6 (Cdh6). Remarkably, overexpression of Tcfcp2l1 in isolated rat metanephric mesenchyme using adenoviral gene transfer resulted in an upregulation of several of these genes and, ultimately, induced the appearance of epithelial tubules. These data indicate that Tcfcp2l1 is sufficient to drive an ectopic distal nephron-specific epithelial gene program in non-epithelial progenitor cells.

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**TH-PO349**

**An NCAM1$^+$ Cell Enriched for Aldehyde Dehydrogenase Activity and Possessing Renal Stem Cell Characteristics Is the Wilms' Tumor Initiating Cell** Naomi Pode Shakked,[1] Sarit Bahar,[1] Peter Tsvetkov,[2] Orit Harari-Steinberg,[1] Benjamin Dekel.[1] *[1]Pediatric Stem Cell Research Institue, Sheba Medical Center, Israel; [2]Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.*

Wilms' tumor (WT), the most common pediatric renal malignancy is suggested to arise from malignant transformation of abnormally persistent renal stem cells which retain proliferative potential but also undergo partial differentiation to form the heterogeneous mature cell populations seen in this tumor. We have previously suggested NCAM1 as a marker for WT stem/progenitor cell based on in vitro assays which demonstrated this cell fraction as highly clonogenic, proliferative and overexpressing the WT "stemness" genes.

Complicating WT in vivo cellular analysis is the inability of fresh or cultured WT cells to form tumors in immunodeficient mice. Here, we have established WT xenografts (Xn) that are characteristically highly proliferating tumors that can be rapidly propagated and sustained by individual cells and are enriched for early renal lineage markers (OSR1/SIX2), poor WT prognostic markers (TOP2A, N-MYC and CRABP2) and the surface marker NCAM1. We utilized this WT reservoir to isolate tumor-initiating cells that recapitulate the entire tumor morphology in immunodeficient mice. Accordingly, sorting experiments of Xn–derived cells and injection to mice at serial dilutions, revealed that the tumor-initiating capacity is solely contained within the NCAM1$^+$ cell population (as few as 500 cells), which gives rise to NCAM1$^-$ tubular cells and can further induce tumors when transplanted into secondary and tertiary recipients, indicative of self-renewal and differentiation capacities.

We go on to show that within the NCAM1 population, CD44 and especially ALDH1 but not PSA-NCAM greatly enrich for the tumor-initiating cells (as few as 200 cells). Importantly, the expression of nephric patterning, stemness and poor prognostic genes in these cell fractions was intimately linked with their tumor forming ability.

In summary, this study definitely identifies a renal stem cell as the cellular origin of WT and affords a novel in vivo system for vast pre-clinical drug studies on WT.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO350**

**Conditional Disruption of *Pkd1* in Mesenchymal Lineage Results in Osteopenia and Polycystic Kidney Disease** Ni Qiu, Zhousheng Xiao, Li Cao, Valentin David, Leigh Darryl Quarles. *College of Medicine, The University of Tennessee Health Science Center, Memphis, TN.*

Selective deletion of *Pkd1* in the osteoblasts using either *Osteocalcin*-Cre or *Dmp1*-Cre results in osteopenia and in kidney using *Ksp*-Cre causes polycystic kidneys. *Pkd1* is also expressed in undifferentiated mesencyme. To broadly examine the function of Pkd1, we respectively used *Col1a1*(3.6)-Cre and *Co1a1*(2.3)-Cre mice to achieve selective inactivation of *Pkd1* at E14.5 and E18.5 in mesenchymal precursors. Bone and kidney samples were analyzed from newborn and 6 week-old control *Pkd1*$^{flox/+}$ and condtional *Col1a1(3.6 or 2.3)*-Cre;*Pkd1*$^{flox/flox}$, *Col1a1(3.6 or 2.3)*-Cre;*Pkd1*$^{flox/null}$ mice, which differed in the timing and magnitude of Pkd1 ablation. Transgenic *Col1a(3.6 or 2.3)*-Cre;*Pkd1*$^{flox/flox}$ and *Col1a(3.6 or 2.3)*-Cre; *Pkd1*$^{flox/null}$ mice displayed osteopenia at 6 weeks-of-age. Histological analyses found that cysts were absent in kidneys and pancreas from newborn of *Col1a1(3.6)*-Cre;*Pkd1*$^{flox/flox}$ mice, but cysts developed in these organs by 6 weeks-of-age. In contrast, *Col1a1(3.6)*-Cre;*Pkd1*$^{flox/null}$ mice had major cyst formation at birth that was associated with increased mortality. Neither transgenic *Col1a(2.3)*-Cre;*Pkd1*$^{flox/flox}$ or *Col1a(2.3)*-Cre; *Pkd1*$^{flox/null}$ mice developed cystic disease. Thus, ablation of Pkd1 in the mesenchymal precursors giving rise to the osteoblast lineage results in osteopenia that is porportionate to the magnitude of Pkd1 reduction rather than the timining of Cre expression. In contrast, the earlier onset and more severe cystic phenotypes in *Col1a1(3.6)*-Cre;*Pkd1*$^{flox/null}$ compared to *Col1a1(3.6 or 2.3)*-Cre;*Pkd1*$^{flox/flox}$ or *Col1a(2.3)*-Cre; *Pkd1*$^{flox/null}$ mice indicates that both gene dose and timing of Pkd1 ablation during embryogenesis influences the severity of cyst formation. The selective deletion of *Pkd1* from mesenchymal lineage results in both defective bone formation and polycystic kidney disease, indicating that *Pkd1* has essential function in mesenchymal precursors to regulate both skeletal and renal development. The long-term survival of *Col1a1(3.6)*-Cre;*Pkd1*$^{flox/flox}$ mice establishes a potential model to study postnatal interventions to retard cyst formation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO351**

**Vangl2, a Gene Encoding a Planar Cell Polarity (PCP) Protein, Is Required for Branching Morphogenesis and Glomerulogenesis in the Mammalian Kidney** Jenny Papakrivopoulou,[1] Laura Yates,[2] David A. Long,[3] John Connolly,[1] Adrian S. Woolf,[4] Charlotte Dean.[2] *[1]UCL Centre for Nephrology, Royal Free Hospital, London, United Kingdom; [2]Mammalian Genetics Unit, Medical Research Council Harwell, Oxford, United Kingdom; [3]Nephro-Urology Unit, Institute of Child Health, University College London, London, United Kingdom; [4]Biomedical Research Centre, University of Manchester, Manchester, United Kingdom.*

Previous studies have highlighted a role for the PCP pathway in maintaining kidney tubule differentiation, with mutations associated with cystogenesis. However, whether the pathway has other roles in kidney development is unclear. The loop-tail (*Lp*) allele results in absence of functional Vang-like protein 2 (*Vangl2*), a core PCP protein. Here we show that homozygous fetuses had neither duplex nor hydronephrotic kidneys. The *Lp/Lp* ureteric bud penetrated adjacent renal mesenchyme normally but the first rounds of *Lp/Lp* ureteric bud/collecting duct branching were reduced *in vivo*. The observed ureteric bud dysmorphology occurred after just-formed metanephroi were explanted and maintained in organ culture for 2 days. In late gestation, the homozygous mutant kidney had a hypoplastic medulla and contained glomerular tufts which were smaller than normal, with less prominent capillary loops. Proliferation rates were reduced in *Lp/Lp* glomeruli but expression of podocyte proteins nephrin and synaptopodin was unchanged. *Lp/Lp* metanephroi contained occasional distorted tubules but an overt polycystic phenotype was not observed. Non viability of homozygous mice due to severe neural tube defects precluded analysis after birth. *Lp*/+ metanephroi displayed a glomerular phenotype midway between wild-types and homozygous mutants and postnatally, after nephrogenesis was complete, heterozygous kidneys had modest reductions in glomerular numbers. *Vangl2* was expressed in podocytes as were other PCP genes including Scribble and Celsr1, themselves also expressed in S-shaped bodies. The data show for the first time that *Vangl2*, and by implication the PCP pathway, is required for early morphogenesis of collecting ducts and complete differentiation of glomeruli.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO352**

**Construction of Lentiviral Vector Encoding HIF-1α and HIF-1α Expression in Human Adipose-Derived Stem Cells** Weiwei Wang, Jinyuan Zhang. *Nephrology Division, Jimin Hospital of Shanghai, Shanghai, China.*

**Objective** Adult stem cells derived from adipose tissue is an attractive cell source. Because adipose tissue is obtained as lipospirate from the patient in a less invasive manner and provides a large quantity of autologous cells, the use of adipose-derived stem cells (ADSCs)is an alternative way of providing seed cells. Hypoxia-inducible transcription factor-1α (HIF-1α) is known as a key regulator in protective role of renal damage by induction of downstream protective genes. Proteins coded by protective genes are sufficient to induce renoprotection. We transfected human adipose-derived stem cells(hADSCs) using lentiviral vector encoding hypoxia inducible factor-1α (HIF-1α) to study its expression level in vitro and to get a stable cell line expressing HIF-1α.

**Methods** The multi-directional differentiation of hADSCs was assessed by alizarin red staining and oil red O staining. HIF-1α gene was sub-cloned into the lentiviral transfer vector. The recombinant lentiviral supernatant were packaged by 293T cells through three plasmids transient co-transfection method using standard lipofectamine reagent. The viral titer was tested by the transfection efficiency of 293T cells. Lentiviral vector carrying HIF-1α transduced to hADSCs in different multiplicity of infection (MOI=1,5,10,15,20). Transfection efficiency was tested by fluorescence-activated cell sorting. HIF-1α gene expression level and protein secretion level of hADSCs were tested by real-time PCR and western blot methods after transfection.

**Results** The alizarin red staining and oil red O staining showed hADSCs could be induced to osteogenic and adipogenic lineages. The viral titer was $3.5\times10^{8}$TU/ml. Transfection efficiency was over 90% under MOI=20 at 7$^{th}$ day after infection. The expression of HIF-1α in transfected hADSCs was increased obviously detected by real-time PCR and western blot compared with vector group. The morphology and viability of transfected hADSCs had no marked change. **Conclusions** We successfully use lentiviral vectors encoding HIF-1α to transfected human adipose-derived stem cells and got a stable human adipose-derived stem cells lines sustained over-expressing HIF-1α.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO353**

**Generation of Inducible Pluripotent Stem Cells from Patients with Autosomal Dominant Polycystic Kidney Disease** Albert Q. Lam, Joseph V. Bonventre. *Department of Medicine, Renal Division, Brigham and Women's Hospital, Boston, MA.*

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common inherited diseases, affecting 1 in 600-1000 individuals. Patients with ADPKD typically present in the 4th and 5th decades of life with bilateral renal cysts, hypertension, and progressive loss of renal function. Mutations in the PKD1 and PKD2 genes encoding polycystin-1 and polycystin-2, respectively, are responsible for nearly 100% of cases of ADPKD. We demonstrate for the first time that inducible pluripotent stem (iPS) cells can be generated from patients with ADPKD by reprogramming their skin fibroblasts with four transcription factors (OCT4, SOX2, KLF4, C-MYC). We have derived, expanded, and characterized six iPS cell lines from fibroblast cell lines of three adult patients with ADPKD. ADPKD-specific iPS cells form compact colonies with embryonic stem (ES) cell-like morphology when co-cultured on a feeder layer of mouse embryonic fibroblasts. ADPKD iPS cells express the hallmarks of pluripotency, as demonstrated by positive alkaline phosphatase staining and positive immunocytochemistry for OCT4, NANOG, Tra-1-60, SSEA-3, and SSEA-4. The expression of pluripotency-promoting genes in ADPKD iPS cells as quantified by RT-PCR is similar to that of human ES cells and human iPS cells from normal controls and elevated compared to the parent fibroblast cell lines. All ADPKD iPS cell lines are capable of differentiating into cell types of all three germ layers in vitro and form teratomas when injected into SCID mice. ADPKD iPS cells remain karyotypically normal after multiple passages. The establishment of pluripotent ADPKD iPS cell lines represents the first step in the process of using human iPS cells for patient-specific ADPKD disease modeling and potential therapeutic testing.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO354**

**Expression of Prox1 in the Renal Cortex during Mouse Development** Yumi Kim, Wan-Young Kim, Eun-Young Park, Jin Kim. *Anatomy and MRC for Cell Death Disease Research Center, The Catholic University of Korea, Seoul, Republic of Korea.*

Prospero-related homeobox1 transcription factor (Prox1), is known to be expressed in various internal organs and is related to their embryogenesis. It is also expressed in lymphatic endothelial cells and plays an essential role in embryonic lymphangiogenesis. However, little is known about Prox1 expression in the development of the lymphatic vasculature of the kidney. This study is intended to examine the expression of Prox1 in the developing mouse kidney with particular emphasis on the lymphatic system of the renal cortex**.** The expression and localization of Prox1 in developing kidneys from 14-, 15-, 16- and 18-day-old fetuses (E14 to E18), 1-, 4-, 7-, 14-, and 21-day-old pups (P1 to P21), and adult C57BL/6 mice were examined using immunohistochemical technique. In the renal cortex, Prox1 was localization in the cell nucleus, and initially expressed on E14 in the LYVE-1$^{+}$ lymphatic endothelial cells. Prox1 was expressed in these cells during the embryonic and postnatal lymphatic development and through to adulthood. However Prox1 was not expressed in a subset of LYVE-1$^{+}$ endothelial cells in glomerular capillaries or arcuate veins. We have reportd previously that LYVE-1 is also expressed transiently in a F4/80$^{+}$/CD11b$^{-}$ macrophages but Prox1 was not coexpressed in these cells. During lymphatic vessel development, LYVE-1$^{+}$/F4/80$^{+}$/CD11b$^{-}$ macrophages may be involved indirectly in active branching process of lymphangiogenesis. These findings suggest that Prox1 plays a major role in lymphangiogenesis in the developing mouse kidney and is required to maintain the lymphatic vasculature during later stages of development and in adulthood.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO355**

**GLI3 Repressor Controls Nephrogenesis and Ureteropelvic Junction Development** Norman D. Rosenblum,[1,2,4] Marian Vicky Staite,[1,2] Brian J. Nieman,[3] Jason Cain.[1] *[1]Prog Dev & Stem Cell Biol, Hosp Sick Child; [2]Dept Physiology, U Toronto; [3]Toronto Centre for Phenogenomics; [4]Div Neph, Dept Peds, U Toronto, Toronto, Canada.*

Hedgehog (HH) signaling controls renal development by increasing GLI transcriptional activators (GLIA) and inhibiting formation of GLI3 transcriptional repressor (GLI3R) (Hu et al, Development, 2006; Cain et al, PLoS ONE, 2009). HH ligands are bound by PTC1, a cell surface protein, that constitutively inhibits GLI signaling in the unbound state. Mutations in *PTC1* cause tissue malformation and cancer in nonrenal tissues. We tested our hypothesis that constitutive active HH signaling is deleterious to renal development in mice with *PTC1* deficiency targeted to the metanephric mesenchyme (MM) (*Rarb2-Cre;Ptc1$^{loxP/-}$*, termed *Ptc1* mutants). Increased HH signaling in MM was confirmed by quantitiative *Ptc1* PCR and expression of the *Ptc1-lacZ* HH signaling reporter. Mutant mice died immediately after birth with a thin renal cortex and massive hydropelvis. Ureteropelvic junction (UPJ) obstruction was demonstrated by intrapelvic dye injection and MRI, the latter demonstrating a 9-fold increase in pelvic volume. As early as E13.5, a cluster of cells marked by ectopic expression of *Raldh2* and *Ptc2* and increased expression of *Bmp4* accumulated at the presumptive UPJ. Reduced cortical volume was associated with decreased formation of NCAM-positive nephrogenic precursors (2.6-fold decrease, p=0.01, n=3) at E13.5 and WT1-positive glomeruli (1.4-fold decrease, p=0.02, n=3) at E18.5, normal *Gdnf, Ret* and *Wnt11* expression and ureteric branching but increased cortical expression of *Foxd1*. The relative contribution of GLIA and GLI3R to the mutant phenotype was defined in mutant mice with deficiency of *Gli2*, the major GLIA, or expression of a single allele of *Gli3$^{\Delta 699}$*, which encodes GLI3R, respectively. Heterozygous *Gli2* deficiency in *Ptc1* mutant mice did not rescue the mutant phenotype. In contrast, expression of *Gli3$^{\Delta 699}$* in *Ptc1* mutant mice increased nephron number to levels observed in WT mice (n=3, p=0.03) and decreased pelvic volume 2.4-fold (n=3, p=0.004) compared to *Ptc1* mutant mice. We conclude that GLI3R is required for nephrogenesis and patterning of the UPJ.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO356**

**Organ-Specific Defects in IGF Signaling and Asymetric Organ Growth in Mice with Intrauterine Growth Retardation** Tatiana Novitskaya, Mark P. De Caestecker. *Nephrology Division, Vanderbilt University, Nashville, TN.*

Intrauterine growth retardation (IUGR) resulting from placental insufficiency affects embryonic patterning of the kidney and gives rise to a decrease in the number of functioning nephrons. However the fetal mechanisms regulating abnormal renal growth in IUGR are unknown. There are currently no established mouse models of placental insufficiency with IUGR that would enable genetic manipulation of the signaling pathways regulating this fetal response. To address this, we have developed a mouse model in which loss of placental Cited1 expression gives rise to late gestational placental insufficiency. These mice have classical asymmetric IUGR with decreased kidney, liver and lung size along with preservation of fetal brain weight. Since IGF signaling plays a critical role in regulating fetal organ growth, we interrogated in IGF signaling pathway in these mice. IGF1 protein levels are reduced in kidney and livers from E18.5 embryos with IUGR compared with wild type littermates, while IGF2 protein expression is selectively decreased in the lung. In contrast IGF1 and IGF2 are increased in brains from embryos with IUGR. Decreased IGF1 in the kidney and liver is associated with reduced activating $Y^{1135/1136}$ phosphorylation of the IGF-1 receptor, while reduced IGF2 expression in the lung is associated with decreased activating $Y^{1135/1136}$ phosphorylation of the insulin receptor (IR). Since IR signals downstream of IGF2 but not IGF1 in the embryo, this suggests that decreased IGF2 in the lungs of embryos with IUGR is functionally significant. In contrast there is no change in IGF1 or IR receptor phosphorylation in the brains of embryos with IUGR. Thus organ-specific loss of IGF1 and IR receptor signaling correlates with asymmetric organ growth retardation and could play a direct role in promoting abnormal kidney, liver and lung growth in IUGR.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO357**

**β1 Integrin Is Required for Ureterovesicular Junction Development** Paul R. Brakeman,[1] Denise K. Marciano,[2] Natalie Spivak,[1] Chao-Zong Lee,[2] Louis Reichardt,[4] Keith Mostov.[3] *[1]Pediatrics, University of California, San Francisco, CA; [2]Medicine, University of California, San Francisco, CA; [3]Anatomy, University of California, San Francisco, CA; [4]Physiology & Biochem/ Biophysics, University of California, San Francisco, CA.*

Integrins are transmembrane receptors that mediate interactions between cells and extra-cellular matrix and are present in signaling complexes at sites of adhesion called focal adhesions (FAs). ß1-containing integrins are known to be critical for renal development. The structure and dynamics of FAs have been well described in 2-dimensional cell culture; however, little is known about FAs in 3-dimensional (3-D) epithelial structures. We have now visualized FAs in 3-D MDCK cultures and in developing renal epithelia *in vivo*. We find that loss of ß1 integrin via shRNA knockdown in MDCK cells in 3-D culture disrupts FA complexes and cellular motility in response to hepatocyte growth factor. In order to study the role of ß1 integrin and FAs *in vivo* during urogenital development, we generated tissue specific knockout mice with disruption of ß1 integrin in the collecting system, the ureteric bud and nephric duct. As recently shown by Zhang et al (2009), we confirm that loss of ß1 integrins in the renal collecting system results in severely hypoplastic kidneys. Further, we observe the novel phenotype that mutant mice develop severe hydroureter owing to a failure of the distal ureter to fuse with and penetrate through the bladder wall, resulting in a markedly abnormal ureterovesicular junction. Together, our results demonstrate that it is possible to study focal adhesion complexes in 3-D epithelial structures and that ß1 integrins are essential for ureterovesicular junction development.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO358**

**Characterization of Renal Injury in Megabladder Mouse, a Unique Animal Model of Congenital Obstructive Nephropathy** Susan E. Ingraham,[1] Kirk M. McHugh.[2] *[1]Pediatric Nephrology, Nationwide Children's Hospital, Columbus, OH; [2]Center for Molecular and Human Genetics, The Research Institute at Nationwide CHildrne's Hospital, Columbus, OH.*

Congenital obstructive nephropathy is the most common cause of chronic renal failure in children, often progressing to end stage renal disease. The transgenic mutant megabladder (mgb) mouse exhibits signs of lower urinary tract obstruction in utero, resulting in the development of congenital hydroureteronephrosis and progressive renal failure following birth. This study examines the development and progression of renal injury in homozygotic mgb mice (mgb-/-). Renal ultrasound was utilized to stratify the disease state of individual mgb-/- mice, while surgical rescue of the phenotype was investigated using cutaneous vesicostomy. The progression of renal injury in these animals was characterized histopathologically using a series of markers that included α-smooth muscle isoactin, TGF-β1, CTGF, WT-1, and Pax2. This analysis indicated that mgb-/- mice are born with pathologic changes in kidney development that progressively worsen in correlation with the severity of hydronephrosis. The initiation and pattern of fibrotic changes observed in mgb-/- kidneys appeared distinctive compared to prior animal models of obstruction, and shared similarities with the development of congenital obstructive nephropathy in humans. These observations suggest that the mgb mouse represents a unique small animal model for the study of congenital obstructive nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO359**

**MicroRNA Regulation of Gene Expression during the Ureteric Bud Outgrowth from the Wolffian Duct** James B. Tee. *Dept of Paediatrics, University of Calgary, Calgary, AB, Canada.*

Embryonic kidney development begins with the outgrowth of the ureteric bud (UB) from the Wolffian duct (WD). Disruptions to this process can lead to renal malformations that are a major contributor to ESRD in children. While recent studies have focused on the gene regulation of this process, little is known about the potential role of governing miRNAs which can serve to silence the expression of target mRNAs. To evaluate this possibility, WDs were extracted with their attaching mesonephros from embryonic day 11 CD-1 mice. UB outgrowth along the duct was induced by the in-vitro administration of glial cell line-derived neurotrophic factor. Biological replicate samples for each condition were concurrently evaluated by whole-transcript gene expression microarray analysis and miRNA array profiling. Comparing the budded with the unbudded WD conditions by T-Test unpaired Benjamini-Hochberg multiple testing correction revealed 16 miRNAs that exhibited a minimum 2-fold increase in expression, ranging from let-7c (2.2-fold change) to miR-29a (16.6-fold change). Utilizing an established miRanda-based algorithm, a generated list of predicted target mRNAs ($p < 0.05$) was cross-referenced with the 5,632 significantly downregulated genes uncovered by the whole-transcript array. This resulted in 1,083 unique gene targets for this set of miRNA that were downregulated in the budded WD, of which the most well-predicted targets are presented. Two downregulated miRNA gene targets had GO terminology referencing either the kidney or ureter: Agtr1b and Pkhd1. Conversely, 7 miRNAs exhibited a 2-fold decrease in expression in the budded WD (ranging from miR-669c (2.2-fold change) to miR-292 (4.5-fold change). Cross-referencing the predicted targets of this subset of miRNAs with the 4,861 significantly upregulated genes on the whole-transcript array revealed 1,022 unique gene targets in the budded WD, of which three referenced similar GO terminology: Nog, Eya1, and Jmjd6. The presented results suggest that microRNAs may be involved in the regulation of gene expression governing the UB outgrowth from the Wolffian duct, and sets the stage for the validation of the most highly-predicted targets in this process.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO360**

**WNT-$Ca^{2+}$ Pathway Activity Peaks during Kidney Development and Restricts Mesenchymal Cell Motility *In Vitro*** Michelle Miller,[1] Paul R. Goodyer,[1] Diana Iglesias.[2] *[1]Human Genetics, McGill University, Montreal, QC, Canada; [2]Pediatrics, McGill University Health Center, Montreal, QC, Canada.*

The non-canonical, calcium-dependent WNT signalling pathway is poorly understood in the context of renal development. In this study, we characterize WNT-$Ca^{2+}$ signalling during normal kidney development using transgenic mice bearing a luciferase reporter driven by the transcription factor NFAT, the major effector of calcineurin activation.

In NFAT-luciferase reporter mice, WNT-$Ca^{2+}$ pathway activity is extremely high in the embryonic kidneys. Reporter activity peaks from E13-E16 and drops significantly by P15 after which WNT-$Ca^{2+}$ signalling as well as nephrogenesis comes to an end. These data suggest that the WNT-$Ca^{2+}$ pathway plays an important role in kidney development.

To understand the function of the pathway, we developed systems *in vitro* and *ex vivo* to specifically manipulate the WNT-$Ca^{2+}$ pathway and used renal mesenchymal cell lines to determine pathway effects on cell migration. When the MK3 cell line (derived from early metanephric mesenchyme) was co-cultured with fibroblasts secreting the classic non-canonical WNT5A, we found that WNT-$Ca^{2+}$ activity was stimulated 2-fold. This increase in pathway activity could be abolished by adding cyclosporine A (CsA), a specific inhibitor of calcineurin, to the co-culture media. E14 kidney explants were also co-cultured with WNT5A-secreting fibroblasts and a 2.5-fold increase in pathway activity was seen, which was again abolished by the addition of CsA. Taken together, these observations indicate that the calcineurin/NFAT pathway is stimulated by WNT5A in the metanephric mesenchyme.

To ascertain whether the NFAT pathway affects cell movement, we performed scratch assays with MK3 cells treated with WNT5A in the presence or absence of CsA. Cell movement was significantly reduced after WNT5A treatment; this inhibitory effect was abolished in the presence of CsA.

We hypothesize that the WNT-$Ca^{2+}$ pathway is stimulated by a non-canonical WNT signal during nephrogenesis and is required for a process that restricts cell movement.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO361**

**Prematurity Leads to Reduced Nephron Number and Hypertension** Ashraf El-Meanawy. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

Reduced nephron number (oligonephronia) is a risk factor for hypertension and chronic kidney disease (CKD). The etiology of oligonephronia and how it leads to hypertension and CKD are poorly understood. Prematurity is a risk factor development of hypertension, metabolic syndrome, and kidney disease later in life. In the USA, one of every 8 children is born prematurely, and incidence in blacks of non-Hispanic origin is twice the incidence in white population. Worldwide, there are 12.5 million premature births annually. The consequences of prematurity are dependent on gestational age as well as other factors including genetic, environmental, and socioeconomic variable. Animal models provide an invaluable resource to study the effect of prematurity on kidney development and the health consequences later in life as it lacks the confounding variable which makes these studies in humans near impossible.

In this report we describe the development of a mouse model for prematurity with evidence of renal developmental abnormality. Adult mice born one or two days early have 20-25% reduction of nephron number compared to full term controls. The premature mice have statistically significant lower Glomerular filtration rate, as measured by real-time inulin clearance. Premature mice also have higher serum creatinine and abnormal urinary protein excretion. The premature mice were found to have a base line statistically significant higher blood pressure.

The renal consequences of prematurity in human has been attributed to the either the etiological factors of prematurity (IGUR and placental aging), or to the insults sustained in the post-natal period in the form of sepsis or exposure to toxic medications. The animal model developed in this study will provide a very useful resource to study effect of prematurity on kidney development and its reflection on hypertension and renal disease in the adult individual. We concluded that loss of placental support prematurely leads to arrest and/or abnormal programming of the developing kidney which leads to reduced nephron number and hypertension in adult animals.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO362

**Deletion of NuRD Component Mta2 Results in Renal Hypoplasia and Abnormal Papillary Development** Darcy R. Denner,[1] Kelly M. Stumpff,[1] Michael I. Rauchman.[1, 2] *[1]Biochemistry and Molecular Biology, St. Louis University, St. Louis, MO; [2]St. Louis VA Medical Center.*

Sall1 is a Zn-finger transcription factor essential for kidney development and when mutated leads to the human developmental disorder Townes-Brocks Syndrome. Our lab discovered that Sall1 recruits the Nucleosome Remodeling and Deacetylase (NuRD) complex via a conserved peptide motif to repress gene expression. Through in vitro binding assays, we have determined that Sall1 associates with NuRD by direct interaction with the metastasis tumor-associated protein 2 (Mta2). We detected all NuRD components by western blotting of E13 embryonic kidney lysates and found that they co-elute with Sall1 by size-exclusion chromatography at an expected size of 1.2 MDa. Like Sall1, Mta2 is highly expressed in the metanephric mesenchyme of the peripheral nephrogenic zone of the developing kidney by in situ hybridization and immunofluorescence. Deletion of *Mta2* in cap mesenchyme using Cre recombinase under control of the *Six2* promoter resulted hypoplastic kidneys in *Six2Cre$^{Tg+}$Mta2$^{flox/flox}$* mutants. This phenotype is similar to that exhibited by specific deletion of *Sall1* in the cap mesenchyme. Mta2 expression is also detected in the maturing renal tubules, collecting duct epithelium and medullary stroma. To determine if *Mta2* plays a role in these regions we deleted Mta2 ubiquitously using the tamoxifen-inducible β-*actinCreER* mouse strain. β-*actinCre$^{Tg+}$Mta2$^{flox/flox}$* mutants (8/11) exhibited renal hypoplasia at E14.5-17.5 similar to that observed in *Six2Cre$^{Tg+}$Mta2$^{flox/flox}$*. However, in contrast to mesenchymal deletion of *Mta2* these mutants also exhibited reduced papillary development. These results suggest multiple roles for the NuRD component Mta2 in developing kidney.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO363

**Hedgehog Controls Ureteric Bud Branching Morphogenesis and Contribute to Reduced Nephron Number in a Mouse Model of Oligomeganephronia** Ashraf El-Meanawy, Cary T. Stelloh. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

Developmental reduction of nephron number is an acknowledged risk factor for renal disease and hypertension. Nephrogenesis proceed through complex process involving myriad of genes and pathways and is dependent on spatiotemporal and quantitative expression of controlling genes. A number of genes and pathways have been shown to control nephron number. In this report we identify Hedgehog signaling as a newly identified regulator of nephron number. The hedgehog regulation of nephron number is exerted through regulation of ureteric bud branching morphogenesis. We exploited the Oligosyndactyly mouse model of oligomeganephronia to identify the genes which can control nephron number. We identified the hedgehog modulating morphogen; hedgehog interacting protein (Hhip), to be a critical gene in controlling nephrogenesis. Hhip is dysregulated in the Os mouse, it is overexpressed early on at embryonic day 14 in the Os embryonic kidneys compared to wild type litter mates. This leads to localized suppression of hedgehog signaling. As a consequence there is suppression of ureteric bud branching morphogenesis. To validate the hypothesis, we used wild type metanephric culture system. Incubating E12.5 cultured kidney explants with recombinant mouse Hhip protein led to significant reduction in ureteric bud branching morphogenesis. We were also able to reproduce these results by transfecting E11.5-E12 embryonic kidney with plasmid expressing Hhip cDNA under control of CMV promotor. Moreover, globally inhibiting the Hedgehog signaling using cyclopamine or the blocking antibody 5E1 produced a similar ureteric bud branching defect. The block of the Hedgehog signal led to the suppressed expression of the mesenchyme specific zinc finger protein Sall1. We conclude that Hhip gene product is an important morphogen which critically regulate local hedgehog signaling in the developing kidney. This leads to regulation of final nephron number by altering ureteric bid branching morphogenesis. The clinical relevance of this finding could be extrapolated from the link of functional defects of other branching organs with SNPS in HHIP gene in human GWAS.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO364

**Pursuing the Human Renal Stem Cells – Isolation and Characterization of Human Fetal Kidney Stem Cell** Sally Metsuyanim,[1,3] Orit Harari-Steinberg,[1] Oren Pleniceanu,[1,2] Tzahi Noiman,[3] Ronald S. Goldstein,[3] Benjamin Dekel.[1,2] *[1]Sheba Center of Regenerative Medicine, Sheba Medical Center, Tel Hashomer, Israel; [2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [3]The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel.*

The ability to isolate human fetal kidney (hFK) stem cells is essential to explore their role in kidney development, regeneration and disease. *In vitro* hFK limiting dilution and colony-forming assays were used to quantitatively measure hFK stem/progenitor cell enrichment and self-renewal. Cell surface markers were screened for their ability to positively or negatively enrich for cells with enhanced growth potential in these assays. Sorted subpopulations were analyzed for tissue regeneration capabilities after grafting in the chick embryo.

Clones developed from single hFK cells at a variable rate (0.5-4%) and could be serially propagated to high-passages (P15) indicative of self-renewal. Nevertheless, these cell clones rapidly adopt a mesenchymal appearance and express a combination of renal progenitor markers (Six2/Osr1 but not Pax2/Wt1) and mesenchymal stem cell markers (CD73, CD105, CD90) and therefore do not resemble epithelial nephron progenitors. FACS sorting of hFK cells demonstrate that specific cell surface markers such as NCAM, PSA-NCAM and aldehyde dehydrogenase (ALDH) that localize to the MM and its early nephron derivatives can be used to highly enrich for expression of the entire battery of renal progenitor genes (Six2/Osr1/Sall1/Pax2/Wt1) as well as for clonogenic capacities, especially NCAM+EpCAM- cells. These cells can be serially passaged in serum-free defined media and when grafted ($10^5 – 0.5X10^6$ cells) in the chick can robustly differentiate to produce multiple nephron tubule structures in contrast with the negative fraction which fails to do so.

In sum, human fetal nephron stem/progenitor cells can be isolated by using this antigenic profile. These cell types are strong candidates for cell therapy; tissue engineering and genetic manipulation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO365

**The Effect of Intrauterine Hypoxia on the Development of the Kidney** Lorine J. Wilkinson,[1] Han Sheng Chiu,[1] Adler (Ali) Leigh Ju,[2] Bree Rumballe,[1] Karen M. Moritz,[2] Melissa H. Little.[1] *[1]Institute for Molecular Bioscience, Brisbane, QLD, Australia; [2]School of Biomedical Sciences, University of QLD, Brisbane, QLD, Australia.*

Perturbations to the intrauterine environment of the fetus are widely accepted as a key factor in the aetiology of adult onset diseases such as hypertension and chronic kidney disease. Intrauterine hypoxia is one of the more common insults to the developing fetus and is known to result in low birth weight. Very little research to date has focused on the effect of fetal hypoxia on kidney development, however a correlation between low birth weight and reduced nephron endowment is well established in both animal models and in humans. In turn, reduced nephron number in the human correlates with hypertension, and renal disease. It is likely that fetal hypoxia will also result in reduced nephron endowment and thereby contribute towards adult onset renal disease. We have begun to examine the affect of hypoxia on the development of the mouse kidney using ex-vivo organ culture as a model. Kidneys were dissected from embryos at 12 dpc and grown in culture for 30 hours in normoxic conditions or with the addition of the hypoxia mimic, cobalt chloride. Cobalt chloride activates the HIFα hypoxia response pathway. Cultured kidneys in normoxic conditions continued to develop and undergo branching morphogenesis and nephrogenesis. In contrast, explants grown in hypoxic conditions failed to develop any nephron structures. Growth and branching of the UB continued, although to a lesser degree than in normoxic cultures. In situ analysis of genes specific for cap mesenchyme, stroma, and UB indicated that these compartments developed normally. However, an early marker of MET, Wnt4, was in most cases completely absent, while the epithelial marker, E-cadherin was confined to UB only. Taken together the results suggest that the major effect of hypoxia on the explants is a block in the mesenchyme to epithelial transition required for nephron formation. This may be due to ER stress associated degradation of key signaling molecules . We are currently investigating whether a similar block in MET occurs in vivo in response to hypoxia.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO366

**Lithium Impairs Kidney Development and Inhibits Glycogen Synthase Kinase-3β in Collecting Duct Principal Cells** Gitte Kjaersgaard,[1] Kirsten Madsen,[1] Niels Marcussen,[2] Boye Jensen.[1] *[1]Cadiovascular and Renal Research, University of Southern Denmark, Odense, Denmark; [2]Clinical Pathology, Odense University Hospital, Odense, Denmark.*

The postnatal rat kidney is highly susceptible to Lithium ($Li^+$), which leads to significant tissue injury. We hypothesized that $Li^+$ impairs development of the kidney through entry into epithelial cells of the distal nephron, inhibition of Glycogen Synthase Kinase-3β (GSK-3β) through phosphorylation on serine9 (pGSK-3β)and subsequent epithelial to mesenchymal dedifferentiation (EMT). GSK-3β immunoreactive protein was associated with collecting ducts in developing and adult human and rat kidney. Total GSK-3β protein abundance was stable in medulla while it decreased in cortex in the postnatal period in rat kidney. In contrast pGSK-3β protein abundance decreased significantly with development

in cortex and medulla. Food pellets containing $Li^+$ were given to female Wistar rats with litters through postnatal (P) days 7-29. At P29, plasma $Li^+$ in offspring was 0.99 mmol/L and quantitative stereological analysis showed reduced total kidney volume and reduced cortex and outer medulla volumes compared to control. At P70, 5 weeks after $Li^+$ withdrawal, stereological analysis showed a persisting reduction of outer medulla volume compared to control kidneys. $Li^+$ treatment (P7-P29) increased pGSK-3β protein level significantly whereas total GSK-3β abundance was unaltered. $Li^+$ treatment increased α-Smooth Muscle Actin (α-SMA) protein level significantly whereas E-cadherin expression was unaltered. In summary, $Li^+$ treatment impairs postnatal development of the kidney cortex and outer medulla and increases pGSK-3β abundance in collecting duct. The data are compatible with the notion that increased GSK-3β activity in the postnatal kidney medulla is necessary for kidney development.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO367**

**The Oxidative Stress Role in Renal Injury Induced in Rats by Losartan Exposition during Lactation** Evelyn Cristina Santana Marin, Cleonice G. Da Silva, Terezila Machado Coimbra. *Physiology, Faculty of Medicine of Ribeirão Preto, Ribeirão Preto, Sao Paulo, Brazil.*

The nephrogenesis of rats is completed between 10 and 15 days after birth. Rats exposed to angiotensin II antagonists during lactation present disturbances in renal function and structure. This study investigates the role of the oxidative stress in rats exposed to losartan during lactation. Male Wistar rats were divided in three groups: control (C), pups of dams that received 2% sucrose, n=15; losartan (LO), pups of dams that received losartan (100 mg/kg/day) diluted in 2% sucrose, n=26; losartan plus tempol, an antioxidant (LOT), pups of dams that received losartan (100 mg/kg/day) diluted in 2% sucrose plus tempol (0.34 g/L), n=22. Losartan and/or tempol were administered in drinking water during lactation. At 21 days after birth blood and urine samples were collected to evaluate the renal function and the animals were killed. The kidneys were removed for histological and immunohistochemical studies. No difference in creatinine serum was observed between the groups. Urinary albumina/creatinine ratio was higher in LO group [0.15 (0.10; 0.55)] compared to C group [0.03 (0.01; 0.07)]. LO group presented higher fractional sodium (3.28%±0.67) and potassium (83.57%±17.31) excretions compared to C group (0.34%±0.08; 37.81%±5.99, respectively). Fractional sodium and potassium excretions were lower in LOT group (1.68% ±0.44 and 57.93%±10.79, respectively) compared to the LO group. Higher number of ED1-positive cells (macrophages/ monocytes) was found in the immunohistochemical studies in LO compared to the C group (23.91±3.15 vs 5.13±0.60 per area of 0.245 $mm^2$). Treatment with tempol attenuated this ED1-positive cells increase in the tubulointerstitial area from renal cortex induced by losartan (10.62±1.21 per area of 0.245 $mm^2$). In conclusion, the use of the antioxidant tempol attenuated the increase of fractional sodium and potassium excretions and the infiltration of macrophages and monocytes in the tubulointerstitial area from renal cortex, showing the role of oxidative stress in renal injury in rats exposed to losartan during lactation. Grant/Research Support: FAPESP

Disclosure of Financial Relationships: nothing to disclose

**TH-PO368**

**Functional Analysis of the *RET Y791F* Mutation in Renal Hypodyspasia** Cecile Jeanpierre,[1] Lamisse Mansour,[1] Marta Truffi,[1] Sophie Saunier,[1] Corinne Antignac,[1,2] Remi Salomon.[1,3] *[1]Inserm U983, Necker Hospital, Paris, France; [2]Department of Genetics, Necker Hospital, Paris, France; [3]Department of Pediatric Nephrology and MARHEA Reference Center, Necker Hospital, Paris, France.*

The RET receptor tyrosine kinase and its ligand GDNF (glial cell line-derived neurotrophic factor) play pivotal roles during early nephrogenesis and enteric nervous system development. *RET* is the most frequently mutated gene in patients with Hirschsprung (HSCR) disease. It is also a proto-oncogene involved in predisposition to medullary thyroid carcinoma (MTC). While HSCR and MTC are associated with inactivating and activating *RET* mutations respectively, some mutations may have concomitant gain and loss of function, as demonstrated for the C620R mutation associated with both HSCR and MTC.

Although *RET* or *GDNF* deficiency cause renal hypodysplasia (RHD) in mouse models, whether and how these genes play a role in human RHD is still poorly characterized. In a cohort of 99 children with RHD from 12 European countries, we have previously identified a heterozygous *RET* Y791F mutation in 6 patients. This mutation is usually reported in MTC and leads to signaling activation. To investigate if the *RET* Y791F mutation could be deleterious for nephrogenesis, we developed a 3D-culture assay in MDCK cells. We demonstrated that this mutation inhibits branching, suggesting that, similarly to the C620R mutation, it could have activating and inactivating effects on different biological processes.

Interestingly, in one family with branchio-oto-renal syndrome, the Y791F *RET* mutation segregated with two other heterozygous mutations affecting *GNDF* (Y197H) and *EYA1* (G427_V428insG). The sole child with RHD presented with the three mutations, suggesting that the *RET* mutated allele is not sufficient to cause RHD. To characterize the epistatic effect of these mutations, we are currently analysing the effect of the mutated form of GDNF, in combination with the *RET* mutation, on branching induction, as well as the consequence of the *EYA1* mutation on transcriptional regulation of *GNDF*.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO369**

**Iron Is Required for Development of Proximal Tubules** Melanie Viltard, Andong Qiu, Rosemary V. Sampogna, Jonathan M. Barasch. *Medicine/ Nephrology, Columbia Presbyterian, New York, NY.*

Iron deficiency is the most common nutritional disorder in the world. It has been reported that during pregnancy, iron deficiency is associated with a higher risk of low birth weight and an increased morbidity and mortality. It is well known that iron is crucial for the growth of any cell, however, the specific steps of development which are controlled by iron are not clear, and embryonic anemia is a confounder. Moreover, because specific defects in vivo have not been determined, in vitro models alone are not directly useful, particularly because the selection and concentration of iron donors for in vitro work is unfounded. The aim of this study was to determine the effects of severe maternal iron deficiency on kidney development. 8 weeks-old C57Bl/6 female mice were fed iron-deficient (2-6ppm) or control diet (220ppm) for 3 weeks before mating up till embryo harvest at E15.5. Iron deficiency was assessed by measuring maternal hematocrit, which fell to 18.5±4.1% compared to 30±3.7%. Fe-deficient embryonic kidneys decreased in size by approximately 40% and embryonic lethality was found. Iron regulatory genes were upregulated in the liver of survivors, evidence of sensing global iron deficiency. However microarray analysis of E15.5 kidneys revealed a surprisingly specific set of down-regulated genes in the kidney; 72% of the downregulated genes were specifically expressed or highly associated with the proximal tubule, and by immunostaining we found that tubules expressing proximal tubule genes megalin, aqp1 and aminopeptidase were markedly decreased in Fe-deficiency compared to control mice. Genes regulating vitamin D production were particularly affected. A 3-D analysis of ureteric bud branching showed that while there were defects in later generations as well as in the formation of the renal pelvis, the general branching program seemed to be maintained. These results suggest that the establishment of the mature proximal tubule required higher levels of iron which result either from higher iron demand or alternatively from poor iron avidity. Hence, iron controls kidney development in a segment specific manner, which is replicated in a parallel set of experiments in vitro.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO370**

**Grainyhead-Like 2 (Grhl2) Targets Cldn8 Expression in Mouse Inner Medullary Collecting Duct (mIMCD-3) Cells** Annekatrin Aue,[1,2] Katharina Walentin,[1,2] Friedrich C. Luft,[1,2] Kai M. Schmidt-Ott.[1,2] *[1]Max-Delbrueck Center for Molecular Medicine, Berlin, Germany; [2]Experimental and Clinical Research Center, Charité-Universitaetsmedizin Berlin, Campus Buch, Berlin, Germany.*

In the nephron, the segment-specific molecular composition of the apical junctional complex determines transport characteristics and paracellular permeability. Proteins of the claudin family are central components of the apical junctional complex by determining structural properties of tight junctions. We have previously identified grainyhead-like 2 (Grhl2), a transcription factor of the CP2 family, as a distal nephron-specific regulator of epithelial gene expression. To assess target genes of Grhl2, we used inner medullary collecting duct cells (mIMCD-3) as an in vitro model of the distal nephron. We used transient lentiviral gene transfer of shRNAs targeting Grhl2 to explore the response of target gene expression to knock-down of Grhl2. Using microarrays, we identified on a genome-wide scale transcripts that are - directly or indirectly - regulated by Grhl2. We combined this approach with chromatin immunoprecipitation using a Grhl2 antibody followed by massively parallel sequencing to identify genome-wide Grhl2-DNA association in mIMCD-3 cells. While we had previously reported and confirm here regulation of E-cadherin via an intronic enhancer, we now report binding of Grhl2 to the core promoter of distal nephron-specific claudin Cldn8. While E-cadherin is only moderately down-regulated following Grhl2 knockdown, Cldn8 is down-regulated more markedly. Our data provide additional evidence that Grhl2 regulates molecular components of the epithelial apical junctional complex by directly targeting their expression. Via the control of Cldn8 expression, Grhl2 may participate in the regulation of distal nephron-specific epithelial properties. Moreover, our data provide evidence that Grhl2 binding at the core promoter exhibits stronger effects on gene expression compared with more distant enhancers, which may constitute one potential mechanism of how genome organization and transcription factor expression contribute to nephron segment-specific gene expression.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO371**

**Lineage Tracing in Adult Human Kidney: Clonal Analysis of Patches Containing Diverse Epithelial Phenotypes** Sagrario Canadillas,[1,2] Nadine T. Gaisa,[1,3] Rosemary Jeffery,[1] Jan U. Becker,[4] Malcolm R. Alison,[1,5] Nicholas A. Wright,[1,6] Richard Poulsom.[1] *[1]Histopathology Lab., London Research Institute - Cancer Research UK, London, United Kingdom; [2]Research Unit (Renal Section), Reina Sofía Hospital – Cordoba University, Cordoba, Spain; [3]Institute of Pathology, RWTH Aachen University, Aachen, Germany; [4]Institut für Pathologie, Medizinische Hochschule Hannover, Hannover, Germany; [5]Diabetes, BICMS - Queen Mary University of London, London, United Kingdom; [6]Digestive Diseases, BICMS - Queen Mary University of London, London, United Kingdom.*

Epithelial cells within adult nephrons are considered by many to self-renew only by division of differentiated cells, although recent evidence supports a common origin of parietal/podocyte and proximal tubule lineages. We aimed to test whether phenotypically

distinct epithelia have a clonal origin. We identified cell fates by lectin histochemistry and this was combined with detection of patches of cells in which mitochondrial cytochrome c oxidase (CCO1) had become suppressed, putatively by mutation or methylation, and thereby had a common origin. Patches of CCO loss existed in tubular epithelium, and were readily detected in formalin fixed paraffin embedded tissues: they were increasingly common in tissue from more elderly persons, consistent with the age-related accumulation of mitochondrial DNA mutations reported in other organs. Mitochondrial genome sequencing will allow the lineage relationship of cells following different fates to be established in sections of frozen tissue, provided there was no influence of methylation. 3D-reconstruction revealed phenotypic boundaries within CCO-null patches, suggesting that a common adult progenitor provides for maintenance of different epithelial cell types, and that the progeny do not migrate far in tubules. Diverse fates within patches could not occur if division of differentiated cells always produced identical daughters. Each daughter cell may respond to neighbouring cells and stromal cues. Our data so far does not exclude or support the hypothesis that nephron regeneration could be assisted by insertion and expansion of progenitors arriving via the interstitium.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO372

**The Distal Nephron Transcription Factor Grhl2 Regulates Composition of the Apical Junctional Complex In Vivo** Katharina Walentin,[1,2] Annekatrin Aue,[1,2] Michael Bader,[1] Jonathan M. Barasch,[3] Friedrich C. Luft,[1,2] Kai M. Schmidt-Ott.[1,2] *[1]Max-Delbrueck Center for Molecular Medicine, Berlin, Germany; [2]Experimental and Clinical Research Center, Charité-Universitaetsmedizin Berlin, Campus Buch, Berlin, Germany; [3]Department of Medicine, Columbia University College of Physicians and Surgeons, New York.*

Differentiation of epithelial cells and morphogenesis of epithelial tubes or layers is closely linked with the establishment and remodeling of the apical junctional complex, which includes adherens junctions and tight junctions. We show that the distal nephron transcription factor grainyhead-like 2 (Grhl2), an epithelium-specific mammalian homolog of Drosophila grainyhead, is essential for adequate expression of the adherens junction gene E-cadherin and the tight junction gene claudin 4 (Cldn4) in several types of epithelia including gut endoderm, surface ectoderm and otic epithelium. We generate Grhl2 mutant mice carrying an inactivated Grhl2 allele as a result of gene trapping to demonstrate perturbed epithelial differentiation in these compartments of homozygous Grhl2 mutant mice coinciding with the occurrence of anterior and posterior neural tube defects and embryonic lethality between E10.5 and E11.5, which is due to a placental defect. While an analysis of metanephric development in these mice is precluded by embryonic lethality, chorioallantoic branching and labyrinthal development is severely impaired in Grhl2 mutant placentas indicating defective epithelial morphogenesis. On the molecular level, we show that Grhl2 specifically associates with a unique conserved cis-regulatory element localized in intron 2 of the E-cadherin gene. Loss of Grhl2 leads to a specific decrease of histone acetylation at the E-cadherin promoter. Together, our data indicate that Grhl2 is directly involved in epithelium-specific gene expression and, thereby, controls epithelial differentiation and epithelial morphogenesis.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO373

**Calcineurin Regulates the Transcription and Functions of the Urinary Tract Smooth Muscle Cells** Qiusha Guo,[1] Matthew J. Coussens,[2] Feng Chen.[1] *[1]Department of Internal Medicine, Renal Division, Washington University in St Louis, Saint Louis, MO; [2]Sigma-Aldrich, Saint Louis, MO.*

Calcineurin is a $Ca^{2+}$-dependent serine/threonine phosphatase and we have previously shown that the inactivation of Calcineurin in the metanephric and ureteric mesenchyme (progenitors of the ureteral smooth muscle cells) leads to defective pyeloureteral peristalsis and obstructive nephropathy. In this study, we have established immortalized cell lines from the developing ureteral smooth muscle cells from control and mutant mice that have calcineurin deficiency in the ureteral smooth muscles. We have shown that the smooth muscle cells with calcineurin deficiency have higher contractile tension. Such abnormal constractile property may interfere with the highly regulated pyeloureteral peristalsis necessary for effective transfer of urine from the kidney to the bladder. By transcription profiling, we have further identified significant expression alterations in a set of genes caused by Calcineurin deficiency in the mutant cells, including some genes that are closely linked to the development of the urinary system or the functions of smooth muscle cells. Furthermore, we have found that calcineurin deficiency also affected the transcriptional responses to Bmp4 signaling in the ureteral smooth muscle cells. These results suggest that Calcineurin regulates the transcription programs in the ureteral smooth muscle cells. Interruption of such regulation may cause alteration in the contractile property of the smooth muscle cells, contributing to ureteral dysfunction.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO374

**Scribble Is Required for Normal Mouse Kidney and Ureter Development** Sung Tae Kim, Jeffrey H. Miner. *Renal Division, Washington University, St. Louis, MO.*

Scribble (Scrib) is a large, multidomain scaffold protein containing 16 leucine-rich repeats and 4 PDZ domains. Scrib acts together with discs-large (Dlg) and lethal giant larvae (Lgl) at cell-cell junctions to regulate epithelial cell polarity and proliferation. Scrib "Circletail" mutant mice carry a single base insertion (c.3182-3183insC) that causes a frame shift, leading to truncation of the Scrib protein. Homozygous Scrib mutants survive to at least E18.5 and exhibit a severe open neural tube defect and failure of abdominal wall closure. Here we studied the urinary system in Scrib mutant embryos. Scrib is broadly expressed in the epithelial cells of embryonic kidney and ureter. Scrib mutant embryos exhibited abnormally shaped kidneys and very short ureters. Although insertion of the distal ureter into the bladder appeared superficially normal, the tip of the distal ureter showed an abnormal, thinned morphology. Both proximal ureters and proximal tubules were dilated at E18.5, though not at earlier stages. Glomerular development, podocyte architecture, and the expression of E-cadherin, beta-catenin, integrins, and laminins were normal, but PKC-zeta and Pals1 expression were decreased in the collecting ducts of Scrib mutant kidneys. Claudin1 protein level was decreased in both kidney and ureter, while ZO2 localization was abnormal in the mutant ureter. Moreover, Dlg1 localization was abnormal in Scrb mutant kidney and ureter, consistent with a direct interaction between the two proteins in normal tissue. Finally, in the mutant kidney cortex there was an increase in the number of Ki-67-positive cells. Taken together, these results suggest that Scribble plays a role in the development of kidney and ureter by regulating cell polarization, migration, and proliferation and in the organization of cell-cell junctions.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO375

**Over-Expression of GTP Cyclohydrolase-I Retards Development of Albuminuria and Ameliorates Renal Injuries in Diabetes** Kengo Kidokoro, Minoru Satoh, Naruya Tomita, Tamaki Sasaki, Naoki Kashihara. *Division of Nephrology and Hypertension, Department of Internal Medicine, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background

Growing evidence indicates that endothelial dysfunction underlies and precedes the developments of diabetic vascular complications, including nephropathy. Increased oxidative stress has been demonstrated to be involved in endothelial nitric oxide (NO) synthase (eNOS) uncoupling via oxidation of tetrahydrobiopterin (BH4), a cofactor required for NO production. We have reported that NAD(P)H oxidase and uncoupled eNOS were major mechanisms of oxidative stress and impaired bioavailability of NO in rat models of diabetes. We hypothesized that transgenic overexpression of GTP cyclohydrolase-1 (GCH1), the rate-limiting enzyme in BH4 biosynthesis, could induce re-coupling of eNOS and ameliorate the development and progression of diabetic renal complications through improvement of NO availability and suppression of oxidative stress as well.

Method and Result

Male diabetic Akita mice were crossed with endothelium-specific GCH1 transgenic mice (GCHtg/AKITA mice) and were bred for 20 weeks. Akita mice developed albuminuria by the age of 12 weeks, whereas urinary excretion of albumin was significantly suppressed in GCHtg/AKITA mice. Productions of reactive oxygen species (ROS) and NO were evaluated by 2',7'-dichlorofluorescein diacetate (ROS marker) and diaminorhodamine-4M (NO marker) with L-arginine using confocal laser microscopy in the kidney tissue. Exacerbated ROS production and diminished bioavailable NO were noted in the glomeruli of Akita mice. Renal mRNA and protein expression of NADPH oxidase components were significantly increased in Akita mice compared to GCHtg/AKITA mice. Biochemical analysis revealed eNOS were uncoupled in Akita mice. On the other hand, in GCHtg/AKITA mice, serum levels of BH4 were preserved and glomerular eNOS were re-coupled.

Conclusion

These data indicate that development of albuminuria in diabetic mice is associated with glomerular endothelial dysfunction caused by reduced BH4 and eNOS uncoupling. Increased endothelial BH4 biosynthesis by transgenic GCH1 overexpression corrects eNOS uncoupling and reduces albuminuria.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO376

**Involvement of Post-Transcriptional Regulation of Tsc-22 by Ybx1, a miR-216a Target, and the E-box Activator Tfe3 in TGF-β Induced Collagen Expression in Mesangial Cells** Mitsuo Kato, Sumanth Putta, Mei Wang, Guangdong Sun, Linda L. Lanting, Rama Natarajan. *Diabetes, Beckman Research Institute of City of Hope, Duarte, CA.*

Transforming growth factor-beta1 (TGF-b) induces extracellular matrix proteins and fibrotic events in kidney cells such as glomerular mesangial cells (MC) and plays a role in the pathogenesis of Diabetic Nephropathy (DN). Although the post-transcriptional regulation of key genes has been implicated in these events, details are not fully understood. Here we show that TGF-b downregulates Ybx1, a target of microRNA-216a (miR-216a) and an RNA binding protein regulating RNA stability and translation. Conversely, miR-216a levels were increased in glomeruli of diabetic mice and in TGF-b-treated mouse MC (MMC). miR-216a inhibited the expression of Ybx1 through its 3'UTR, suggesting that Ybx1 is a direct target of miR-216a. Phosphorylation of Ybx1 by Akt, a key mechanism of inhibition of the translational repressor activity of Ybx1, was also observed in MMC treated with TGF-b. Ybx1 was colocalized with processing bodies (P-bodies) in MMC and formed a ribonucleoprotein complex with its target *Tsc-22* mRNA. Ybx1-Tsc-22 mRNA interaction was reduced by TGF-b, as well as by miR-216a or Ybx1 shRNA, but increased by miR-216a inhibitor. Tsc-22 protein levels were enhanced by TGF-b due to reduction in Ybx1 levels. Chromatin immunoprecipitation (ChIP) assays revealed increased occupancies of Tsc-22 and Tfe3, an E-box activator, on far-upstream enhancer E-boxes of collagen type I alpha-2 (Col1a2) by TGF-b treatment. Co-IP assays confirmed, for the first time, the physical interaction of Tsc-22 and Tfe3 in TGF-b-treated MMC. Since both Tsc-22 and Tfe3 have typical leucine zipper (LZ) structures, these two proteins may interact with each

other through these LZ and enhance Col1a2 expression through far-upstream enhancer E-boxes. These results demonstrate that post-transcriptional regulation of Tsc-22 mediated through Ybx1, a target of miR-216a, along with Tsc-22 interactions with the E-box activator Tfe3 at enhancer E-boxes, constitute a key novel mechanism of TGF-b-induced Col1a2 expression in MMC related to the pathogenesis of DN.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO377**

**Acceleration of Diabetic Glomerulopathy in db/db Mice in Chronic Hypoxia** Naoki Takahashi,[1] Hideki Kimura,[1] Kazuko Kamiyama,[1] Tomomi Kurose,[2] Hidehiro Sugimoto,[2] Toshio Imura,[2] Daisuke Mikami,[1] Kenji Kasuno,[1] Haruyoshi Yoshida.[1] *[1]Division of Nephrology, Fukui University, Fukui, Japan; [2]Division of Clinical Laboratories, Fukui University Hospital, Fukui, Japan.*

Background: Although hypoxia in the tubulointerstitial area has been considered one of the key mediators of disease progression, the role of hypoxia for diabetic glomerulopathy has not been elucidated.

Purpose: We examine whether chronic hypoxia induces the characteristic severe diabetic glomerulosclerosis (including nodular formation and microaneurysms) in db/db mice.

Methods: 8 w.o. male db/db mice were bred in a normobaric hypoxic chamber (12%$O_2$). The hypoxic group (H-group) was kept in this chamber for 16 weeks (n=4), the hypo-normoxic group (HN-group) was kept in this chamber for 12 weeks prior to 4-week normoxia (n=3) and the control group (N-group) was bred in room air (n=3). Mice were sacrificed at 24 w.o. for histological analysis.

Results: The antecedent experiments on blood and urine chemistry with 8-10 mice in each group have shown that chronic hypoxia induced prominent erythrocytosis and accelerated increase of albuminuria. However, hypertension and increases of serum Cr and UN were not observed. In N-group the moderate diffuse mesangial sclerosis was observed. In H-group hypertrophic glomeruli with microaneurysms were observed and the tuft area significantly increased in comparison to N-group (108700±6400 vs. 87700±2600 pixels, $p<0.01$). In HN-group the nodular formation was observed. Immunohistologically, the percentage of CD34 positive area in glomeruli was significantly decreased in H-group in comparison to N-group (9.4±2.7 vs. 17.0±2.2%, $p<0.01$). The number of WT-1 positive cells were also significantly decreased in H-group in comparison to N-group (1.0±0.1 vs. 1.5±0.1, $P<0.01$). The number of podocyte had negative correlations to the degree of microaneurysms ($r=-0.26$), nodular formation ($r=-0.35$), respectively ($p<0.01$).

Conclusion: These results suggest that chronic hypoxia may cause podocyte loss and microaneurysms due to endothelial dysfunction, and that hyperglycemia with preconditioned endothelial injury by hypoxia may induce the nodular formation in db/db mice.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO378**

**The Succinate Receptor GPR91 Controls Cell and Mitochondrial Metabolism and Signaling of Diabetic Nephropathy** James L. Burford, Jahoon Koo, Janos Peti-Peterdi. *Dept. of Physiology, University of Southern California, Los Angeles, CA.*

The succinate receptor GPR91 is a newly established direct link between hypoxia/hyperglycemia and the activation of the renin-angiotensin system (RAS). In contrast to the proximal tubule (PT), cells of the distal nephron, connecting segment (CNT) and collecting duct (CD) have a high rate of glycolysis and mitochondrial ROS production. Also, the CNT-CD is the major source of (pro)renin in diabetes. Since renal GPR91 expression is the highest in the CNT-CD, we hypothesized that GPR91 signaling may be an important determinant of cell and mitochondrial metabolism and consequently a trigger of diabetes pathology. Using multiphoton microscopy we directly visualized mitochondrial membrane potential ($\Psi$)in vivo using MitoTracker-Red in distal (D) and proximal (P) tubules in control (C) and STZ-diabetic (DM) wild type (WT) and GPR91-/- mice (n=4). The D/P ratio of MitoTracker-Red fluorescence intensity was 2.3±0.2 in C WT, 1.7±0.1 in C GPR91-/-, 2.6±0.2 in DM WT, and 1.6±0.1 in DM GPR91-/- mice indicating significantly higher $\Psi$ in D versus P nephron segments, which is further increased in DM and is dependent on GPR91. Consistent with these findings, immunoblot analysis of whole kidney homogenates showed a 25% reduction in GLUT-1 and GAPDH expression in both C and DM GPR91-/- versus WT kidneys. Immunofluorescence staining of a-SMA, a marker of epithelial-mesenchymal transformation, VEGF, and MCP-1 were most intense in the CNT-CD and their expression was entirely GPR91-dependent in DM. Immunoblot analysis confirmed significantly elevated levels of TGF-beta, the key mediator of tissue fibrosis in DM (2.1±0.2) compared to C (normalized to 1), which was almost completely abolished in DM GPR91-/- mice (1.3±0.2). In conclusion, GPR91 signaling is a novel feedback mechanism between mitochondrial and cell metabolism and may explain the high glycolytic and mitochondrial activity in D versus P nephron segments. In DM, the increased glucose uptake and glycolysis via succinate accumulation may overactivate GPR91 and trigger pathological signaling particularly in cells of the distal nephron-CD that ultimately results in diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO379**

**Glomerular Mitochondrial Dysfunction and Oxidative mtDNA Damage Are Hallmarks of Susceptibility for Podocyte Depletion and Diabetic Nephropathy** Gabriella Casalena, Ilse S. Daehn, Haiying Qi, Erwin P. Bottinger. *Nephrology/Medicine, Mount Sinai School of Medicine, New York, NY.*

Susceptibility to diabetic nephropathy (DN) is in part determined by genetic factors. Oxidative stress and mitochondrial dysfunction are key factors promoting DN. Our previous transcriptomic analysis suggested that podocyte depletion, a hallmark of progressive DN, may be associated with oxidative phosphorylation and mitochondrial dysfunction in diabetic DBA/2J (D2) inbred mice with susceptibility to DN, but not in DN-resistant C57BL/6J (B6) inbred mice [ASN 2009, abstract: 554886].

HYPOTHESIS: Genetic susceptibility to podocyte depletion and DN in mice correlates with mitochondrial dysfunction and oxidative stress.

PURPOSE: To examine mitochondrial function and mitochondrial DNA (mtDNA) oxidation in diabetic and non-diabetic DN-resistant B6 and DN-susceptible D2 inbred mouse strains.

METHODS: Type I diabetes was induced in D2 and B6 mice with multiple, low-dose streptozotocin(STZ) injections. Glomerular and tubular fractions were collected from diabetic and non-diabetic control animals at days 1, 8 and 24 after the onset of hyperglycemia.

RESULTS: Blood glucose levels were comparable (> 400 mg/dl) between diabetic B6 and D2 mice. Uncoupled (FCCP-stimulated) oxygen consumption was significantly reduced at day 8 of hyperglycemica in glomeruli from STZ-D2 mice, but not in STZ-B6 mice, when compared to control mice. Oxidative DNA damage was quantitated by QPCR in mtDNA and nuclear DNA (nDNA). mtDNA lesions were significantly increased in day 1 STZ-D2, and further increased by 10-fold in day 24 STZ-D2 mice, compared to STZ-B6 mice, whilst oxidation of nDNA was not detected in either strain. Increased mtDNA damage accumulation, observed specifically in STZ-D2 mice, was confirmed by increased 8-oxoguanine in STZ-D2 mice compared to STZ-B6 mice.

CONCLUSIONS: Functional in vivo studies demonstrate that mitochondrial dysfunction and oxidative mtDNA damage are detectable in early-onset T1D specifically in inbred DN-susceptible D2 mice with diabetes-induced podoycte depletion, but not in DN-resistant B6 mice without podocyte depletion.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO380**

**Altered Endothelial-Podocyte Crosstalk Mediates Albuminuria in Diabetic eNOS$^{-/-}$ Mice** Darren A. Yuen,[1] Yanling Zhang,[1] Kathryn E. White,[2] Kim Connelly,[1] Darren J. Kelly,[3] Takamune Takahashi,[4] Raymond C. Harris,[4] Andrew Advani.[1] *[1]Department of Medicine, St. Michael's Hospital, Toronto, ON, Canada; [2]EM Research Services, Newcastle University, Newcastle upon Tyne, United Kingdom; [3]Department of Medicine, St. Vincent's Hospital, Melbourne, Australia; [4]Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN.*

Endothelial dysfunction may predispose to albuminuria in diabetic nephropathy through direct effects on glomerular endothelial cells or crosstalk with neighbouring podocytes. In experimental diabetes, anti-angiogenic therapy may improve renal function despite clinical experience to the contrary. The present study sought to unravel the relationship between endothelial dysfunction, podocyte injury and albuminuria in diabetic eNOS$^{-/-}$ mice, a model characterized by abnormal angiogenesis. Fluorescent microangiography demonstrated glomerular capillary volume was increased 2-fold in streptozotocin (STZ)-diabetic eNOS$^{-/-}$ mice in comparison to wildtype (cap. vol. [x10$^4$µm$^3$], control 5.0±0.5, STZ-eNOS$^{-/-}$ 11.4±1.9 [$p<0.01$]). Treatment with the VEGFR-2 inhibitor, vatalanib caused an increase in BP and AER in wildtype, while STZ-eNOS$^{-/-}$ mice had heavy albuminuria that was unaffected by vatalanib (AER [µg/24h], vehicle 923±225, vatalanib 891±252), even though VEGFR-2 inhibition normalized capillary volume (cap. vol. [x10$^4$µm$^3$], 5.4±0.9 [$p<0.01$]). Increased albumin excretion occurred in STZ-eNOS$^{-/-}$ mice as early as 2 weeks after diabetes induction (AER [µg/24h], eNOS$^{-/-}$ 87±12, STZ-eNOS$^{-/-}$ 566±57 [$p<0.001$]), when endothelial morphology was preserved. At this timepoint, there was significant podocyte injury including vacuoles and electron dense deposits with areas of foot process fusion and effacement. Similar early podocytopathy was observed in db/dbeNOS$^{-/-}$ mice. These observations indicate that a) the primary cause of albuminuria in diabetic eNOS$^{-/-}$ mice is a podocytopathy that occurs with the onset of hyperglycemia and b) normalization of glomerular capillary growth in diabetes does not restore permselectivity. Altered endothelial-podocyte crosstalk is likely to be a major mechanism by which endothelial injury causes albuminuria in diabetes.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO381**

**Lipid Phosphatase SHIP2 Downregulates Insulin Signaling and Promotes Apoptosis in Podocytes** Mervi E. Hyvonen,[1,2] Pauliina H. Saurus,[1] Anita A. Wasik,[1] Eija Heikkila,[1] Marika Havana,[1] Moin Saleem,[3] Harry B. Holthofer,[1,4] Sanna H. Lehtonen.[1] *[1]Haartman Institute, University of Helsinki, Finland; [2]Hospital for Children and Adolescents, University of Helsinki, Finland; [3]Southmead Hospital, University of Bristol, United Kingdom; [4]Dublin City University, Ireland.*

Podocyte injury plays an important role in the development of diabetic nephropathy. Podocytes are insulin responsive cells and develop insulin resistance, but the mechanisms are not known. Considering the finding that insulin resistance associates with proteinuria in diabetic patients, we hypothesize that disturbances in insulin response can affect podocyte function and aimed to investigate the regulation of insulin signaling in podocytes. We found by yeast-two-hybrid screening of a rat glomerular library that CD2AP, an adaptor protein essential for the glomerular filtration barrier, binds to SH2-domain-containing inositol polyphosphate 5-phosphatase 2 (SHIP2), a negative regulator of insulin signaling and a molecule associated with metabolic syndrome. The in vivo interaction of CD2AP and SHIP2 was confirmed by co-immunoprecipitation showing that CD2AP binds to the non-tyrosine phosphorylated form of SHIP2. By pull-down assay we found that the strongest interaction with SHIP2 is mediated by the 3rd SH3 domain of CD2AP. In order to investigate the role of SHIP2 in the regulation of insulin signaling in podocytes, we overexpressed SHIP2 in cultured human podocytes and analyzed Akt activation in response to insulin. In SHIP2 overexpressing podocytes Akt was not activated in response to insulin stimulation, and also, SHIP2 overexpression induced apoptosis in differentiated podocytes. We found that SHIP2 localizes in podocytes, and is upregulated in glomeruli of obese Zucker rats and diabetic db/db mice, animal models of insulin resistance and type 2 diabetes. These results indicate that SHIP2 downregulates insulin signaling in podocytes and promotes podocyte apoptosis. The upregulation of SHIP2 in Zucker rat glomeruli prior to the onset of proteinuria shows that it is not a secondary effect to the podocyte injury but rather might contribute to the development of the disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO382**

**TNF-α Affects Podocyte Expression of Collagen IV and Integrin α3: Role of PI3K and MCP-1** Jingyi Fan,[1] Choon Hee Chung,[2] Eun-Young Lee,[3] Petr Pyagay,[1] Amy Wang,[1] Sheldon C. Chen.[1] *[1]Medicine/Nephrology, Northwestern University, Chicago, IL; [2]Internal Medicine, Yonsei University, Wonju, Korea; [3]Internal Medicine, Soon Chun Hyang University, Cheonan, Korea.*

The inflammatory cytokine, tumor necrosis factor-α (TNF-α), is believed to play a role in diabetic kidney disease, but specific effects of TNF-α regarding nephropathy-relevant parameters have not been studied in the podocyte. Cultured mouse podocytes treated with recombinant TNF-α showed a surprisingly robust increase (~900%) in the secretion of monocyte chemoattractant protein-1 (MCP-1), induced in a dose- and time-dependent manner. The signaling mechanism partly relies on the phosphatidylinositol 3-kinase (PI3K) pathway but does not involve the ERK or p38 mitogen-activated protein kinase (MAPK) pathways. The NF-κB transcription factor may be involved, based on the successful prevention of TNF-α-induced MCP-1 with the specific inhibitors MG132 and CAPE, but the p65 subunit of NF-κB did not appear to be involved, because shRNA knockdown of p65 failed to suppress the stimulation of MCP-1. Other TNF-α effects on podocyte biology included an increase in collagen IV production and a decrease in integrin α3 expression. The collagen IV increase was preventable by an inhibitor of the MCP-1 receptor, CCR2, demonstrating that the MCP-1/CCR2 axis plays an intermediary role between TNF-α and collagen IV, but not for integrin α3. Furthermore, TNF-α and MCP-1 were assayed in the *db/db* mouse model of type 2 diabetic nephropathy. Though TNF-α and MCP-1 levels in the plasma or kidney parenchyma were unchanged vs. control after eight weeks of diabetes, the urinary excretions of TNF-α and MCP-1 were both significantly elevated in the *db/db* mice. Both urinary measures were highly correlated with the severity of diabetic albuminuria.

We posit that podocytes, exposed to elevated TNF-α concentrations in the urinary space, increase collagen IV and decrease integrin α3 expression, potential contributors to glomerular basement membrane thickening and podocyte detachment. Collagen IV production is stimulated by TNF-α-induced MCP-1 acting on its CCR2 receptor, while MCP-1 itself is partially signaled by the PI3K pathway.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO383**

**Glucose-Changed Bioenergetic Profiles in Podocytes** Nicole Stieger,[1] Kirstin Worthmann,[1] Stefan Engeli,[2] Hermann G. Haller,[1] Mario Schiffer.[1] *[1]Division of Nephrology, Medical School Hannover, Hannover, Germany; [2]Institute of Clinical Pharmacology, Medical School Hannover, Hannover, Germany.*

Background:Diabetic nephropathy is the most common cause of chronic renal failure in the industrialized countries. Depletion of podocytes plays an important role in progression of diabetic glomerulopathy.Various factors in the diabetic milieu lead to serious podocyte stress driving the cells towards cell cycle arrest, hypertrophy,detachment and apoptosis. Mitochondria are responsible for glucose metabolism and energy supply in podocytes. Recent studies indicate that mitochondrial dysfunction is a key factor in diabetic nephropathy.In the present study we investigated the glucose metabolism of podocytes under diabetic conditions.

Methods:The oxygen consumption rates(OCR) of murine podocytes were measured using the Seahorse Bioscience XF24 Extracellular Flux Analyzer. Mitochondrial function was characterized by addition of respiratory chain inhibitors. In additional experiments either Glucose or Insulin or both compounds were injected to measure the acute effect on podocyte metabolism. Cells were cultured either permanently under high glucose conditions or exposed to high glucose for 48 h.

Results:We compared the bioenergetic profiles of podocytes under normal, high glucose or mannitol conditions. 48 h high glucose exposure shows no effect on the OCR rates in comparison to the mannitol control. In contrast, cells cultured permanently in high glucose showed significantly increased baseline rates compared to the mannitol controls and dependent on this fact there was major decrease after the injection of oligomycin. Interestingly, the cells under high glucose condition showed an increase in OCR rates after FCCP injection corresponding to a higher mitochondrial oxidative capacity. Rotenone reduced both OCR rates to the same level indicating no changes in non-mitochondrial respiration. Normalisation of the hyperglycaemic milieu back to normal medium for 48 h did not cause any changes in OCR in comparison to the corresponding chronic profile.

Conclusion:In summary, we provide evidence that the diabetic milieu changes glucose metabolism and mitochondrial function in podocytes.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO384**

**TRB3 Is Stimulated in Diabetic Kidneys, Regulated by the ER Stress Marker CHOP and Is a Suppressor of Podocyte MCP-1** Robyn A. Cunard,[1,2,3] Elizabeth Morse,[2] Jana Schroth,[3] Donald Pizzo,[3] Shinichi Okada,[3] Satish P. Ramachandrarao,[3] Volker Vallon,[1,2,3] Kumar Sharma.[1,2,3] *[1]Research and Nephrology-Hypertension, Veterans Affairs San Diego Healthcare System, San Diego, CA; [2]Veterans Medical Research Foundation, San Diego, CA; [3]Medicine, University of California, San Diego, La Jolla, CA.*

Diabetic Nephropathy is the leading cause of renal failure in the United States, and it is associated with podocyte loss and defective podocyte function. TRB3 is a kinase-like protein, that modulates cellular signaling pathways and transcriptional events. Our studies demonstrate that TRB3 expression is increased in the kidneys of Type 1 and Type 2 diabetic mice. TRB3 is expressed in conditionally immortalized podocytes, and its expression is enhanced by reactive oxygen species (ROS) such as H2O2 and superoxide anion (via the xanthine/xanthine oxidase reaction), as well as the free fatty acid (FFA), palmitate. C/EBP homologous protein (CHOP) is a transcription factor that is associated with the Endoplasmic Reticulum (ER) Stress Response and we provide further evidence that CHOP expression increases in diabetic mouse kidneys. CHOP expression is also enhanced in podocytes treated with ROS and FFA and chromatin immunoprecipitation studies reveal that ROS augment recruitment of CHOP to the proximal TRB3 promoter. MCP-1/CCL2 is a chemokine that contributes to the inflammatory injury associated with diabetic nephropathy and we demonstrate that TRB3 can inhibit basal and stimulated podocyte production of MCP-1. In summary, enhanced ROS and/or FFA associated with the diabetic milieu induce podocyte CHOP and TRB3 expression. Because TRB3 inhibits MCP-1, manipulation of TRB3 expression could provide a novel therapeutic approach in diabetic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO385**

**Nicorandil Ameliorates Glomerular and Tubulointerstitial Injury in an Advanced Diabetic Nephropathy Mouse Model** Katsuyuki Tanabe, Wataru Kitagawa, Christopher J. Rivard, Richard J. Johnson, Takahiko Nakagawa. *Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Diabetic nephropathy is one of the major complications in patients with diabetes and associated with end-stage renal diseases as well as cardiovascular diseases. Recently, we have reported that diabetic endothelial nitric oxide synthase (eNOS) knockout mice showed severe renal lesions which resemble human diabetic nephropathy, suggesting endothelial nitric oxide deficiency might be a key factor to cause the progression of advanced diabetic nephropathy. Nicorandil is well-known anti-anginal drug. This agent not only releases NO but also open ATP-dependent K channel, leading to the improvement of endothelial dysfunction. Here, we examined if nicorandil slows the progression of advanced diabetic nephropathy in our mouse model. Eight week-old male eNOS-KO mice received intraperitoneal injections of 50mg/kg of streptozotocin for 5 consecutive days to induce diabetes. Nicorandil was administered at the dosage of 30mg/kg for 8 weeks. Urinary nitrate+nitrite and cyclic GMP excretion were significantly increased by nicorandil administration. While it did not lower blood pressure, nicorandil significantly ameliorated microalbuminuria (0.57±0.12 vs 0.17±0.10, μg/mgCre) and urinary 8-OHdG excretion (7.9±1.0 vs. 6.3±1.0 μg/mgCre). Likewise, mesangiolysis and glomerulosclerosis were significantly inhibited by nicorandil treatment. Immunohistochemistry demonstrated that mesangial deposition of type IV collagen, interstitial accumulation of type III collagen, and glomerular/tubulointerstitial F4/80-positive monocyte/macrophage infiltration were significantly prevented by nicorandil treatment. These results indicate that nicorandil can be a novel therapeutic option against advanced diabetic nephropathy in the presence of severe endothelial dysfunction.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO386**

**Purinergic $P2X_7$ Receptors Are Implicated in the Macrophage Recruitment and TGF-β Secretion of Diabetic Nephropathy in Mice** Maurilo Leite, Marfiza Meirelles, Claudio Bernardazzi, Husten Da Silva Carvalho, Andre Barreira, Christina Maeda Takiya. *Universidade Federal do Rio de Janeiro, Brazil.*

Purinergic $P2X_7$ receptors ($P2X_7R$) are involved in the pathogenesis of several kidney diseases. In the present study we attempted to investigate the role of $P2X_7$ activation on the pathogenesis of diabetic nephropathy in streptozotocin (STZ)-treated mice. Four groups of mice included wild type (WT) C57/Bl6 (group 1), $P2X_7$ knockout (-/-) (group 2), both treated with STZ 60 mg/kg/day (5 days); Groups 3 and 4, WT and $P2X_7$(-/-), respectively, were treated with vehicle for STZ (citrate buffer). The STZ-treated mice were kept with serum glucose between 300-600 mg/dl. The mice were sacrificed after 20 weeks of STZ induction or vehicle, the urine collected for albumin/creatinine (alb/cr), and the kidneys prepared for histology, immunostaining for macrophages (F4/80) and TGF-β, PCR and Western blot analyzes for $P2X_7R$ and interleukin-1β (IL-1β). Results: There was no statistical difference of alb/cr (mg/mg) between groups 1 and 2, but both were higher than groups 3 and 4 (14.4±4.7; 13.6±4.4 vs 0.8±0.7 and 0.7±0.6, respectively, P<0.05). The mean glomerular volume ($\mu m^3 x 10^{-3}$) was higher in group 1 compared to 2 (145.4±21.8 vs 128.7±17.4, P<0.001). Interstitial macrophages count (cells/field) was higher in group 1 compared to 2 (16.5±9.3 vs 6.7±4.6, respectively, P<0.05), whereas groups 3 and 4 were lower than groups 1 and 2 (0.2±0.4 and 0.6±0.8, P<0.001). The surface density of TGF-β staining (% of total surface) was higher in group 1 compared to 2 (37.2±5.1 vs 12.4±3.6, P<0.001), both higher than 3 and 4 (0.7±0.1 and 0.6±0.1, P<0.001, relative to 1 and 2). PCR and Western blot analyzes for $P2X_7R$ and IL-1β revealed mRNA for IL-1β only in group 1. Conclusion: $P2X_7R$(-/-) mice showed less glomerular lesion, less interstitial macrophages and lower expression of TGF-β in renal tissue. Neither the expression of $P2X_7R$, nor its mRNA, could be detected at this time. However, IL-1β mRNA, but not the protein, was detected only in wild type mice. These results constitute evidence that $P2X_7R$ activation and downstream IL-1β signaling are partly involved in the pathogenesis of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO387**

**R3h-Domain Containing Like Protein Is a Novel Regulator of Glomerular Basement Membrane** Takahiro Ishikawa,[1] Minoru Takemoto,[2] Yoshihiro Akimoto,[3] Kunimasa Yan,[4] Karl Tryggvason,[5] Christer Betsholtz,[5] Koutaro Yokote.[1,2] *[1]Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Chiba, Japan; [2]Division of Diabetes, Metabolism and Endocrinology, Chiba University Hospital, Chiba, Japan; [3]Department of Anatomy, Kyorin University School of Medicine, Tokyo, Japan; [4]Department of Pediatrics, Kyorin University School of Medicine, Tokyo, Japan; [5]Department of Medical Biochemistry and Biophysics, Division of Matrix Biology, Karolinska Institutet, Stockholm, Sweden.*

We have identified R3h-domain containing like (R3hdml) as a podocytes specific gene. This study aimed to elucidate the function of the R3hdml protein in the glomeruli. In order to investigate the function of the R3hdml protein in vivo, we created R3hdml null mice. In addition, we used cultured podocytes to investigate the functions of R3hdml. Light microscopic analysis revealed that the R3hdml null mice had no observable glomerular developmental defects. However, an electron microscopic study revealed thickening of the glomerular basement membrane (GBM) and effacement of the podocyte foot processes. We examined the alteration of the GBM components and found that the expression of fibronectin mRNA in the glomeruli of the R3hdml null mice was significantly higher than that in the glomeruli of the controls. Because the fibronectin gene is one of the target genes of the transforming growth factor-ß (TGF-ß), we examined the effect of TGF-ß on fibronectin expression in the presence and absence of R3hdml in the cultured podocytes. When the expression of R3hdml was knocked down by siRNA in cultured podocytes, the expression of fibronectin, which was induced by TGF-ß, was significantly increased compared to the controls. The expression of R3hdml was also regulated by TGF-ß. To conclude, we identified R3hdml as a novel podocyte-specific protein that might play a role in the regulation of GBM possibly through the modulation of TGF-ß signaling.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO388**

**EP1 Receptor Deletion in Mice; Implications for Diabetic Nephropathy** Jean Francois Thibodeau,[1,2] Chris R. Kennedy.[1,2] *[1]Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, ON, Canada; [2]Cellular & Molecular Medicine, University of Ottawa, Ottawa, ON, Canada.*

Prostaglandin (PG) levels and expression of their receptors is enhanced in various clinical and experimental kidney diseases, including diabetic nephropathy (DN). $PGE_2$, the most abundant renal PG signals via its E-Prostanoid (EP)1-4 receptors, activating G-protein coupled signaling pathways. However, the functional relevance of EP receptors in kidney disease remains unclear. To this end, we employed gene-targeted EP1 receptor knockout male mice ($EP1^{-/-}$) on a FVB/N background and induced DN using either the low-dose streptozotocin (STZ) model of type 1 diabetes (5 day i.p., 50 mg/kg bw; Na-citrate as vehicle) or by breeding $EP1^{-/-}$ mice with transgenic OVE26 type 1 diabetic mice with β-cell calmodulin overexpression. Mice were followed for 16 weeks. Although induction of STZ-diabetes was without effect on systolic blood pressure, both STZ-wt and STZ-$EP1^{-/-}$ mice became equivalently hyperglycaemic (>35 mmol/L) and were hyperfiltering to similar levels at 16 weeks post-STZ, as measured by FITC-inulin clearance (STZ-wt, 26.1 ± 3.5 vs wt, 10.3 ± 2.1 $\mu l.min^{-1}.g.bw^{-1}$ , p<0.01; STZ-$EP1^{-/-}$, 28.6 ± 3.9 vs $EP1^{-/-}$, 11.1 ± 2.5 $\mu l.min^{-1}.g.bw^{-1}$, p<0.01). However, consistent with our previously reported urine concentrating defect in $EP1^{-/-}$ mice, STZ-$EP1^{-/-}$ mice produced a significantly more dilute urine than STZ-wt mice (STZ-wt, 1506 ± 96 vs STZ-$EP1^{-/-}$, 1170 ± 117 mOsm/kg.H20, p<0.01) that was accompanied by greater polyuria (STZ-wt, 24 ± 2 vs STZ-$EP1^{-/-}$, 32 ± 2 ml/24h, p<0.01). Importantly, a diabetes-induced glomerular filtration barrier defect was more prominent in STZ-$EP1^{-/-}$ mice (STZ-wt, 164 ± 9 vs wt, 33 ± 9; STZ-$EP1^{-/-}$: 341 ± 183 vs $EP1^{-/-}$, 32 ± 7 µg albumin/mg creatinine). Albuminuria was likewise exacerbated in $EP1^{-/-}$ OVE26 mice (OVE26: 443±139 vs OVE26 $EP1^{-/-}$3301±1119 µg albumin/mg creatinine; p<0.05). Lastly, DN-induced renal hypertrophy was more severe in STZ-$EP1^{-/-}$ mice as compared to STZ-wt (STZ-wt, 20.5 ± 0.9 vs STZ-$EP1^{-/-}$, 23.1 ± 0.6 mg/kg bw p<0.05). Taken together, these data suggest a protective role for the PGE2 EP1 receptor in the context of early DN progression.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO389**

**New Mouse Model for Diabetic Kidney Disease Expressing Human CD36 in Tubular Epithelial Cells** Seon-Ho Ahn,[1,2] Katalin Susztak.[1] *[1]Nephrology/ Internal Medicine, Albert Einstein College of Medicine, Bronx, NY; [2]Nephrology/ Internal Medicine, Wonkwang University College of Medicine & Hospital, Iksan, Jeonbuk, Korea.*

Diabetic nephropathy (DN) is one of the major causes of end-stage renal disease. It has been proposed that CD36, which is a scavenger receptor and fatty acid transporter, plays an important role in development of diabetic complications. Hyperglycemia regulates translation efficiency of CD36 in human macrophages and atherosclerotic vascular lesions, stimulating enhanced fatty acid uptake. We previously reported decreased CD36 expression in diabetic mouse kidneys, while its expression is increased in patients with diabetic nephropathy. Mechanistic studies showed that CD36 mediates tubule cell apoptosis and damage in diabetic kidneys. We hypothesized that such species specific differences could be responsible for the resistance of mice to diabetic tubular damage and progressive diabetic kidney disease. To test this concept, we developed a new mouse with tubule specific inducible expression of CD36. We generated mice where CD36 expression is under the control of the 'Tet'O operator. We crossed these animals with the Pax8 rtTA mouse, expressing the tetracycline transactivator in the renal tubules, and animals were placed on food containing doxycycline at 4 weeks of age. Out of the four founder lines, we found increased CD36 mRNA expression in 3 lines and increased protein expression in 2 transgenic lines. Gene expression studies indicated key differences in levels of lipid and glucose metabolic enzymes. We found a two-fold decrease in fatty acid metabolizing enzyme, medium-chain acyl CoA dehydrogenase, while the expression of key transcriptional regulator, PPAR gamma was increased four-fold in animals with increased expression of CD36.

In conclusion, it is expected that inducible transgenic humanized CD36 expression model will help to understand the role of CD36 and lipid metabolism in diabetic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO390**

**SOD1, but Not SOD3, Deficiency Causes Diabetic Nephropathy through Reduction of Glomerular Endothelial Nitric Oxide in C57BL/6-*Ins2$^{Akita}$* Mice** Hiroki Fujita,[1,2] Hiromi Fujishima,[1] Keiko Takahashi,[2] Matthew D. Breyer,[2] Raymond C. Harris,[2] Yuichiro Yamada,[1] Takamune Takahashi.[2] *[1]Division of Endocrinology and Metabolism, Akita University Graduate School of Medicine, Akita, Japan; [2]Division of Nephrology, Vanderbilt University Medical Center, Nashville, TN.*

Superoxide dismutase (SOD) is a major defender against superoxide. We have reported that renal SOD1 (cytosolic CuZn-SOD) and SOD3 (extracellular CuZn-SOD) isoenzymes are remarkably down-regulated in KK/Ta-*Ins2$^{Akita}$* diabetic mice, which exhibit progressive diabetic nephropathy (DN), but not in DN-resistant C57BL/6- *Ins2$^{Akita}$* (B6-Akita) diabetic mice (JASN 20: 1303, 2009). To determine the role of SOD1 and SOD3 in DN, we generated SOD1, SOD3, and SOD1/3 double knockout B6-Akita mice and investigated their renal phenotype up to the age of 20 weeks. Increased glomerular superoxide levels were observed in $SOD1^{-/-}$ $SOD3^{+/+}$ and $SOD1^{-/-}$ $SOD3^{-/-}$ B6-Akita mice but not in $SOD1^{+/+}$ $SOD3^{-/-}$ B6-Akita mice. In parallel with glomerular superoxide excess, $SOD1^{-/-}$ $SOD3^{+/+}$ and $SOD1^{-/-}$ $SOD3^{-/-}$ B6-Akita mice exhibited increased urinary albumin levels and histopathologically progressive mesangial expansion, yet the severity of DN did not differ in these two groups. Significant differences were not observed in blood glucose, blood pressure, body weight, kidney weight or GFR among $SOD1^{+/+}$ $SOD3^{+/+}$, $SOD1^{-/-}$ $SOD3^{+/+}$, $SOD1^{+/+}$ $SOD3^{-/-}$, and $SOD1^{-/-}$ $SOD3^{-/-}$ B6-Akita mice. Interestingly, glomerular endothelial nitric oxide (NO) levels were markedly reduced in $SOD1^{-/-}$ $SOD3^{+/+}$ and $SOD1^{-/-}$ $SOD3^{-/-}$ B6-Akita mice, indicating that excessive superoxide scavenged NO. In conclusion, the present study demonstrates that deficiency of SOD1 rather than SOD3 predominantly reduces renal defense capacity against superoxide and accelerates diabetic renal injury, possibly via endothelial dysfunction in DN-resistant mice.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO391**

**(Pro)renin Receptor – COX-2 Interaction in Diabetes Induced Podocyte Injury** Huifang Cheng,[1] Xiaofeng Fan,[1] Gilbert W. Moeckel,[2] Raymond C. Harris.[1] *[1]Nephrology/Medicine, Vanderbilt University Medical School, Nashville, TN; [2]Pathology, Yale University Medical School, New Haven, CT.*

Our animal studies suggested that increased COX-2 expression in podocytes promotes diabetic glomerular injury, but the underlying mechanisms remain undetermined. To investigate the impact of high glucose on podocytes, we incubated differentiated wild type (POD $_{WT}$) or COX-2 over-expressing podocytes (POD $_{COX-2}$) with normal glucose (5.5 mM), high mannitol (30 mM) or high glucose (30 mM, HG) for 48 hours. HG selectively induced further COX-2 up-regulation in POD $_{COX-2}$ (3.2±0.2 fold of wild type control), along with cytoskeleton disorganization and down-regulation of α-actinin 4. HG also induced increased apoptosis in POD $_{COX-2}$.

Since previous studies have demonstrated that most RAS components exist in differentiated podocytes, we further investigated their role in HG-induced podocyte injury. In response to high glucose incubation, intracellular and medium renin activity in POD $_{COX-2}$ increased from 1.3±0.1 to 2.5±0.2 ng Ang I/mg protein and from 1.1±0.1 to 1.8 ±0.2 fold, respectively (n=4, P<0.05). HG also induced ERK and P38 phosphorylation significantly in POD $_{COX-2}$. HG increased (Pro)renin mRNA in POD $_{COX-2}$ to 4.4±0.2 fold POD $_{WT}$ (n=4, P<0.05), and COX-2 inhibition decreased HG-stimulated (Pro)renin mRNA expression in POD $_{COX-2}$, as well as inhibiting HG-induced ERK and P38 activation, apoptosis and cytoskeleton disorganization. Similarly, SiRNA down-regulation of (Pro)renin in POD $_{COX-2}$ inhibited HG-induced ERK and P38 phosphorylation and partially prevented the apoptosis and cytoskeleton alterations induced by HG. AT1 receptor blockade also inhibited HG-induced phospho-ERK and phospho-p38 expression, and reduced apoptosis. In transgenic mice selectively over-expressing COX-2 in podocytes, streptozotocin-induced diabetes up-regulated glomerular (Pro)renin receptor expression, which was inhibited by the administration of the COX-2 specific inhibitor, SC58236. These results indicate a possible role for the (Pro)renin receptor in mediation of diabetic podocyte injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO392**

**Targeting Intracellular Angiotensin II To Block High Glucose Effects on Mesangial Matrix** Rekha Singh, Bilquis Basith, David J. Leehey. *Hines VA Medical Center, Hines, IL.*

High glucose has been shown to increase both extracellular and intracellular Ang II levels, which are accompanied by increased TGF-b1 and matrix proteins in mesangial cells. Our recent work with human mesangial cells (HMC) showed that angiotensin converting enzyme inhibitors failed to block high glucose-induced increase in intracellular Ang II (int-Ang II), TGF-b1 and matrix proteins. These findings led us to hypothesize that int-Ang II may play an important role in mediating the stimulatory effect of high glucose on TGF-b1 and matrix proteins. To test this, we investigated whether blockade of int-Ang II by Ang II type 1 (AT1) receptor antagonists could normalize TGF-b1 and matrix under high glucose conditions. Cultured HMC (ScienCell, CA) were incubated with 5 mM (NG) or 25 mM (HG) glucose for 5 days. Since candesartan binds to cell membrane AT1 receptors (extracellular) and losartan can bind to both extracellular and intracellular AT1 receptors, cells were co-incubated with HG and $10^{-4}$M candesartan alone or in combination with losartan. HG significantly increased TGF-b1, collagen IV and fibronectin, and these effects were partially blocked by candesartan. In contrast, the combined treatment of candesartan and losartan completely normalized TGF-b1 and matrix proteins. In further studies, HMC were pretreated with $10^{-4}$M candesartan to block extracellular AT1 receptors and transfected with Ang II ($10^{-6}$M) to increase int-Ang II levels. In some experiments, losartan ($10^{-4}$M) was added to the transfection mixture to deliver losartan intracellulary. Transfection of cells with Ang II increased int-Ang II in a time- and dose- dependent manner and significantly increased TGF-b1, collagen IV and fibronectin. Candesartan did not block int-Ang II effects on TGF-b1 or matrix proteins whereas intracellular delivery of losartan attenuated int-Ang II-induced increase in TGF-b1, collagen IV and fibronectin. These results suggest that int-Ang II has a role in HG-induced stimulation of TGF-b1 and matrix and that targeting int-Ang II action may be necessary to completely block high glucose effects on TGF-b1 and mesangial matrix.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO393**

**RAS Blockade Normalizes Angiotensin Converting Enzyme-2 Expression and Prevents Hypertension, Tubulointerstitial Fibrosis and Tubular Apoptosis in Akita Angiotensinogen-Transgenic Mice** John S. D. Chan,[1] Fang Liu,[1] Nicolas Godin,[1] Shiao-Ying Chang,[1] Chao-Sheng Lo,[1] Isabelle Chenier,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] Shao-Ling Zhang.[1] *[1]Res. Ctr., CHUM-Hotel Dieu Hosp., Montreal, QC, Canada; [2]Res. Ctr., Maisonneuve-Rosemont Hosp., Montreal, QC, Canada; [3]Pediatr Nephrol Unit, Mass Gen Hosp., Boston, MA.*

We investigated the impact of renin-angiotensin system (RAS) blockade on angiotensin converting enzyme-2 (ACE2) expression and prevents hypertension, tubulointerstitital fibrosis and renal proximal tubular cell (RPTC) apoptosis in Akita (a model of type 1 diabetes) angiotensinogen (Agt)-transgenic (Tg) mice. Akita Agt-Tg mice were created by cross-breeding Akita mice with our Agt-Tg mice, which specifically overexpress Agt in their RPTCs (Kidney Int, 2006). Non-Akita littermates served as controls. Blood glucose, systolic blood pressure (SBP) and albuminuria were monitored weekly from 7 until 16 weeks of age. Kidneys were processed for histology and apoptosis studies. Renal proximal tubular ACE2, pro-fibrotic and pro-apoptotic gene and protein expression were quantified by respective real time-qPCR and Western blotting. Akita Agt-Tg mice developed hyperglycemia and significantly higher SBPs, kidney/body weight ratios and albuminuria, compared to non-Akita littermates. Kidneys from Akita Agt-Tg mice displayed progressive hydronephrosis, renal hypertrophy, tubulointerstitial fibrosis and tubular apoptosis as compared to non-Akita littermates. Down-regulation of ACE2 expression and increased expression of transforming growth factor-beta1 receptor II, collagen type IV, plasminogen activator inhibitor-1 and active caspase-3 were evident in RPTCs of Akita Agt-Tg mice. Finally, treatment with RAS blockers (perindopril and losartan) normalized ACE2 expression, prevented hypertension and albuminuria and attenuated kidney abnormalities, pro-fibrotic and pro-apoptotic gene expression in Akita-Agt Tg mice. These results demonstrate that RAS blockade effectively prevents hypertension, albuminuria, tubulointerstitial fibrosis and tubular apoptosis in diabetes via inhibition of intrarenal RAS activation and normalization of ACE2 expression in the kidney.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO394**

**Role of Blood Pressure and Renin-Angiotensin System (RAS) in Development of Diabetic Nephropathy (DN) in eNOS-/- db/db Mice** Suwan Wang,[1] Haichun Yang,[2] Xiaofeng Fan,[1] Takamune Takahashi,[1] Raymond C. Harris,[1] Mingzhi Zhang.[1] *[1]Medicine, Vanderbilt University, Nashville, TN; [2]Pathology, Vanderbilt University, Nashville, TN; [3]Nashville, TN.*

We previously reported that deletion of eNOS in db/db mice (eNOS-/- db/db) leads to significant and early onset albuminuria, arteriolar hyalinosis, mesangiolysis and focal segmental and nodular glomerulosclerosis, similar to human diabetic nephropathy. Blood pressure was higher in eNOS-/- db/db mice than db/db mice (146 ± 6 vs. 120 ± 3 mmHg). eNOS-/- db/db mice (8 wks old, albuminuria 837 ± 79 µg/mg Alb/Cr) were randomly divided into 3 groups: vehicle, treatment with the ACEI, captopril or "triple therapy" (hydralazine, resperine, HCTZ), and the animals were sacrificed after treatment for 12 wks. Captopril treatment led to significant decreases in blood pressure (102 ±5 mmHg), albuminuria (432 ± 101 vs. 2574 ± 974 µg/mg Alb/Cr of vehicle), and glomerulosclerosis index (0.37 ± 0.03 vs. 1.26 ± 0.29 of vehicle), along with less macrophage infiltration and decreased expression of nitrotyrosylated proteins (a marker of oxidative stress), Kim-1 (a marker of renal injury) and CTGF. Triple therapy reduced blood pressure to similar levels in captopril-treated mice (106 ± 4 mmHg). However, triple therapy was less effective in reducing albuminuria (1204 ± 180 µg/mg Alb/Cr) and glomerulosclerosis index (0.58 ± 0.07, P<0.01 vs. captopril group), along with less reduction of macrophage infiltration, nitrotyrosylated proteins, Kim-1 and CTGF. In vehicle treated eNOS-/- db/db, there was increased expression of $p22^{phox}$, a component of NADPH oxidase. Captopril significantly reduced $p22^{phox}$ expression, but triple therapy did not. Therefore, ACEI is more effective than triple therapy in reducing the progression of DN in eNOS-/- db/db mice, indicating an additional role for RAS inhibition in addition to blood pressure control.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO395**

**Activation of Local Renin Angiotensin System in Glomerular Endothelial Cells by High Glucose** Hui Peng, Yan-Fang Xing, Cheng Wang, Zengchun Ye, Hua Tang, Yan-Ru Chen, Canming Li, Tan-Qi Lou. *Department of Nephrology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, GD, China.*

Objective: To explore the effect of high glucose on local renin angiotensin system (RAS) in glomerular endothelial cells (GEnCs) and its probable mechanism in diabetic kidney disease.

Methods: Rat GEnCs were cultured and then exposed for 12h, 24h, 48h and 72h to culture medium containing: control group (5mM, NG), high glucose group ( 30mM, HG), captopril group ( HG+ captopril 1mM), chymostatin group (HG+chymostatin 50uM) . NG+mannitol (25mM), NG+captoril(1mM) and NG+chymostatin(50uM) were control groups. Captopril and chymostatin were pre-incubated for 1h. Then culture medium and cell lysate were collected. ELISA, Real-time PCR, Western blot and immunofluorescence were employed to examine AngII concentration in cell lysate and culture medium, mRNA and protein expression of RAS components and localization of RAS components.

Results: (1) Compared with control group, exposure to HG for 12h increased AngII in cell supernatant (P<0.001). However, there was no difference in intracellular AngII generation between these two groups (P=0.94). Exposure to HG for 72h, AngII levels significantly increased both in culture medium and cell lysate compared with control cells(P<0.001). However, it did not increased when exposure for 24h or 48h (P>0.05). Captopril and chymostatin respectively inhibited HG induced AngII both in culture medium and cell lysate (P<0.05). (2) Both angiotensinogen and renin mRNA were significantly increased when cells were exposed to HG for 72h (P<0.001), while ACE mRNA were unchanged (P>0.05). (3) Angiotensinogen protein in GEnCs was significantly increased when exposed to high glucose for 72h (P<0.001), while AT1R protein expression was significantly down-regulated (P<0.05). AT2R and renin protein were unchanged. Interestingly, redistribution of AT2R from cell nucleus to cytoplasm was observed by immofluorescence.

Conclusion: we suggest that high glucose can activate the local RAS by upregulating angiotensinogen expression in glomerular endothelial cells. It may through ACE and non-ACE pathways.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO396**

**Dose Dependent Anti-Inflammatory Effect of Irbesartan Determined by Antibody Microarray in Patients with Chronic Kidney Disease** Jie Ni, Linli Lv, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China.*

**Objective:** The non-hemodynamic effect of angiotensin II type 1 receptor antagonist in delay the progression of chronic kidney disease (CKD) has still been unclear. In this study, we investigated the influence of irbesartan on the urinary excretion of cytokines in hypertensive patients with CKD determined by a novel antibody microarray.

**Methods:** Sixteen patients were randomly divided into 2 groups (A and B group). After 8 weeks wash-out, irbesartan was given (300 mg·d$^{-1}$ in group A and 150 mg·d$^{-1}$ in group B) for 8 weeks. After 8 weeks, the dosage in group A and B has been changed to 150 mg·d$^{-1}$ and 300 mg·d$^{-1}$ respectively. Blood pressure (BP) and 24 hour proteinuria were examined before and after the treatment. Urinary excretion of cytokines was determined by human inflammation antibody aarry (Raybio®). A two-fold changes in spot intensity was considered significant change.

**Results:** It was shown that irbesartan (150 mg·d$^{-1}$) treatment induced the urinary excretion of GCSF, GM-CSF, IFN-γ, IL-1α, IL-11, IL-12p40, MCP-2, MIP-1α two to nine folds decrease, while increasing the dosage of irbesartan to 300mg·d$^{-1}$, the excretion of GCSF, GM-CSF, IL-12p40, MCP-2, MIP-1α further decreased in group A. In group B, only MIP-1α was found a decreasing in urinary cytokine excretion, and there is no significant difference when the dose changed to 150 mg·d$^{-1}$ for another 8 weeks. After treatment, the levels of EOTAXIN, EOTAXIN-2, GCSF, GM-CSF, ICAM-1, IFN-γ, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IL-11, IL-12p40,IL-12p70, IL-15, IL-16, MCP-2, M-CSF, MIG, MIP-1α, MIP-1β, MIP-1δ, RANTES, TGF-β1 and TNF-α in group B were significantly increased compared to group A, while there is no cytokine decreasing in group B compared to group A. The level of BP and 24 hour urine protein in both groups dropped similarly.

**Conclusion:** This study firstly demonstrates the dose dependent renoprotective effects of irbesartan on patients with CKD via the inhibition of urinary excretion of cytokines.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO397**

**ACE/ACE2 Expression in Kidney from NOD (Non-Obese) Diabetic Mice with Early Diabetic Kidney Disease** Marta Riera,[1] Julio Pascual,[1] Judit Rigol,[1] Eva Marquez,[1] Daniel Batlle,[2] Maria Jose Soler.[1] *[1]Nephrology, Hospital del Mar. Parc de Salut Mar. Fundació IMIM, Barcelona, Spain; [2]Northwestern University Feinberg School of Medicine., Chicago.*

It has been previously shown that renin angiotensin system(RAS) is altered in diabetic kidney disease. RAS has not been studied in non-obese diabetic(NOD)mice, a model that develops autoimmune diabetes that mimics human type 1 diabetes. We studied kidney histology as well as angiotensin converting enzyme(ACE)-2 and ACE expression in kidney from NOD mice at 21 days after diabetes development and compared with the respective non-obese resistant(NOR) mice controls.

Blood glucose(BG) and urinary albumin excretion(UAE) at the end of the study was significantly increased in NOD(n=7) as compared to NOR mice(n=6)(BG:571.1±20.3 vs 112±4.9 mg/dL;UAE:91.4±27 vs 8.6±3μgalbumin/mg creatinine,p<0.05). Glomerular size was increased in NOD as compared to NOR mice (8.324±271 vs 6.238±226um2,p<0.05). We did not observe differences in podocyte number/glomerulus and optical microscopy lesions. ACE2 and ACE activity were increased in plasma from NOD as compared from NOR mice(ACE2:31.2±4.78 vs 11.6±2.3RFU/μL/h; ACE:335.2±10.8 vs 262.3±4.8RFU/μg S/μL;p<0.05). In tubules from NOD, ACE2 protein was determined by Western Blot(WB) and activity expression was increased as compared to NOR mice(WB:0.93±0.05 vs 0.78±0.04 ACE2/βactin; activity: 63.2±14.5 vs 29.8±5.5 RFU/μgprot/h,p<0.05). By immunostaining, the glomerular expression of ACE2 and ACE were significantly increased in NOD as compared with NOR mice (ACE2: 38.5 ± 6.2% vs 16.8 ± 2.6; ACE: 24.6±6.02% vs 6.4±2.26; p<0.05). We conclude that in NOD mice after 21days of new-onset diabetes, UAE and glomerular size are increased. However, we did not observe podocyte loss or other diabetic kidney lesions. In this early model of diabetic kidney disease, ACE2 and ACE expression are increased in plasma, tubules and glomeruli. In conclusion, the NOD mice develops albuminuria and glomerular hypertrophy as the earliest manifestation of kidney disease and at this stage ACE2 in plasma and kidney expression are increased likely as a mechanism to counterbalance increased ACE expression within the kidney.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO398**

**Urinary Excretion of ACE2 – A Biomarker of Diabetic Kidney Disease?** Jan A. Wysocki,[1] Karla Evora,[1] Minghao Ye,[1] Maria Jose Soler,[2] Anastasia Z. Kalea,[1] Daniel Batlle.[1] *[1]Division of Nephrology & Hypertension, Northwestern University, Chicago, IL; [2]Hospital del Mar, Barcelona, Spain.*

ACE2, an Ang II-dissipating enzyme, is abundantly present on apical membrane of renal cortical tubules but also in renal vasculature and glomeruli. We have previously shown that kidney cortex ACE2 activity is increased in diabetic mice whereas glomerular ACE2 expression is decreased by immunostaining. The aim of this study was to examine urinary ACE2 activity in two diabetic models: the *db/db* and STZ-treated mice with established kidney lesions and increased urinary albumin excretion.

Enzymatic ACE2 activity was measured in urine using Mca-APK-Dnp as a substrate. Urine ACE2 activity was not measurable in *Ace2* KO, whereas in WT controls it was about 10-fold higher than in serum. Female mice carrying only one functional allele of the *ace2* gene (*ace2*$^{+/-}$) exhibited a reduced urinary ACE2 activity as compared to WT mice (30-40% of the WT).

In diabetic *db/db* mice, urine ACE2 activity was markedly higher than in *db/m* (137±26 vs. 17±4 RFU/ug creat/hr, respectively, *p<0.001*). Urinary ACE2 excretion was also strikingly increased in diabetic STZ-mice as compared to non-diabetic controls (99±11 vs. 8.6±0.7 RFU/ug creat/hr, respectively, p<0.001). Serum and kidney ACE2 activity were significantly elevated in both, the *db/db* [1.7-fold (p<0.005) and 3.3-fold (p<0.05) higher than in *db/m*, respectively] and STZ mice [2-fold (p<0.01) and 1.7-fold (p<0.005) higher than in control, respectively]. The differences in serum and kidney, however, were small as compared to the large differences in the urine. To examine whether the increase in urinary ACE2 excretion stems from an increase in renal or serum ACE2, recombinant (r)ACE2 was administered via osmotic minipumps. rACE2 administration resulted in amplification of serum but not urinary ACE2 activity suggesting that kidney but not serum ACE2 is the primary source of ACE2 found in the urine.

We conclude that in diabetic mice, there is a marked increase in urinary ACE2 activity and suggest a possibility that this enzyme may be a biomarker of diabetic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO399**

**Diferuloylmethane (Curcumin) Augments Renoprotective Effects of Angiotensin Blockade in Diabetic Nephropathy by Ameliorating Mitochondrial Oxidative Stress** Sharma S. Prabhakar, Kevin Wyatt McMahon, Shobha Pandey. *Medicine, Texas Tech University Health Sciences Center, Lubbock, TX.*

The treatment of diabetic nephropathy (DN) remains suboptimal since the pathogenesis remains incompletely understood. We recently demonstrated that curcumin slows renal disease in obese ZSF rats, a model of type II diabetes by inhibiting VEGF and TGF-β in the kidney (Pandey, 2009). It is currently unclear if these effects are additive to the renoprotection with angiotensin blockade, an established treatment to slow DN. To examine our hypothesis, we studied ZSF rats (8th to 26th week) given either plain water (control) or curcumin (1mg/ml) or losartan (25 mg/L) or losartan plus curcumin in drinking water. Body weights and daily water intake was monitored weekly. Endogenous creatinine clearance and urinary protein excretion rates were measured at the beginning and end of the study. Expression of TGF-β and VEGF in kidneys harvested at euthanesia and urinary levels of 8-isoprostane, and 8-OHdG (8-hydroxy deoxyguanosine), markers of lipid peroxidation and mitochondrial oxidative stress respectively were measured at the end of the study.

Characteristics of ZSF rats at 26 weeks

| Group | BW (gms) | Ccr (L/Kg BW/d) | Uprot (mg/G cr) | 8 isoprostane (pg/G creat) | 8-OHdG (ng/Kg BW |
|---|---|---|---|---|---|
| Control | 716±32 | 3.7±0.31 | 1254± 211 | 65±13 | 5423±459 |
| curcumin | 702±37 | 4.4±0.32* | 623±154* | 53±11† | 2325±271* |
| losartan | 695±34 | 4.1±0.28† | 836±198† | 46±9* | 3875±366 † |
| losartan+curcumin | 678±29 | 4.6±0.27** | 516±138** | 47 ±11* | 2176±298** |

*P<0.01 vs. control, ** P<0.001 vs. control, †P<0.05 vs.control

Results: Rats tolerated curcumin, losartan and the combination well as evidenced by weight gains and water intake. There was a greater reduction in proteinuria and preservation of renal function with the combination than either agent alone along with a consistent inhibition of renal VEGF and TGF-β. Curcumin more significantly inhibited 8-OHdG than losartan and augmented the renal protection from losartan. We conclude that the additional renoprotective effects of curcumin derive from effective inhibition of mitochondrial oxidative stress, a better indicator of total body oxidative stress than lipid peroxidation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO400**

**BMP4/Smad1 Signaling Is a Critical Therapeutic Target for Diabetic Nephropathy** Takeshi Matsubara,[1] Hideharu Abe,[2] Otoya Ueda,[3] Tatsuya Tominaga,[2] Akira Mima,[2] Kojiro Nagai,[2] Kazuo Torikoshi,[1] Makoto Araki,[1] Chisato Goto,[3] Masahiko Kinosaki,[3] Kou-Ichi Jishage,[3] Naoshi Fukushima,[3] Noriyuki Iehara,[1] Atsushi Fukatsu,[1] Hidenori Arai,[4] Toshio Doi.[2] *[1]Department of Nephrology, Kyoto University, Kyoto, Japan; [2]Department of Nephrology, University of Tokushima, Tokushima, Japan; [3]Chugai Pharmaceutical Co., Ltd, Shizuoka, Japan; [4]Department of Human Health Sciences, kyoto University, Kyoto, Japan.*

Accumulation of α1/α2 type IV collagen (Col4) in mesangium is the hallmark of diabetic nephropathy. We have shown that Smad1 transcriptionally regulates Col4 and α smooth muscle actin (αSMA) *in vitro*, and that overexpression of Smad1 accelerates matrix expansion in diabetic mice . Our study, however, demonstrated that overexpression of Smad1 by itself did not exacerbate nephropathy, but induction of diabetes resulted in marked matrix expansion along with prominent Smad1 phosphorylation. Trying to identify a regulatory factor of Smad1, we found that bone morphogenetic protein 4 (BMP4) was increased under diabetic condition *in vitro*. Therefore, the aim of this study was to examine the role of BMP4/Smad1 for diabetic nephropathy. Glomerular expressions of BMP4 and its receptor, quantified by qPCR from isolated glomeruli, were increased along with phospho-Smad1 in streptozotocin (STZ)-induced diabetic mice at 36 weeks. Immunohistochemistry showed that BMP4 was increased in podocytes, whereas its receptor, ALK3 was localized mainly in mesangium. Next, STZ-mice (Wild type and Smad1-Tg) were treated with either a neutralizing antibody against BMP4 (αB) or control IgG from 20 to 36 weeks. As a result, diabetic mice treated with αB showed less Smad1 phosphorylation, mesangial expansion, and Col4 accumulation than those with control IgG. In mesangial cells, overexpression

of constitutive active Smad1 increased transcriptional activity of αSMA, whereas no activation was found in cells overexpressing an inactive mutant of Smad1. BMP4 increased the expression of αSMA, which was blocked by Dorsomorphin, an inhibitor of Smad1. In conclusion, phosphorylation of Smad1 by BMP4 is crucial and blocking this signal could be a critical therapeutic strategy for diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO401**

**Biglycan Deficiency Attenuates Diabetic Nephropathy** Patricia Wilson,[1] Joel Thompson,[1] Liliana Schaefer,[2] Shuxia Wang,[1] Lisa R. Tannock.[1] *[1]Internal Medicine, University of Kentucky, Lexington, KY; [2]Allgemeine Pharmakologie und Toxikologie, Goethe University Frankfurt, Frankfurt am Main, Germany.*

Hyperlipidemia and renal lipid accumulation has been shown to exacerbate diabetic nephropathy, although the mechanism by which renal lipid accumulates is unknown. Glomerulosclerosis is characterized by increased deposition of mesangial matrix including proteoglycans as well as increased renal deposition of apolipoproteinB and/or E. We recently reported that there is a striking co-localization between glomerular biglycan and apolipoproteinB, and regression analyses demonstrated a correlation between renal biglycan and apolipoproteinB content, suggesting that renal biglycan content influences renal apolipoproteinB accumulation. The goal of this study was to determine if renal biglycan mediates renal lipid accumulation. Biglycan deficient or biglycan wildtype mice on a hyperlipidemic background (LDL receptor deficient) were made diabetic with streptozotocin then fed a diet containing 0.12% g/kg cholesterol for 26 weeks. Biglycan deficiency had no effect on survival, body weight, renal weight, blood pressure, or glycated hemoglobin levels. Biglycan deficient mice had no difference in plasma triglyceride or cholesterol levels compared to biglycan wildtype mice, but biglycan deficient mice had significantly less glomerular lipid accumulation. Although diabetic biglycan deficient mice had elevated TGF-beta levels compared to diabetic biglycan wildtype mice (13.0±3.4 vs 4.9±2.3 ng/ml, p=0.02), the diabetic biglycan deficient mice had less matrix expansion than diabetic littermate controls and did not develop renal hypertrophy. Furthermore, diabetic biglycan deficient mice had significantly less elevation in urinary albumin excretion compared to diabetic biglycan wildtype mice (204±17 vs 410±12 mg/g creatinine, p<0.001). Thus, biglycan deficiency attenuates renal lipid accumulation and appears to be protective against development of diabetic nephropathy, despite the elevated TGF-beta levels.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO402**

**SREBP-1 Activation by Glucose Mediates TGFβ Upregulation in Mesangial Cells** Lalita Uttarwar, Bo Gao, Alistair J. Ingram, Joan C. Krepinsky. *Medicine/Nephrology, McMaster University, Hamilton, ON, Canada.*

Glomerular matrix accumulation is a hallmark of diabetic nephropathy. Recent studies have shown that overexpression of the transcription factor SREBP-1 induces pathology reminiscent of diabetic nephropathy, and SREBP-1 upregulation has been seen in diabetic kidneys. We thus studied whether SREBP-1 is activated by high glucose (HG) and mediates its profibrogenic responses.

In primary rat MC, HG (30mM) activated SREBP-1 by 30min, as shown by the appearance of the cleaved active form of SREBP-1 (nSREBP-1) in the nucleus, and by EMSA. This was confirmed by HG-induced activation of an SREBP-1 response element (SRE)-driven GFP construct. Activation was dose-dependent, and not induced by an osmotic control. Cleavage by proteases S1P and S2P were required for activation, since their inhibition by AEBSF prevented SREBP-1 activation. SCAP, the ER-associated chaperone for SREBP-1, was also necessary since its inhibition by fatostatin also blocked SREBP-1 activation. Signaling through the EGFR/PI3K pathway, which we have previously shown to mediate HG-induced TGFβ1 upregulation, was upstream of SREBP-1 activation. Inhibitors of EGFR (AG1478) and PI3K (LY294002, wortmannin) all blocked HG-induced SREBP-1 activation. By northern analysis, we next showed that both fatostatin and AEBSF prevented HG-induced TGFβ1 upregulation. Using a TGFβ1 promoter-luciferase construct, we showed that HG-induced promoter activation was inhibited by both fatostatin as well as a dominant negative SREBP-1a. By ChIP analysis, we confirmed that HG-induced activation of SREBP-1 led to its binding to a putative SREBP-1 binding site (SRE) in the TGFβ1 promoter (within -200bp of the start site).

Thus, HG-induced TGFβ1 activation by SREBP-1 in MC is mediated through EGFR/PI3K activation and processing of SREBP-1 through SCAP-mediated transport to the Golgi for proteolytic cleavage. SREBP-1 activation mediates TGFβ1 upregulation through its binding to its response element in the promoter. SREBP-1 thus provides a potential novel therapeutic target for the treatment of diabetic nephropathy, which will be evaluted in further studies.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO403**

**High Glucose-Stimulated UBF-1 Activation Increases Ribosome Biogenesis for Enhanced Protein Synthesis in Glomerular Epithelial Cells** Meenalakshmi M. Mariappan,[1,2] Kristin D'Silva,[1] Myung-Ja Lee,[1] Kavithalakshmi Sataranatarajan,[1,2] Goutam Ghosh-Choudhury,[1,2] B. S. Kasinath.[1,2] *[1]University of Texas Health Science Center, San Antonio, TX; [2]South Texas Veterans Health Care System, San Antonio, TX.*

We have reported that high glucose increases efficiency of mRNA translation in renal epithelial cells that leads to increase in matrix laminin synthesis. We tested the hypothesis that high glucose expands the capacity for protein synthesis by inducing ribosomal biogenesis as indicated by rDNA transcription in glomerular epithelial cells (GECs). High glucose (30mM), but not equimolar mannitol, significantly increased global protein synthesis and laminin gamma1 and fibronectin expression. This was associated with induction of rDNA transcription as indicated by increase in luciferase activity of a construct consisting of an rDNA promoter-driven IRES-luciferase that was expressed in GEC. High glucose induced Ser388 phosphorylation of upstream binding factor1 (UBF1, a rDNA transcription factor) along with increased phosphorylation of Erk and p70S6kinase. Inactivation of Erk, p70S6kinase or mTOR by inhibitors or by dominant negative enzyme expression blocked high glucose-induced UBF1 phosphorylation. In resting state, nuclear p19ARF combines with UBF1 and prevents it from binding to rDNA promoter and RNA polymerase I (Pol I). High glucose reduced nuclear content of p19ARF, promoted dissociation of UBF1-p19ARF complex and association of UBF1 with RPA194, a subunit of Pol I. Inhibition of Erk, p70S6kinase, mTOR and UBF1 by dominant negatives abolished high glucose induction of laminin gamma1 synthesis, protein synthesis, and, rDNA transcription. In contrast, expression of active S388D mutant of UBF1 in 5mM glucose mimicked the effect of high glucose on protein synthesis. Renal cortex from rodents with type 1 and type 2 diabetes showed increased phosphorylation of UBF1, Erk, p70S6kinase and mTOR, coinciding with renal hypertrophy and matrix accumulation. We conclude that high glucose promotes ribosomal biogenesis by promoting Erk/mTOR/p70S6kinase-UBF1-Pol I axis in GECs. This was confirmed by activation of UBF1 in kidneys of rodents with type 1 or type 2 diabetes.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO404**

**Rap1b GTPase Ameliorates Glucose-Induced Mitochondrial Dysfunction Via Regulating Mitochondiral Biogenesis, Intra-Mitochondrial Dynamics and Antioxidant Gene Expression** Xuejing Zhu,[#1] Fu-You Liu,[#1] Guanghui Ling,[#1] Li Xiao,[#1] Hong Liu,[#1] Yinghong Liu,[#1] Youming Peng,[#1] Yashpal S. Kanwar,[#2] Lin Sun.[#1,2] *[1]Department of Nephrology, Second Xiangya Hospital of Central South University, Changsha, Hunan, China; [2]Department of Pathology and Medicine, Northwestern University Medical School, Chicago.*

Our previous studies have demonstrated that the GTPase Ras-proximate-1 (Rap1b) ameliorates glucose-induced mitochondrial dysfunction in renal tubular cells (JASN 2008). In the present study, we observed that Rap1 expression was decreased in renal proximal tubular cells of patients with diabetic nephropathy (DN), which was associated with the tubular atrophy and decreased tubular functions. This indicated that abnormal expression of Rap1b was related to tubular cell damage in DN. In this study, we investigated whether Rap1b is relevant to tubular cell survival in DN. To address this question, subcellular localization of the Rap1 and its role in mitochondrial biogenesis and mitochondrial gene expression in renal tubular cells was investigated under high glucose (HG) ambience was evaluated. Rap1b was localized to mitochondrial cristae by Immunoelectronmicroscopy. Compared to control (5 mM), 30 mM D-gluocse (HG) induced mitochondrial dysfunction, including altered mitochondrial morphology and membrane potential, as measured by TMRE staining. ATP levels were also decreased. However, over- expression of Rap1b partially reversed such abnormalities. In addition, HG increases overproduction of mitochondrial superoxide, as analyzed by Mito Sox staining with confocal microscopy. Furthermore, HG decreased the expression of mitochondrial fusion gene (MFN) and antioxidant enzymes. Whereas, increased expression of mitochondrial fission gene, DMN, apoptosis related genes, and protein expression of caspase-3 and caspase-9 were observed. These effects were partially reduced by transfection with Rap1b as assayed by QPCR, Western blotting and immunofluorescence microscopy. These data indicate that Rap1b ameliorates glucose-induced mitochondrial dysfunction by maintaining a balance in the favor of biogenesis genes and antioxidant enzymes in the mitochondria of renal tubular cells.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO405**

**Free Symmetrical Dimethylarginine Increases Collagen IV by Altering the AT2-VEGF Signaling Axis. Potential Role in Diabetic Nephropathy** Denis Feliers, Yves Gorin, B. S. Kasinath. *UTHSCSA, San Antonio, TX.*

Circulating symmetric dimethylarginine (sDMA) is increased and intrarenal renin-angiotensin system is activated in diabetes. We studied whether sDMA interferes with AngII in regulation of collagen IValpha5 (col4A5) expression in glomerular endothelial cells (GEndos) and diabetic kidneys. In GEndos, use of losartan and PD123319, AT1 And AT2 antagonists, showed that AngII induced increase in col4A5 by was mediated by AT1 and inhibited by AT2. AngII stimulation of VEGF and VEGFR2 activation was mediated by AT2 only. AT2 repression of col4A5 was prevented by sFlt1, a specific VEGF inhibitor. AngII stimulation of eNOS activation and NO production (a known anti-fibrotic factor) was blocked by inhibition of either AT2 or VEGF. This suggests that activation of an

AT2-VEGF axis represses col4A5 through eNOS activation. sDMA suppressed col4A5 repression, but not increased VEGF and VEGFR2 signaling induced by AT2 activation. It also suppressed VEGF-induced eNOS activation and NO production, but not VEGFR2 signaling leading to eNOS activation. sDMA caused eNOS uncoupling and superoxide anion production in response to VEGF. All these effects were blocked by preventing cellular uptake of sDMA with excess arginine. This shows that sDMA interferes with NO production by uncoupling eNOS and leads to oxidative stress in response to activation of the AT2-VEGF axis. In normal mice administration of losartan and PD123319 showed that AT2 but not AT1 repressed col4A5 expression in the kidney. In OVE26 mice with type 1 diabetes, however, both receptors contributed to increase of col4A5. In nondiabetic mice, VEGF expression as well as VEGF-R2 and eNOS activation were mediated by AT2 alone, demonstrating the existence of a AT2-VEGF axis that could repress col4A5. In diabetic kidneys, the AT2-VEGF axis contributed to col4A5 increment in spite of VEGF-R2 and eNOS activation, reminiscent of data from GEndos in culture. This suggests that alteration of eNOS activation and NO production by free sDMA could contribute to increased col4A5 in diabetic kidneys. By altering signaling by the AT2-VEGF axis, circulating sDMA could contribute to matrix expansion in diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO406

**Farnesoid X Receptor Deficiency Accelerates Diabetic Nephropathy in FVB/N Mice** Tao Jiang, Cydney Lynn Urbanek, Nathaniel L. Solis, Hannah Danielle Santamaria, Moshe Levi, Xiaoxin X. Wang. *Medicine, University of Colorado Denver.*

The pathogenesis of diabetic nephropathy remains poorly defined, and animal models that represent the human disease have been lacking. We have demonstrated recently that farnesoid X receptor (FXR) agonist has renal protective role in db/db mice of type 2 diabetes model and that FXR deficiency potentiates diabetic nephropathy in nephropathy-resistant C57BL/6 mice. In this study we are further testing the effect of FXR deficiency in FVB/N mice, a strain known to be susceptible to diabetic nephropathy. The diabetes was caused with Akita mice. We kept male mice on chow diet until 20 wk old. Although the severity of hyperglycemia in diabetic FXR KO mice was similar to diabetic wild-type mice, the diabetic FXR KO mice on FVB background developed kidney injury including increased proteinuria (15 fold vs non-diabetic FXR KO mice; 3 fold vs diabetic wild-type mice), massive glomerulosclerosis, severe tubule atrophy and fibrosis, and increased extracellular matrix protein deposition. Furthermore diabetic FXR KO mice have increased oil red o positive lipid deposits in both the glomeruli and tubular epithelial cells. We have also determined that diabetic FXR KO mice have increased macrophage infiltration. In summary, diabetic FXR KO mice in the FVB background provide a novel model of diabetic nephropathy and support a role for FXR deficiency in modulating diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO407

**High Glucose Induces Human Glomerular Endothelial-to-Mesenchymal Transition through TGF-β1 Pathway in Diabetes Mellitus** Hui Peng, Canming Li, Yan-Ru Chen, Cheng Wang, Zengchun Ye, Bo Peng, Tan-Qi Lou. *Devision of Nephrology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China.*

Background: Emerging evidence suggests that endothelial-to-mesenchymal transition (EndMT) contributes to kidney fibrosis. Our aim is to explore if EndMT exists in human diabetic nephropathy (DN), and also to investigate if high glucose induces EndMT in glomerular endothelial cells (GEnCs) and the mechanisms in DN. Methods: Primary human GEnCs were exposed for 24 hours to culture medium containing: (1) no additions (control), (2) high glucose (HG, 30mM), (3) high glucose with TGF-β1 receptor kinase inhibitor LY-364947 (1mM), (4) TGF-β1 (10ng/ml), (5) mannital control. Immunofluorescent staining was performed to detect the expression of CD31, fibroblast specific protein-1 (FSP-1), and α -smooth muscle actin (α-SMA) in GEnCs, five human diabetic kidney sections and three normal renal controls (from renal carcinoma patients). Phenotypic changes of GEnCs were observed using phase contrast microscope. Protein and mRNA expression of FSP-1, α-SMA, TGF-β1 was measured by Western blotting and real-time PCR respectively. Total TGF-β1 in cell supernatant and cell lysate were detected by ELISA. Results: Double staining of FSP-1 with CD31 as well as α-SMA with CD31 revealed EndMT also existed in the glomerulis of human diabetic kidney. In vitro study showed GEnCs changed their phenotype from cobble-like to fibroblast-like cells in HG, which did not happen in mannitol groups. It also showed that α-SMA with CD31 double positive percentage was significantly increased in HG and TGF-β1 group when comparing with control($P<0.05$). Real-time PCR and Western blotting results indicated FSP-1 and α-SMA mRNA and protein significantly increased in HG and TGF-β1 groups compared with control($P<0.05$). TGF-β1 both in cell supernatant and cell lysate were significantly increased in HG compared with control($P<0.01$). When pre-incubated with LY-364947, EndMT induced by HG was partly decreased ($P<0.05$). Conclusions: Our results indicate that EndMT exists in the glomerulis of human diabetic kidney. High glucose may contribute to the EndMT of GEnCs through TGF-β1 signaling pathway.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO408

**High-Glucose Induces CTGF Which Leads to Activation of TrkA and Subsequent Renal Cell Migration** Maria Fragiadaki,[1] Aleksandra Wright,[1] Yoshifumi Itoh,[2] Nadia Wahab,[1] Roger M. Mason.[1] *[1]Renal Medicine, Imperial College London, London, United Kingdom; [2]Kennedy Institute of Rheumatology, Imperial College London, United Kingdom.*

High-glucose plays an important role in the development of diabetic nephropathy (DN) through a variety of molecular mechanisms. These include activation of connective tissue growth factor (CTGF), a profibrotic cytokine, which is upregulated in glomeruli in DN and in mesangial cells exposed to high glucose *in vitro*. We previously identified TrkA as a CTGF receptor and showed that CTGF binding to TrkA activates downstream cell signalling. To investigate whether this induces a biological response in mesangial and tubular epithelial cells we investigated whether stimulation with high-glucose promotes cell migration via this pathway. Migration was measured using a transwell migration assay and a scratch wound assay with live cell imaging. We show that glucose-stimulated migration is dependent on induction of CTGF and CTGF-mediated activation of TrkA. Knockdown of CTGF with siRNA or neutralising anti-CTGF antibodies, abolished both the activation of TrkA and cell migration in high glucose conditions, as did siRNA knockdown of TrkA. Moreover, pharmacological inhibition of TrkA (K252a, or GW441756) had similar effects. The N-terminal half of CTGF was responsible for CTGF-TrkA mediated cell migration. We also studied the expression and activation of TrkA in kidney biopsies from patients with DN and in control kidneys from patients with thin basement membrane disease. TrkA is not expressed in the control kidney but is strongly expressed and activated in tubular epithelial cells in DN patients. Overall these results indicate that glucose-induced CTGF expression in DN may activate TrkA receptor signalling and downstream cellular responses during disease development.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO409

**Urinary Connective Tissue Growth Factor Is Associated with Tubular Dysfunction in Diabetic Nephropathy** Jan Willem Leeuwis,[1] Karin G. Gerritsen,[1] Tri Q. Nguyen,[1] Maarten P. Koeners,[2] Jaap A. Joles,[2] Stephan J. L. Bakker,[4] Roel Goldschmeding,[1] Robbert J. Kok.[3] *[1]Pathology, University Medical Center, Utrecht, Netherlands; [2]Nephrology, University Medical Center, Utrecht, Netherlands; [3]Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands; [4]Nephrology, University Medical Center, Groningen, Netherlands.*

Connective tissue growth factor (CTGF) plays a key role in the development of diabetic nephropathy (DN). Urinary CTGF (uCTGF) is elevated in DN and relates to markers of disease severity. It is unknown which processes contribute to elevated uCTGF. We investigated how tubular dysfunction affects uCTGF in type 1 DN.

We examined uCTGF and tubular reabsorption marker $\beta_2$-microglobulin ($\beta_2$M) in patients with type 1 DN, and studied renal handling of endogenous CTGF (eCTGF) and recombinant CTGF (rCTGF) in mice with streptozotocin-induced DN. rCTGF and FITC-inulin were infused by minipumps. Fractional excretion (FE) of rCTGF was used as a measurement for tubular reabsorption of CTGF.

In patients, uCTGF was independently associated with $\beta_2$M ($\beta=0.41$, $P<0.001$), and not with glomerular damage marker IgG1.

Diabetic mice had increased urinary eCTGF and FE of rCTGF ($P<0.001$), which correlated tightly ($r=0.95$, $P<0.001$). Approximately 40% of uCTGF could be accounted for by decreased reabsorption of filtered plasma eCTGF, indicating that the remaining uCTGF had a direct renal source. *In situ* hybridization showed that CTGF was mainly located in glomeruli and medullary tubuli. While medullary CTGF mRNA in DN did not correlate with directly kidney-derived uCTGF, both glomerular CTGF mRNA and FE of rCTGF showed a strong correlation with directly kidney-derived uCTGF ($r=0.86$ and $0.91$, $P<0.001$). However, it should be kept in mind that glomeruli-derived CTGF will undergo tubular reabsorption and tubular dysfunction will result in a major increase in uCTGF from this source.

Our data indicate that in DN, uCTGF is strongly dependent on reduced tubular reabsorption of both filtered plasma CTGF and glomeruli-derived CTGF. This should be taken into account when using uCTGF as a biomarker and in research addressing its pathogenic role.

*(JWL and KGG contributed equally)*

Disclosure of Financial Relationships: nothing to disclose

### TH-PO410

**Transcriptional Regulation of MicroRNAs-192 and -194 by TGF-β1. Implications for the Pathogenesis of Diabetic Nephropathy** Robert H. Jenkins, John Martin, Aled O. Phillips, Timothy Bowen, Donald Fraser. *Institute of Nephrology, School of Medicine, Cardiff University, Cardiff, Wales, United Kingdom.*

Tubulointerstitial fibrosis is a key determinant of progressive chronic kidney disease (CKD). Epithelial-to-mesenchymal transition of proximal tubular epithelial cells (PTC) primarily due to the cytokine transforming growth factor-β1 (TGF-β1) is integral to this process. Several recent studies show that microRNA-192 (miR-192) has functionally important targets in the kidney, and implicate alterations in miR-192 expression downstream of TGF-β1 in the pathological changes in diabetic nephropathy. The data is complex, however, with evidence for both induction and suppression of miR-192 by TGF-β1 in

renal cells in vivo and in vitro under differing contexts. The aim of the current study was to define the mechanisms by which miR-192 expression is regulated. Initial screening demonstrated that miR-192 is found in a restricted subset of human tissues, including kidney, as part of a family of miRs sharing a similar expression pattern, which also includes miR-194. miR-192 and miR-194 are processed from the same primary transcript (pri-192/194). miR quantification in primary human cells showed expression in proximal tubular cells (PTC), mesangial cells and podocytes, with predominant expression in PTC. Incubation of PTC with TGF-β1 suppressed pri-192/194 expression for time points from 12 to 96h, suggesting transcriptional regulation by TGF-β1. A series of luciferase reporter gene constructs incorporating a nested set of pri-192/194 promoter fragments identified a TGF-β1 responsive element in the pri-192/194 promoter, and in silico analysis suggested the presence of a Hepatocyte Nuclear Factor binding site. Electrophoretic mobility shift assay demonstrated HNF binding to this sequence. Screening of human tissues showed strong correlation between HNF family member expression and miR-192/194 expression. Taken together, these data identify transcriptional regulation of miRs -192 and -194 by TGF-β1, and implicate Hepatocyte Nuclear Binding Factors in this process.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO411**

**Post-Transcriptional Regulation of Transforming Growth Factor-β1 by microRNA** John Martin, Robert H. Jenkins, Aled O. Phillips, Timothy Bowen, Donald Fraser. *Institute of Nephrology, School of Medicine, Cardiff University, Cardiff, United Kingdom.*

TGF-β1 is an important mediator of progressive renal tubulointerstitial fibrosis in diabetic nephropathy. There is substantial evidence for post-transcriptional regulation of TGF-β1 synthesis in various in vitro and in vivo contexts. We have shown previously that in proximal tubular cells, TGF-β1 synthesis is controlled independently at the level of translation by stimuli of relevance to the pathogenesis of diabetic nephropathy (glucose, insulin and PDGF). The mechanisms underlying this remain incompletely defined. The purpose of this work was to investigate the potential role for microRNA, recently identified post-transcriptional regulators of gene expression, in regulation of TGF-β1. In vitro experiments were performed with primary proximal tubular cells (PTC) and with the cell line, HK-2.

MicroRNA exert their regulatory action via binding to sites in the 3'Untranslated Region (3'UTR) of target genes. The TGF-β1 3'UTR is reported as 543 nucleotides, but an alternative polyadenylation site leading to a significantly shorter transcript has also been reported. Absolute quantification using reference standards showed that, while both UTR lengths are detectable in a broad range of tissues, the short form of the TGF-β1 3'UTR predominates in the kidney and elsewhere. Incorporation of the short UTR into a luciferase reporter gene significantly reduced reporter protein synthesis without major effect on RNA, suggesting post-transcriptional inhibition. In silico approaches identified two microRNAs with multiple potential binding sites within the TGF-β1 UTR. miR transfection inhibited endogenous PTC TGF-β1 synthesis, and in separate experiments, decreased TGF-β1 3'UTR reporter activity, confirming direct targeting of TGF-β1. This work identifies important post-transcriptional regulation of TGF-β1 by its 3'UTR, and identifies microRNAs that regulate synthesis of this key pro-fibrotic cytokine in the kidney.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO412**

**Toll-Like Receptor 4 Mediates High Glucose Induced Tubular Inflammation in Diabetic Nephropathy** Miao Lin,[1] Wo-Shing Au,[1] Loretta Y. Y. Chan,[1] Joseph C. K. Leung,[1] Kwok Wah Chan,[2] Hao-Jia Wu,[1] Gary Chan,[1] Kar Neng Lai,[1] Sydney C. W. Tang.[1] *[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong, China; [2]Department of Pathology, The University of Hong Kong, Queen Mary Hospital, Hong Kong, China.*

Tubulointerstitial inflammation is a hallmark of diabetic nephropathy, and governs overall renal prognosis. We recently showed that high glucose (HG) stimulated tubular proinflammatory signals via MAPK and PKC signaling (*Nephrol Dial Transplant* 2009). Here, we studied whether Toll-like receptor 4 (TLR4), a key mediator of immune responses and inflammatory signaling, is involved in tubular inflammation of diabetic nephropathy. Immunohistochemical analyses of human renal biopsies revealed that TLR4 were expressed in proximal and distal tubules, with more intense staining in kidneys with histologically proven diabetic nephropathy (n=8) compared with normal controls (n=10). In vitro, we showed that HG induced TLR4 overexpression in cultured human proximal tubular epithelial cells (PTEC) in a time- and dose- dependent manner, resulting in upregulation of IL-6, CCL-2 mRNA expression and protein secretion via IκB phosphorylation and NF-κB p65 nuclear translocation. Knocking down TLR4 expression with TLR4-specific siRNA in PTEC resulted in a significant decrease in HG-induced IκB/NF-κB activation (76-80%, $p<0.01$) compared with the scrambled control. The associated downstream proinflammatory cytokine IL-6, chemokine CCL-2 mRNA level and protein synthesis were also substantially attenuated by transfection with TLR4 siRNA (68%-74%, all $p<0.01$). In conclusion, our findings suggest a novel TLR4-mediated pathway through which hyperglycemia may contribute to tubular inflammation in the diabetic kidney.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO413**

**Diabetes Attenuates the Blood Pressure Responses to Nitric Oxide (NO) Synthesis Inhibition: Potential Mechanisms and Relationship to Nephropathy Susceptibility** Karen A. Griffin,[1] Aaron Polichnowski,[1] Hector Licea-Vargas,[1] Jianrui Long,[2] Geoffrey A. Williamson,[2] Anil K. Bidani.[1] *[1]Medicine, Loyola Univ and Hines VA Hosp, Maywood, IL; [2]Electrical and Computer Engineering, Illinois Institute of Technology, Chicago, IL.*

We and others have shown that the Sprague-Dawley (SD) rats from Charles River (CR), exhibit reduced BP responses to L-NAME as compared to the SD rats from Harlan (H). Given the postulated importance of NO in the pathogenesis of DN, BP radiotelemetry was used to examine the effects of ~2 weeks of streptozotocin (STZ) diabetes *per se* on the dose response relationship of BP to L-NAME in control (CR, n=7; H, n=10) and diabetic rats (CR, n=8; H, n=9). Results mean ± SEM.

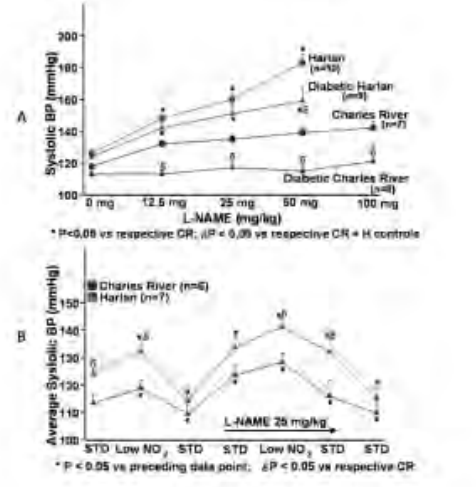



Fig. 1A shows that diabetes blunts the BP responses in both CR and H rats suggesting an increase in NO availability in early diabetes, with the effects being particularly prominent in CR rats (each L-NAME dose was given for 1 wk). Evidence that vascular nitrite ($NO_2$) may be an additional NO source in diabetes is provided by the significant and reversible increases in BP produced by the substitution of a low $NO_2$ for a standard (STD) diet for 72 hrs in diabetic (CR, n=6; H, n=7) (Fig. 1B), but not control rats (data not shown). Similar BP effects of a low $NO_2$ diet were seen during concurrent L-NAME administration (Fig. 1B). After 4 months of partially insulin-treated STZ diabetes (blood glucose 300-400 mg/dl), average systolic BP was also modestly but significantly lower in diabetic CR (n=6) vs. H (n=8) rats (112±2 vs 122±2 mmHg). However, much larger differences were seen between CR vs. H rats for proteinuria (23±4 vs. 77±13 mg/24hr; $p<0.001$) and percent glomerulosclerosis (1±0.2 vs. 9±3; $p<0.01$). The strong correlation between the BP response to L-NAME phenotype and the severity of DN suggest its potential utility as a predictor of DN susceptibility.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO414**

**Generation of a Conditional eNOS Allele for the Assessment of Cell Specific eNOS Function** Rosie T. Jiang,[1] Suwan Wang,[1] Keiko Takahashi,[1] Hiroki Fujita,[2] Matthew D. Breyer,[1] Raymond C. Harris,[1] Takamune Takahashi.[1] *[1]Nephrology, Vanderbilt University Medical Center, Nashville, TN; [2]Endocrinology and Metabolism, Akita University Graduate School of Medicine, Akita, Akita, Japan.*

Nitric oxide (NO) generated by eNOS enzyme plays a major role in vascular homeostasis. Further, recent studies of eNOS knockout mice have shown that abnormalities in eNOS expression and activity play a central role in the pathogenesis of diabetic nephropathy (DN). However, there remain uncertainties about the precise role of eNOS in DN. First, eNOS is present in a variety of non-endothelial cell types, including hematopoietic cells, cardiac cells, and renal tubular cells. Second, eNOS knockout mouse have been shown to exhibit congenital vascular and renal anomalies with high frequency, including septal defects, abnormal aortic valve development, and focal renal scars. Thus, a conditional knockout approach to target endothelial eNOS in adult diabetic mice is required to determine the role of endothelial eNOS in DN. To this end, we designed and generated a conditional allele (flox allele) in which the exons 9-12, encoding the sites essential to eNOS function, are excised by Cre-loxP recombination. Correct targeting events were confirmed by a series of Southern blot analysis and genomic PCR. Mice homozygous for floxed eNOS allele (flox/flox mouse) are healthy with morphologically normal kidneys, and their blood pressure and tissue eNOS protein levels do not differ from those in wild-type littermates. To evaluate the exons-deleted eNOS allele (lox allele), we also generated mice homozygous for lox allele (lox/lox mouse) by crossing with Sox2-Cre mice. Compared with wild-type littermates, the lox/lox mice showed higher blood pressure levels that are comparable to eNOS -/- mice, and Western blot analysis showed deletion of the targeted eNOS protein sequences in tissues. Taken together, these findings demonstrate that conditional targeting of eNOS gene can be achieved in our floxed eNOS mouse. This model should provide a

useful reagent for elucidating cell or tissue-specific eNOS function. Endothelial specific eNOS targeting study is currently underway.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO415

**Elevated Tissue Factor Expression Contributes to Exacerbated Diabetic Nephropathy in Mice Lacking eNOS Fed a High Fat Diet** Nobuyuki Takahashi, Feng None Li, Chih-Hong Wang, Nobuyo Maeda. *Pathology, University of North Carolina at CH, Chapel Hill, NC.*

Human endothelial nitric oxide synthase (eNOS) polymorphisms that lower its expression are associated with advanced diabetic nephropathy (DN), and the lack of eNOS accelerates kidney damage in diabetic mice. Diabetes is associated with fibrin deposition. Lack of nitric oxide and fatty acids stimulate the NF-kB pathway, which increases tissue factor (TF). To test the hypothesis that TF contributes to the severity of DN in the diabetic eNOS-/- mice fed a high fat (HF) diet. We made eNOS-/- and wild type mice diabetic with streptozotocin. Half of them were placed on a HF diet. Blood glucose levels were not affected by either the diet or eNOS genotype. The lack of eNOS in the diabetic mice increased urinary albumin excretion, glomerulosclerosis, interstitial fibrosis, and glomerular basement membrane thickness. HF by itself did not affect DN in the wild type mice, but significantly enhanced DN in the eNOS-/- mice. More than half of diabetic eNOS-/- mice on HF died prematurely with signs of thrombotic complications. Diabetic kidneys contained fibrin and TF, and their levels were increased by the lack of eNOS and by HF in an additive fashion. The HF diet increased the kidney expression of inflammatory genes, including cell adhesion molecules, interleukin-1b, and interleukin-6. The increase in TF preceded the development of DN, and administration of an anti-mouse TF neutralizing antibody to diabetic mice reduced the increase in the expression of inflammatory genes. Together, these data indicate a causal link between TF and the exacerbation of DN in the eNOS-/- mice. The condition is significantly worsened by enhanced inflammatory responses to a HF diet via TF.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO416

**Amelioration of Diabetes-Induced Renal Injury by Sodium Nitroprusside Is Associated with Glycogen Synthase Kinase 3 beta Activation** Meenalakshmi M. Mariappan,[1,2] Kavithalakshmi Sataranatarajan,[1,2] Goutam Ghosh-Choudhury,[1,2] B. S. Kasinath.[1,2] *[1]University of Texas Health Science Center, San Antonio, TX; [2]South Texas Veterans Health Care System, San Antonio, TX.*

We have previously shown that unphosphorylated glycogen synthase kinase 3beta (GSK 3beta) inhibits protein synthesis by phosphorylating eIF2Bepsilon (eIF2Be), a regulator of mRNA translation; high glucose-induced matrix protein synthesis in renal cells is mediated by Ser9 phosphorylation and inactivation of GSK 3beta (JBC, 2008). We hypothesized that activation of GSK 3beta by sodium nitroprusside (SNP) mitigates renal injury in diabetes. Male mice were divided into: Control (Con), Con+SNP (200ug/kg/day,IPx 3wks), Diab (induced by STZ), Diab+SNP. SNP did not affect blood glucose, body wt and blood pressure in non-diabetic or diabetic mice. Renal hypertrophy (45% increase in kidney/body wt ratio, $p<0.001$) seen in diabetes was reduced by 30% by SNP ($p<0.01$). Urinary albumin excretion, increased by 5-fold in diabetes ($p<0.001$), was reduced by 40% by SNP ($p<0.01$). Renal cortical matrix proteins fibronectin, laminin beta1 and laminin gamma1, increased by 2-fold in diabetes ($p<0.05$), were reduced by SNP ($p<0.05$). Diabetes increased inactivating Ser9 phosphorylation of renal cortex GSK 3beta by 4-fold ($p<0.01$) that was robustly decreased by SNP ($p<0.001$) indicating activation of the kinase. Neither diabetes nor SNP affected phosphorylation of GSK3 alpha isoform, indicating specificity for beta isoform. Diabetes promoted dephosphorylation of eIF2Be and SNP treatment fully restored it indicating its inhibition. Diabetes increased phosphorylation of Akt and Erk, upstream regulators of GSK 3beta; this was also reduced by SNP. SNP did not induce significant changes in above paremeters in control mice. Our study shows: 1. Diabetes-induced hypertrophy and matrix protein accumulation are associated with decrease in GSK 3beta phosphorylation and inactivation. 2. SNP ameliorates diabetes-induced renal hypertrophy, albuminuria and matrix protein increment. 3. SNP inhibits diabetes-activated Akt and Erk, resulting in activation of GSK 3 beta and inactivation of eIF2Be to inhibit protein synthesis in the kidney.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO417

**When Should We Stop CRRT in AKI? Analysis of an International ICU Cohort** Dinna N. Cruz,[1] Roberto Fumagalli,[2] Manuel E. Herrera-Gutiérrez,[3] Paola Inguaggiato,[4] Detlef Kindgen-Milles,[5] Aníbal Defensor Marinho,[6] Rene Robert,[7] Claudio Ronco.[1] *[1]S Bortolo Hosp, Vicenza, Italy; [2]Milano Bicocca Univ, Monza, Italy; [3]Carlos Haya Hosp, Malaga, Spain; [4]S. Croce e Carle Hosp, Cuneo, Italy; [5]Univ Hosp, Dusseldorf, Germany; [6]Santo Antonio General Hosp, Porto, Portugal; [7]Univ Hosp, Poiters, France.*

**Introduction**

There is no consensus on timing of RRT initiation and discontinuation (DC) for acute kidney injury (AKI) in the ICU. Our aim was to identify predictors of successful DC.

**Methods**

We studied ICU patients started on CRRT in the DoReMi study. We excluded patients who died or were discharged from ICU while on RRT. Among those who stopped RRT, we classified them as "failed DC" (F) if they required repeat RRT or died ≤7 days after RRT was first stopped; "successful DC" (S) if they remained RRT-free for >7 days. We compared the 2 groups by uni- and multivariate analysis to identify independent predictors of "S".

**Results**

Of 294 patients who stopped first RRT cycle, 186 were classified as "S" while 108 as "F". At RRT-DC, S patients had lower SOFA, higher platelets, $PaO_2$, MAP and urine output. Serum Cr at RRT-DC was same in both groups. The S group had lower hospital mortality (25.9% vs 77.2% $p<0.01$). On multivariate analysis, urine output >400 ml/d was the strongest predictor for successful "weaning".

Table 1: Analysis for successful weaning from Acute RRT

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | OR(95%CI) | p | OR(95%CI) | p |
| **pH>7.35 at start RRT** | 0.43(0.26-0.70) | <0.001 | 0.46(0.27-0.79) | 0.005 |
| **$PaO_2$ at RRT-DC** | 1.01(1.00-1.02) | 0.075 | 1.01(1.00-1.02) | 0.002 |
| **Urine Output>400 ml/d at RRT-DC** | 2.77(1.36-4.68) | <0.001 | 2.69(1.52-4.77) | <0.001 |
| **MAP at RRT-DC(mmHg)** | 1.03(1.01-1.04) | <0.001 | 1.03(1.01-1.04) | 0.002 |
| **CRRT first cycle(days)** | 1.08(1.02-1.14) | 0.006 | 1.09(1.02-1.15) | 0.006 |

**Conclusion**

Over 60% of CRRT patients can be successfully weaned from RRT for >7 days. Adequate urine output was the strongest predictor for successful discontinuation.

Disclosure of Financial Relationships: Honoraria: Speaker Honoraria for Biosite/Inverness Medical.

### TH-PO418

**Early Start of Renal Replacement Therapy in Critically Ill Patients May Improve Renal Outcome but Not Survival** Matthias Klingele, Anne Lerner-Gräber, Danilo Fliser, Timo Speer. *Department of Internal Medicine, Nephrology, Saarland University Medical Centre, Homburg / Saar, Germany.*

**Methods**

Observational study of all patients requiring RRT in 2 internal medicine and 4 surgical intensive care units of our tertiary medical centre between 03/2007 and 08/2009. Patients being on chronic RRT before admission to the hospital were excluded from analysis. Serum creatinine and urea levels at the start of RRT, mortality and recovery of kidney function were assessed during hospitalization and in a follow-up period of 511 ± 249 days after discharge.

**Results**

In total, 583 patients and 5,694 RRTs were included for analysis. The in-hospital mortality was 68.4% (n=399), whereas the mortality rate in survivors after discharge who were not lost to follow-up was 25.7% (n=140). There was no statistically significant difference in serum creatinine and urea levels at initiation of RRT between those patients who died and those who survived. After discharge, in 20 survivors no recovery of renal function after AKI could be observed and they required RRT due to persistent renal insufficiency. In these patients serum creatinine (261 ± 151 vs. 475 ± 282 µmol/L; $p=0.000$) as well as serum urea (20.8 ± 11 vs. 30.8 ± 16.5 mmol/L; $p=0.007$) levels were significantly higher at initiation of RRT compared to those in whom renal function recovered after discharge.

**Conclusion**

In critically ill patients with AKI requiring RRT on the intensive care unit, lower serum creatinine and urea levels at the initiation of RRT indicate better renal outcome after hospital discharge, while mortality is not affected. This observation supports the notion that an earlier start of RRT in critically ill patients with AKI may preserve kidney function.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO419

**Behavior of the Middle and Large Solutes during SLED and SLED-*f*** Pei-Chen Wu,[1] Vincent Wu,[1] Hung-Bin Tsai,[2] Pi-Ru Tsai,[3] Wen-Je Ko,[3] Kwan-Dun Wu.[1] *[1]Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; [2]Internal Medicine, Buddhist Tzu Chi General Hospital, Chiayi, Taiwan; [3]Surgery, National Taiwan University Hospital, Taipei, Taiwan.*

**Introduction:** It remains challenging for nephrologists to evaluate the dose of renal replacement therapy (RRT) in critically ill patients with AKI. Since SLED and SLED-*f* have gained increasing popularity in the ICUs while few studies have focused on their dialysis efficiency, we examine the dialysis dose by these modalities.

**Methods:** We prospectively enrolled 7 patients with AKI and with MRSA infection receiving vancomycin treatment. These patients were randomly assigned to either SLED or SLED-*f*, and exchanged their RRT on the following day. The extent of solute (urea, vancomycin, and β2-microglobulin) removal was measured from direct dialysate quantification (DDQ). We examined the behavior of solutes in the perfused compartment during dialysis by the EKR and double-pool kinetic model.

**Results:** These patients (mean age 63.7±11.5; 4 of them female) had ultrafiltration 2.4±1.0 kg during SLED and 1.7±1.2 kg during SLED-*f*. The solute clearance, calculated by EKR and double-pool kinetic model separately, showed good correlation with DDQ.

Solute Behavior Predicted by EKR and Double-Pool Kinetic Model During SLED-f and SLED

| Mode | SLED-f (urea) | SLED (urea) | SLED-f (vancomycin) | SLED (vancomycin) | SLED-f (B2M) | SLED (B2M) |
|---|---|---|---|---|---|---|
| Patient Number | 7 | 7 | 3 | 3 | 5 | 3 |
| Post-Dialysis Weight (kg) | 66.4±14.1 | 65.8±13.2 | 70.4±15.7 | 69.0±13.9 | 68.9±16.0 | 61.8±13.5 |
| Ultrafiltration (kg) | 1.7±1.2 | 2.4±1.0 | 2.7±0.8 | 2.9±0.9 | 2.0±1.3 | 2.0±0.7 |
| EKR (ml/min) | 132.5±32.3 | 131.4±30.5 | 95.3±24.9 | 84.5±19.4 | 36.6±4.6 | 26.6±3.3 |
| R | 0.990 | 0.980 | 0.948 | 0.945 | 0.917 | 0.902 |
| dpK (ml/min) | 106.3±26.2 | 94.0±24.7 | - | - | 24.1±2.1 | 18.6±3.2 |
| R | 0.981 | 0.966 | - | - | 0.891 | 0.880 |

**Conclusion:** The EKR and double-pool kinetic model could specifically predict the behavior of urea, a small molecular weight solute, as well as middle and large molecules such as vancomycin and β2-microglobulin during SLED and SLED-*f*. Not surprisingly, SLED-*f* offers higher solute clearance than SLED does.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO420

**High Cut-Off (HCO) Renal Replacement Therapy for Removal of Myoglobin in Severe Rhabdomyolysis and Acute Kidney Injury** Nils Heyne,[1] Martina Guthoff,[1] Katja C. Weisel,[2] Hans-Ulrich Haering.[1] *[1]Department of Endocronology and Diabetology, Angiology, Nephrology and Clinical Chemistry, University of Tübingen, Tübingen, Germany; [2]Department of Hematology, Oncology and Immunology, Unversity of Tübingen, Tübingen, Germany.*

Rationale: Rhabdomyolysis is associated with the release of myoglobin into the circulation, promoting acute kidney injury (AKI) by cellular toxicity, altered renal hemodynamics and heme pigment tubular obstruction. In rhabdomyolysis patients, dialysis-dependent AKI doubles mortality. Myoglobin removal by standard blood purification techniques has limited efficacy and prognostic impact. We describe the use of high cut-off (HCO) protein permeable filters for rapid extracorporeal elimination of myoglobin in severe rhabdomyolysis.

Methods: With an in vivo molecular cut-off at 45 kD, HCO filters (Gambro HCO1100, Theralite) are effective in removing 17.8 kD myoglobin. Elimination kinetics (n = 118) across standard and HCO filters were intraindividually assessed in 9 patients with severe rhabdomyolysis and AKI using continuous and intermittent renal replacement therapy (CVVHD, batch hemodialysis (SLEDD), hemodialysis (HD)). Clearances rates were normalized per m² of membrane surface area.

Results: Median myoglobin clearance on CVVHD was 1.1 [interquartile range 0.8 - 1.2] ml/min. Standard high-flux hemodialysis resulted in a median myoglobin clearance of 2.2 [1.7 - 3.9] ml/min. Up to 20-fold higher clearances were obtained using HCO filters in both continuous (SLEDD: 20.2 [17.9 - 24.3] ml/min) and intermittent (HD: 40.2 [37.5 - 42.7] ml/min) dialysis techniques. Using full size (2.1 m²) HCO filters, median absolute myoglobin clearances in excess of 70 ml/min (HD) were achieved, resulting in rapid and highly effective reduction of plasma myoglobin concentration. Albumin losses across HCO filters require substitution over time.

In conclusion, HCO renal replacement therapy for the fist time allows the safe and highly efficient elimination of myoglobin in severe rhabdomyolysis and acute kidney injury. Limiting exposure of the kidneys to myoglobin may improve renal recovery and patient prognosis.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO421

**The "Gap" between Prescribed and Delivered CRRT Dose: Analysis of an International ICU Cohort** Dinna N. Cruz,[1] Roberto Fumagalli,[2] Manuel E. Herrera-Gutiérrez,[3] Paola Inguaggiato,[4] Detlef Kindgen-Milles,[5] Aníbal Defensor Marinho,[6] Rene Robert,[7] Claudio Ronco.[1] *[1]S Bortolo Hosp, Vicenza, Italy; [2]Milano Bicocca Univ, Monza, Italy; [3]Carlos Haya Hosp, Malaga, Spain; [4]S. Croce e Carle Hosp, Cuneo, Italy; [5]Univ Hosp, Dusseldorf, Germany; [6]Santo Antonio General Hosp, Porto, Portugal; [7]Univ Hosp, Poiters, France.*

**Introduction**

CRRT dose is a modifiable factor in the ICU management of acute kidney injury (AKI). In the DoReMi study, the median prescribed CRRT dose (RxD) was 34.3 while delivered (DD) was 27.1ml/kg/hr. Other studies also show that DD is lower than RxD. Our aim was to identify factors contributing to the "gap" between RxD and DD.

**Methods**

We studied 338 patients treated with CRRT in the DoReMi study. We calculated "%dose gap" as 100*((RxD-DD)/RxD). We grouped patients into "low gap" (≤15%, n=108) and "high gap" (>15%, n=220). We performed uni- and multivariate analysis to identify independent predictors of high CRRT dose gap.

**Results**

The "high gap" group was younger, had lower pH, higher WBC & platelet counts. They had longer "downtime" (no. of hours/day in which CRRT is interrupted), more likely to have femoral access, and used higher % of pre-dilution replacement fluid. On multivariate analysis, %pre-dilution and CRRT downtime were significant independent predictors of a high dose gap.

Table 1: Predictors of "high" CRRT dose gap

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| | OR(95%CI) | p | OR(95%CI) | p |
| Age(yrs) | 0.98(0.97-0.99) | 0.008 | 0.98(0.97-1.00) | 0.053 |
| CVVH or HVHF modality | 0.62(0.40-0.98) | 0.042 | 1.82 (0.93-3.56) | 0.080 |
| pH<7.2 | 1.57(0.98-2.51) | 0.059 | 2.86(1.23-6.67) | 0.015 |
| Downtime(hrs) | 2.07(1.62-2.65) | <0.001 | 1.92(1.50-2.46) | <0.001 |
| Pre-dilution>67% | 3.29(2.05- 5.29) | <0.001 | 4.20(2.14-8.28) | <0.001 |

**Conclusions**

Other than CRRT downtime, the use of high %pre-dilution for replacement fluid may be a significant, and previously unrecognized, contributing factor to the CRRT dose gap. Physicians should take this into consideration when prescribing CRRT.

Disclosure of Financial Relationships: Honoraria: Speaker Honoraria for Biosite/ Inverness Medical.

## TH-PO422

**Effect of Type of Anticoagulation on Delivered Dialysis Dose of CRRT** Rolando Claure-Del Granado,[1] Etienne Macedo,[1] Sharon Soroko,[1] Glenn M. Chertow,[2] Jonathan Himmelfarb,[3] T. Alp Ikizler,[4] Emil P. Paganini,[5] Ravindra L. Mehta.[1] *[1]University of California, San Diego; [2]Stanford University School of Medicine; [3]Kidney Research Institute, University of Washington; [4]Vanderbilt University Medical Center; [5]Cleveland Clinic Foundation.*

***Background:*** Efficiency of CRRT depends on circuit longevity which is influenced by anticoagulation and the operational characteristics of the modality. Effluent volume is a surrogate for dose delivered, however comparisons across modalities are scant. We evaluated the effect of anticoagulation (AC) on the efficacy of 3 CRRT modalities on delivered dose. We hypothesized that the choice of AC and modality affect delivered dose of dialysis. ***Methods:*** We analyzed data from 1,730 sessions of 24 hrs in 244 critically ill pts from 5 centers included in the PICARD study, with at least 48 hours on CRRT (CVVH, CVVHD or CVVHDF). Citrate (TSC), heparin (Hep) or no anticoagulant (No-AC) was used to prevent filter clotting. Delivered dose was calculated by using stdKt/V adjusted for each type of therapy (Artif Organs 30:178-185;2006). ***Results:*** Among modalities CVVHDF had the highest delivered dose when citrate was used as AC. Filter longevity is significantly lower with heparin and non AC circuits and resulted in the use of a higher number of filters to achieve the same delivered dose(Table 1).

Comparisons of delivered stdKt/V with use of heparin, citrate or no anticoagulation among different types of CRRT

| **Type of anticoagulant** | | | | |
|---|---|---|---|---|
| | Mean filter life [hrs] (# of filters) | CVVH * | CVVHD * | CVVHDF * |
| **Citrate** | 54.5 (173) | 1.7 (0.59-2.9) | - | 8.2(5.9-10.4) |
| **Heparin** | 21.1 (824) | 2.85(1.8-4.1) | 5.6(4.6-7.4) | 8.3(5.8-10.8) |
| **No anticoagulant** | 23.1 (403) | 7.3(5.3-9.0) | 5.9(4.5-7.3) | 8.5(6.3-11.2) |
| **p value** | < 0.001 | < 0.001 | 0.584 | 0.062 |

*Median (IQR) stdKt/V

***Conclusions:*** Computation of stdKt/V allows comparison of delivered dose across modalities. Choice of AC influences the dose delivered with citrate AC providing the highest dose per filter used. Dose studies should consider AC type as a factor influencing delivered dose.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO423

**Hemodiafiltration with High Flux Polyphenylene Membrane (Phylther®). A Much Cheaper Alternative to HCO Dialysis for Kappa Light Chain Cast Nephropathy** Simon Desmeules, Mohsen Agharazii. *Medicine, CHUQ, Quebec, QC, Canada.*

Introduction : Addition of extended High Cut-Off (HCO) dialysis to uninterrupted chemotherapy might improve renal recovery in patients with cast nephropathy (CN). Reported Free Light Chain (FLC) reduction ratios average 65-71%. Unfortunately, HCO dialysers costs are prohibitive. We report the reduction ratios (RR) of FLC and clinical outcome of 6 patients with renal failure due to multiple myeloma (MM), using hemodiafiltration (HDF) with various types of dialysers.

Methods: Retrospective and prospective data collection among patients with acute renal failure due to MM needing dialysis. Only one filter per session were used. HDF was performed with either online fluid generation (predilution ol-HDF(34-49L)) or a biofiltration system using Hemosol B0 5L bags as an post filter infusion fluid (9-14L). FLC were measured by nephelometry (FREELITETM, The Binding Site, San Diego, USA) before and at the end of the each hemodiafiltration session using the slow flow/stop pump technique. Results: A total of 116 HDF sessions were evaluated. 5/6 patients had kappa FLC elevation among which, 4 had biopsy proven cast nephropathy (creatinine 5.6-13.8 mg/dL). One patient had lambda CN. Table summarizes FLC and beta2-microglobuline RR. Our results demonstrate that ol-HDF with Phylther®HF22SD achieves kappa FLC RR comparable to HCO dialysis/HDF. All 4 patients with biopsy proven kappa FLC CN recovered dialysis-independent renal function within 5-100 d (creatinine 0.76-2.9 mg/dL). Regarding lambda FLC clearance, HCO HDF with Theralite offers better clearances.

| | | reduction ratio | | | reduction ratio | |
|---|---|---|---|---|---|---|
| | n | kappa | beta2 | n | lambda | Beta2 |
| HCO 1100 | 14 | 70.2% | na | | | |
| TheraLite | | | | 34 | 63.0% | 69.6% |
| HF22SD | | | | | | |
| olHDF | 21 | 79.6% | 67.1% | 4 | 35.0% | 56.2% |
| bagHDF | 20 | 50.2% | 50.1% | | | |
| Nephral500 | 7 | 45.3% | na | | | |
| Polyflux 210H | 16 | 25.8% | na | | | |

Conclusion: Our experience suggests that in patient with dialysis dependant kappa FLC cast nephropathy, HDF with high flux Phylther® HF22SD resulted in a similar degree of kappa FLC RR as compared to HCO, and in addition to early chemotherapy resulted in renal function recovery.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO424**

**Factors Affecting the Adequacy of Hemodialysis in Acute Setting – A Follow-Up Study** Jasmeet Kaur, Manjusha Rajamohanty, Manpreet Singh, Kaushik Manthani, Mersema Abate, Nand K. Wadhwa. *Division of Nephrology, SUNY, Stony Brook, NY.*

Urea reduction ratio (URR) of ≥ 65% is considered optimal hemodialysis (HD) treatment in chronic setting. Prescribed HD treatment, more often may not be adequate and may also not be delivered as prescribed in the acute setting. We previously reported that 34% of acute HD treatments had URR < 65% due to inadequate HD prescription and delivery (Am J Kid Dis 51: B28, 2008). We initiated a protocol where parameters were set to provide an adequate HD treatment. The patients with AVF/AVG should be prescribed a BFR of 450 ml/min and with catheters a BFR of 350 ml/min. URR was measured each HD treatment. We collected data on 389 HD sessions in 67 patients from March 2008 to November 2009. Three hundred thirty six (86%) HD sessions had URR ≥65% while 53 (14%) sessions had URR < 65%. One hundred sixty four (42%) HD sessions were done using AVF/AVG and 225 (58%) done using catheters. Mean BFR of ≥300ml/min was delivered in 350 (90%) of HD sessions.

Patient Characteristics and URR

| Variable | URR ≥ 65% | URR < 65% | P value |
|---|---|---|---|
| Age (years) | 61 ± 15 | 57 ± 17 | NS |
| Weight (Kg) | 75.1 ± 20.7 | 87.6 ± 24.8 | < 0.001 |
| AVF-AVG/Catheter | 148/190 | 19/32 | NS |
| HD duration prescribed (hours) | 3.9 ± 0.2 | 3.7 ± 0.5 | NS |
| HD duration delivered (hours) | 3.9 ± 0.3 | 3.6 ± 0.5 | NS |
| BFR prescribed (ml/min) | 393.0 ± 54.1 | 361.6 ± 66.1 | < 0.05 |
| BFR delivered (ml/min) | 365.9 ± 54.6 | 328.5 ± 68.0 | NS |
| UF prescribed (liters) | 3.0 ± 9.7 | 2.5 ± 0.9 | NS |
| UF delivered (liters) | 2.3 ± 0.8 | 2.6 ± 1.2 | < 0.05 |

Patients with URR <65% had significantly higher body weight. Prescribed and delivered BFR were significantly lower in HD using catheters compared to AVF/AVG. Prescribed and delivered BFR were significantly lower in the group with URR < 65% compared to the group with URR ≥ 65%. In both groups, delivered BFR was significantly lower than the prescribed rate. Sub optimal HD (URR < 65%) decreased to 14% of the HD sessions compared to 34% in our previous study. In conclusion, we as prescribers not only prescribe adequate dialysis but also need to ensure proper delivery in order to improve dialysis treatment in acute setting.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO425**

**Optimal Timing of CRRT and Clinical Outcomes in Critically Ill Patients with AKI** Yong Chul Kim, Kwon Wook Joo, Yon Su Kim, Curie Ahn, Jin Suk Han, Suhnggwon Kim, Dong Ki Kim. *Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.*

**Introduction** We evaluated retrospectively the optimal timing of CRRT stratified by various clinical and laboratory parameters and its association with clinical outcomes in patients with rapidly progressive AKI.

**Methods** A retrospective study was performed on the data of 658 AKI patients received CRRT in intensive care unit (ICU) of Seoul National University Hospital from October 2007 to January 2010. Rapidly progressive AKI was defined as >2-fold increase of serum creatinine or >50% reduction of hourly urine output during 24h prior to initiation of CRRT. Data included RIFLE criteria, SOFA score, APACHE II score, and number of organ failures. Timing of CRRT was stratified into 'early' and 'late' by median value of BUN and creatinine levels at the start of CRRT, and also by median urine output during 6h, 12h, and 24h before the initiation of CRRT. The clinical outcomes assessed included duration of RRT, ICU stay, hospital stay and 90 day-mortality.

**Results** There were no significant differences in outcomes of patients between early and late group stratified by median value of creatinine at the start of CRRT. However, in terms of BUN, 90 day mortality rate was significantly higher for late group (BUN 54 > mg/dL) in univariate analysis (P=0.013), but not in multivariate analysis. When the patients were stratified by urine output before CRRT, patients with lower urine output during 6h (early >110ml) and 12h (early >260ml) before CRRT had significant higher multivariate-adjusted, 90 day-mortality. (6h: OR 1.45, 95% CI 0.99-2.15, P=0.005, 12h: OR 1.69, 95% CI 1.14-2.39, P=0.008). Finally, when CRRT was started at 'Failure' stage of RIFLE criteria compared with 'Injury' stage, the multivariate adjusted OR for death was 1.74 (95% CI 1.15-2.64). Duration of RRT, ICU stay and hospital stay had no significant differences between 'early' and 'late' group.

**Conclusion** Our data suggest that early CRRT may have survival benefit in critically ill patients with rapidly progressive AKI, and urine output is the most important parameter for the decision to start CRRT.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO426**

**Influence of DECAP and DECAP+CVVH Treatment on Hemodinamics and Respiratory Pressures** Anthi Panagiotou,[1,3] Gramaticopolo Silvia,[2] Dinna N. Cruz,[1,3] Nicola Marchionna,[1,3] Matteo Floris,[1,3] Alessandra Brendolan,[1] Federico Nalesso,[1,3] Monica Zanella,[1] Francesco Garzotto,[1,3] Pasquale Piccinni,[2] Claudio Ronco.[1,3] *[1]Nephrology, S Bortolo Hosp, Vicenza, Italy; [2]Intensive Care Unite, S Bortolo Hosp, Vicenza, Italy; [3]IRRIV, Vicenza, Italy.*

Introduction: In Acute Lung Injury (ALI) it is important to limit alveolar pressure and shear stress by applying low tidal volumes (TV) at the cost of increased arterial blood $CO_2$ and respiratory acidosis. Extracorporeal CO2 removal (DECAP) is a method to achieve ultra-low TV in mechanically ventilated (MV) patients with severe respiratory failure avoiding respiratory acidosis. The extracorporeal CO2 removal cartridge can be combined with a hemofilter for renal replacement therapy (RRT). This coupled system has been used in AKI + ALI, MV patients, reducing ventilatory pressures expressed as Peak Inspiratory Pressure (PIP), ameliorating hemodinamics and respiratory acidosis.

Methods: Four patients (3 F, 1 M) admitted to the ICU for septic multiorgan failure were treated with DECAP and DECAP+CVVH. Duration of DECAP treatment ranged from 24 to 48 hours while for DECAP+CRRT was 12hours. Blood gas analysis, respiratory and hemodinamics parameters were recorded at time 0, 1st hour and after 6 hours.

Results: During DECAP treatment alone and CVVH+DECAP treatment it was possible to reduce the PIP, normalize pH with an improvement in hemodinamics without adverse events.

DECAP AND DECAP+CVVH DATA

| DECAP DATA | | | | | | | |
|---|---|---|---|---|---|---|---|
| | t0 | 1st hr | 6th hr | | | | |
| Max PIP (cmH2O) | 32 | 29 | 29 | | | | |
| pH | 7,22 | 7,39 | 7,35 | | | | |
| Medium BP (mmHg) | 82 | 89 | 87 | | | | |
| pCO2 (mmHg) | 121 | 77 | 80 | | | | |
| | | | | | | | |
| DECAP+CVVH DATA | | | | | | | |
| pH | t0 | 1st hr | 6th hr | pCO2 (mmHg) | t0 | 1st hr | 6th hr |
| A | 7,31 | 7,4 | - | A | 92 | 67 | - |
| B | 7,33 | 7,36 | 7,36 | B | 47,2 | 45,1 | 54 |
| C | 7,22 | 7,23 | 7,39 | C | 74,1 | 65 | 43,9 |
| | | | | | | | |
| PIP (cmH2O) | t0 | 1st hr | 6th hr | Medium BP (mmHg) | t0 | 1st hr | 6th hr |
| A | 30 | 28 | - | A | 53 | 84 | 73 |
| B | 33 | 29 | 27 | B | 58 | 88 | 80 |
| C | 39 | 37 | 29 | C | 108 | 92 | 89 |

Conclusions: DECAP system combines CO2 removal and protective ventilation, protecting the lung by a further reduction in TV, managing acidosis with a minimally invasive system and side effects. In AKI patients the same system can be used in combination with a hemofilter using a single venous access to perform standard CRRT.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO427**

**24-Hour Sustained Low Efficiency Dialysis (SLED) with Near-Automated Regional Citrate Anticoagulation (RCA) Incorporating Online Ionic Dialysance (OLC) and Optical Hematocrit Monitoring: The First 1000 Hours Experience** Balazs Szamosfalvi, Stan Frinak, Lenar T. Yessayan, Jerry Yee. *Division of Nephrology and Hypertension, Henry Ford Health System, Detroit, MI.*

**Purpose:** Evaluation of a 24-h SLED-RCA protocol with OLC that provides highly effective anticoagulation with a calcium infusion (QCa) prediction algorithm which maintains normal systemic ionized Ca (iCa) in all patients.

**Methods:** 8 critically ill patients, 5 with severe liver dysfunction were treated with 24-h SLED-RCA for total >1000 treatment hours. The system includes a 1.5 $m^2$ high-flux hemodialyzer; zero calcium, 140 Na and 32 bicarbonate dialysate with optional phosphate supplement 3.2 mg/dL; a dialysis machine with OLC measurement and an optical hematocrit (Hct) sensor. Flow rates were QB=60 mL/min; QD=400 mL/min; Qcitrate=150 mL/h of Acid Citrate Dextrose-A; and net ultrafiltration as indicated. The QCa was infused into the venous limb of the circuit and adjusted hourly as a function of online Hct and a <24 h-old albumin level. Systemic iCa was sampled every 6 h.

**Results:** The high Qcitrate to QB ratio completely abrogated circuit clotting without adverse electrolyte or acid-base effects. The algorithm QCa accurately adjusted systemic iCa between 0.9 and 1.3 for all patients. Sodium and bicarbonate approximated normal values during treatment. OLC was feasible and stable in the range of 40-57 mL/min. Optical hematocrit correlated well with laboratory values. Ultrafiltration was well tolerated and effective over several days. Access recirculation was noted in one patient but did not affect QCa dosing. Systemic citrate accumulation did not occur even after days of SLED-RCA as evidenced by systemic total calcium to iCa ratio. Nurse satisfaction with the modality was very high.

**Summary:** The >95% citrate extraction on the dialyzer prevented systemic citrate accumulation even in shock liver patients. Predictive QCa dosing based on albumin and Hct resulted in normal systemic iCa and a very simple to perform RCA protocol. We conclude that 24-h SLED-RCA is a safe alternative to traditional CRRT and holds great promise to expand the use of 24-hour renal support in the ICU without an increase in costs.

Disclosure of Financial Relationships: Consultancy: Baxter, Inc.Research Funding: Fresenius; Honoraria: Renal Research Institute; Patent: Automated system for the delivery of regional citrate anticoagulation (patent application).

**TH-PO428**

**Liver Dysfunction Predicts Development of Citrate Toxicity in Patients on Continuous Renal Replacement Therapy** Scott E. Liebman,[1] Joseph J. Guido,[2] Rebeca D. Monk.[1] *[1]Medicine, University of Rochester, Rochester, NY; [2]Preventitive Medicine, University of Rochester, Rochester, NY.*

**Background-** Citrate use during continuous renal replacement therapy (CRRT) may lead to toxicity if citrate entering the systemic circulation is not sufficiently metabolized. Liver failure is considered a risk factor for citrate toxicity, but supporting data are scant. We hypothesized that patients who develop citrate toxicity have more severe liver dysfunction compared with patients who do not.

**Methods-** This is a retrospective study of 372 patients receiving CRRT (typically continuous venovenous hemodiafiltration, starting with predilutional citrate-containing replacement fluid at one liter per hour, and dialysate at one liter per hour) at the University of Rochester between 1/1/2006 and 7/24/2009. Citrate toxicity was defined as one or two occurrences of a total to ionized calcium ratio of ≥2.5 (T/I ratio). Relevant pre-CRRT laboratory values were compared between toxic and non-toxic patients.

**Results-** Of 372 patients, 116 had one elevated T/I ratio (31%) and 59 had at least two (16%). Patients developing citrate toxicity by both definitions had a significantly higher AST, INR, albumin, lactate and total bilirubin compared with those who did not. Ammonia was also higher in patients with two elevated T/I ratios.

Demographic factors predicting the development of citrate toxicity on univariate analysis included female gender. Toxic patients were more likely to be younger (by two T/I ratios) and African American (by one T/I ratio).

Albumin, INR, lactate and total bilirubin remain significant in the multivariate stepwise logistic regression model.

**Conclusions-** Pre-CRRT laboratory values indicative of more severe liver dysfunction are associated with the development of citrate toxicity on CRRT. Female gender, age, and African American race may also be predictive.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO429**

**In Vitro Evaluation of Teicoplanin Adsorption Kinetics onto Polysulfone Membrane** Marco Sartori,[1] Dinna N. Cruz,[1] Giuliano Dall'olio,[1] Francesco Garzotto,[1] Mirella Zancato,[2] Claudio Ronco.[1] *[1]S Bortolo Hosp; [2]University of Padua.*

Teicoplanin is an alternative drug for sepsis due to Gram(+) Vancomycin-resistant infection. Septic patients can develop severe acute kidney injury requiring Renal Replacement Therapy. Therefore it is important to understand drug removal by RRT to help guide dosing.

METHODS

Mock hemopefusion (HP) was performed for 120 min using 5L of Teicoplanin solution (71.0 µg/ml). After 5 min of priming, this solution was circulated in a closed circuit simulating HP (Qb 300mL/min) using a 1.5m2 polysulfone membrane. Drug levels were measured at 15,30,60,120 min from arterial(Cpin)and venous(Cpout)ports. After 120 min of HP, mock zero-balance hemofiltration (HF) was then performed (Qb 300mL/min,Quf 60mL/min) with 0.9%NS as replacement fluid. Samples were taken from arterial, venous and ultrafiltrate(Cpuf)ports at same intervals. Teicoplanin levels were measured using fluorescence polarization immunoassay. Teicoplanin removal kinetics,adsorption,sieving coefficient(SC) were calculated.

FINDINGS

At end of HP, Teicoplanin level (Cpout) was 38.9µg/mL. Ratio of Cpout/Cpin rose rapidly during priming and first 30 min of HP, then plateaued (Fig 1), likely due to drug adsorption in the first 30 min. Furthermore, SC was stable at 1.09, 1.03, 1.01 and 1.01 after 15, 30, 60 and 120 min of HF, supporting this presumption. On mass balance analysis, the sum total amount of Teicoplanin retrieved from the system at the end of HP (M-outflow 221.8 mg)was notably less than that introduced into the system (494.9 mg), suggesting that 273.1 mg (55%) were adsorbed onto the polysulfone membrane (Fig 2).

CONCLUSION

In this in-vitro study,the estimated adsorption was more than half the dose. This should be considered when Teicoplanin is used in patients on RRT. A supplemental dose at RRT initiation may be needed to avoid potential underdosing.

Figure 1

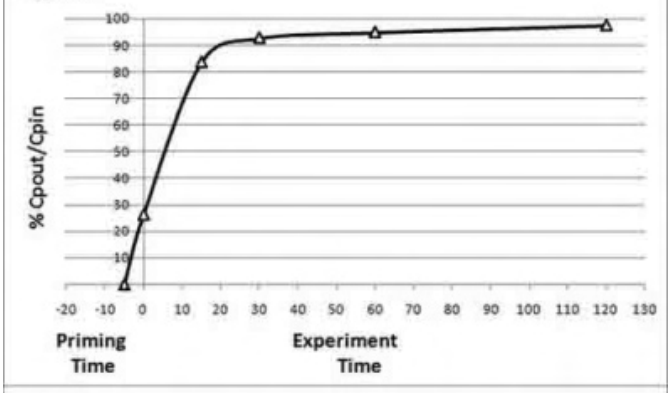
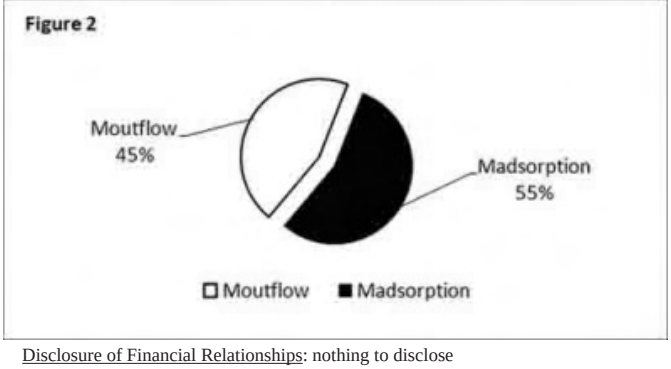



Figure 2




Disclosure of Financial Relationships: nothing to disclose

**TH-PO430**

**Is It True That Fluid Overload Is Independent Risk Factor of Mortality in Critically Ill Patients Regardless of Disease Severity?** Ihm Soo Kwak, Sang Heon Song, Jung Sub Kim, Harin Rhee, Eun Young Seong, Soo Bong Lee, Il Young Kim, Jungmin Son. *Department of Internal Medicine, Pusan National University School of Medicine, Busan, Korea.*

**Purpose**: Recent studies suggested that fluid accumulation is associated with adverse outcome in critically ill patients. **Method**: From January 2007 to December 2008, a total of 146 patients who underwent CRRT in ICU were evaluated. Percent fluid overload(%FO) was defined as total fluid input minus output (3days before CRRT start(%FO1) and from CRRT initiation to ICU discharge(%FO2)) divided by body weight. Also, we calculated APACHIE IV score from clinical data. **Results**: Mean renal replacement time was 74±77.8 hours and 64patients survived(43.8%). Mean %FO1 and total %FO were significantly lower in survivors than non-survivors(2.8% vs. 5.4%, p=0.001 and 4.9% vs. 9.4%, p=0.004, respectively). Patients' characteristics stratified by fluid overload status are summarized in Table 1. For the low APACHIE IV(below 89)group, %FO1 was higher in non-survivors than survivors(2.2±3.5 vs. 5.0±5.4, p=0.013). But for the high APACHIE IV group(above 89), total %FO was higher in non-survivors than survivors (4.9±8.8 vs. 9.4±9.4, p=0.047). **Conclusion**: Our study shows fluid overload was associated with mortality, but it was not an independent risk factor. According to the results of subgroup analysis, we suggest that high APACHIE IV group has much more difficulties in correcting fluid overload during CRRT.

Table 1. Patients' characteristics based on fluid status

| Baseline Characteristics | Non fluid overload(n=74) | Fluid overload(n=72) | P |
|---|---|---|---|
| MAP (mmHg) | 80.8 (17.4) | 74.8 (14.8) | 0.025 |
| Urine output (ml/day) | 1242.0 (1220.3) | 653.6 (854.1) | 0.001 |
| Tachycardia | 29 (39.2%) | 44 (61.1%) | 0.008 |
| Peak Cr (mg/dl) | 5.0 (3.0) | 3.9 (1.7) | 0.007 |
| CRP (mg/dl) | 12.2 (12.4) | 16.7 (12.6) | 0.034 |
| AST (IU) | 328.1 (810.7) | 729.4 (1309.8) | 0.028 |
| ALT (IU) | 171.2 (371.4) | 367.9 (688.0) | 0.035 |
| APACHE IV | 83.6 (25.5) | 93.5 (24.1) | 0.017 |
| Cardiovascular failure | 51 (68.9%) | 64 (88.9%) | 0.003 |
| Ventilator use | 45 (60.8%) | 56 (77.8%) | 0.026 |

Disclosure of Financial Relationships: nothing to disclose

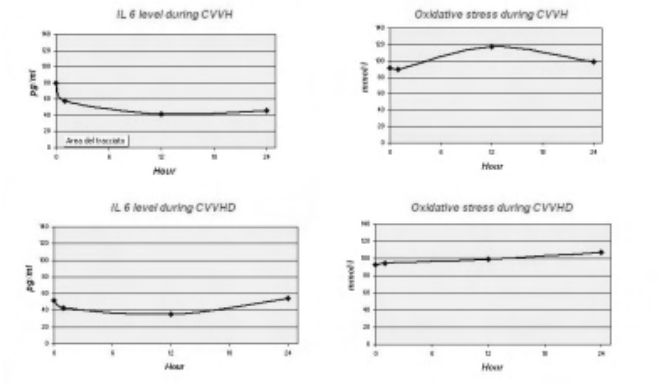
**TH-PO431**

**IL-6 Decrese and Oxidative Stress Evaluation during Continuous Renal Replacement Therapy with a New High Polysulfone-Based Hemofilter (CUREFLO®)** Matteo Floris,[1,3] Nicola Marchionna,[1,3] Alessandra Brendolan,[1] Federico Nalesso,[1,3] Monica Zanella,[1] Pasquale Piccinni,[2] Massimo De Cal,[1,3,4] Grazia Maria Virzì,[1,3] Dinna N. Cruz,[1,3] Claudio Ronco.[1,3] *[1]Nephrology, S Bortolo Hosp, Vicenza, Italy; [2]ICU, S Bortolo Hosp, Vicenza, Italy; [3]IRRIV, Italy; [4]University of Padua.*

BACKGROUND: The Oxidative Stress (OS) and the balance between pro- and anti- inflammatory cytokines may contribute to unfavourable outcomes in ICU patients; death is even higher when associated with Acute Kidney Injury (AKI). Continuous Renal Replacement Therapy (CRRT) can reduce the inflammatory status. We reported the variation of an inflammatory biomarker, the Interleukine 6 (IL6) and oxidative stress with advanced oxidation protein products (AOPP)in a patient requiring CRRT admitted in ICU for AKI.

METHODS: we performed 11 treatments, 6 Continuous Veno Venous Hemofiltration (CVVH) and 5 Continuous Veno Venous Hemodialysis (CVVHD) using ACF-130W hollow-fiber dialyzer (1.3 sqm, Asahi Kasei Kuraray Medical co.Ltd. Patients were treated by CRRT using the Multifiltrate Fresenius machine. The treatment conditions were: CVVH (Qb 200 ml/min; Qr 1500 ml/h in postdiluition; Qf -100 ml/h) or CVVHD (Qb 200 ml/min; Qd 2500 ml/h; Qf -100 ml/h). Samples were collected before the sessions and after 1, 12 and 24h from the beginning. We measured the decrease in IL-6 and oxidative stress after every session.

RESULTS: As shown, the CVVH treatment is related with a major reduction of IL 6 level.




Furthermore AOPP increase during both treatments, especially in CVVHD mode.

CONCLUSION: Our data suggest that this filter is able to remove Il-6 by convection but not by diffusion and adsorption. The study of oxidative stress demonstrated that during all treatments AOPP increased more in CVVHD than in CVVH as it can be explained by the IL-6 (and other cytokines) removal by convection only.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO432**

**Hypophosphatemia – Major but Relatively Unrecognized Complication during CRRT** Norio Hanafusa,[1] Kousuke Negishi,[1] Kent Doi,[1] Naoki Yahagi,[2] Eisei Noiri,[1] Toshiro Fujita.[1] *[1]Department of Hemodialysis and Apheresis, The University of Tokyo Hospital, Tokyo, Japan; [2]Department of Emergency and Critical Care Medicine, The University of Tokyo Hospital, Tokyo, Japan.*

**Introduction:** Several investigations have shown that hypophosphatemia frequently occurs during CRRT especially in higher dose of CRRT [NEJM 361: 1627, 2009]. In Japan we often deliver the reduced dose of CRRT. Therefore we investigate the incidence of hypophosphatemia during CRRT, the causative factors and its relation with mortality.

**Settings:** The study was conducted in a retrospective observational manner. We investigated all patients who underwent CRRT in the ICU at our tertiary teaching hospital during 2007. We collected data from medical charts for CRRT therapy and laboratory data including serum phosphate concentration.

**Results:** During the study period, 106 CRRT courses were identified. Among them, the serum phosphate of only 57 patients was measured. Most who were measured experienced hypophosphatemia (median 2.0 mg/dl) during the course, though they were received reduced dose of CRRT; effluent flow rate was 19.7±8.86ml/kg/hr. The time up to the measurement was inversely related with the lowest value of phosphate. In the multivariate analysis, the lowest value of phosphate predicted 30 days mortality (OR 0.38 95%CI 0.08-0.98 $p$=0.045) after adjustment of age, SOFA score, ESRD or not, and initial creatinine value.

**Discussion:** The incidence of hypophosphatemia was high also in this population, though the reduced dose of CRRT. The serum phosphate level was not determined in many cases, which indicates that hypophosphatemia during CRRT might seem not so familiar to physicians in the ICU. Moreover the reason remains unclear but hypophosphatemia itself was related with the survival. These factors might affect the non-superiority of higher dose of CRRT. Because appropriate supplementation of phosphate can avoid hypophosphatemia, nephrologists should take efforts to make ICU physicians aware of the significance of hypophosphatemia during CRRT.

**Conclusion:** Results of this study reconfirmed the high incidence of hypophosphatemia. Nephrologists might have to raise awareness of hypophosphatemia during CRRT.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO433**

**Clinical Characteristics and Prognostic Predictors in Acute Kidney Injury Treated by Continuous Renal Replacement Therapy, Retrospective Study** Jong Woo Seo, Hyun-Jung Kim, Dong Jun Park, Dong Won Lee, Hyeon Jeong Lee, Se-Ho Chang. *Internal Medicine, Gyeoungsang National University Hospital, Jinju, Republic of Korea.*

Continuous renal replacement therapy (CRRT) has been used widely for treating critically ill patients with AKI because of the hemodynamic tolerance. We performed this study to identify clinical characteristics and prognostic predictors of mortality in critically ill patients with AKI treated by CRRT at intensive care units in our hospital.

We analyzed the data of 181 patients who were treated by CRRT from Jan, 2007 to Feb, 2010. We excluded patients treated by CRRT within 24 hrs.

Of the 181 cases who treated by CRRT, 117 were males. The average age was 61.4 years. Type 2 diabetes mellitus was the most frequent comorbid illness (49%), followed by hypertension (38%), chronic kidney disease (CKD) (31%), liver cirrhosis (21%), heart failure (12%) and malignancy (19%). Causes of AKI included sepsis in 100 (55.2%), surgery in 34 (18.8%), cardiogenic shock in 13 (7.2%), rhadomyolysis in 11 (61%), drug intoxication in 4 (2.2%), trauma in 3 (1.7%), and hepatorenal syndrome in 2 (1.1%) patients. The overall mortality was 68%. The average APACHE II score was 49.27±11.11. Univariate analyses suggested APACHE II scores, comorbid illness of CKD, heart failure and sepsis, serum bicarbonate started CRRT after 48hours were significantly lower in survivors. Multivariate analysis revealed that APACHE II scores (Odd ratio : 1.12; 95% Cl 1.05-1.19; p = 0.01), prothrombin time (Odd ratio : 1.52; 95% Cl 1.22-1.88; p = 0.01), predialysis oliguria (Odd ratio : 13.32; 95% Cl 1.19-148.46; p = 0.03), presence of sepsis (Odd ratio : 4.04; 95% Cl 1.11-14.61; p = 0.03), and serum bicarbonate started CRRT after 48hrs (Odd ratio : 0.05; 95% Cl 0.37-0.68; p = 0.01) were independent predictors for the prediction of mortality.

This study showed that independent prognostic predictors for mortality in AKI patients treated with CRRT were APACHE II scores, prothrombin time, predialysis oliguria, presence of sepsis and serum bicarbonate after 48hrs started CRRT. Using these prognostic predictors in critically ill patients will be made for the appropriate circumstances.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO434**

**Evaluation of Filter Patency, Acid-Base and Electrolyte Balance of a Prismaflex-Based Regional Citrate Anticoagulation Protocol for Continuous Renal Replacement Therapy** Dmytro Khadzhynov, Ina Lieker, Torsten Slowinski, Hans-Hellmut Neumayer, Harm Peters. *Department of Nephrology, Charite Campus Mitte, Charite Universitaetsmedizin, Berlin, Germany.*

**Purpose:** Regional citrate anticoagulation has been shown to be an excellent alternative to heparin anticoagulation for patients requiring CRRT. Here, we evaluate a new commercially available citrate protocol involving the actual PrismaFlex dialysis device and an isotonic citrate solution (Prismocitrate) (Gambro GmbH, Germany).

**Methods:** According to the manufacture´s information, this citrate protocol was based on a PrismaFlex ST100 set (AN69®ST membrane) mounted on the Prismaflex dialysis device, a blood flow 120 ml/min, 1.8 l/h Prismocitrate (10 mmol/l citrate) in pre-dilution mode and a 0.8 l/h dialysate flow employing Prism0Cal. Separate i.v. infusions of potassium, calcium and magnesium were initiated according to the manufacture´s protocol. Blood pH, bicarbonate, base excess and ionized calcium levels were measured before and at least every 6 h after start of CRRT, magnesium levels before and every 24 h, respectively. Scheduled hemofilter run time was 72 h.

**Results:** A series of 25 CCRT treatments in 16 patients was included. Of the 25 hemofilters started, only 5 reached the scheduled run time (20%). 20 treatments stopped prematurely (9 because of filter clotting, 10 because of Prismaflex hardware or software problems). Mean filter running time achieved was 33.2±24.4 h (46% of the scheduled time). During CCRT, 70% of the blood bicarbonate levels were lower than normal range (21-28 mmol/L), 65.7% of ionized calcium measurements were outside the normal range (1.1-1.3 mmol/L) and 56.4% of the magnesium readings were above the normal range (0.75-1.06 mmol/l). In addition, mean bicarbonate decreased from 23 to 20 mmol/l and mean base excess from -1.5 to -4 mmol/L, resp.. Substitution of bicarbonate was necessary in 6 treatments.

**Conclusion:** The present study shows that the evaluated Prismaflex/Prismocitrate-based regional citrate anticoagulation protocol yields not satisfying filter running times. Furthermore, the control of blood acid base status, calcium and magnesium homeostasis is of poor quality.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO435**

**Prophylactic Hemodiafiltration after Coronary Angiography** Maria Joao Carvalho Azevedo Rocha, António Cabrita. *Nephrology, Hospital de Santo António - Centro Hospitalar do Porto, Porto, Portugal.*

Contrast induced nephropathy (CIN) is a form of acute kidney injury (AKI) that can be prevented.Small elevations of plasma creatinine have a negative impact in patient outcome bringing a lot of attention to prophylactic strategies.Convective or diffusive

techniques for contrast elimination have been proposed as a possible form of prophylaxis in patients with chronic kidney disease (CKD).Our purpose was to evaluate the impact of prophylactic hemodiafiltration(PHDF) on incidence and severity of AKI in patients submitted to coronary angiography (CA).We performed an observational case control study. We performed 1 4 hour session of PHDF (auto-flux,postdilution mode,0 ultrafiltration) in 19 patients immediately after CA.The control group 19 patients submitted to CA with no PHDF.Isotonic saline (1mL/Kg/h in the 12 h previous and after CA) and N-acetylcistein (1200mg before and after CA) were administered to all patients.Fifteen patients required a central venous catheter (CVC),4 had an arteriovenous fistula.There was no statistically significant difference between case and control groups with respect to prevalence of diabetes(89% vs74%:p=0,2),incidence of moderate to severe cardiac dysfunction (63% vs 52%; p=0,7),admission plasma creatinine(Pcr)(2.08±0.58 mg/dL vs 2.23±0.49 mg/dL;p=0,8), dose of contrast evaluated by need of angioplasty 84%vs73%;p=0,43).Mean age was higher in the control group (72 ± 9.24 vs 65±10 years; p=0.01)

PCr was analyzed post-procedure days 3 to 5.AKI was defined according to Acute Kidney Injury Network criteria.The incidence of AKI was higher in the case group but did not have statistical significance (47% vs 68%; p=0,2).Average Pcr elevation was higher in the control group (0.36±0.3mg/dL vs 1.58±1.72 mg/dL; p=0.006).No patient in the case group needed further dialysis, 3 patients in the control group did, 1 needed 14 sessions and 2 needed 2.There were no complications related to the CVC.PHDF did not reduce the incidence of AKI,however the smaller elevations of Pcr and absence of need for further dialysis,suggest less severe forms of AKI. Since our patients had only moderate renal dysfunction on admission we cannot exclude benefit in more severe CKD.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO436

**Predictors of Post-Hospital Discharge Outcomes in Patients with Acute Renal Failure** Lawand A. Saadulla, Christin M. Spatz, Nasrollah Ghahramani. *Hershey, Penn State University/Hershey Med Center, Hershey, PA.*

**Introduction:** Predictors of post-hospital discharge outcome among patients with acute renal failure (ARF) requiring renal replacement therapy (RRT) are not well known. We investigated potential predicting variables among a group of patients at our institution.

**Methods:** Between January 2004 and December 2005, 898 adult patients were diagnosed with ARF, of whom 278 required RRT. Post-hospital discharge follow-up data (for at least one year; average: 50.5 ± 17.4 months) was available on 35 patients. Twenty-one had been on continuous renal replacement therapy (CRRT) and 14 on hemodialysis (HD). Six of the CRRT group and 9 of the HD group were dialysis dependent at time of discharge (DC). One patient in each group died in follow-up. Ten of the CRRT group and 11 of the HD group progressed to chronic kidney disease (CKD). Among this group, 5 from the CRRT group and 6 from the HD group developed end stage renal disease (ESRD). Multivariate regression analysis was used to examine the association between outcomes (CKD, ESRD, death, complete recovery of renal function) and independent variables: age, treatment group, sepsis, acidosis, Sequential Organ Failure Assessment (SOFA) score, dialysis-dependence at DC, and creatinine at DC.

**Results:** Progression to CKD and to ESRD were both associated with dialysis dependence at time of discharge (r= 0.354; p= 0.037 and r=0.53; p= 0.001, respectively). Post-discharge mortality was associated with sepsis at time of ARF (r=0.603; p=0.0001), while complete recovery of renal function was negatively associated with age (r=-0.32; p=0.008) and SOFA score (r= -0.4; p=0.005).

**Conclusions**: Among patients with ARF, dialysis-dependence at time of discharge is a strong predictor of progression to CKD and ESRD. Younger patients with ARF and those with lower SOFA scores are more likely to have complete recovery of renal function in follow-up.

Disclosure of Financial Relationships: nothing to disclose

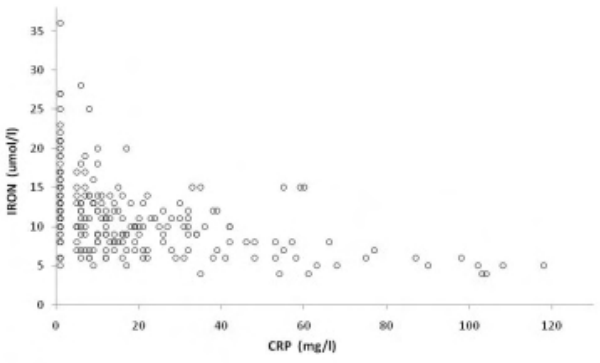
### TH-PO437

**Determinants of Circulating Iron in Haemodialysis Patients** Damien Ashby,[1] Georgina Henrietta Aldous,[1] Mark Busbridge,[2] Kevin G. Murphy,[2] Stephen R. Bloom,[2] David Taube,[1] Tom Cairns,[1] Neill D. Duncan,[1] Frederick W. K. Tam,[1] Peter Choi.[1] [1]*Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom;* [2]*Investigative Medicine, Imperial College, London, United Kingdom.*

Erythropoiesis is dependent on efficient transport of iron to the bone marrow, and low levels of circulating iron are associated with anaemia in renal patients. The hepatic peptide hepcidin limits iron transport, and elevated levels in renal failure are thought to restrict iron availability.

Plasma iron and related parameters were measured in a stable group of haemodialysis patients. Hepcidin was measured by radioimmunoassay.

In 100 haemodialysis patients (66 male, aged 25-87) mean+/-sd plasma iron was 11.4+/-4.6umol/l, and correlated negatively with haemoglobin (R=0.272, p<0.001). By multiple linear regression, plasma iron was independently predicted by TIBC (beta=0.405, p<0.001), CRP (beta=-0.367, p<0.001, figure 1), reticulocytes (beta=-0.293, p<0.001), ferritin (beta=0.254, p<0.001) and hepcidin (beta=-0.132, p=0.03).




Only baseline hepcidin was predictive of the change in plasma iron over the next month (R=-0.151, p=0.05)

Plasma iron levels are influenced by multiple factors, including iron stores, bone marrow activity and inflammatory state. High hepcidin levels are associated with subsequent deterioration in iron availability, suggesting a role in the pathogenesis of anaemia and erythropoietin resistance in haemodialysis patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO438

**Comparison of Therapeutic Efficacy of Epoetin alfa vs. Epoetin beta in Haemodialysis Patients** Alice Loughnan, Galil Rahman Ali, Sumith C. Abeygunasekara. *Nephrology Department, Broomfield Hospital, Broomfield, United Kingdom.*

PURPOSE: Evaluate the therapeutic efficacy of epoetin alfa compared to epoetin beta in maintenance haemodialysis patients.

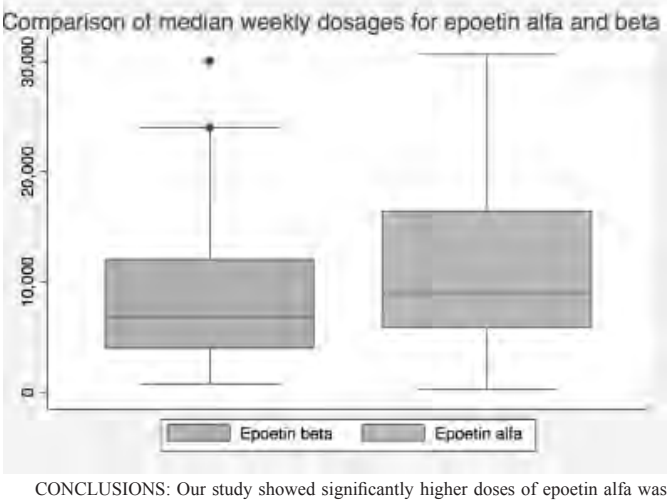
METHOD: In May 2009, the epoetin product for all dialysis patients in our unit changed from epoetin beta to epoetin alfa. Current evidence suggests equal efficacy at the same dose. We compared epoetin dosage required to maintain target haemoglobin in the same group of patients.

Patients selected for the study were all those who had their haemoglobin maintained at 11-12.5g/dl by haemodialysis and epoetin. Iron levels were monitored and ferritin kept within range of 200-500 g/l. In all patients, we examined haemoglobin concentration, mean dose of epoetin per week and EPO index (weekly epoetin dose/ mean monthly HCT).

Data excluded was; conversion month, and if within the study period patients developed signs of infection, bleeding, required blood transfusion, under dialysis or required hospital admission.

Values are stated as mean (standard deviation) or median (inter quartile range) where appropriate. The data for each patient before and after regimen change was compared using paired t-test or Wilcoxons signed rank sum test as appropriate (Stata corp).

RESULTS: Out of 128 patients, 79 were eligible. The mean (SD) for haematocrit concentration achieved before and after epoetin change was 0.343 (0.312) % and 0.348(0.241) % respectively (paired T test p=0.1504). Weekly epoetin doses increased from 6733.333 (4000- 12000) IU/wk to 9000(5833.333- 16375) IU/wk (p=0.0001). EPO index similarly increased from 20465.19 (12549.12- 33389.63) IU/wk/% to 27073 (15086.55-46875)IU/wk/% for epoetin beta and epoetin alfa respectively (p=0.0003).




CONCLUSIONS: Our study showed significantly higher doses of epoetin alfa was needed to maintain target haemoglobin. We recommend a prospective randomised control trial to compare the two products.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO439**

**Comparison of Efficacy and Safety for Anemia Management between Darbepoetin alpha and Epoetin beta for Chronic Hemodialysis Patients**
Tadashi Kuji,[1] Gen Yasuda.[2] *[1]Yokodai Central Clinic; [2]2nd Internal Medicine, Yokohama City University, Yokohama, Kanagawa, Japan.*

**Introduction and Aims**: Erythropoiesis-stimulating agents (ESAs) are effective in the management for anemia of chronic kidney disease. Darbepoetin alpha is a longer half-life and unique biological activity ESA that can be administered to patients less frequently. The European equimoler conversion factor recommended 1 μg darbepoetin alpha corresponded to 200 IU epoetin beta. We performed a comparison of the efficacy of 1 μg darbepoetin alpha corresponded to 300 IU epoetin beta for chronic hemodialysis patients.

**Methods**: A total of 281 patients of intermittent hemodialysis were enrolled. One hundred twenty-five patients were for darbepoetin alpha and one hundred fifty-six were for epoetin beta. Hematcrit levels were checked once a week on Monday or Tuesday. When hematocrit levels were below 32%, ESAs were administered intravenously at the end of hemodialysis (On/Off method). One group was administered 30 μg of darbepoetin alpha once a week. Another were administered 3000 IU of epoetin beta in every hemodialysis, three times a week. CBC and blood chemistry were checked every other week for 44 weeks totally. Variabilities of hematocrit level weekly were calculated. **Results**: The weekly ratio of ESAs was 1 μg darbepoetin alpha corresponded to 289 IU epoetin beta. There are no differences from baseline CBC, blood chemistry and adequacy of hemodialysis between two groups. Hematocrit levels were well managed with ESAs. No patients need transfusion during this study. Hematocrit levels were 32.1±2.7% and 32.9±3.1% (p=NS) in darbepoetin alpha group and in epoetin beta group, respectively. There are no differences of variabilities of hematocrit level weekly (p=NS) and ESAs administrative weeks (p=NS) in both groups. **Conclusion**: In this study, 1 μg darbepoetin alpha could keep hematocrit level corresponded to 300 IU epoetin beta and no variability differences of weekly hematocrit in both agents. Darbepoetin alpha have benefits for treatment costs and nursing times by reducing frequency of administration for management of anemia for chronic hemodialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO440**

**Effects of the Difference in the Timing of Darbepoetin Alfa (DA) Administration on Cardiac Function and Endothelial Progenitor Cells in Hemodialysis (HD) Patients**
Shuzo Kobayashi,[1] Hidekazu Moriya,[1] Takayasu Ohtake,[1] Sumi Hidaka,[1] Eisei Noiri,[2] Yuko Shibuya.[3] *[1]Nephrology, Immnology, and Vascular Medicine, Shonan Kamakura General Hospital, Kamakura, Kanagawa, Japan; [2]Nephrology, Tokyo University, Tokyo, Japan; [3]Nephrology, NTT Nippon Kanto Teishin Hospital, Tokyo, Japan.*

**BACKGROUND** DA can treat anemia by once-weekly (QW) administration, while little is known about the differential effects of the timing of DA administration on cardiac function between the first and the last in a week. Endothelial progenitor cell cells (EPC) in circulating peripheral blood (CPB) which are mobilized by erythropoietin stimulating agent (ESA) are known to be related with cardiovascular events.

**METHODS** We conducted prospective, randomized, open-label, multicenter trial for 48 weeks including 61 maintenance HD patients. Patients with well controlled Hb levels by rHuEPO were switched to DA administration once-weekly, and the timing was divided into two groups: DA was administered on the first dialysis day in a week, Group A (n=34) or on the last dialysis day, Group B (n=27). CD34-positive cells, a population that includes EPC in CPB were analyzed by flowcytometry.

**RESULTS** There was no significant difference in the patients' demographic characteristics including pre-HD BPs, Hb levels, and dosage of DA throughout 48 weeks. At 24 weeks, the changes in LVMI before and after DA administration showed statistically significant difference between two groups (+8.84 vs. -11.59 g/m$^2$ in Group A and Group B, respectively:P<0.05). EF (%) was also significantly increased from 63±8.8 to 66±10 (P<0.05) in Group B, while it was not significantly changed in Group A. At 48 weeks, there was a significant difference in the changes of EF compared with values before and after DA treatment (-4.03 vs. +1.65 % in Group A and Group B, respectively: P<0.005). The number of CD34$^+$ cells at 48 weeks after DA was higher in Group B than that in Group A (Median: 0.495/μl vs. 0.890/μl).

**CONCLUSIONS** Administering DA on the last dialysis day in a week significantly improved LVMI and %EF, compared with those on the first dialysis day. The underlying mechanisms may suggest the difference of EPC-mobilizing effects of ESA.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO441**

**Switch of ESA Therapy from CERA to Darbepoetin-alpha in Chronic Hemodialysis Patients: A Multicenter Experience**
Pascal Meier. *Nephrology, CHCVs, Sion, Switzerland.*

CERA, a continuous erythropoietin receptor activator and darbepoetin-alpha (DA), are approved for the treatment of anemia in patients with end-stage renal disease (ESRD). The molecular differences of both erythropoiesis-stimulating agents (ESAs) may enable a reduced dosing frequency as well as different treatment dosage. We aimed to assess the impact of a switch from CERA to DA in unselected chronic hemodialysis (HD) patients on change in hemoglobin (Hb), the proportion of patients reaching target guideline levels and on the monthly requirement of medication. Twenty-one HD patients from three dialysis centers were eligible and were switched from CERA iv (QM) to DA iv (QW, Q2W or QM). As no data were published on the equimolar conversion from one to the other ESA, intra-individual evaluation was compared 12 weeks before with 12 weeks after the switch. The dose of the ESA and the Hb levels were analyzed for the whole period. During treatment with CERA, 66.7% of the 21 patients achieved an Hb level ≥ 110 g/L. After switching to DA, the mean Hb level increased significantly from 114±12.2 g/L to 119.5±15.9 g/L (p = 0.007) and the percentage of patients with Hb levels ≥ 110 g/L was 71.4% at 12 weeks. Furthermore, the weekly median required ESA doses was 38.5 mcg [10-100] for CERA and 30 mcg [10-130] for DA. This corresponded to a 28% increase of CERA to reach a median Hb level of 116 g/L. The overall cost decreased by 22% [17-29] after switching from CERA to DA (p = 0.02). After the switch from CERA to DA using an equimolar conversion rate, more patients reached an Hb level of ≥ 110 g/L despite a dose decrease of 22%. Previous data showed that after the reverse switch from DA to CERA according to the manufacturers guidance a significant drop in Hb level and an increased requirement for transfusions were observed. Thus the conversion rate for a switch from CERA to DA seems to be lower than the equimolar dose, and might be in the range of 1 mcg CERA to 0.7-0.9 mcg DA. The results of this pilot evaluation should be further substantiated by a larger data pool in order to be able to provide stronger dosing guidance for such a drug switch.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO442**

**Cost Savings after Implementation of an Anemia Management Protocol in a Hemodialysis Unit**
Emily Charlesworth,[1,2] Robert M. Richardson,[1,2] Marisa Battistella.[1,2] *[1]Nephrology, University Health Network, Toronto, ON, Canada; [2]University of Toronto, Toronto, ON, Canada.*

**Background:** National guidelines recommend the use of anemia management protocols to guide treatment and promote consistency. **Objectives**: We aimed to determine if an anemia management protocol would improve the proportion of hemodialysis patients within the target hemoglobin range and to determine whether within-patient hemoglobin (Hgb) variability would be reduced. Secondary objectives included whether the protocol would reduce the amount and cost of darbepoetin alfa (DBO) and intravenous iron (iv iron) used. **Methods:** An anemia management protocol was created and implemented in the hemodialysis unit at the Toronto General Hospital in December 2009. A retrospective observational review in 174 patients was conducted in two six-month blocks- one before and one after implementation of the anemia protocol. Data collection included baseline characteristics, DBO and iv iron doses and relevant lab parameters including hemoglobin, transferrin saturation and ferritin. Differences between means and proportions were analyzed using the two-sided paired t-test and the chi-square test respectively and were reported by the Fisher's exact test for significance. **Results:** The number of Hgb measurements in the target range of 110-125g/L increased from 44.3% to 46.0% (p=0.48) after protocol implementation. Hemoglobin variability decreased from 33% to 28% (p=0.29) after protocol implementation. The mean weekly dose of DBO was reduced from 34.56 ± 31.12mcg to 31.11 ± 28.64mcg post-protocol implementation (p=0.011), which translated to a cost savings of $41,649 over six months. The mean monthly iv iron dose also decreased from 139.56 ± 98.83mg pre-protocol to 97.65 ± 79.05mg post-protocol (p<0.005), a cost savings of $18,594 over the same time period. The percentage of patients with target ferritin and Tsat (200-500ug/L ; 20-50%) remained the same during the study period. **Conclusions:** Anemia management protocols may increase the number of patients in target and decrease variability in hemodialysis patients. These protocols do significantly reduce costs of anemia therapy in the hemodialysis population.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO443**

**Effect of Rapamycin on Peritoneal Dialysis Related Peritoneal Membrane Changes**
Vega Goedecke, Joon-Keun Park, Jan Menne, Marcus Hiss, Hermann G. Haller. *Nephrology and Hypertension, Medical School Hannover, Hannover, Germany.*

Peritoneal dialysis is a well established renal replacement therapy. One of its major limitations are the negative long-term effects of dialysis on the peritoneal membrane, which may subsequently even lead to technique failure. Dialysis-associated peritoneal changes are characterized by cell proliferation, inflammation and fibrosis. Since rapamycin is an immunosuppressive agent with antiproliferative properties, we tested its effect on peritoneal membrane changes during dialysis in an animal model.

We used C57BL/6J mice (n=12) and performed daily peritoneal dialysis for 4 weeks using twice daily intraperitoneal injections of a sterile pre-warmed dialysis solutions (Physioneal, 3,86%, Baxter). We used 75 ml/kg body weight, on average 1,5 ml, of dialysis fluid for each injection. A treatment group received rapamycin at a concentration of 1,5 mg/kg body weight mixed with the peritoneal dialysis fluid on every single day of treatment. Control groups received the equivalent amount of normal saline (0,9%), rapamycin only or no treatment, respectively. Peritoneal membrane thickness was significantly reduced with rapamycin mixed with dialysate fluid.

In the group treated with dialysate and rapamycin, CTGF expression was markedly reduced compared to dialysate alone. Rapamycin seems to ameliorate dialysis fluid related effects on the peritoneal membrane, such as thickening of the mesothelial layer, expression of CTGF and expression of α-smooth muscle actin in the mesothelial zone.

Rapamycin itself shows no negative effects on the peritoneal membrane.

These results demonstrate that rapamycin prevents the deleterious changes of peritoneal dialysis and suggests a potential therapeutic use of rapamycin for the prevention of dialysis related changes of the peritoneal membrane.

Disclosure of Financial Relationships: Research Funding: Wyeth.

**TH-PO444**

**Relation of N-Terminal pro-B-Type Natriuretic Peptide (NT-proBNP) and Left Atrial Volume Index to Left Ventricular Function in Chronic Hemodialysis (HD) Patients** Tetsuya Ogawa, Kosaku Nitta. *Department of Medicine, Kidney Center, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan.*

**Aim:** The left atrium volume (LAV) is associated with adverse cardiovascular (CV) outcomes in general population. The aim of the present study was to investigate the relation of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and LAV to left ventricular (LV) function in chronic HD patients.

**Methods:** A total of 117 patients (74 men and 43 women) were enrolled as subjects. Echocardiography was performed to evaluate the LAV index (LAVi) and left ventricular mass index (LVMI). Diastolic left ventricular function was estimated as E/E' by tissue doppler imaging with cardiac ultrasonography. Serum NT-proBNP was measured at the time of echocardiographic measurements.

**Results:** By univariate analysis, LAVi was positively associated with serum NT-proBNP levels (r=0.418, p<0.0001) and echocardiographic parameters including LVDd (r=0.489, p<0.0001), LVDs (r=0.375, p<0.0001), LVMI (r=0.538, p<0.0001), and E/E' (r=0.494, p<0.0001). Multiple regression analysis showed that LAVi (F=24.372, p<0.0001) and E/E' (F=23.473, p<0.0116) were significant predictors for serum NT-proBNP levels, and LVMI (F=46.807, p<0.0001) was a best predictor for LAVi among associated factors.

**Conclusion:** These findings suggest that both serum NT-proBNP and LAVi is a good biomarker for predicting the LV remodeling in chronic HD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO445**

**Plasma Gelsolin Is Associated with Inflammatory Biomarkers in Chronic Hemodialysis Patients** Georges Ouellet,[1, 2] Laura Rosales,[1] Viktoriya Kuntsevich,[2] Boris Medvedovsky,[2] Stephan Thijssen,[1, 2] Peter Kotanko,[1, 2] Nathan W. Levin.[1, 2] *[1]Renal Research Institute; [2]Beth Israel Medical Center, New York.*

Plasma gelsolin (pGSN) is an extracellular protein mainly secreted by striated muscle cells, which binds actin and buffers inflammatory mediators. pGSN depletion in acute injury is associated with a poorer prognosis. However, data are limited in situations of chronic inflammation. We aimed to characterize levels of pGSN in prevalent hemodialysis (HD) patients, and to test the hypothesis that pGSN level is associated with inflammatory biomarkers in this population.

Stable maintenance HD patients were recruited. Pre-HD pGSN was measured with the 2C4 Plasma Gelsolin ELISA kit (Critical Biologics, Cambridge, MA). C-reactive protein (CRP) and neutrophil-to-lymphocyte ratio (NLR) were obtained by routine laboratory techniques and interleukin-6 (IL-6) was measured with the Quantikine ELISA kit (R&D, Mpls, MN) at Spectra Clinical Research (Rockleigh, NJ). Univariate and multivariate linear regression analysis was performed with pGSN as the dependent variable.

We studied 153 patients (mean age 61±15 years; median dialysis vintage 2.3 (range 0.2-20.0) years; 52% male; 42% Blacks, 52% diabetic). pGSN distribution was skewed (median 6353 mU/mL, IQR 5244-7640). In univariate analysis, pGSN correlated inversely with CRP, IL-6 and NLR and positively with serum albumin (table 1). Age and CRP were significant predictors of pGSN in multivariate regression analysis with age, gender, race, access type, IL-6, CRP, serum albumin and NLR as independent variables.

Table 1. Pearson (r) and Spearman ($r_s$) correlations for pGSN and inflammatory markers

| | Log CRP | IL-6 | Serum albumin | Log NLR |
|---|---|---|---|---|
| pGSN | r=-0.316 P<0.001 | $r_s$=-0.317 P<0.001 | $r_s$=0.247 P=0.001 | r=-0.228 P=0.014 |

In stable prevalent HD patients, pGSN is inversely associated with CRP, IL-6 and NLR, and directly associated with serum albumin. Our data suggest that markers of inflammation influence pGSN and are thus consistent with current knowledge in situations of acute inflammation. Whether lower pGSN is a predictor of mortality in prevalent HD patients remains to be determined.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO446**

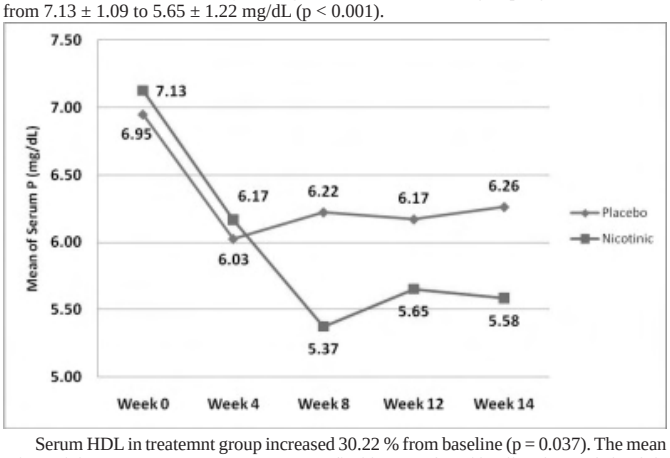
**Effectiveness and Safety of Extended-Release Nicotinic Acid for the Reduction of Serum Phosphorus Level in Maintenance Hemodialysis Patients** Ouppatham Supasyndh,[1] Rachanon Srisawadwong,[2] Pornanong Aramwit.[2] *[1]Nephrology, Phramongkutklao Hospital and College of Medicine, Bangkok, Thailand; [2]Pharmacy Practice, Chulalongkorn University, Bangkok, Thailand.*

**Background:** Inadequate control of serum phosphorus (P) in dialysis patient leads to elevation of the calcium-phosphate product. This plays a pivotal role in vascular calcification, cardiovascular disease and death. Experimental and clinical studies found that nicotinic acid and its metabolite nicotinamide decrease P level in dialysis patients by inhibition of the Na-Pi-2b sodium-dependent phosphate co-transporter in the GI tract.

**Objective:** To evaluate the effectiveness and safety of extended-release (ER) nicotinic acid for the reduction of serum phosphorus level in dialysis patients.

**Method:** A randomized placebo- controlled trial was conducted for 18 weeks. The 28 hemodialysis patients with serum P > 5.5 mg/dL after 4 weeks of diet control period were randomized to receive once daily ER nicotinic acid at bedtime for 12 weeks. The initial daily dose of ER nicotinic acid was 375 mg and titrated once weekly to 500, 750 and 1,000 mg, as tolerated. Control group was received placebo in the same manner. All patients still received standard P binder. Blood chemistries were tested every 4 weeks. Data was analyzed using the intention-to-treat principle.

**Result:** At the end of study, average serum P of treatment group significant reduced from 7.13 ± 1.09 to 5.65 ± 1.22 mg/dL (p < 0.001).




Serum HDL in treatemnt group increased 30.22 % from baseline (p = 0.037). The mean tolerated dose was 723.21 ± 302.95 mg. Hot flushing was found in all patients of nicotinic acid group. Common GI adverse effects and dizziness were found in both groups.

**Conclusion:** We can conclude that extended-release nicotinic acid is effective and safe in reducing serum phosphorus as add-on standard therapy in hemodialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO447**

**Effect of Glucocorticoid on Phenotypic Transition of Human Peritoneal Mesothelial Cells and Fibroblasts** Yang Hee Jang, Eun Sun Ryu, Mina Yu, Dong-Ryeol Ryu, Seung-Jung Kim, Kyu Bok Choi, Duk-Hee Kang. *Nephrology, Ewha Womans University, School of Medicine, Seoul, Korea.*

There were several reports demonstrating that glucocorticoid restored the deterioration of peritoneal water transport and protected the peritoneum from the development of encapsulating peritoneal sclerosis in animal model of PD. On the other hand, glucocorticoid was known to activate TGF-β and other profibrotic proteins in hepatocytes. In this study, we investigated the effects of glucocorticoid on TGF-β-induced EMT in HPMCs. EMT was evaluated by morphological changes and the expressions of E-cadherin and α-SMA after stimulation of TGF-β with or without pretreatment of dexamethasone (1-10 μM) or hydrocortisone (1-10 μM). To examine the effect of steroid on TGF-β-induced cell signaling pathway, phosphorylation of ERK1/2 and p38 MAPK were also assessed. We also examined the effect of glucocorticoid on mesenchymal-to-epithelial transition (MET) of human peritoneal fibroblast and TGF-β-treated HPMCs. TGF-β (>1 ng/ml) induced a morphological transformation from cuboidal and cobble stone appearance to spindle shaped scattered fibroblast-like cells at 48hrs, which was inhibited by pre-treatment of dexamethasone (5 μM) and hydrocortisone (10 μM). TGF-β down-regulated E-cadherin and up-regulated α-SMA expression at 48 hrs, which were significantly attenuated by pre-treatment with dexamethasone (5 μM) or hydrocortisone (10 μM). Furthermore, TGF-β-induced morphological changes and the expression of E-cadherin/α-SMA were reversible with a removal of TGF-β and subsequent exposure to dexamethasone or hydrocortisone, suggesting the reversal of TGF-β-induced EMT by glucocortocoid even after the development of morphologic changes. TGF-β significantly increased phosphorylation ERK1/2 and p38 MAPK in HPMCs from 5 minutes of stimulation, which was also blocked by dexamethasone or hydrocortisone. Neither dexamethasone nor hydrocortisone induced MET of human peritoneal fibroblasts.

In conclusion, glucocorticoid prevented or reversed TGF-β induced EMT in HPMCs. These results suggest glucocorticoid may be one of the possible therapeutic strategies to prevent peritoneal fibrosis in PD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO448**

**Continuous Glucose Monitoring in Insulin Treated Type 2-Diabetic Hemodialysis Patients** Katsushige Abe,[1,2] Rie Abe,[1,2] Nobuyuki Abe,[2] Kenichi Saruwatari.[1] *[1]Renal Medicine, Jinikai Hospital, Oita, Japan; [2]Internal Medicine, Abe Diabetic Clinic, Oita, Japan.*

**Background:** Diabetes mellitus is the most common cause of end-stage renal disease (ESRD) in Japan. The diabetic patient with ESRD exhibits greater fluctuations in glucose due to decreased kidney function and to dialytic therapies and has greater cardiovascular problems. The aim of this study was to analyze, with the use of 4 day continuous glucose monitoring system (CGMS), the influence of dialysis therapy on daily glucose profile in insulin treated diabetic patients with hemodialysis therapy.

**Methods:** Twenty insulin treated type 2 diabetic patients undergoing maintenance hemodialysis were evaluated. CGMS was performed to examine the difference in glycemic variability during the dialysis in 2 days with and 2 days without dialysis.

**Results:** The average insulin requirement was 13±7 IU/day in the day with dialysis and 15±7 IU/day in the day without dialysis. 24-h mean glucose values and their SDs were significantly higher during the day with dialysis than without dialysis. During the dialysis, glucose concentration decreased and return to 100-150mg/dl at the end of dialysis. The incidence of hypoglycemia (<70 mg/dl) and hyperglycemia (>180mg/dl) were significantly higher in the day with dialysis.

**Conclusion:** Glucose values were significantly higher and more glucose variability was observed on dialysis days in insulin treated dialysis patients. CGM could be a useful tool to evaluate glycemic control in dialysis patients and it should be used for medication adjustments around dialysis days to optimize glycemic control and avoid hypoglycemia.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO449

**Effect of Hemodialysis on microRNA Expression in Serum of Uremia** Hong Ye, Ping Wen, Huiqin Zhong, Lei Jiang, Junwei Yang. *Center for Kidney Disease, the 2nd Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China.*

**Purpose:** Hemodialysis is the main theray strategy of ESRD patients, which has many complications, such as cardiovascular diseases and metabolic bone disease. However, the mechanisms of these complications are not completely elucidated. During the hemodialysis, with the eliminating of toxins, internal environment of the patients may change. The effects of hemodialysis on the patients' metabolisms have not been evaluated. Recently, the development of Systematic Biology provide us a new approach to explore new molecules. MicroRNA is a class of endogenous ~22nt small non-coding RNA, which are important regulators of cell differentiation, proliferation, mobility, and apoptosis. It has been shown that microRNA play important roles in many diseases such as tumor. Since it can not be cleared by hemodialysis, this study aims to observe the expression microRNA before and after hemodialysis.

**Method:** Twenty patients undergoing routine hemodilysis therapy are choosen for objects. Blood samples before and after hemodialysis are collected for microarry analysis.

**Results:** The microRNA spectrum of ESRD patients are significantly different in plasma and blood cell. After hemodialysis therapy, unique peak of some microRNA was observed and some microRNAs can not be detected.

**Conclusions:** MicroRNA expression can be affected by hemodialysis, the new microRNAs secreted after hemodialysis may relate to the metabolisms change in ESRD patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO450

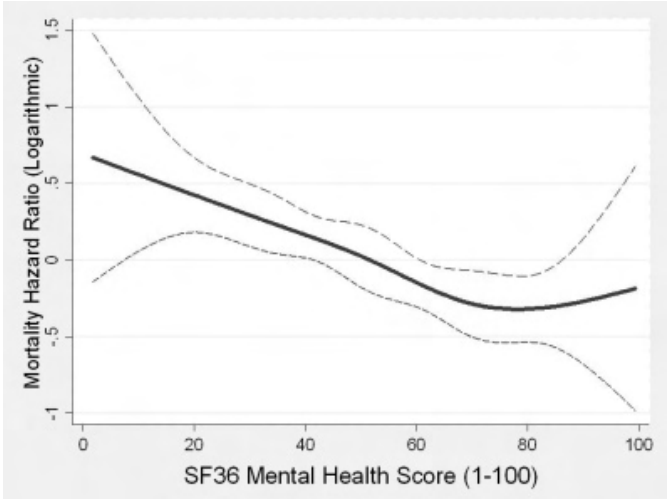
**Mental Health Score of SF-36 Quality of Life and 5-Year Mortality in Maintenance Hemodialysis Patients** Usama Feroze,[1] Ramanath B. Dukkipati,[1] Deborah A. Benner,[2] Allen R. Nissenson,[2] Csaba P. Kovesdy,[3] Joel D. Kopple,[1] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]DaVita, Lakewood, CO; [3]Salem VA Medical Center, Salem, VA.*

Hemodialysis (HD) patients (pts) have a high mortality and often suffer from mental health (MH) derangements. The We hypothesized that MH is a predictor of survival in HD pts. Mortality predictability of MH, assessed via SF36 questionnaires quality of life was examined in 705 HD pts over 2001-07 period using Cox models and cubic splines. HD patients age across the above 4 increasing (improving) MH score quartiles were 55.2±13.2, 53.6 ±14.4, 52.5 ±15.1, 52.7 ±16 yrs old. Fully adjusted death hazard ratio (HR) (and 95%CI) for the lowest (worse) compared to the highest (best) MH quartile showed 67% higher death risk: HR 1.67(1.14-2.41). Cubic spline graph confirmed the almost linear association:

Death HR of quartiles of SF36 MH score in 705 HD pts

| Mental Health Quartiles | T1 (n=177) HR(%CI) | T2 (n=176) HR(%CI) | T3 (n=177) HR(%CI) | Trend p |
|---|---|---|---|---|
| Unadjusted | 1.8(1.2-2.5) P=0.001 | 1.6(1.1-2.3) P=0.008 | 1.1(0.79-1.63) P=0.5 | 0.000 |
| Case-mix* | 1.5(1.06-2.1) P=0.02 | 1.5(1.05-2.12) P=0.02 | 1.08(0.75-1.56)P=0.68 | 0.005 |
| Previous + MICS# | 1.63(1.13-2.3) P=0.009 | 1.52(1.05-2.2) P=0.026 | 0.97(0.64-1.4)P=0.87 | 0.001 |
| Previous + inflammation†(full mode) | 1.62(1.12-2.34) P=0.01 | 1.66(1.14-2.4) P=0.008 | 0.99(0.66-1.5)P=0.95 | 0.001 |

Reference group: 4th (best) MH score quartile



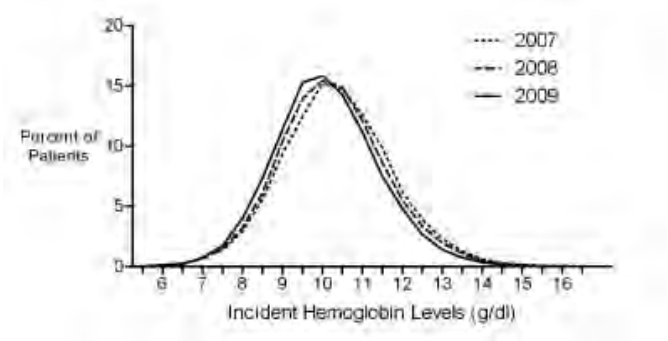

Hence, better MH appears associated with greater survival in HD pts.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO451

**The Effect of Pre-ESRD Care on Incident Patient Outcomes** Joe Weldon, Karen M. Spach, Mahesh Krishnan. *DaVita, Denver, CO.*

Starting in 2007, significant changes to ESA product labeling and new data have decreased ESA use in the pre-dialysis setting. However, this has had an unintended effect on outcomes for incident ESRD patients. MEDCAC recently discussed lower Hb targets in CKD.We sought to understand how anemia management in pre-ESRD has changed and to quantitate the impact that such potential revisions in payment policy may have on ESRD outcomes. Patients receiving ≥12 ICHD treatments and no other modality treatments in the 1st 30 days of dialysis were identified in a large US dialysis provider's database. We performed an analysis using Hb results in both the first 30 and next 30 days (n=29,969). Initial Hb levels were grouped into 0.5g/dL increments and charted over time, and a matched analysis for Hb increment to first month's ESA dose was completed. Initial Hb levels and ESA requirements were compared by Chi square test. The % of patients entering dialysis with Hb levels of <10 g/dL has increased in the past 3 years; 32.6% in 2007, 36.1% in 2008 and 40.8% in 2009, p<0.0001 between each set of years (Figure).




ESA requirements in the 1st month (ranging from a median 30 day ESA dose of 0 U to 176,000 U) correlated with incident Hb (from <6.5 mg/dL to 16 mg/dL). The dose prescribed to those patients has also declined year over year for the same incident Hb increment. The changes to pre ESRD-related ESA use in the last 3 years have had a predictable decline in incident Hb outcomes for patients new to dialysis. This downward trend in progressively lower Hb is likely to continue given the current controversies in ESA use. The amount of ESA required in the 1st month of dialysis was proportional to initial Hb on dialysis with lower Hbs requiring higher doses. Over time both first hemoglobin on dialysis and 1st month ESA dose have decreased. Significant changes in pre-ESRD ESA coverage may have an effect on anemia outcomes for new ESRD patients.

Disclosure of Financial Relationships: Employer: DaVita Inc.

## TH-PO452

**Long-Term Tolerance and Efficacy of Cinacalcet for the Treatment of Secondary Hyperparathyroidism (SHPT) in Peritoneal Dialysis Patients** Jesus Montenegro, José Ignacio Cornago, Maria Isabel Gallardo, Paula García, Ainhoa Hernando, Isabel Martínez, Rosa Muñoz. *Hospital de Galdakao-Usansolo, Galdakao, Spain.*

Purpose: SHPT is relatively uncommon in PD patients, generally because of positive peritoneal Ca balances and the fact that most patients are controlled with regular measures: phosphorous-chelating agents and vitamin D. A small number of patients, however, due to inefficacy of or intolerance to classic treatment, have to resort to cinacalcet treatment.

Methods: We describe our experience with cinacalcet in PD patients with SHPT.

Results: Since it was approved, of the 129 patients treated with CAPD in the last few years, 12 received Cinacalcet for 12 months to maintain PTH at over 300 pg/ml; all of them were resistant to or did not tolerate standard treatment. CKD etiology was normal and time in CAPD was 24 months, with a mean age of 60 years and 40% women. The Ca of the bath was 7 mg/dl. Before starting cinacalcet and after 1, 3, 6, 9 and 12 months, we collected: PTH, Calcium, Ca++, P, alkaline phosphatase, GOT, GPT and albumin. PCR-n, Kt/V of urea, residual renal function and peritoneal Ca and P balances were calculated.

| | 0 months | 1 month | 6 months | 12 months |
|---|---|---|---|---|
| PTH pg/ml | 509±158 | 287±164* | 174±108* | 199±91* |
| Total Ca mg/dl | 9.66±0.41 | 9.06±0.67* | 9.48±0.73* | 9.30±0.52* |
| Ca++ mg/dl | 4.98±0.33 | 4.64±0.29* | 4.89±0.38 | 4.65±0.25* |
| P mg/dl | 5.63±1.62 | 5.37±1.25 | 4.86±1.03* | 5.40±1.28 |
| Mimpara mg/d | 0 | 30 | 30 (15-90) | 25 (15-60) |
| *=p<0.05 | | | | |

Most of them took calcitriol 0.25 mcg/d and a few were taking oral paricalcitol 1 mg/d. The most common P-chelating agent was Ca salts. Peritoneal Ca balances were usually positive, depending on the degree of ultrafiltration.

Conclusions: It can be concluded that cinacalcet is efficient in maintained reduction of PTH. Cinacalcet was well tolerated in the long term by most of the patients. There were no hypocalcemia episodes, possibly because of the positive Ca balances. Gastrointestinal symptoms were few and mild, related to the dose. Since cinacalcet became available, no patient resistant to classic SHPT treated has needed a parathyroidectomy.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO453

**Clinical Advantages of Acetate Free Dialysate Containing Citrate** Takahiro Kuragano, Takeshi Nakanishi. *Hyogo College of Medicine, Department of Internal Medicine, Division of Kidney and Dialysis, Nishinomiya, Hyogo, Japan.*

Background: Dialysate for hemodialysis (HD) treatment had inevitably contained acetate as a buffer. We evaluated clinical advantages of acetate free dialyzate containing citrate (AFCD) over acetate containing dialysate (AD) on acid-base balance, anemia, nutritional condition, and mineral and bone disease in HD patients. Method: 29 HD patients were treated with AD for 4 month (first AD) switched AFCD for 4 month (AFCD), and returned to AD for next 4 month (second AD). Result: HCO3- levels did not changed in the patients with normal HCO3- (≥ 20mEq/L). While in the patients with low HCO3-, it was significantly increased in AFCD. In the patients with target hemoglobin (Hb) (≥10g/dL), Hb were maintained, even if dose of ESA decreased in AFCD. In the patients with low Hb, it was significantly increased in AFCD without increasing ESA and iron doses. In the patients with normal albumin (≥3.8g/dL), it did not change. While in the patients with lower albumin, it was significantly increased in AFCD. In the patients with normal parathyroid function (≥60pg/mL), intact-parathyroid hormone (int-PTH) did not changed. While in the patients with hypoparathyroidism, int-PTH were significantly increased in AFCD. These improvements of acidosis, anemia, nutrition, and bone turnover indexes in AFCD totally dissipated in the second AD.

table 1

| | First AD period | AFCD period | Second AD period |
|---|---|---|---|
| HCO3- (≥20mEq/L) | 22.4±2.4 | 23.2±1.8 | 20±1.2 |
| HCO3- (<20mEq/L) | 18.4±2 | 22.1±2.8* | 18.1±1.8 |
| Hb (≥10g/dL) | 10.8±0.4 | 10.7±0.3 | 10.9±0.4 |
| Hb (10<g/dL) | 9.2±0.2 | 10.4±0.3* | 10.1±0.2 |
| Albumin (≥3.8g/dL) | 4.0±0.2 | 3.9±0.3 | 3.8±0.2 |
| Albumin (<3.8g/dL) | 3.6±0.2 | 3.9±0.3* | 3.6±0.2 |
| Int-PTH (≥60pg/mL) | 132±22 | 151±18 | 136±22 |
| Int-PTH (<60pg/mL) | 38±3 | 58±3* | 40±2 |

*P<0.05: Compared with first AD period.

Conclusion: HD treatment with AFCD may improve the patients with intractable metabolic acidosis, hyporesponsiveness of ESA, malnutritional condition, and low turnover bone disease, which had not been normalized in the condition of HD using AD.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO454

**Phosphorus (PO4) Clearances Based on Blood (BFR) and Dialysate Flow Rates (DFR) during Thrice Weekly In-Center 8-Hour Hemodialysis (3x8HD)** Laura K. Troidle, Fredric O. Finkelstein. *Renal Research Institute, Metabolism Associates, New Haven, CT.*

PO4 levels are strong predictors of mortality in HD patients. Recently, we showed that PO4 is steadily removed during a 3x8HD (Troidle et al, HDI 13:487, 2009) using 400 mL/min BFR and 600 ml/min DFR. More traditionally, blood flows during 3x8HD are run at 200 to 250 cc/min. Thus, we wondered if the 400 BFR results in higher PO4 clearances than a 200 BFR. Patients in our 3x8HD who had a fistula/graft were invited to participate. All were dialyzed with the Fresenius180NR Optiflux dialyzer. Serum PO4 and creatinine samples were taken at treatment start and at 1,2,4,6, 8 hours, and, 30 minutes post treatment. Dialysate samples were obtained at 1,2,4,6 and 8 hours. The study was performed on each patient for two consecutive treatments using a 400 BFR at the first treatment and a 200 BFR at the second. PO4 clearances were obtained by using D/P x DFR where D/P is the dialysate to plasma PO4 ratio. 6 patients participated; 4 patients were male.

(A) Serum PO4 Levels (mg/dL) (B) PO4 Clearances (cc/min)

| **(A)** | PRE | 1H | 2H | 4H | 6H | 8H | POST |
|---|---|---|---|---|---|---|---|
| 400 BFR | 4.6 +/- 1.8 | 3.1 +/- 1.3 | 2.6 +/- 1.1 | 2.4 +/- 0.7 | 2.1 +/- 0.7 | 1.9 +/- 0.6 | 2.4 +/- 0.7 |
| 200 BFR | 4.4 +/- 2.4 | 3.3 +/- 1.9 | 3.5 +/- 1.9 | 3.2 +/- 1.3 | 2.9 +/- 1.2 | 2.8 +/- 1.3 | 3.1 +/- 1.5 |
| P VALUE | 0.55 | 0.79 | 0.07 | 0.09 | 0.05 | 0.02 | 0.10 |
| **(B)** 400 BFR | | 133 +/- 27 | 136 +/- 23 | 122 +/- 25 | 105 +/- 28 | 103 +/- 31 | |
| 200 BFR | | 96 +/- 31 | 99 +/- 31 | 101 +/- 35 | 90 +/- 33 | 88 +/- 40 | |
| P VALUE | | 0.03 | 0.002 | 0.04 | 0.13 | 0.16 | |

H=Hour

Table 1 shows serum PO4 levels and PO4 clearances at each time point. The data indicates significantly higher PO4 clearances using a 400 BFR during the first 4 hours; clearances remain higher at 6 and 8 hours, but are not statistically significant. Serum PO4 levels are lower after the 1st hour with the higher BFR but the reduction is not statistically significantly until 6 hours. Thus, it is important to maximize BFR during 3x8HD, particularly for the initial few hours of treatment to optimize PO4 removal.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO455

**Does Sevelamer Hydrochloride Affect Trace Element Levels in Chronic Kidney Disease Patients Treated by Regular Haemodialysis?** Kristin Vibeke Veighey, John William Booth, Andrew Davenport. *UCL Centre for Nephrology, Royal Free Hospital, London, United Kingdom.*

Introduction

Zinc, copper and selenium are essential micronutrients, incorporated into many metallo-enzymes and proteins involved in cell metabolism, production of neurotransmitters and regulatory enzymes controlling oxidative stress.

Previous reports have suggested that ion exchange resins can bind trace elements, and sevelamer in particular binds both copper and zinc, particularly at acid pH.

We decided to audit trace elements in our haemodialysis cohort, to determine whether those patients prescribed sevelamer hydrochloride had lower plasma levels of zinc and copper.

Methods

Trace elements were measured prior to the midweek haemodialysis session in 210 stable adult outpatients attending a University Hospital haemodialysis centre.

Results

Patient characteristics in those prescribed sevelamer hydrochloride vs not. (Patients taking oral zinc/intravenous trace element supplements excluded)

| | No sevelamer group | Sevelamer group |
|---|---|---|
| Number | 138 | 44 |
| Age years | 58.6 +/-1.6 | 58.9 +/-2.5 |
| % male | 53.6 | 52.3 |
| % diabetic | 35.5 | 43.2 |
| % caucasoid | 62.2* | 40.9 |
| vintage months | 28 (12-57) | 45.5 (20-82) |
| urine volume ml/day | 150 (0-915)* | 0 (0-197) |
| zinc umol/l | 11.6 +/-0.2 | 12.0 +/-0.03 |
| copper umol/l | 16.8 +/-0.4 | 16.7 +/-0.7 |
| selenium umol/l | 0.82 +/-0.02 | 0.91 +/-0.03 |
| bicarbonate mmol/l | 23.7 +/-0.3 | 23.2 +/-0.3 |
| chloride mmol/l | 100.2 +/-0.3 | 100.7 +/-0.2 |
| calcium mmol/l | 2.25 +/-0.02 | 2.26 +/-0.03 |
| magnesium mmol/l | 0.96 +/-0.01 | 0.98 +/-0.02 |

* p <0.05, ** p<0.01 vs Sevalemer group.

Conclusions

Although in-vitro studies have suggested that ion exchange resins, including sevelamer hydrochloride could bind trace elements and micronutrients, we found no difference in copper or zinc levels. Indeed rather than have micronutrient deficiency, the sevelamer group had significantly greater selenium values.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO456

**Pulverized Lanthanum Carbonate Hydrate Pulverized Administration: A Comparison of Serum Phosphorous Levels before and after Administration in Tube Feeding and Oral Feeding Hemodialysis Patients** Sato Megumi, Yuzuru Sato. *Nephrology, Satojunnkannkikanaika, Matsuyama City, Ehime, Japan.*

[Objectives]

Lanthanum carbonate hydrate(LC), a therapeutic agent for hyperphosphatemia, is basically chewed before swallowing. In this study, pulverized LC was administered to tube feeding patients and dental prosthesis patients who can't chew it. We investigated the effects on serum phosphate levels before and after administration.

[Methods]

Subjects were 7 oral feeding patients and 4 tube feeding hemodialysis patients with hyperphosphatemia in whom the dietary intake and drug intake could be confirmed. Pulverized LC was stirred into a small amount of water and administered either oral ingestion or by infusion immediately after food. We investigated the serum phosphate level, adjusted calcium level, sevelamer hydrochloride(SH) dose and calcium carbonate(CC) dose before administration, and three months and six months after administration.

[Results]

The LC dose was 750 mg/day in each person. The enteral nutrient of tube feeding subjects consisted of 1200 kcal/day of energy content and 600 mg/day of phosphorous content. In oral feeding patients, the energy intake was 1900 kcal/day and the phosphorus content was no more than 900 mg/day. The serum phosphate level decreased significantly

from an average of 6.5 ±1.3 mg/dl immediately before LC administration to an average of 5.1 ±0.4 mg/dl three months after administration (P<0.05). After six months, the serum phosphate level decreased significantly from 6.5 ±1.3 mg/dl before administration to an average of 5.1 ±0.4 (P<0.05). The adjusted calcium level wasn't affected before and after administration. The SH dose decreased from an average of 750 mg/day before LC administration to 273 mg/day three months after administration, and 136 mg/day six months after administration, and the CC dose was an average of 193 mg/day before LC administration, and zero after administration.

[Conclusion]

Among hemodialysis patients with hyperphosphatemia, we concluded that in tube feeding patients and in patients not able to chew due to dental prosthesis etc., serum phosphate control was made easier by the pulverized administration of LC.

Disclosure of Financial Relationships: Employer: Satojunnkannkikkanaika Dr.Yuzuru Sato PhD.

## TH-PO457

**Free Triiodothyronine Levels and Inflammation in US Chronic Hemodialysis Patients** Georges Ouellet,[1,2] Laura Rosales,[1] Stephan Thijssen,[1,2] Peter Kotanko,[1,2] Nathan W. Levin.[1,2] *[1]Renal Research Institute; [2]Beth Israel Medical Center, New York.*

A relationship between free triiodothyronine (fT3) and inflammation has been shown in Caucasian hemodialysis (HD) patients. We aimed to investigate this relationship further in an ethnically diverse US HD population.

Stable maintenance HD patients with no history of thyroid disease were recruited. fT3 was measured pre-HD by direct chemiluminescence (ADVIA Centaur, Deerfield, IL). C-reactive protein (CRP) and complete blood count were obtained by routine laboratory techniques; interleukin-6 (IL-6) was measured with the Quantikine ELISA kit (R&D, Mpls, MN). Univariate and multivariate linear regression models with fT3 as the dependent variable were constructed.

We studied 141 patients (age 61±15 years; median vintage 2.4 years (range 0.2-20.0); 55% male; 43% Blacks, 52% diabetic). fT3 levels were not normally distributed (median 2.4 pg/mL, range 1.7-3.5). fT3 decreased with age (r=-0.327, p<0.001). In univariate analysis, fT3 was negatively associated with CRP, neutrophil-lymphocyte ratio (NLR), epoetin resistance index (ERI) and directly associated with serum albumin. There was no relation to pre-HD body temperature. A negative correlation between fT3 and IL-6 was observed only in non-black patients ($r_s$=-0.221, P=0.042). Multivariate analysis including age, race, gender, CRP, NLR, IL-6, ERI and serum albumin, identified a set of independent predictors of fT3 (Table 1).

Table 1. Independent predictors of fT3 ($R^2$= 0.264; P<0.001)

| Variable | Coefficient | Standard error | Std. coefficient | P value |
|---|---|---|---|---|
| Age | -0.006 | 0.002 | -0.259 | 0.001 |
| Black race | -0.175 | 0.053 | -0.256 | 0.001 |
| NLR | -0.027 | 0.012 | -0.194 | 0.019 |
| IL-6 | -0.006 | 0.003 | -0.160 | 0.045 |
| Albumin | 0.163 | 0.071 | 0.177 | 0.023 |

In stable maintenance HD patients, we observed that low fT3 was associated with inflammatory markers, age and black race. Low fT3 has been reported to be associated with inflammation in Caucasian HD patients. Our results corroborate these findings in an ethnically diverse cohort of US HD patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO458

**Serum Lactate Amendment (SLC) in Hemodialysis Patients (HD) and Its Relationship with Intrahemodialysis Hypotension (HDHypo)** Emilio E. Gonzalez-Parra,[1] Pablo Justo Avila,[1] Beatriz Fernández,[1] Catalina Martin Cleary,[1] Secundino Cigarran,[2] Jesus Egido.[1] *[1]Nephrology, Fundacion Jimenez Diaz, Madrid, Spain; [2]Nephrology, Hospital de Burela, Lugo, Spain.*

sLC is an anaerobic oxidation carbohydrates intermediate. It has been associated with non oclusive mesenteric ischemia (NOMI) in HD. The incidence of NOMI is 1.04% of patients/yr and with high mortality. There have been no changes in the sLC in HD high-flow. HDHypo correlate with high rate of NOMI.

OBJECTIVES

1 .- Determine sLC baseline and its modification by HD, in the first HD of the week.

2 .- Observe the changes of the SLC at the beginning of HD in the intervening day.

3 .- Check if symptomatic hypotension arising fromsLc and increase that impact.

MATERIALS AND METHODS

18 patients (10 f., 8 m), age of 71.8 ± 14.55 (42 to 87 yrs), and hypotension usual events. We measured sLc (normal 5.7-22 mg / dl) and blood gas test before and after a HD, and before next HD. 12 patients had HDHypo, and 6 no. All with a conventional high flux HD. None of the patients had abdominal pain.

RESULTS

Hypotension intraHD was 1.05 (0-2), with a UF rate over the weight of 3.11 ± 0.9%. Basal sLc was higher than normal and it decreased with HD (20.94 vs 13.11), and increased before next HD (16.00). The CO3H HD increased (22.54 vs 28.71 mEq/L) with decreasing values in next HD (22.64 mEq/L). 4 cases sLC rose and 14 fell. No relationship between the number of HDHypo number and the UF rate with increased sLC. Basal sLC in first group was increased, and levels above baseline are manteined 48 hours later, whereas second group did not reach baseline values. Patients with sLC increases had HDHypo. The systolic blood pressure was higher in non sLc increased group (p<.0001). No correlation with vascular calcification.

CONCLUSIONS

1 .- The sLc values HD patients are hight before HD with a significant decrease after HD, which increases again before the next HD.

2 .- sLC increased in 4 of the 12 cases with symptomatic hypotension (33.33%), which do not regain baseline values at the beginning of the next HD, yet maintain acidosis.

3 .- 100% of patients with increase the sLC without abdominal pain had HDHypo, and correlate with postHD systolic blood pressure.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO459

**Sodium Thiosulfate Therapy for Calciphylaxis in Hemodialysis Patients** Debra Meade, Eduardo K. Lacson, Weiling Wang, Cindy A. Premo, Melanie Cousins, J. Michael Lazarus, Jeffrey L. Hymes. *Fresenius Medical Care, North America, Waltham, MA.*

We previously reported a case series of 16 HD patients diagnosed with calciphylaxis and treated with intravenous sodium thiosulfate (IV-ST) in Fresenius Medical Care North America (FMCNA) facilities in 2008. We extend the report to 53 patients initiating therapy by 6/30/09.

Calciphylaxis was diagnosed clinically, with 25 of 53 cases (47%) supported by skin biopsy. Most (70%) had lesions in the lower extremities and commonly affected areas included abdomen, buttocks, and hands. Initial therapy was increased phosphate binder dose in 62% (with 45% using non-calcium binders and/or lowering dialysate calcium), along with starting cincacalcet (58%), wound debridement (48%), stopping Vitamin D (34%) and parathyroidectomy (17%). At the start of IV-ST, mean age was 51.6±12.3 years, 72% female, 62% white, 60% with diabetes, and mean vintage 4.4±4.7 years. The median dose was 25g (100 ml) per treatment, given for 50±51 treatments (range: 2-201) over 176±200 days (range: 3-954). Eight patients (15%) reported initial nausea and vomiting but only 1 patient stopped therapy after 2 doses. One patient reported temporary bad taste/periorbital tingling and another questionable decreased hearing. In 39 of 53 patients (74%) skin lesions improved (10 with marked improvement and 13 had complete resolution). Ten (19%) remained on therapy as of 12/31/09 so mean values for 43 patients who completed therapy are shown:

| IV-ST Treatment | Sodium (mEq/L) | IDWG (kg) | Post-Wt (kg) | Calcium (mg/dL) | Phosphorus (mg/dL) | iPTH (pg/ml) | Anion Gap (mEq/L) | Bicarbonate (mEq/L) |
|---|---|---|---|---|---|---|---|---|
| Baseline | 138.9 | 3.1 | 95.8 | 8.7 | 6.1 | 512.9 | 18.0 | 22.8 |
| During | 139.2 | 3.1 | 94.2 | 8.6 | 5.7 | 515.8 | 21.2 | 22.2 |
| Stopped | 138.4 | 3.2 | 93.5 | 8.3* | 5.9 | 650.6 | 16.7** | 23.0 |

* p=0.002 compared to baseline and p=0.01 vs. during; ** p=0.005 compared to during – all else not significant; IDWG = Inter-dialytic Weight Gain, iPTH = Intact Parathyroid Hormone

Anion gap increased during IV-ST then declined after stopping (along with calcium).

Updated information from FMCNA physicians who volunteered clinical data on a larger cohort confirmed that therapy with IV-ST is well tolerated and effective for most HD patients with calciphylaxis. Although encouraging, findings are subject to reporting bias due to incomplete (33.5% of treated patients) data capture.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO460

**Isotopic Detection To Study Muscle Wasting in ESRD** Biruh Workeneh, William E. Mitch. *Nephrology Division, Baylor College of Medicine, Houston, TX.*

Clinically significant muscle wasting affects up to 70% of the millions of patients with advanced CKD and the process accelerates in patients with ESRD. Muscle wasting results in significant weakness, dependency and decreased quality of life. Unfortunately, muscle wasting is difficult to study in CKD patients because significant interstitial edema is often present making interpretation of traditional modalities (CT, DXA, MRI) difficult. This problem can be overcome by measuring radioactive isotopes naturally present in the body, namely potassium and nitrogen, which reflect body cell mass and muscle stores, respectively. Reconstruction of body composition from the elemental level is reliable because it minimizes assumptions related to tissue density, hydration and other factors compared with traditionally employed modalities. Methods: We conducted an observational study involving 5 adult subjects (3 men; 2 women) using 3 imaging modalities: 1) DXA scan; 2) TBK (total body potassium); and 3) TBN (total body nitrogen). Subjects were studied post-dialysis and studies were repeated again 6 months later. Results: The characteristics of these subjects including weight (73±13kg), BMI (26±4) or albumin (3.8±.3g/dL) provided no evidence that a significant loss of lean body mass was present. At baseline, 1 subject showed to have sufficiently excess body fat to be considered obese, which was identified by both DXA and TBK/TBN. In contrast, 2 subjects had significant depletion of muscle mass (-1.8 standard deviations from age/gender matched controls) identified by the TBK/TBN measurements that DXA analysis did not capture. Three out of the 4 subjects who underwent repeat testing showed significant losses in lean mass, which corresponded with significant reductions in total body potassium and nitrogen. Conclusions: Our findings are consistent with previous reports that show patients with ESRD suffer from accelerated loss is muscle mass. The degree of lost protein stores varies considerably among patients and high resolution techniques, like TBK/TBN, can be extremely useful in the study of muscle wasting and can complement other methods to assess in-vivo responses to potential interventions.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO461**

**Does Inflammation Independently Contribute to Malnutrition in Chronic HD Patients? A Longitudinal Study** Ilia Beberashvili,[1] Inna Sinuani,[1] Ada Azar,[2] Leonid Feldman,[1] Zhan Averbukh,[1] Joshua Weissgarten.[1] *[1]Nephrology, Assaf Harofeh Medical Center, Zerifin, Israel; [2]Nutrition Department, Assaf Harofeh Medical Center, Zerifin, Israel.*

The present report describes a prospective longitudinal study of interleulin-6 (IL-6) levels and nutritional parameters to determine whether high IL-6 levels play a decreasing role in the nutritional status in a cohort of prevalent clinically stable hemodialysis patients.

IL-6, dietary energy and protein intake, biochemical markers of nutrition and body composition (anthropometry and bioimpedance analysis) were measured at baseline and at 6, 12, 18 and 24 months following enrollment, in 96 prevalent hemodialysis patients. Observation of this cohort was continued over 2 additional years. In total, the study period extended 35±17 months. Changes in repeated measures were evaluated, with adjustment for baseline differences in age, sex, diabetes status, dialysis vintage and history of cardiovascular disease.

At baseline, no statistically significant differences in dietary protein or energy intake, laboratory nutritional markers and body composition parameters (anthropometry and BIA derived) between groups according to tertiles of baseline IL-6 were observed. Significant reductions of fat mass index (linear estimate: -0.1460±0.06 kg/m$^2$; p=0.017), fat free mass index (linear estimate: -0.1974±0.04 kg/m$^2$; p=0.0001) and phase angle (linear estimate: -0.1061±0.03$^0$; p=0.0001) with time were observed. A longitudinal decrease in body composition parameters was not influenced by IL-6 (IL-6-by-time interactions were not significant). Finally, cumulative incidences of survival were affected by the baseline serum IL-6 levels (p=0.006 by log-rank test).

Our results suggest that while IL-6 is a robust predictor of death, inflammation alone does not independently contribute to uremic anorexia or modify nutritional status in chronic HD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO462**

**Hormonal Regulation of Energy Homeostasis in Hemodialysis Patients: An Anorexigenic Profile That May Predispose to Adverse Cardiovascular Outcomes** Manish Suneja, John B. Stokes, Victoria S. Lim. *Department of Medicine, university of Iowa Hospital and Clinics, Iowa City, IA.*

To assess hormonal control of energy homeostasis in uremia, we profiled peptides around the circadian clock with a built-in perturbation of a 40-hr fast in 10 hemodialysis patients [HD] with no co-morbid illnesses and 8 normal controls (C). We measured energy and protein balance,quantitated appetite and body composition. Baseline leptin (ng/ml) showed a higher nocturnal peak in HD {15±4 (C) and 36±9 (HD), p=0.08}. Fasting led to rapid decline in both groups with similar disappearance rate constants, suggesting similar metabolic rates in both groups. On re-feeding, leptin tended to have a higher peak in HD patients. These results suggest that leptin in HD is higher because of greater secretion. Poly-peptide Y (PYY), a gut hormone, showed higher levels in the HD during the entire time of the study and showed little circadian variation. Acylated-ghrelin (AG), a orexigenic gut hormone, was only mildly lower in HD at baseline, but the differences became more prominent before lunch and supper after fasting as the HD patient lost the pre-meal spike. Alpha-MSH levels were not different between the two groups. Neuropeptide Y (NPY), showed higher levels in HD at baseline and stayed elevated at all times during the study. Nor-epinephrine (NE, pg/ml) levels tended to be higher in HD at all time points. NPY and PYY showed some removal by hemodialysis. Energy and protein balance as well as lean and fat mass were not different in the two groups. High leptin and PYY combined with a low ghrelin indicate an anorexigenic profile, yet HD patients had good appetite. These data demonstrate that HD patients with no co-morbid illnesses have good appetite and nutrition status. We hypothesize that impaired para-sympathetic function in HD patients could explain both the high PYY and the absence of anorexia. The hormone profile of high leptin, elevated PYY, and NPY may enhance vasoconstriction, the low ghrelin may impair vasodilation. The increased NPY and NE may reflect heightened sympathetic activity. All these abnormalities can potentially, predispose HD patients to adverse cardiovascular events.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO463**

**Thyroid Function in Japanese Patients with End-Stage Renal Disease Undergoing Hemodialysis** Toru Sanai,[1] Ken Okamura,[2] Hiroki Miyazaki,[1] Tomoya Kishi,[1] Motoaki Miyazono,[1] Koichi Node.[1] *[1]The Department of Cardiovascular and Renal Medicine, Faculty of Medicine, Saga University, Saga, Japan; [2]The Department of Medicine and Clinical Science, Kyushu University, Fukuoka, Japan.*

**Background/Methods.** Thyroid function was evaluated in 145 Japanese end-stage renal disease (ESRD) patients on hemodialysis comparing with the data of 158 control in different age group such as young (13-39 years), old (40-69) and very old (over 70). **Results.** Although free thyroxine ($fT_4$) was stable, age-dependent slight increase in thyroid-stimulating hormone (TSH) and decrease in free triiodothyronine ($fT_3$) were observed both in control and ESRD, especially in very old patients aged 70 or more. In each age group, fT4 and fT3 were lower and TSH was higher in ESRD with minimal change in $fT_3/fT_4$. Excluding the data of very old group and those with apparently abnormal TSH (undetectable or over 4.5 U/ml), reference values for ESRD (n=91) compared with control (n=143) were as follows; fT4 1.0 +/- 0.2 vs 1.2 +/- 0.2 ng/dl (P<0.05), fT3 2.3 +/- 0.4 vs 3.2 +/- 0.7 pg/ml (P<0.05), TSH 1.73 (0.55-5.45) vs 1.12 (0.31-4.65) mU/l (P<0.05), and fT3/fT4 2.4 +/- 0.6 vs 2.7 +/- 0.7 (not significant). Compared with these reference values, apparent thyroid dysfunction among 145 ESRD patients was suggested as follows; thyrotoxicosis in 2 (1.4%), latent hypothyroidism in 17 (11.6%), overt hypothyroidism 8 (5.6%) and low $T_3$ syndrome in 23 (15.9%) of the patients. **Conclusion.** Slight but significant abnormality in thyroid function was confirmed in ESRD patients. Decrease in both serum $fT_4$ and $fT_3$ levels with slight increase in serum TSH level was the characteristic abnormality in ESRD, which was quite different from typical non-thyroidal illness pattern or low $T_3$ syndrome.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO464**

**Hemodialyzer Design May Affect Iron and Anemia Parameters in Long-Term Hemodialysis Patients** Hiba Hamdan, Andrew I. Chin, Thomas A. Depner. *Internal Medicine, Division of Nephrology, University of California at Davis, Sacramento, CA.*

**Background**: We made a change in hemodialyzers within our community-based dialysis units. Improved rinse back with the new filters was noted by the clinical staff. We speculated that less blood was being lost and that anemia parameters may be improved. **Purpose**: To evaluate whether or not anemia parameters were improved upon switch to a new hemodialysis filter. **Methods**: A retrospective cohort review of anemia parameters in our dialysis clinic patients, comparing transferrin saturation (TSat%), ferritin, hemoglobin (Hgb), platelets, spKt/V, total dose of erythropoietin (EPO), and total dose of IV iron administered, between 4-month intervals before and after the switch in high-flux, re-use dialyzers. Paired t-test was used to compare 4 month average values, before and after the switch. **Results**: We evaluated 207 chronic hemodialysis patients, mean age was 57.7 years, 55% male. There was a statistically significant improvement in iron parameters (TSat & ferritin), pre-dialysis Hgb, and spKt/V after the switch.

Laboratory data before and after dialyzer change

| | Before dialyzer change | After dialyzer change | p value |
|---|---|---|---|
| TSat% | 22.9 ± 7.6 | 25.0 ± 9.5 | .009 |
| Ferritin | 691 ± 327 | 798 ± 358 | <0.001 |
| Hgb | 11.6 ± 0.8 | 11.7 ± 0.7 | 0.02 |
| Albumin | 3.8 ± 0.4 | 3.8 ± 0.4 | 0.3 |
| spKt/V | 1.52 ± 0.27 | 1.61 ± 0.23 | <0.001 |

There was no significant difference in EPO dose (units/kg/HD treatment), total administered IV iron, or heparin dose between the 2 time periods. Serum albumin also remained unchanged before and after the dialyzer change, suggesting that patients remained clinically stable between the time periods. **Conclusion**: Maintaining adequate iron stores is a key aspect of anemia management in the care of hemodialysis patients. We noted that a switch to a different hemodialyzer improved anemia parameters. Therefore, dialyzer design may have a significant impact on iron loss. Whether these noted differences are simply due to improved rinse back and hence lower blood loss, or if they are partially due to improved bio-compatibility of the new dialyzer, remains to be seen.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO465**

**Hemoglobin Variability in Peritoneal Dialysis Patients; a Comparison of Epoetin Beta and CERA** Sally Ann Fonseca, Maarten W. Taal, Richard J. Fluck, Georgina Chandler, Nicholas M. Selby. *Department of Renal Medicine, Royal Derby Hospital, Derby, United Kingdom.*

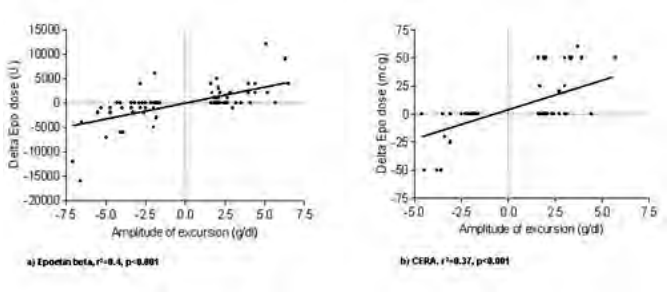
The extent to which hemoglobin (Hb) cycling occurs in peritoneal dialysis patients is unclear. It is also uncertain whether the half-life of erythropoietin stimulating agents (ESA) impacts on this. We therefore performed a retrospective cohort study of our peritoneal dialysis population before and after the entire programme was changed from epoetin beta (Neorecomon®) to CERA (Mircera®).

All patients treated with an ESA and peritoneal dialysis were screened for inclusion. Hb concentrations were measured monthly, each study period was 12mths. A significant Hb excursion was defined as a change in serum Hb of >1.5g/dl sustained for more than four weeks. Each complete Hb cycle consisted of two consecutive excursions in opposite directions.

A total of 79 patients were included (41 period one, 38 period two, 15 patients in both groups). There was a trend to fewer patients on CERA experiencing Hb excursions (26 patients, 68.4%) compared to those on epoetin beta (36 patients, 87.8%, p=0.054). There were also signficantly fewer dose changes in the CERA group (0.8 ± 0.7 per patient) as compared to epoetin beta (2.0 ± 1.7 per patient, p=0.0007). However there was no difference between the frequency of those with complete cycles.

Factors associated with Hb excursions were the number of dose changes, ESA dose, hospital admission and infection. There was a positive correlation between delta ESA dose and amplitude of Hb excursion, suggesting that the dose changes were causal, rather than reactive (see figure).

In conclusion, Hb cycling occurs in peritoneal dialysis patients and seems largely due to current practice in ESA dosing plus the effects of intercurrent illness. The longer half-life of CERA may offer a slight advantage in reducing Hb variability, possibly because of fewer dose changes per patient.

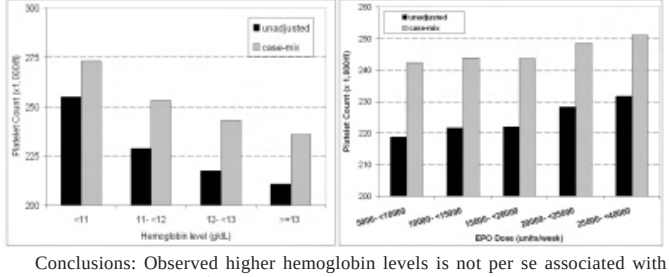



Disclosure of Financial Relationships: nothing to disclose

### TH-PO466

**Dissociation of Relative Thrombocytosis Associated with Higher ESA Dose vs. Hemoglobin Levels in Hemodialysis Patients** Elani Streja,[1] Csaba P. Kovesdy,[3] Lilia R. Lukowsky,[1] Ramanath B. Dukkipati,[1] Allen R. Nissenson,[2] Keith C. Norris,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]DaVita, Lakewood, CO; [3]Salem VA MC, Salem, VA; [4]Charles Drew University, Los Angeles, CA.*

Background: Recent randomized controlled trials indicated increased thromboembolic events and mortality upon targeting higher hemoglobin levels using higher doses of erythropoietin stimulating agents (ESA). It is not clear whether the high mortality is as a result of high ESA per se or high hemoglobin level. We, hence, examined the association of the latter 2 factors with relative thrombocytosis (increased platelet count), which is a predictor of increased thromboembolic events and death. Methods: Using linear regression models, we separately examined the associations between ESA dose and hemoglobin levels with13-week week (calendar quarter) averaged platelet count during July to Dec 2001 in a cohort of 40,697 maintenance hemodialysis (MHD) patients from in all DaVita clinics. Models were adjusted for case-mix. Results: MHD patients were 47%women; 46% diabetics; 34% African Americans, respectively. The 13-week averaged platelet count was 229 x103/µl. In unadjusted, and case-mix adjusted models, incrementally higher hemoglobin levels were associated with lower platelet count, whereas incrementally higher ESA doses were associated with higher platelet count (see Figures).




Conclusions: Observed higher hemoglobin levels is not per se associated with thrombocytosis, but only if associated with higher ESA dose or conditions that require higher ESA dose.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO467

**Conversion to Mircera in a UK Haemodialysis Unit: Effects on Haemoglobin Targets and Staff Time** Riaz Bavakunji,[1] Belinda Dring,[1] Amanda Pulfer,[2] Sheila Cooper,[2] Mark A. J. Devonald,[1] Simon Roe.[1] *[1]Nottingham University Hospital; [2]pH Associates.*

**Aims:** To study effects of converting IV epoetin alfa (Eprex) to IV Methoxy polyethylene glycol-epoetin beta (Mircera) with respect to achieving target haemoglobin (Hb), stability of Hb and nursing time required for drug administration in a UK satellite haemodialysis unit.

**Methods:** 60 patients receiving haemodialysis at a satellite unit were converted from Eprex to Mircera using a computerised decision support conversion algorithm and monitored for 12 months. Hb was measured monthly and percentage of patients within target (10.5-12.5 g/dL) recorded, along with mean Hb and Mircera dose. Mean times required for administration of Eprex and Mircera were calculated.

| Variables | Month -1 | Month 1 | Month 12 |
|---|---|---|---|
| Mean (+SD)Hb(g/dL) | 11.47(1.59) | 11.35(1.63) | 11.69(1.10) |
| Mean (95% CI) change in Hb(g/dL), from baseline | - | 0.15(-0.13-0.43) | 0.52(0.18-0.86) |
| Patients within target range, percent(n) | 81(35/43) | 71(41/60) | 86(25/29) |
| Mean ferritin( g/L) | 655 | 642 | 609 |
| Mean Mircera dose( g/month) | - | 150 | 200 |

**Results :** For those with complete data, 71%(41/60), and 86%(25/29) achieved target Hb of 10.5-12.5g/dL at 1 and 12 months respectively (Table 1). At 12 months 14% had Hb of <10.5g/dL and 28% >12.5g/dL. The initial drop in Hb recovered by 3 months (55-71% achieved Hb between 10.5-12.5g/dL from month 2 onwards. Mean dose of Mircera increased over 12 months from 150 to 200g/month. In any month post-conversion, an average of 69% patients (range 58-80%) did not require a change in dose of Mircera.

Mean nursing time required per patient for administration of Eprex was 3 min 14 sec(38min 48sec/patient/month based on a most common administration Eprex schedule of 4000 IU x3/week) compared with 3min 56sec/patient/month for Mircera. Seven patients attributed new, non-specific symptoms to the conversion and were changed back to Eprex.

**Conclusions:** Conversion to Mircera resulted in an initial dip in Hb followed by increase and stabilization with 86% of patients within target at 12 months. Nurse time saving of 34min 52sec/patient/month was calculated. 7/60 patients attributed non-specific symptoms to Mircera.

Disclosure of Financial Relationships: Other Relationship: Part of this study co-ordinated by pH Associates Ltd via an unconditional educational grant from Roche Products.

### TH-PO468

**Determining Maximum Clinically Effective Dose of Epoetin Alfa in ESRD Anemia Management** Adam Gaweda,[1] George R. Aronoff,[1] Alfred A. Jacobs,[1] Michael Brier.[2] *[1]Department of Medicine, University of Louisville, Louisville, KY; [2]Department of Veterans Affairs.*

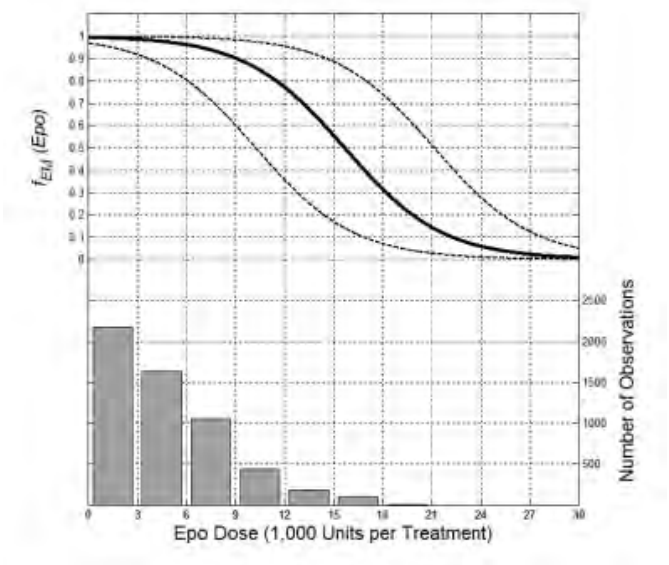
Secondary analysis of the CHOIR study indicates that increasing Hemoglobin (Hb) with excessive ESA doses may be harmful. Furthermore, the new Medicare reimbursement system provides an incentive to reduce the ESA dose. We performed a retrospective cohort study to answer the question: What is the maximum clinically effective ESA dose?

We studied a population of 209 ESRD patients treated at the Kidney Disease Program, University of Louisville, Louisville, KY, between 1996 and 2001, who received Epoetin alfa (Epo). We collected their monthly Hb and weekly Epo and estimated their erythropoietic sensitivity using the following model:

$\Delta Hb(k) = \Sigma^{N}_{i=1} \beta_i f_{EM}(Epo(k-i)) \Delta Epo(k-i)$

This model relates monthly change in Hb, $\Delta Hb(k)$, to past monthly changes in Epo dose, $\Delta Epo(k-i)$. $\beta_i$ represents erythropoietic sensitivity, $f_{EM}(Epo(k-i))$ represents the change in erythropoietic sensitivity with respect to the Epo dose per treatment received at month k-i. The inflection point of $f_{EM}$ represents 50% of the maximum erythropoietic sensitivity. We estimated this model using mixed effect nonlinear regression in MATLAB®.

Statistically, the best model incorporates three past Epo dose changes (N=3). The $\beta$ coefficients for this model are $\beta_1 = 0.28$, $\beta_2 = 0.13$, $\beta_3 = 0.09$ (all, $p < 0.001$). The mean inflection point for $f_{EM}$ is 15.7 ($p < 0.001$).




Top: Mean $f_{EM}$ (thick line) and its 95% Confidence Interval (dotted lines). Bottom: Data histogram.

The mean erythropoietic sensitivity decreases by 10% at 9,000, by 50% at 16,000, and by 90% at 22,000 Units Epo per treatment. The results of this study can be used by the physician to determine the stopping point for dose escalation when no clinically significant effect is expected.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO469

**Evaluating the Impact of Change in Pre-Dialysis Weight on a Change in Measured Hemoglobin (Hb)** Joe Weldon, Tracy Jack Mayne, Steven M. Wilson, Mahesh Krishnan. *DaVita Inc., Denver, CO.*

The literature has shown an association between low Hb, higher ESA doses and increased morbidity and mortality in dialysis patients, but many have questioned the role of confounding factors. Interdialytic weight gain is associated with increased morbidity and mortality. Increased volume status could produce spuriously low Hb measures via simple dilution, triggering ESA dose increases and confounding associations between both variables and outcomes. We examined the association between interdialytic weight gain, measured

Hb, and ESA dose. **Methods:** Retrospective analysis of 164866 hemodialysis patients dialyzed between January 1 and December 31 of 2009 at a large dialysis organization. Sequentially paired Hb values were created within a 30 day period. For each treatment corresponding to those dates, change in ESA and Hb were recorded and categorized by change in pre dialysis wt. Multiple observations per patient were used. **Results:** Prior mean Hb ranged from 11.2 to 11.7 g/dL and prior mean weekly ESA dose from 20k to 26k Units. Table 1 shows change in Hb and ESA dose by interdialytic weight gain category. To control for multiple measurements on many patients, the weighted Pearson product moment correlation was calculated on mean scores. The correlation was r = -0.17 between change in pre-dialytic weight and change in Hb, and r = 0.03 between change in pre-dialytic weight and change in ESA dose.

| Change in Pre-Dialytic Weight | Matched Hb Draws | Change in Hb (g/dl) Mean±SD | Change in Weekly ESA U from Prior Draw |
|---|---|---|---|
| >2.0 to 10.0 kg | 422,528 | -0.29±0.86 | 6.8% |
| 0.5 to ≤2.0 kg | 1,467,172 | -0.09±0.73 | 1.0% |
| >-0.5 to >0.5 kg | 1,544,905 | +0.07±0.70 | -1.3% |
| -0.5 to ≤-2.0 kg | 1,473,124 | +0.23±0.74 | -2.5% |
| <2.0 to -10.0 kg | 478,550 | +0.39±0.91 | 0.4% |

**Conclusions:** Changes in pre-dialysis weight were associated with reciprocal changes in both Hb and, in sequence, ESA dose. Given the magnitude of these effects, changes in pre-dialysis weight may be a clinically meaningful confounder of the relationship between Hb, ESA dose and morbidity & mortality in dialysis patients.

Disclosure of Financial Relationships: Employer: DaVita Inc.

## TH-PO470

### The Influence of CMV and EBV Serostatus on Haemoglobin (Hb) and Erythropoietin Stimulating Agent (ESA) Responsiveness in Patients with CKD Stage 5

Cherrise Baldeo,[2] Wael F. Hussein,[1] Mark D. Denton.[1] *[1]Renal Unit, Beaumont Hospital, Dublin, Ireland; [2]Royal College of Surgeons Ireland, Dublin, Ireland.*

**Background.** CMV seropositivity has been reported to be a major determinant of ESA resistance in patients with CKD. We wished to examine whether CMV or EBV serostatus influenced Hb and / or ESA dose in our population of CKD stage 5 patients.

**Methods** We collected data on CMV and EBV serology, age, sex and Hb on 1417 patients presenting for a renal transplant at our institution between 2000 to 2009. We examined whether CMV and EBV serostatus influenced Hb values pre transplantation. We next examined 117 patients on the transplant waiting list. CMV and EBV serostatus, Hb, ESA dose, iron studies, B12/folate, albumin, PTH, diabetes, vascular access type, and dialysis adequacy were collected. Multivariate analysis was performed to determine factors associated with increased ESA dosage.

**Results:** We found no effect of CMV or EBV serostatus on Hb or ESA dosage. Firstly, analysis of 1417 patients showed no difference in mean Hb between the various CMV / EBV serostatus groups. Analysis of variance showed that only increasing age and female sex were associated with lower haemoglobin values. Secondly, analysis of patients currently on the transplant pool also showed that there was no difference in mean Hb between the CMV or EBV groups. There was a trend for increased ESA dose in patients who were CMV seropositive but this was not significant. Analysis of a subpopulation of patients not on ESA also showed no difference in mean Hb between CMV / EBV groups. Analysis of haemodialysis patients alone, CMV positivity was associated with a higher mean Hb and a lower mean ESA dose. On multivariate analysis only low serum iron was associated with ESA resistance.

**Conclusion:** Our results contrast with those of a recent report linking ESA resistance to CMV seropositivity. Our study benefitted from a large population size and completeness of data collection. We found no association between CMV or EBV serostatus on Hb or ESA dosage. These conflicting results may be due to 1) differences in patient demographics (younger, and fewer diabetic patients) and 2) differences in target Hb and mean ESA dosage.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO471

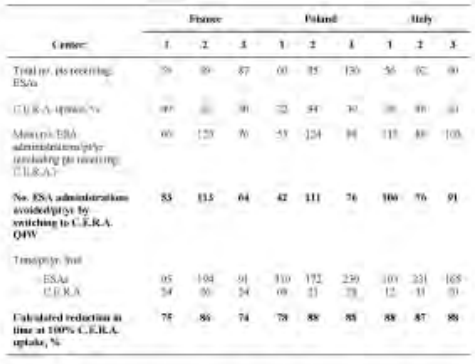
### Time Savings Associated with Once-Monthly (Q4w) C.E.R.A.: A Time and Motion Study Conducted in Dialysis Centers in France, Poland, and Italy

Wieslaw Klatko,[1] Giuseppe Villa,[2] Jean-Claude Glachant,[3] Erwin De Cock.[4] *[1]Nephrology Department, Specjalistyczny Szpital Wojewódzki, Ciechanów, Poland; [2]Fondazione Salvatore Maugeri, Pavia, Italy; [3]Service Néphrologie – Hémodialyse, Bourg en Bresse, France; [4]United Biosource Corporation.*

**Purpose:** Anemia management in CKD is time consuming for healthcare professionals and patients. The challenge for hemodialysis centers is to improve efficiency while maintaining high standards of quality of care. Simplifying anemia management may reduce resource usage, allowing healthcare staff to devote more time to other important CKD care needs. We report results from a multinational, multicenter time and motion study that documents healthcare personnel time savings for anemia management after the introduction of Q4W maintenance therapy with a continuous erythropoiesis receptor activator (C.E.R.A.) in hemodialysis centers in France, Poland, and Italy.

**Methods:** The time spent on frequent anemia management tasks (preparation and distribution, injection, and record-keeping) was timed by trained observers and recorded on case report forms. The time per patient per session was used to calculate the annual time per patient and per center.

**Results:** Key parameters that determined time for anemia management with traditional ESAs and C.E.R.A., and that impacted on time reductions with 100% C.E.R.A. uptake, are shown below.



| Center: | France 1 | France 2 | France 3 | Poland 1 | Poland 2 | Poland 3 | Italy 1 | Italy 2 | Italy 3 |
|---|---|---|---|---|---|---|---|---|---|
| Total nr. pts receiving ESAs | 79 | 89 | 87 | 60 | 85 | 130 | 56 | 82 | 90 |
| C.E.R.A. uptake, % | 97 | 96 | 90 | 92 | 94 | 93 | 98 | 98 | 94 |
| Mean ESA administrations/pt/yr (excluding pts receiving C.E.R.A.) | 60 | 120 | 90 | 53 | 124 | 94 | 115 | 89 | 108 |
| **No. ESA administrations avoided/pt/yr by switching to C.E.R.A. Q4W** | **55** | **115** | **64** | **42** | **111** | **76** | **106** | **76** | **91** |
| Time/pt/yr, hrs | | | | | | | | | |
| ESAs | 95 | 198 | 91 | 310 | 372 | 230 | 169 | 231 | 165 |
| C.E.R.A. | 24 | 50 | 24 | 98 | 93 | 58 | 42 | 58 | 41 |
| **Calculated reduction in time at 100% C.E.R.A. uptake, %** | **75** | **86** | **74** | **78** | **88** | **85** | **88** | **87** | **88** |

**Conclusion:** Modeled 100% uptake of C.E.R.A. Q4W maintenance therapy was shown to offer substantial annual time savings on frequent tasks related to anemia management of 74-86% in France, 78-88% in Poland and 87-88% in Italy, allowing healthcare resources to be reallocated to other important CKD therapy needs and improving overall patient care. This confirms findings from a previous study using time and motion methodology.[1]

1. Saueressig U et al. Blood Purif 2008; 26: 537-546.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO472

### Normalisation of CRP and LDL-C Levels Is Related to Reduction of Cardiovascular Morbidity and Mortality in Haemodialysis Patients on Rosuvastatin Treatment – A *Post Hoc* Analysis of the AURORA Trial

Bengt C. Fellstrom,[1] Hallvard Holdaas,[2] Alan G. Jardine,[4] Roland E. Schmieder,[3] Ingar Holme,[5] Faiez Zannad.[6] *[1]Nephrology, Uppsala; [2]Nephrology, Oslo; [3]Nephrology, Erlangen; [4]Cardiovasc Res Center, Glasgow; [5]Preventive Cardiology, Oslo; [6]Inserm, Nancy.*

**Purpose:** C-reactive protein (CRP) is an important marker for cardiovascular (CV) events in patients on dialysis.We analysed CRP and LDL-C levels in haemodialysis patients randomised to rosuvastatin 10 mg or placebo in the AURORA trial. to determine if there was a treatment effect related to reduction in LDL-C or CRP.

**Methods** : Levels of CRP and LDL-cholestrol at 3 or 12 months post-randomization were analysed in both treatment groups, and split points of CRP = 2 mg/ l and LDL-C = 1.8 mmol/l were used..

**Results** : Of 2776 patients randomised in AURORA, 813 had CRP <2 mg/L on treatment, 1418 had LDL-C <1.8 mmol/L, and 509 had both CRP <2 mg/L and LDL-C <1.8 mmol/L.In the subgroup of 813 patients in whom CRP was < 2 mg/liter after 3 or 12 months of statin/placebo therapy, LDL-C emerged as a significant risk factor for CV events (HR 1.53 , P= 0.014 ). In this subgroup, rosuvastatin treatment lead to reduced risk of major CV events compared to placebo (HR= 0.71, P= 0.018). Subgroups based on CRP or LDL-cholesterol levels at baseline were not predictive of treatment effects by rosuvastatin upon any of the endpoints. However, in patients who had post-randomisation CRP < 2 mg/l and LDL< 1,8 mmol/l ( 509 / 2516 patients (20%) ), rosuvastatin treatment was associated with a significant lower risk of major cardiovascular events (HR= 0.53 , P=0.0005), of cardiovascular death (HR = 0.54, p= 0.0012) of combined endpoint of major CV event or patient death (HR= 0.64, p = 0,0035) and of all cause mortality (HR = 0.71 , p= 0.014). Non-CV death did not differ in the rosuvastatin low post randomization CRP (HR= 0,88, P = 0,61). **Conclusion** : CRP is an important marker for CV events and death and LDL-C is a risk factor for major CV events in haemodialysis patients with CRP <2 mg/L on treatment. A significant risk reduction was observed in patients in whom CRP and LDL-C levels were normalised with rosuvastatin.

Disclosure of Financial Relationships: Consultancy: Astrazeneca, Roche, Novartis, WyethResearch Funding: Astrazeneca, Roche, NOVARTIS , Merck; Honoraria: Astrazeneca, Roche, NOVARTIS, Wyeth; Scientific Advisor: Roche, Wyeth.

## TH-PO473

### Cardiovascular Events Following an Infectious Hospitalization in Patients on Dialysis

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**Purpose:** To examine whether an infectious hospitalization increases the subsequent risk of a cardiovascular (CV) event in older patients on dialysis.

**Methods:** The source population was assembled from the United States Renal Data System and included patients aged 65 to 100 years newly starting dialysis between 1/1/00 and 12/31/02. Patients were followed until transplant, death or study end 12/31/04. All discharge diagnoses were examined to determine whether an infection occurred during hospitalization. Only principal discharge diagnoses were examined to ascertain CV events (unstable angina, myocardial infarction, transient ischemic attack or stroke). We used the

case series method to estimate the relative incidence of a CV event within 90 days following an infectious hospitalization compared to periods without infection.

**Results:** The source population included 138,665 patients, of whom 16,874 had at least one CV event and were included in the analysis. The risk of a CV event was increased by 25% in the first 30 days following an infection, and decreased over the first 90 days. Each type of infection examined (septicemia, pulmonary, and genitourinary) was associated with an increased risk of CV events.

Table 1. Age-Adjusted Relative Incidence (RI) of a CV Event Following Infectious Hospitalization

| Risk Period | All Infections | Septicemia | Pulmonary | Genitourinary |
|---|---|---|---|---|
| Days | RI (95% CI) | RI (95% CI) | RI (95% CI) | RI (95% CI) |
| 1-30 | 1.25 (1.18,1.33) | 1.15 (1.02,1.28) | 1.20 (1.08,1.34) | 1.20 (1.08,1.34) |
| 31-60 | 1.17 (1.09,1.26) | 1.18 (1.04,1.34) | 1.10 (0.98,1.25) | 1.10 (0.97,1.25) |
| 61-90 | 1.08 (1.00,1.17) | 1.21 (1.06,1.39) | 1.10 (0.96,1.25) | 1.01 (0.88,1.16) |
| 1-90 (combined) | 1.18 (1.13,1.24) | 1.17 (1.09,1.27) | 1.14 (1.06,1.23) | 1.12 (1.04,1.21) |

**Conclusions:** The first 30 days following an infection is a high risk period for CV events and may represent an opportunity for risk reduction in older patients on dialysis.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO474

**Carotid Intima-Media Thickness Is Associated with the Severity of Coronary Artery Disease and Cardiovascular Prognosis in Hemodialysis Patients** Shinji Tanaka, Naobumi Mise, Tokuichiro Sugimoto. *Nephrology, Mitsui Memorial Hospital, Chiyoda-ku, Tokyo, Japan.*

**Purpose:** This study aimed to investigate the association between carotid intima-media thickness (IMT) and the angiographic severity of coronary artery disease (CAD), and to clarify prognostic factors for cardiovascular mortality in chronic hemodialysis (HD) patients.

**Methods:** This retrospective cohort study comprised 64 consecutive HD patients (44 male, mean age 65 ± 8 years, 34 (53.1%) diabetics, mean dialysis vintage 98 ± 90 months) who underwent elective coronary angiography for suspected CAD between July 2002 and March 2004. IMT was measured by ultrasonography within a month before the angiography. All patients were followed until the end of 2009, and the study endpoint was death from cardiovascular disease.

**Results:** Coronary angiography revealed no significant stenosis in 24 (37.5%) patients, single-vessel disease in 14 (21.9%), and multi-vessel disease in 26 (40.6%). There was a consistent incremental increase in mean IMT with increasing severity of CAD: 0.71 ± 0.16 mm in zero-vessel, 0.84 ± 0.17 mm in single-vessel, and 1.05 ± 0.30 mm in multi-vessel disease patients (zero vs. single: p=0.016, single vs. multi: p=0.023).

There were 29 cardiovascular deaths (45.3%) during the follow-up period of 50 ± 30 months. In univariate analysis using Cox proportional hazard model, IMT (p<0.01), the severity of CAD (p=0.068), and age (p<0.01) influenced cardiovascular mortality (p<0.1). Multivariate analysis including these 3 variables demonstrated that IMT (hazard ratio per 0.1 mm increase: 1.19, 95% confidence interval 1.04-1.35, p=0.014) and age (hazard ratio per 1 year increase: 1.06, 95% confidence interval 1.01-1.12, p=0.037) were significant predictors of cardiovascular mortality.

**Conclusions:** IMT was associated with the angiographic severity of CAD in HD patients. In addition, IMT was more strongly related to cardiovascular prognosis than was CAD severity in this population.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO475

**Repeat Coronary Revascularization of Dialysis Patients in the US after Surgical Versus Percutaneous Coronary Intervention** Charles A. Herzog, David T. Gilbertson, Craig Solid. *CVSSC, USRDS, Minneapolis, MN.*

**Introduction:** There are few published data on the comparative risk of repeat coronary revascularization of dialysis pts following surgical versus percutaneous coronary .intervention (PCI).

We searched the records of the United States Renal Data System database to identify 12,792 dialysis pts having coronary artery bypass surgery (CAB), drug eluting stents (DES), or bare metal stents (BMS) in 2004-2006. Event-free survival for repeat coronary revascularization (PCI or CAB) and the combined event of repeat revascularization or death was estimated by Kaplan-Meier method and independent predictors were examined in a comorbidity-adjusted Cox model.

**Results:** There were 3,455 CAB pts, 7,486 DES pts, and 1,851 BMS pts. The tables show event-free survival and predictors for repeat revascularization and the combined endpoint of revascularization/death (age < 65, male, white, hemodialysis, time on dialysis < 2 years, no comorbidity, CAB is reference) with hazard ratio (HR). The risk of repeat revascularization is markedly lower in CAB patients, while DES and BMS patients have similar rates of repeat coronary revascularization. The combined event of repeat coronary revascularization or death is least likely to occur in CAB pts.

**Conclusion:** Our data indicate that dialysis pts receiving CAB (vs PCI) have a lower rate of repeat coronary revascularization. DES and BMS pts have comparable rates of repeat coronary revascularization.

Event-Free Survival(%)

| | Repeat Revascularization | | | Repeat Revascularization/Death | | |
|---|---|---|---|---|---|---|
| Months | CAB | DES | BMS | CAB | DES | BMS |
| 1 | 98.3 | 94.3 | 94.4 | 86.4 | 88.5 | 85.2 |
| 6 | 95.1 | 85.5 | 84.2 | 71.5 | 69.6 | 62.9 |
| 12 | 92.0 | 79.0 | 78.7 | 62.0 | 55.5 | 48.3 |
| 24 | 85.9 | 69.8 | 69.2 | 46.5 | 36.1 | 32.0 |
| 36 | 81.3 | 62.4 | 60.9 | 32.6 | 22.5 | 19.3 |
| 48 | 78.0 | 55.8 | 55.7 | 21.2 | 12.8 | 10.8 |

Predictors of:

| | Repeat Revasc. | Repeat Revasc/Death |
|---|---|---|
| Variable | HR (95% CI) | HR (95% CI) |
| Age 65-74 | 0.86 (0.79, 0.94) | 1.10 (1.05, 1.16) |
| Age 75+ | 0.81 (0.74, 0.90) | 1.37 (1.30, 1.45) |
| Female | 0.95 (0.88, 1.03) | 0.95 (0.91, 0.99) |
| Black | 0.93 (0.86, 1.01) | 0.87 (0.83, 0.91) |
| CHF | 1.04 (0.96, 1.13) | 1.23 (1.17, 1.28) |
| Peritoneal dialysis | 0.97 (0.81, 1.16) | 1.33 (1.21, 1.47) |
| Diabetes | 1.12 (1.00, 1.25) | 1.10 (1.04, 1.17) |
| **BMS (vs. CAB)** | **2.58 (2.26, 2.94)** | **1.45 (1.36, 1.55)** |
| **DES (vs. CAB)** | **2.46 (2.22, 2.74)** | **1.27 (1.21, 1.34)** |

Disclosure of Financial Relationships: Consultancy: Amgen, CorMedix; Ownership: Cambridge Heart, Boston Scientific, Johnson & JohnsonResearch Funding: Amgen, NIH (NIDDK); Honoraria: UpToDate; Other Relationship: RoFAR (Roche Foundation for Anemia Research) Board of Trustees Member.

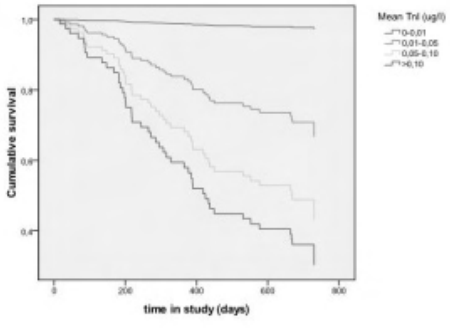
## TH-PO476

**Troponin I as a Cardiac Biomarker for Cardiovascular Mortality in Patients on Chronic Haemodialysis** Stefanie Vogels,[1] Volkher Scharnhorst,[1] Daniel A. Geerse,[1] Constantijn Konings,[1] Frank Van der Sande,[2] Jeroen Kooman,[2] Karel M. Leunissen.[2] *[1]Internal Medicine, Catharina Hospital, Eindhoven, Netherlands; [2]Nephrology, University Hospital, Maastricht, Netherlands.*

***Background:*** The high prevalence of cardiovascular mortality in patients on chronic haemodialysis is well established. Biomarkers like BNP and Troponin T are correlated with cardiovascular (CV) mortality, but this relationship is much less clear for troponin I (TnI). With a more sensitive test for TnI we found a strong correlation between TnI and cardiovascular mortality.

***Materials and methods:*** Plasma TnI levels of 206 chronic haemodialysis patients in our centre were measured every 3 months for a period of 24 months. We used a TnI-ultra assay on an Advia Centaur analyzer. Cardiovascular morbidity and mortality were assessed over a period of 30 months.

***Results:*** 206 patients were divided in 4 groups by their mean TnI according to the reference values of TnI used in our hospital. Group 1 (n=59) had a TnI level <0.01µg/l; group 2 (n=94) had TnI levels of 0.01-0.05µg/l; group 3 (n=29) 0.05-0.10µg/l and group 4 (n=24) had a TnI level ≥0.10µg/l. Forty-nine patients (23.8%) died; 31 (63.3%) due to CV disease. In group 1 one patient (1.7%) died of a CV cause. Group 2 had 21 deaths (22.3%); 11 (52.4%) of a CV cause. In group 3 13 patients (44.8%) died; 8 (61.5%) due to CV causes. Fourteen patients (58.3%) in group 4 died. Eleven of them (78.6%) died of a CV event. Twenty patients had a heart attack; per subgroup: n=2 (3.4%), n=9 (9.6%), n=3 (10.3%) and n=6 (25.0%) respectively. In a univariate analysis a high TnI was related with a significant higher risk for myocardial infarction (p=0.007) and cardiovascular death (p=0.001).




In a multivariate analysis TnI remained a strong independent risk factor.

***Conclusion:*** TnI can be a prognostic biomarker for (cardiovascular) mortality in patients on chronic haemodialysis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO477**

**Accelerated Progression of Vascular Calcification in Paediatric CKD and Dialysis Patients Is Associated with Baseline Vessel Calcification** Rukshana C. Shroff,[1] Ambrose M. Gullett,[1] Melanie Hiorns,[2] John Deanfield,[3] Lesley Rees,[1] Catherine M. Shanahan,[4] Kimberly Rita Hassen.[1] *[1]Nephrology Unit, Great Ormond Street Hospital, London, United Kingdom; [2]Radiology Unit, Great Ormond Street Hospital; [3]Vascular Physiology Unit, Institute of Child Health; [4]Cardiovascular Division, King's College London.*

Vascular calcification is thought to begin early in CKD and progress rapidly on dialysis, but there are few studies demonstrating this. We compared vascular changes as seen on imaging with a quantitative and histological assessment of the vessel calcium [Ca] load on arterial biopsy to study progression of vascular changes.

39 children (13 pre-dialysis CKD 4-5; 26 dialysis) had carotid intima-media thickness [cIMT], pulse wave velocity [PWV] and coronary artery calcification [CAC] scoring on CT and an arterial biopsy at the time of renal transplantation or PD catheter insertion. The Ca load in the vessel wall was quantified and vessel histology performed. 32 children (19 dialysis, 13 transplants) had repeat imaging after 10.8±6.6 months. Changes in vascular measures and their correlation with baseline arterial biopsy was noted.

At initial measurement cIMT was increased in 85% of dialysis patients and showed a positive correlation with increased vessel Ca load ($p<0.003$). Only 2 of 13 pre-dialysis patients had an increased cIMT despite an increased vessel Ca load in all ($p=0.7$). On follow-up dialysis patients had an increase in cIMT (0.54±0.07 to 0.68±0.04mm; $p=0.02$), PWV (6.4±0.2 to 7.3±0.8 m/sec; $p=0.005$) and CAC increased in 2 children with baseline CAC and was found in 3 others. cIMT progression correlated with baseline vessel Ca load ($p=0.004$, $r=0.58$), vascular smooth muscle cell number and apoptosis. Only 4 dialysis patients with the highest vessel Ca loads showed an increase in PWV and CAC. No correlation was seen between vessel alkaline phosphatase or osteogenic marker runx2 and imaging studies. Pre-dialysis patients had a deterioration in PWV (6.1±0.3 to 7.0±0.2 m/sec), but cIMT and CAC were unchanged and did not correlate with vessel Ca load.

Calcification is rapidly progressive on dialysis and strongly correlates with baseline vessel wall changes.

Disclosure of Financial Relationships: Honoraria: Genzyme.

**TH-PO478**

**Increased Circulating PIVKA-II (Protein Induced by Vitamin K Absence II) Levels in Dialysis Patients and Its Relation to Calcification, Outcome and Vitamin K2 Administration** Georg Schlieper,[1] Leon J. Schurgers,[2] Ralf Westenfeld,[3] Cees Vermeer,[2] Jurgen Floege,[1] Thilo Krueger.[1] *[1]Nephrology, RWTH University Hospital, Aachen, Germany; [2]CARIM, Maastricht University, Mastricht, Netherlands; [3]Cardiology, Heinrich-Heine University, Duesseldorf, Germany.*

End-stage renal disease patients exhibit a clinical vitamin K deficiency as indicated by increased serum levels of uncarboxylated osteocalcin and - as recently shown - by increased PIVKA-II (protein induced by vitamin K absence II) levels. Recent experimental and clinical data suggest that vitamin K antagonism by warfarin use is associated with increased cardiovascular calcifications.

In this study we addressed the question whether PIVKA-II levels were associated with cardiovascular calcifications (Adragao and extended score) and mortality in 188 hemodialysis (HD) patients (59 ± 11 years). In addition, we tested whether vitamin K2 (MK-7) administration over 6 weeks was able to lower PIVKA-II levels in 50 HD patients. Patients on warfarin were excluded because of its vitamin K-antagonising effect.

PIVKA-II levels were increased (> 2 ng/mL) in 121 (64%) of the HD patients (median 2.98; range 0.45 – 318 ng/mL), indicating hepatic vitamin K deficiency. PIVKA-II levels correlated well with those of desphosphorylated uncarboxylated matrix gla protein (MGP; $r = 0.62$; $p < 0.0001$) and to a lesser extent with desphosphorylated carboxylated MGP ($r = 0.19$; $p = 0.01$). In contrast to MGP levels PIVKA-II levels did not show an association with cardiovascular calcifications. High PIVKA-II levels (> 2 ng/mL) were not associated with an increased mortality risk (log-rank test: $p = 0.67$; Cox regression: HR 1.00, CI 0.53 – 1.89, $p = 1.00$). Daily vitamin K2 administration (45, 135 or 360 µg/d) in HD patients lowered PIVKA-II levels by 32%, 39% and 50%, respectively (all $p<0.01$).

HD patients display a profound vitamin K deficiency. Elevated PIVKA-II levels likely reflect hepatic vitamin K deficiency whereas MGP levels seem to serve as more robust marker for vascular vitamin K deficiency. Whether vitamin K treatment can improve survival needs to be tested in future studies.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO479**

**Predictors of Survival in Dialysis Patients with Acute Myocardial Infarction: Findings from the USRDS AMI Special Study** Samy M. Riad,[1] Sally K. Gustafson,[2] Xinyue Wang,[2] David T. Gilbertson,[2] Charles A. Herzog.[1,2] *[1]Dept of Nephrology, U of MN, Minneapolis, MN; [2]USRDS, Minneapolis, MN.*

**Background:** Acute myocardial infarction (AMI) in dialysis pts is associated with poor survival. This study aims to identify predictors of survival in dialysis pts prior to AMI and to examine the association between survival and different revascularization techniques.

**Methods and Results:** 3049 US prevalent dialysis pts hospitalized for AMI 4/1/1998-6/30/2000 were identified by cross-matching United States Renal Data System (USRDS) database and the Third National Registry of Myocardial Infarction (NRMI 3). Of the 3011 data abstraction forms sent to the 18 renal networks, 1696 were sufficiently complete for analysis. Average age was 66.4 years old and average years on dialysis 2.7 years. 69% were white and 47% were females. Diabetes (DM) and dysrhythmia were prevalent in 72.5% and 65.5% respectively. At 1 yr post-AMI, 62% of the cohort had died. The impact of independent predictors on survival was examined in a Cox Proportional Hazards model. Beta Blocker (BB) use was associated with a favorable 1-yr all-cause mortality (hazard ratio (HR) of 0.8, $p=0.003$). As compared to pts who dialyzed via catheter, fistula use was associated with favorable outcome (HR=0.76, $p=0.008$), as was graft use (HR=0.82, $p= 0.016$). Compared to pre-dialysis systolic blood pressure (PDSBP) between 120-179 mmHg, PDSBP < 120 mmHg was more hazardous (HR=1.46, $p\leq 0.0001$) while PDSBP ≥ 180 mmHg (HR=0.7, $p=0.004$) had better survival. Coronary artery bypass graft surgery (CABG) and percutaneous coronary intervention (PCI) within 30 days of AMI were examined in time-independent and time-dependent Cox models. In the time-independent model, PCI and CABG were similarly associated with favorable outcome. However, in all time-dependent models, CABG lost its significance (HR = 0.86, P = 0.35), while PCI maintained its protective association (HR=0.67, $p=0.0005$).

**Conclusion:** BB use prior to AMI, and PCI within 30 days of AMI are associated with favorable one year survival in dialysis patients. Validation of these observational findings by randomized clinical trials is needed.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO480**

**Study of the Autonomic Response in Hemodialysis Patients at Different Levels of Fluid Overload** Manuela Ferrario,[1] Ulrich Moissl,[2] Francesco Garzotto,[3] Maria Gabriella Signorini,[1] Dinna N. Cruz,[3] Flavio Basso,[3] Alessandra Brendolan,[3] Federico Nalesso,[3] Ciro Tetta,[2] Sergio Cerutti,[1] Claudio Ronco.[3] *[1]Department of Bioengineering, Politecnico di Milano, Milano, Italy; [2]Fresenius Medical Care GmbH, Bad Homburg, Germany; [3]Nephrology, San Bortolo Hospital, Vienza, Italy.*

**Background**

This work aims at studying the autonomic nervous system (ANS) response to hemodialysis (HD) treatments in a population of end stage renal disease (ESRD) patients which are characterized by different conditions in terms of blood pressure (BP) and fluid overload (FO).

**Methods**

80 patients were recruited from the dialysis unit of San Bortolo Hospital, Vicenza. Whole body bioimpedance spectroscopy measurements were performed for each patient before HD and 24 hr ECG Holter were recorded starting from the period immediately before HD. Patients were classified according to the FO values and the systolic blood pressure (SBP) measured before HD [Wabel et al., NDT, 2008]. Time and frequency domain indices for heart rate variability (HRV) were determined for the first 30 minutes and last 30 minutes of HD, the first hour after HD, and night (12.00 p.m.-4 a.m.) [Task Force, Circulation, 1996].

**Results**

Significant differences were obtained in fluid overloaded but normotensive patients (Group IV) with respect to fluid overloaded and hypertensive patients (Group I) and normohydrated and normotensive patients (Group N+Dx), see Figure 1. In particular, SDNN, RMSSD, SDSD, pNN50%, indices were significantly higher in Group IV with respect to the other groups.

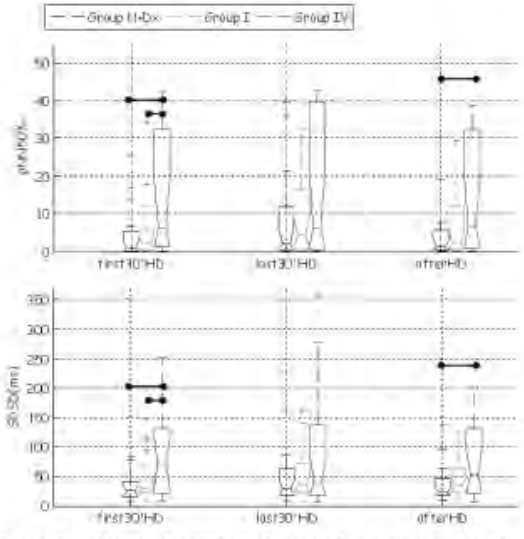



Figure 1. Upper Panel: Boxplot of the pNN50% values. Lower panel: Boxplot of the SDSD values for the 3 groups. The lines mark the significant differences between the groups (ANOVA p-value<0.05 and Bonferroni multicomparisons test p-value<0.05).

**Conclusion**

Overhydrated patients with hypertension (Group I) could be affected by a blunted parasympathetic activity, which may contribute to hypertension.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO481

**Secular Trends in Cardiovascular Mortality Rates of Patients Receiving Dialysis Compared to the General Population** Matthew A. Roberts,[1] Kevan R. Polkinghorne,[2] Stephen P. McDonald,[3] Francesco L. Ierino.[1] *[1]Nephrology, Austin Health, Victoria, Australia; [2]Nephrology, Monash Medical Centre, Victoria, Australia; [3]Nephrology, The Queen Elizabeth Hospital, South Australia, Australia.*

We analysed cardiovascular mortality rates (CVMR) of dialysis patients over time and compared these to the general population.

CVMR were calculated from ANZDATA Registry data for Australian patients receiving dialysis from 1992-2005, and from Australian Bureau of Statistics data for the comparable general population. Patients were included if dialysis was the first renal replacement therapy, and censored at transplantation or three years from commencing dialysis. Age-stratified CVMR were reported per hundred person-years and Relative Risks (RR) calculated relative to the Australian population rates. Secular changes in CVMR were analysed with negative binomial regression.

The analysis included 24,352 patients with 68,407 person-years of follow up and 5,134 cardiovascular deaths. CVMR declined over time in the general population (not shown). Age-stratified CVMR declined in patients on dialysis aged 55-74 years but the corresponding RR increased over this time (Table). The RR of dialysis patients increased markedly with younger age, a trend which did not alter over time. The risk associated with dialysis compared to the general population increased over time (p=0.044 for interaction). CVMR in Australia were comparable to those in the United States and Europe.

Although cardiovascular mortality rates are falling, the excess cardiovascular risk in dialysis patients compared to the general population is increasing over time.

Age-stratified CVMR and RR (95% CI) in 1992-1994 and 2004-2005

| Era: | CVMR per 100 person-years | | RR | |
|---|---|---|---|---|
| Age | 1992-1994 | 2004-2005 | 1992-1994 | 2004-2005 |
| 35-44 | 2.5 (1.4-4.4) | 3.3 (2.3-4.9) | 122 (69.1-214) | 192 (130-284) |
| 45-54 | 3.5 (2.4-5.3) | 3.2 (2.4-4.4) | 46.9 (31.4-70.0) | 69.0 (51.0-93.4) |
| 55-64 | 7.3 (5.8-9.1) | 6.0 (5.0-7.3) | 26.3 (20.9-33.1) | 49.2 (40.6-59.5) |
| 65-74 | 9.4 (7.7-11.4) | 6.6 (5.6-7.8) | 10.5 (8.6-12.7) | 16.6 (14.1-19.5) |
| 75-84 | 14.2 (9.8-20.6) | 10.2 (8.8-11.8) | 4.8 (3.3-6.9) | 6.4 (5.5-7.4) |

Disclosure of Financial Relationships: nothing to disclose

## TH-PO482

**Long-Term Survival of Dialysis Patients in the US after Surgical Versus Percutaneous Coronary Revascularization** Charles A. Herzog, David T. Gilbertson, Craig Solid. *CVSSC, USRDS, Minneapolis, MN.*

**Introduction:** There are few published data on the comparative long-term survival of dialysis patients undergoing surgical versus percutaneous coronary revascularization in the era of drug-eluting stents (DES).

We searched the records of the United States Renal Data System database to identify 10,941 dialysis pts having coronary artery bypass surgery (CAB) or DES in 2004-2006. Long-term survival was estimated by Kaplan-Meier method and independent predictors of death were examined in a comorbidity-adjusted Cox model.

**Results:** There were 3,455 CAB pts and 7,486 DES patients. The tables show survival and predictors of death, (age < 65, male, white, hemodialysis, time on dialysis < 2 years, no comorbidity, CAB is reference) with hazard ratio (HR), as well as estimated survival within CAB patients related to utilization of internal mammary grafts [IMG(+) or IMG (-)]. DES patients have better survival at 12 months, but after 18 months CAB patients have better outcome. CAB patients receiving internal mammary grafts (68% of CAB pts, n=2356) do significantly better than those without (Log-Rank p-value = 0.0005); the risk of death is 14% lower in IMG(+) CAB vs IMG(-) CAB pts (HR 0.86, (95%CI 0.78, 0.95)).

**Conclusion:** Our data suggest that drug-eluting stents provide the best first year survival, but unadjusted long-term survival is best in CAB patients receiving internal mammary grafts.

| | Survival (%) | | Survival (%) for CAB | |
|---|---|---|---|---|
| Months | CAB | DES | IMG (+) | IMG (-) |
| 1 | 88.0 | 93.8 | 88.6 | 86.8 |
| 6 | 75.5 | 81.6 | 77.4 | 71.4 |
| 12 | 68.0 | 70.3 | 70.3 | 63.0 |
| 24 | 55.0 | 52.3 | 57.1 | 50.5 |
| 36 | 43.9 | 39.6 | 45.8 | 39.9 |
| 48 | 34.6 | 30.6 | 35.8 | 31.9 |
| Predictors of Death | | | | |
| | Death | | | |
| Variable | HR (95% CI) | | | |
| Age 65-74 | 1.24 (1.16, 1.32) | | | |
| Age 75+ | 1.66 (1.55, 1.77) | | | |
| Female | 1.02 (0.96, 1.07) | | | |
| Black | 0.85 (0.80, 0.90) | | | |
| CHF | 1.41 (1.33, 1.50) | | | |
| Peritoneal dialysis | 1.30 (1.16, 1.46) | | | |
| Diabetes | 1.08 (0.99, 1.16) | | | |
| **DES (vs. CAB)** | **1.05 (0.99,1.11)** | | | |

Disclosure of Financial Relationships: Consultancy: Amgen, CorMedix; Ownership: Cambridge Heart, Boston Scientific, Johnson & JohnsonResearch Funding: Amgen, NIH (NIDDK); Honoraria: UpToDate; Other Relationship: RoFAR (Roche Foundation for Anemia Research) Board of Trustees Member.

## TH-PO483

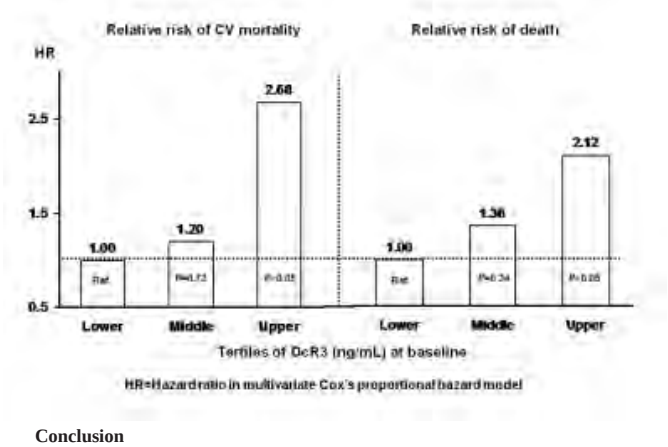
**Serum Decoy Receptor 3 (DcR3) Predicts Cardiovascular and All-Cause Mortality among Chronic Hemodialysis (HD) Patients** Ta-Wei Hsu,[1] Der-Cherng Tarng.[2,3] *[1]Division of Nephrology, Department of Internal Medicine, National Yang-Ming University Hospital, Yilan, Taiwan; [2]Division of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan; [3]Department and Institute of Physiology, National Yang-Ming University, Taipei, Taiwan.*

**Aim**

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in HD patients. Novel but not traditional risk factors are far more prevalent in this population and contribute significantly to atherosclerosis and CVD. DcR3, belonging to the tumor necrosis factor receptor superfamily, is a soluble receptor considered to play a role in immune modulation. Increased serum DcR3 levels have been reported in patients with renal failure. However, whether serum DcR3 is a biomarker for predicting outcome in HD patients has not been elucidated.

**Methods and Results**

Three-hundred and sixteen patients (155 men and 161 women; mean age 59 ± 13 years) undergoing maintenance HD (vintage 84 ± 63 months) were studied. The baseline serum DcR3 level was 2.29 ± 2.20 ng/mL. Serum DcR3 was significantly correlated with albumin (r= -0.321, P<0.001), hs-CRP (r= 0.223, P<0.001), hemoglobin (r= -0.128, P=0.033), and ferritin (r= 0.129, P=0.032). All patients were further stratified into the tertiles of serum DcR3 at baseline. The follow-up duration of this study was 54 months. Kaplan-Meier survival curves showed that patients with DcR3 upper tertile had worse outcome for CV mortality (P=0.003) and all-cause death (P<0.001). A multivariate Cox regression analysis demonstrated that DcR3 upper tertile independently had higher risks toward CV death with a hazard ratio of 2.68 (P <0.05) and overall death with a hazard ratio of 2.12 (P <0.05), respectively.




**Conclusion**

Serum DcR3 level is a surrogate biomarker of malnutrition-inflammation complex and independently predicts the CV and all-cause mortality among chronic HD patients.

Disclosure of Financial Relationships: nothing to disclose

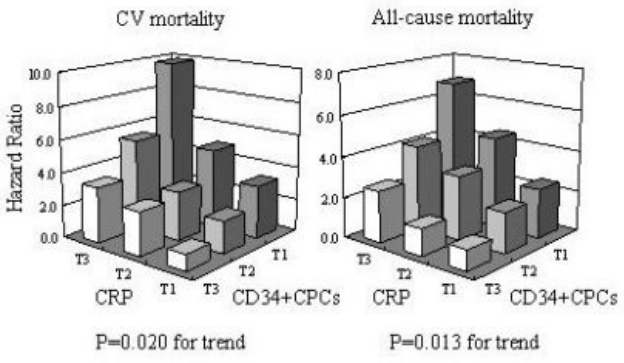
## TH-PO484

**Association of CD34+ Circulating Progenitor Cells and C-Reactive Protein with Cardiovascular and All-Cause Mortality in Hemodialysis Patients** Shoichi Maruyama,[1] Takenori Ozaki,[1] Waichi Sato,[1] Naotake Tsuboi,[1] Masashi Mizuno,[1] Hiroshi Takahashi,[2] Hirotake Kasuga,[2] Enyu Imai,[1] Yasuhiko Ito,[1] Seiichi Matsuo.[1] *[1]Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Department of Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Japan.*

Background: Circulating progenitor cells (CPCs) are believed to act to maintain the endothelial function. C-reactive protein (CRP) has also been reported to predict cardiovascular (CV) events. We investigated the association between CD34+ CPCs and CRP and their role with prediction of mortality in hemodialysis (HD) patients.

Methods: The number of CD34+ CPCs was determined in 216 HD patients. The patients were divided into tertiles according to CD34+ levels and serum CRP levels. Follow-up period was 5 years.

Results: CRP levels were 6.1±9.4 mg/l, 3.1±4.1 mg/L and 2.1±2.0 mg/L in T1, T2 and T3 of CD34+ CPCs levels (p=0.0010), and were negatively correlated with CD34+ CPCs levels (r=0.31, p<0.0001). Adjusted hazard ratio (HR) of reduced CD34+ CPCs was 3.38 (95%CI 1.24-9.25, p=0.016 for T1 vs. T3) for CV mortality and 2.71 (95%CI 1.20-6.13, p=0.017 for T1 vs. T3) for all-cause mortality. Among tertiles of CRP, adjusted HR of elevated CRP levels was 4.46 (95%CI 1.46-9.57, p=0.021 for T3 vs. T1) for CV mortality and 3.69 (95%CI 1.56-8.72, p=0.0064 for T3 vs. T1) for all-cause mortality. In the joint setting of CD34+ CPCs and CRP, the risk of CV and all-cause mortality was 9.8 (p=0.020) and 6.9 (p=0.013) in the T1 of CD34+ CPCs with T3 of CRP compared with the T3 of CD34+ CPCs with T1 of CRP even after adjustment, respectively.




Conclusions: Reduced number of CD34+ CPCs and elevated CRP levels may interactively increase mortality risk in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO485**

**Risk Factors for Non-Peridialytic Sudden Cardiac Death in Hemodialysis Patients** Patrick H. Pun, Ruediger W. Lehrich, John Paul Middleton. *Medicine, Nephrology, Duke University, Durham, NC.*

We have previously found that dialysis-specific factors such as exposure to low potassium dialysate and low pre-dialysis blood pressure are potent risk factors for sudden cardiac death (SCD) occurring within dialysis facilities, but whether or not these factors are also predictive of the majority of SCD events that occur outside of dialysis facilities is unclear. We sought to examine the role of dialysis-specific factors in determining the risk of out-of-clinic SCD in a large cohort of hemodialysis patients.

We examined a cohort of hemodialysis patients (N=1632) from among 43,200 US Davita hemodialysis patients who did not experience adverse events in the outpatient dialysis clinic during the observation period 2002-2005. Using standardized medicare death reporting forms, we determined that 172 subjects experienced SCD outside the dialysis clinic during the observation period. We examined the clinical and dialytic characteristics of patients over a 90-day period among those who experienced SCD compared to those that died from other causes or were alive at the end of the observation period.

Patients who suffered a SCD were older (69 vs 64 years), and more likely to carry a diagnosis of coronary artery disease (44% vs 29%), congestive heart failure (38% vs 31%), and peripheral vascular disease (29% vs 18%). After accounting for differences in baseline comorbidities and patient demographics, mean 90-day dialysate potassium concentration (OR 1.4 per 1 meq/L decrease, 95% CI 1.1-2.0), pre-dialysis mean arterial blood pressure (OR 1.2 per 10 mmHg decrease, 95% CI 1.01-1.33), mean serum creatinine (OR 1.09 per 1 mg/dl decrease, 95% CI 1.01-1.17) and exposure to aspirin (OR 1.6, 95% 1.1-2.3) and statin medications (OR 0.6, 95% CI 0.4-0.8) were independently predictive of SCD.

This analysis suggests that potentially modifiable risk factors for SCA in dialysis clinics such as exposure to low potassium dialysate and low pre-dialysis blood pressure are also consistent predictors of SCD occurring outside of dialysis clinics. These and other identified risk factors may be helpful in targeting patients at highest risk for SCD for preventive strategies.

Disclosure of Financial Relationships: Research Funding: Satellite Health Care, Davita Clinical Research; Scientific Advisor: Genzyme.

**TH-PO486**

**Echocardiography at Dialysis Initiation: Use and Association with Mortality among Medicare Beneficiaries in the United States** Rajiv Saran,[1] Brett Lantz,[2] Dori Bilik,[2] Bruce M. Robinson,[2] Richard Hirth,[1] Jeffrey Pearson,[2] J. M. Messana.[1] *[1]Kidney Epidemiology and Cost Center, University of Michigan, Ann Arbor, MI; [2]Arbor Research Collaborative for Health, Ann Arbor, MI.*

Current guidelines recommend performing an echocardiogram (echo) in all patients (pts) at dialysis initiation (KDOQI 2005). We examined echo use in incident pts to evaluate whether the practice was associated with lower first-year mortality. Medicare pts age≥65 years initiating dialysis in 2004-08 were included (N=190902) and linked to facilities using the CMS-2728. Echo use was determined from inpatient, outpatient, skilled nursing facility, and carrier claims. Pts were found to meet the guideline if an echo was performed within 90 days before/after starting dialysis. Pts were "converted" if an echo was not performed in the 90 days prior to initiation (N=121538) but was performed ≤ 90 days after. By these criteria, 49% (N=93329) of pts met the guideline and 20% (N=23965) of pts were converted. The percent of pts meeting the guideline rose from 46% to 50% from 2004 to 2008 (p<.001); similarly, the conversion rate rose from 18% to 20% (p<.001). Echo practice patterns also varied by facility (N=4393). The median facility had 50% meeting guidelines (top quintile > 62%; bottom quintile < 38%) and a 20% conversion rate (top quintile > 30%; bottom quintile < 11%). First-year mortality was somewhat elevated among pts meeting the guideline (HR=1.05, p<.001) and among converted pts (HR=1.07, p<.001) even after adjustment for demographics and comorbidities. After limiting to 2005-07 (N=3924), facilities in the top quintile of meeting the guideline had higher adjusted first-year mortality versus the lowest quintile (RR=1.08, p<.001) as did the top quintile for conversions (RR=1.06, p<.001). Despite the 2005 KDOQI guideline, echo use at dialysis start became only slightly more common from 2004-08. Although echo use was associated with elevated first-year mortality, this finding may be due to treatment-by-indication bias or sub-optimal use of echo data to guide treatment decisions. Further research is necessary to evaluate the effectiveness of routine echocardiography in new dialysis pts.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO487**

**Obesity Is a Risk Factor for Mortality, Especially among Younger Dialysis Patients** Ellen K. Hoogeveen,[1] Nynke Halbesma,[2] Friedo W. Dekker,[2] Elisabeth W. Boeschoten.[3] *[1]Internal Medicine, Jeroen Bosch Hospital, Den Bosch, Netherlands; [2]Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands; [3]Clinical Epidemiology, Hans Mak Institute, Naarden, Netherlands.*

Obesity is an important risk factor for cardiovascular disease and mortality in dialysis patients. However, differences in mortality between young and elderly dialysis patients have not been well addressed. Therefore, our aim was to investigate whether the association of BMI and mortality differs between younger (<65 y) and older (≥65 y) dialysis patients.

In a prospective multi-centre cohort study, all dialysis patients (>18 y) starting with their first dialysis treatment were included and followed until death, transplantation or a maximum of 7 years (NECOSAD). We divided patients into eight categories based on their baseline BMI and age: <20 (7.5%), 20 to 25 (47%), 25 to 30 (34.5%) and ≥30 (11%) kg/m$^2$. Cox regression analysis was performed to calculate hazard ratios (HR) associated with BMI groups, using a normal BMI (20-25 kg/m$^2$) in the younger patients as the reference category. Analyses were adjusted for age, sex, treatment modality and smoking.

In total, 1749 patients were included (age (mean (SD)) younger patients: 49 y (11), older patients: 73 y (5) years, BMI: 25 (4) kg/m$^2$, 62% male, 62% HD). Overall, the 7-years mortality was 67%. Compared to a normal BMI and <65 y at baseline, the adjusted HRs (95%-CI) by BMI category were for the younger patients: 2.16 (1.37-3.40), 1 (reference), 0.89 (0.65-1.23), 1.60 (1.11-2.30), and for the elderly 1.64 (1.03-2.63), 1.18 (0.84-1.66), 1.18 (0.84-1.65), 1.06 (0.71-1.59).

We conclude that both young and elderly dialysis patients with underweight have an about 2-fold increased risk factor for mortality, although this maybe due to reverse causality. However, obesity is about 1.5-fold stronger risk factor for mortality in young compared to elderly dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO488**

**Outcomes of Elderly Diabetic Patients Starting on Hemodialysis** Gero D. von Gersdorff,[1] Mathias Schaller,[1] Thomas Benzing,[1] Claudia Barth.[2] *[1]Nephrology, Cologne University Medical Center, Cologne, Germany; [2]KfH - Curatorium for Dialysis and Kidney Transplantation, Neu-Isenburg, Germany.*

**Introduction**: We sought to identify factors associated with survival in elderly diabetic patients (DM) starting on hemodialysis. **Methods:** Incident patients who survived at least 90 days were eligible and were divided into age groups ≤ 55 years, 56 – 75 years, and > 75 years. Survival was calculated for each age group using the Kaplan-Meier method. Cox regression models, which included interaction terms for age and diabetes, were constructed to determine hazard ratios of survival. All calculations were two-sided with significance at p < 0,05. **Results**: There were 12290 incident patients and 27% of <55y, 55% of 56-75 y, and 52% of >75y were diabetic. 34%, 34%, and 49%, respectively, were women. Unadjusted mortality difference was marked between DM and n-DM patients ≤55y, but was no longer present >75y (p= 0,24). Cox regression models with multiple adjustments confirmed that there was a significant interaction of age with diabetes, and that diabetes was not a risk factor anymore in elderly incident dialysis patients (p=0,07). 1899 DM and 1770 n-DM patients >75y were therefore analyzed further (mean age 80,5y vs. 81,1y; 54% vs. 43% female; weight 73 vs. 67 kg; all p<0,001). There were more comorbidities among DM patients. Laboratory parameters at baseline were similar. Mortality was not different between groups of DM based on HbA1c of < 5,5%, 5,5 – 7%, and > 7% (p=0,969). However, a small number of patients (N=581) showed large SD >1 of HbA1c and a significantly increased mortality, suggesting that wide swings in HbA1c measurements may be associated with worse prognosis. **Conclusions**: In patients who started hemodialysis over age 75 years, there was no difference in survival between diabetic and non-diabetic subjects. No association was found between mean or median HbA1c and mortality. Wide swings in HbA1c, however, were associated with worse prognosis. This may reflect sicker patients in whom there is increased erythrocyte- and hemoglobin turnover, or frequent periods of hypo- or hyperglycemia. In elderly patients HbA1c is to be used cautiously for management of diabetes on dialysis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO489**

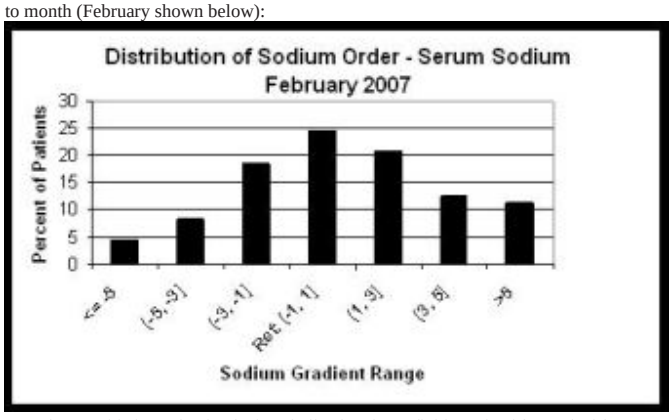
**Sodium Gradient & Hospitalization for Fluid Overload in Hemodialysis Patients** Eduardo K. Lacson, Weiling Wang, J. Michael Lazarus, Raymond M. Hakim. *Fresenius Medical Care, North America, Waltham, MA.*

**Introduction:** Sodium load is associated with fluid retention in hemodialysis patients. We hypothesized that the sodium gradient (between serum vs. dialysate) may impact salt/fluid retention and hospitalization risk for fluid overload.

**Methods**: All chronic HD patients treated from January 1 to March 31, 2007 at legacy Fresenius Medical Care North America facilities with serum sodium data (~89%) were included. The weighted mean of the prescribed dialysate sodium was computed monthly

and the gradient determined. Interdialytic weight gain (IDWG) was recorded. Case-mix (age, gender, race, DM, BSA, and vintage) and vascular access were identified as of 1/1/07 and hospitalizations (≥1 day) primarily due to fluid overload (including heart failure and acute pulmonary edema) were tracked. Cox models were constructed based on the sodium gradient for each of the three study months.

**Results**: Mean age (N=71,767) was 61.3±14.9 years, with 54.3% males, 49.9% white, 41.3% black and 52.5% diabetic, with vintage of 3.6±3.7 years and 44% had fistulas, with 28% grafts, and 28% catheters. The distribution of sodium gradient was similar from month to month (February shown below):




The monthly hazard ratios for hospitalization due to fluid overload for Jan/Feb/Mar was consistently high at 1.54/2.17/1.92 for gradient >5 meq/L and after case-mix adjustment were 1.48/2.08/1.86, respectively (all p<0.003). A gradient >5 meq/L was also associated with +0.4 kg higher IDWG each month (p<0.0001).

**Conclusion**: A positive sodium gradient >5 meq/L, seen in 11%-14% of patients, was consistently associated with greater IDWG and a 50-100% increased hazard rate for hospitalization due to fluid overload. Physicians need to pay more attention to the sodium component of dialysate prescriptions. Minimizing the sodium gradient could potentially decrease hospitalizations for fluid overload.

Disclosure of Financial Relationships: Employer: I am an employee of Fresenius Medical Care, North America.

## TH-PO490

### Use of High Magnesium Dialysate Does Not Abrogate Intradialytic Haemodynamic Instability or Haemodialysis-Induced Myocardial Stunning

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Haemodialysis (HD) induced ischaemic cardiac injury (myocardial stunning) is common, associated with adverse cardiovascular (CV) outcomes, mortality, and appears important in development of HD-related heart failure. Intradialytic hypotension is a critical modifiable determinant of myocardial stunning. Magnesium (Mg) is reportedly important in maintaining intradialytic blood pressure, with cardiovascular effects which could protect against demand myocardial ischaemia. This study aimed to compare the effects of high vs low dialysate Mg on intradialytic haemodynamics and myocardial stunning.

20 stable prevalent HD patients entered a randomised cross-over trial of low vs high (0.5 vs 1.0 mmol/L) dialysate Mg. Patients were studied after 2 weeks of standard HD at each Mg concentration. Dialysate composition differed only by Mg; other HD parameters were unchanged. Serial echocardiography (pre-HD, peak stress and post-HD) assessed myocardial stunning, measured by left ventricular regional wall motion abnormalities (RWMAs). Noninvasive haemodynamics and biochemistry were assessed.

Pre-dialysis serum Mg was higher with high dialysate Mg (1.42±0.20 vs 1.05±0.13mmol/L, p<0.0001). Low Mg dialysate was associated with intradialytic fall in serum Mg (-0.19±0.09 mmol/L); intradialytic serum Mg rose (+0.02±0.13 mmol/L) with high Mg dialysate (p<0.0001). There was no difference in mean number of peak stress RWMA per patient (3.4±1.9 vs 3.7±1.9, p=0.5). UF volume, a critical determinant of stunning, did not differ between high and low dialysate Mg studies (1.5±0.9 vs 1.5±0.7 litres, p=0.6). There was no advantage from high Mg dialysate with respect to intradialytic blood pressure stability or CV performance.

Serum Mg does not appear to critically modulate the propensity to haemodialysis-induced myocardial ischaemia. This study provides no evidence for use of high Mg dialysate to treat haemodynamic instability during HD, and suggests commonly-used lower dialysate Mg concentrations remain useful to optimise serum Mg without significant adverse haemodynamic effects.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO491

### Plasma Level of Fibroblast Growth Factor-23 and Intracranial Artery Calcification in Hemodialysis Patients

Yuko Iwasa,[1] Shigeru Otsubo,[2] Aiji Yajima,[1] Naoki Kimata,[3] Kosaku Nitta,[2] Takashi Akiba.[3] *[1]Nephrology, Towa Hospital, Tokyo, Japan; [2]Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan; [3]Blood Purification, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.*

***Objective*** Intracranial artery calcification has been reported to be an independent risk factor for ischemic stroke. Fibroblast growth factor (FGF) 23 is involved in the regulation of mineral metabolism, which may in turn affect vascular calcification. We investigated the relationship between FGF-23 and intracranial artery calcification in hemodialysis patients.

***Materials and Methods*** Brain computed tomographic examinations were performed in 107 patients received maintenance hemodialysis therapy. For comparison, 43 general population were also studied. FGF-23 levels were determined using an enzyme-linked immunosorbent assay.

***Results*** Intracranial artery calcification was found in 94 of the 107 patients. The plasma FGF-23 levels tended to be higher among the hemodialysis patients with intracranial artery calcification(5868.3±9441.2pg/mL) than among those without calcification(4211.6±8273.8pg/mL). Intracranial calcifications were more frequently found among the 58 hemodialysis patients who were under the age of 70 years(60.9±6.5years, 87.9%) than among the 43 age and gender-matched control subjects(61.0±6.2years, 53.5%, p=0.0003). The prevalence of calcification were as follows in the hemodialysis patients under 70 years of age and the 43 general population, respectively: vertebral artery(65.5% vs. 25.6%, p=0.0002), internal carotid artery(62.1% vs. 18.6%, p<0.0001), basilar artery(34.5% vs. 34.9%, ns), anterior cerebral artery(0% vs. 2.3%, ns), middle cerebral artery(24.1% vs. 20.9%, p=0.0203), and posterior cerebral artery(5.2% vs. 4.7%, ns).

***Conclusion*** The results showed a much higher rate of intracranial artery calcification among hemodialysis patients than among the general population and the more frequently involved sites of calcification were internal carotid artery and vertebral artery, which are relatively large intracranial arteries. FGF-23 level tended to be higher among hemodialysis patients with intracranial artery calcification than among those without calcification.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO492

### High Serum Prolactin (PRL) Predicts Mortality in Male Hemodialysis (HD) Patients

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Introduction. In the general population, hyperprolactinemia has been associated with an adverse cardiovascular risk profile. Elevated PRL levels are usually found in 25 to 75 % of HD patients, due to both increased production and decreased metabolic clearance. The current study was undertaken on male HD patients to examine a) the possible associations between PRL levels and cardiovascular markers, including arterial stiffness (AS), and b) the relationship between hyperprolactinemia and subsequent mortality due to all causes and cardiovascular disease (CVD).

Methods. One hundred and eleven HD men were assessed at baseline and were followed-up until a maximum of 51 months after the start of the study (median follow-up time 37 months).

Results. The median PRL value in our patient cohort was 16.3 ng/ml (interquartile ranges 8.4 - 32.9). PRL levels were inversely related to serum albumin, hemoglobin and smoking habits and were positively related to serum phosphorus, C-reactive protein (CRP), body mass index (MBI), prevalence of diabetes mellitus and pulse wave velocity (r=0.250; p<0.01), a reliable index of AS. During follow up, 49 deaths occurred, 28 (57%) of which were caused by CVD. As compared to those below the median value, patients with PRL levels above the median had an greater risk of CVD and all-cause mortality (crude hazard ratio: 2.24 [95% CI, 1.02 to 4.88] and 2.02 [95% CI, 1.13 to 3.61], respectively), even after adjustment for age, MBI, serum albumin and phosphorus, CRP, clinical history of CVD at baseline, presence of diabetes and smoking habits. The association of PRL levels with CVD mortality, but not with all-cause mortality, lost its significance after adjusting for pulse wave velocity

Conclusions. Our results indicate that PRL levels are inversely associated with all-cause and CVD mortality in HD men and that AS might be a potential mechanism underlying this association.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO493

### Risk Factors and Course of Peripheral Arterial Disease (PAD) in Incident Chronic Dialysis Patients (CDP)

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*Background*: PAD is a major risk factor of morbidity and mortality in CDP. The impact of conventional and uremia associated risk factors for PAD in CDP is not well defined.

*Aim*: To assess course of PAD in incident CDP and the role of potential risk factors.

*Method*: Prospective multicenter observational study over 24 month including all patients entering a chronic dialysis program. PAD was defined by ankle / brachial index (ABI) ≤ 0.9 and/or clinical signs. Primary endpoints were death, newly developed PAD

stage IV or therapeutic intervention for PAD; sec. endpoints: non lethal CV – complications, progression of preexisting PAD (ABI). Data are given as mean±SD; the impact of various risk factors was analysed by Cox proportional hazards survival analysis. Significance level p<0.05.

*Results*: 233 patients from 5 centres (m=64%; age 68.2±12.2; 10.5% PD) were included, 53.3% diabetics; 18.1% current smoker. Preexisting coronary artery disease was found in 39.5%. At start of dialysis 37.6 % had PAD. Mean ABI was 1.1±0.4 and did not change over 24 mo. The percentage of CDP with ABI <0.9 increased from 20 to 25%, ABI 0.9-1.1 decreased from 51.6 to 46.2% of pts. Primary endpoints were observed in 56.8%, sec. endpoints in 43.2% of pts. Fifty-five pts. died, 7 developed stage IV PAD, 21 had therapeutic interventions for PAD. Progression of PAD occurred in 57 pts., non-lethal CV-events in 6. Incidence of primary and sec. endpoints was higher throughout the first 12 months of study. Primary endpoints were associated with older age and neg. with S-Alb., a trend was shown for diabetes and blood pressure (n.s.). Fifty % of CDP not taking CaPB died compared to 25.3% on CaPB. Sec. endpoints did not correlate with any of the covariates tested, including S-chol., triglyceride, CRP, fibrinogen, t-homocystein, Ca, $PO_4$, iPTH, the use of CSEI or platelet inhibitors.

*Conclusions*: PAD in incident CDP was more frequent then reported previously. Primary endpoints developed in more than 50% of the patients, predominantly during the first year after start of dialysis. Only age, and S-Alb. and the intake of Ca-$PO_4$ binders were associated with primary endpoints.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO494

**Short and Long Term Blood Pressure Measurements and Cardiovascular Mortality in Hemodialysis Patients** Robert Ekart,[1] Radovan Hojs,[2] Sebastjan Bevc,[1] Vojko Kanic,[3] Karl Pecovnik.[1] *[1]Department of Dialysis, University Clinical Centre Maribor, Maribor, Slovenia; [2]Department of Nephrology, University Clinical Centre Maribor, Maribor, Slovenia; [3]Department of Cardiology, University Clinical Centre Maribor, Maribor, Slovenia.*

**Purpose of study**: Arterial hypertension (AH) is common and contributes to the high cardiovascular (CV) mortality in hemodialysis (HD) patients. The best method and timing for measuring blood pressure (BP) is still uncertain. It is unknown which BP measurement better defines the influence on CV mortality. The purpose of study was to analyze association between single BP measurements on HD day and ambulatory BP measurements (ABPM) and CV mortality in HD patients.

**Methods:** In our prospective study 73 HD patients (44 males and 29 females; mean age 54.2 years; 19-78 years;SD±13.3) were included. Thirty-two (43.8%) patients were habitual smokers and 11 (15.1%) patients were diabetics. BP was measured with a standard mercury sphygmomanometer before and after the HD session. 48-hour ambulatory BP measurements (ABPM) were performed after the end of HD session using a non-invasive ABPM monitor (Spacelabs 90207, USA). Using echocardiography (ATL HDI 3000) left ventricular mass (LVM) was measured and LVM index (LVMI) was calculated. Using B-mode ultrasonography (ATL HDI 3000), carotid intima-media thickness (IMT) was measured.

**Results:** The mean predialysis systolic/diastolic BP was 147/84 mmHg, mean postdialysis systolic/diastolic BP was 138/81 mmHg and the mean 48-hour systolic/diastolic ABPM was 135/80 mmHg. The mean LVMI was 148.9 g/m$^2$ (69-308;SD±49,44) and carotid IMT 0,78 mm (0,4-1.3;SD±0.2). During a follow-up period (mean 1795 days; 23-3664 days; SD±1063.5) 28 patients (38.3%) died, 16 (57%) of them of CV causes. In a Cox regression model that included age, gender, smoking, diabetes, sensitive CRP, albumin, haemoglobin, troponin T, LDL cholesterol, HDL cholesterol, triglycerides, Ca, P, carotid IMT and LVMI only 48-hour systolic ABPM (P=0.037) and 48-hour diastolic ABPM (P=0.006) turned out to be independent predictors of CV death.

**Conclusion**: Only 48-hour ABPM and not single BP measurements before or after HD were associated with CV mortality in HD patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO495

**Relation between Intradialytic Changes in Systolic Blood Pressure and Sodium Gradient in Chronic Hemodialysis Patients** E. Lars Penne,[1,2] Len A. Usvyat,[1] Samer Rateb Abbas,[1,2] Olga Sergeyeva,[1] Jochen G. Raimann,[1,2] Stephan Thijssen,[1,2] Nathan W. Levin,[1,2] Peter Kotanko.[1,2] *[1]Renal Research Institute, New York; [2]Beth Israel Medical Center, New York.*

Intradialytic hypertension has been associated with increased hospitalization and mortality rates in chronic hemodialysis (HD) patients. Intradialytic sodium loading has been proposed as a contributor of intradialytic hypertension. The aim of the present analysis was to evaluate the relation between intradialytic blood pressure changes and sodium gradient ($grNa^+$), defined as the difference between the dialysate sodium and the serum sodium concentration ($grNa^+ = dNa^+ - sNa^+$).

Chronic HD patients from nine Renal Research Institute clinics that did not use dialysate sodium profiling between Sep 1 2009 and Dec 31 2009 were included. The relation between the average $grNa^+$ and intradialytic changes in SBP (ΔSBP) during this period were analyzed with linear regression models, adjusting for patient characteristics (sex, age, race, diabetes, dialysis vintage) and ultrafiltration rate (UFR). In addition, odds ratios (OR) for an increase in SBP (ΔSBP > 0 mmHg) during treatment were estimated.

We studied 560 patients (age 61±15 [±SD] yr, 53% males and 49% blacks). Mean pre- and post-dialysis blood pressures were 149±19/77±12 and 139±18/72±10 mmHg, respectively. The mean $grNa^+$ was -2.2±2.7 mEq/L ($grNa^+$ > 0mEq/L in 19% of the patients). The adjusted $grNa^+$ was positively related to ΔSBP (B=0.5; 95% CI 0.1 to 1.0, P=0.02). Dialysis vintage (P=0.02), diabetes (P=0.004) and UFR (P=0.001) were inversely related to ΔSBP. SBP increased in 126 patients (23%). Increases in SBP were more often observed in patients with a positive gradient (OR = 2.1; 95% CI 1.3 to 3.3).

These data identify a positive sodium gradient as a contributing and potentially modifiable factor for intradialytic hypertension, which can probably be explained by intradialytic sodium loading. Whether intradialytic hypertension can be treated by lowering the dialysate sodium concentration in these patients needs further study.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO496

**Uremic Cardiomyopathy Is Associated with Reduced Cardiac High Energy Phosphate Metabolism: A Phosphorus Magnetic Resonance Spectroscopy Study** Rajan Kantilal Patel,[1] Patrick Barry Mark,[1] Kathryn K. Stevens,[1] Emily P. Mcquarrie,[1] Alan G. Jardine.[1] *[1]Renal Research Group, BHFGCRC, University of Glasgow, Glasgow, United Kingdom; [2]Department of Cardiology, Western Infirmary, Glasgow, United Kingdom.*

**Purpose:** Premature cardiovascular (usually sudden) death is the commonest cause of death in end stage renal disease (ESRD) patients and associated with uremic cardiomyopathy (comprising left ventricular hypertrophy (LVH), systolic dysfunction (LVSD) and LV dilation). High energy phosphate (HEP) metabolism, quantified using $^{31}$P magnetic resonance spectroscopy, is reduced in diabetic and heart failure patients. Phosphocreatine: β ATP ratio (PCr: β ATP) relates to cardiac metabolic activity. **Aim**: To compare resting HEP metabolism in ESRD patients and hypertensive LVH patients with normal renal function and assess associations with abnormalities of uremic cardiomyopathy. **Methods:** 53 ESRD and 30 hypertensive patients with LVH and normal renal function (LVH only) underwent cardiac MRI and $^{31}$P magnetic resonance spectroscopy of their LVs. Left ventricular dimensions were measured. PCr: β ATP ratios were calculated from $^{31}$P-MR spectra obtained from the LV. No spectra were obtained from areas of reduced/absent LV motion.**Results:** There were no significant differences in LV mass, chamber sizes and ejection fraction between patient groups. PCr:β ATP was significantly higher in LVH patients compared to ESRD patients (1.6±0.4 vs. 1.3 ±0.5 respectively; p=0.007). PCr: β ATP was significantly lower in ESRD patients with LV systolic dysfunction (n=10; no LVSD 2.0±0.5vs LVSD 1.2±0.2; p=0.05) and LV dilation (n=16; no LV Dilation 1.79±0.4 vs LV Dilation 0.98±0.8; p=0.01). LVH was not associated with significant difference in PCr: β ATP. **Conclusion:** Despite similar myocardial mass, ESRD patients have lower HEP metabolism compared to LVH patients. Lower PCr: βATP ratio is associated with features of uremic cardiomyopathy and may contribute to higher risk of sudden cardiac death.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO497

**Effect Modification by C-Reactive Protein and Interleukin-6 on the Relationship between Asymmetric Dimethyl-Arginine (ADMA), Death and Cardiovascular Events in End Stage Renal Diseases (ESRD) Patients** Giovanni Tripepi, Patrizia Pizzini, Sebastiano Cutrupi, Carmine Zoccali, Francesca Mallamaci. *CNR-IBIM, Clin. Epid. and Physiopath. of Renal Dis. and Hypertension, Reggio Calabria, Italy.*

Asymmetric dimethyl-arginine (ADMA) and inflammation have been implicated in atherosclerotic complications, death and cardiovascular (CV) events in patients with end stage renal diseases (ESRD). Inflammation amplifies the effect of ADMA on the severity of atherosclerosis in ESRD (*JASN 13: 490-496, 2002*) but it remains unclear whether inflammation and ADMA interact in the high risk of death and CV events in this population. In a cohort of 225 hemodialysis patients we investigated the interaction of CRP and IL-6 with ADMA as predictors of death and CV events (average follow-up: 42 months). The effect modification of CRP and IL-6 on the relationship between ADMA and study outcomes (additive model) was analysed by dividing the study population according to the corresponding median values of these biomarkers.

Circulating levels of ADMA (median: 2.4 μMol/L, inter-quartile range: 1.6-3.8 μMol/L), CRP (7.4 mg/L, 3.4-16.4 mg/L) and IL-6 (5.0 pg/mL, 2.7-9.2 pg/mL) were significantly inter-related (r ranging from 0.13 to 0.33, P<0.05). During the follow-up, 112 patients died and 104 had fatal and non fatal CV events. Both on unadjusted and adjusted Cox regression analyses, a biological interaction was found between inflammation biomarkers and ADMA for predicting death and fatal and non fatal CV events in ESRD patients. Indeed, the adjusted hazard ratios (HR) for death (HR: 2.08, 95% CI: 1.16-3.73) and CV outcomes (HR: 2.11, 95% CI: 1.17-3.83) of patients with increased CRP and ADMA were higher than those expected in the absence of interaction under the additive model (HR: 1.05 and HR: 1.79, respectively) and this was also true when the same analysis was carried out by stratifying according to IL-6.

These data support the hypothesis that inflammation amplifies the ADMA associated risk of death and CV events in ESRD patients. These analyses further emphasize the need of interventional studies to clarify the nature (causal/non causal) of these relationships.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO498**

**Impact of Arterial Stiffness on Adverse Cardiovascular Outcome and Mortality in Peritoneal Dialysis Patients** Murat H. Sipahioglu,[1] Hamit Kucuk,[1] Fatih Oguz,[2] Aydin Unal,[1] Bulent Tokgoz,[1] Oktay Oymak,[1] Cengiz Utas.[1] *[1]Nephrology, Erciyes University Medical Faculty, Kayseri, Turkey; [2]Cardiology, Erciyes University Medical Faculty, Kayseri, Turkey.*

Our aim was to determine whether arterial stiffness is an independent risk predictor of mortality and adverse cardiovascular (CV) outcomes in peritoneal dialysis (PD) patients.

Methods

This is a prospective, observational cohort study with 2 years of follow-up. A cohort of 156 PD patients was studied with mean follow-up of 19.2 (± 6.4) months. Aortic stiffness index β (ASI β) (as surrogate marker of arterial stiffness) were calculated using transthoracic echocardiography from the formulae: β=ln (Systolic BP/Diastolic BP)/[(SystolicDiameter-DiastolicDiameter)/DiastolicDiameter].

Results

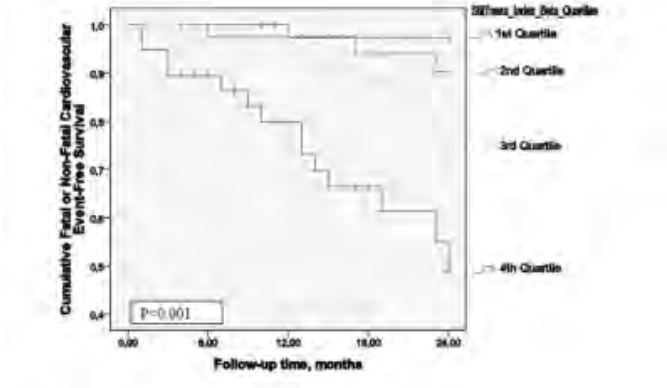
Baseline demographic, clinical and echocardiographic parameters were shown in Table 1.

Baseline Clinical and Biochemical Characteristics

| | |
|---|---|
| Age (years) | 48.2 ±13.7 |
| Sex (M:F) | 76:80 |
| Duration of dialysis (months) | 41.2 ±33.5 |
| Comorbid DM | 31 (20%) |
| Hb (g/dL) | 11.2 ±1.8 |
| Alb (g/dL) | 3.2 ±0.4 |
| LDL (mg/dL) | 123 ±39.9 |
| Total Kt/V | 2.48 ±0.78 |
| Residual GFR (ml/min1.73 m2 | 5.8 ±3.9 |
| D/P Creatinine | 0.70 ±0.11 |

DM:Diabetes mellitus

Twenty-five (16.0%) patients had died, 10 deaths were CV causes. Fifteen patients had non-fatal CV events. In the fully adjusted multivariable Cox regression analysis (age, gender, alb, hb, DM, comorbid CVD, LVMI, residual GFR, D/P creatinine, LDL were covariates) ASI β independently predicted fatal and non-fatal CV event and CV mortality [HR:1.244 (95 %CI 1.107-1.399)], [HR:1.298 (1.048-1.609)], but not all-cause mortality. Cumulative fatal or nonfatal CV event-free survival for ASI β-quartiles was shown.




Conclusion

To our knowledge, these results provide the first direct evidence that arterial stiffness is an independent risk predictor of adverse CV outcome and CV mortality in PD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO499**

**Pre-Dialysis Dietitian Care and Survival during the First Year on Dialysis** Yelena Slinin,[1] Haifeng Guo,[2] David T. Gilbertson,[2] Kristine E. Ensrud,[1] Allan J. Collins,[2] Areef Ishani.[1,2] *[1]Minneapolis VA Medical Center, Minneapolis, MN; [2]Chronic Disease Research Group, Hennepin County Medical Center, Minneapolis, MN.*

The National Kidney Foundation recommends management by a registered dietitian for patients with chronic kidney disease.

We aimed to determine whether dietitian care prior to ESRD is associated with normoalbuminemia at dialysis onset and lower 1 year mortality among incident hemodialysis patients.

Patients ≥20 years of age who initiated hemodialysis between 6/2005, and 6/2007 in the US and had pre-dialysis dietitian care reported on the Medical Evidence Form were included (N=156,440). Multivariate regression analyses were performed to identify associations with serum albumin (SA) ≥ 4gm/dL at dialysis initiation and to compare time to death on dialysis.

Dietician care was present for: none, 0-12 and > 12 months for 88%, 9%, and 3% of patients respectively and was tightly linked to pre-dialysis nephrology care. Adjusted odds ratios (95% CI) of having SA≥ 4gm/dL at the time of dialysis initiation was 1.11(1.07-1.16) and 1.15(1.08-1.23) for patients who had pre-dialysis dietitian care for 0-12 months and >12 months, respectively, compared to patients without dietitian care. The association between pre-dialysis dietitian care and survival during the first year on dialysis was explained by the difference in pre-dialysis nephrology care in the total cohort, but significant in the cohort limited to patients with nephrology care.

Table. Pre-dialysis Dietitian Care and Risk of Death during the First Year

| | Multivariable Model | | |
|---|---|---|---|
| | Not Including Nephrology Care | Including Nephrology | Limited to Patients with Nephrology Care |
| Variables | HR (95% CI) | | |
| Dietitian care | | | |
| None | 1 | 1 | 1 |
| 0-12 months | 0.95 (0.91-0.98) | 0.99 (0.95-1.03) | 1.02 (0.98-1.06) |
| > 12 months | 0.85 (0.79-0.91) | 0.97 (0.90-1.04) | 0.91(0.85-0.98) |

Pre-dialysis dietitian care is independently associated with SA≥ 4gm/dL at initiation. Pre-dialysis dietitian care for > 12 months is associated with lower mortality during the first year on dialysis in patients with pre-dialysis nephrology follow-up.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO500**

**The Effect of Sevelamer Hydrochloride (SE) on Arterial Stiffness and Left Ventricular Function. A Prospective Cohort Study** Yusuke Tsukamoto,[1] Soichiro Iimori,[2] Sei Sasaki.[1] *[1]Department of Nephrology, Shuwa General Hospital, Kasukabe-shi, Saitama-ken, Japan; [2]Department of Nephrology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.*

[Background] We previously reported that dialysis patients on SE resulted a reduced incidence of cardiovascular (CV) death [ASN09#TH-PO284]. This study analyzed the same cohort to demonstrate the etiology of lower CV death in SE by studying a change of arterial stiffness and left ventricular (LV) function. [Methods] Hemodialyzed patients (n=484) were enrolled between April/2005 and March/2008. CKD-MBD was treated according to the K/DOQI Bone and Mineral CPG. SE was the first choice of P-binder, unless GI side effect prevented. Since there was a significant difference of demographics between SE and Non-SE group at the end, these two groups were matched by propensity score (adjusted by age, vintage, gender and diabetes). In SE group, SE alone was 34 patients and 138 patients also with calcium carbonate. Cinacalcet was not used. Oral or IV vitamin D was given to 71% in SE (n=172) and 53% in non-SE (n=172) (p<0.001). Brachial-ankle pulse wave velocity (PWV) and echocardiography were recorded annually at same interval. [Results] (1) HDL cholesterol increased from 46±15 to 48.8±15.0 mg/dL in SE (p<0.01), but did not change in Non-SE (43.4±12.8 vs 45±13). LDL cholesterol increased from 83±24 to 92±31 mg/dL in Non-SE (p<0.001) but not in SE. LDL cholesterol level inversely correlated with SE dose. (2) In SE, mean arterial pressure did not change (112 vs 112 mmHg), but changed from 109 to 115 mmHg in Non-SE (p<0.0001). (3) PWV did not change (1750±453 vs 1799±497 cm/s) in SE, but changed from 1734±635 to 1829±677 cm/s in Non-SE (p<0.05). (4) LV mass index did not change either in SE or Non-SE. (5) Abnormal LV wall motion was recorded in 45.6% of SE and 54.4% of Non-SE at the beginning (p<0.05). There was a significant improvement in SE than in Non-SE (Mann-Whitney, Z=2.15, p<0.05). [Conclusion] There was an improvement of lipid abnormality, arterial mean pressure, arterial stiffness and LV wall motion in SE group when compared with Non-SE. That may explain the reason of reduced incidence of CVD death in hemodialyzed patients on SE.

Disclosure of Financial Relationships: Honoraria: I accepted a honoraria for the lectureship from Chugai Pharmaceutical Co., Japan and Kyowa-Kirin Pharmaceutical company.

**TH-PO501**

**Heart Failure Symptoms Are Not Reliable in Diagnosing Low Ejection Fraction in Incident Hemodialysis Patients: The Predictors of Arrhythmic and Cardiovascular Risk in ESRD (PACE) Study** Tariq Shafi,[1] Bernard G. Jaar,[1] Stephen M. Sozio,[1] Julia J. Scialla,[1] Pooja C. Oberai,[1] Zarqa Tariq,[1] Lucy A. Meoni,[1] Wen Hong Linda Kao,[1] Rulan S. Parekh.[2] *[1]Johns Hopkins University, Baltimore, MD; [2]University of Toronto, Toronto, ON.*

Congestive heart failure (CHF) in hemodialysis patients is an independent predictor of mortality and treatment of CHF with beta-blockers improves survival. Guidelines recommend echocardiograms (echo) after dialysis initiation and re-evaluation with an echo if "symptoms of CHF" occur. However, the validity of CHF symptoms in predicting low left ventricular ejection fraction (EF) among dialysis patients is not known. Our objective was to determine if symptoms of CHF in dialysis patients are associated with low EF measured on echo. The PACE study is an ongoing prospective cohort study of incident in-center hemodialysis patients in the Baltimore area. We administered Kansas City Cardiomyopathy Questionnaire (KCCQ), a valid and reliable health status measure of CHF in non-dialysis patients, to PACE participants. Higher KCCQ scores (range 0-100) indicate worsening symptoms. EF was measured by an echo performed on the interdialytic day. Low EF was defined as EF ≤40%. We used linear and logistic regression to assess the association between symptoms of CHF and EF. Information on EF and KCCQ was available for 111 PACE participants. Mean age was 54 yrs, 58% male and 64% African American. Mean EF was 61%; 6.3% had EF <40%. Among those with a self-reported history of CHF (n=17) compared to those without, there was no difference in EF (60% vs. 62%; p=0.6) or score on the symptoms domain of KCCQ (61±15 and 60±19; p=0.8). For each 20 higher symptom score, EF decreased by 3% but did not reach statistical significance (p=0.06). Symptom score was also not associated with low EF (odds ratio per 20 higher score, 1.47; p=0.5). Clinical symptoms of CHF in dialysis patients may not be a reliable indicator of EF. Sole reliance on symptoms to identify development of low EF may result in under-diagnosis, under-treatment and potentially, increased mortality. Routine echo should be considered as a part of dialysis care and monitored as a clinical performance improvement target.

Disclosure of Financial Relationships: Honoraria: Novartis.

**TH-PO502**

**Left Ventricular Dyssynchrony: A Potential Contributor to Left Ventricular Dysfunction in Chronic Kidney Disease?** Shirley Yumi Hayashi,[1,2] Marcelo M. Nascimento,[1] Astrid Seeberger,[1] Britta Lind,[2] Miguel C. Riella,[3] Britta Lind,[2] Lars-Åke Brodin,[2] Bengt Lindholm.[1] *[1]Baxter Novum & Renal Medicine Karolinska Institute, Stockholm, Sweden; [2]School of Technology and Health KTH, Royal Institute of Technology, Stockholm, Sweden; [3]Nephrology, Pontifícia Universidade Católica do Paraná, Curitiba, Brazil.*

Sudden death is common in chronic kidney disease (CKD) and could be associated with abnormalities in cardiac synchronicity. The aim of this study was to analyze cardiac synchronicity in patients with different CKD stages. Tissue doppler echocardiography (TDE) was used to evaluate LV synchronicity in 147 patients; 44 HD (29 men, 56.3±13.8 yr), 50 PD (28 men, 59.2±16.3yr) and 54 CKD stages 3-4 patients (34 men, 60.5±11.3yr) Left ventricular dyssynchrony (LVD) was defined as a regional difference in time to peak systolic myocardial velocity (PSv) or peak longitudinal strain (PSLS), between different LV walls > 105 ms in at least 2 of the following techniques; manual and automatic measurements of time to PSv using TDE and tissue synchronization imaging (TSI), respectively, and time to PSLS using speckle tracking imaging (STI). Twelve LV segments, six basal and six midventricular were evaluated. Abnormal LV synchronicity was present in 55 % of tho whole cohort with no difference in the prevalence between HD (52%), PD (53%) and CKD3-4(60%)($p>0.05$). Patients with LVD presented with significantly higher left atrium diameter (43.5±6.6 vs. 40.8±5.5, $p<0.05$), LV end diastolic diameter (50.4±7.8 vs. 47.5±6.9, $p<0.05$) and LV mass (245.7±81.0 vs. 206.2±70.9, $p<0.01$) compared to patients without LVD. LVD patients also showed significantly lower LV isovolumetric contraction velocities (2.5±1.6 vs. 3.4±1.6, $p<0.001$), PSv (4.3±1.4 vs. 5.4±1.6, $p<0.0001$), early diastolic myocardial velocities (4.4±1.8vs. 5.3±2.3, $p<0.01$), increased LV end diastolic pressure (22.3±14.4 vs. 15.7±10.5, $p<0.0001$) and a higher prevalence of pulmonary hypertension (30% vs. 9%, $p<0.05$). In conclusion, LV dyssynchrony was highly prevalent in CKD patients regardless to CKD stage showing a possible role in the mechanism of LV dysfunction in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO503**

**The Analysis of Competing Events like Cause-Specific Mortality – Beware of the Kaplan-Meier Method** Marion Verduijn,[1] Diana C. Grootendorst,[1] Friedo W. Dekker,[1] Kitty J. Jager,[2] Saskia le Cessie.[1] *[1]Leiden University Medical Center, Amsterdam, Netherlands; [2]Academic Medical Center, Amsterdam, Netherlands.*

**Introduction**

Kaplan-Meier analysis is a popular method used for analyzing time-to-event data, but profoundly overestimates the cumulative mortality probabilities in case of competing endpoints such as cardiovascular (CV) and non-CV mortality. This study compares application of 1) Kaplan-Meier analysis and 2) competing risk analysis for estimation of cumulative probabilities of CV and non-CV mortality in dialysis patients.

**Methods**

Data were used of 617 elderly incident dialysis patients from the Dutch NECOSAD cohort (>70 years). CV mortality was defined as death due to myocardial infarction or ischemia, hyper- or hypokalaemia, haemorrhagic pericarditis, hypertensive cardiac failure, other causes of cardiac failure, cardiac arrest, fluid overload, cerebro-vascular accident, haemorrhage from ruptured vascular aneurysm or mesenteric infarction, uncertain or unknown cause of death. All other causes of death were considered non-CV mortality. Follow-up was maximized at 5 years.

**Results**

Within 5 years of follow-up, 202 patients (32.7%) died due to CV causes (14.7 per 100 person-years), and 201 (32.6%) patients due to non-CV causes (14.5 per 100 person-years). The 5-year cumulative all-cause mortality probability estimated by Kaplan-Meier analysis was 78.3%. The 5-year cumulative mortality probability estimated using Kaplan-Meier analysis was 51.8% for CV causes and 55.1% for non-CV causes, suggesti ng that total (all-cause) mortality was 106.9%. In contrast, the 5-year cumulative probability for CV mortality estimated by competing risk analysis was 38.8% and for non-CV mortality 39.5%. The sum of CV and non-CV mortality from this model was 78.3%, equal to the cumulative 5-year all-cause mortality as obtained from Kaplan-Meier analysis.

**Conclusion**

This study demonstrates that, in contrast to the Kaplan-Meier method, application of competing risk analysis yields unbiased estimates of the cumulative probabilities for cause-specific mortality. Therefore, mortality from competing endpoints should not be reported from Kaplan-Meier based methods, but from competing risk analysis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO504**

**Regression of Left Ventricular Mass Following Conversion from Conventional Hemodialysis to In-Centre Nocturnal Hemodialysis** Ron Wald, Andrew T. Yan, Jeffrey Perl, Howard Leong-Poi, Marc B. Goldstein. *Department of Medicine and the Keenan Research Centre in the Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, ON, Canada.*

Background: Left ventricular mass (LVM) is closely associated with adverse outcomes in patients receiving chronic hemodialysis. Conversion from conventional hemodialysis (CHD, 3x/week, 4 hrs/session) to home nocturnal hemodialysis (5-6x/week, 7-8 hrs/session) may reduce LVM. However, a minority of patients with ESRD are eligible for home hemodialysis. For other individuals, intensification of dialysis with in-centre nocturnal hemodialysis (INHD, 3x /week, 7-8 hrs/session in the dialysis unit) may confer a similar cardiovascular benefit.

Methods: We evaluated changes in LVM following INHD conversion in individuals who received INHD for at least 6 months. In our primary analysis, LVM on the first echocardiogram performed at least 6 months post-conversion was compared to LVM on the study that most closely preceded conversion. In a secondary analysis, we restricted the cohort to patients with post-conversion echocardiograms > 12 months since starting INHD. To determine the effect of conversion to INHD on LVM over time, we performed a longitudinal analysis using a mixed model that incorporated all LVM data on patients who had at least two evaluable echocardiograms (n=41).

Results: 61 patients converted to INHD, of whom 37 were eligible for the primary analysis. Mean age at conversion was 49±12 yrs, median time on CHD was 4.0 (IQR 1.4-8.3) yrs and 30% were women. Principal causes of ESRD were glomerulonephritis (35%) and diabetes mellitus (32%). Mean pre-conversion LVM was 219±66 gm and following conversion, LVM declined by 32±58 gm ($p=0.002$). Among patients whose follow-up echocardiogram occurred at least 12 months following conversion (n=31), LVM declined by 40±56 gm ($p=0.0004$). The rate of change of LVM decreased significantly from 1 gm/yr before conversion, to -13 gm/yr in the post-conversion period (difference -12 gm/yr, $p<0.0001$).

Conclusion: Conversion to INHD from CHD is associated with a regression in LVM, which may portend a more favourable cardiovascular outcome.

Disclosure of Financial Relationships: Scientific Advisor: I have participated in advisory boards for Amgen and Gilead.; Other Relationship: I have received an unrestricted educational grant from Amgen.

**TH-PO505**

**Effects of Sodium Thiosulfate on Vascular Calcification in ESRD: A Pilot Study** James A. Delmez,[1] Eduardo Slatopolsky,[1] Anton Cabellon,[1] Lisa de las Fuentes,[1] Andrew J. Bierhals,[2] Prashanth Podaralla,[1] Santhosh Mathews,[1] Victor G. Davila-Roman.[1] *[1]Medicine, Washington University School of Medicine, Saint Louis, MO; [2]Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, MO.*

**Purpose:** Vascular calcification (VC) in ESRD patients is usually progressive and associated with increased mortality. Sodium thiosulfate (TS), an antioxidant that increases the solubility of Ca, may affect VC. The purpose of the study was to assess the effects of IV TS on VC of the coronary, carotid, and thoracic aorta and on L1-L2 vertebrae bone density. **Methods:** ESRD patients with initial Agatston scores >50 were imaged by 64-slice MDCT of the neck, chest, and lumbar spine at baseline and after 5 months of intravenous TS (12.5-18.75 gm given after each HD). Images were analyzed by 2 independent blinded observers. Annualized rate of change was calculated as the difference between the square roots of the follow-up and baseline calcium volumes; by definition, Progressors had annualized change ≥2.5 $mm^3$. Variables are mean ± SD. **Results:** Subjects completing the entire protocol (N=22) were 86% black, 59±10 years old, 41% diabetic, with a HD vintage of 67±52 months. Mean annualized change of the coronary, carotid, and thoracic aorta and L1-L2 vertebrae were not significant in the entire cohort. In subgroup analyses, Progressors (N=14; annualized change 12.3±7.1%) and Non-progressors (N=8; annualized change -3.2±3.7%) had similar baseline coronary artery scores (1813±1700 and 1622±1114, respectively; P=NS). There was no correlation between annualized change of the coronary arteries and anion gap (reflecting TS levels), fetuin, phosphorus, or CRP levels during treatment. The annualized change of the coronary arteries correlated with that of the thoracic aorta Ca (r=0.61, p=0.003) but not the carotid arteries or L1-L2. Although nausea was common, there were no serious adverse events related to TS treatment. **Conclusion:** TS treatment may safely decrease the rate of progression of VC in ESRD patients without effecting L1-L2 calcium. A large randomized, controlled study to further assess the safety and efficacy of this treatment should be performed.

Disclosure of Financial Relationships: Research Funding: Genzyme Corp.; Honoraria: Genzyme Corp.

**TH-PO506**

**Acute Coronary Disease and VDR Activation in Hemodialysis Patients** Luca Emilio Bernardi, Paola Padovese, Claudio Minoretti. *Nephrology and Dialysis, Sant'Anna Hospital, Como, CO, Italy.*

BACKGROUND

25-OH Hydroxyvitamin D levels inversely associate with the risk of developing coronary artery calcification (De Boer, JASN 2009), while active serum vitamin D levels show inverse correlation with coronary calcifications (Watson, Circulation 1997).

AIM

To study VDR activators potential to prevent coronary artery disease (CAD) in hemodialysis (HD) patients.

MATERIAL AND METHODS

We studied 578 patients who underwent dialysis treatment since 1/1/2002 until 31/12/2009 in our Center. 81/578 patients (14,01%) underwent coronary angioplasty during follow up time.

Table 1

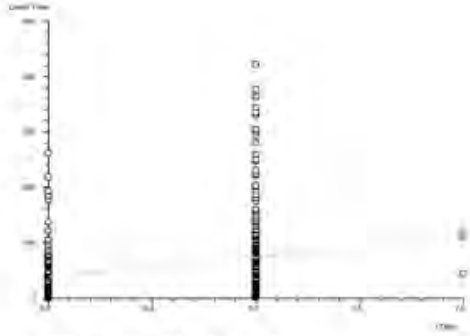
| | | |
|---|---|---|
| Patients Number 578 | Male 350 (60.55%) | Female 228 (39.45%) |
| | Coronary Angioplasty 81 | No Angioplasty 497 |
| | Dead at follow up 346 | Alive at follow up 232 |
| HD starting age (months±standard error) 66.12±0.58 | Median 69 | Range 16-91 |
| Death time since starting HD (months±standard error) 62.76±3.83 | Median 41 | Range 1-422 |

RESULTS

Cross tabulation with VDR activators as first classifier and coronary angioplasty as second classifier showed a significant statistical difference between groups (p=0.0077).

The relationship between mortality and VDR activators showed a correlation coefficient significantly different from zero (P<0.0001).

Figure 1



Legend: **mortality and VDR activators**
First column = No VDR activator therapy
Second column: oral or endovenous calcitriol therapy
Third column: calcitriol plus paricalcitol

Oral calcitriol was inversely related with coronary artery disease (two sided p = 0.0011), while multiple linear regression model showed a reduction in mortality rate using oral and endovenous calcitriol and paricalcitol (p=0,0374, p=0.0881 and p<0.0001 respectively).

DISCUSSION

VDR activation seems to protect dialysis patient from both overall mortality and coronary artery disease. Paricalcitol seems to be more effective in our population in reducing overall mortality, while the lack in reducing coronary artery disease as calcitriol should be related to the relatively recent introduction of paracalcitol in our center, reducing a potential effect in early population.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO507

**LDL Size Reduction as a Possible Risk Factor for the Prevalence of Coronary Artery Diseases in Hemodialysis Patients** Hideki Kimura,[1] Ryoichi Miyazaki,[2] Shinya Masunaga,[3] Akihiro Shimada,[3] Toshio Imura,[3] Daisuke Mikami,[1] Kenji Kasuno,[1] Naoki Takahashi,[1] Tsutomu Hirano,[4] Haruyoshi Yoshida.[1] *[1]Div of Nephrol, Sch of Med, Univ of Fukui, Fukui, Japan; [2]Dept. of Internal Med., Fujita Memorial Hosp, Fukui, Japan; [3]Dept of Clin Lab, Univ of Fukui Hosp, Fukui, Japan; [4]Division of Diabetes and Metabolism, Showa Univ Sch of Med, Tokyo, Japan.*

Smaller LDL size has recently been reported as a non-traditional lipid risk factor for coronary artery disease (CAD). Cholesteryl ester transfer protein (CETP) and the hepatic lipase (HL) C/T mutation may promote LDL size reduction via the CETP-mediated exchange of CE for TG and subsequent HL-mediated TG hydrolysis in LDL. However, little is known about LDL size status and its relationship with CAD prevalence in hemodialysis (HD) patients who are at high risk for atherosclerosis.

We determined CETP levels, HL genotype, and LDL size in a total of 236 HD patients aged over 30 years and investigated the determinants of LDL size and its association with CAD prevalence.

The HD patients had a significant negative correlation between LDL size and serum TG levels, and a similar LDL size to the healthy subjects. In the HD group, HDL-C was an independent positive determinant of LDL size, while log10 (TG) was an independent negative determinant in the high (≥2.1 μg/ml) but not low (<2.1 μg/ml) CETP group. In the patients with hypertriglyceridemia, the high CETP group had a significantly smaller LDL size than the low CETP group (256.9 ± 3.7 Å vs. 264.7± 6.6 Å). Among the patients with above-median TG levels, the CC genotype and CETP were independent negative determinants of LDL size, as these factors enhanced the negative slope of the regression line relating serum TG levels and LDL size. In the whole group and the high CETP group, the patients with CAD had a significantly smaller LDL size than those without CAD (259.2 Å vs. 262.0 Å and 258.3 Å vs. 261.5 Å). Finally, multivariate stepwise logistic regression analyses showed that DM and LDL size reduction were identified as independent risks factor for CAD prevalence.

These suggest that LDL size reduction mediated by TG, CETP, and HL genotype may serve as a risk factor for CAD in HD patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO508

**Impact of Circulating Soluble Receptor for Advanced Glycation End Product (sRAGE) and the Pro-Inflammatory RAGE Ligand (EN-RAGE, S100A12) on Mortality in Hemodialysis Patients** Ayumu Nakashima, Juan J. Carrero, Abdul Rashid Tony Qureshi, Tetsu Miyamoto, Björn Anderstam, Peter F. Barany, Olof Heimburger, Peter Stenvinkel, Bengt Lindholm. *Divisions of Renal Medicine and Baxter Novum, Department of Clinical Science, Karolinska Institutet, Stockholm, Sweden.*

**Background:** The soluble receptor of advanced glycation end products (sRAGE) may exert anti-inflammatory protective roles on the vasculature. In contrast, the RAGE ligand S100A12 (also known as EN-RAGE) contributes to inflammation and the development of atherosclerosis in animal models. Whether alterations at this level contribute to the increased mortality observed in dialysis patients is currently unknown.

**Methods:** This prospective study included 184 prevalent hemodialysis (HD) patients and 50 healthy controls matched for age and gender. Plasma concentrations of S100A12 and sRAGE were studied in relation to risk profile and mortality after a median follow-up period of 41 months.

**Results:** S100A12 and sRAGE levels were significantly elevated in HD patients compared to controls. S100A12 had a strong positive correlation with CRP and IL-6, whereas sRAGE negatively associated with CRP. S100A12, but not sRAGE, was independently and positively associated with clinical cardiovascular disease (CVD). During follow-up, 85 (33 CVD-related) deaths occurred. Whereas sRAGE did not predict mortality, S100A12 was associated with both all-cause (per log10 ng/ml hazard ratio [HR] 1.93, 95% confidence interval [CI] 1.18 to 3.15) and CVD-related (HR 3.23, 95% CI 1.48 to 7.01) mortality, even after adjustment for age, sex, vintage, and co-morbidity. Further adjustment for inflammation made the prognostic value of S100A12 disappear for all-cause mortality, while it still persisted for CVD-related mortality.

**Conclusions:** Circulating S100A12 and sRAGE are both elevated in HD patients. However, only S100A12 associates with mortality, which only partly is explained by its links with inflammation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO509

**Prognostic Values of C-Reactive Protein Levels on Clinical Outcome after Endovascular Therapy in Hemodialysis Patients with Peripheral Artery Disease** Hirotake Kasuga,[1] Tetsuya Yamada,[2] Nobumasa Nakamura,[1] Ryo Takahashi,[1] Keiko Kimura,[1] Takanobu Toriyama,[1] Seiichi Matsuo,[3] Hirohisa Kawahara.[1] *[1]Internal Medicine, Division of Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Japan; [2]Seto Kyoritsu Clinic, Seto, Japan; [3]Nephrology, Nagoya University Hospital, Nagoya, Japan.*

**Background:** Endovascular therapy (EVT) has become the common therapeutic standard for peripheral artery disease (PAD). However, the high restenosis rate after EVT remains a major problem in patients on HD. Recent studies suggest that C-reactive protein (CRP) reflects vascular wall inflammation and can predict adverse cardiovascular events. We evaluated the possible prognostic values of CRP on clinical outcomes after EVT in HD patients.

**Methods**: A total of 234 HD patients successfully undergoing EVT for PAD were enrolled and were followed-up for up to 5 years. Serum CRP levels were measured prior to EVT. They were divided into tertiles according to serum CRP levels; tertile 1 (T1): < 1.4 mg/l, T2: 1.4 – 6.0 mg/l, and T3: ≥ 6.0 mg/l). We analyzed the incidence of major adverse cardiovascular events (MACE) as a composite endpoint including all-cause death, target lesion revascularization (TLR) after EVT, and amputation.

**Results:** During follow-up period (33±21months), 76 TLR (32%) and 35 amputations (15%) were performed, and 53 patients died (23%). The event-free survival rate from MACE for 5 years was 60.2%, 50.0%, and 25.1% in the T1, T2 and T3, respectively (p<0.0001). In addition, freedom rate from TLR was 72.5%, 64.1% and 44.5% (p=0.0014), and all-cause survival rate was 81.5%, 65.2% and 59.3% in the T1, T2 and T3, respectively (p=0.0078). The difference of limb salvage rate was not significant (88.7%, 77.3% and 74.8% in T1, T2 and T3, respectively, p=0.15). Even after adjustment for other risk factors, pre-procedural CRP levels were a significant predictor for MACE after EVT (HR 1.93, 95%CI 1.07-3.49 for T2 vs. T1 and HR 3.08, 95%CI 1.75-5.41 for T3 vs. T1, p=0.0004 for trend). Elevated CRP levels were also independently associated with TLR and mortality after EVT, respectively.
**Conclusions:** Elevated pre-procedural serum CRP levels could strongly predict MACE after EVT in HD patients with PAD.

Disclosure of Financial Relationships: nothing to disclose

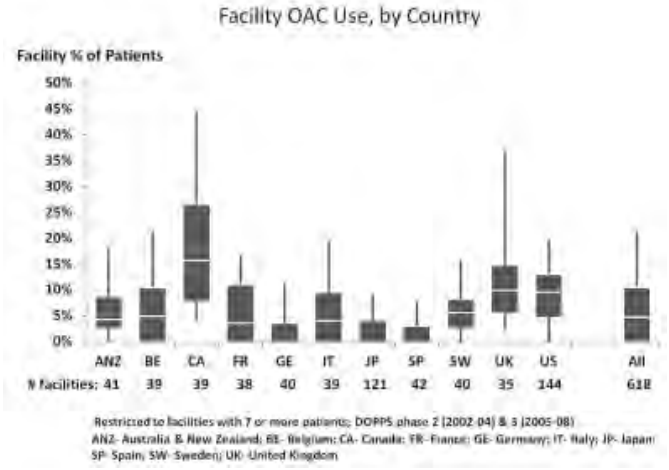
## TH-PO510

**Oral Anticoagulants (OAC): Prescription Patterns and Outcomes in the Dialysis Outcomes and Practice Patterns Study (DOPPS)** Manish M. Sood,[1] Jyothi R. Thumma,[2] Francesca Tentori,[2] Brenda W. Gillespie,[3] Shunichi Fukuhara,[6] David C. Mendelssohn,[4] Kevin Chan,[5] Bruce M. Robinson.[2,3] *[1]St. Boniface Gen. Hosp., Winnipeg, MB, Canada; [2]Arbor Research, Ann Arbor, MI; [3]Univ. of Michigan, Ann Arbor, MI; [4]Humber River Regional Hosp., ON, Canada; [5]FMC North America, MA; [6]Kyoto Univ., Japan.*

Indications for OAC use in hemodialysis (HD) remain controversial. We report OAC prescription and clinical events in the international DOPPS cohort.

The study sample comprised 37,863 HD patients (pts) with no mechanical heart valves from 925 facilities in the US, Canada (CA), Japan (JP), Europe + Australia and New Zealand [Eur/ANZ] in 1996-2008. Associations between baseline OAC prescription and bleeding events, stroke, and mortality were assessed in fully adjusted Cox models.

OAC prescription was 18% in CA, 10% in the US, 5% in Eur/ANZ, and 3% in JP. OACs were more commonly used in pts with atrial fibrillation (40% in CA, 34% in the US, 20% in Eur/ANZ and 13% in JP); OAC use in pts using a catheter was more common in CA (22%) vs. the US ( 8%) and Eur/ANZ (6%). Among patients on OACs, low dose warfarin (<10.5 mg/week) was prescribed to a minority of pts (31% in CA; 29% in JP; 17% in Eur/ANZ and the US). The % of facility pts on OACs varied in each country.




Older age, low serum albumin, cardiovascular and lung disease were associated with OAC use. Compared those not on OAC, OAC patients had a higher risk of bleeding events (hazard ratio: 1.19 [95% confidence interval: 1.04-1.37]; p=0.01), all-cause (1.16 [1. 08-1.25]; p<.0001) and cardiovascular mortality (1.13 [1.01-1.26]; p=0.03) and stroke (1.13 [0.92-1.38]; p=0.25).

OAC use varies internationally and is associated with poor clinical outcomes, despite extensive case-mix adjustment. Health care providers should consider the potential harms of OAC therapy in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO511**

**Contrasts between Risk Factors for Stroke and Other Cardiovascular (CV) Events in Hemodialysis (HD) Patients: Data from the DOPPS** Christian Combe,[1] Jyothi R. Thumma,[2] Jonathan W. Bazeley,[3] Brenda W. Gillespie,[3] Hiroyasu Yamamoto,[5] Werner Kleophas,[4] Martin P. Gallagher,[6] Friedrich K. Port,[2] Bruce M. Robinson,[2,3] Patricia De Sequera.[7] *[1]CHU de Bordeaux, France; [2]Arbor Research; [3]Univ. of Michigan; [4]Dialysezentrum Karlstrasse, Germany; [5]Jikei Univ. School of Medicine, Japan; [6]Concord Repatriation & Gen. Hosp., Australia; [7]Hosp. Infanta Leonor, Spain.*

CV morbidity and mortality rates are high in HD patients (pts). Studies of therapeutic interventions in clinical trials for composite CV endpoints have led to conflicting results, which may be due to non-homogeneous associations of risk factors (RF) with different types of CV events.

Data were from 41,208 HD pts followed for a median of 1.5 years in the DOPPS, an international prospective cohort study. Cox regression was used to evaluate associations of different types of events with traditional and non-traditional RF (listed in table).

The number of events ranged from 1,202 (Amp) to 9,863 (ACM). Associations between RF and events are reported in Table.

| | ACM | CVM | SD | MI | Stroke | HF | Amp |
|---|---|---|---|---|---|---|---|
| *Traditional risk factors (hazard ratio (HR)):* | | | | | | | |
| Age (per 1 yr) | 1.03[A] | 1.03[A] | 1.03[A] | 1.03[A] | 1.03[A] | 1.01[A] | 1 |
| Male (vs. female) | 1.09[A] | 1.11[B] | 1.09 | 1.21[A] | 0.88[B] | 1 | 1.34[A] |
| Black (vs. non-black) | 0.91[B] | 0.90 | 0.93 | 0.75[A] | 0.84 | 0.98 | 1.09 |
| Active smoker [C] | 1.12[A] | 1.05 | 1.07 | 1.06 | 0.95 | 1.19[A] | 1.03 |
| SBP (per 10 mmHg) | 0.96[A] | 0.95[A] | 0.95[A] | 0.98[B] | 1.06[A] | 0.99 | 1.01 |
| BMI (per 1) | 0.97[A] | 0.97[A] | 0.97[A] | 0.98[A] | 0.97[A] | 0.99[B] | 0.99 |
| Diabetes (yes vs. no) | 1.19[A] | 1.35[A] | 1.36[A] | 1.34[A] | 1.46[A] | 1.11[B] | 0.60[A] |
| HDL (per 10 mg/dl) | 0.97[B] | 0.98 | 0.94 | 0.95 | 1.04 | 0.98 | 1.05 |
| LDL (per 30 mg/dl) | 0.98 | 0.95 | 0.95 | 1.01 | 0.94 | 0.96 | 0.98 |
| *Non-traditional risk factors (HR):* | | | | | | | |
| Albumin (per 1 g/dl) | 0.70[A] | 0.79[A] | 0.79[A] | 0.80[A] | 0.87[A] | 0.91[A] | 0.83[A] |
| WBC (per 1000/ml) | 1.04[A] | 1.04[A] | 1.03[A] | 1.05[A] | 1 | 1.03[A] | 1.06[A] |
| $PO_4$ (per 1 mg/dl) | 1.07[A] | 1.12[A] | 1.12[A] | 1.11[A] | 1.08[A] | 1.08[A] | 1.10[A] |
| Calcium (per 1 mg/dl) | 1.10[A] | 1.12[A] | 1.11[A] | 1.16[A] | 1.02 | 1.03 | 1.06 |
| PTH (per 200 pg/ml) | 1.03[A] | 1.04[A] | 1.03[A] | 1.03[A] | 1 | 1.01 | 0.99 |
| Hgb (per 1 g/dl) | 0.95[A] | 0.97[A] | 0.95[A] | 0.99 | 0.97 | 0.95[A] | 0.93[A] |

Elevated risk | Reduced risk | Weak or no association (p≥0.05)

[A] p<0.001; [B] p=0.001 - <0.05

-Models adjusted for above RF, 13 summary comorbidities, years on dialysis, ferritin, WBC, potassium, creatinine, catheter; stratified by phase, country and accounted for facility clustering.
-ACM=all-cause mortality; CVM=CV mortality; SD=sudden death; Amp=amputation; MI, Stroke, HF = death or hospitalization from myocardial infarction, stroke, or heart failure, respectively.
-CVM includes death from MI, stroke, HF, and sudden death
[C] Reference: Previous smoker (>1 yr since quit) or non-smoker

Key findings include: male sex associated with lower risk of stroke, but higher risk of other CV events. Black race associated with lower risk for MI than other events. Smoking associated with higher risk of ACM and HF. Higher WBC associated with higher risk of all events except stroke. Higher SBP associated with lower risk for all events except stroke. Treated categorically, SBP <130 associated with elevated mortality for all events except stroke (HR 0.87, p=0.045) and SBP ≥160 associated with elevated mortality for stroke (HR 1.28, p=<.0001) and HF (HR 1.14, p=0.002), but not other CV events.

In HD pts, different types of CV events are associated with specific RF patterns: Stroke is associated with a specific RF pattern, with higher risk in females and with high SBP, in contrast to other CV events.

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**TH-PO512**

**Lowering Dailysate Calcium Concentration from 3.0 mEq/L to 2.5 mEq/L Attenuates the Progression of Abdominal Aortic Calcification in Patients on Chronic Hemodialysis** Kazuhiro Yamada, Shouichi Fujimoto, Yuji Sato, Kazuo Kitamura. *Department of Internal Medicine, Circulatory and Body Fluid Regulation, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan.*

Background: Vascular calcification is an independent determinant of cardiovascular events in patients on chronic hemodialysis (HD). However, the relation between dialysate Ca concentration and vascular calcification has not been clarified. This study was planned to clarify the effect of lowering dailysate Ca concentration from 3.0 mEq/L to 2.5 mEq/L on the progression of abdominal aortic calcification in HD patients.

Methods: We enrolled 44 HD patients with dialysate containing 3.0 mEq/L of Ca, and randomly 26 HD patients (lowering group) lowered the their dialysate Ca concentration from 3.0 mEq/L to 2.5 mEq/L after about three years. Eighteen patients (control group) continued HD with dialysate containing 3.0 mEq/L of Ca. All patients underwent abdominal computed tomography (CT) three times at an interval of approximately three years. The aortic calcification index (ACI) was quantified morphometrically using abdominal CT films (range of ACI, 0–240). The progression rate of aortic calcification was calculated as ΔACI/year.

Results: The first ACI and ΔACI/year was no significant difference in lowering and control group (first ACI: 42.7 ± 42.8 vs 57.4 ± 38.1, first ΔACI/year: 6.7 ± 6.4 vs 8.5 ± 6.4). In lowering group, the second ΔACI/year, after lowering dialysate Ca concentration, was significantly decreased compared with the first ΔACI/year (6.7 ± 4.3 vs 4.9 ± 5.1, p < 0.0263, Wilcoxon signed rank test). On the other hand, in control group the second ΔACI/year was significantly increased compared with the first ΔACI/year (8.5 ± 6.4 vs 12.1 ± 7.2, p < 0.0249). In stepwise multivariate analysis the progression of ACI after lowering dailysate Ca concentration was negatively and independently associated with lowering dailysate Ca concentration, age and serum Ca concentration just after HD, and positively associated with serum phosphate.

Conclusion: Lowering dailysate Ca concentration from 3.0 mEq/L to 2.5 mEq/L attenuated the progression of abdominal aortic calcification in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO513**

**Higher Plasma Fibroblast Growth Factor-23 Level Is Associated with Lower Body Mass Index and Dyslipidemia in Dialysis Patients** Jessica B. Kendrick,[1] Alfred K. Cheung,[2,4] James S. Kaufman,[3] Tom H. Greene,[4] William L. Roberts,[4] Gerard John Smits,[1] Michel B. Chonchol.[1] *[1]University of Colorado Denver Health Sciences Center, Denver, CO; [2]VASLCHCS, Salt Lake City, UT; [3]VA Boston Healthcare System, Boston, MA; [4]University of Utah, Salt Lake City, UT.*

Purpose: Fibroblast growth factor-23 (FGF-23) has recently been associated with death in dialysis patients. Since FGF-23 shares structural features with the FGF-19-subfamily members (FGF-19, FGF-21) that exert hormonal control of fat mass and glucose metabolism, we hypothesized that FGF-23 would be associated with metabolic factors that predispose to increased cardiovascular risk.

Methods: Study was conducted among 654 patients receiving dialysis who participated in the Homocysteine in Kidney and End Stage Renal Disease study. FGF-23 levels were measured in stored plasma samples. Linear regression was used to examine the cross-sectional associations of plasma FGF-23 with body mass index (BMI), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) and total triglycerides (TG).

Results: Participants had a mean age of 60±11 years and median dialysis vintage of 1.4 years. Mean (SD) serum phosphorus and the median [IQR] FGF-23 levels were 5.6±1.8 mg/dL and 4212 [1411-13816] RU/mL, respectively. The mean (SD) BMI, TC, LDL-C, HDL-C and TG were 27±6 kg/m2, 156±39 mg/dL, 84±32 mg/dL, 41±14 mg/dL and 160±107 mg/dL. An increase in log10 FGF-23 was associated with lower BMI (β= -1.11 95% CI -1.91, -0.31; p=0.008), total cholesterol (β= -6.46 95% CI -11.96, -0.96; p=0.02), LDL-C (β= -4.73 95% CI -9.23, -0.1; p=0.04) and HDL-C (β= -2.14 95% CI -4.08, -0.20; p=0.03), after adjusting for age, gender, race, serum albumin, calcium, phosphorus, 25-hydroxyvitamin D, calcitriol, and parathyroid hormone levels. No significant relationship was observed between FGF-23 and TG (p=0.80).

Conclusions: Higher FGF-23 levels are associated with lower BMI and dyslipidemia in dialysis patients. These findings suggest the relationship between FGF-23 and death in dialysis patients may be mediated through risk factors unrelated to mineral metabolism.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO514**

**FGF-23 Is Associated with Cardiac Troponin, Osteocalcin and Mortality in Prevalent Hemodialysis Patients** Rachel M. Holden,[1] David P. Beseau,[2] Michael A. Adams,[2] Jocelyn S. Garland,[1] Alexander R. Morton,[1] Robert N. Foley.[3] *[1]Medicine, Queen's University, Kingston, ON, Canada; [2]Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada; [3]Chronic Disease Research Group, Minneapolis, MN.*

FGF-23 is elevated in patients with ESKD and is linked with adverse patient outcomes including mortality and left ventricular hypertrophy. We determined the correlates of FGF-23 (including cardiac troponin T (cTNT) and osteocalcin (OC)) and determined its association with mortality over 3.5 years of follow-up in 103 prevalent hemodialysis patients. Linear regression was used to examine the association between FGF-23 and age, gender, dialysis vintage, albumin, calcium, phosphate, hemoglobin, Kt/V, PTH, cholesterol, triglycerides, cTNT and total and uncarboxylated OC. Unadjusted comparisons by tertile of FGF-23 were also performed and adjusted odds ratios were calculated for being in the highest tertile of FGF-23. Cox proportional hazards models were used to examine the effect of FGF-23 (per 1000 RU/ml) as well as patient characteristics and laboratory variables on mortality. The mean age was 61.2(15.5), 33% were female and the mean dialysis vintage was 4.19 (4.6) years. The mean FGF-23 level was 2732±4506 RU/ml and the median [IQR] was 1259 (491,2885) RU/mL. In multivariable analysis including adjustment for age and phosphorus, independent predictors of FGF-23 concentrations included dialysis vintage, total OC, uncarboxylated OC and cTNT. The phosphate and age-adjusted odds ratio (CI) of being in the highest tertile of FGF-23 included total OC (1.02(1.0,1.04). There were 57 deaths in 103 patients over 3.5 years of follow-up. In the fully-adjusted model, the significant predictors of mortality included FGF-23 (HR 1.09 per 1000 units increase: 1.01, 1.17, p=0.02), age and albumin. FGF-23 is an independent biomarker that predicts mortality in ESKD. The independent association between FGF-23 and cTNT is a novel finding that may relate to hypertrophic changes of left ventricular geometry that have been associated with these biomarkers. The association between higher levels of OC and FGF-23, both products of the osteoblast, is a novel finding and requires further study.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO515**

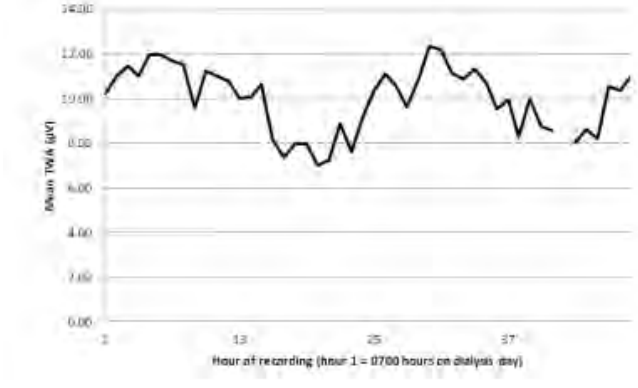
**Diurnal Variation of Ventricular Repolarization in Dialysis Patients** Darren Green,[1] Velislav N. Batchvarov,[2] Ganepola Arachhige Chandrkumara Wijesekara,[1] David I. New,[1] Philip A. Kalra.[1] *[1]Department of Renal Medicine, Salford Royal Hospital, Salford, United Kingdom; [2]Division of Cardiac and Vascular Sciences, St. George's Hospital, London, United Kingdom.*

Sudden cardiac death (SCD) is a major cause of death in dialysis patients, with a peak incidence on dialysis days. T wave alternans (TWA) measures beat to beat variation in t wave amplitude on ECG and is associated with risk of ventricular arrhythmia. It is recognized to be useful in risk stratification for SCD in other patient groups but not yet dialysis patients. Modified Moving Average (MMA) TWA is recorded via ambulatory ECG to give continual TWA. This may show change in risk relative to timing of dialysis.

We recorded 48 hour, 12 lead Holter ECG on 19 chronic haemodialysis patients from the onset of dialysis. This is the first patient group to have undergone MMA TWA testing. We previously reported an increase in TWA during dialysis with a return to baseline 2 hours after dialysis. Here we describe further analysis from this cohort.

For this analysis, the hourly group mean of TWA was sorted by time of day rather than by time from onset of recording. Figure 1 shows the hourly group mean TWA over a 48 hour period from 0700 hours on the dialysis day. One aberrant peak has been removed (mean TWA = 12.90 µV at 0100 hours on day 2, caused by a prolonged rise in a single patient). Figure 1 shows a peak TWA at approximately 1200 hours with a trough in measurement 12 hours later, on both days.

Figure 1




This is the first evidence of diurnal variation in TWA in any patient group. Its occurrence in dialysis patients may indicate instability of ventricular repolarisation specific to this group, though similar analysis in other at-risk populations is necessary for validation. This may provide some support for the high incidence of SCD in these patients as abnormal repolarisation is a potential trigger for ventricular arrhythmia.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO516**

**Differentiated Role for Novel Biomarkers in Cardiovascular Calcification in ESRD** Theresa Gross,[1] Markus Ketteler,[2] Jurgen Floege,[1] Leon J. Schurgers,[3] Thilo Krueger,[1] Georg Schlieper,[1] Vincent Brandenburg.[4] *[1]Nephrology, RWTH University Hospital Aachen, Aachen, Germany; [2]Nephrology, Klinikum Coburg, Coburg, Germany; [3]CARIM, University of Maastricht, Maastricht, Netherlands; [4]Cardiology, RWTH University Hospital Aachen, Aachen, Germany.*

**INTRODUCTION AND AIMS:** Cardiovascular calcification is associated with high mortality in ESRD patients. Novel serum/plasma biomarkers may help establishing the diagnosis and estimating severity of the disease.

**METHODS:** We investigated 66 chronic HD patients (52% female) with MSCT scans for coronary artery calcification (CAC) and aortic valve calcification (AVC). We analyzed various potential serum / plasma biomarkers regarding their association with CAC and AVC, respectively: adiponektin, fetuin-A, osteoprotegerin (OPG), uncarboxylated matrix-gla protein (ucMGP), osteocalcin, CRP, PTH, alk. phos., sclerostin, and YKL-40.

**RESULTS:** CAC defined as Agatston score > 100 was present in 65%, any degree of AVC in 38% of pts. We stratified pts according to CAC (> 100 vs < 100 Agatston score) and AVC (present vs absent), respectively, into two groups each. There was no strong association between CAC and AVC: pts with CAC > 100 had no AVC in 50% of cases; on the contrary pts without AVC had a median CAC of 169 (range 0 - 1542). Regarding biomarkers, intergroup comparison for CAC revealed significant ($p<0.05$) higher OPG (5.8 +/- 2.6 vs 4.1 +/- 2.6 pmol/L) and YKL-40 levels (288 +/-91 vs 230 +/- 98 ng/mL) for pts with CAC>100 vs pts with CAC <100. In pts with AVC vs pts without AVC, serum sclerostin was elevated: 1.83 +/- 0.84 vs 1.35 +/- 0.72 ng/mL ($p<0.05$). Regression analyses revealed a sign. positive association between AVC and sclerostin serum levels.

**CONCLUSIONS:** Cardiovascular calcification is highly prevalent in chronic HD patients. The occurrence of ACV and CAC do not correlate in our HD pts. Concerning the association to cardiovascular calcification, the biomarkers applied show a different behaviour regarding their association to CAC and AVC. Of the novel biomarkers, high YKL-40 and OPG were associated with the presence of CAC, while sclerostin was associated with the presence and the severity of AVC in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO517**

**Prevalence, Period Trends and Survival of Dialysis Patients with Peripheral Arterial Disease in the United States: 1995-2004** Austin G. Stack,[1] Kieran Hannan,[2] Catherine A. Wall.[3] *[1]Regional Kidney Centre, Letterkenny General Hospital, Letterkenny, Donegal; [2]Cavan General Hospital, Cavan; [3]Nephrology Department, Adeliade and Meath Hospitals, Tallaght, Dublin, Ireland.*

Peripheral Arterial Disease (PAD) is a major risk factor for death in dialysis patients. The principal aim was to assess trends in prevalence and associated mortality in the U.S. dialysis population.

Methods: Data on all new dialysis patients (N=1,003,305) between 1995-2004 were obtained from the US Renal Data System, a national registry of the U.S. End Stage Kidney Disease population. Data on patient characteristics recorded at dialysis onset was linked with mortality data from the CMS Death Notification files. Patients were followed until 4/10/2006. Annual Mortality was calculated for those with and without PAD by year of incidence from 1995-2004. Multivariable Cox regression was used to estimate 2- year mortality hazard (RR) ratios for each calendar year (with 2000 as referent) in sequentially adjusted models. The final multivariable model was adjusted for demographic, socioeconomic, and comorbid factors (n=21). Analyses were conducted using SAS V 9.12.

Results: The prevalence of PAD has remained relatively stable between 14-15 % from 1995-2004. Over 50% of patients were dead within 2 years of dialysis initiation. The adjusted mortality risk has decreased at most by 14% since 1998 and 7% since 2000.

| Year | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 |
|---|---|---|---|---|---|---|---|---|---|---|
| PAD (%) | 13.8 | 14.4 | 15 | 15 | 14.6 | 14.5 | 14.5 | 14.1 | 14.3 | 14 |
| Annual Mortality | | | | | | | | | | |
| With PAD (%) | 55.4 | 55.4 | 56.6 | 57.5 | 56.1 | 56.2 | 55.7 | 55.7 | 54.9 | 52.5 |
| Without PAD (%) | 36.5 | 37.2 | 38.2 | 38.4 | 38.5 | 38.6 | 38.3 | 38.7 | 38.3 | 36.3 |
| RR Death | | | | | | | | | | |
| Unadjusted | 0.97 | 0.97 | 1.01 | 1.04 | 1 | 1.00 (ref) | 0.99 | 0.99 | 0.97* | 0.94** |
| Adjusted | 1.04* | 1.03* | 1.03 | 1.07** | 1.03 | 1.00 (ref) | 0.97 | 0.97 | 0.97 | 0.93** |

Conclusions: Although peripheral arterial disease remains a common diagnosis at dialysis initiation, mortality rates in these patients remain exceedingly high. Nevertheless, an encouraging trend of improved survival is observed in recent cohorts.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO518

**Increased Hospital Admissions on Mondays and Tuesdays in Hemodialysis Patients** Eduardo K. Lacson, Shu-Fang Lin, J. Michael Lazarus, Raymond M. Hakim. *Fresenius Medical Care, North America, Waltham, MA.*

**Introduction:** Sudden deaths in hemodialysis (HD) patients occur more frequently on Mondays and Tuesdays, after the longest interdialytic period on a thrice weekly treatment schedule (3Tx/Wk). We hypothesized that hospitalization for all causes and more specifically for conditions due to fluid overload occur in a similar pattern.

**Methods:** All 70,374 chronic in-center 3Tx/Wk HD patients who were active in all Fresenius Medical Care, North America legacy facilities and survived during the entire quarter from January 1 to March 31, 2009 were included. All hospitalizations were tracked, tagging those primarily due to fluid overload, including congestive heart failure and pulmonary edema. Events were mapped to the day of the week of occurrence.

**Results**: More patients (60% vs. 40%) dialyzed on the MWF than TThS schedule. There were 18,381 patients (26%) with 26,602 hospitalization events during the quarter, proportionately distributed between the MWF and TThS HD schedule. 5,509 of 15,489 MWF events (36%) occurred on Mondays while 4,129 of 11,098 TThS events (37%) occurred on Tuesdays – more than the (proportionate) 14% expected. Furthermore, 44% of hospitalizations due to fluid overload occurred either on Mondays (for MWF) or Tuesdays (for TThS). Cumulative fluid overload hospitalizations during the longest interdialytic period (i.e. including the weekend events), percentages increased further to 49% and 52%, respectively. Almost 1 in 5 patients with hospitalization for fluid overload had a similar event in the prior quarter.

**Conclusions**: Disproportionately more hospitalization events occur on Mondays or Tuesdays after the 2-day interdialytic period of a thrice weekly HD schedule. The proportion of hospitalizations primarily due to fluid overload is even higher. Additional HD treatment during the long interdialytic period may help prevent hospitalization for fluid overload in patients with recurrent events.

Disclosure of Financial Relationships: Employer: I am an employee of Fresenius Medical Care, North America.

### TH-PO519

**Use and Costs of ACEIs, ARBs and Renin Inhibitors in U.S. Adult Dialysis Patients with Medicare Part D in 2007** Wendy L. St. Peter,[2,3] Christopher Powers,[1] Eric D. Weinhandl,[2] Benjamin L. Howell,[1] James P. Ebben,[2] Diane L. Frankenfield.[1] *[1]ORDI, CMS, Baltimore, MD; [2]USRDS Coordinating Center, MMRF, Minneapolis, MN; [3]College of Pharmacy, University of MN, Minneapolis, MN.*

**Background:** Angiotensin-converting enzyme inhibitors (ACEIs) and angiontensin receptor blockers (ARBs) use have been associated with improved survival in dialysis patients (pts) in several small clinical trials. However, current prevalence of use is largely unknown, as relevant studies predate implementation of the Medicare Part D drug benefit. **Methods:** Use and costs of ACEIs, ARBs, and renin inhibitors (RIs) in prevalent dialysis pts in the U.S. in 2007 were assessed using Part D data in Medicare's Chronic Condition Warehouse linked to administrative data from the United States Renal Data System. All adult pts alive on December 31, 2007 with Part D coverage during all of 2007 were included (n=169,443). **Results:** ACEIs were the most commonly used agents (38%), followed by ARBs (21%), and RIs (0.2%). The use of ACEIs was slightly higher in pts with diabetes as a comorbidity (41%) or hypertension as primary ESRD cause (39%). ACEI use was greater in younger (<65 yr), Black, American Indian/Alaska Native, and Hispanic pts. ARB use was greater in female, Asian/Pacific Islander, and Hispanic pts, as well as in those with shorter dialysis duration. Pts in independent facilities were less likely to use ACEIs and more likely to use ARBs than pts receiving care in large dialysis organizations. There was considerable variation in use across ESRD Networks, ranging from 32 to 44% for ACEIs and 15 to 27% for ARBs. During 2007, the total cost per person per year was about $157, $494, and $232 for ACEIs, ARBs, and RIs, respectively. The average out-of-pocket cost per prescription was $3.07, $10.40, and $8.16 for ACEIs, ARBs, and RIs, respectively. **Conclusions:** Disparities in use existed for some patient groups, by some facility characteristics and by geographic region. ARBs were more than three times the cost of ACEIs and were used extensively. Further research is necessary to determine whether step therapy was applied in patients receiving ARBs.

Disclosure of Financial Relationships: Consultancy: Ono PharmaResearch Funding: Chronic Disease Research Group receives research funding from Amgen, Mitsubishi Tanabe Pharma America, Inc.; Honoraria: American College of Clinical Pharmacy, Medical Communications Media, Foundation for Managed Care Pharmacy.

### TH-PO520

**Decline in Glomerular Filtration Rate during Predialysis Phase and Survival on Chronic Renal Replacement Therapy** Mikko Haapio,[1] Jaakko Helve,[2] Carola Gronhagen-Riska,[1] Patrik Finne.[2] *[1]Division of Nephrology, Helsinki University Central Hospital, Helsinki, Finland; [2]Finnish Registry for Kidney Diseases, Helsinki, Finland.*

INTRODUCTION

Estimated glomerular filtration rate (GFR) at RRT start has been shown to correlate with mortality on RRT, with lower values paradoxically associating with better survival. Knowledge is very limited, however, on the impact of GFR decline pattern during the predialysis phase on consequent survival on RRT. We aimed at exploring the effect of GFR slope on survival of patients on chronic RRT.

METHODS

Using the database of the Finnish Registry for Kidney Diseases, we conducted a cohort study of all incident patients 20 years or older entering chronic RRT in Finland in 1998, with follow-up until 31 December 2008. Of these 457 patients, we included those (n=365) with 2 or more serum creatinine measurements (at approximately 12 and 3 months, and 1–2 weeks prior to RRT start), and calculated their slopes of estimated GFR with Modification of Diet in Renal Disease formula. According to GFR slopes (in ml/min/1.73m$^2$ per year), patients were divided into tertiles: most rapid decline (over 9.1, n=121), intermediate group (3.7–9.1, n=122), and slowest decline (3.6 or less, n=122). Survival probabilities were calculated with the Kaplan-Meier method, with death as the event, and Cox proportional hazards regression was used to perform multivariable modelling of survival probabilities.

RESULTS

Median survival time of the 365 patients was 5.5 (95%CI 4.3–6.6) years. Compared to the patient group with the slowest GFR decline, the age and sex-adjusted relative risk of death was 1.22 (95% CI 0.89–1.67, P=0.221) and 1.64 (1.20–2.24, P=0.002) in patients with a GFR slope from 3.7 to 9.1, and over 9.1 ml/min/1.73m$^2$ per year. When including only patients with all 3 creatinine measurements (n=324) available, and after adjustment for age and sex, the patients with the steepest decline had 1.98-fold risk (95% CI 1.40–2.81, P<0.001) of death compared the patients with the slowest GFR decline (P<0.001).

CONCLUSIONS

Rapid decline in GFR before entering chronic RRT is associated with increased mortality on RRT.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO521

**Survival Analysis in Incident Dialysis Patients: A National Cohort Study in Taiwan** Chih-Chiang Chien. *Nephrology, Chi-Mei Medical Center, Tainan, Taiwan.*

**Background:** The incidence and prevalence of ESRD in Taiwan ranked first in the world. However, most reports for mortality in dialysis patients were from Western countries. The study is aim to investigate the impact on survival in dialysis patients based on the Taiwan's National Health Insurance (NHI) claim data.

**Patients and methods:** We selected all ESRD patients who initiated dialysis treatment in 1999. Patients had to be more than 20 years. Follow-up began at the first reported data of renal replacement therapy to the date of death or at the end of database (December 31, 2003). A total of 5302 incident HD and 341 incident PD patients were enrolled. The Cox proportional hazards model was applied to identify factors that predict survival.

**Results:** The PD group had a better survival status than did the HD group (66.57% vs. 55.88%, $p = 0.01$). In non-diabetic patients, survival rate did not differ significantly between HD and PD. However, all-cause mortality rate was significantly higher for diabetic PD patients than for diabetic HD patients [PD vs HD; HR 1.65, 95% CI: 1.29-2.10]. The interaction of PD patients and diabetes mellitus (DM) was significantly associated with all-cause mortality.

Mortality risk increased with age. In HD group, comparing with young-age (20-44 years), the hazard ratio of all-cause mortality were 2.54 for middle-age (45-64 years) and 5.38 for old-age (≥65 years) ( $p < 0.01$). In PD group, the trend was much more apparent; comparing with young-age, the hazard ratio of all-cause mortality were 4.13 for middle-age and 14.30 for old-age ($p < 0.01$).

DM was the only significant predictor of mortality in young-age patients. However, DM, congestive heart failure and cerebrovascular accident were significant predictor of mortality in middle- and old-age patients.

**Conclusion:** This is the first study to use Taiwan's NHI claim data to investigate the

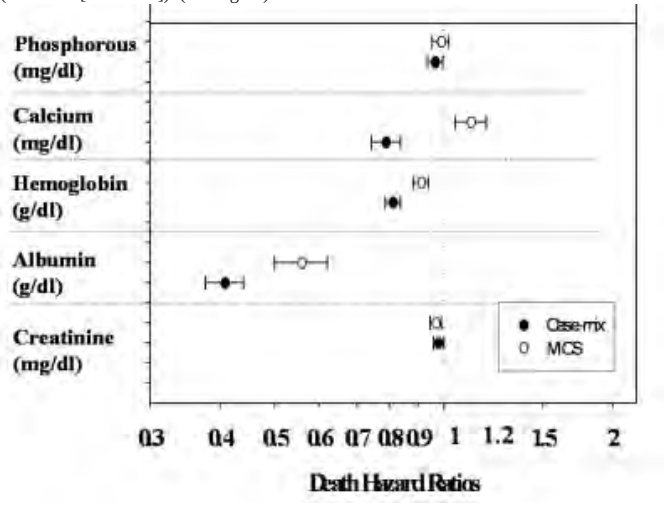
impact on survival in dialysis patients. Age and DM were significantly associated with all-cause mortality. Mortality risk increased with age in both HD and PD patients. In non-diabetic patients, survival rate did not differ significantly between HD and PD patients; however, HD provided a better survival rate than PD in diabetic patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO522

**Laboratory Measures as Predictors of the First 3-Month Mortality in Incident Hemodialysis Patients** Lilia R. Lukowsky,[1,2] Leeka I. Kheifets,[2] Onyebuchi A. Arah,[2] Allen R. Nissenson,[3] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]Epidemiology, UCLA, Los Angeles, CA; [3]DaVita, Lakewood, CO; [4]Salem VA MC, Salem, VA.*

**Background:** All-cause mortality is among maintenance hemodialysis (MHD) patients is much higher during the first 3 months of initiation of MHD treatment. We examined the association of first 90-day mortality with several laboratory measures in the first 3 months of treatment. **Methods:** Using data on 20,348 incident MHD patients, who started MHD treatment between 7/2001 and 6/2006, we calculated the hazard ratio (HR) for death (and 95% confidence intervals) given selected laboratory measures in the first 3 months using Cox proportional models adjusted for case-mix and predictors of malnutrition-inflammation complex syndrome (MICS). **Results:** There were 2,058 deaths (10.1% of the entire population) during the first 90 days. The mean age for all MHD patients in cohort (vs. those who died) was 62±17 years (vs.71±13 years). Women comprised 45% (vs. 46% who died), African Americans 23% (vs. 18%), and Hispanics 13% (vs. 10%) of the sample. Among laboratory markers, each 1.0 g/dl higher levels for serum albumin was associated with 37% greater survival (death HR 0.63 [0.52-0.75]) and for hemoglobin with 8% greater survival (HR=0.92 [0.87-0.97]). (see Figure)



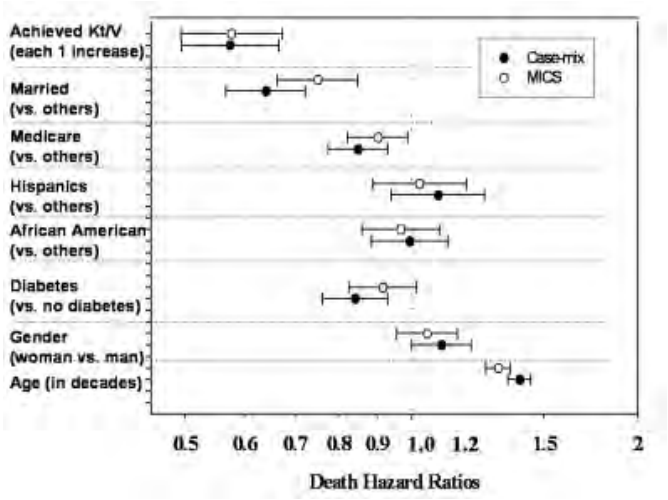

**Conclusions:** Among MHD patients, higher serum albumin and higher blood hemoglobin levels were the strongest predictors of the greatesr first 3-month survival. Serum phosphorus and creatinine did not appear to play a major role whereas serum calcium showed inconsistent association with mortality during this early period.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO523

**Comparing Demographic Factors as Predictors of the First 90-Day Mortality in Incident Hemodialysis Patients** Lilia R. Lukowsky,[1,2] Leeka I. Kheifets,[2] Onyebuchi A. Arah,[2] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]Epidemiology, UCLA, Los Angeles, CA; [3]DaVita, Lakewood, CO.*

**Background:** Mortality among dialysis patients in the United States is exceptionally high (~20% per year). It is even higher during the first year, in especially in the first 90 days of initiation of maintenance hemodialysis (MHD) treatment. We examined the associations between the first 90-day mortality and several patient demographics and compared and achieved dialysis dose in the first 90 days. **Methods:** Using data on 20,348 incident MHD patients, who started MHD treatment between 7/2001 and 6/2006, we calculated the hazard ratio (HR) of death (and 95% confidence intervals) in the first 90 days using Cox proportional models adjusted for case-mix and predictors of malnutrition-inflammation complex syndrome (MICS). **Results:** There were 2,058 deaths (10.1% of the entire population) during the first 90 days. The mean age for all MHD patients in cohort (vs. those who died) was 62±17 years (vs. 71±13 years), Women comprised 45% (vs. 46% who died), African Americans 23% (vs. 18%), Hispanics 13% (vs. 10%). Death HR during the first 90 days was <1.0 for higher achieved Kt/V, married status and diabetes mellitus, whereas it was >1.0 for older age (see Figure).




**Conclusions:** Risk factors of the first-90-day mortality among incident MHD patients include lower achieved dialysis dose, a surrogate catheter (vs. arteriovenous fistula or graft), non-married status, no diabetes mellitus, and older age. Race, ethnicity and gender do not appear to have substantial associations with the first-90-day mortality.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO524

**Discriminators of Death and Survival in the First Year of Hemodialysis: Threshold Values and Hierarchical Importance – The United States Renal Data System (USRDS)** Robert N. Foley,[1,2] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

High first year mortality is an ongoing public health issue in the US. In this regard, knowing optimally discriminating threshold values of suspected risk factors, high-yield subgroups and hierarchical importance could help gauge the appropriateness of renal replacement therapy and identify interventions. Using data at initiation of dialysis from the USRDS Medical Evidence Form, we identified thresholds with maximum sensitivity and specificity ($Max_{Sn+Sp}$) predictions for death in the first year in 156,924 adults who began hemodialysis in 2005 and 2006, and then applied classification tree methodology to define subgroups, interactions, and hierarchical rank. For the overall population, age > 66 years was the best discriminator between death and survival (sensitivity 0.63/specificity 0.66), followed by congestive heart failure (0.45/0.72), GFR ≤ 10 mL/min/1.73m$^2$ (0.55/0.60), access with a catheter (0.70/0.43), immobility (0.21/0.91) and albumin ≤ 3.2 g/dL. In a classification tree with nodes defined by $Max_{Sn+Sp}$, the first two iterations produced four age bands, ≤ 53, 54-66, 67-76 and > 76 years; within these age bands, the best discriminators between death and survival were catheter use (0.68/0.47), serum albumin ≤ 3.1 g/dL (0.61/0.53), catheter use (0.68/0.47) and catheter use (0.71/0.45), respectively. After four iterations, best discriminators included older age (applicable to subgroups totaling 100% of the study population), catheter use (applicable to 100%), congestive heart failure (applicable to 55.3%), low serum albumin levels (applicable to 53.2%), immobility (applicable to 29.0%), diabetes (applicable to 5.1%) and white race (applicable to 4.8%). Initiation of dialysis with a catheter appears to be the most important modifiable discriminator of mortality within the first year of hemodialysis therapy.

Disclosure of Financial Relationships: Consultancy: Affymax,Amgen,Ortho,Luitpold, Merck, Novartis.

### TH-PO525

**Potentially Modifiable Patient Characteristics and First-Year Hemodialysis (HD) Mortality: Results from the DOPPS** Bruce M. Robinson,[1,2] Jinyao Zhang,[1] Yun Li,[2] Hal Morgenstern,[2] Ronald L. Pisoni,[1] Brian D. Bradbury,[3] Masafumi Fukagawa,[4] Peter G. Kerr,[5] Leslie Ng,[3] Hugh C. Rayner,[6] Friedrich K. Port,[1,2] Rajiv Saran.[2] *[1]Arbor Research Collaborative for Health, Ann Arbor, MI; [2]Univeristy of Michigan; [3]Amgen, Thousand Oaks, CA; [4]Tokai University, Japan; [5]Monash Medical Centre, Australia; [6]Birmingham Heartlands Hospital, United Kingdom.*

Hemodialysis (HD) patients have especially high mortality risk in the early dialysis period. This study aims to identify the most important potentially modifiable patient characteristics in the first year of dialysis.

We analyzed 5,942 incident HD patients (enrolling within 30 days of first dialysis) in the DOPPS, a prospective cohort study (1996-2004) in 12 countries. Multivariable logistic regression was used to model the odds ratio of first-year mortality by patient characteristics at study enrollment. For categorical exposures, cut-points used were clinically accepted values and/or best fitting functional forms. Attributable fractions (AFs) were calculated from patient-specific predicted risks to estimate the proportion of first-year deaths attributable to each exposure in this population. The AF estimates can be interpreted as the maximum potential impact of eliminating each exposure.

Elevated first-year mortality was associated with older age (p<0.001), non-black race (p =0.04), higher BMI (p =0.05) and 7 of 13 comorbidities (p <0.05). Findings for potentially modifiable patient characteristics are in Table.

| Lab Value or Other Patient Characteristic [1] | Exposure (Reference) | Exposure Prevalence (%) | Odds Ratio (p value) for 1st Year Mortality | Attributable Fraction for 1st Year Mortality (%) |
|---|---|---|---|---|
| Creatinine [2] | < (vs. ≥) 6 mg/dL | 38.8 | 1.47 (<0.0001) | 9.6 |
| Albumin | < (vs. ≥) 3.5 g/dL | 52.6 | 1.38 (0.0007) | 8.2 |
| White blood cells | > (vs. ≤) 10k | 21.3 | 1.61 (<0.0001) | 6.2 |
| Phosphate | >5.5 (vs. 3.5-5.5) mg/dL | 44.2 | 1.28 (0.01) | 4.6 |
| Phosphate | <3.5 (vs. 3.5-5.5) mg/dL | 10.4 | 1.37 (0.01) | 2.0 |
| Ferritin | > (vs. ≤) 500 ng/mL | 15.7 | 1.35 (0.02) | 1.9 |
| Catheter | yes (vs. no) | 56.5 | 1.49 (<0.0001) | 15.6 |
| Systolic blood pressure | < 130 (vs. ≥130) mm Hg | 23.1 | 1.55 (<0.0001) | 7.0 |
| Pre-ESRD nephrology care | no or ≤1month (vs. >1 month) care | 24.4 | 1.41 (0.0001) | 5.4 |
| Residual kidney function (RKF) [3] | no (vs. yes) | 18.0 | 1.47 (0.0002) | 4.5 |

Logistic regression model accounted for facility clustering and adjusted for all variables listed plus age, sex, race, BMI, 13 comorbidities, other labs (below), country, and study phase.
Comparable estimates were found in a Cox model including censored patients.

[1] Potentially modifiable variables with p ≥0.05 for association with 1st year mortality (not shown above) were: hemoglobin, PTH, calcium, and TSAT.

[2] Creatinine (Cr) after starting dialysis; correlation (r) with RKF=0.01

[3] RKF estimated by urine output > 200 mL/day

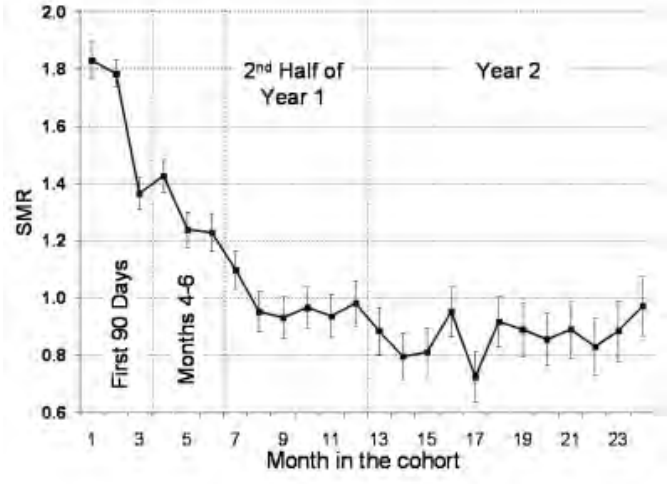
These results indicate that a substantial proportion of the first-year mortality among HD patients could potentially be reduced by addressing modifiable factors. Highest priority targets should include decreasing catheter use and avoiding or treating malnutrition/ inflammation, when possible.

Disclosure of Financial Relationships: Research Funding: The DOPPS is administered by Arbor Research Collaborative for Health and is supported by scientific research grants from Amgen (since 1996), Kyowa Hakko Kirin (since 1999, in Japan), Genzyme (since 2009), and Abbott (since 2009), without restrictions on publications.

## TH-PO526

**Relative Mortality Trends in the First 2 Years among Incident Hemodialysis Patients** Lilia R. Lukowsky,[1,2] Leeka I. Kheifets,[2] Onyebuchi A. Arah,[2] Allen R. Nissenson,[3] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center, Harbor-UCLA, Torrance, CA; [2]Epidemiology, UCLA, Los Angeles, CA; [3]DaVita, Lakewood, CO; [4]Salem VA MC, Salem, VA.*

**Background:** All-cause mortality among maintenance hemodialysis (MHD) patients is much higher during the first year of initiation of MHD treatment then in subsequent years. We examined the mortality trends during the first 24-months among a group of incident MHD patients. **Methods:** Using data from 524 DaVita clinics (prior to Gambro acquisition) on 21,772 incident MHD patients from who started MHD treatment between 7/2001 and 6/2006 in a DaVita clinic, we calculated the standardized mortality ratio (SMR) [and 95% confidence intervals] using the entire DaVita MHD cohort over the same period (~140,000 MHD patients) as the reference group. We standardized to age, gender, race (whites vs. non-whites), and diabetes status. **Results:** The incident MHD patients had a mean age of 62.5±15.9 years and included 45% women, 24% African Americans and 14% Hispanics. The SMR were the highest during the first several months but decreased after 6 to 8 months and remained relatively stale during year 2. The highest SMR was in the first month [1.82 (1.74 -1.91)], followed by Months 2, 3 and 4 with SMRs of 1.77 (1.69 -1.86), 1.36 (1.27-1.44), and 1.42 (1.37-1.51) respectively. The SMRs for months 12 and 24 were similar and close to unity, i.e., 0.98 (0.89-1.06) and 0.97 (0.88-1.05), respectively.




**Conclusions:** Among incident MHD patients, mortality is up to 80% higher in the first few months, but it declines over the subsequent 6 to 8 months to reaches a relatively steady state by the end of first year. Interventions to improve outcomes during the first 3 to 4 months need to be examined.

Disclosure of Financial Relationships: nothing to disclose

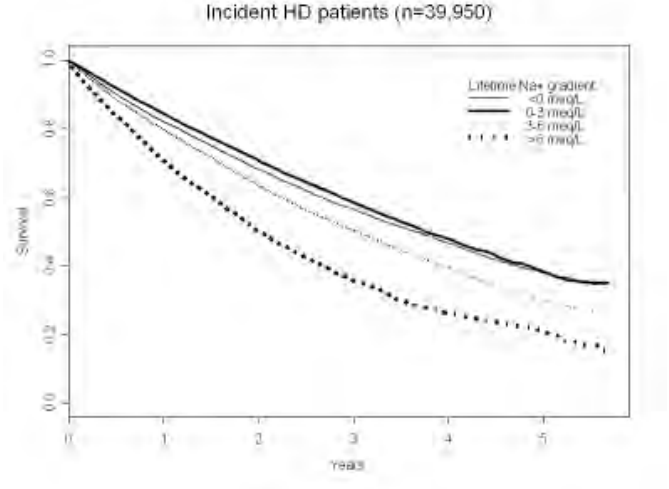
## TH-PO527

**The Relationship of Na+ Gradient and Mortality in Incident Hemodialysis Patients** John Rogus,[1,2] Eduardo K. Lacson,[2] Peter Kotanko,[1] Nathan W. Levin.[1] *[1]Renal Research Institute, New York, NY; [2]Fresenius Medical Care North America, Waltham, MA.*

Objective: To evaluate the impact of Na+ flux during hemodialysis, we examined the relationship of the Na+ gradient (dialysate concentration minus plasma concentration) and mortality. We hypothesized that mortality would be positively correlated with Na+ gradient.

Methods: All 39,950 adult (age >18 years) black/white incident in-center chronic HD patients with Na+ data at Fresenius Medical Care, North America facilities admitted during 2001 — 2004 were included. Deaths and censoring events (i.e., transplant or transfer out of FMCNA) were followed up to December 31, 2006.

Results: Compared to patients with a negative Na+ gradient, those with gradients from 0-3, 3-6, and >6 had hazard rate ratios (HRR) of 0.94 (95%CI: 0.90-0.97), 1.20 (95%CI: 1.15-1.25) and 1.77 (95%CI: 1.68-1.88), respectively (all p<0.001). Similar results were obtained after adjusting for case-mix variables (i.e., age, race, gender, and diabetes status) and after adjusting for case-mix variables plus nutritional status (i.e., baseline albumin).




Conclusion: Our findings are consistent with a hypothesis that higher Na+ gradients may increase mortality, presumably due to outcomes related to fluid overload. However, causality cannot be proven from this observational study and prospective studies are required to test this hypothesis.

Disclosure of Financial Relationships: Employer: Fresenius Medical Care; Renal Research Institute.

**TH-PO528**

**Association between Dependency and Depressive Symptoms with Mortality in Hemodialysis Patients** Joaquin Manrique, Carolina Purroy, Maria Jesus Sorbet, Juan Jose Unzue, Jesus Arteaga. *Nephrology, Hospital de Navarra, Pamplona, Navarra, Spain.*

The presence of depressive symptoms is high in patients on hemodialysis (HD). According to the population ages, their limitations in performing daily activities affect the their well-being as well as increase the burden on caregivers and the use of health services. There are some scales useful to detect depressive symptoms and to define physical dependency useful in HD.

In this prospective study, we measured the proportion of patients undergoing chronic outpatient HD who presented any degree of dependency and/or depressive symptoms and identified the clinical characteristics of this population at most risk. Their dependence were measured by the Barthel scale (BS) performed by nursing HD staff. We assessed it association with presence of depressive symptoms (by Beck Depression score, BDs), and mortality after one year follow-up (Kaplan-Meier survival)

A cohort of 115 patients has been studied and 97 were followed for 12 months. The 44.1% of patients had depressive symptoms assessed by BDs (BDs>or=8). We observed a significant association between BS and BDs ($r=-0.5$; $p<0.001$). Patients with greater disability (Bs<90) had worse depressive score ($p<0.001$). We observed worse nutritional status assessed by albumin and phosphorus in depressive patients compared to non depressive, and lower weight gain ($p<0.05$). Female, diabetic and patients not on waiting list showed higher BDI ($p<0.05$)

After 12 months of follow-up, survival rates were 80.5% for BDs≥8 and 94.3% for BDs < 8 ($p=0.036$), and 71.4% for dependent and 95.5% for no dependent patients ($p<0.001$). Other studies variables, including age, gender, and time on dialysis, were not significantly associated with mortality.

Our study shows that the disability in self-care and the presence of depressive symptoms are common among patients on haemodialysis. Both of them may act as independent risks factors for mortality. Strategies could be helpful to routinely identify those dialysis patients at risk of functional impairment and depression to limit their disabilities. Self-report scales and those performed by nursing staff could improve the integral treatment of those patients

Disclosure of Financial Relationships: nothing to disclose

**TH-PO529**

**The Association of Baseline, New-Onset, and Persistent Depressive Symptoms with Mortality in ESRD Patients** Tessa O. Van den Beukel,[1,2] Marion Verduijn,[1] Elisabeth W. Boeschoten,[3] Raymond T. Krediet,[4] Friedo W. Dekker,[1] Sandra Van Dijk.[5] *[1]Dept. of Clinical Epidemiology, Leiden University Medical Center, Leiden; [2]Dept. of Nephrology, Sint Lucas Andreas Hospital, Amsterdam; [3]Hans Mak Institute, Naarden; [4]Dept. of Nephrology, Academic Medical Center, Amsterdam; [5]Dept. of Medical Psychology, Leiden University Medical Center, Leiden, Netherlands.*

*Introduction*

Among dialysis patients it is suggested that depressed patients have decreased survival compared to those who are non-depressed. The aim of this study is to examine the survival of dialysis patients with depressive symptoms (DS) at baseline only, new-onset DS, and persistent DS compared to patients without DS.

*Methods*

1078 patients participating in the NECOSAD study, a prospective multicenter cohort study of hemodialysis and peritoneal dialysis patients in the Netherlands, completed the SF-36 mental health scale, which was used as a measure of DS, 3 and 12 months after starting dialysis. Cox regression analyses were used to examine survival.

*Results*

After an average of 3.8 years of follow-up 40 percent of the patients had died. Patients with persistent DS had the highest mortality risk, both unadjusted and adjusted for sociodemographic and clinical characteristics (Table 1).

Hazard ratios (HR) for mortality for dialysis patients with depressive symptoms (DS) at baseline only (n=96), new-onset DS (n=98), and persistent DS (n=117) compared to dialysis patients without DS (n=767).

| | Unadjusted | | Adjusted [1] | |
|---|---|---|---|---|
| | HR | 95% CI | HR | 95% CI |
| DS baseline only | 1.47 | 1.07 – 2.03 | 1.37 | 0.97 – 1.93 |
| DS new onset | 1.77 | 1.31 – 2.39 | 1.39 | 1.02 – 1.89 |
| DS persistent | 2.45 | 1.87 – 3.20 | 1.83 | 1.36 – 2.45 |

[1] Adjusted for age, sex, education, marital status, Davies comobidity index, primary kidney disease, dialysis modality, serum albumin, and hemoglobin.

*Discussion*

Among dialysis patients persistent DS are related to an increased mortality risk. In the dialysis population DS may be masked by physical symptoms, because symptoms can be attributed to the renal disease or treatment instead of depression it selves. This may have implications for recognition and treatment of depression.

Disclosure of Financial Relationships: nothing to disclose

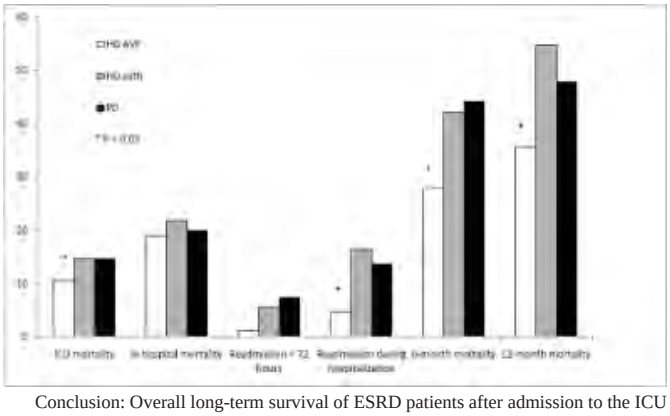
**TH-PO530**

**Long-Term Outcomes of End Stage Renal Disease Patients Admitted to the ICU** Manish M. Sood,[1] Lisa M. Miller,[1] Paul Komenda,[1] Martina Reslerova,[1] Claudio Rigatto.[1] *[1]Nephrology, University of Manitoba, Winnipeg, MB, Canada; [2]Critical Care, University of Manitoba, Winnipeg, MB, Canada.*

Introduction: End stage renal disease (ESRD) patients admitted to the ICU have poor survival and high rates of readmission however little evidence exists on long-term outcomes. We set out to investigate the long-term (6 month and 12 month) survival of ESRD patients admitted to the ICU and whether differential survival could be explained by dialysis modality and vascular access.

Methods: We compared the admission characteristics, outcomes and readmission rates of 619 ESRD (95 peritoneal dialysis (PD), 334 hemodialysis with a catheter (HD CVC), 190 hemodialysis with an AV fistula (HD AVF)) patients admitted to 11 ICU's in Winnipeg, Manitoba, Canada. Parametric and non-parametric tests were used as appropriate to determine differences in baseline characteristics. Multivariable logistic regression was used to assess outcomes between the groups.

Results: The 6 and 12-month crude survival was 62 and 52% respectively. Modality and vascular access were associated with an increased odds ratio of death (6 month: PD OR 2.05, HD CVC OR 1.87, 12-month: PD OR 2.22, HD CVC OR 2.12) compared to patients on HD with an AVF. In 3 different, multivariate adjusted models, this association persisted with odds ratio for death of 2.09-2.62 for PD and 1.83-2.37 for HD CVC.



Conclusion: Overall long-term survival of ESRD patients after admission to the ICU is poor. Being on peritoneal dialysis or being dialyzed with a catheter was independently associated with an increased mortality.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO531**

**All-Cause Mortality in a French Cohort of Incident Hemodialysis Patients from 2007 to 2010: KDOQI vs KDIGO** Denis Fouque,[1] Hubert Roth,[2] Gerard M. London,[3] Thierry P. Hannedouche,[4] Guillaume Jean,[5] Jean-Louis Bouchet,[6] Tilman B. Drueke.[7] *[1]Nephrology, Herriot, Lyon, France; [2]univ Fourier, Grenoble, France; [3]H. Manhes, Fleury Merogis, France; [4]H. Civils, Strasbourg, France; [5]CRAT, Tassin, France; [6]cl. St Augustin, Bordeaux, France; [7]inserm, Amiens, France.*

PhotoGraph is a prospective observational French cohort in CKD-5D patients. We report data from 4018 incident pts followed from July 1 2007 to January 1 2010 using a Cox model, aged 67.3±15.1 yr, sex ratio 1.56 (m/f), 31% diabetic.

Predictors of all-cause mortality

| K/DOQI | HR | 95% CI | p |
|---|---|---|---|
| Phosphorus>5.5 mg/dl | 1.07 | 0.84-1.37 | 0.59 |
| Phosphorus<3.5 mg/dl | 1.24 | 0.95-1.62 | 0.11 |
| PTH>300 pg/ml | 0.75 | 0.58-0.97 | 0.032 |
| PTH<150 pg/ml | 1.09 | 0.85-1.39 | 0.49 |
| Calcium>9.5 mg/dl* | 1.45 | 1.15-1.82 | 0.001 |
| Calcium<8.4 mg/dl* | 1.19 | 0.84-1.67 | 0.32 |
| KDIGO | | | |
| Phosphorus>4.3 mg/dl | 1.06 | 0.84-1.33 | 0.65 |
| Phosphorus<2.8 mg/dl | 1.59 | 1.11-2.28 | 0.012 |
| PTH>585 pg/ml | 1.00 | 0.68-1.47 | 0.99 |
| PTH<130 pg/ml | 1.12 | 0.89-1.42 | 0.33 |
| Calcium>10.2 mg/dl | 1.31 | 0.67-2.57 | 0.42 |
| Calcium<8.6 mg/dl | 0.80 | 0.65-0.996 | 0.046 |
| | | | |
| Phosphate binder (Y/N) | 0.58 | 0.47-0.71 | <0.001 |

models adjusted for age, gender, BMI, diabetes, CVD history, albumin and hemoglobin; *albumin-corrected calcemia

Basal hypercalcemia (KDOQI>9.5 mg/dl) and hypophosphatemia (KDIGO<2.8 mg/dl) were associated with increased mortality, elevated iPTH (KDOQI>300 pg/ml) and hypocalcemia (KDIGO<8.6 mg/dl) with better survival. The different prognosis of calcemia may be explained by correcting (KDOQI) or not (KDIGO) total calcium by albumin. There is a strong independent association between the use of phosphate binders (69% pts received a calcium-based and/or non calcium-based binder) and improved survival. Nutrition, inflammation and anemia were also predictors of mortality. These results from

an up to date cohort highlight the role of modifiable factors with the potential to improve patient outcome, namely optimal management of nutritional status and early start of phosphate binder treatment.

Disclosure of Financial Relationships: Consultancy: genzyme, amgen, danoneResearch Funding: amgen, baxter; Honoraria: genzyme, novo nordisk, amgen, fresenius kabi; Scientific Advisor: danone, fresenius kabi.

### TH-PO532

**Mortality Patterns and Day of the Week – The United States Renal Data System (USRDS)** Robert N. Foley,[1,2] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

Hemodialysis in many countries is delivered on a thrice weekly basis, with intervals of 2 (Monday-Wednesday or Tuesday-Thursday), 2 (Wednesday-Friday or Thursday-Saturday) and 3 (Friday-Monday or Saturday-Tuesday) days between sessions. All things being equal, including configuration of renal replacement therapy (RRT) services, one would expect that deaths in RRT would be equally spaced, with one seventh (14.3%) of all deaths occurring on each day of the week. We hypothesized that asymmetrical spacing of dialysis treatments may have unintended mortality consequences. To address this hypothesis, we examined annual trends from 1980 to 2007 in the proportions of deaths that occurred in RRT patients on different days of the week (1.35 million deaths). Relative deviation from expected proportional mortality for each day of the week was calculated as 100 x (observed percentage of deaths - 14.3)/14.3. In 1980, ranks of relative deviations from expected proportional mortality were as follows +21.1% on Mondays, +4.7% on Fridays, -0.8% on Sundays, -1.4% on Tuesdays, -2.9% on Thursdays -9% on Wednesdays, and -11.5% on Saturdays. Corresponding arrays in 1990, 2000 and 2007 were as follows: 1990 - +9.7% on Wednesdays, +8.3% on Mondays, +1.8% on Tuesdays, +0.2% on Fridays, -3.5% on Saturdays, -5.9% on Thursdays and -10.8% on Sundays; 2000 - +10.5% on Mondays, +3.9% on Tuesdays, -0.7% on Thursdays, -0.8% on Fridays, -1.3% on Wednesdays, - 5.4% on Saturdays and -6.4% on Sundays; 2007 - +7.9% on Mondays, + 3.4% on Tuesdays, + 1.4% on Fridays, +1.0% on Wednesdays, -3.2% on Thursdays, -4.2% on Saturdays and -6.4% on Sundays. The pattern of meaningfully higher mortality on Mondays and Tuesdays and meaningfully lower mortality on Sundays appears to have become established from 1996: mortality was highest on Mondays in every year between 1996 and 2007, second highest on Tuesdays in every year except 1997 (replaced by Fridays), and lowest on Sunday in every year except 1999 (replaced by Thursdays). Day-to-day mortality appears to differ substantially in RRT patients in the US, compatible with the hypothesis that long interdialytic intervals may have adverse consequences.

Disclosure of Financial Relationships: Consultancy: Affymax,Amgen,Ortho,Luitpold, Merck, Novartis.

### TH-PO533

**The Survival of Asian Americans Is Superior to Other Race Groups Receiving Haemodialysis and Peritoneal Dialysis Treatment in the United States: A Trend Analysis from 1995-2006** Austin G. Stack,[1] Catherine A. Wall,[2] Hoang Thanh Nguyen.[3] *[1]Regional Kidney Centre, Letterkenny General Hospital, Letterkenny, Ireland; [2]Renal Department, Adelaide and Meath Hospitals, Tallaght, Dublin, Ireland; [3]University of Texas MD Anderson Cancer Center, Houston, TX.*

Prior studies have demonstrated superior survival of Asian Americans over Whites and African Americans. Whether this survival advantage is present in both peritoneal dialysis (PD) and haemodialysis (HD) and the extent to which these survival differences have changed over time has not been fully explored.

Methods: We compared mortality differences among Asians, Blacks and Whites using data from the US Renal Data System. National incidence data were available on all new patients (N=823,753) between May 1995 and December 2004 and all patients were followed until Oct 2006. Mortality rates over 2 years were compared among race groups (White=referent) across 3 calendar periods (1995-1998, 1999-2001, and 2002-2004) and separately for PD and HD subgroups with the generation of adjusted hazard ratios[1] (HR) 95% Confidence intervals) using multivariable Cox regression analysis. Additional models examined mortality trends for the entire Asian population over 3 calendar periods.

Results: Mortality risks for Asians were significantly lower than Blacks and Whites across 3 calendar periods as shown in table below. Recent cohorts experienced better survival compared with earlier cohorts.

| | Calendar Period | 1995-1998 | 1999-2001 | 2002-2004 |
|---|---|---|---|---|
| | Number | (N=256,575) | (N=278,906) | (N=289,384) |
| | Race Group | | | |
| All Patients | Asian | 0.82 (0.80-0.85) | 0.79 (0.77-0.81) | 0.80 (0.78-0.82) |
| (N=823,753) | Black | 0.93 (0.92-0.93) | 0.95 (0.94-0.96) | 0.95 (0.95-0.96) |
| | White (referent) | 1.00 | 1.00 | 1.00 |
| PD Patients | Asian | 0.72 (0.65-0.80) | 0.79 (0.71-0.89) | 0.72 (0.65-0.80) |
| (N=42,952) | Black | 0.94 (0.91-0.96) | 0.91 (0.88-0.94) | 0.93 (0.90-0.96) |
| | White (referent) | 1.00 | 1.00 | 1.00 |
| HD Patients | Asian | 0.83 (0.81-0.86) | 0.79 (0.77-0.81) | 0.81 (0.79-0.83) |
| (N=780,801) | Black | 0.93 (0.92-0.94) | 0.95 (0.94-0.96) | 0.96 (0.95-0.96) |
| | White (referent) | 1.00 | 1.00 | 1.00 |
| | Calendar Period | 1995-1998 | 1999-2001 | 2002-2004 |
| Mortality Trends | | | | |
| Asians | All (29,453) | 1.00 (referent) | 0.85 (0.79-0.92) | 0.84 (0.78-0.90) |
| | PD (2,662) | 1.00 (referent) | 1.07 (0.79-1.44) | 0.73 (0.53-0.99) |
| | HD (24, 195) | 1.00 (referent) | 0.84 (0.78-0.91) | 0.84 (0.78-0.91) |

Conclusions: The survival of Asian-Americans who reach end-stage kidney disease and receive dialysis is superior to other race groups and demonstrates a trend of continued improvement. The factors (internal and external) that confer this survival advantage require further exploration.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO534

**The Influence of Ethnicity on Survival of Incident Dialysis Patients in the Netherlands** Tessa O. Van den Beukel,[1,2] Marion Verduijn,[1] Carl E. H. Siegert,[2] Adriaan Honig,[3] Elisabeth W. Boeschoten,[4] Raymond T. Krediet,[5] Friedo W. Dekker.[1] *[1]Dept. of Clinical Epidemiology, Leiden University Medical Center, Leiden; [2]Dept. of Nephrology, Sint Lucas Andreas Hospital, Amsterdam; [3]Dept. of Psychiatry, Sint Lucas Andreas Hospital, Amsterdam; [4]Hans Mak Institue, Naarden; [5]Dept. of Nephrology, Academic Medical Center, Amsterdam, Netherlands.*

*Introduction*

Ethnic minority patients on dialysis have better survival rates compared to whites. The reasons for this finding are not exactly understood and European studies are limited. This study examined whether ethnic survival differences could be explained by different patient characteristics, and in particular by psychosocial factors.

*Methods*

We analyzed data of the NECOSAD study, an observational prospective cohort study of incident hemodialysis and peritoneal dialysis patients in the Netherlands. Ethnicity was classified as white, black, and Asian according to patients' country of origin. Other data collected at baseline included demographic, clinical, and laboratory characteristics, as well as quality of life (QoL) and depressive symptoms (DS), both determined using the SF-36 questionnaire.

*Results*

1775 patients were white, 45 black, and 108 Asian. Characteristics that were significantly different between the ethnic groups were age, marital status, rGFR, diabetes mellitus, erythropoietin use, hemoglobin, calcium, and the presence of DS. No ethnic differences were found in QoL. Hazard ratios for mortality (HR) for whites compared to blacks and Asians were 3.0 (95 % CI 1.5–5.7) and 1.2 (95% CI 1.0–1.6 ), respectively. After adjustment for a range of potential explanatory variables, including QoL and DS, the HRs were 2.9 (95 % CI 1.4–6.0) and 1.1 (95% CI 0.8–1.6), respectively.

*Conclusions*

This study demonstrates increased survival for black compared to white dialysis patients, and slightly increased survival for Asian compared to white dialysis patients in the Netherlands. Adjustment for demographic, clinical, laboratory, and psychosocial characteristics did not materially influence ethnic survival differences, despite significant differences in baseline characteristics between ethnic groups.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO535

**Racial Disparities in ESRD Outcomes for African Americans with Diabetes Mellitus: USRDS Data** Mark E. Williams,[1] Gurprataap Singh Sandhu,[2] Alexander S. Goldfarb-Rumyantzev.[2] *[1]Renal Unit, Joslin Diabetes Center, Boston, MA; [2]Renal Division, Beth Israel Deaconess Medical Center, Boston, MA.*

Eliminating racial health disparities is a national health objective for 2010. Racial disparities in the prevalence of kidney disease are well-known, and African American (AA) with diabetes (DM) have a 4X excess burden of ESRD in the U.S. AA maintenance hemodialysis patients have greater survival compared to other races, and unadjusted USRDS data indicate that AA survival advantage with diabetes as primary cause of ESRD increases with time on dialysis. We analyzed USRDS data for all incident and prevalent dialysis (HD and PD) patients with the diagnosis of DM from 1990 to 2007 using a classic Cox model analysis adjusted for age, comorbidities, duration of nephrology care, ESRD cause, access type, education level, serum albumin, hemoglobin, dialysis type, and BMI. Data were also analyzed without and with HgbA1c as a variable. Parameters affecting the mortality hazard

ratios included age, Charlson comobidity index, dialysis type, serum albumin and creatinine, education index, and race. The mortality hazard ratio for AA was .73 compared to whites (W). Statistics for major cause of death by race (Cardiac: AA 51%, W 54%; Infection: 18% AA, W 14.5%; Cerebrovascular: AA 6%, W 5%; Malignancy AA 4%, W 3%) differed by chi-square analysis (<.001). AA with DM and ESRD have reduced mortality on dialysis, and the disparity appears to equal or exceed that of the general USRDS dialysis population. Longitudinal data are needed to further analyze this finding.

Disclosure of Financial Relationships: nothing to disclose

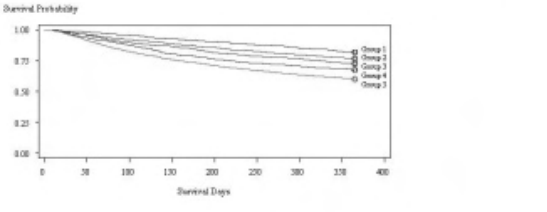
### TH-PO536

**Incident Arteriovenous Fistula (AVF) and Associated Mortality: A Comparison of Medicare Claims and CMS-2728 Data** Eiichiro Kanda,[1] T. Christopher Bond,[1] Jenna O. Krisher,[2] William M. McClellan.[1] *[1]Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA; [2]ESRD Network 6, Raleigh, NC.*

**Background and Objectives:** The Centers for Medicare & Medicaid Services (CMS) 2728 are used to develop quality improvement interventions. We compared the predictive validity for mortality of an incident AVF on the CMS-2728 (2728-AVF) or from Medicare claim (Med-AVF).

**Methods:** Data was collected for patients beginning hemodialysis between June 1, 2006 and May 31, 2007 in North Carolina, South Carolina and Georgia (ESRD Network 6); 10,401 patients (aged 66 years and older). Patients were followed for one year after beginning hemodialysis. Incident AVF was categorized as: Group 1, yes 2728-AVF and yes Med-AVF (881, 8.5%); Group 2, either 2728-AVF or maturing AVF and no Med-AVF (1138, 10.9%); Group 3, maturing 2728-AVF and Med-AVF (837, 8.1%); Group 4, no 2728-AVF or maturing 2728-AVF and yes Med-AVF (1163, 11.2%); Group 5, no 2728-AVF and no Med-AVF (6382, 61.4%). Mortality was evaluated by adjusted Cox Proportional Hazard Model (PHM). Race was included in analyses as a variables that predict health care quality and outcomes, such as access to care and differential treatment. **Results:** The mean age was 76.0; 41.2% diabetes; 52.0% male; 67.5%, black; a history of myocardial infarction 30.7%; and cerebrovascular disease 13.9%. Mortality was 34.5% (18.2% (Group 1) to 40.0% (Group 5)). Higher survival was noted for Groups 1, 2 and 3 who had a 2728-AVF or maturing AVF (p<0.0001).

Figure 1 Survival Probability Associated with Incident AVF Placement Persons diagnosed as ESRD in Network 6 area (6/1/06-5/31/07)




| Group | AVF Status per 2728 (2728-AVF) | AVF Status per Medicare Billing (Med-AVF) |
|---|---|---|
| 1 | Yes-in place | Yes-in place |
| 2 | Yes-in place or maturing | No |
| 3 | Yes-maturing | Yes-in place |
| 4 | No | Yes-in place |
| 5 | No | No |

The hazard ratio (HR) (95% confidential interval [CI]) for groups 1 to 4 compared to Group 5 were: 0.59 (0.48-0.71); 0.60 (0.53-0.69); 0.80 (0.69-0.92); 0.92 (0.82-1.0). **Conclusion:** The association between mortality may be stronger for incident access measured by 2728-AVF than for Med-AVF and supports use of 2728 data for quality assurance activities by the Networks.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO537

**Circulting Cell Free DNA (CFD) in Hemodialysis (HD) Patients Is Associated with Risk Factors and Mortality** David Tovbin,[1] Moshe Zlotnik,[1] Amir Abd Elkadir,[3] Jony Sheynin,[3] Amos Douvdevani.[2] *[1]Nephrology, Soroka Medical Center; [2]Nephrology Lab, Soroka Medical Center; [3]Biomedical Engineering, Ben-Guriun University of the Negev, Beer-Sheva, Israel.*

Circulating cell-free DNA (CFD) is present in healthy individuals and increases in hemodialysis (HD) and in various disorders including inflammation and diabetes (DM), which are important risk factors in HD patients. We hypothesized that CFD is an integrative marker of damage and thus has prognostic value. Therefore, we assessed CFD in HD patients and its relation to clinical/laboratory parameters and prognosis. In a prospective study, 31 chronic HD patients with no acute disease were evaluated for serum CFD levels before and after HD, using the reported rapid non-cumbersome inexpensive fluorometric assay developed in our laboratory. Follow-up CFD levels were assessed at 18 months in 20 patients. Pre-HD-CFD levels were higher than in healthy subjects (582±305 Vs 431± 221 ng/ml, p<0.05) and increased after HD (832±296 ng/ml, p<0.01). Post-HD CFD were high (>850 ng/ml) in 35% of patients and at 18 months in 80%, with 47% increase in levels (p<0.0001). Post-HD-CFD was higher in the DM subgroup (1004±278 Vs 737±268 ng/ml in non-DM, p<0.05), correlated positively to post-HD interleukin-6 (IL-6) levels (p<0.05, r=0.51) and negatively to creatinine (p<0.01,r=-0.54). No correlation was found with serum albumin, urea, glucose, hemoglobin, leukocyte count and dialysis-related parameters. Pre-HD-CFD levels correlated with neutrophil number (p<0.05, r=0.36), but showed less prominent correlation then post-HD-CFD for other parameters. Post-HD-CFD was positively correlated with death at one year (p<0.01, r=0.46) and 5/6 patients who died within one year had elevated CFD levels. Conclusions: CFD in HD patients is associated with diabetes, inflammation, low muscle mass reflected by serum creatinine and mortality. The increase after HD and the stronger associations with post–HD levels suggest combined effect of patients' state and the dialysis process. We suggest that CFD is an integrative marker of pathologic processes and a correlate of outcome. Its' detection is a cheap applicable tool for identifying patients at risk and follow up.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO538

**N-Terminal Pro-B-Type Natriuretic Peptide and Interleukin-6 Associate Synergistically in Increasing the Risk of Mortality in Maintenance Hemodialysis Patients** Hirokazu Honda,[1] Keiko Takahashi,[2] Tetsuo Michihata,[3] Hiroaki Ogata,[4] Fumihiko Koiwa,[5] Eriko Kinugasa,[4] Kanji Shishido,[2] Tadao Akizawa.[1] *[1]Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan; [2]Kawasaki Clinic, Kawasaki, Japan; [3]Ebara Clinic, Tokyo, Japan; [4]Department of Internal Medicine, Showa University Northern Yokohama Hospital, Yokohama, Japan; [5]Division of Nephrology, Department of Medicine, Showa University Fujigaoka Hospital, Yokohama, Japan.*

INTRODUCTION AND AIMS: N-terminal pro-B-type natriuretic (NT-proBNP) and interleukin (IL)-6 are risk factors for cardiovascular disease (CVD), protein-energy wasting (PEW) and mortality in prevalent hemodialysis (HD) patients. Aim of the study was to estimate the predictive ability of NT-proBNP and interleukin-6 for CVD, PEW and mortality in those patients.

METHODS: Blood samples were obtained from 383 prevalent HD patients (mean age 65 year, men 63%, diabetes mellitus state (DM) 32%) measuring high-sensitive (hs) CRP, IL-6 and NT-proBNP for this prospective cohort study. PEW was defined by subjective global assessment (SGA). Clinical CVD was ascertained from hospital record. After baseline estimation, patients were followed for 36 months.

RESULTS: NT-proBNP was correlated with BMI (rho -0.27, p<0.0001), hsCRP (rho 0.18, p=0.0005), IL-6 (rho 0.26, p<0.0001). Receiver operative characteristics curves showed that predictive values of NT-proBNP and IL-6 for CVD were similar, however, NT-proBNP was fair and more accurate predictor for PEW than IL-6. In four combined NT-proBNP and IL-6 groups classified according to the median values, prevalence of CVD and PEW was higher in the group with high NT-proBNP and high IL6 than in other groups. Kaplan-Meier analysis demonstrated that patients with high NT-proBNP and high IL6 exhibited a higher all-cause mortality (Long-rank $\chi 2$=18.1, p=0.0004). This finding was also confirmed by Cox hazard model adjusted for confounders (HR: 2.28, 95% confidence interval: 1.00 to 5.91, p<0.05).

CONCLUSIONS: High NT-proBNP together with high IL-6 compared to elevated NT-proBNP or IL-6 alone might be strong predictor for mortality in prevalent HD patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO539

**Red Blood Cell Macrocytosis: A Novel Marker of Survival in Stable Hemodialysis Patients** Karthik K. Tennankore, Steven D. Soroka, Bryce A. Kiberd. *Department of Medicine, Division of Nephrology, Dalhousie, Halifax, NS, Canada.*

BACKGROUND: Red blood cell macrocytosis is a common finding in chronic hemodialysis (CHD) patients; however its significance is unknown. We analyzed the prevalence, distribution, associations and impact on survival of macrocytosis in stable CHD patients.

METHODS: We studied 150 CHD patients at our center. Patients were excluded if they received a blood transfusion within two months or were inpatients at the time of study. Macrocytosis was defined as a mean cellular volume (MCV) > 97 fl averaged over 3 months. All patients were on folate supplementation.

RESULTS: The mean MCV was 99.1+/- 6.3 fl, (range 66-120 fl). MCV was normally distributed. 92 (61%) patients had an MCV > 97 fl, 70 (47%) > 99 fl, 45 (30%) > 102 fl and 24 (16%) > 105 fl. Patients were not B12 or folate deficient. Five patients were on antiepileptic medication.

In a logistic regression analysis, both MCV >102 and >105 fl were associated with older age, antiepileptic use and higher ratios of darbepoeitin alpha dose/(kgBW*hemoglobin). There were 18 deaths at six months in the study. Both mean MCV >102 fl and >105 fl were associated with a statistically significant increase in all cause mortality (log rank p=0.002 and <0.001, respectively).

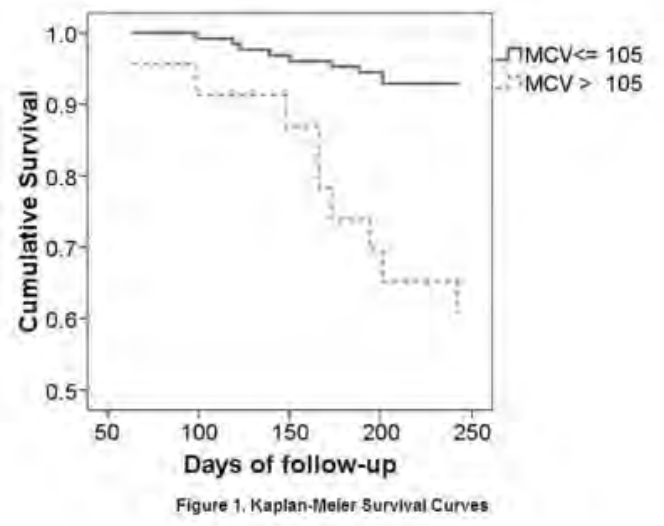



Figure 1. Kaplan-Meier Survival Curves

There was a trend for increased mortality in patients with an MCV > 99 fl (log rank p = 0.067). Adjusting for age, duration of HD, gender, comorbid conditions (diabetes, coronary artery disease, stroke, peripheral vascular disease), HD access, urea reduction ratio, darbepoeitin alpha dose, mean hemoglobin, albumin and ferritin, an MCV > 105 fl was associated with increased mortality (HR 6.13, 95% CI 2.10-17.89 p = 0.01).

CONCLUSION: Macrocytosis may be a novel independent predictor of mortality in stable CHD patients. A longer duration of follow-up will be necessary to confirm this association.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO540

**Neutrophil-to-Lymphocyte Ratio: A Novel Predictor of Survival in Chronic Hemodialysis Patients?** Georges Ouellet,[1,2] E. Lars Penne,[1,2] Len A. Usvyat,[1] Stephan Thijssen,[1,2] Nathan W. Levin,[1,2] Peter Kotanko.[1,2] *[1]Renal Research Institute; [2]Beth Israel Medical Center, New York.*

Neutrophil-to-lymphocyte ratio (NLR), defined as the neutrophil count divided by lymphocyte count, is increasingly recognized as a marker of systemic inflammation and as a prognostic factor for survival in the cardiologic and oncologic literature. Its prognostic value in chronic hemodialysis (CHD) patients is unknown. We aimed to study NLR as a predictor of survival in this population.

Incident CHD patients at RRI clinics between 1/1/2001 and 7/31/2008 were studied. Causes of death were classified as cardiovascular (CV), infection-related (INF), or other. NLR was measured 90 days after dialysis initiation. Patients were stratified in NLR tertiles (T1 to T3). Cox regression analysis was performed with age, gender, race, diabetic status, serum albumin, epoetin resistance index (ERI), NLR and vascular access type as covariates.

We analyzed data from 3707 patients (mean age 61±15 years, 55% male, 48% Black, 52% diabetics, 52% fistulas, 22% grafts, 27% catheters). Median NLR was 3.1 (IQR 2.1 to 4.5). NLR > 3.9 was a significant predictor of mortality in 3 adjusted models (Table 1). Patients in the highest NLR tertile (≥3.9) were at increased risk of death for CV (HR=1.40; 95% CI: 1.14-1.71; P=0.001) and INF (HR=1.70; 95% CI: 1.15-2.50; P=0.008) causes.

Table 1. Cox regression models of NLR and overall mortality

| | NLR tertile | HR | 95% CI | | P value |
|---|---|---|---|---|---|
| Model 1: Adjusted for age, sex, race and diabetic status | T2 | 1.24 | 1.06 | 1.46 | 0.009 |
| | T3 | 1.71 | 1.45 | 2.00 | <0.001 |
| Model 2: Model 1 + serum albumin | T2 | 1.20 | 1.02 | 1.40 | 0.03 |
| | T3 | 1.47 | 1.25 | 1.73 | <0.001 |
| Model 3: Model 2 + ERI and vascular access | T2 | 1.15 | 0.98 | 1.35 | 0.1 |
| | T3 | 1.40 | 1.20 | 1.65 | <0.001 |

T1: NLR< 2.42; T2: 2.42≤NLR<3.90; T3: NLR≥3.90; reference group: T1

This study identifies NLR as a novel and robust predictor of all-cause mortality in incident CHD patients. A NLR greater than 3.9 is associated with an increased risk for CV and INF causes. NLR is an inexpensive and readily available marker that may help to identify incident CHD patients with increased risk of death.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO541

**Relationship of Body Size and Blood Pressure: Impact on Survival in Incident Hemodialysis Patients** Olga Sergeyeva, Len A. Usvyat, Nathan W. Levin, Peter Kotanko. *RRI, NY, NY.*

**Background:**

In hemodialysis(HD) patients (pts) survival improves with body size. Here we explore the relationship between survival, weight and blood pressure (BP).

**Methods:**

All RRI in-center HD patients (pts) who started HD btw Jan 1, 2000 and Jan 2009 with at least 13 txts were studied. Pts' pre-HD systolic (preSBP) and diastolic (preDBP) BP were recorded per txt in the 1st year of HD. preSBP, post-HD weight (postWt) and pulse pressure as % of preSBP (prePPP = 100%*[preSBP - preDBP]/ preSBP) were averaged over the first 30 days; the individual linear slope of preSBP change ($preSBP_{slope}$) was computed over the first year.

Pts were stratified in quartiles (Qt) of postWt: Qt 1 (<63kg), 2 (63-74kg), 3 (74-88kg), 4 (>74kg) and by $preSBP_{slope}$: increased>1mmHg/month; decreased>1mmHg/month; stable: change ± 1mmHg/month.

**Results:**

7077 pts were included (56% male, 53% white, 39% black, 53% diabetic, age [SD]: 62.2 [15.8] years). Pts in postWt Qt 1 had the lowest preSBP and highest prePPP; no significant differences btw $preSBP_{slope}$ were observed although the slope was more positive in higher postWt Qt. Low preSBP, declining preSBP and high prePPP were associated with poorer survival.

Cox proportional hazards models with PreSBP, prePPP, SBP slope, and PostWt Qt adjusted for age, gender, race, and DM status were constructed. PostWt Qt remained a significant predictor of survival (HR [95%CI]: Qt 1: 1.34 [1.08-1.70], Qt 2: ref. group, Qt 3:0.74 [0.57-0.96], Qt 4:0.63 [0.47-0.84].

| Post Wt Qt | Avg preSBP [mmHg] | Avg prePPP [%] | Avg preSBP Slope [mmHg/yr] |
|---|---|---|---|
| 1 | 145.7 | 48.2 | 0.856 |
| 2 | 147.6 | 47.8 | 0.335 |
| 3 | 146.8 | 47.6 | 2.856 |
| 4 | 148.6 | 47.3 | 2.68 |

| significant differences observed | 1 & 2; 1 & 4 | 1 & 3; 1 & 4 | none |
|---|---|---|---|

| | preSBP<120 | | preSBP>150 | |
|---|---|---|---|---|
| Post Wt Qt | HR | p-value | HR | p-value |
| 1 | 1.26 | 0.41 | 1.57 | 0.02 |
| 2 | ref | 1 | ref | 1 |
| 3 | 0.96 | 0.82 | 0.59 | 0.04 |
| 4 | 1.03 | 0.94 | 0.60 | 0.12 |

In pts with preSBP<120 mmHg survival did not differ between PostWt Qt. However, in pts with preSBP>150 mmHg, survival was less in smaller compared to larger pts.

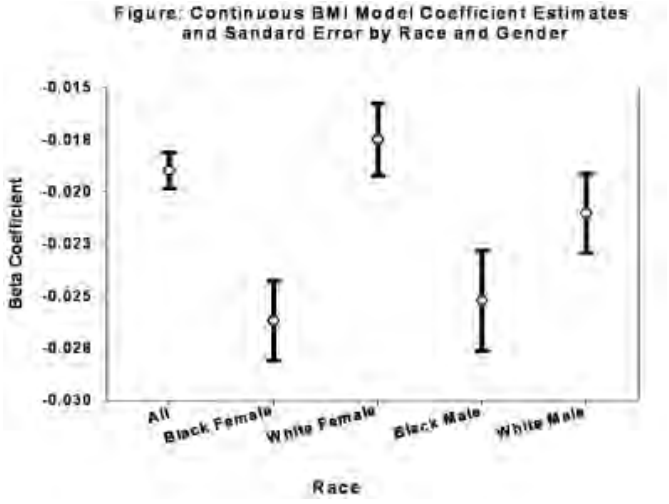
**Conclusion:** The overall beneficial effect of larger size on survival disappears in pts. with systolic BP<120mmHg. In pts with SBP>150 mmHg the benefit of larger body size was still evident. Body size remains a critical determinant of survival.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO542

**Racial and Gender Differences of the Survival Advantage of Obesity in Maintenance Hemodialysis Patients** Joni L. Ricks,[1] Csaba P. Kovesdy,[2] Joel D. Kopple,[1] Keith C. Norris,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor -UCLA, Torrance, CA; [2]Salem VA MC, Salem, VA; [3]King-Drew School of Medicine, Los Angeles, CA.*

**Background:** High body mass index (BMI) is associated with lower death risk in maintenance hemodialysis (MHD) patients, among whom African-Americans (AA) have superior survival. It is not known whether the survival advantage of high BMI differ across race and gender. **Methods:** In the cohort of 122,869 MHD patients including 39,090 AAs and 53,098 Whites from all DaVita dialysis clinics between 7/2001 to 6/2006 with survival follow-up till 6/2007 Cox survival regression models were examined across race and gender strata including after adjustment for case-mix and malnutrition-inflammation complex syndrome (MICS). **Results:** Among AA and White men and women, the mean BMI values were 26.6, 28.1, 26.4 and 27.1 kg/m$^2$, age (mean±SD) was 55.8±4.6, 59.8±15.1, 65.2±15.2, and 65.8±14.9yrs old and 39%, 49%, 41%, and 45% diabetic, respectively. Across all 4 groups, higher BMI was associated with better survival rates. Overall, for each 1 kg/m$^2$ rise in BMI there was approximately 1.9% improvement in mortality. Within race and gender strata, the survival advantage of 1 kg/m$^2$ higher BMI was the largest among AA women (2.6%) and AA men (2.5%), whereas lower survival benefit was observed in white men (2.1%) and white women (1.7%) (See Figure).



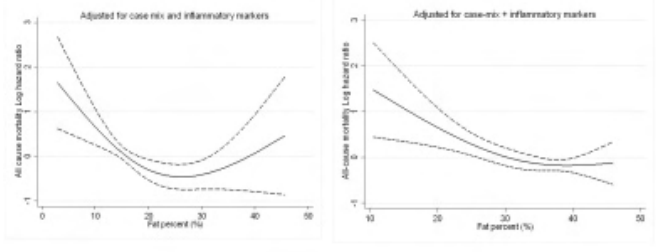

**Conclusions:** Survival advantage of obesity is most prominent among AA men and women and the weakest among White women. The racial and gender differences of obesity paradox deserve further investigations.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO543

**Gender Differences in the Survival-Predictability of Lean vs. Fat Mass in Maintenance Hemodialysis Patients** Nazanin Noori,[1] Ramanath B. Dukkipati,[1] Rachelle Bross,[1] Antigone Oreopoulos,[2] Deborah A. Benner,[3] Joel D. Kopple,[1] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]Univ . Alberta, Edmonton, AB, Canada; [3]DaVita, Lakewood, CO.*

**Background:** Larger body size is associated with greater survival in maintenance hemodialysis (MHD) patients. However, it is not clear whether lean body mass (LBM) or fat mass (FM) are equal predictors of survival in men and women. **Methods**: In 732 MHD patients we categorized men (n=391) and women (n=341) separately into four quartiles of Near-infrared (NIR) measured LBM and FM. Cox proportional models were used to estimate death hazard ratios (HR) [and 95% confidence intervals (95%CI)] after adjustment for case-mix over 5 years (2001-06). **Results:** In women the highest quartiles of FM, FM% and LBM were all associated with greater survival: HR=0.38 (0.20-0.71), 0.57 (0.32-1.03) and 0.34 (0.17-0.67), respectively. In men the highest quartiles of FM and FM%, but not LBM, were associated with lower mortality: HR=0.56 (0.27-0.96), 0.45 (0.23-0.88) and 1.17 (0.60-2.27), respectively. Figure compares cubic spline survival models in men and women.




**Conclusions:** In MHD patients higher fat mass in both genders and higher lean mass in women appear protective. The gender differences of body composition and their impact on survival warrant additional studies.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO544

**Association of Malnutrition and Mortality in Hemodialysis Patients: 5-Year Prospective Cohort Study** Yoshihiko Kanno,[1] Tsuneo Takenaka,[2] Tadashi Yoshida,[1] Matsuhiko Hayashi,[1] Hiromichi Suzuki.[2] *[1]Apheresis and Dialysis Center, Keio University, School of Medicine, Tokyo, Japan; [2]Department of Nephrology, Saitama Medical University, School of Medicine, Iruma, Saitama, Japan.*

346 Japanese patients receiving hemodialysis were followed up for 5 years according to their status on dietary intake. Their mean age at registration was 62 ± 11, mean age of history on hemodialysis was 10 ± 6 years, and 32.9% of them had diabetes mellitus. The status of dietary intake was assessed using self-administered diet history questionnaire (DHQ) with dietician interview. At the end of 5-year observation, 313 of 346 patients were completed the study protocol. Total survival was 78.2% (male 76.0%, female 81.4). The patients were divided into 3 groups according to the event during the study period; Death by any reason [D], Admission by any reason [A], No event [N]).

Dietary intake at the start of the study.

| | | Energy (kcal/kg) | Protein (g/kg) | Salt (g) | Phosphate (mg) |
|---|---|---|---|---|---|
| No Event | Total | 28.3±8.1 | 0.96±0.31 | 6.3±2.7 | 778±255 |
| | Male | 27.4±0.8 | 0.92±0.29 | 6.3±2.8 | 784±257 |
| | Female | 29.5±1.2 | 1.01±0.33 | 6.2±2.7 | 768±255 |
| Admission | Total | 27.3±9.6 | 0.90±0.37 | 5.5±3.0 | 719±283 |
| | Male | 26.9±10.3 | 0.86±0.37 | 5.8±4.0 | 743±293 |
| | Female | 27.8±8.7 | 0.95±0.37 | 5.3±2.4 | 689±270 |
| Dead | Total | 25.3±8.7* | 0.85±0.35* | 5.3±2.7* | 686±283 |
| | Male | 24.4±7.9 | 0.78±0.26 | 5.0±2.7 | 666±243 |
| | Female | 27.0±10.1 | 0.99±0.47 | 5.9±3.0 | 726±352 |
| Total | Total | 27.3±8.8 | 0.91±0.34 | 5.8±2.9 | 738±272 |
| | Male | 26.5±8.5 | 0.87±0.31 | 5.9±3.0 | 743±268 |
| | Female | 28.4±9.1 | 0.99±0.37 | 5.8±2.7 | 731±280 |

As shown in table. Dietary intake of energy, protein and salt were significantly lower in D group compared with A and N groups. As food material, intake of meat and animal fat were also significantly lower in D group than other two groups. It is suggested that shortage of calorie and protein intake may result in malnutrition and can be a predictor of prognosis of dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO545

**Impact of Seropositivity for Hepatitis C Virus Core Antigen on Long-Term Mortality in Patients on Maintenance Hemodialysis** Akihiko Kato,[1] Hideo Yasuda,[2] Hiroyuki Suzuki,[2] Yoshihide Fujigaki,[2] Tatsuo Yamamoto,[3] Akira Hishida.[2] *[1]Division of Blood Purification, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; [2]First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; [3]Faculty of Health Promotional Sciences, Department of Health and Nutritional Sciences, Hamamatsu University, Hamamatsu, Shizuoka, Japan.*

Serological status positive for anti-HCV antibodies (HCV-Ab) is independently associated with poor prognosis in hemodialysis (HD) patients. However, HCV-Ab cannot distinguish between patients with active infection and those who have recovered from infection. Although measurement of HCV core antigen (HCVcAg) is shown useful to detect active HCV infection, there was no study to assess the impact of HCVcAg seropositivity on clinical outcomes in HD patients. In this study, we measured serum HCVcAg using an immunoradiometric assay (IRMA) in 405 HD patients (age: 63±13 years, time on HD: 126±104 months), and examined whether HCVcAg seropositivity is associated with mortality during the 93-month follow-up. There were 82 (20.2%) patients who had been positive for HCV-Ab, and 57 (69.5%) of them had been positive for HCVcAg. A significant increase was found in serum ALT (21±10 vs. 10±6 IU/L, p<0.01) and AST (19±9 vs. 13±8 IU/L, p<0.01), while there was a significantly lower level of platelet count (14.8±4.7 vs. 19.5±6.7 x$10^4$/μL, p<0.01) in patients positive for HCVcAg when compared with those for negative. During the follow-up, 113 (39.1%) out of the 289 patients who had been younger than 75 years old had expired, 57 out of them were due to non-cardiovascular (non-CV) causes. Univariate Cox hazards analysis revealed that HCVcAg seropositivity was associated with total (hazards ratio (HR): 1.67 [1.07-2.59], p<0.03) and non-CVD death (HR: 2.24 [1.24-4.02], p<0.01). After adjusting for co-morbid parameters, HCVcAg was also associated with total (HR: 1.81 [1.08-3.03], p<0.03) and non-CV mortality (HR: 2.12 [1.11-4.06], p<0.03). It follows from these findings that HCVcAg seropositivity is more sensitive to identify persistent HCV infection, and associated with long-term mortality in HD patients.

Disclosure of Financial Relationships: nothing to disclose

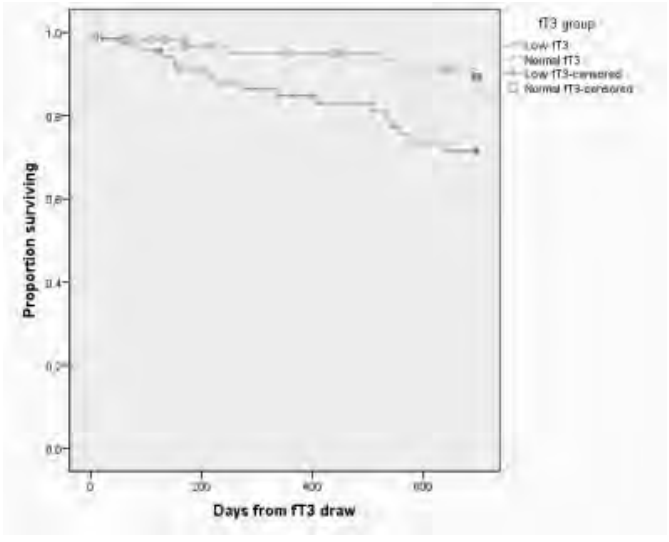
### TH-PO546

**Free and Total Triiodothyronine Levels and 2-Year Survival in Chronic Hemodialysis (HD) Patients** Rebecca Apruzzese,[1] Sudhi Tyagi,[1] Anja Kruse,[1,3] Georges Ouellet,[1,2] Laura Rosales,[1] Len A. Usvyat,[1] Mary Carter,[1] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]RRI, NYC; [2]Beth Israel Medical Center, NYC; [3]Bern University Hospital, Bern.*

Background: Low levels of total (T3) and free (fT3) triiodothyronine are independent predictors of mortality in White chronic HD patients (pts) (Zoccali, 2006; Carrero, 2007). This study aimed to examine the relationship between survival, T3 and fT3 in Black and Non-Black US HD pts.

Methods: Cross-sectional T3 and fT3 samples were obtained pre-HD from pts in 1 NYC RRI clinic, June 2008. 1.5 yr followup fT3 sampling was done on a subset of original cohort and compared by paired T-Test. T3 and fT3 were measured by direct chemiluminescence (T3:ADVIA T3 Lite Reagent and Solid Phase; fT3: ADVIA Centaur assay). Pts were stratified by levels of fT3 (low > 2.3; normal - 2.3-4.2pg/ml). T3 was analyzed as a continuous variable because 98.7% of the data were in the normal range. Survival was analyzed with the Kaplan-Meier method and Cox proportional hazard model adjusted for race, gender, diabetes, age, and vintage.

Results: We studied 155 pts (age 61±15 yrs (mean±SD); HD mean vintage 3.76±3.8; 52% male; 39% diabetics; 62% black. 1.5 yr follow-up fT3 data for 33 pts did not differ significantly from baseline. Kaplan-Meier analysis showed reduced 2 yr survival in pts with low fT3 (P=0.015; log-rank test). In multivariate Cox analysis normal levels of fT3 predicted improved survival (HR 0.38 [95% CI 0.15 to 0.96], P= 0.042). T3 showed borderline significant associations with survival (HR 0.21 [95% CI 0.04 to 1.05], P=0.057). No differences between Blacks and Non-Blacks were observed.




Conclusion: Results show low fT3 levels are associated with poor survival in a diverse cohort of US HD pts corroborating findings obtained in Whites. fT3 levels did not significantly change over 1.5 yrs, rendering it a valuable predictor of survival.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO547

**Increased Rate of Hospitalization in Adult Patients with ESRD Secondary to Systemic Lupus Erythematosus (SLE)** Sangeeta D. Sule,[1] Barbara A. Fivush,[1] Alicia M. Neu,[1] Susan L. Furth.[2] *[1]Pediatrics, Johns Hopkins Univ, Baltimore, MD; [2]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA.*

**Background:** To assess whether the risk of hospitalization is higher in patients with ESRD secondary to SLE compared to patients with ESRD secondary to other causes.

**Methods:** Data from the United States Renal Data Systems (USRDS) 2004 Hospitalization File were used to compare hospitalization rates among pediatric (age < 19 yrs) and adult patients who initiated dialysis between Jan 1, 2000 through Dec 31, 2004. Patients were censored at transplant or the end of follow-up. Hospitalization rate ratios were compared by Poisson regression.

**Results:** 9935 patients were included in the analysis. There was an increased prevalence of female and Black race patients in those with SLE (Table 1). Pediatric patients with SLE had a trend toward higher rate of hospitalization, although it did not reach statistical significance (IRR: 1.2, 95% CI: 0.9-1.5). Despite being much younger (mean age 40 vs 58 yrs (p<0.001), adult patients with SLE were hospitalized more frequently than adults with other causes of ESRD (IRR: 1.3, 95% CI: 1.2-1.4). The most frequent reason for hospitalization for adult patients with SLE was cardiovascular disease (myocardial disease) and malignant hypertension.

Table 1

| | SLE causing ESRD | | Other Causes of ESRD | |
|---|---|---|---|---|
| | Peds N=64 | Adults N=134 | Peds N=948 | Adults N=8189 |
| Mean Age (years [SD]) | 16 [2.5] | 40 [14.8] | 13.6[4.9] | 58 [15.6] |
| % Black Race | 66 | 55 | 35 | 38 |
| % Female | 79 | 83 | 44 | 47 |
| Hospitalization Rate Ratio | 1.2 | 1.3 | -- | -- |

**Conclusions:** Although pediatric patients with SLE do not have a significantly increased risk of hospitalization, this data supports that adult patients with SLE do. It will be important to understand the impact of cardiovascular disease in both the pediatric and adult population with SLE and its impact on morbidity and hospitalization risk.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO548

**Risks of All Malignancies, Liver, Kidney, and Urinary Tract Cancers before and after Dialysis in ESRD Patients – Data from Taiwan National Health Insurance** Shang-Jyh Hwang,[1,2] Ming-Yen Lin,[1] Li-Tzong Chen,[3] H. C. Chen.[1,2] *[1]Division of Nephrology, Kaohsiung Medical University Hospital, Taiwan; [2]Faculty of Renal Care, College of Medicine, Kaohsiung Medical University, Taiwan; [3]National Health Research Institute, Taiwan.*

**Background:** We aimed to estimate the risks of malignancies for dialysis patients in period before and after dialysis.

**Method:** Patients initiating dialysis over 90 days in 1997-2004 from National Health Insurance is considered as dialysis group (DG) and matching Control group (CG), was from general populations, by birth year and sex. Time was backward and forward calculated from the dialysis date to the first date of malignancies diagnosed or first of 1997 and Sept. 30 of 2004. Cox regression model was used and two sides p value less than 0.05 was considered as significant.

**Results:** The risks of all malignancies, liver cancer, renal cancer, transitional cell carcinoma, upper urinary tract cancer, and bladder cancer were higher in DG group than in CG group,in periods before and after dialysis.

Table 1. Hazard ratios of various malignancies in dialysis group compared to control group before and after dialysis

| Sites (ICD9 code) | Before dialysis | | After dialysis | |
|---|---|---|---|---|
| | Hazard Ratio (95% CI) | p-value | Hazard Ratio (95% CI) | p-value |
| All malignancy (140-208) | 2.25 (2.09, 2.43) | <0.001 | 1.66 (1.57-1.75) | <0.001 |
| Liver cancer (155) | 2.06 (1.61, 2.62) | <0.001 | 1.78 (1.54-2.07) | <0.001 |
| Renal cancer ( 189 or 189.0) | 11.91 (5.54, 25.61) | <0.001 | 9.65 (6.22-14.99) | <0.001 |
| Transitional cell carcinoma (188 or 189.1, 189.2) | 5.73 (4.72, 7.96) | <0.001 | 7.74 (6.04-9.92) | <0.001 |
| Upper urinary tract cancer (189.1 or 189.2 ) | 15.79 (6.44-38.71) | <0.001 | 10.78 (6.35-18.28) | <0.001 |
| Bladder cancer (188) | 4.47 (3.13-6.40) | <0.001 | 7.16 (5.41-9.47) | <0.001 |

Full Model adjusted by age, sex, dialysis year, and geographic.

**Conclusions:** The study demonstrates that dialysis ESRD patients had higher risks of malignancies than the general populations even before dialysis.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO549

**Factors That Determine Survival in Patients with Multiple Myeloma Who Receive Dialysis** Rebecca Jane Roberts,[1] Dominic Summers,[2] Murugan Sivalingam,[1] Ken Farrington.[1] *[1]Nephrology, Lister Hospital, United Kingdom; [2]Department of Surgery, Addenbrooke's Hospital, United Kingdom.*

Registry data indicate that patients with end stage renal failure and multiple myeloma have a median survival of approximately 11 months. However some patients exceed survival expectations and may live several years. Early recognition of factors that are associated with better prognosis would inform clinical decisions and enhance care. The aim of this study was to investigate factors that predict survival advantage in patients with multiple myeloma who require dialysis.

Methods: The case notes of 91 patients who presented between 1991 and 2009 with myeloma (diagnosed according to International Myeloma Working Group criteria) who received dialysis were reviewed. The influence on survival of: presentation blood parameters (haemoglobin, white cell count, serum calcium, creatinine and albumin); patient age (median, 68, range 37-88) and gender (58:33 M:F ratio); era of diagnosis (before or after 2000); and renal recovery was assessed using Cox regression methods.

Results: The unadjusted factors that were associated with improved survival were renal recovery, haemoglobin (Hb) and albumin of 25-35g/L. On performing adjusted cox regression analysis, only renal recovery and Hb were independent predictors of improved survival. The association between renal recovery and survival was striking; the hazard ratio (HR) for death in those requiring dialysis versus those whose renal function recovered was 4.9 (95% CI 2.4-10.1, p=<0.001). An Hb of <10g/dl at presentation was linked to poorer prognosis (adjusted HR 1.64 (CI 1-2.70) p= 0.05).

Conclusion: Reversal of renal failure offers a key survival advantage to patients with a diagnosis of multiple myeloma who require dialysis. While this association may simply reflect a subgroup of patients with less severe myeloma and a reversible precipitant (such as hypercalcaemia or infection), it raises the question of whether aggressive therapies to improve renal function, such as chemotherapy and removal of nephrotoxic immunoglobulin, would improve survival.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO550

**Survival on Dialysis in the Systemic Amyloidoses** Jennifer H. Pinney,[1,2] Helen J. Lachmann,[1] Prayman Sattianayagam,[1] Simon Gibbs,[1] Carol J. Whelan,[1] Ashutosh D. Wechalekar,[1] Philip Hawkins,[1] Julian D. Gillmore.[1] *[1]National Amyloidosis Centre, Division of Medicine, UCL Medical School, London, United Kingdom; [2]Centre for Nephrology, Division of Medicine, UCL Medical School, London, United Kingdom.*

Systemic amyloidosis frequently involves the kidneys and may lead to end stage renal disease (ESRD). The prognosis on dialysis depends on the amyloid type but is reportedly poor in both AA and AL amyloidosis. We report outcome among all dialysis-dependent patients with systemic amyloidosis evaluated at the UK National Amyloidosis Centre (NAC) between 1983 and 2010. Survival was estimated by Kaplan-Meier plots, and patients were censored at renal transplantation.

Two hundred and forty nine of 1094 (23%) patients with renal AL amyloidosis, 158 of 425 (37%) patients with AA amyloidosis, 50 of 89 (56%) with fibrinogen Aa-chain amyloidosis (AFib), 11 of 58 (19%) with apolipoprotein AI amyloidosis (AApoAI), and 3 of 17 (18%) with lysozyme amyloidosis (ALys) reached ESRD. Median age at start of dialysis in AL, AA, AFib, AApoAI and ALys was 62, 47, 60, 50 and 62 years respectively. Median time from diagnosis of amyloidosis to ESRD in AL, AA, AFib, AApoAI and ALys was 1.0, 3.8, 4.5, 6.6 and 8 years respectively. Median survival on dialysis in AA amyloidosis was 45.5 months; 1 year mortality was 14% with an annual mortality of 10%. Median survival on dialysis in AL amyloidosis was 38.9 months and was heavily influenced by presence of cardiac amyloid deposits (P<0.0001). Median survival on dialysis in AFib was 116 months and 1 and 5 year mortality was 5% and 20% respectively. A single patient with AApoAI died after 67 months of dialysis and the remainder received renal transplants a median of 32 months after starting dialysis. Two patients with ALys were on dialysis for 7 and 30 months respectively and the third received a renal transplant after 3 months of dialysis which was functioning at censor.

Survival on dialysis is substantially better in AA and AL amyloidosis than previously reported and is better still among patients with hereditary renal amyloidosis who reach ESRD. These findings highlight the importance of correctly diagnosing the amyloid fibril type in patients with renal amyloidosis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO551**

**Novel Measures of LDL and HDL Subfractions and Mortality in Maintenance Hemodialysis Patients** Nazanin Noori,[1] Michael Caulfield,[2] Allen R. Nissenson,[3] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]Quest Diagnostics, Irvine, CA; [3]DaVita, Lakewood, CO; [4]Salem VA, Salem, VA.*

Measuring subfractions of lipoproteins via novel ion mobility method may better risk-stratify maintenance hemodialysis (MHD) patients (pts). We examined mortality predictability of LDL and HDL subfractions in 235 MHD pts over 6 yrs (2001-07). HDL subfractions did not correlate with mortality. Cox regression was adjusted for case-mix (including vintage, Charlson score & Kt/V), malnutrition-inflammation complex (albumin, creatinine, phos, calcium, ferritin, hemoglobin, nPCR, & BMI) and inflammatory markers (CRP, IL-6, & TNFα). The highest quartiles of VERY SMALL and LARGE particles of LDL were associated with highest and lowest death risk, respectively (see Table):

Death hazard ratios according to quartiles of LDL subfractions concentration

| Very Small LDL quartiles | Q2(n=60) | Q3(n=59) | Q4(n=58) | p for trend |
|---|---|---|---|---|
| concentration (nmol/L) | 57-87 | 88-121 | >121 | |
| Unadjusted | 0.84(0.42-1.70) | 0.93(0.45-1.91) | 1.54(0.82-2.89) | 0.13 |
| Case-mix | 0.79(0.39-1.61) | 0.97(0.47-2.03) | 1.75(0.95-3.33) | 0.05 |
| | | | | |
| + MICS | 0.65(0.29-1.46) | 0.89(0.40-1.99) | 2.15(1.02-4.56)* | 0.02 |
| + inflammation | 0.65(0.29-1.49) | 0.84(0.37-1.94) | 2.14(1.00-4.62)* | 0.02 |
| Large LDL quartiles | Q2(n=54) | Q3(n=58) | Q4(n=58) | p for trend |
| concentration (nmol/L) | 44-76 | 77-105 | >105 | |
| Unadjusted | 0.70(0.37-1.34) | 0.88(0.47-1.62) | 0.45(0.22-0.92)* | 0.05 |
| Case-mix | 0.76(0.38-1.52) | 0.96(0.51-1.84) | 0.53(0.25-1.12) | 0.18 |
| | | | | |
| + MICS | 0.45(0.21-0.98) | 1.07(0.52-2.21) | 0.49(0.21-0.98)* | 0.36 |
| + inflammation | 0.45(0.21-0.98) | 1.14(0.55-2.37) | 0.47(0.20-0.99)* | 0.37 |

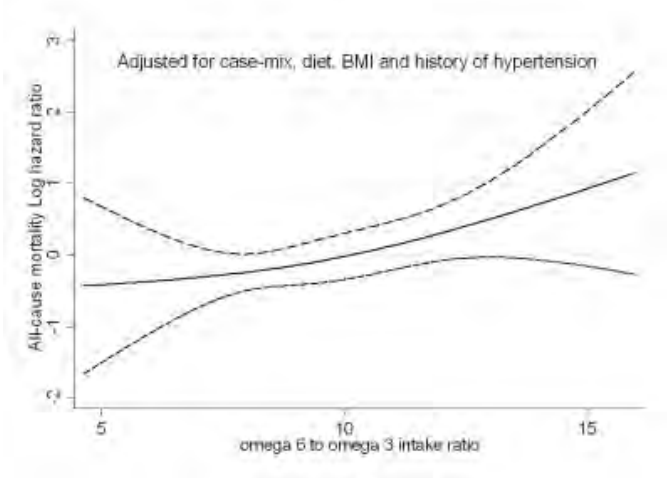
Hence, lower concentrations of VERY SMALL LDL and higher concentrations of LARGE LDL appear associated with greater survival, which needs to be verified in additional prospective studies.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO552**

**Dietary Omega-3 Fatty Acid, Ratio of Omega-6 to Omega-3 Intake and Survival in Long-Term Hemodialysis Patients** Nazanin Noori,[1] Sameer B. Murali,[1] Ramanath B. Dukkipati,[1] John J. Sim,[2] Usama Feroze,[1] Rachelle Bross,[1] Deborah A. Benner,[3] Joel D. Kopple,[1] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Hrabor-UCLA Medical Center, Torrance, CA; [2]Kaiser Permanente, Los Angeles, CA; [3]DaVita, Lakewood, CO; [4]Salem VA, Salem, VA.*

**Background:** Human beings evolved on a diet with an equal proportion of omega-6 to omega-3 poly-unsaturated fatty acid (PUFA). In Western diets this ratio has dramatically increased up to 15 times higher. Given the evidence that half of all CKD deaths are attributed to cardiovascular disease, the anti-inflammatory and cardioprotective benefits of omega-3 PUFA may play an important role in modulating these processes to reduce mortality. **Methods:** Using 3-day diet record, we examine the survival predictability of dietary omega-3 PUFA and the ratio of omega-6 to omega-3 PUFA at the start of the cohort of 145 hemodialysis patients who were followed for up to 6 years (2001-07). **Results:** There was no correlation between omega-3 PUFA intake and mortality. However, the lowest (vs. highest) quartile of dietary omega-6 to omega-3 ratio was associated with decreased mortality hazard ratio (95% CI) in the analyses adjusted for age and gender [0.54 (0.21-1.38), p trend=0.14], case-mix plus diet [0.37 (0.14-1.08), p trend=0.04] and case-mix plus diet plus BMI plus history of hypertension (HTN) [0.39 (0.14-1.18),p trend=0.06]. Spline survival models confirmed the associations (Figure).




**Conclusions:** Higher omega-6 to omega-3 PUFA intake ratios are associated with increased death risk in hemodialysis patients, even after adjustments for energy, saturated fatty acids, trans fat, cholesterol and fiber intakes. These findings, if verified in additional, prospective studies, suggest that the low dietary ratios of omega 6 to omega 3 PUFA should be recommended to MHD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO553**

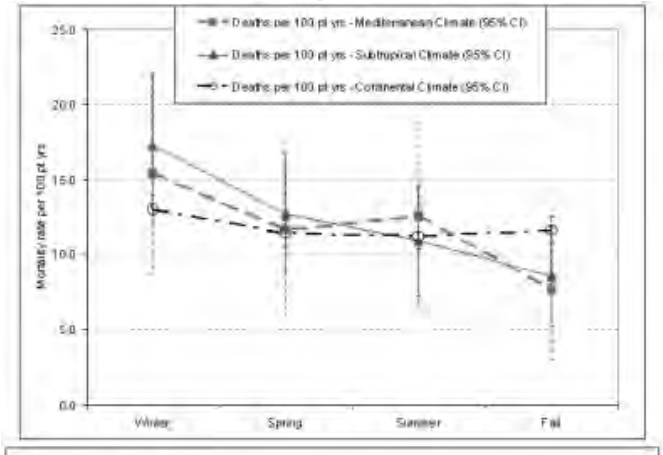
**Seasonal Variability in Mortality of Chronic Hemodialysis Patients** Len A. Usvyat,[1] Frank Van der Sande,[2] Jeroen Kooman,[2] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Renal Research Institute, NY, NY; [2]Maastricht University Medical Centre, Maastricht, Netherlands.*

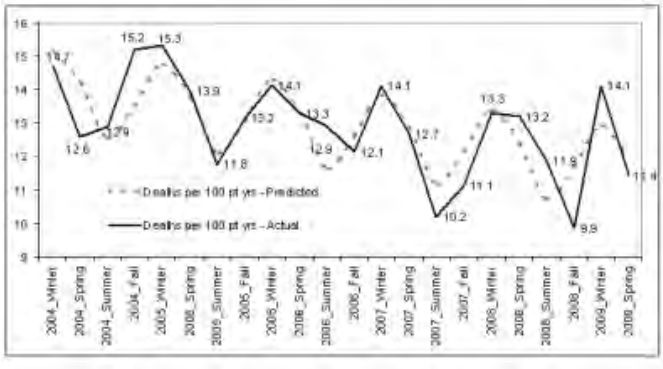
In the general population, mortality varies seasonally, but it is unknown whether this phenomenon is present in hemodialysis (HD) patients (pts). The aim of the present study was to assess seasonal variations in mortality across diverse climatic US regions.

We reviewed records of HD pts treated in RRI clinics b/n Apr 1, 2004 and Mar 31, 2009. 15,056 pts were studied (55% male, 45% black, 45% white, 48% diabetic). Seasons were defined on a calendar basis. All cause mortality was noted per season and expressed as deaths [95% confidence interval] per 100 pt yrs (D100PY).

Cosinor analysis was conducted to test for seasonality in mortality. Given that RRI clinics are located in 6 states, they were grouped in distinct climate groups: continental (14,015 pts in NY, CT, MI, and IL), mediterranean (316 pts in CA), and subtropical (737 pts in NC).

Irrespective of climatic zone all cause mortality was highest in winter (14.2 [13.3 to 15.1] D100PY), followed by spring (13.1 [12.2 to 14.0] D100PY), fall (12.3 [11.4 to 13.2] D100PY), and summer (11.9 [11.1 to 12.7] D100PY). The results were further confirmed by comparing seasonal variations across climates with highest mortality in winter ($p<0.05$ for all geographies); mortality was lowest in summer in the continental climate ($p<0.05$) and in fall in the mediterranean and subtropical climates (NS).




Cosinor analysis over a 5 yr period demonstrated a seasonal component of mortality: highest in winter and lowest in summer. Overall decline in mortality over a 5 yr period was observed.

This study demonstrates a significant seasonal influence on mortality in chronic hemodialysis pts with highest mortality in the winter and lowest mortality in the summer. Seasonal effects of mortality should be considered in the design and analysis of clinical trials.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO554**

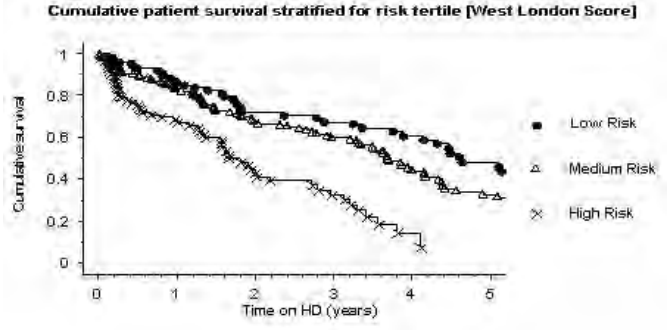
**Prognosis of Elderly Patients on Haemodialysis – A Novel Comorbidity Score** Albert J. Power, Kakit Chan, Seema Singh, David Taube, Neill D. Duncan. *Imperial College Kidney & Transplant Institute, West London Renal & Transplant Centre, Hammersmith Hospital, London, United Kingdom.*

Although a number of scoring systems have been developed and validated in patients with ESRD to predict survival they are complex and address all age groups. The purpose of this study was to develop a simple scoring system to predict prognosis in elderly hemodialysis [HD] patients.

All incident patients >75 yrs on HD alone Jan 2000-Dec 2009 were examined. The Charlson Comorbidity Index [CCI] and its adapted form for ESRD [Hemmelgarn, 2003] was calculated using standardised definitions and cardiac failure defined as LV ejection fraction <35% and/or NYHA Class III/IV. Weibull and logistic regression models were used to test associations and 2yr predictive power.

Mean CCI was 4.5±2.0, adapted CCI 3.7±3 [n=410, mean age 80.0±3.5 yrs, 64% male, mean MDRD eGFR 8.4±3.3ml/min]. Cumulative survival [inc. first 90d] was 80%, 63%, 56% & 32% at 1, 2, 3 & 5 yrs; mean survival 3.5 yrs [2.3 yrs, USRDS 2005, 8.5 yrs in age-matched UK population].

Four factors associated with worse survival: cardiac failure [HR 1.70, 95%CI 1.09-2.64, p<0.001], age per yr [HR 1.06, 95%CI 1.00-1.12, p=0.01], male [HR 1.56, 95%CI 1.00-2.45, p=0.05] and metastatic malignancy [HR 3.66, 95% CI 1.14-11.7, p<0.05] and predicted 2yr survival [AUC=0.69]. Ethnicity, diabetes, eGFR and other coded comorbidities had no effect on survival. Higher CCI & adapted CCI also associated with worse survival [p=0.03 & p<0.001].




This succinct prognostic model in the elderly on HD is unique in the literature to our knowledge. Cardiac function appears to be the prime determinant of survival and needs accurate definition at an ideal dry weight. Other factors known to affect survival in other age groups do not appear to be significant. The favourable performance of our model requires larger studies for comprehensive validation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO555**

**Assessment of 5-Year Survival on Hemodialysis (HD) in Brazil: A Cohort of 3,149 Incident Patients** Jorge Strogoff-de-Matos,[1] Frederico Ruzany,[3] Adrian Marcos Guinsburg,[2] Ana Beatriz Barra,[3] Marcos Sandro Fernandes de Vasconcelos,[3] Eufronio D'Almeida,[3] Marcos Hoette,[3] Jocemir R. Lugon.[1] *[1]Medicine / Nephrology, Universidade Federal Fluminense, Rio de Janeiro, RJ, Brazil; [2]Fresenius Medical Care, Buenos Aires, BA, Argentina; [3]Fresenius Medical Care, Rio de Janeiro, RJ, Brazil.*

Brazil has the third largest contingent of patients on maintenance HD worldwide. However, little is known regarding survival rate and predictors of mortality risk in that population, which are the objectives of this study.

Incident patients on HD from 01/01/2000 to 06/30/2004 from 26 dialysis facilities distributed among 7 out of 26 states of Brazil were followed up until 06/30/2009. Data were extracted from Latin America FCM Registry. Primary outcome was all cause mortality. Data were censored at 5 years of followed-up. Survival was calculated by Kaplan-Meier method; curves were compared by Log-Rank test. Cox proportional model was used to assess associations of demographic, clinical and laboratory variables with the risk of death.

A total of 3,159 patients were included in this analysis. Patients were 52 ± 16 years old, 57.8% males, 20.1% diabetics, 4.2% HCV+, 1.3% HBV+, and 0.5% HIV+. Out of those, 2101 patients dropped out before completing the 5-year follow-up (47% death, 31% change of center, 16% kidney transplant, 3% migration to peritoneal dialysis, 3% recovery of renal function, and 1% abandonment of treatment). The global 5-year survival rate was 58.5%. Age (HR= 1.04 per year, P <0.0001), and diabetes (HR= 1.61, P <0.0001) were associated with increased risk of death, whereas serum albumin (HR= 0.70 per g/dL, P <0.0001) and body mass index (HR= 0.98 per Kg/m2, P= 0.018) were protective.

The mortality rate on HD in this Brazilian cohort was found to be relatively low, but the population is younger and has a lower prevalence of diabetes than the ones reported for developed countries.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO556**

**Social Adaptability Index Application in Dialysis Population** Gurprataap Singh Sandhu,[1] Anna Barenbaum,[2] Hongying Tang,[1] Preeti Rout,[1] Bradley C. Baird,[3] Mark E. Williams,[1] Alexander S. Goldfarb-Rumyantzev.[1] *[1]Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA; [2]Tel Aviv University, Tel Aviv, Israel; [3]University of Utah, Salt Lake City, UT.*

Background. Patient groups associated with disparities in health care are usually defined on the basis of race, gender or geographic location. Social Adaptability Index (SAI) calculated based on education, marital status, income, employment and substance abuse has been strongly associated with clinical outcome in other patient populations.

Methods. We used data from trhe USRDS to evaluate the role of SAI in survival of patients on dialysis in Cox model. We also studied subgroups based on age, race, sex, comorbidites and diabetic status.

Results. We analyzed 3,396 patients (age of ESRD onset 56.9±16.1 years, 54.2% males, 64.2% White, 30.3% African American). Mean SAI was 7.1±2.5 (range 0 to 12 points). SAI was higher in Whites (7.4±2.4) than African Americans (6.5±2.5) [ANOVA, p<0.001] and greater in men (7.4±2.4) than in women (6.7±2.5) [T-test, p<0.001]. In Cox model adjusted for potential confounders SAI was associated with decreased mortality (HR of 0.97, [95% CI 0.95-0.99], p=0.005). Subgroup analysis demonstrated association of SAI with survival in most of the subgroups.

The SAI association with mortality in the entire population and study subgroups by proportional hazards model

| | Hazard ratio (95% CI) | p |
|---|---|---|
| SAI in the entire study population | 0.97 (0.95-0.99) | 0.005 |
| SAI in males | 0.95 (0.93-0.97) | <0.001 |
| SAI in females | 0.97 (0.94-0.99) | 0.027 |
| SAI in patients 18-40 years old | 0.87 (0.80-0.94) | 0.002 |
| SAI in patients 40-65 years old | 0.95 (0.92-0.98) | 0.003 |
| SAI in patients >65 years old | 0.98 (0.95-1.01) | 0.216 |

Potential limitations of the study include reverse causality, possible misclassification, deficient psychometric data and retrospective design.

Conclusion. SAI is significantly associated with mortality in dialysis patients. SAI could be used to identify individuals at risk for inferior clinical outcomes.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO557**

**Survival of Patients on Renal Replacement Therapy (RRT) after Intensive Care Unit (ICU) Admission** Bhadran Bose,[1] Roy Cherian,[1] Michael Steele,[2] Thomas T. Titus.[1] *[1]Department of Nephrology, Gold Coast Hospital, Southport, Queensland, Australia; [2]Faculty of Health Science & Medicine, Bond University, Gold Coast, Queensland, Australia.*

**Aim:** To report the survival rates of patients on renal replacement therapy admitted to ICU and its relationship to their co-morbidities (Charlson Co-morbidity index), SAPS-2, APACHE II, APACHE III and APACHE III risk scores on admission.

**Background:** Survival of patients on RRT after an ICU admission is believed to be poor given the high co-morbidity in these patients. We hypothesised that survival in these patients was related to their co-morbid status and the severity of their illness at the time of admission to the ICU rather than the presence of End Stage Renal Disease (ESRD).

**Methods:** A retrospective cohort study was undertaken evaluating the hospital charts of 53 ESRD patients who were admitted to Gold Coast Hospital ICU from July 2003 – June 2008.

**Results:** Out of 4825 admissions to ICU, there were 65 admissions with ESRD (1.3%) with 53 patients. The major causes of ICU admission was post- surgery (32.3%), pulmonary oedema (15.4%) and sepsis (13.8%). 89.2% of admissions were discharged from the ICU to the ward and 75.4% were discharged alive from the hospital. SAPS-2, APACHE II, APACHE III, APACHE III risk scores showed statistically significant differences between survivors and non survivors at the time of discharge from ICU and from hospital. Charlson co morbidity score did not show any statistically significant difference between survivors and non-survivors at the time of ICU and hospital discharge.

From the initial admission to ICU, 54% of patients were alive at one year. APACHE III and APACHE III risk scores at the first ICU admission were predictive of survival at one year using logistical regression analysis**.**

**Conclusions:** Survival of ESRD patients after ICU admission appears to be related to the severity of illness necessitating ICU admission as measured by the acute physiological scores. APACHE III and APACHE III risk scores correlated with survival at one year. Charlson co-morbidity scores did not differentiate survivors from non-survivors.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO558**

**Urinary Biomarkers Predict Renal Recovery in Critically Ill Patients with Renal Support: Result from BioMaRK Study** Nattachai Srisawat,[1] Xiao Yan Wen,[1] Minjae Lee,[1,2] Lan Kong,[1,2] Mark L. Unruh,[1,3] Kevin W. Finkel,[4] Anitha Vijayan,[5] Emil P. Paganini,[6] Mohan Ramkumar,[3,7] Paul M. Palevsky,[1,3,7] John A. Kellum.[1,3] *[1]CRISMA Center, Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA; [2]Dpt Biostatistics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; [3]Dpt Medicine, University of Pittsburgh, Pittsburgh, PA; [4]University of Texas Medical School at Houston, TX; [5]Washington University in St. Louis, MO; [6]Cleveland Clinic Foundation, Cleveland, OH; [7]VA Pittsburgh Healthcare System, Pittsburgh, PA.*

Introduction: Predicting renal recovery after acute kidney injury (AKI) is challenging and there are no clinical methods available. The discovery of biomarkers to predict recovery could represent an important new prognostic tool for management of AKI.

Methods: We conducted the Biological Markers of Recovery for the Kidney (BioMaRK) study as an ancillary to the VA/NIH Acute Renal Failure Trial Network (ATN) study, a prospective, multicenter randomized trial comparing two intensities of renal support. We collected urine on days 1, 7, and 14 from 76 patients who developed AKI and received renal replacement therapy. We explored whether urinary NGAL (uNGAL), HGF (uHGF), Cystatin C (uCystatin C), IL-18 (uIL-18), or NGAL/MMP-9 (uNGAL/MMP-9) could predict renal recovery. We defined recovery as alive and free of dialysis by day 60.

Results: Patients who had higher uCystatin C on day 1 (7.27 [6.50-8.33] vs 6.60 [4.97-7.58] ng, P=0.02), lower uHGF on days 7 and 14 (2.97 [2.24-3.63] vs 3.48 [3.04-4.44], P=0.01, 2.24 [1.35-2.95] vs 3.40 [2.87-4.79] ng, P=0.04, respectively), all per mg creatinine, and decreasing uNGAL and uHGF in the first fourteen days had greater odds of renal recovery, (P = 0.01 and P = 0.003, respectively). A prediction model combining urinary biomarkers with clinical markers resulted in an area under the receiver operator characteristics curve of 0.94 for predicting renal recovery at day 60.

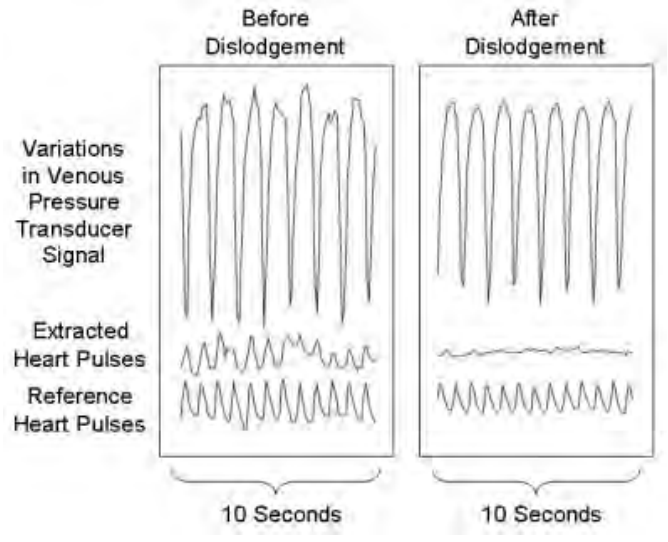
Conclusion: This study demonstrates that biomarkers may be useful for predicting renal recovery after AKI especially together with clinical data.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO559**

**Successful Detection of Venous Needle Dislodgement Using Extracted Heart Pulses Obtained from Venous Pressure Monitoring** Marten Segelmark,[1] Lena Mattsson,[2] Sarok Said,[2] Bo Olde,[3] Kristian Solem.[3] *[1]Division of Nephrology, Department of Clinical Sciences, Faculty of Medicine, Lund University, Lund, Sweden; [2]Department of Nephrology, University Hospital, Lund, Sweden; [3]Research Department, Gambro Lundia AB, Lund, Sweden.*

Accidental venous needle dislodgement (VND) during dialysis is a rare, but serious clinical event. Modern dialysis machines rely on pressure monitoring to detect VND. However, better venous needle monitoring (VNM) is needed because in practice it may be difficult to set alarm limits adequately to assure safety. We have developed a new VNM method, which is based on detection of heart pressure pulses passing from the patient via the blood access to pressure transducers in the blood line. The pressure signal contains the heart pulses but is dominated by pressure variations from the blood pump. Our algorithm eliminates the blood pump variations and extracts the heart pulses. Disappearance of the heart pulses from the venous pressure indicates VND. Ten treatments with intact access connection and 3 treatments with controlled VND were clinically evaluated. The venous needle was deliberately disconnected from the access during 30 s while the arterial needle was still connected and the blood pump was still running. Pressure data from the extracorporeal circuit was acquired and a pulse oximeter was used as reference for the heart pulses. The method was capable of detecting the heart pressure pulse in all treatments despite varying heart rate (53-112 bpm), blood flow (320-500 ml/min) and relative amplitude of pulse to pump (1/250-1/3). All VND events were successfully detected within 20 s after the needle was disconnected. Variations in the venous pressure signal, the extracted heart pulses, and the reference of the heart pulses are illustrated in the figure, before and after a VND.




Disclosure of Financial Relationships: Research Funding: Funding from GAMBRO AB, about $30.000 per year from 2008-2011.

**TH-PO560**

**Are Differences in Incident Fistula Use across Dialysis Facilities Explained by Casemix and Community Characteristics?** Janet R. Lynch, Michael P. Lilly, William M. McClellan. *Fistula First Breakthrough Initiative Data Committee, Mid-Atlantic Renal Coalition, Midlothian, VA.*

**Purpose.** K/DOQI Guidelines recommend incident AVF use ≥ 50%, yet US facilities vary and most fall short. We aimed to see if patient risk and community factors explain differences. **Methods.** US patient-level Medical Evidence Report and county-level Area Resource File data were used. We sorted facilities into quartiles based on 2008-2009 facility incident AVF use and examined patient risk (per published risk of maturation failure formula) across quartiles using Cochran-Armitage for linear trends. Kruskal-Wallis was used to compare facility community characteristics. **Results.** 4,587 facilities received ≥ 12 new start adult HD patients. Quartile facility AVF use range, facility and patient counts: Q1 (0-7.3%, 1247, 48000); Q2 (7.3-13.2%, 1150, 52891); Q3 (13.2-19.5%, 1146, 50459); Q4 (19.5-66.7%, 1144; 46103). Percent prior nephrology care, high and very high risk increased with increasing quartiles. The median number of office-based physicians varied from 203.1 to 206.1 (p=.007) but did not follow a trend. Total general surgeons per 100,000 population increased from 11.6 - 12.2 (p=.003) and percent poverty in surrounding county decreased from 15.0 - 13.3 (p<.0001) as quartiles increased. **Conclusion.** We found no evidence that case complexity, per risk formula, explains low incident AVF use rates. Higher use facilities have a more complex casemix and higher percent patients with prior care, reinforcing the importance of nephrologist referral and evaluation of all patients for AVF placement. External factors are also related.

Patient Characteristics by Facility Incident AVF Use Quartile

| | Q1 | Q2 | Q3 | Q4 | p-value |
|---|---|---|---|---|---|
| Prior Nephrology Care | | | | | |
| % Any | 39.5 | 52.1 | 59.0 | 67.7 | <.0001 |
| % ≥ 6 Months | 29.7 | 40.6 | 45.8 | 55.4 | <.0001 |
| Risk | | | | | |
| Low | 22.4 | 21.4 | 21.5 | 21.9 | ns |
| Moderate | 47.4 | 45.6 | 45.2 | 43.4 | <.0001 |
| High | 27.9 | 30.5 | 30.7 | 32.2 | <.0001 |
| Very High | 2.3 | 2.5 | 2.6 | 2.5 | .03 |

Risk Score = 3 + 2*(Age≥65) + 3*PVD + 2.5*CAD -3*White; Score: <2.0, low, 2.0-3.0, moderate, 3.1-7.9, high, >8, very high; Risk formula attributed to Lok et al, JASN, 2006

Disclosure of Financial Relationships: nothing to disclose

**TH-PO561**

**Stenosis Length Predicts Percutaneous Transluminal Angioplasty (PTA) Failure after First Radiologic Salvage** Alexandra Romann,[1] Pascal Rheaume,[2] Mercedeh Kiaii,[3] Ravi Sidhu,[2] Monica C. Beaulieu.[1,3] *[1]BC Provincial Renal Agency, Vancouver, BC, Canada; [2]Divison of Vascular Surgery, UBC, Vancouver, BC, Canada; [3]Divison of Nephrology, UBC, Vancouver, BC, Canada.*

The most common AV fistula (AVF) complication is stenosis. Percutaneous transluminal angioplasty (PTA) has generally replaced surgical procedures to treat AVF stenosis. There is little data examining post-intervention patency after PTA, and even less data identifying the factors that predict success of this endovascular approach.

The aim of this study was to (1) assess patency after PTA of dysfunctional AVFs and (2) identify factors predictive of PTA failure after first radiological salvage.

A retrolective analysis was conducted in patients with an AVF creation between Jan 2005 – Jan 2008 and at least one PTA to maintain or re-establish patency. Follow-up data

was collected until Jan 31, 2009. Demographic data and information about fistula creation, use, interventions and failure were extracted. Characteristics of the stenosis and success of the intervention were re-assessed by one investigator (PR) blinded to the initial report.

161 of 375 (43%) AVFs created during the study required at least one angioplasty. The post-intervention primary patency was 31%, 21% and 21% at 1, 2, and 3 yrs. Post-intervention secondary patency was 76%, 66% and 64%.

Multivariate Cox Proportional Hazards analysis of post-intervention primary patency, adjusted for age and gender, identified the following predictors of requiring a 2nd PTA: outflow vein lesion (HR=2.268, p=0.0343), multiple lesions (HR=1.660, p=0.0379) and stenosis > 2 cm (HR=2.024, p=0.0020).

Adjusted multivariate competing risks analysis, (age, gender), recognized stenosis > 2 cm to be a good predictor of requiring a 2nd PTA (HR=1.814, p=0.0089) after further addressing the competing risk of AVF failure, study end date or death.

In summary, length, number, and location of stenotic lesions predict poor outcomes. Repeated angioplasties may not be effective for these lesions. Future studies are needed to determine the optimal approach to treating these lesions, especially once they require several repeated PTAs.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO562

**Venography or Ultrasound: Which Mapping Technique Is Superior?** Deuzimar Kulawik,[1] Kelly M. Mayo,[3] Jifeng Ma,[4] Jeffrey J. Sands,[2] Arif Asif.[5] *[1]FMQAI: The Florida ESRD Network, Tampa, FL; [2]FMC, Celebration, FL; [3]FMQAI, Tampa, FL; [4]FMQAI, Tampa, FL; [5]Intervention Nephrology, University of Miami Miller School of Medicine, Miami, FL.*

**Objective**: Both venography and vascular ultrasound based mapping techniques provide objective assessment of the vasculature prior to the creation of an arteriovenous fistula (AVF). While these vessel mapping (VM) techniques have been known to increase AVF rates, whether one technique is superior to the other has not been systematically studied. In this retrospective analysis we investigated the role of venography and ultrasound on AVF rates separately and in combination.

**Methods:** Incident Medicare end stage renal disease patient claims from the State of Florida (1/1/2006 to 12/31/2009) were used to identify VMs. The type of vascular access that was used for the first outpatient dialysis was determined by the CMS form 2728. VMs were identified by CPT codes used for venography and ultrasound mapping techniques: G0365 '93930', '93931', '93970', '93971', '36005','75822','75820'. Chi-square test and logistic regression were used to ascertain the association of types of vessel mapping and AVF rates.

**Results:** After controlling for age, body mass index, gender and race, venography resulted in significant higher AVF rates (51.30%) compared to ultrasound (33.97%). A combination of the two mapping techniques did not increase AVF rates above and beyond what was achieved by venography alone.

**Conclusion:** The results suggest that venography results in higher AVF rates compared to ultrasound. This result may not be causal and possibly related to the small number of surgeons or differences in the reasons for use of the two techniques.

Impact of Type of VM on AVF Rates.

| | Venography VM | Both Types of VM | VM Ultrasound |
|---|---|---|---|
| Denominator | 154 | 262 | 4846 |
| AVF rate | 51.30% | 49.24% | 33.97% |
| Odds Ratio | Reference | 0.939 | 0.507 |
| 95% CI of Odds Ratio | Reference | 0.627-1.404 | 0.367-0.703 |
| P-Value | | 0.07 | < .0001 |

Disclosure of Financial Relationships: nothing to disclose

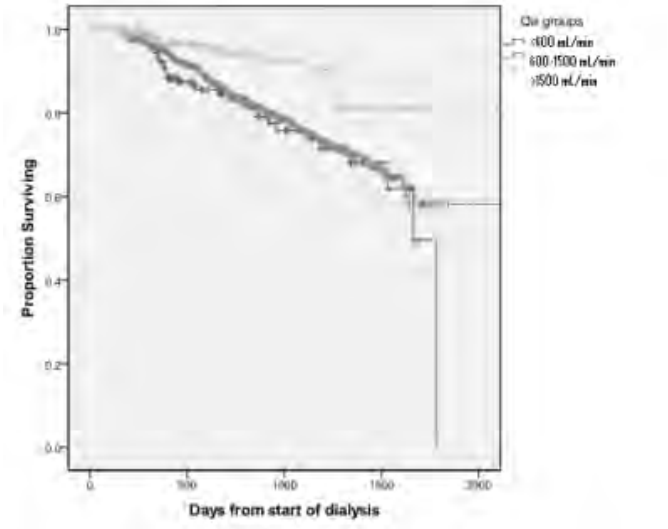
## TH-PO563

**Relationship between Survival and Access Flow in Hemodialysis Patients** Laura Rosales,[1] Len A. Usvyat,[1] Mary Carter,[1] Nathan W. Levin,[1, 2] Peter Kotanko,[1, 2] Stephan Thijssen.[1, 2] *[1]Renal Research Institute; [2]Beth Israel Medical Center, New York.*

Sufficient access blood flow (Qa) is vital in hemodialysis (HD). Complications derived from low and high Qa relate to morbidity and mortality. We investigated the association of Qa and survival in incident HD patients (pts).

We reviewed retrospectively records in all RRI in-center pts who started HD between Jan 2001 and Dec 2009 and survived the first 6 months. Only pts with arterio-venous fistula (AVF) or graft (AVG) who had 3 Qa measurements by online clearance were included. Pts were followed until death or censoring. Mean Qa for the first 6 months was computed and pts stratified into 3 groups: Qa<600mL/min, Qa 600-1500 mL/min, and Qa >1500 mL/min.

Data on 1006 pts (64% males, age (SD): 62.6 (14.6) years, 52% diabetic; 42% Black; 51% White) were analyzed. Number of pts: Qa <600 mL/min 149 (15%); 600-1500 mL/min 687 (68%) and Qa >1500 mL/min 170 (17%). In Kaplan-Meier analysis, mean survival time was 1410 days (95% CI: 1299-1521) in pts with Qa<600 mL/min, 1689 days (95% CI: 1607-1770) for Qa 600-1500 mL/min, and 1875 days (95% CI: 1758-1993) for Qa>1500 mL/min. In the highest Qa group, survival was significantly better compared to the other groups (Figure 1).




In Cox proportional hazards analysis, adjusted for age, gender, diabetic status, race, and access type, pts with Qa>1500 had a HR of 0.49 (95% CI:0.25-0.95) compared to the Qa 600-1500 group; again, survival in pts with low Qa did not differ from those in the Qa 600-1500 group.

In this large population of HD pts using AVF or AVG as vascular access, pts with Qa>1500 mL/min had a significantly lower risk of death compared to those with Qa of 600 to 1500 mL/min or <600 mL/min. Qa <600 mL/min was not associated with higher death rates than those between 600 and 1500 mL/min.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO564

**Clinical Significance of Early Post-Operative Venography of Vascular Access before First Needling in Hemodialysis Patients** Jayoung Lee,[1] Hyun Gyung Kim,[2] Hye Eun Yoon,[3] Yong-Soo Kim,[1] Chul Woo Yang,[1] Young Soo Kim,[2] Young Ok Kim.[2] *[1]Nephrology, Seoul St' Mary Hospital, Seoul, Republic of Korea; [2]Nephrology, Uijeongbu St. Mary's Hospital, Uijeongbu, Republic of Korea; [3]Nephrology, Incheon St' Mary Hospital, Incheon, Republic of Korea.*

Introduction: Venography has been a standard method to detect vascular access dysfunction (VAD) under maintenance hemodialysis (HD). However, there has been few data about the venography verifing VAD before 1st needling. This study was performed to define clinical significance of early post-operative venography of vascular access before 1st needling in HD patients.

Methods: From August, 2004 to April, 2010, 300 patients received vascular access operation in an academic tertiary care center. The type and location of vascular access was decided by surgeon's physical examination. Venography was performed 4~6 weeks after the operation and before 1st needling for HD. In patients with severe stenosis with more than 50% of luminal diameter, percutaneous angioplasty (PTA) or re-operation was done. In patients with no or mild stenosis, vascular access was used for HD. VAD was evaluated 1 year after the operation.

Results: Mean age of the patients was 56±13year. Male and female were 148 and 152, respectively. Vascular access composed 237 AVFs (192 radio-cephalic, 25 braciho-cephalic, and 20 brachio-basilic) and 63 AVGs. Venography revealed 31.3% (n=94) of severe stenosis, 28% (n=84) of mild stenosis and 40.7% (n=122) of good patency. For 31.4% (n=94) with severe stenosis, PTA and re-operation were performed in 70.2% (n=66) and 17% (n=16), respectively. All patients who received PTA showed immediate success. Although AVG group (n=63) showed higher incidence of severe stenosis than AVF group (237), there was no statistical difference (41.2% vs. 28.9%, p=0.06). Out of 86 patients with mild stenosis, 65 patients were followed for 1 year. VAD occurred in 13 patients (20%) and 11 patients received successful PTA. Out of 122 patients with normal venography, 102 patients were followed for 1 year and no one had VAD in 1 year.

Conclusion: Early post-operative venography before first needling is helpful to detect and treat early VAD in HD patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO565

**Does the Risk of Maturation Failure Explain Arteriovenous Fistula (AVF) Use in Incident Hemodialysis (HD) Patients?** Michael P. Lilly, Janet R. Lynch, William M. McClellan. *Fistula First Breakthrough Initiative Data Committee, Mid-Atlantic Renal Coalition, Midlothian, VA.*

**Background.** The US falls short of the Fistula First goal for 50% incident AVF use. Some suggest certain patient characteristics predict difficult AVF construction and poor maturation. **Methods.** We examined AVF use with logistic regression and CMS Medical Evidence Report data for all US new start adult HD patients between July 1, 2005 and December 31, 2009 with ≥ 6 months nephrologist care. Risk was assessed using a validated prediction rule for maturation failure (Lok et al, JASN 2006: Risk Score = 3 + 2*(Age≥65)

+ 3*PVD + 2.5*CAD - 3*White; Score: <2.0, low risk, 2.0-3.0, moderate, 3.1-7.9, high, >8, very high) alone and with other risk factors. **Results.** Of 196,936 patients, 43.8% had a placed AVF (23.3% in use) and 63.6% were low or moderate risk. Placed AVF varied (41.6%, very high risk to 46.2%, low risk, F=46.4, p<.0001) with differences among risk classes for placed/unused (F=9.3, p<.0001) and in-use (F=91.3, p<.0001). Logistic regression revealed lower odds of AVF use for moderate, OR (95% CI) = 0.903 (0.876, 0.930); high, OR (95% CI) = 0.871 (0.841, 0.901); very high, OR (95% CI) = 0.779 (0.742, 0.861) risk patients vs. low. Odds were lower for women, Hispanics, co-morbid CHF and diabetes, cardiac disease, CVA/TIA, low BMI. Odds were higher for diabetes or hypertension as ESRD etiology, co-morbid hypertension, high BMI, and Medicare coverage. Despite associations, c statistic was 0.603 and pseudo $R^2$ = 0.034. **Conclusion.** Risk factors have limited ability to predict incident AVF use. Even patients judged at highest risk can have successful AVF construction and initiate dialysis via a functioning AVF.

Frequency (%) of AVFs at Incident HD By Risk Category, Adults with Nephrologist Care ≥ 6 Mos, 7/1/2005 – 12/31/2009

| | Low (n) | Moderate (n) | High (n) | Very High (n) |
|---|---|---|---|---|
| Placed AVF | 46.2 (18,087) | 43.8 (37,649) | 42.6 (28,234) | 41.6 (2,251) |
| Placed But Unused | 20.6 (8,053) | 20.1 (17,309) | 20.9 (13,838) | 22.6 (1,225) |
| In-Use | 25.6 (10,034) | 23.7 (20,340) | 21.7 (14,396) | 19.0 (1,026) |
| Other | 53.9 (21,108) | 56.2 (48,362) | 57.4 (38,081) | 58.4 (3,164) |

Disclosure of Financial Relationships: nothing to disclose

## TH-PO566

**Choice of Vascular Access among Incident Hemodialysis Patients: A Decision and Cost-Utility Analysis** Hui Xue,[1] Eduardo K. Lacson,[2] Weiling Wang,[2] Gary C. Curhan,[1] Steven M. Brunelli.[1] *[1]Department of Medicine, Renal Division, Brigham and Women's Hospital, Boston, MA; [2]Fresenius Medical Care, North America, Waltham, MA.*

Introduction: Arteriovenous fistula (AVF) are the preferred hemodialysis (HD) vascular access type. However, supporting data have not considered morbidity and mortality resulting from bridge catheter exposure during failed/prolonged maturation. These analyses compare predicted outcomes and costs among incident HD assigned to receive AVF or arteriovenous grafts (AVG).

Methods: Analogous Markov models were created, one each for AVF and AVG. Patients entered consideration at time of first access creation simultaneous with dialysis initiation.

Subsequent outcomes were determined probabilistically; transition probabilities, utilities and costs were gathered from published sources; the timing and likelihood of access maturation were measured in a contemporary cohort of incident HD patients.

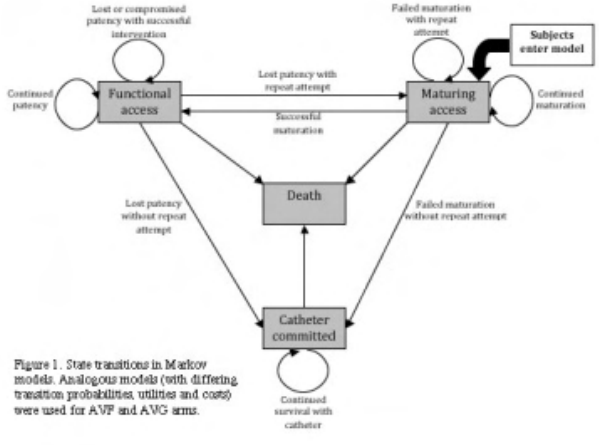



Figure 1. State transitions in Markov models. Analogous models (with differing transition probabilities, utilities and costs) were used for AVF and AVG arms.

Results: Mean survival was 39.2+/-0.8 and 36.7+/-1.0 months for AVF and AVG, respectively: difference 2.6 months (p<0.001). Quality-adjusted survival was 36.1+/-0.8 and 32.5+/-0.9 QALMs for AVF and AVG, respectively: difference 3.6 QALMs (p<0.001). One-way sensitivity analysis indicated that AVG was the preferred strategy among patients in whom the probability of AVF maturation was <41% of that for the overall AVF cohort (i.e., patients at high risk for maturation failure). The incremental cost effectiveness ratio (95% CI) for AVF relative to AVG was $446 (166, 720) per quality-adjusted life year saved.

Conclusion: AVF is associated with greater overall and quality-adjusted survival than AVG at an acceptable cost. Observed differences were much less pronounced than might be expected from prior literature, suggesting that individualized planning for patients at high risk for AVF maturational failure might improve outcomes.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO567

**Fistula First Initiative (FFBI) Improves Fistula (AVF) Use, Reduces Catheter Use and Does Not Cause Increased Immature AVF** Vo D. Nguyen. *Nephrology, Memorial Nephrology Associates, Olympia, WA.*

FFBI has led to 54.8% prevalent AVF use in February 2010. There is concern that creating AVF in HD patients with high co-morbidities leads to increased immature AVF and catheter (CVC). We used FFBI data from 2003 to 2010 to determine if increasing AVF rates leads to increased immature AVF and CVC use. **Methods**. Monthly census of vascular access was collected by all dialysis centers (4234 centers). Percent immature AVF is defined as maturing AVF (difference between AVF placed -AVF used) divided by total AVF used.

| Date | #AVF in use | #AVF placed | #Maturing AVF | %Maturing/in use AVF | % Total catheter | %Graft |
|---|---|---|---|---|---|---|
| July 2003 | 49 786 | 59 370 | 9584 | 19.25% | 26.9% | 40.1% |
| July 2004 | 99 823 | 118 006 | 18 183 | 18.2% | 27% | 35.6% |
| July 2005 | 114 429 | 136 926 | 22 497 | 19.7% | 28% | 31.8% |
| July 2006 | 130 476 | 156 514 | 26 038 | 19.95% | 28.5% | 27.8% |
| July 2007 | 149 694 | 177 537 | 27 843 | 18.6% | 27.7% | 24.6% |
| July 2008 | 168 075 | 193 957 | 25 882 | 15.4% | 26.3% | 22.7% |
| July 2009 | 186 306 | 214 430 | 28 124 | 15.1% | 25.5% | 21.2% |
| Feb 2010 | 195 462 | 223 519 | 28 057 | 14.4% | 24.4% | 20.6% |

**Conclusion:** FFBI has improved vascular access outcome: AVF in use increased dramatically, while percent immature AVF, graft and catheter decreased.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO568

**Low Rate of Incident Fistulas in a Veterans Administration Healthcare System Are Due to Poor Processes of Care** Timmy C. Lee,[1,2] Elisha I. Lancaster,[1] Prabir Roy-Chaudhury,[1,2] Charuhas V. Thakar.[1,2] *[1]Internal Medicine, University of Cincinnati, Cincinnati, OH; [2]Veterans Administration Medical Center, Cincinnati, OH.*

According to the 2009 USRDS the AVF incident rate is only 15%. The K/DOQI guidelines for vascular access targets greater than 50% incident AVFs. The purpose of this study was to determine the proportion of patients meeting specific benchmarks (initial nephrology referral, pre-operative vessel mapping, surgical referral, and access placement) necessary in pre-dialysis vascular access care and associated GFR when these benchmarks were reached. We performed a retrospective study at the Cincinnati Veterans Administration (VA) using a database of identified patients with a GFR ≤30 from 2006 to 2007 seen in our CKD clinics and performed a 2-year follow-up. We identified 344 patients who met the above criteria. 98% were male, 76% white, 58% diabetics, and 37% had peripheral vascular disease. 83% (n=285) had an initial nephrology consult from a primary care physician. Median GFR at the time of initial nephrology clinic referral was 28. 29% (n= 99) of patients had referral for pre-operative access mapping during the follow-up period. Median GFR at the time of vein mapping referral was 15 with only 2.0% of patients having a GFR ≥25 at the time of vein mapping referral. 20% (n=69) of patients had referral to a surgeon for vascular access surgery, and 79.7% (n=55) of patients referred for surgery had documented permanent access placement. Among those patients with vascular access surgery, median GFR at the time of vascular access placement was 11. Only 13% of the patients with vascular access placement had GFR ≥ 20 at the time of vascular access placement. Among the 344 patients 24% (n=84) initiated hemodialysis during the follow-up period. The patient distribution of access type at the initiation of dialysis was 26% AVFs, 1% AVGs, and 73% catheters. Even in a VA health care system, we see delayed referral for pre-operative vascular mapping and surgical evaluation, placement of AVF at low GFRs, and low rates of AVFs at dialysis initiation. Focusing on improving these processes of care measures may increase incident AVF use.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO569

**Vascular Access Blood Flow Measurement: Variability Is Real** Janet Lynn Graham, Swapnil Hiremath, Peter Magner. *Division of Nephrology, Kidney Research Centre, The Ottawa Hospital, Ottawa, ON, Canada.*

Background: The gold standard for diagnosing AV fistula stenosis is conventional angiography (fistulogram). The best known non-invasive predictor of stenosis and risk of occlusion is the measurement of fistula blood flow. The recommendation is to intervene if access flow decreases by >20% . The standard measurement of fistula blood flow is calculated using an ultrasound dilution technique with a Transonic access monitor (Transonic Corp) utilizing the Krivitski formula. An alternative method of access flow measurement uses changes in dialysate conductivity. This is an option available on standard Fresenius haemodialysis machines.

Objectives: To assess the reproducibility of access flow measurements with both Fresenius and Transonic measurements in individual patients over multiple measures.

Methods: A cohort of dialysis patients with AV fistula had paired measures with the Fresenius machine and the Transonic machine. The repeated measures with both Fresenius and Transonic machines were performed at 20 consecutive, routinely scheduled dialysis treatments to assess intra-individual variability of access flow and factors that influence this. Results: A total of 40 patients had repeated access blood flow measurements using the Transonics and Fresenius measurement device. 9/40 patients had Fresenius flows >2000 mL/min and were excluded from the analysis of variability. 60/517 (11.2%) pairs of consecutive readings with the Transonic method showing a 20% drop compared with 100/528 (18.9%) using the Fresenius method (p <0.001). 77/577 (13.8%) readings with the Transonic varied >20% from the average reading compared to 131/542 (24.2%) with Fresenius (p <0.001). 6/31 (19.4%) of patients had more than 20% of the values that were >20% different from the average value using the Transonic method when compared to 18/31 (58.1%) with Fresenius (p <0.001).

Conclusion: Both methods of access blood flow measurement demonstrate high variability but the Fresenius method demonstrates a significantly greater variability which may result in excessive interventions.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO570**

**Pressure Gradients in Hemodialysis Fistulae: A New Non Invasive Method**

David H. King,[1] Graeme Taylor,[2] Mo Al-Qaisi,[1] Sumith C. Abeygunasekara,[1] Yiannis Panayiotopoulos,[3] Galil Rahman Ali.[1] *[1]Renal Unit, Broomfield Hospital, Chelmsford, Essex, United Kingdom; [2]Department of Physics, Guy's and St Thomas Hospital Trust, London, United Kingdom; [3]Department of Surgery, Broomfield Hospital, Chelmsford, Essex, United Kingdom.*

Introduction: BP gradients in hemodialysis fistulae can identify venous or arterial stenoses *(Sullivan et al 1993)*. Gradients can be obtained non invasively.

Method: An algorithm[3] combining arm BP with Doppler U/S spectral data has been tested against direct pressure measurement in an in vivo animal model *(slope = 0.93 ,zero intercept +0.12mmHg, r =.94, range 34 to 82mmHg).*

A prototype instrument[4] computes a pressure gradient plot by sampling at multiple locations.

Aim: Demonstrate the clinical utility of the instrument on a-v fistulae.

Results: 39 sets of measurements were made on 33 native fistulae. Doppler data was recorded from the mid brachial artery and the basilic or cephalic vein in the upper arm. In several cases multiple sites were sampled.

Table 1 shows mean, SD and SD % for MAP, computed arterial pressure Pa/MAP, computed venous pressure Pv/MAP and brachial artery flow Q ml/min (Duplex measurement).

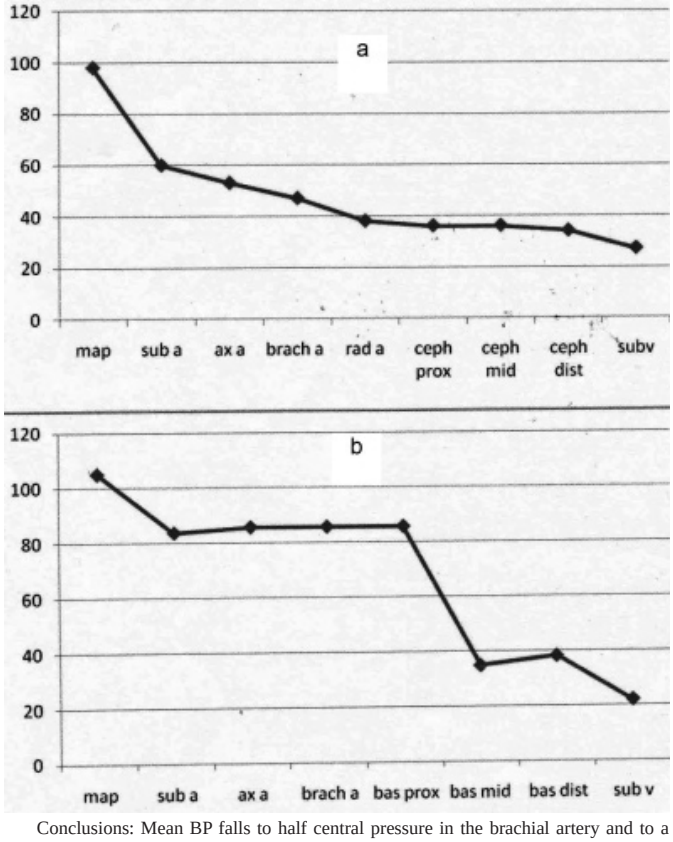
Table 1

| | MAP mmHg | Pa/MAP | Pv/MAP | Q ml/min |
|---|---|---|---|---|
| Mean | 97.1 | .48 | .34 | 1099 |
| SD | 16.0 | .08 | .08 | 467 |
| SD% | 16 | 17 | 24 | 42 |
| N | 39 | 39 | 39 | 31 |

Fig 1a shows the BP gradient recorded in a normal functioning fistula

Fig 1b shows the BP gradient in a failing fistula with a 70% stenosis

**Figure 1**




Conclusions: Mean BP falls to half central pressure in the brachial artery and to a third in the vein.Noninvasively obtained mean BP gradients may provide a clinically useful monitoring method.

[3]DK and MA are joint holders of the provisional international patent P45995GB-PCT.

[4]DK and GT are co developers of the prototype instrument.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO571**

**The Impact of Vascular Access (VA) Practices on Dialysis Facility Costs and Clinical Outcomes**

Richard Hirth,[1] Marc Turenne,[2] K. Sleeman,[1] Wei Zhang,[1] Robin L. Sands,[1] J. M. Messana.[1] *[1]KECC, U Mich, Ann Arbor, MI; [2]Arbor Research Collaborative for Health, Ann Arbor, MI.*

The Fistula First initiative brought greater focus on the desirability of increased use of arteriovenous fistulae (AVF) for hemodialysis VA. Dialysis Facility Reports (DFR) show that AVF use rose from 35% in 2004 to 50% in 2008. Increased AVF use may improve outcomes and lower costs of providing dialysis through reductions in complications and missed treatments.

Using data from DFRs, Medicare Cost Reports, and Medicare claims, we estimated cross-sectional and time series models of the relationship between VA and septicemia rates, standardized mortality ratios (SMRs), and costs per dialysis session. Cross-sectional models controlled for year fixed effects, patient characteristics (age, sex, race, body size, insurer, comorbid conditions) and facility characteristics (size, chain/ hospital status, rural location). Time series models used year-to-year changes within facilities to estimate the relationship between VA and outcomes. An advantage of the time series approach is that it eliminates non-time varying, unobserved confounders. Both sets of models showed generally statistically significant associations between %AVF and the outcome measures and yielded similar results. The table shows the effect sizes scaled to a 15% increase in AVF (magnitude of increase observed in the sample period) along with p-values.

| Outcome Measure | Cross-Section Model | Time Series Model |
|---|---|---|
| Composite Rate (CR) Dialysis Costs | - $1.22 (.06) | - $0.96 (.13) |
| Separately Billable (SB) Drugs and Labs | - $1.06 (.003) | - $1.05 (.001) |
| Total Costs (CR + SB) | - $2.16 (.004) | - $2.09 (.01) |
| SMR | - .062 (<.0001) | - .054 (<.0001) |
| % of Patients with Septicemia | - .78% (<.0001) | - .84% (<.0001) |

Increased AVF use between 2004 and 2008 was associated with modest decreases in cost per treatment. The clinical effects are quite meaningful, with SMRs falling more than 5%, and occurrences of septicemia in the current or previous three months falling by about 8% (.8% reduction relative to a 10.1% occurrence rate).

Disclosure of Financial Relationships: Honoraria: Fresenius Medical Care, Davita.

**TH-PO572**

**Better Agreement between Incident Arteriovenous Fistula Status on the Centers for Medicare & Medicaid CMS-2728 Form and Medicare Claims Identifies Facilities with Better Quality of Pre-Dialysis Care**

Eiichiro Kanda,[1] Jenna O. Krisher,[2] William M. McClellan,[1] T. Christopher Bond.[1] *[1]Epidemiology, Emory University, Rollins School of Public Health, Atlanta, GA; [2]ESRD Network 6, Raleigh, NC.*

**Background and Objectives:** This study investigates the agreement between CMS-2728 and Medicare claims data for pre-ESRD AVF placement.

**Methods:** CMS-2728 forms were linked to Medicare in- and out-patient claims for 10,401 incident ESRD patients aged 65 years or older at the onset of hemodialysis between June 1, 2006 and May 31, 2007 in North Carolina, South Carolina and Georgia (ESRD Network6). Records were matched to the first Medicare claim for AVF placement during the year prior to beginning dialysis. CMS-2728 AVF and Medicare claims were classified (Yes, No). We assessed percent agreement between CMS-2728 data and Medicare claims by Cohen's kappa coefficient for 375 facilities. The association between agreement and pre-ESRD care (nephrologist referral, degree to which albumin, hemoglobin and erythropoietin met clinical guidelines) was evaluated with regression models adjusted for facility mean age, race, and gender. Race was included in models as a variable that predict health care quality and outcomes (access to care, insurance status, and differential treatment).

**Results:** Facility rate of agreement between CMS-2728 and Medicare billing was 77.8%, median 80.0%, 25th to 75th range (70.0% to 86.7%); mean kappa statistic was 0.45 (modest agreement); median 0.46 (range 0.27 to 0.63). As CMS-2728 and Medicare claims became more congruent, the proportion of incident patients with AVF and nephrologist referral increased.

Table 1 Relationship between the Degree of Agreement between 2728Data and Medicare Billing Data and Incident AVF Rate in Network 6 Area (6/1/06-5/31/07)

| 2728/claims agree Kappa group | All | No agreement | Slight 0-0.2 | Modest 0.2-0.4 | Moderate 0.4-0.6 | Strong >0.6 | ANOVA P-value |
|---|---|---|---|---|---|---|---|
| Facilities (n) | 375 | 31 | 39 | 75 | 108 | 122 | - |
| Patients/center | 16.2 | 13.3 | 15.9 | 16.4 | 18.0 | 15.3 | 0.002 |
| Average value of % agreement | 77.8% | 59.6% | 61.5% | 72.0% | 79.4% | 89.9% | <0.0001 |
| Neph referral | 61.0% | 59.3% | 54.1% | 64.1% | 60.3% | 62.4% | 0.174 |
| Incident AVF | 13.7% | 9.1% | 10.9% | 13.4% | 14.2% | 15.4% | 0.01 |

**Conclusion:** Agreement between AVF placement by CMS-2728 and Medicare claims varies among facilities. Higher AVF placement and nephrologist referral rates tended to predict better congruence between the two data sources.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO573**

**Effective Bedside Screening for Fistula (AVF) Stenosis Should Be Taylored to the Site of Arteriovenous Anastomosis** Nicola Tessitore,[1] Valeria Bedogna,[1] Giovanni Lipari,[2] Giancarlo Mansueto,[3] Antonio Lupo,[1] Albino Poli.[4] *[1]Hemodialysis Unit Ospedale Policlinico, Nephrology Div.; [2]Surgical Science Dpt.; [3]Radiology Inst.; [4]Public Health Dpt., University of Verona, Verona, Italy.*

Given different sites of stenosis and access blood flow rates (Qa), diagnostic tools and criteria for AVF stenosis might conceivably vary according to the anastomosis site.

To test this hypothesis, we assessed the performance of several bedside tests (physical examination(PE), blood pump flow/arterial pressure(Qb/AP), venous pressure at Qb 200 ml/min(VP200) and Qb 300 ml/min(VP300), derived static venous pressure(VAPR), and Qa measurement) in diagnosing angiographically-proven >50% stenosis in an unselected population of 114 hemodialysis patients (82 m, 32 f, aged 63±16 y) with mature AVFs (41 at the wrist [dAVF],73 more proximally at the mid-forearm or the elbow region [pAVF]).

The prevalence of inflow stenosis(upstream from the venous needle)(STin) was uninfluenced by the anastomosis site (41% in dAVF vs 38% in pAVF), while that of outflow stenosis(downstream from the venous needle)(STout) was higher in pAVF (20% vs 2%,p=0.01).

In dAVF, the best test for STin was Qa<650 ml/min (90%[76-97] accuracy, 82%[57-96] sensitivity, 96%[79-100] specificity). In pAVF, the best test for STin was the combination of positive PE and Qa<900 ml/m (85%[73-91] accuracy, 71%[51-87] sensitivity, 91%[79-97] specificity), which significantly improved specificity in comparison with PE (76%[60-87]) and Qa<900 ml/min (73%[58-85])(p=0.02) without unduly reducing sensitivity. A positive PE and VAPR>0.5 were both equally highly diagnostic for STout (86%[76-93] and 85%[75-92] accuracy, 80%[52-95] and 87%[59-98] sensitivity, 88%[77-95] and 84%[73-93] specificity). Combining PE and VAPR>0.5 afforded a non-significant improvement in specificity.

Our study shows that an effective screening program for detecting and locating stenosis with an accuracy of 85% or more can be implemented during dialysis by tailoring the choice of the screening procedures to the site of the arteriovenous anastomosis, Qa being the best choice for wrist AVF, and PE followed by Qa or VAPR measurement for more proximally-located accesses.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO574**

**Single Centre Experience of Routine Post-Operative Doppler of Vascular Access for Dialysis** Krish S. Raman,[1] Anuradha Jayanti,[1] Arvind Ponnusamy,[1] Lourinti Fletchman,[1] Zulfikar Ali Pondor,[1] Babatunde Adeniyi Campbell,[2] Afshin Tavakoli,[2] Alistair G. Cowie,[1] Rosemary L. Donne.[1] *[1]Renal Medicine, Salford Royal Hospital, Salford, United Kingdom; [2]Surgery, Manchester Royal Infirmary, Manchester, United Kingdom.*

**Introduction**

The UK Vascular Access Joint Working party report recommends that at least 70% of incident haemodialysis patients should start dialysis via an AV fistula and that an early post-operative review of fistula function is performed. In our unit a single radiologist performs a post-operative doppler ultrasound at 8-12 weeks.

**Aim**

To assess whether our current practice is effective in ensuring a functioning fistula at dialysis start.

**Methods**

Prospective data collection on incident haemodialysis patients identified those who had fistula surgery prior to dialysis start. These patients were analysed retrospectively recording details of surgery, doppler results and further action taken.

**Results**

64 patients who had prior AV fistula surgery commenced haemodialysis during the 12 months before 1st May 2010. 56 patients had a Doppler of the fistula. 51 of these were within 8-12 weeks and the remainder occurred later because of cancellations or non-attendance. 90% of the patients who attended for a Doppler started dialysis via an AV fistula.

27/56 patients had a normal scan and 26 of these commenced dialysis with an AV fistula. 29/56 patients (52%) had an abnormal scan. Of these, 32% had a further scan but no intervention, 15% had radiological intervention and 63% required further surgery. 86% of these 29 patients commenced dialysis via an AV fistula. The remaining 14% commenced dialysis via a line, which in the majority occurred within 8 weeks of fistula creation. Of 96 patients starting maintenance haemodialysis during this period, 64 patients (66.6%) started dialysis through AV fistula and none on grafts.

**Conclusion**

Post-operative doppler successfully identified problems with fistula maturation allowing action to be taken to minimise dialysis catheter use. In the most challenging patients, with difficult vessels or low GFR, catheter use may have been avoided by a post-operative doppler within 2 weeks followed by urgent intervention.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO575**

**The Pragmatic Association of Vessel Mapping Timing and AVF Placement** Deuzimar Kulawik,[1] Jeffrey J. Sands,[2] Kelly M. Mayo,[3] Jifeng Ma,[4] Arif Asif.[5] *[1]FMQAI, Tampa, FL; [2]FMC, Celebration, FL; [3]FMQAI, Tampa, FL; [4]FMQAI, Tampa, FL; [5]Univ of Miami, Miami, FL.*

**Objective**: The primary purpose of vessel mapping (VM) is to identify vessels suitable for AVF placement. While the role of vessel mapping on AVF rates has been well studied, the impact of the timing of VM prior to the initiation of hemodialysis on AVF rates has not been systematically studied.

**Methods:** Incident Medicare end stage renal disease patient claims from the State of Florida (1/1/2006 to 12/31/2009) were used to identify VMs. The type of vascular access that was used for the first outpatient dialysis was determined by the CMS form 2728. VMs were identified by CPT codes: G0365 for overall vessel mapping; '93930', '93931', '93970', '93971' for ultrasound VM; '36005','75822','75820' for venography VM. Chi-square test and logistic regression were used to ascertain the association of timeliness of vessel mapping and AVF rates.

**Results:** Consistent with previous studies, VM increased AVF rates (VM=35.17% versus no VM=27.10%, p<0.0001). The length of time interval from VM to the initiation of hemodialysis had a significant impact on AVF rates. Vessel mapping performed 2 months to a year prior to the initiation of hemodialysis had the highest impact on increasing AVF rates (Table 1). Patients with VM within 30 days of the initiation of hemodialysis had similar AVF rate than the patients who did not have VM at all.

**Conclusion:** The results suggest that VM has the greatest impact on improving AVF rates when performed within 2 months to a year prior to the initiation of hemodialysis.

Impact of Timing of VM on AVF Rates

| | No VM | VM within 0-30 days | VM within 31-60 days | VM within 61-90 days | VM within 91-180 days | VM within 181-365 days | VM >365 days |
|---|---|---|---|---|---|---|---|
| Denominator | 12755 (70.63%) | 2077 (11.5%) | 495 (2.7%) | 392 (2.2%) | 687 (3.8%) | 768 (4.3%) | 884 (4.9%) |
| AVF rate | 27.1% | 26.5% | 33.3% | 42.9% | 46.6% | 44.5% | 36.2% |
| Odds Ratio | Reference | 0.969 | 1.344 | 2.018 | 2.344 | 2.160 | 1.524 |
| 95% CI of Odds Ratio | Reference | (0.872, 1.076) | (1.110, 1.627) | (1.646, 2.474) | (2.008, 2.737) | (1.864, 2.504) | (1.321, 1.758) |

Disclosure of Financial Relationships: nothing to disclose

**TH-PO576**

**The Viability of Arteriovenous Access for Hemodialysis in Elderly Versus Middle-Aged Patients** Samir Parikh, Chadwick E. Barnes, Anil K. Agarwal. *Nephrology, The Ohio State University, Columbus, OH.*

Purpose: Arteriovenous fistula (AVF) is considered the most preferable vascular access (VA) for hemodialysis (HD), followed by arteriovenous graft (AVG). Catheters are the least desirable VA. Recently, the success and appropriateness of AVF and AVG in older patients has been debated due to the limited life span of the patient and the VA as well as the efforts required to develop and maintain these VA. The purpose of this study is to compare the success rates of AV accesses in older and younger cohorts of HD patients.

Methods: We are retrospectively reviewing the AV accesses placed between 1998 and 2008 (N=1200) at our center for rates of primary and cumulative patency, numbers of interventions required to establish and maintain patency, time before first intervention, time from access creation to first use and several patient demographics. Findings in patients ≥70 years of age and a group of patients aged 30 to 55 years are being compared.

Results: We have analyzed data from 2007 and 2008 thus far. Of a total of 235 AV accesses placed during this period, 32 were in elderly patients, and 4 were lost to follow up. We randomly selected 35 patients, ages 30-55 years, as controls.

| DEMOGRAPHICS | Average Age(yrs) | Sex(%male) | Race (%Black) | Access (%fistulas) | Diabetes (%) | Tobacco (%) | |
|---|---|---|---|---|---|---|---|
| Elderly (n=28) | 76.9 | 60.7 | 17.9 | 78.6 | 53.6 | 39.3 | |
| Control (n=35) | 45.5 | 68.6 | 68.6 | 65.7 | 54.3 | 42.9 | |
| | | | | | | | |
| RESULTS | Cumulative patency(%) | Interventions prior 1st use (avg) | Total Inter-ventions (avg) | Time to 1st intervention (mos) | Time to 1st use (mos) | Deceased (%) | Primary failure (%) |
| Elderly (n=28) | 67.9 | 1.29 | 4.17 | 7.05 | 4.18 | 32.1 | 21.5 |
| Control (n=35) | 80 | 1.26 | 3.03 | 3.67 | 4.3 | 23 | 20 |

Conclusions: Our preliminary analysis demonstrates that AV accesses in patients ≥70 years of age have a high cumulative patency rate similar to younger patients. The primary failure rate, number of interventions prior to first access, and total number of interventions are also similar. Our preliminary data suggest that permanent VA is a viable form of HD access for advanced age patients. A complete analysis from 1998 to 2008 is in progress to be presented at the meeting.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO577**

**Superficialization of the Radial Artery – The Novel Option of the Vascular Access Creation on the Forearm** Waclaw Weyde, Tomasz Golebiowski, Mariusz Kusztal, Jozef Penar, Madziarska Katarzyna, Magdalena Krajewska, Krzysztof Letachowicz, Miroslaw Banasik, Beata Strempska, Marian Klinger. *Nephrology and Transplantation Medicine, Wroclaw Medical University, Wroclaw, Poland.*

The standard approach in patients (pts) with clotted forearm arteriovenous fistula (AVF) is the creation of the next vascular access on upper arm using own patient vessels or prosthetic graft. We have elaborated the new procedure based on the superficialization of the radial artery which diameter extends during fistula maturation from 1.5–2.5 mm up to 5-6 mm. This enlargement remains even after clotting of AVF.

Material and methods

The procedure of radial artery superficialization was undertaken in 6 chronic HD pts (age 68±16 y, 2 F, 4 M) dialyzed by forearm AVF for 27±26 months. In 4 pts the forearm veins were completely obstructed, in 2 pts the central outflow stenosis required AVF closure. In these persons the procedure was performed after arm edema subsidence. Preoperatively the diameter and patency of radial and ulnar arteries were evaluated by Doppler ultrasound. In local anesthesia, 10cm long skin incision above radial artery was made. All collaterals were ligated to allow the artery mobilization, and finally it was placed in subcutaneous pocket. At the end the vessel bed and the skin was sutured. The attempt was successful in 5 pts, complicated by transient hematoma in one case. In 1 female patient the procedure was abandoned due to small radial artery diameter (4mm) detected intraoperatively. The superficialized radial artery is punctured uneventfully during HD sessions in 5 pts by period from 4 up to 9 months without pain complains. The plethysmography examination (PVL device; Biomedix, USA) did not reveal any signs of hand's ischemia.

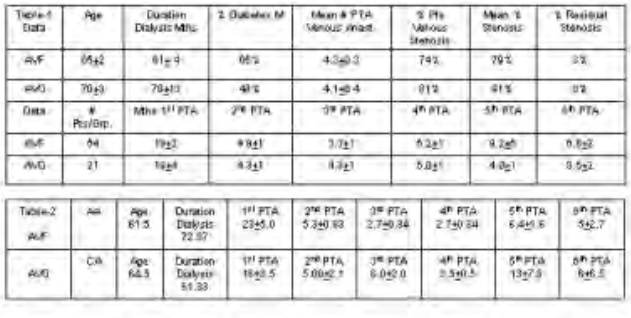
Conclusions:1.The described superficialization of the radial artery is the new procedure enabling the maintenance of the forearm HD access in patients with irreversible clotted wrist AVF and/or with untreatable central outflow stenosis. 2.The procedure is particularly suitable for patients with compromised cardiac function.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO578**

**Serial PTA of Dialysis Access: Comparative Responsiveness of AVF and AVG Venous Outflow Tracts to Repeated Angioplasty** James A. Tumlin. *Division Nephrology, University Tennessee College Medicine Chattanooga, Southeast Renal Research Institute, Chattanooga, TN.*

The failure of dialysis accesses remains a leading cause of morbidity and cost among ESRD populations. The onset of intimal hyperplasia and smooth muscle cell infiltration leads to progressive narrowing of the lumen, impaired blood flow and ultimately thrombosis of both AVF and AVGs. Percutaneous angioplasty (PTA) can restore blood flow, but is limited by elastic recoil and recurrent stenosis. Few studies have investigated the efficacy of or time between serial PTA of individual lesions within venous outflow tracts. To investigate this, we have prospectively studied 75 ESRD patients with stenotic dialysis accesses (54 AVF:21 AVG) undergoing serial PTA of venous outflow tracts. The primary endpoint is the time between serial PTAs and percent recoil of lesions within the venous outflow tract.



As in Table-1, 74% of patients with AVF and 81% with AVG developed a stenosis in the venous outflow. There was no difference in age, duration of dialysis or percentage of diabetics between AVF or AVG. The mean time from insertion to 1st PTA was 19±2 and 19±4 months for AVF and AVG respectively (P=NS). There was no difference in the mean number of angioplasties to venous outflow tracts for AVF 4.3±0.3 and AVG 4.1±0.4 respectively (p=NS). There was no difference in time between the 1st and 6th PTA; averaging 5.8 and 5.2 months for AVF and AVG respectively (P=NS). Moreover, the percent dilation in response to PTAs did not diminish over time. Lastly, there was no difference between African Americans and Caucasians or diabetic and non-diabetic patients in the number or frequency of serial PTA (table-2). In summary, we find that serial PTAs of AVF and AVG remains effective through the 6th PTA without significant elastic recoil. The current data suggests that serial PTA to the venous outflow is safe and effective.

Disclosure of Financial Relationships: Consultancy: Questcore PharmaceuticalsResearch Funding: Questcore Pharmaceuticals.

**TH-PO579**

**Clinical Predictors of Arteriovenous Fistula (AVF) Patency** Carrie Grafft, James T. McCarthy, John J. Dillon, Robert C. Albright, Bernice (Bonnie) M. Jenson, Karl A. Nath. *Nephrology, Mayo Clinic, Rochester, MN.*

The AVF is the optimal access for hemodialysis (HD). Unfortunately, the rate of primary failure is high and complications associated with placement are rarely reported. We analyzed the outcomes of AVF placement and predictors of patency with a retrospective cohort study of AVFs placed at Mayo Clinic Rochester from January 2006 through December 2008. We examined the primary and secondary failure rate, plus hospitalizations and complications associated with AVF placement. The complications included infection, bleeding, steal syndrome, aneurysm, nerve injury, seroma, and subclavian stenosis. Primary failure was defined as AVF abandonment prior to use for HD; secondary failure was abandonment after use for HD. Primary patency was the interval from AVF placement to intervention or abandonment, and secondary patency was the interval from placement to abandonment. The first AVF for each patient was used for this analysis. Primary patency and secondary patency were determined using Kaplan-Meier survival curves, and Cox-proportional hazard models were used to determine predictors of patency.

During the studied time period, 293 patients had AVF created. The primary failure rate was 37.1% after exclusions (patients never requiring HD; died before use; transplanted prior to AVF use, or lost to follow up). Of the AVF that were suitable for dialysis, 11.4% failed during the median follow-up time of 379 days. A complication occurred in 21.2% of patients and a hospitalization was associated with fistula creation in 12.3% of the patients. Artery size was the only predictor of both primary and secondary patency in our cohort, and diabetes predicted primary patency only.

The decision to create AVF for HD will need to be individualized for each patient, but should be the primary HD vascular access if possible. Age, sex, gender, BMI, diabetes, cardiovascular disease, or time on HD (vintage) should not preclude AVF placement. More stringent criteria for artery size may need be considered prior to AVF creation to improve patency rates.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO580**

**Use of Low Dose Aspirin Does Not Prolong Post Dialysis Needle Site Holding Time for Hemostasis** Sorour Rahgoshay,[1] Alejandro Solano Bayardo,[1] Saadia Sherazi,[1] Geetha Koushik,[1] Anum Choudhry,[2] Michael Disalle,[1] Wajid M. Choudhry.[3] *[1]Internal Medicine, Unity Health System, Rochester, NY; [2]MHS, Rochester, NY; [3]Department of Nephrology, Unity Health System/University of Rochester, Rochester, NY.*

**Background**

Aspirin is underutilized in dialysis patients. There is generally a fear of excessive bleeding from AVG/AVF in dialysis patients who are treated with aspirin. We studied the effect of low dose aspirin (81mg) on post dialysis needle site holding time (NSHT).

**Method**

A longitudinal cross over study of patients receiving outpatient dialysis using AVF/AVG at Unity Health System Rochester NY from Dec. 2009 to May 2010. Patients were divided into two arms. "On Aspirin" and "Off Aspirin". At the end of dialysis treatment needles were removed and needle sites were held under pressure to record time to hemostasis.(NSHT). The dose of heparin used during individual dialysis treatment remained unchanged as these patients served their own controls. Patients were studied for 2 weeks on aspirin followed by 1 week of wash out period. They were then studied for another 2 weeks as off aspirin arm. Patients were excluded if they required aspirin for secondary prevention or had any contraindication to aspirin use.

**Results**

There were 38 patients who completed the study, no side effects were noted. Data was analyzed by using paired T test. Average NSHT for venous site was 11.07±3.8 minutes in on aspirin arm compared with 11.24 ±3.9 minutes in off aspirin (p 0.74)

Average NSHT for arterial site was 10.8±3 min in on aspirin arm compared with 11.7± 4.7 min in off aspirin (p 0.07) as shown.

Post Dialysis Needle site Holding time (NSHT) in ptients "On-Aspirin" and "Off- Aspirin"

| | No. of NSHT observed | Venous site Holding Time (Minutes) | Arterial site Holding Time (Minutes) |
|---|---|---|---|
| On Aspirin | 228 | 11.07 ± 3.8 | 10.8 ± 3.0 |
| Off Aspirin | 228 | 11.24 ± 3.9 | 11.7 ± 4.7 |
| Statistical Significance | | NS | NS |

NS= Not statistically significant NSHT=Needle site holding time

**Conclusion**

In our study population we did not find any difference in NSHT in patients receiving low dose aspirin compared when they were off aspirin.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO581**

**Patient Attitudes towards the Arteriovenous Fistula: A Qualitative Study on Vascular Access Decision Making** Wang Xi,[1] Lori E. Harwood,[1] Michael Diamant,[1] Kerri Gallo,[1] Jessica M. Sontrop,[2] Jennifer J. Macnab,[2] Louise M. Moist.[1] *[1]Nephrology, London Health Sciences Center, University of Western Ontario, London, ON, Canada; [2]Epidemiology and Biostatistics, University of Western Ontario, London, ON, Canada.*

The incident and prevalent use of arteriovenous fistula (AVF) in Canadian hemodialysis patients is declining and up to 30% of eligible patients refuse the creation or cannulation of an AVF. The objective of this qualitative study was to understand patient attitudes, beliefs, preferences and values around vascular access (VA). We conducted semi-structured interviews with 13 patients, 6 of whom had previously used an AVF and 7 who continued to dialyze with a catheter. Transcribed interviews were reviewed by 3 persons with agreement on the following 3 main themes that impacted decision making around VA: (1) impact of previous personal or vicarious experiences with the fistula. Patients suggested cannulation, bleeding, time commitment, and appearance to be important deterrents against obtaining an AVF; (2) knowledge transfer and informed decision making. Patients identified knowledge transfer from other patients to be as important as knowledge transfer from health care workers, that information on VA was presented but not understood, and that timing of information was crucial with information overload at the start of dialysis; and (3) maintenance of status quo and outlook on life. This was an important determinant of VA as patients' stated they live day to day and maintain the status quo without being influenced by the mortality risks with a catheter. They expressed being overwhelmed with decision making and change preferring to maintain their current state. These themes allow us to understand the reasoning process behind patient AVF refusal and to identify opportunities in timing and formatting of information transfer, assisting in decision making, and ensuring a patient-centered approach to use of the fistula.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO582**

**The Creation of Native Fistula as First Vascular Access in Elderly Patients: An Italian Experience** Chiara Venturelli,[1] Giovanni Cancarini,[2] Giuliano Brunori.[1] *[1]Division of Nephrology, "S.Chiara" Hospital, Trento, Italy; [2]Division and Institution of Nephrology, University of Brescia Spedali Civili, Brescia, Italy.*

Background: In the last decade, the number of elderly patients (≥65 years) who started hemodialysis increased. These patients have an elevated incidence of comorbidities that can make difficult to create a vascular access using native vessels. The type of vascular access (fistula, graft, catheter) plays an important role in the results of dialysis treatment. The ideal vascular access remains the arterovenus fistula (AVF). Graft (AVG) and catheter (CVC) should be considered as 'second choice' for a higher incidence of complications.

Methods: we analyzed the patients who started hemodialysis in 2 tertiary care centres of northern Italy between 1st January 2006 to 31th December 2008 (Trento and Brescia). Dialysis population is 600 patients in an area of 1.100.000 people. Patients were divided in two group: patients ≥65 years (E) and <65 years (Y). The analysis between the two group was performed using demographic characteristic (age, gender, risk factors), access function, type of vascular access. As vascular access we considered AVF, AVG, CVC. We assay the survival rate of patient and vascular access.

Results: In a 3-year period 336 patients started HD: 208 E and 128 Y. As first vascular access for HD in the total population we have 191 (57%) wrist AVF, in 80 (24%) elbow AVF, 15 (4%) AVG, 50 (15%) CVC.

Type of access in E vs Y

| | E | Y |
|---|---|---|
| AVF | 157 (75.5%) | 114 (89%) |
| AVG | 9 (4.3%) | 6 (4.7%) |
| CVC | 42 (20.2%) | 8 (6.3%) |

The primary patency of AVF was similar in Y and E, for AVG patency was 100% in Y and 44.4% in E (p=0.018).Using Cox analysis for vascular access survival, we did not observe any statistically significant difference in E vs Y for all the risk factors tested. Analyzing patients survival rate with Cox analysis we find a grater mortality rate in patients that had used CVC as first vascular access (p<0.0001).

Conclusions: According to NFK DOQI Clinical Practice Guidelines, we suggest that AVF can be considered the preferred types of access because of the low incidence of complications and better survival. AVF can be created also in elderly patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO583**

**Increased Hemoglobin Variability as a Risk Factor for Vascular Access Dysfunction in Chronic Hemodialysis Patients** Tsutomu Koike,[1] Fumihiro Tomoda,[1] Taizo Nakagawa,[1] Hidenori Yamazaki,[1] Hexing Liu,[1] Satoshi Kagitani,[1] Hiroshi Inoue,[1] Hitoshi Hirata,[2] Masanari Kageyama.[2] *[1]The Second Department of Internal Medicine, University of Toyama, Toyama, Japan; [2]Jonan Naika Clinic, Toyama, Japan.*

Objective: Increased Hb level leads to the occurrences of vascular access thrombosis in hemodialysis (HD) patients. Recently, increased variability of hemoglobin (Hb) has been highlighted as a risk of mortality of HD patients. However, the influences of Hb variability (HbV) on vascular access have not yet been explored. In the present study, therefore, the associations of HbV with occurrences of vascular access dysfunction (VAD) were evaluated in HD patients. Design and Methods: In 70 chronic HD patients, HbV was estimated at the prospective follow-up of 1 year by 4 parameters including standard deviation (SD) of Hb, residual SD (RSD) of Hb, and numbers of Hb cycling defined by cycles which amplitude > 1.5 g/dL and duration > 8 weeks, and numbers of excursion defined by half of one cycle. The parameters of HbV and the other factors affecting VAD were compared between 29 patients complicated with VAD defined by venous stenosis or thrombosis (D group) and the remainders without VAD (N group). Results: SD and RSD of Hb were significantly elevated in D group compared with N group (0.86±0.09 g/dL, 0.70±0.06 g/dL vs 0.67±0.03, 0.54±0.01). The numbers of Hb cycling and excursion were also increased in D group than in N group (0.74±0.20 per patient/year, 1.84±0.21 per patient/year vs 0.31±0.02, 1.31±0.14). However, averaged levels of Hb, weekly erythropoiesis stimulating agent (ESA) doses and responsiveness to ESA defined by the weekly ESA doses divided by Hb, did not differ between the two groups. There was no difference in calcium × phosphate product, C-reactive protein, interdialytic weight gain and blood pressure during HD between both groups. In multiple logistic regression analysis, SD of Hb and weekly ESA doses were independent determinant factors for VAD, as shown by odds ratio of 12.01 (95% CI 1.38-209.49) and 0.997 (95% CI 0.993-0.999), respectively. Conclusions: In chronic HD patients, the occurrences of VAD might be associated with HbV in addition to the used dosage of ESA.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO584**

**Costs and Outcomes of Endovascular Treatment of Thrombosed Arteriovenous Fistulas** Luis Coentrao,[1] Pedro Bizarro,[1] Carlos Alexandre Ribeiro,[2] Ricardo Neto,[1] Manuel Pestana.[1] *[1]Nephrology R&D Unit, Hospital S. João, Faculty of Medicine, Porto, Portugal; [2]Financial Managment Unit of Medicine, Hospital S. João, Porto, Portugal.*

**Background:** Previous studies that have examined the procedure of choice for arteriovenous fistula (AVF) thrombectomy have focused exclusively on clinical outcomes and, in the absence of costing data, current guidelines do not take into consideration economic issues. In the present study, we determined the costs and outcomes of endovascular treatment of thrombosed AVF in a single centre.

**Methods:** Retrospective cohort of 44 consecutive hemodialysis patients who underwent percutaneous declotting of a completely thrombosed AVF by manual catheter-directed thromboaspiration over a period of 1 year (January 1 to December 31, 2008). Patients were followed-up for a period of 1 year. Success, complications and secondary interventions were recorded according to consensus definitions. A comprehensive measure of total vascular access costs was obtained. Costs are reported in US 2009 Dollars.

**Results:** The mean patient age was 66 years, 66% were male and 35% had diabetes. The average time on dialysis was 4.9 years and the mean fistula age was 4.5 years. Fifty five percent of the patients had forearm AVF. Technical success was achieved in 42/44 following percutaneous thromboaspiration. The primary and secondary patency rates were 63% and 78% at 1 year, respectively. Primary patency rate at 12 months was significantly better for forearm AVF (70% *vs.* 43%; $P = 0.047$). The mean cost of all vascular access care during year 1 was $3459 (median $2115; IQR $2115 to $19505). The mean cost for maintaining a forearm and an upper arm AVF was $2595 (median $2115; IQR $2115 to $4653) and $4580 (median $2724; IQR $2115 to $19035) per patient-year at risk, respectively ($P = 0.015$).

**Conclusion:** Endovascular treatment of thrombosed AVF is a cost-effective procedure for the maintenance of vascular access in hemodialysis patients. Percutaneous thrombectomy efficiency is superior in forearm AVF.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO585**

**Design of the Hemodialysis Fistula Maturation (HFM) Study** Gerald J. Beck,[1] Alfred K. Cheung,[2] Laura M. Dember,[3] Harold I. Feldman,[4] Jonathan Himmelfarb,[5] Thomas S. Huber,[6] John W. Kusek,[7] Prabir Roy-Chaudhury,[8] Miguel A. Vazquez,[9] Charles E. Alpers,[5] Michelle Robbin,[10] Joseph Vita,[3] The HFM Study Group.[7] *[1]Cleveland Clinic; [2]U Utah; [3]Boston U; [4]U Penn; [5]U Wash; [6]U Florida; [7]NIDDK; [8]U Cincinnati; [9]U Texas SW; [10]UAB.*

The DAC Fistula Study found that only 40% of new arteriovenous fistulas (AVFs) matured adequately for use (*JAMA* 2008). Consequently, the NIDDK-sponsored HFM Study was initiated to identify predictors and causes of AVF maturation failure. The goal is to enroll 600 patients undergoing AVF creation. Detailed data on demographics, surgery, clinical factors, vascular anatomy and histology, and vascular biology and physiology are collected pre-operatively, intra-operatively and/or post-operatively, and patients are followed under routine clinical care until AVF maturation or abandonment. Participating sites include 6 clinical centers (Boston U, U Cincinnati, U Florida, U Texas SW, U Utah, U Wash), the Data Coordinating Center (Cleveland Clinic), and the Ultrasound (U Alabama), Vascular Function (Boston U) and Histology (U Wash) cores.

Unique features of the study protocol include 1) pre-operative physiological examination of the vasculature with measurements of arterial flow-mediated dilation, arterial pulse wave velocity, and venous capacitance in the planned surgical arm; 2) detailed documentation of attributes of the surgical personnel and surgical procedures at the time of AVF creation; and 3) serial post-operative ultrasounds, including one within 2 days, to define the course of development of the AVF and changes in adjacent blood vessels. These and other factors will be correlated with each other and with the careful, uniform characterization of subsequent AVF function. Additionally, samples of serum, plasma, DNA and vein used for AVF creation are being stored for future biomarker and mechanistic studies. We anticipate that

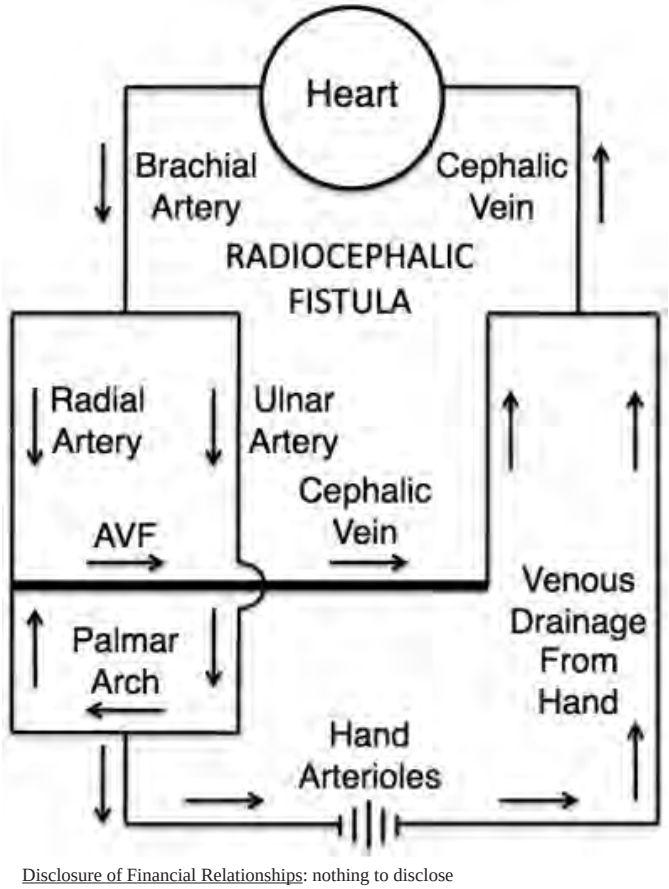
this multifaceted assessment will identify clinically useful early predictors of AVF outcome as well as potential targets for interventions to improve maturation. The enrollment period began in May 2010 and the study is expected to be completed in 2013.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO586**

**Mathematical Model of Arteriovenous Fistula (AVF): A Powerful Method To Improve Understanding of the Hemodynamics of AVF Failure** William D. Paulson,[1] Arif Asif,[2] Loay H. Salman,[2] Steven A. Jones.[3] *[1]Medicine, Charlie Norwood VA Med. Center, Augusta, GA; [2]Medicine, U. of Miami Miller School of Med., Miami, FL; [3]Biomedical Engineering, Louisiana Tech U., Ruston, LA.*

Failure of AVFs is an important cause of morbidity in dialysis patients. Analysis of the hemodynamics of AVF failure should improve understanding of the mechanisms, diagnosis, and treatment of this problem. In this study, we developed mathematical models of radiocephalic (see figure) and brachiocephalic AVFs that extend a previous model of the synthetic graft (Jones SA: J Biomech Eng 2005). The AVF model includes the cephalic V. anastomosed end to side to either the brachial or radial A., the ulnar A., palmar arch, and a vein that drains the hand and forms a Y connection to the cephalic V. We set mean arterial pressure = 93 mmHg, central venous pressure = 5 mmHg, Hct = 36%. Luminal diameters were based on data from angiographic evaluation of AVFs. Stenosis was placed at the arteriovenous anastomosis of the AVF. Equations from the engineering literature were used to predict circuit blood flows and pressure drops. The equations defined whether flow is laminar entry-flow, laminar, or turbulent, as indicated by the previous in vitro model. Turbulent flow was applied when the Reynolds No. was ≥1500. Equations for turbulent flow and stenosis were modified to reflect the effect of upstream events on downstream segments. The model defines relations between stenosis, flow, intra-access pressures, and luminal diameters. The radiocephalic case predicts reversal of radial A. flow with possible steal that resolves as stenosis progresses at the anastomosis. We conclude that the model provides a powerful method to improve understanding of the hemodynamics of AVF failure by allowing control of circuit conditions while changing values of selected variables at will.




Disclosure of Financial Relationships: nothing to disclose

**TH-PO587**

**Predictors of Fistula Maturation in the Dialysis Access Consortium (DAC) Fistula Trial** Laura M. Dember,[1] Michael Allon,[2] Bradley S. Dixon,[3] Bo Hu,[4] Milena Radeva,[4] Tom H. Greene,[5] Gerald J. Beck,[4] John W. Kusek,[6] Harold I. Feldman,[7] The DAC Study Group.[6] *[1]Boston Univ.; [2]Univ. Alabama Birmingham; [3]Univ. Iowa; [4]Cleveland Clinic; [5]Univ. Utah; [6]NIDDK; [7]Univ. Pennsylvania.*

**Background:** A high proportion of new fistulas fail to mature adequately for use. Identifying risk factors for maturation failure should facilitate development of interventions to improve fistula outcomes.

**Methods:** We performed a secondary analysis of the DAC Fistula Trial in which 877 subjects from 9 centers were randomized to clopidogrel or placebo for 6 weeks after fistula creation and followed to ascertain use of the fistula for dialysis. Demographic, clinical, and surgical predictors of fistula usability were identified using forward stepwise regression with adjustment for randomized treatment group and surgical center.

**Results:** Older age, female sex, forearm fistula site and aspirin use at baseline were associated with fistula usability failure (see Table). The relationship between aspirin and usability failure was not attenuated by including cardiovascular disease in the model and may reflect either aspirin use before surgery or the protocol-required discontinuation of aspirin for 6 weeks after surgery. Surgical center was also an independent predictor of usability (adjusted OR 2.94, P=0.02).

**Conclusion:** Among DAC Fistula Trial participants, age, sex, aspirin use, fistula location and surgical center were independent predictors of fistula usability. Further exploration of the relationships between fistula outcome and both aspirin use and modifiable surgical factors is warranted.

Predictors of Fistula Usability Failure

| Predictor | Adjusted Odds Ratio for Failure | 95% CI | P Value |
|---|---|---|---|
| Age (per year)* | 1.02 | 1.00-1.03 | 0.01 |
| Female sex* | 3.58 | 2.51-5.12 | <.0001 |
| Black race | 1.32 | 0.92-1.89 | 0.13 |
| Absence of diabetes mellitus | 1.27 | 0.90-1.78 | 0.17 |
| Forearm fistula* | 2.40 | 1.72-3.35 | <.0001 |
| Aspirin use* | 1.55 | 1.01-2.36 | 0.04 |
| Cardiovascular Disease | 0.97 | 0.66-1.43 | 0.87 |

*P<0.05 in univariate analysis

Disclosure of Financial Relationships: Consultancy: ShireResearch Funding: Proteon Therapeutics.

**TH-PO588**

**Long Term Results of Percutaneous Transluminal Angioplasty (PTA) for Artero-Venous Fistula (AVF) Stenosis Treatment: A Single Centre 5 Years Experience** Emanuele Mambelli,[1] Elena Mancini,[1] Alberto Bazzocchi,[2] Marco Veronesi,[1] Maria Grazia Facchini,[1] Francesco Losinno,[2] Antonio Santoro.[1] *[1]Nephrology, Dialysis, Hypertension, S. Orsola-Malpighi, Bologna, Italy; [2]Radiological Sciences Department, S. Orsola-Malpighi, Bologna, Italy.*

Percutaneous Transluminal Angioplasty is a standard care for AVF stenosis in hemodialysis (HD) patients. However some concern remains over frequency and severity of re-stenosis. The aim of this work was to evaluate the long term results of PTA for the treatment of AVF stenosis in the HD population of our Centre.

We retrospectively analyzed the case records of 531 HD patients considering: type of AVF; use of cutting balloon (CB); incidence of procedure-related complications; number, site and time of restenosis after PTA. Primary patency was the time interval between PTA and the day of access failure or reintervention or end follow-up. Secondary patency included all further PTA procedures until the day of access failure. Death or renal transplant were considered end of follow-up.

Sixty-eight patients (12.8%; 49 M; 69±12 yrs) were treated with 115 PTA performed with a high pressure balloon; CB was used in case of not satisfactory results. Stent placement was avoided. Seventeen patients (25%) showed recurrence of stenosis and were submitted to 64 PTA procedures (7 using CB). Primary treated stenosis were distributed as follows: 2 arterial, 24 juxta-anastomosis vein, 8 draining vein, 3 central vein. The corresponding re-stenosis rate per region was: 17/24 (70.8%), 5/8 (60.3%), 2/3 (66.7%), respectively. On a patient-based analysis, primary stenosis incurred after 205±160 days, restenosis (or new stenosis) after 205±246 (p=NS), 154±116 and 245±243 days, respectively for proximal and distal AVF (p=NS). Only two out of 7 stenosis tretated with CB had restenosis (p=0.05 vs PTA without CB). The only treatment complication was a venous cleft with hematoma. PTA is confirmed as an effective treatment to extend life-time of AVFs. Time of restenosis after PTA is higly variable but generally shorter for proximal AVF. The introduction of CB seems to be a promising event, suggesting that its shrewd use might improve the long-term results of some PTA procedures.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO589**

**The Factors Related with Arteriovenous Fistula Patency According to the Hemodialysis Duration** Ju-Young Moon,[1] Young-Il Jo,[2] Sang-Woong Han,[3] Tae Won Lee,[5] Kyung-Hwan Jeong,[5] Sug Kyun Shin,[4] Chun-Gyoo Ihm.[5] *[1]Internal Medicine, Kyung-Hee Univ EWN Medical Center, Seoul, Korea; [2]Internal Medicine, Konkuk University Hospital, Seoul, Korea; [3]Internal Medicine, Hanyang University Guri Hospital, Guri, Korea; [4]Internal Medicine, NHIC Ilsan Hospital, Ilsan, Korea; [5]Internal Medicine, Kyung Hee University Medical Center, Seoul, Korea.*

The patency of arteriovenous access is important for stable and effective hemodialysis (HD), and long-term technical survival is best achieved with a native arteriovenous fistula (AVF). However, maintaining the AVF patency remains a challenging problem. This study was designed to determine the independent prognostic factor for AVF patency according to the HD duration.

We enrolled patients with end-stage renal disease (ESRD) who were treated on maintenance HD in 2010 at several hospitals with available complete medical records. The primary study end point was unassisted patency of the AVF, which was defined as the time from the first fistula surgery to the first AVF failure. An AVF failure was defined as an event that required percutaneous intervention or surgery to revise or replace the fistula, which occurred at least 2 months after fistula formation.

We enrolled 526 patients, mean age was 54±3.4 and the mean duration of dialysis was 40±23 months. Total 111 cases (21.1%) of AVF failure events occurred. The factors related with AVF patency ware different according to the HD duration. Using Cox-adjusted model, we observed a significant correlation between the incidence of AVF failure and surgeon and diabetes in the first half of patients. The last half of patients with AVF failure was related with hyperphosphatemia (mean serum phosphorus >5.5 mg/dL during HD).

The different factor was associated with the development of AVF failure according to the HD duration, and a preventive role of hyperphosphatemia control will be examined more precisely.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO590**

**Angiography after Surgical Thrombectomy of Dialysis Grafts: Findings, Procedures and Outcomes** Luis Resende,[1] Artur P. Mendes,[2] Carlos Lucas,[2] Celia Gil,[2] Jose Diogo Barata.[2] *[1]Nephrology, Hospital Central do Funchal, Funchal, Portugal; [2]Nephrology, Hospital de Santa Cruz, Carnaxide, Lisboa, Portugal.*

**Introduction:** Thrombosis is a common complication of vascular access for haemodialysis. Surgical thrombectomy alone fails to identify the underlying vascular lesion. Percutaneous balloon angioplasty as an adjunctive therapy is associated with better graft outcome.

**Methods:** From 1st January 2007 to 31st December 2009, 883 angiographies were performed; 93 (10,5%) were done after dialysis graft surgical thrombectomy. Demographic and clinical data of patients, angiography findings, procedures and outcomes were reviewed.

**Objectives:** Evaluate the angiography findings after surgical thrombectomy of dialysis grafts and determine the factors that influence graft rethrombosis and primary patency.

**Results:** A total of 93 procedures were performed in 78 patients (52,7% males, 67.7±14.3 years, 93.5% caucasians, 34,4% diabetics, 64.2±39.4 months on dialysis, 89.2% upper arm grafts, 18.9±17.5 months graft age).

Vascular stenosis was present in 77 procedures (82.8%) (central - 29%; axillary - 14%; venous anastomosis - 52.7%; intra-graft - 21.5%; multiple - 32.2%). Percutaneous balloon angioplasty was performed in 68,8%. Residual stenosis after angioplasty remained in 24.7% and clinical graft pulsatility in 12.9%.

On univariate analysis, higher graft age (p=0.004), previous graft thrombosis (p<0.001) and axillary stenosis (p=0.015) were significant predictors for graft rethrombosis.

Graft primary patency after angiography was 61.3% and 45% at 3- and 6 months, respectively. According to Kaplan-Meyer survival curve analysis, graft primary patency was lower in black race (p=0.033), diabetics younger than 60 years (p=0.038), episodes of previous thrombosis (p=0.001), axillary stenosis on angiography (p=0.004) and clinical pulsatility after angioplasty (p=0.035). Patients submitted to baloon angioplasty presented a better graft survival (p=0.033).

**Conclusions:** Elevated number of vascular access stenosis, mainly venous anastomosis stenosis. Axillary stenosis were associated with a greater risk of graft rethrombosis. Grafts submitted to balloon angioplasty had a better primary patency rate.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO591**

**Impact of Ultrasonographic Mapping in Construction and Patency of Arteriovenous Fistula in End-Stage Kidney Disease Patients** Maria Joao Carvalho Azevedo Rocha, António Cabrita. *Nephrology, Hospital de Santo António - Centro Hospitalar do Porto, Porto, Portugal.*

Ultrasonographic mapping (UM) of vessels prior to construction of AVF increases the number of distal arteriovenous fistulas formed and improves overall patency rates.

Our purpose is to evaluate the impact in site of fistula and patency rates in our patients after the introduction of our UM programme in 2008.

We retrospectively evaluated demographic and clinical data of 343 patients who attended our clinic for programming a vascular access for dialysis. All patients had a thorough physical examination. In 180 patients (52.5%) UM of arm and forearm was performed by a trained nephrologist. Inner diameter of veins and arteries was evaluated as well as distensibility of veins. The minimum diameter considered adequate was 2 mm for both arteries and veins.

Site of AVF was then proposed and construction was carried out by a team of vascular surgeons. Patency and adequate development were evaluated at 48h and 1 month after construction.

There was no significant difference in mean age (62,96±15,27y vs 64,54±15,75 y; p=0,34), sex distribuition (42% vs 58% female patients; p=0,44) or prevalence of diabetes (51% vs 38%; p=0,24) between the two groups (UM and no-UM).

The AVF sites didn't differ significantly among both groups with the exception of humero-basilic (HB) AVF proposed and constructed in 11.1% of patients with UM and 3,7% of patients with no UM (p=0,013). The other sites were the following (UM vs no UM): wrist radiocephalic AVF (25% vs 22,7%; p=0,70), forearm radiocephalic AVF (5,6% vs 10,4%; p=0,10), elbow braquiocephalic AVF (58,3% vs 63,2%; p=0,38).

The rate of primary failure was significantly different between both groups, 23,3% failure in the UM group and 36,2% in the non UM group (p=0,013).

Ultrasonographic vascular mapping improved primary patency rates of AVF constructed at our center. Its introduction caused an increased number of HB fistulas allowing for more AVF construction in patients otherwise not considered for this type of vascular access.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO592**

**Factors Influencing Failure To Create Vascular Access in Municipal Hospital Incident Hemodialysis Patients** Lada Beara Lasic,[1,2] Trina D. Banerjee,[1] Nathan Thompson,[1] Frank Modersitzki,[2] David S. Goldfarb.[1,2] *[1]Nephrology, NYU Medical Center, New York, NY; [2]Nephrology Section, New York Harbor VAMC, New York, NY.*

**BACKGROUND**. A high percentage of municipal hospital (MH) end stage renal disease (ESRD) patients who start hemodialysis (HD) are racial/ethnic minorities with low socio-economic status; a significant proportion is uninsured with poor English language skills. These factors are associated with decreased access to pre-dialysis care, which leads to higher percentage of central venous catheter (CVC) use, rather than arterio-venous fistula (AVF) or graft (AVG) at first HD. We sought to determine factors associated with failure to create vascular access in a MH incident dialysis population.

**METHODS**. Retrospective chart reviews were done of incident HD patients from 2005-2007. Hospitalizations and deaths were recorded in the first 90 days after hemodialysis was started. Results were compared to 2007 USRDS population incident dialysis patients.

**RESULTS.** Compared to USRDS, MH patients are younger (51.6 vs. 64.4 y), have higher percentage of racial/ethnic minority (26% vs. 4.6% of Asian, 42.9 vs. 13.56% Hispanic, 24 vs. 21% African American), have higher % of glomerulonephritis as cause of ESRD (23% vs. 6.8%), start dialysis with lower hemoglobin (9.5 vs. 10.2 g/dl) and lower estimated glomerular filtration rate (7.9 vs. 10.9 ml/min/1.73m$^2$). Higher % of MH patients start dialysis with no insurance (18.6 vs. 7.7%) or with Medicaid (42.8 vs. 24.8%) and with CVC (97.3% vs. 80.6%). Primary cause of failure to create vascular access was patient/disease related in 65% and physician/hospital related in 35%. 51% of patients were hospitalized in the first 90 days, 30% hospitalizations were related to CVCs.

**CONCLUSIONS.** Municipal hospital patients differ from USRDS population but their care is inferior. Timely nephrology and vascular surgery referral and shorter time to access creation is needed. 21% of patients were 19-39 years old, consistent with 2007 USRDS data showing increasing ESRD incidence rates for racial/ethnic minorities age 30-39 y. Better care of the growing, young minority ESRD patients is warranted.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO593**

**Utility of Access Flow Monitoring in Predicting Access Thrombosis in ESRD Patients** David B. DaRocha-Afodu, Jennifer J. Tan, Rajesh Agarwala, Sumit Mohan, Herman L. Anderson, Velvie A. Pogue, Jen-Tse Cheng. *Medicine, Harlem Hospital Center/Columbia University, New York, NY.*

Malfunction of vascular access is a challenge in ESRD patients on chronic hemodialysis. Recently CMS started reimbursement for access flow (Qa) surveillance with transonic hemodialysis monitoring systems to evaluate vascular access dysfunction. There is a paucity of data on the optimal value of Qa that best predicts thrombosis of native AV fistulas (AVF) and AV grafts (AVG). We have a monthly vascular monitoring protocol using the transonic ultrasound dilution HD-02 monitor. We reviewed Qa measurements for 101 hemodialysis patients with either an AVF or AVG at our dialysis center retrospectively for the past 30 months to assess the utility of Qa in predicting access thrombosis. Our cohort was 71.3% male, 26.7% diabetic, mean age 54±15.8 yrs. 75 patients (74.3%) had AVF and 26 patients (25.7%) had AVG. A total of 25 patients (27.7%) had a first thrombotic event: 8 events occurring with AVF and 17 with AVG. Our study suggests a significantly lower incidence of access thrombosis among patients with AVF (65.4 vs. 10.7%, p<0.001). There was no difference between the lowest Qa in accesses that did and did not have a thrombosis- among patients with AVF (1028±780 vs 731±331 mL/min, *p=ns*) and AVG (649±509 vs 778±435 mL/min, *p=ns*). In addition, neither diabetes nor location of the access influenced flow rates or outcomes. Our findings suggest that the use of Qa transonic monitoring systems does not improve the diagnostic yield in predicting thrombosis in both AVF and AVG. Larger multicenter studies need to be done to formulate specific guidelines regarding vascular access surveillance.

Incidence of first thrombotic events: AVF vs AVG

| Access | Clotted | Patent | Total | % clotted |
|---|---|---|---|---|
| AVF | 8 | 67 | 75 | 10.7 |
| AVG | 17 | 9 | 26 | 65.4 |
| Total | 25 | 76 | 101 | 24.8 |

chi-square ($\chi 2$ ) test p <0.001

Disclosure of Financial Relationships: nothing to disclose

### TH-PO594

**Interrupted Time Series Analysis of an End Stage Renal Disease (ESRD) Network-Initiated Intervention To Improve the Prevalent Arteriovenous Fistula Rate in a Group of Facilities** Janet R. Lynch,[1] Nancy Gregory,[1] Nancy Armistead,[1] Stephen L. Seliger.[2] *[1]Mid-Atlantic Renal Coalition, Midlothian, VA; [2]Department of Nephrology, University of Maryland School of Medicine, Baltimore, MD.*

**Purpose.** ESRD Network 5 aimed to improve prevalent arteriovenous fistula (AVF) rates in a small dialysis organization (SDO) serving about 1,000 patients through 9 and 7 facilities in each of regions 1 and 2, respectively. **Methods.** Technical assistance in the Fistula First (FF) Change Concepts (CC) was used to stimulate improvement. Monthly vascular access data were tracked and pre- and post-intervention rates compared. Interrupted time series (ITS) regression corrected for autocorrelation and using maximum likelihood estimation used 15 months pre and 14 months post QI project initiation data. **Results.** There was no difference in pre (October 2007, 31.3%) and post (December 2008, 34.6%) region 1 AVF rates (p-value=.235). Region 2 improved significantly in the same time period (35.3% to 43.5%, p-value=.008). ITS revealed the AVF rate for region 2 was increasing prior to the intervention (0.38/month, p-value<0.0001) but increased more rapidly afterward (an incremental 0.13/month, p-value=.03 for a total 0.51/month average increase), $R^2$=.98. **Conclusions.** Barriers remain to improving AVF rates. Unlike region 2, region 1 staff reported problems identifying accessible surgeons willing to place AVFs (CC #4). Region 2 facilities made changes (e.g. added vascular access managers - CC #1) and the nurse leader was actively involved in improvements. Network initiated QI interventions, in partnership with natural groupings of facilities, where leadership is actively involved and change concepts are successfully employed, can result in improved quality.

Interrupted Time-Series Regression of Prevalent AVF Rates, Adjusted for Autocorrelation

| | Coefficient | Standard Error | t-statistic | p-value |
|---|---|---|---|---|
| Intercept | 31.1 | 0.33 | 93.5 | <.0001 |
| Baseline trend | 0.38 | 0.03 | 11.95 | <.0001 |
| Trend after intervention | 0.13 | 0.06 | 2.34 | 0.03 |

Disclosure of Financial Relationships: nothing to disclose

### TH-PO595

**Prospective Evaluation of Primary Patency of Hemodialysis Vascular Access after Angiography and Angioplasty** Marco Mendes,[1,2] Vasco Baptista Fernandes,[2] Ines Aires,[1,2] Fernanda Gomes,[1] Nilza Gonçalves,[3] Manuel A. Ferreira,[1,2] Fernando Nolasco.[2] *[1]Hemodial, Vila Franca de Xira, Portugal; [2]Hospital Curry Cabral, Lisbon, Portugal; [3]KeyPoint, Lisbon, Portugal.*

Efforts have been done to prevent and solve hemodialysis (HD) vascular access (VA) dysfunction.

During 36 months (m) we prospectively evaluated indications for angiography and the primary patency (PP) (median time to new intervention or thrombosis) of VA, in all our patients (pts) (mean population: 166 HD pts)

The PP was evaluated by Kaplan-Meier method, covariates were adjusted by Cox Regression and results expressed by Hazard Ratio (HR) (SPSS-15.0.).

We conducted monthly determinations of VA flow (Qa) using the BTM-Blood Temperature Monitor-Fresenius Medical Care.

Predefined indications for angiography were: A-V fistula (AVF)Qa < 200 ml/min; PTFE graft < 600 ml/min or a Qa drop > 50% in 2 consecutive months (65.2% pts); significant high venous pressure (VP) and/or edema of VA limb (25.8% pts) and thrombosis of the VA (9.1% pts).

According to these criteria 67 pts were submitted to angiography (mean age 66.7 years; 46.3% male). Hypertension (58%) and diabetes (44%) were the two major co-morbidities. In 51% of cases, VA was an AVF and in 49% a PTFE graft. Antiplatelet drugs were used in 52.2% of pts.

The most frequent angiographic findings included: graft-vein anastomosis stenosis (49.2%), AVF stenosis (14.4%) and central vein stenosis (12.3%.) Angiography was normal in 18.5% of cases.

A successful angioplasty was performed in 66.1% of the pts; 13.8% were referred to surgery.

After angioplasty, thrombosis occurred in 47.0% of cases, mainly in PTFE VA (60.6% vs 33.3%) (p=0.026).

The median PP of VA was 18 m (9.6-26.4 m) for AVF, 12 m (6.9-17.0 m) for PTFE graft and 18 m (13.1-23.0 m) for VA of pts under antiplatelet therapy. PTFE grafts were associated with worse PP results (HR=2.1, IC95% [1.1; 4.4]).According to these results, surveillance programmes of VA disfunction (specially Qa analysis by BTM) and angiographic intervention contribute to a longer survival of VA. The use of antiplatelet agents appears to be protective and prolong VA patency in grafts. Larger cost-benefit analysis are needed to confirm these results.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO596

**Improving AVF Rates in Obese Patients: The Role of Vessel Mapping** Deuzimar Kulawik,[1] Jifeng Ma,[2] Jeffrey J. Sands,[3] Kelly M. Mayo,[4] Arif Asif.[5] *[1]FMQAI, Tampa, FL; [2]FMQAI, Tampa, FL; [3]FMC, Celebration, FL; [4]FMQAI, Tampa, FL; [5]Univ Miami, Miami, FL.*

**Background:** The rising prevalence of obesity has been concomitant with the increasing prevalence of CKD population. Due to poor visibility of the veins, traditionally obese patients have not been considered good candidates for an AVF. We investigated the role of vessel mapping (VM) on AVF rates by using BMI.

**Methods:** Incident Medicare ESRD claims from 18,020 Florida patients (1/1/2006 to 12/31/2009) were used to identify CKD patients with and without VMs. BMI was used to categorize patients into underweight (<18.5), normal (18.5-24.9), overweight (25 -29.9) and obese (≥30) categories. The type of VM that was used for the first outpatient dialysis was determined by the CMS form 2728. VMs were identified by CPT codes: G0365, '93930', '93931', '93970', '93971','36005','75822','75820'. Chi-square test and logistic regression were used to ascertain the association of VM, BMI and AVF rates.

**Results:** Significantly increased AVFs rates were observed across all ranges of BMI when preoperative VM was performed. When VM was performed, the AVF rates for underweight, normal, overweight and obese patients were 28.4%, 35.4%, 36.0%, 35.1%, respectively. These differences were not statistically significant. However these values were significantly higher for patients with similar BMI with no VM. The adjusted odds ratio for AVF is 1.46 (95% CI 1.36, 1.56) in favor of VM.

**Conclusion:** The study demonstrates significantly higher AVF in each BMI category with VM and demonstrates that patients with increased BMI have the same likelihood of receiving AVF as patients with normal BMI.

Association BMI / AVF Rates

| | | Under weight | Normal Weight (BMI=18.5-24.9) | Overweight (BMI=25 -29.9) | Obesity (BMI>30) |
|---|---|---|---|---|---|
| No VM | Denominator | 507 | 4203 | 3678 | 4370 |
| No VM | AVF rate | 18.7% | 25.4% | 27.1% | 29.7% |
| VM | Denominator | 176 | 1650 | 1505 | 1931 |
| VM | AVF rate | 28.4% | 35.4% | 36.0% | 35.1% |
| Odds ratio (95% CI) | | 1.72 (1.16, 2.59) | 1.61 (1.42, 1.82) | 1.51 (1.33, 1.72) | 1.28 (1.15, 1.44) |
| P-Value | | 0.007 | <0.0001 | <0.0001 | <0.0001 |

Disclosure of Financial Relationships: nothing to disclose

### TH-PO597

**Defective Membrane Expression of Na-HCO3 Cotransporter NBCe1 Is Associated with Migraine** Masashi Suzuki,[1] George Seki,[1] Shoko Horita,[1] Hideomi Yamada,[1] Takashi Igarashi,[2] Toshiro Fujita.[1] *[1]Internal Medicine, Tokyo University, Tokyo, Japan; [2]Pediatrics, Tokyo University, Tokyo, Japan.*

The $Na^+$-$HCO_3^-$ cotransporter, encoded by SLC4A4, has three splice variants: NBCe1A, NBCe1B, and NBCe1C, mediating distinct physiological roles in the systemic and/or local pH regulation. Homozygous mutations in SLC4A4 cause proximal renal tubular acidosis (pRTA) and ocular abnormalities. In this study, we found two sisters with pRTA, ocular abnormalities, and hemiplegic migraine. Genetic analysis ruled out pathological mutations in the known genes for familial hemiplegic migraine, but identified a novel homozygous 65-base-pair deletion (Δ65bp) in the C-terminus of NBCe1, corresponding to a codon change S982NfsX4 for NBCe1A. Several heterozygous members of this family also presented glaucoma and migraine with or without aura. Despite the normal electrogenic activity in Xenopus oocytes, the Δ65bp mutant showed almost no transport activity due to a predominant cytosolic retention in mammalian cells. Furthermore, the co-expression experiments mimicking the heterozygous status uncovered a dominant negative effect of the mutant through the formation of hetero-oligomer complexes with wild-type NBCe1, which could potentially explain the occurrence of migraine and glaucoma in the heterozygous members. Among other pRTA pedigrees with distinct NBCe1 mutations, we identified 4 additional homozygous patients with migraine: hemiplegic migraine with episodic ataxia in L522P, migraine with aura in Δ2311A, and migraine without aura in R510H and R881C. Evaluation of the corresponding NBCe1B mutants revealed a remarkable coincidence between the lack of plasma membrane expression and the occurrence of migraine, which could not be attributed to a chance coincidence. Furthermore, functional analysis in HEK293 cells confirmed that NBCe1B mutants that lack plasma membrane expression had almost no transport activities, while the other mutants reaching the plasma membrane had reduced transport activities corresponding 30-40% of the wild-type activity. These results demonstrate that the near total loss of NBCe1B activity in astrocytes can potentially cause migraine through dysregulation of synaptic pH.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO598

**Functional Analysis of SNP Mutations Leading to Single Amino Acid Substitution in NBCe1** Osamu Yamazaki, Hideomi Yamada, Masashi Suzuki, Shoko Horita, Ayumi Shirai, George Seki, Toshiro Fujita. *Internal Medicine, Tokyo University, Tokyo, Japan.*

Homozygous mutations in SLC4A4 encoding the electrogenic Na-HCO3 cotransporter cause proximal renal tubular acidosis associated with ocular abnormalities. Although up to 11 SLC4A4 mutations have been identified, the mechanism of NBCe1 inactivation due to the individual mutations has not been completely clarified. Recently, rare mutations in renal sodium transporters such as NCCT and NKCC2 have been shown to affect the level

of blood pressure in general population. In the present study we investigated the impact of SNP (Single Nucleotide Polymorphism) mutations on NBCe1 function. We identified 4 SNP mutations in NBCe1A, resulting in the single amino acid substitution E122G, S356Y, K558R, and N640I. Among them, the frequency of rare allele was reported only for N640I (2.6%). Functional analysis in Xenopus oocytes with two-electrode voltage clamp method revealed that E122G, S356Y, N640I had the activity comparable to that of wild-type NBCe1A. However, K558R had a significantly reduced activity corresponding to 47% of that of wild-type ($p<0.01$). Functional analysis in HEK293 cells with the pH-sensitive dye BCECF also revealed the reduction in transport activity of K558R (45% of wild-type, $p<0.01$), but the activities of other mutants were comparable to the wild-type NBCe1A activity. Immunohistological analysis with confocal microscopy revealed that these SNP mutants were predominantly expressed in the plasma membrane of HEK293 cells. In addition, these SNP mutants were predominantly expressed in the basolateral membrane in the polarized MDCK cells. Biotynylation Western blotting in HEK293 cells confirmed that the surface expression of these SNP mutants was comparable to that of wild-type. These results indicate that, K558R, the only one SNP in the transmembrane domains, inactivates the NBCe1 function without changing the membrane expression in polarized and non-polarized epithelia. Because NBCe1 plays a major role in renal proximal sodium and bicarbonate reabsorption, K558R SNP may be associated with disturbance in systemic acid-base balance or changes in blood pressure.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO599

**The C-Terminal Tails of the NBCe1-A Dimer Are Structured: Relevance to CAII Binding** Quansheng Zhu, Liyo Kao, Debra Newman, Ira Kurtz. *Division of Nephrology, UCLA, Los Angeles, CA.*

NBCe1-A mediates basolateral $Na^+$-coupled $HCO_3^-$ efflux in the proximal tubule. The C-terminal tail of NBCe1-A contains a $D^{986}NDD^{989}$ motif that was predicted to interact with intracellular carbonic anhydrase II (CAII) to form a metabolon thereby enhancing $HCO_3^-$ transport. Previous *in vitro* studies have either supported or refuted the existence of the interaction. In order to study this question, we examined the structural properties of the NBCe1-A C-terminal tail *in vivo*. Wild-type NBCe1-A expressed in HEK cells appeared as both monomers and dimers when separated on SDS-PAGE. Surprisingly, the dimerization was eliminated when the cells were pre-treated with NEM (a cysteine reactive regent) indicating that none of the native NBCe1-A cysteines mediates dimerization. Surprisingly, the dimers on SDS-PAGE were found to be due to the spontaneous cross-linking of endogenous cytoplasmic Cys992 in the detergent. Further studies revealed that the residues neighboring Cys992 (10 on each side) could not be cross-linked. Therefore, the two C-terminal monomer tails in the NBCe1-A dimer must be located in close proximity (2 Angstrom) at Cys992 *in vivo*. Importantly, the predicted CAII binding site is near Cys992, raising the question as to how CAII (28 kDa) can bind to this spatially confined region. Asn987 an uncharged residue within the predicted CAII binding site was substituted with cysteine and its accessibility to BM labeling was tested in the absence and presence of over-expressed CAII. In these experiments, no differences in the level of BM labeling were observed. In conclusion, our data demonstrate that the C-terminal tail of the NBCe1-A dimer is highly structured and may sterically prevent CAII binding.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO600

**C-Terminal Transmembrane Region of NBCe1-A: Structural Properties and Functional Role** Quansheng Zhu, Rustam Azimov, Liyo Kao, Debra Newman, Ira Kurtz. *Division of Nephrology, University of California, Los Angeles, Los Angeles, CA.*

NBCe1-A and AE1 belong to the SLC4 transport family of membrane proteins that mediate $HCO_3^-$ transport. The C-terminal transmembrane region of AE1 is implicated in anion translocation, and has a unique topology with two re-entrant loops. Although NBCe1-A and AE1 share 40% sequence homology in the C-terminal transmembrane region, we recently showed that their topology differs. To clearly determine the *in vivo* folding of this potential functional important region of NBCe1-A, we performed substituted cysteine scanning mutagenesis of NBCe1-A expressed in HEK cells covering the entire C-terminal transmembrane region (Ala-800 to Lys-967). The topologic location of the introduced cysteine residues was determined by whole cell labeling with the membrane permeant biotin maleimide (BM) and a membrane impermeant MTS-TAMRA cysteine reactive reagent. The results show that regions M813-G828 and D960-K967 are only accessible to BM labeling, indicating their intracellular location. M858 is accessible to both BM and TAMRA and S925-A929 only to TAMRA labeling, indicating their extracellular location. Surprisingly, the P888-R905 region is inaccessible to BM whole cell labeling but partially accessible to TAMRA labeling in isolated plasma membranes, suggesting its cryptic cytosolic location. Substitution of NBCe1-A P868-L967 residues (a functionally important region in AE1) had only a minimal affect on transport function. In summary, our data demonstrate that the C-terminal transmembrane region of NBCe1-A is tightly folded and significantly differs from AE1. This region of NBCe1-A has several interesting features including: 1) highly exposed and cryptic cytosolic loops; and 2) sharp turn between transmembrane segment (TM) 11 and 12, and a cryptic extracellular facing loop between TM 13 and 14. In comparison to AE1, the average size of NBCe1-A C-terminal TMs is greater. Unlike AE1, the C-terminal region is not functionally involved in ion translocation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO601

**Measurement of Oligomerization State of NBCe1-A in Kidney Tissue Via Spatial Fluorescence Intensity Fluctuation Analysis** Mikhail Sergeev,[1] Antoine Guillaume Godin,[1] Paul W. Wiseman,[1] Liyo Kao,[2] Natalia Abuladze,[2] Ira Kurtz.[2] *[1]Department of Physics, McGill University, Montreal, QC, Canada; [2]UCLA School of Medicine for Health Sciences, UCLA, Los Angeles, CA.*

The electrogenic sodium bicarbonate cotransporter NBCe1-A plays an important role in absorbing sodium bicarbonate across the basolateral membrane of the proximal tubule. In the present study, we developed an in situ measurement methodology to determine the oligomeric state of NBCe1-A without requiring tissue disruption using biochemical methods. We first used fluorescent moment image analysis and spatial intensity distribution analysis (SpIDA) to study the oligomeric state of NBCe1-A in cultured mammalian cells expressing NBCe1-A. Both methods allow for unbiased quantitative measurement of fluorescent particle densities and oligomerization states within individual images acquired with confocal laser scanning microscopy. We used cells expressing GPI-anchored monomeric eGFP to quantify the quantal brightness of eGFP. We then examined the basal membrane of cells expressing eGFP-tagged NBCe1-A. Utilizing the recovered values of the eGFP monomeric quantal brightness, we show that NBCe1-A exists on the cell membrane predominantly as a monomer and negligibly as higher order oligomers. As an alternative approach, we performed spatial fluctuation analysis on Alexa488 dye immobilized on cover slips. We then used an Alexa488-α-bungarotoxin conjugate to label cells expressing an NBCe1A-bungarotoxin binding peptide construct. Spatial fluctuation analysis revealed a similar distribution of aggregates as shown for the eGFP data. We then performed SpIDA and moment analysis on immunolabeled NBCe1-A in rat kidney tissue. As a control, we expressed wild type NBCe1-A in HEK293 cells and immunolabeled the cotransporter with the same primary and secondary antibodies used for kidney tissue staining. In rat kidney, SpIDA and moment analysis show that NBCe1-A is present on the proximal tubule basolateral membrane in predominantly dimeric and rarely higher order oligomeric states. Our results demonstrate for the first time that proteins can have a different oligomeric state in native tissue and in heterologous expression systems.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO602

**Bicarbonate Translocation of the Sodium/Bicarbonate Transporter SLC4A4 (NBCe1)** Soojung Lee, Inyeong Choi. *Physiology, Emory University School of Medicine, Atlanta, GA.*

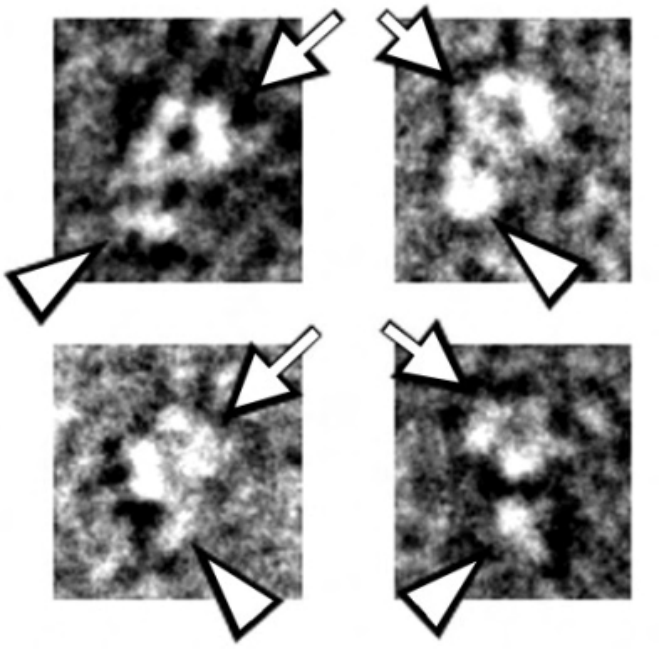
NBCe1 plays an essential role in the basolateral $HCO_3^-$ reabsorption in the proximal tubules. We previously demonstrated that the conserved substitution of $Asp^{555}$ in transmembrane domain (TM) 5 with a Glu (D555E) causes the transporter to carry $Cl^-$ in the absence of $HCO_3^-$. To further examine the anion selectivity of this residue, we replaced TM5 of NBCe1 with the corresponding TM of NBCn1, which contains a Glu instead of Asp. Measured by two-electrode voltage clamp, the chimeric transporter in *Xenopus* oocytes produced electrogenic $HCO_3^-$ currents ($I_{HCO3}$). However, the chimeric transporter did not produce $Cl^-$ currents ($I_{Cl}$). Sequence comparison showed that the residues downstream of $Asp^{555}$ (i.e., 558-562) are weakly conserved between NBCe1 and NBCn1. NBCe1 has KKMIK at this region, while NBCn1 has EKLFD (745-749) at the corresponding region. To test whether the lack of $I_{Cl}$ in the above chimeric transporter is associated with these residues, we replaced EKLFD in the chimeric transporter to **K**KLFD, EKLF**K** and **K**KLF**K**. We found that mutants **K**KLFD and **K**KLFK produced $I_{Cl}$, whereas the mutant EKLFK did not. Thus, $Glu^{745}$ in EKLFD of NBCn1 (or $Lys^{558}$ in KKMIK of NBCe1) contributes to anion selectivity. In other experiments, we examined the effect of $Na^+$ depletion on D555E-mediated $I_{Cl}$ and $I_{HCO3}$. D555E induced $I_{Cl}$ in the absence of $CO_2/HCO_3^-$ but $I_{HCO3}$ in the presence of $CO_2/HCO_3^-$. Interestingly, $Na^+$ depletion from the bath solution caused D555E to produce $I_{Cl}$ in the presence of $CO_2/HCO_3^-$, implying that D555E does not select $HCO_3^-$ if $Na^+$ is absent. $Na^+$ is likely bind to the protein before $HCO_3^-$ binds. Our study is significant for providing direct structural and molecular insights into the mechanism for electrogenic $Na/HCO_3$ cotransport of NBCe1 that plays an essential role in renal $HCO_3^-$ reabsorption.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO603

**Three-Dimensional (3D) Model of Erythrocyte Full-Length Band 3 (Anion Exchanger 1, AE1)** Alexander Pushkin,[1] Ivo Atanasov,[2] Kirill Tsirulnikov,[1] Peng Ge,[2] Nathaniel Magilnick,[1] Z. Hong Zhou,[2] Ira Kurtz.[1] *[1]Medicine/Nephrology, D. Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Microbiology, Immunology and Molecular Genetics, D. Geffen School of Medicine at UCLA, Los Angeles, CA.*

AE1 plays an essential role in transporting $HCO_3^-$ in erythrocytes and in the renal collecting duct. In erythrocytes AE1 mediates $Cl^-/HCO_3^-$ exchange and thereby contributes to the systemic transport of $HCO_3^-$ derived from metabolically produced $CO_2$. AE1 also is involved in the structural integrity of erythrocytes. In kidney alpha intercalated cells, an N-terminal splice variant of AE1 mediates transcellular $HCO_3^-$ transport, and mutations in AE1 result in distal renal tubular acidosis. The structure of AE1 is currently unknown. In this study, the low-resolution three-dimensional (3D) structure of AE1 was generated using electron tomography of negatively stained AE1 dimers. Selected electron micrographs of AE1 dimers are shown in the figure 1.



The dimers were purified under non-denaturing conditions from bovine erythrocyte ghosts. Two major components are seen in AE1 dimers: 1) doughnut-like (arrows); and 2) asymmetric shapes (arrowheads). We hypothesize that the doughnut-like and asymmetric components are membrane and cytoplasmic domains respectively. The membrane domain has a central pore that may be involved in ion transport. This is the first 3D model of full-length native AE1 protein. This model will help to generate a high resolution 3D structure of AE1 using cryoelectron microscopy.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO604**

**The Receptor Protein Tyrosine Phosphatase Gamma (RPTPγ) Is Located at the Basal (but Not Lateral) Membrane of Proximal Tubules** Lara A. Skelton, Walter F. Boron. *Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH.*

RPTPγ is a receptor protein tyrosine phosphatase that possesses an N-terminal, extracellular carbonic anhydrase like domain (CALD) that shares between 35-40% homology with α-type carbonic anhydrases. Yuehan Zhou, who perfused single proximal tubules (PTs), has shown that RPTPγ-null mice are unable to alter their rate of $HCO_3$ reabsorption in response to isolated increases in basolateral [$CO_2$] or [$HCO_3$]. In this work we determined the immunolocalization pattern of RPTPγ along the nephron of mouse kidney by developing a rabbit polyclonal antibody (pAb) to the CALD. The chosen epitope is a 17-amino acid peptide sequence unique to the CALD of RPTPγ. The affinity-purified pAb recognizes a 180-kDa band by western blot of whole-kidney lysates from WT but not RPTPγ-null mice, consistent with the predicted molecular weight of the RPTPγ monomer plus N-linked glycosylation. Next, we used a PLP-based fixative to perfuse wild type and RPTPγ-null mice and harvested the kidneys for cryosectioning. The 5 μM sections were co-labeled with a monoclonal antibody to the α-subunit of the Na-K ATPase as a basolateral marker, and the RPTPγ pAb followed by alexa-dye conjugated secondary antibodies and imaged on an Olympus Fluoview confocal microscope. For PTs of WT kidneys, immunostaining with the RPTPγ pAb revealed a punctate pattern along the basal membrane of most PT cells. However, RPTPγ was completely absent from the lateral infoldings of the basolateral membrane, as well as from the apical membrane. RPTPγ-null kidneys yielded only a diffuse background immunostaining pattern. Thus RPTPγ appears to be trafficked to a highly specialized region of the basal side of the cell. In thick ascending limb and collecting ducts, we observed RPTPγ immunostaining at the basal membrane, as in the PT, but also a more vesicular pattern between the nucleus and the basal membrane. We hypothesize that along the PT, the basal localization of RPTPγ allows the CALD to the sense acid-base status of the interstitial fluid in contact with the blood rather than the interstitial fluid in contact with the lateral infoldings of the PT cells.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO605**

**Receptor Signaling Mechanisms Involved in Inhibition of $HCO_3^-$ Absorption by Lipopolysaccharide (LPS) in Medullary Thick Ascending Limb (MTAL)** David W. Good, Thampi George, Bruns A. Watts III. *Medicine, Univ TX Medical Branch, Galveston, TX.*

Recently we demonstrated that LPS inhibits $HCO_3^-$ absorption in the MTAL through distinct TLR4-dependent signaling pathways in the basolateral and apical membranes. These studies established that bacterial molecules act directly through Toll-like receptors to impair the transport function of renal tubules. Here we examined molecular mechanisms involved in the inhibition of $HCO_3^-$ absorption by basolateral LPS. Addition of LPS to the bath decreased $HCO_3^-$ absorption by 35% in isolated, perfused mouse and rat MTALs. The inhibition by bath LPS was eliminated by the MEK/ERK inhibitors U0126 and PD98095. LPS induced a 1.8-fold increase in ERK phosphorylation, as assessed in microdissected MTALs by confocal immunofluorescence using antiphospho-ERK1/2 antibody. LPS had no effect on ERK phosphorylation or $HCO_3^-$ absorption in MTALs from $TLR4^{-/-}$ mice. Analysis of TLR4 adapter proteins showed that LPS-induced ERK activation was eliminated in MTALs from $MyD88^{-/-}$ mice but preserved in tubules from $Trif^{-/-}$ mice. Surprisingly, inhibition of $HCO_3^-$ absorption by bath LPS, but not inhibition by lumen LPS, was eliminated in MTALs from $TLR2^{-/-}$ mice, despite normal expression of TLR4 in $TLR2^{-/-}$ MTALs. LPS also did not activate ERK in $TLR2^{-/-}$ MTALs. Immunoprecipitation of TLR2 from inner stripe of outer medulla resulted in coprecipitation of TLR4, as shown by immunoblot analysis using two different TLR4 antibodies. The extent of the association between TLR2 and TLR4 was increased by stimulation with LPS. We conclude that basolateral LPS inhibits $HCO_3^-$ absorption in the MTAL through activation of a TLR4/MyD88/ERK-dependent signaling pathway that depends on a novel interaction of TLR4 with TLR2. In previous studies, we found that activation of basolateral TLR2 by bacterial lipoproteins inhibits $HCO_3^-$ absorption through a signaling pathway distinct from that activated by LPS. Thus, basolateral TLR2 functions in a dual capacity to inhibit $HCO_3^-$ absorption in the MTAL, both through interaction with TLR4 to mediate ERK signaling by Gram-negative LPS and through an independent signaling pathway activated by Gram-positive bacterial lipoproteins.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO606**

**Mutations That 'Restore' Enzymatic Activity to the Carbonic Anhydrase-Like Domain (CALD) of Receptor Protein Tyrosine Phosphatase Gamma (RPTPγ): Importance of the Shuttle His and $CO_2$ Binding Site** Lara A. Skelton,[1] Raif Musa-Aziz,[1,2] Xue Qin,[1] Walter F. Boron.[1] *[1]Physiol & Biophys, Case Western Reserve Univ, Cleveland, OH; [2]Physiol & Biophys, Univ of Sao Paulo, Sao Paulo, SP, Brazil.*

In an accompanying abstract we demonstrate that the putative $CO_2$/$HCO_3$ sensor RPTPγ is present in the basal membrane of the proximal tubule. RPTPγ is a single-pass membrane protein with a 730-aa extracellular N terminus that includes a 250-aa carbonic anhydrase-like domain (CALD) that is 30-40% identical to canonical α-type carbonic anhydrases (CAs) and a 680-aa intracellular C terminus that has phosphatase activity. Although it retains the overall fold of catalytically active CAs, CALD lacks CA activity. Previously we reported the 'restoration' of some CA activity to CALD after returning: (1) the 2 missing His residues (E149H and Q175H, analogous to CAIV's H115 and H140) that, along with H151, are required for $Zn^{++}$ coordination, and (2) the $H^+$-shuttle His (K122H, analogous to CAIV's H88). Here, we expressed mutated RPTPγ in *Xenopus* oocytes and pushed a pH-sensitive microelectrode against the oocyte surface ($pH_S$). We exposed cells to $CO_2$/$HCO_3$ and monitored the rising $pH_S$ spike caused by $CO_2$ influx ($H^+ + HCO_3 \rightarrow CO_2 + H_2O$), taking the RPTPγ-dependent $\Delta pH_S$ as a proxy for CA activity. Restoring two CALD residues to improve $CO_2$ access (F177V and F178H) greatly increased $\Delta pH_S$. However, on this 5x-mutant background, reverting either E149H or Q175H to wt eliminates CA activity, presumably because of impaired $Zn^{++}$ co-ordination. On the 5x-mutant background, reverting K112H to wt reduces CA activity but only slightly, suggesting that the shuttle His is not so important. Reverting F178H to wt slight enhances CA activity probably by improving the native local structure. In the wt CALD, F177 is positioned at the mouth of the $CO_2$ binding cavity, where canonical CAs would have the smaller Val residue. The Phe may reduce CA activity by hindering $CO_2$ access to the hydrophobic $CO_2$ binding site or causing other local structural changes.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO607**

**Effect of Chronic Metabolic Acidosis on the Proteome of the Brush Border Membrane of the Proximal Convoluted Tubule (PCT)** Scott J. Walmsley, Norman P. Curthoys. *Biochemistry and Molecular Biology, Colorado State Univ, Fort Collins, CO.*

During chronic metabolic acidosis (CMA), increased catabolism of glutamine within the PCT results in increased production of $NH_4^+$ and $HCO_3^-$ ions that aid in restoration of blood pH. In humans and rats, the residual carbons from glutamine are largely converted to glucose. The latter process is mediated, in part, by increased expression of the enzymes unique to gluconeogenesis. Previous proteomic analyses of brush border membrane vesicles isolated from purified rat renal PCTs ($BBMV_{PCT}$) versus vesicles from cortex ($BBMV_{CTX}$) was performed using an ion trap (LTQ) mass spectrometer and spectral counting. This comparison indicated that $BBMV_{PCT}$ are enriched for 11 enzymes of glycolysis and gluconeogenesis. Of these, fructose-1,6-bisphosphatase-1 (FBP1), was previously localized to the apical membrane. The current study used a QTOF mass spectrometer (Agilent 6520) with a C18-HPLC-chip to perform a global analysis of the effect of CMA on protein expression in $BBMV_{PCT}$. Vesicles were isolated from normal and 7-d acidotic rats (n=4/group), solubilized with 7 M urea/1M thiourea, and digested with trypsin. The samples were analyzed using two approaches. $MS^2$ spectra were identified using SEQUEST and changes were quantified by comparing the sum of the total ion current (TIC) for all of the spectra per identified protein (Total proteins: 128 normal, 140 acidotic). Using the retention time and mass, aligned peptide arrays were quantified by comparing the mean peak intensities ($I_M$) of the ion chromatogram of the identified peptide. The two approaches produced very similar results. The NaPi2 transporter decreased 3.2 fold by TIC ($p=0.04$) and 6.3 fold by $I_M$ ($p=0.004$) in response to CMA. By contrast, γ-glutamyltranspeptidase increased 2.8 fold by $I_M$ and 2.4 fold ($p=0.016$) by TIC. This response was validated (2.9 fold) by enzyme assay ($p<0.001$). Surprisingly, enolase1 ($I_M$: 4.0, TIC: 4.3) and FBP1 ($I_M$: 4.0, TIC: 4.6)

were both significantly decreased during CMA. The latter results suggest that enzymes of gluconeogenesis are normally sequestered in the apical region of the cell and translocated to the cytoplasm and activated in response to CMA.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO608**

**High Sodium Intake Increases *In Vivo* $HCO_3^-$ Absorption in the Loop of Henle (LOH) through Activation of Basolateral $Na^+/H^+$ Exchanger (NHE1)** Oriana Petrazzuolo, Roberto Scanni, Sara Damiano, Francesco Trepiccione, Miriam Zacchia, Giovambattista Capasso. *Internal Medicine, SUN, Naples, Italy.*

It has been reported that sodium intake may affect renal tubular acidification. Previous data have also demonstrated that most of the bicarbonate transport along the loop of Henle (LOH) is localised in the medullary thick ascending limb (mTAL) and it is mainly driven by luminal $Na^+$-$H^+$ exchanger (NHE3) and partially by $H^+$-ATPase. In the present study we have investigated the effect of high sodium diet (HSD)(0.28 M NaCl for 14 days in the drinking water) on: 1. bicarbonate transport along the LOH; 2. mRNAs and proteins abundance of acid-base transporters expressed along the mTALs. Rats fed HSD became hypertensive and showed a reduction in GFR as measured by inuline clearances (0.72±0.04 vs 0.95±0.01 ml.min$^{-1}$ 100g b.w.)($p<0.01$). To measure LOH net bicarbonate reabsorption ($JHCO_3$), superficial loops were perfused at 40 nl.min$^{-1}$ from late proximal to early distal tubules by in vivo micropuncture. Perfusate was an end-like proximal solution containing $^3$H-methoxy-inulin. Total [$CO_2$] in the collected fluid was measured by microcalorimetry. In the presence of similar LOH perfusion rate (39.1±0.9 vs 38.7±0.9 nl/min), and with 13 mM bicarbonate in the perfusate, $JHCO_3$ was 160±5 pmol.min$^{-1}$ (n=20) (mean±ES) in control animals and 192±5 pmol.min$^{-1}$ (n=22) ( $p<0.05$) in rats fed HSD. Increased $JHCO_3$ was found in HSD animals also when the luminal bicarbonate was raised to 24 and 38 mM, thus demostrating a load dependence of $JHCO_3$ . For the molecular biology experiments, total RNA and proteins were extracted from mTALs, identified and microdissected from collagenase treated kidneys. mRNAs and proteins abundance were measured by real rime PCR and immunoblotting, respectively. NHE3 and NHERF1 were unchanged both at mRNA and protein levels; the same holds for B1- and A4 subunits of $H^+$-ATPase. At variance NHE1/β- actin protein abundance was significantly enhanced in mTALs isolated from HSD (0.84±0.02 o.d.) as compared to control animals (0.65±0.01 o.d.) ($p<0.001$). Thus, a high salt intake enhances LOH bicarbonate transport by increasing the expression and abundance of NHE1.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO609**

**Sepsis Impairs $HCO_3^-$ Absorption in the Medullary Thick Ascending Limb (MTAL) through a Two Hit Mechanism** Bruns A. Watts III, Thampi George, Edward R. Sherwood, David W. Good. *Univ TX Medical Branch, Galveston, TX.*

Sepsis is associated with renal tubule dysfunction and dysregulation of systemic electrolyte balance but the underlying mechanisms are incompletely understood. Recently we demonstrated that $HCO_3^-$ absorption by the MTAL is inhibited by LPS through activation of TLR4, indicating that bacterial molecules act directly through innate immune receptors to impair the transport function of renal tubules. In the present study, we used a cecal ligation and puncture (CLP) model of polymicrobial sepsis to examine the effects of sepsis on MTAL $HCO_3^-$ absorption. MTALs from sham-operated and CLP mice were dissected and perfused in vitro at 18 h after surgery. The basal rate of $HCO_3^-$ absorption was reduced 30% in MTALs from CLP mice compared with sham controls ($p<0.05$). The reduced $HCO_3^-$ absorption rate was associated with a 2-fold increase in ERK phosphorylation in the CLP MTALs. Activation of ERK decreases $HCO_3^-$ absorption in the MTAL through inhibition of NHE1. To test the role of basolateral $Na^+/H^+$ exchange in mediating inhibition by CLP, we examined the effects of bath amiloride, which inhibits $HCO_3^-$ absorption in the MTAL through inhibition of NHE1. Addition of 10 μM amiloride to the bath decreased $HCO_3^-$ absorption by 26% in MTALs from sham controls but had no effect in MTALs from CLP mice. These findings suggest that CLP reduces basal $HCO_3^-$ absorption by increasing ERK activity and inhibiting NHE1. Adding LPS to the bath decreased $HCO_3^-$ absorption by 48% in MTALs from CLP mice compared with a decrease of only 28% in tubules from sham controls ($p<0.05$). The inhibition by bath LPS was eliminated by the MEK/ERK inhibitor U0126 in MTALs from both sham and CLP mice. These results indicate that CLP sepsis impairs $HCO_3^-$ absorption in the MTAL through a two hit mechanism involving a decrease in basal $HCO_3^-$ absorption rate and potentiation of inhibition by LPS. These effects would impair the correction of metabolic acidosis that contributes to sepsis pathogenesis. The ERK signaling pathway appears to play a role in both inhibitory effects and thus represents a potential therapeutic target to treat and prevent sepsis-induced renal tubule dysfunction.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO610**

**Multiple Molecular Determinants in the Carboxyl-Terminus Regulate ER Exit and Cell-Surface Expression of NKCC2** Nancy Zaarour,[1, 2, 3] Sylvie Demaretz,[1, 2, 3] Nadia Defontaine,[1, 2, 3] Kamel Laghmani.[1, 2, 3] *[1]INSERM, UMRS 872 - Equipe 3- ERL7226; [2]Universite Paris-Descartes; [3]Universite Paris-VI, Paris, France.*

Mutations in the apical Na-K-2Cl co-transporter, NKCC2, cause type I Bartter syndrome (BS1), a life-threatening kidney disease. Yet the mechanisms underlying the regulation of NKCC2 mutants in renal cells are scarcely known. We previously showed that BS1 mutations denying NKCC2 of its distal C-terminal tail and/or interfering with the highly conserved LLV motif, located at positions 1081-1083, result in ER exit defect of the co-transporter. The aim of the present study was to investigate whether there are carboxyl-terminal residues, other than LLV, that are essential for NKCC2 trafficking. Sequence analysis revealed that the distal C-terminal region of NKCC2 is enriched in di-leucine like motifs. These adjacent hydrophobic residues have been reported to function as ER export signals. Consequently, we mutated these residues to alanines and expressed the mutant proteins in OKP and HEK cells. Using cell surface biotinylation, we show that $^{1050}$VL$^{1051}$, $^{1064}$LL$^{1065}$ and $^{1072}$IL$^{1073}$ motifs had no effect on NKCC2 expression. In contrast, double mutations of $^{1037}$LL$^{1038}$ or $^{1048}$LI$^{1049}$ disrupted glycosylation and cell surface expression of NKCC2. Using microscopic confocal imaging, we further demonstrate that $^{1037}$LL$^{1038}$ and $^{1048}$LI$^{1049}$ mutants were not expressed at the cell surface and were localized to the ER as indicated by co-localization with the ER marker calnexin. To determine if the ER localization of NKCC2 mutants reflected a defect in maturation, we analyzed the biosynthetic processing of NKCC2 by pulse-chase analysis. In contrast to WT NKCC2, the conversion to the mature form was not detectable during the chase period indicating failure in ER exit of the mutated proteins. Altogether, these data are consistent with a model whereby multiple molecular determinants in the carboxyl-terminus regulate ER exit and cell-surface expression of NKCC2. The exact characterization and identification of the molecular mechanisms of the motif-facilitated ER export is essential for the development of therapeutic strategies targeting NKCC2 transport from the ER to the cell surface.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO611**

**Identification of a Candidate Functional Partner for Vacuolar $H^+$-ATPase: SLC26A11 (KBAT = Kidney Brain Anion Transporter) Co-Localizes with $H^+$-ATPase in the Kidney Collecting Duct and Mediates Electrogenic Transport of Chloride** Jie Xu,[1, 3] Sharon L. Barone,[1, 2] Hong C. Li,[1, 3] Kamyar A. Zahedi,[1, 2] Manoocher Soleimani.[1, 2, 3] *[1]Medicine, University of Cincinnati, Cincinnati, OH; [2]Center on Genetics of Transport, University of Cincinnati, Cincinnati, OH; [3]Research Services, Veterans Administration, Cincinnati, OH.*

Chloride plays an important role in regulating vacuolar $H^+$-ATPase activity across specialized cellular and intracellular membranes. Here we report the identification of a transporter which mediates electrogenic transport of chloride, and demonstrates co-localization, co-regulation and co-trafficking with V $H^+$-ATPase in the kidney collecting duct. Immunoblotting identified Slc26a11 as a ~72 kDa band in kidney membrane proteins and immunofluorescent labeling detected its expression in a subset of cells in the collecting duct. Double immunofluorescent labeling with AQP2 indicated that Slc26a11 is expressed in cells distinct from principal cells in the collecting duct. Double immunofluorescence labeling with $H^+$-ATPase demonstrated remarkably identical co-localization of Slc26a11 with $H^+$-ATPase along the length of the collecting duct: Slc26a11 and $H^+$-ATPase co-localized on the apical membrane of A-intercalated and basolateral membrane of B intercalated cells. Mouse Slc26a11 has two distinct variants, a 593 aa or long, and a 421 aa or short variant; the long variant is predominantly expressed in the kidney and other epithelial tissues whereas the short variant is predominantly expressed in the brain. Functional studies in transfected COS7 cells demonstrated that KBAT can mediate the electrogenic transport of $^{36}$chloride. Immunofluorescent studies indicated co-trafficking of KBAT and $H^+$-ATPase from subapical to the apical membrane of A-intercalated cells in potassium depletion and metabolic acidosis. We propose that KBAT facilitates $H^+$-ATPase-mediated acid secretion in the collecting duct through direct stimulation and/or by dissipating the membrane potential resulting from $H^+$ secretion with electrogenic chloride transport.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO612**

**Regulation of Vacuolar $H^+$-ATPase Localization and Activity by PKA and AMPK Phosphorylation of the A Subunit in Kidney Cells** Nuria M. Pastor-Soler,[1] Rodrigo Alzamora,[1] Fan Gong,[1] Ramon Fabio Thali,[2] Christy Smolak,[1] Hui Li,[1] Yolanda Joho-Auchli,[3] Rene A. Brunisholz,[3] Dietbert Neumann,[2] Kenneth R. Hallows.[1] *[1]Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Department of Biology, Institute of Cell Biology, ETH Zurich, Zurich, Switzerland; [3]Functional Genomics Center Zurich (FGCZ), University of Zurich, Zurich, Switzerland.*

Vacuolar $H^+$-ATPases (V-ATPases) are protein complexes that are highly expressed at the apical membrane in kidney type A intercalated cells where they contribute to luminal collecting duct acidification. We have shown that acute apical V-ATPase accumulation in intercalated cells depends on active soluble adenylyl cyclase. We also recently showed that the metabolic sensor AMP-activated protein kinase (AMPK) prevents PKA-mediated V-ATPase apical accumulation in kidney intercalated cells. Moreover, we showed that both

kinases phosphorylate the V-ATPase A subunit (ATP6V1A) in vitro and in a kidney cell line and that PKA-dependent phosphorylation at residue Ser-175 of the A subunit contributes to V-ATPase apical accumulation and activity in kidney cells. We hypothesized that direct phosphorylation of the A subunit by AMPK prevents apical V-ATPase accumulation and thus reduces pump activity in kidney intercalated cells. Indeed, we have identified a novel AMPK phosphorylation site on the V-ATPase A subunit by mass spectrometry. Mutation of this phosphorylation site significantly reduced A subunit phosphorylation by AMPK both in vitro and in kidney cells. V-ATPase apical membrane accumulation in a cell line of intercalated cell characteristics (Clone C) was enhanced by a phosphorylation-deficient A-subunit mutant at this novel AMPK site. These results suggest that PKA and AMPK phosphorylation sites on the A subunit may be involved in the inter-regulation of V-ATPase activity and subcellular localization by acid-base stimuli via PKA and by metabolic stress via AMPK. (Supported by NIH, ASN, and AHA).

Disclosure of Financial Relationships: nothing to disclose

## TH-PO613

**Expression Profile and Regulation of an H/K-ATPase α2 (*Atp12a*) Gene-Reporter Transgene in Mice** Zhiyuan Yu,[1] Pedro E. Cruz,[2] Qun Kong,[1] Brian Poindexter,[1] Bruce C. Kone.[1] *[1]Medicine, University of Texas-Houston Medical School, Houston, TX; [2]Medicine, University of Florida College of Medicine, Gainesville, FL.*

The *Atp12a* gene helps maintain K and acid-base balance. Atp12a is basally expressed at high levels in distal colon surface epithelium, and at lower levels in collecting duct principal cells. With dietary K deprivation, Atp12a expression increases in collecting duct, but not distal colon. Previously, we developed transgenic mice carrying an insertional *Atp12a* promoter-reporter gene. These mice exhibited transgene expression in principal cells, but surprisingly not in distal colon (Am J Physiol 286: F1171, 2004). This result suggested that sequences outside the promoter region direct distal colon-specific expression. Accordingly, we sought to develop a model system with which to test the molecular basis for differential regulation of the *Atp12a* gene in these tissues. The recombineering system was used to introduce a humanized *Renilla reniformis* GFP (hrGFP) cassette in-frame with the *Atp12a* coding region just before the *Atp12a* stop codon contained within a 188 kb BAC clone. The construct placed hrGFP under control of the entire natural genomic sequence context for the 23.6 kb *Atp12a* gene. The *Atp12a*-hrGFP BAC recombination and genotyping were confirmed by restriction enzyme, PCR, and Southern analysis. Patterns of *Atp12a*-hrGFP transgene expression were examined by qRT-PCR and deconvolution fluorescence microscopy. Of 10 major organs examined, *Atp12a*-hrGFP transgene mRNA was abundant in distal colon and kidney, with low levels in lung. In kidney, hrGFP fluorescence colocalized with aquaporin-2 immunoreactivity, consistent with expression in principal cells. hrGFP fluorescence also localized to the surface epithelium of distal colon. Dietary K restriction comparably induced endogenous Atp12a and *Atp12a*-hrGFP mRNAs in kidney medulla, but did not alter the abundance in distal colon. Thus, these *Atp12a*-hrGFP BAC transgenic mice faithfully mimic the differential expression and regulation of the endogenous *Atp12a*2 gene in kidney and distal colon and provide a critical model to dissect the cell- and stimulus-specific regulation of this gene.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO614

**Modulatory Role of Renal Complex Sulfatides in the Control of Acid-Base Balance by the Kidney** Paula Stettner,[1] Soline Bourgeois,[2] Christian Marsching,[3] Milena Traykova-Brauch,[1] Tjeerd Petrus Sijmonsma,[1] Roger Sandhoff,[1,3] Carsten A. Wagner,[2] Richard Jennemann,[1] Elisabeth Groene,[1] Hermann-Josef Groene.[1] *[1]Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Germany; [2]Institute of Physiology, University of Zuerich, Zuerich, Switzerland; [3]Instrumental Analytics and Bioanalytics, Mannheim University of Applied Sciences, Mannheim, Germany.*

Glycosphingolipids (GSL) are amphipathic molecules localized in the outer layer of the plasma membrane of all eukaryotic cells. GSL mediate several cellular functions, including cellular growth, differentiation, and cell-cell interactions. The mouse kidney expresses mainly uncharged neutral GSL and sulfated GSL (sulfatides). The function of renal sulfatides has not been defined. We postulated an influence on ionic transport in tubular epithelia. Mouse models with renal tubular epithelium specific (Pax8) deletion of genes encoding the key enzymes involved in synthesis of glucosylceramide based GSL (Ugcg, UDP-glucose:ceramide glucosyltransferase), sulfatides (Cst, cerebroside sulfotransferase), and combination of both, were generated.

Mice with renal Ugcg, - Cst, and - Ugcg//Cst deletion displayed a normal life span and morphology of the kidney. However, metabolic experiments revealed a significantly lower urinary pH. HCl loading (9d) induced a more severe and persistent hyperchloremic acidosis in Ugcg//Cst double deleted mice (blood pH controls: 7.2+/- 0.1 vs. mutants: 7.0 +/-0.1; n=7-8, p<0.05). The increase of urinary ammonium was significantly less in Ugcg//Cst deficient animals. Enzymes involved in ammoniagenesis were not differentially expressed and NHE3 activity was not altered as compared to control mice.

The pH phenotype could be referred to the absence of the complex sulfatides SM3 and SB1a as these are the only GSL which are lost in all 3 mutant mice. MALDI-tissue-MS demonstrated SM3 to be localized in cortical collecting ducts (CCDs). In vitro microperfused CCDs of HCl challenged GSL-deficient mice showed a reduced NH3 permeability. With this findings, we propose a modulatory function for SM3, and possibly SB1a, in the control of systemic acid-base balance by generating a plasma membrane permeable for NH3 in CCDs.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO615

**Mechanisms of Type 4 (Hyperkalemic) Renal Tubular Acidosis** Kahori Hori,[1] Takanori Nagai,[1] Yuichiro Izumi,[2] Yushi Nakayama,[3] Yukiko Hasuike,[1] Masayoshi Nanami,[1] Yukiko Yasuoka,[4] Yoshinaga Otaki,[1] Akito Tanoue,[5] Katsumasa Kawahara,[4] Takeshi Nakanishi,[1] Hiroshi Nonoguchi.[1] *[1]Division of Kidney and Dialysis, Dept of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [2]LKEM, NHLBI, National Institutes of Health, Bethesda, MD; [3]Nephrology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan; [4]Physiology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan; [5]Pharmacology, National Research Institute for Child Health, Tokyo, Japan.*

The main role of kidney is to maintain water/sodium and acid-base balance. We found that vasopresin V1a receptor knockout (KO) mice shows hyporeninemic hypoaldosteronism, reduced renal function, metabolic acidosis with resiratory compensation, and hyperkalemia, showing the characteristics of type 4 (hyperkalemic) renal tubular acidosis (RTA). We examined the mehcanisms of type 4 RTA in V1aR KO mice. Although urine pH was slightly lower in V1aR KO than wild type (WT) mice (6.29±.03 and 6.45±.03, respectively, ns), urinary excretion of ammonium was significantly lower in V1aR KO than WT mice (0.13±.01 and 0.16±.01 μEq/μg.Cr, respectively, p<0.05). The administration of fludrocortisone to V1aR KO mice ameliorated metabolic acidosis by increasing urinary ammonium excretion (0.39±.04 μEq/μg.Cr,) while decreasing titratable acid excretion. Fludrocortisone slightly increased urinary ammonium excretion in WT mice (0.24±.04 μEq/μg.Cr,). Urine pH was closely related with urinary amonium excretion both in KO and WT mice. In V1aR KO mice, HK-ATPase and Rhcg exressions were decreased but H-ATPase expression was increased. Fludrocortisone decreased H-ATPase and increased HK-ATPase and Rhcg expressions in the kidney. These data show that 1)decreased response to aldosterone in type 4 RTA is caused by reduced function of V1aR 2) low urine pH in V1aR KO mice is caused by upregulation of H-ATPase 3) decreased Rhcg and HK-ATPase expression causes low urinary ammonium excretion 4)Urine pH is not a determinant of urinary ammonium excretion but is related with the amount of urinary ammonium excretion 5) vasopressin and aldosterone has major role for urinary ammonium excretion.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO616

**Impaired Cellular Response to Acid Challenge in Proton Sensor Null Mice** Xuming Sun,[1,2] Lisa M. Stephens,[1] Raymond B. Penn,[3] Thomas D. DuBose,[4] Snezana Petrovic.[1,2] *[1]Medicine, University of Cincinnati, Cincinnati, OH; [2]Research Service, Veterans' Affairs Medical Center, Cincinnati, OH; [3]Medicine, University of Maryland Baltimore, Baltimore, MD; [4]Medicine, Wake Forest University, Winston Salem, NC.*

Deletion of proton receptor GPR4 results in metabolic acidosis and impairs net acid secretion. To define cellular mechanisms of this phenotype, we examined expression of acid-base transporters by real-time RT-PCR in kidneys of GPR4-/- and GPR4+/+ before/after acid loading with $NH_4Cl$, and identified the numbers of intercalated (ICs) and principal cells (PCs) in collecting ducts (PCs as AQP2-positive, ICs as $H^+$-ATPase-positive, A-ICs as AE1-positive, B-ICs as pendrin-positive/Rhbg-negative, and nonA-nonB ICs as pendrin/Rhbg-positive). We analyzed 10 (100x100μm) fields/section and expressed results as avg# cells/field. We examined corresponding nephron segments in kidney sections from 3 GPR+/+ and 3 GPR-/- before/after acid loading. mRNA expression of AQP2, ATP6V0A4, AE1, SLC26A7 and pendrin was similar at baseline. AE1 mRNA nearly doubled in acid loaded GPR4+/+, but not in acid loaded GPR4-/-. Table 1 shows that acidosis increased A-IC numbers and decreased B-IC and nonA-nonB IC numbers in GPR4+/+. GPR4-/- lacked this response, likely because the mice are partially adapted to chronic acidosis. Baseline GPR4-/- show IC-profile akin to acid loaded GPR4+/+. (ICs/PCs in GPR4+/+ and -/- were similar.)

| TABLE 1 | ICs | A-ICs | B-ICs | nonA-nonB ICs |
|---|---|---|---|---|
| Baseline GPR4+/+ | 26.5±0.6 | 15.3±1.4 | 6.4±0.25 | 4.8±0.26 |
| Acid loaded GPR4+/+ | 30.3±0.9 | 23.7±2.3 | 3.9±0.4 | 2.6±0.21 |
| p value | 0.09 | 0.003 | 0.01 | 0.003 |
| Baseline GPR4-/- | 27.1±1 | 20.2±1.6 | 5.1±0.35 | 1.7±0.4 |
| Acid loaded GPR4-/- | 25.3±1 | 20±1.9 | 3.6±0.4 | 1.8±0.3 |
| p value | 0.3 | 0.9 | 0.04 | 0.8 |
| Baseline GPR4+/+ | 26.5±0.6 | 15.3±1.4 | 6.4±0.25 | 4.8±0.26 |
| Baseline GPR4-/- | 27.1±1 | 20.2±1.6 | 5.1±0.35 | 1.7±0.4 |
| p value | 0.7 | 0.02 | 0.05 | 0.007 |
| Acid loaded GPR4+/+ | 30.3±0.9 | 23.7±2.3 | 3.9±0.4 | 2.6±0.21 |
| Acid loaded GPR4-/- | 25.3±1 | 20±1.9 | 3.6±0.4 | 1.8±0.3 |
| p value | 0.02 | 0.2 | 0.6 | 0.2 |
| Baseline GPR4-/- | 27.1±1 | 20.2±1.6 | 5.1±0.35 | 1.7±0.4 |
| Acid loaded GPR4+/+ | 30.3±0.9 | 23.7±2.3 | 3.9±0.4 | 2.6±0.21 |
| p value | 0.07 | 0.2 | 0.09 | 0.2 |

While acidosis in GPR4-/- may be caused by defective transporter activity/trafficking, our data also suggest a cellular response that may alleviate the severity of acidosis in GPR4-/-. Expression of other proton receptors OGR1 and TDAG8 was equal in GPR4+/+ vs. -/-. It is possible that other sensory pathways compensate for GPR4 loss, and/or additional $H^+$ transporters like $H^+$-$K^+$ATPase take part in alternative signaling pathways.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO617**

**Induced but Constrained Expression of GPR4 Mediates Acid-Induced HK$\alpha_2$ Expression** Juan Codina,[1] Snezana Petrovic,[2] Raymond B. Penn,[3] Thomas D. DuBose.[1] *[1]Internal Medicina/Nephrology, Wake Forest University School of Medicine, Winston-Salem, NC; [2]Division of Nephrology, University of Cincinnati, Cincinnati, OH; [3]Department of Medicine, University of Maryland School of Medicine, Baltimore, MD.*

Preliminary experiments from our laboratory demonstrate that PKA activity (measured as phosphorylation of Ser157 of the vasodilator-stimulated phosphoprotein, VASP) is increased by transfection of HEK293 cells with rat or human GPR4. PKA activity is potentiated when the cells are treated with acute acidosis (3-30 minutes). Moreover, chronic acidosis induced up-regulation of HK$\alpha_2$ protein in cells stably transfected with HK$\alpha_2$/NK$\beta_1$ and transiently transfected with GPR4. However, these effects required modest levels of GPR4 expression, because typical transfection methods (1 µg pCDNA3GPR4/3.5 cm dish) promoted expression of poorly glycosylated (assessed by sensitivity to glycosidase F and Endo H), non-functional GPR4 that accumulated in intracellular compartments (revealed by ICC and immunoprecipitation with antibodies using CHAPS solubilized proteins). Transfecting HEK293 cells with a low concentration of plasmid (<100 ng pCDNA3GPR4/3.5 cm dish) enabled plasma membrane GPR4 expression, increased basal VASP phosphorylation, acid-induced (media pH ~ 6.8) VASP phosphorylation, and increased expression of HK$\alpha_2$ by chronic acidosis (via reduction of media [$HCO_3^-$] for 16-24 hours). Additional findings suggest assembly of GPR4 with $\beta_2$-arrestin-GFP that correlates with the level of expression. These results support the view that acid pH (protons) activates GPR4 to increases PKA activity and regulate the expression of a proton transporter important in the control of acid-base balance.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO618**

**Acid Retention Mediates Augmented Kidney Endothelin and Aldosterone Production in Subjects with Moderately Reduced GFR** Donald E. Wesson,[1,2] Jan Simoni,[3] Kristine Broglio.[4] *[1]Internal Medicine, Texas A&M College of Medicine, Temple, TX; [2]Internal Medicine, Scott and White Healthcare, Temple, TX; [3]Surgery, Texas Tech University Health Sciences Center, Lubbock, TX; [4]Statistics, Texas A&M University, College Station, TX.*

Oral $NaHCO_3$ but not NaCl slows GFR decline in subjects with moderately reduced eGFR (CKD stage 2 = 60-90 ml/min) without metabolic acidosis (Kid Int, 2010). Animals with 2/3 nephrectomy have no metabolic acidiosis but have acid retention with GFR decline (Kid Int 2009). Because kidney endothelin-1 (ET) and aldosterone (aldo) mediate GFR decline in animals with partial nephrectomy, we tested if subjects with eGFR 60-90 ml/min (n=40) compared to normal eGFR (> 90 ml/min) (N=40), each without metabolic acidosis, have acid retention that increases kidney ET and aldo measured by urine ET (UET) and aldo (Ualdo) excretion. eGFR 60-90 subjects had higher UET (5.5 ± 1.2 vs. 3.5 ± 1.7 ng/g creatinine [cr], $p < 0.0001$) and Ualdo (36.7 ± 8.7 vs.13.1 ± 2.8 µg/g cr, $p < 0.0001$). UET (4.9 ± 1.2 ng/g cr, $p = 0.028$) and Ualdo (25.7 ± 5.9 µg/g cr, $p < 0.0001$) were each lower in separate eGFR 60-90 subjects (n=40) after 30 days of oral $NaHCO_3$. By contrast, separate eGFR 60-90 subjects after 30 days of oral NaCl (n=40) had lower Ualdo (32.7 ± 8.2 µg/g cr, $p = 0.038$) but not lower UET. Ualdo was lower in $NaHCO_3$-ingesting than NaCl-ingesting eGFR 60-90 ($p < 0.0001$). Baseline urine net acid excretion (NAE) was not different among groups. Acid retention was assessed by measuring urine NAE after an acute oral $NaHCO_3$ bolus. We assumed that greater acid retention would manifest as greater acid tritration of the ingested $NaHCO_3$ bolus, yielding lower urine $HCO_3$ excretion and less urine NAE reduction. $NaHCO_3$ bolus reduced urine NAE less (i.e., post bolus NAE was higher, $p < 0.0001$) in eGFR 60-90 than > 90 subjects, supporting greater acid retention in eGFR 60-90. The data show that UET and Ualdo excretion are increased in CKD stage 2 eGFR and that daily $NaHCO_3$, but not NaCl, reduces each. The data suggest that GFR decline in CKD stage 2 eGFR is mediated by increased kidney ET and aldo production induced by acid retention.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO619**

**Renal Adaptation to Metabolic Acidosis Is Impaired in a Rat Model of Autosomal Dominant Polycystic Kidney Disease (ADPKD)** Remy Buerki,[1] Nilufar Mohebbi,[1,2] Andreas L. Serra,[2] Carsten A. Wagner.[1] *[1]Institute of Physiology, University of Zurich, Zurich, Switzerland; [2]Division of Nephrology, University Hospital Zurich, Zurich, Switzerland.*

ADPKD is associated with renal tubular acidosis. Here, we used an animal model for ADPKD (Han:SPRD rat (Cy/+)) and investigated regulation of key proteins in renal acid-base handling. Wild type rats served as controls. All animals were treated at baseline with standard diet and consecutively challenged with HCl diet for 2 days to induce metabolic acidosis. Under baseline conditions, both groups showed similar arterial blood pH but lower $HCO_3^-$ and $pCO_2$ in ADPKD rats. After 2 days HCl diet, ADPKD animals showed a much more severe metabolic acidosis when compared to controls. At baseline, urinary pH was significantly lower in Cy/+ rats. However, after an acid load with HCl, control and ADPKD rats acidified their urine to the same pH indicating preserved ability to acidify urine. Urinary ammonium excretion was much lower in Cy/+ rats compared to controls. Using realtime RT-qPCR, the kidney specific phosphate transporters NaPi-IIa and NaPi-IIc were significantly down-regulated whereas Pit-2 was up-regulated in Cy/+ rats. Also, relative mRNA abundance of the $Na^+/H^+$ exchanger NHE3 and the $Cl^-/HCO_3^-$ exchanger AE1, as well as the glutamine transporter SNAT3 were reduced. Immunoblotting for NaPi-IIa, NaPi-IIc, NHE3 and AE1 showed down-regulation in Cy/+ rats. In conclusion, cyst formation in Han:SPRD rat model causes renal acidosis and results in more severe metabolic acidosis upon acid-loading. Cy/+ rats showed normal urinary acidification but reduced expression of key transport proteins involved in ammoniagenesis and bicarbonate regeneration.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO620**

**Regulation of AE4 Expression in β-ICs by Acid/Base Status** Jeffrey M. Purkerson, Aya Nakamori, George J. Schwartz, Eric V. Heintz. *Pediatrics, University of Rochester Medical Center, Rochester, NY.*

Adaptation of the rabbit CCD to acidosis is characterized by a reduction in bicarbonate secretion by β-intercalated cells (β-ICs) and increased proton secretion by α-ICs. To identify mechanisms that underlie changes in $H^+/HCO_3$ flux in the CCD, we examined IC phenotypes in normal (urine pH: 8.1±0.1, $HCO_3$:27± 1 mM), 3 day acid (urine pH:4.7±0.2, $HCO_3$:16±1), and rabbits immediately transitioned from 3 day acid to alkali loading for 16-18 h (recovery: urine pH: 7.8±0.1, $HCO_3$:29±1). Recent studies have identified decreased size and intensity of apical pendrin (PND) caps and increased basolateral distribution of AE1 that may reflect increased endocytosis and exocytosis of the respective anion transporter, as well as decreased expression of PND mRNA and protein expression as mechanisms for adaptation of ICs to metabolic acidosis. In β–ICs a reversible redistribution was observed for B1-V-ATPase, as the percentage of cells exhibiting a predominantly apical B1 distribution increased from 33% in normal rabbit kidney sections to 80% in acidotic animals and returned to 29% upon recovery. We have initiated a study of other anion exchangers that may contribute to $HCO_3$ transport in these cells. Confocal microscopy of microdissected CCDs revealed that AE4 expression in β-ICs occurs subapically in a ring-like pattern. Staining of kidney sections failed to demonstrate co-expression of AE4 and AE1. Consistent with expression of AE4 in β-ICs, the relative abundance of AE4 and pendrin mRNA was cortex>outer medulla>>inner medulla. Regulation of AE4 mRNA abundance mirrored PND expression. Specifically, PND and AE4 mRNA levels decreased 2-3 fold after 3 day acidosis; 14- and 5-fold, respectively, following 7 day acidosis. Examination of AE4 localization in kidney sections revealed a predominant lateral distribution of AE4 in normal and acidotic rabbits, whereas upon recovery from acidosis there was an apparent shift toward apical expression. Conclusion: AE4 is expressed in the CCD and may act in conjunction with pendrin to regulate $HCO_3$ secretion in β-ICs during alkali-loading.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO621**

**Effect of Sodium-Free Diet on Renal Ammonia Metabolism and the Role the Ammonia Transporter Family Member, Rh C Glycoprotein** Hyun-Wook Lee,[1] Jill W. Verlander,[1] Jesse M. Bishop,[1] Mary E. Handlogten,[1] David Weiner.[1,2] *[1]Division of Nephrology, Hypertension and Transplantation, University of Florida, College of Medicine, Gainesville, FL; [2]Nephrology and Hypertension Section, NF/SGVHS, Gainesville, FL.*

Sodium depletion commonly leads to metabolic alkalosis, but the mechanisms are incompletely understood. The current studies were designed to address the mechanism and to determine the role of the ammonia transporter, Rhcg, in this response in the mouse. A $Na^+$-free diet for 7 days increased urinary ammonia excretion from 115 ± 14 to 336 ± 58 µmol/d (n= 8; $p<0.03$). Urine pH did not change significantly (pre, 6.02 ± 0.04; post, 6.16 ± 0.06, p=NS). In contrast to metabolic acidosis, where increased PEPCK and PDG expression contribute to increased renal ammonia metabolism, a $Na^+$-free diet did not increase expression of either PEPCK or PDG. However, glutamine synthetase expression decreased significantly in the cortex, suggesting that decreased proximal tubule glutamine synthetase-mediated ammonia degradation might contribute to increased net renal ammonia production. Also in contrast to metabolic acidosis, $Na^+$-free diet did not alter significantly renal expression of either of the ammonia transporter family members, Rh B Glycoprotein (Rhbg) or Rh C Glycoprotein (Rhcg). To confirm the lack of role of Rhcg in the increased renal ammonia excretion, we examined the effect of collecting duct-specific Rhcg deletion on the response to a $Na^+$-free diet. Rhcg deletion did not alter changes in either urinary ammonia or urine pH. We conclude: 1) a $Na^+$-free diet increases urinary ammonia excretion, which likely contributes to the metabolic alkalosis commonly observed with $Na^+$ depletion; 2) altered glutamine synthetase expression may contribute to increased renal ammonia excretion in response to a $Na^+$-free diet; and, 3) the mechanisms of increased ammonia excretion in response to a $Na^+$-free diet fundamentally differ from those observed in response to metabolic acidosis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO622**

**Angiotensin II-Stimulated Ammonia Production by Proximal Tubules from Acidotic Mice Is Modulated by Increased Expression of AT2 Receptors**
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AT1 receptor (AT1R) activation by angiotensin II (Ang II) increases ammonia ($tNH_3$) production (AP) rates by isolated perfused S2 proximal tubules (S2 PTs). As shown previously, Ang II increased AP more in S2 PTs from short-term (18-h) acid-loaded mice than in those from non-acid- loaded controls and this was associated with increased AT1R and reduced AT2 receptor (AT2R) protein levels in brush border membranes (BBMs). In this study, we examined the effects of Ang II and the AT2R blocker PD123319 on AP by S2 PTs and the expression of AT1R and AT2R proteins in BBMs from long-term (7-d) acid-loaded or control mice. Mice (N=5 per group) were given 0.3 M $NH_4Cl$ in 2% sucrose drinking water or sucrose water alone for 7 d. The acid-loaded mice had lower plasma total $CO_2$ and pH levels (acidosis) and excreted higher urinary $tNH_3$ levels than control mice. As shown before, S2 PTs from acidotic mice displayed higher AP rates than controls. Ang II added to the lumen increased AP rates in S2 PTs from acidotic and control mice by comparable amounts (Increase in AP: 9.8±1.0 pmol/min/mm (Control) vs 10.3±1.1 (Acidotic) (mean±SEM)) and was not accentuated as previously observed in S2 PTs from 18-h acid-loaded mice. When PD123319 was added with Ang II, AP rates increased in S2 PTs from acidotic (+Ang II no PD 50.2±1.2; +Ang II +PD 61.2±1.4, $p<0.01$) and control (+Ang II no PD 29.6±1.5; +Ang II +PD 36.2±1.2; $p<0.05$) mice. Western blot analysis demonstrated that both AT1R and AT2R protein levels were higher in BBMs from acidotic mice compared to controls (AT1R: 2.2±0.3-fold increase vs control, $p<0.05$; AT2R: 1.9±0.1-fold increase, $p<0.05$). Thus, the comparable stimulatory effects of Ang II on AP in S2 PTs from acidotic and control mice despite increased AT1R protein levels in BBMs may have resulted from the concurrent increase in AT2R BBM levels with the 7-d acid load. We conclude that AT2Rs attenuate the AT1R-mediated stimulatory effect of Ang II on AP in S2 PTs from acidotic and control mice while reduced AT2R levels with short-term acid loading accentuate this AT1R-mediated effect.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO623**

**Both Luminal and Basolateral $NH_3$ Permeabilities Are Decreased in Rhcg-Null Mice**
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We previously showed that disruption of the rhesus protein Rhcg in mouse leads to defective renal handling of ammonia and incomplete dominant renal tubular acidosis. Here we investigated the role of Rhcg in $NH_3$ and $NH_4^+$ handling by the apical and basolateral membranes of cells lining the collecting duct (CD).

Rhcg related staining was detected at the light microscopy level in mouse kidneys both at the luminal and basolateral sides of principal and intercalated cells. Immunogold electron microscopy further demonstrated the presence of Rhcg in the luminal membrane and in the membrane lining the basolateral interdigitations of type A intercalated cells. We assessed total transepithelial, luminal, and basolateral ammonia membrane permeabilities (MP) on microperfused CD after an acute 2 day HCl load. Transepithelial $NH_3$ MP was reduced by 80% and 40% respectively in CDs from Rhcg-/- and +/- mice compared to control. Luminal $NH_3$ MP was drastically reduced both in CDs from Rhcg-/- and +/- mice but only CDs from Rhcg+/- mice exhibited also a decrease in apical $NH_4^+$ MP. Finally, in the presence of ouabain and furosemide and in the absence of extracellular potassium (to block NKCC1 and Na,K-ATPase mediated $NH_4^+$ fluxes), basolateral $NH_3$ MP was also decreased in CDs from Rhcg-/- mice.

In conclusion, we demonstrate for the first time that $NH_3$ permeates the basolateral membrane by non-ionic diffusion and that this process is in part dependent on the presence of Rhcg. We also show that haplo-insufficiency in Rhcg is associated with impaired $NH_3$ transport in the CD. These data further enlighten the functional role of Rhcg in $NH_3$ transport across the epithelium of the collecting duct.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO624**

**$NH_3$ Permeability of Human RhAG, RhBG, and RhCG**
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Mammalian Rhesus (Rh) proteins include the erythroid RhAG, RhCE, and RhD, as well as the non-erythroid RhBG and RhCG, which are mainly expressed in kidney and liver. In human erythrocytes, the Rh proteins are highly abundant membrane proteins forming the Rh complex, which probably consists of a trimer of 2 molecules of RhAG plus RhCE and/or RhD, plus further accessory proteins of unknown function [e.g., CD47, LW, glycophorin B (GPB)]. RhAG, RhBG, and RhCG can function as gas channels, mediating the movement of $NH_3$ and, at least in the case of RhAG, $CO_2$. This study explored the $NH_3$ permeability of mammalian Rh proteins expressed in *Xenopus* oocytes and began to determine the contributions of the proteins within the erythroid Rh complex. We used microelectrodes to monitor surface-pH ($pH_S$), intracellular-pH ($pH_i$), and membrane potential ($V_m$) as we added 0.5mM $NH_4Cl$ ($pH_o$=7.5) to the extracellular fluid (ECF). An ultra-fine micromanipulator positioned the flat tip (20mm diameter) of the $pH_S$ electrode until it just touched the oocyte surface, and then further advanced the tip ~40mm. Periodically we retracted this electrode for calibration in the bulk ECF. Adding 0.5mM $NH_4Cl$ caused no $pH_i$ changes, only small depolarizations, and a rapid fall in $pH_S$ ($\Delta pH_S$) that was followed by a slow recovery. The magnitude of $\Delta pH_S$ is a semiquantitative index of maximal $NH_3$ influx, uncorrected for protein expression at the membrane. The $\Delta pH_S$ with $NH_3$ addition was 0.096±0.015 (n=4) in RhAG oocytes, 5 times the value of $H_2O$-injected control oocytes (0.020±0.001, n=4). The $\Delta pH_S$ values were 0.063±0.004 (n=6) for RhBG and 0.085±0.001 (n=9) for RhCG, but only 0.030±0.005 (n=6) for RhCE. The combinations RhAG+RhCE and RhAG+GPB were no different from RhAG alone. Furthermore, the combination RhCE+GPB was no different from RhCE alone, GPB alone or $H_2O$. Thus, we have no evidence that RhCE enhances the $NH_3$ permeability of RhAG, nor that RhCE has $NH_3$ permeability alone or in combination with GPB. However, using the $pH_S$ approach, we can confirm that RhAG, RhBG, and RhCG all have significant $NH_3$ permeability.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO625**

**Rh B Glycoprotein (Rhbg) Is Necessary for Increased Renal Ammonia Excretion during Hypokalemia in the Mouse**
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Rhbg is an $NH_3$-specific transporter and Rhbg-mediated $NH_3$ transport contributes to increased renal ammonia excretion during metabolic acidosis. Hypokalemia increases renal ammonia excretion and urine pH, suggesting increased collecting duct $NH_3$ secretion. We reported previously that the ammonia transporter, Rh C Glycoprotein (Rhcg), does not contribute to the increased urinary ammonia excretion induced by hypokalemia in the mouse, suggesting other mechanisms are involved. Thus we examined the role of renal Rhbg in ammonia excretion during hypokalemia by quantifying Rhbg protein expression and urinary ammonia excretion in control mice (C) and mice with intercalated cell-specific Rhbg deletion (IC-Rhbg-KO) generated using Cre-loxP techniques. We induced hypokalemia by feeding mice a $K^+$ free diet for 3 days. In C mice, dietary $K^+$-restriction increased urinary ammonia excretion and urine pH within 24 hrs. After 3 days, Rhbg protein expression increased in the renal cortex and outer medulla. IC-Rhbg-KO did not alter basal rates of urinary ammonia excretion, or urine pH on either diet. Potassium deficiency increased urinary ammonia excretion in IC-Rhbg-KO on each day, but the increase was significantly less in IC-Rhbg-KO than C mice (day 1; C, 291±25 vs IC-Rhbg-KO, 186±17 μmol $d^{-1}$, $P\leq0.05$; day 2; C, 333±33 vs IC-Rhbg-KO, 231±29 μmol $d^{-1}$, $P<0.05$;day 3; C, 374±28 vs IC-Rhbg-KO, 249±33 μmol $d^{-1}$, $P<0.05$ ; n=8 each group). Unlike C mice, IC-Rhbg-KO mice did not increase urine ammonia concentration during $K^+$-restriction; the increase in urinary ammonia excretion was produced by increased urine volume. We conclude: 1) hypokalemia increases renal ammonia excretion at least in part by increasing intercalated cell Rhbg-mediated $NH_3$ transport; 2) intercalated cell Rhbg is necessary for the normal increase in ammonia excretion response during hypokalemia; and 3) because Rhbg has a specific role in ammonia excretion in the mouse in hypokalemia, whereas Rhcg does not, these two ammonia transporters serve distinct roles in renal ammonia excretion.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO626**

**Regulation of Frizzled-Dependent Planar Cell Polarity and Wnt Signaling by the (Pro)renin Receptor**
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Frizzled (Fz) is a seven-pass transmembrane receptor that acts in both Wnt/β-catenin and Wnt/planar cell polarity (PCP) pathways. Both pathways are essential for numerous developmental processes and are deregulated in many human diseases. A prerequisite for PCP signaling is the asymmetric subcellular distribution of Fz in epithelial cells. However, the regulation of Fz asymmetry is currently not well understood. Here, we describe that the transmembrane protein CG8444 (here termed VhaPRR) is needed for PCP signaling in Drosophila melanogaster. VhaPRR is an accessory subunit of the vacuolar (V)-ATPase proton pump. It also functions as a receptor for (pro)renin (PRR) in mammals and has recently gained importance as a potential target in anti-hypertensive therapy. We show that VhaPRR function is tightly linked with Fz but not other PCP core proteins. Fz fails to localize asymmetrically at the plasma membrane and accumulates in cytoplasmic vesicels in the absence of VhaPRR. This is accompanied by prehair mispositioning and mispolarization in pupal wing cells. In addition, VhaPRR forms a protein complex with Fz receptors and interacts genetically with Fz in the Drosophila eye. VhaPRR also acts as a modulator of canonical Wnt signaling in larval and adult wing tissue. Its loss leads to an expansion of the Wnt/Wingless (Wg) morphogen gradient and reduction of Wg target gene expression. The requirement for additional V-ATPase subunits suggests that proton fluxes contribute to normal Fz receptor function and signaling. Our findings have broad implications on the relationship between membrane trafficking and signaling events, and may also suggest that novel PRR-targeting drugs may need to be critically assessed to avoid serious side effects.

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**TH-PO627**

**Mineralocorticoid Receptor Degradation Is Promoted by Hsp90 Inhibition and the Ubiquitin-Protein Ligase CHIP** Olivier Staub, Nourdine Faresse. *Department of Pharmacology & Toxicology, University of Lausanne, Lausanne, Switzerland.*

The mineralocorticoid receptor (MR) plays a crucial role in the regulation of Na+ balance and blood pressure, as evidenced by gain of function mutations in the MR of hypertensive families. In the kidney, aldosterone binds to the MR, induces its nuclear translocation, and promotes a transcriptional program leading to increased transepithelial Na+ transport via the epithelial Na+ channel ENaC. In the unliganded state, MR is localized in the cytosol and part of a multiprotein complex, including Hsp90, which keeps it ligand binding competent. 17-AAG is a benzoquinone ansamycin antibiotic that binds to Hsp90 and alters its function. We investigated whether 17-AAG affects the stability and transcriptional activity of MR and consequently Na+ reabsorption by renal cells. 17-AAG treatment lead to reduction of MR protein level in epithelial cells in vitro and in vivo, thereby interfering with aldosterone-dependant transcription. Moreover, 17-AAG inhibited aldosterone-induced Na+ transport, possibly by interfering with MR availability for the ligand. Finally, we identified the ubiquitin-protein ligase CHIP as a novel partner of the cytosolic MR, which is responsible for its polyubiquitylation and proteasomal degradation in presence of 17-AAG. In conclusion, 17-AAG may represent a novel pharmacological tool to interfere with Na+ reabsorption and hypertension.

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**TH-PO628**

**Impaired Aldosterone Responsiveness in Corticosteroid Binding Globulin (CBG) Deficient Mice** Jens G. Leipziger,[1] Helle A. Praetorius,[1] Anders Nykjaer,[3] Mads Vaarby Sorensen.[1] *[1]Physiology and Biophysics, Aarhus University, Aarhus, Denmark; [2]Medical Biochemistry, Aarhus University, Aarhus, Denmark.*

Corticosteroid Binding Globulin (CBG) is the relevant plasma carrier protein for corticosterone. CBG is assumed to keep the steroids inactive and to define the amount of free hormone acting on target tissues. Previous findings have shown that the delivery of corticosterone to peripheral tissues is insufficient in $CBG^{-/-}$ mice despite elevated free plasma corticosterone. In the large intestine glucocorticoids synergistically enhance the pro-absorptive effects of aldosterone (aldo). In addition, CBG is also known to carry about 20% of the total plasma aldo. We therefore hypothesized that $CBG^{-/-}$ mice show altered responsiveness to aldo. Methods: We used $CBG^{-/-}$ and $CBG^{+/+}$ mice to test aldo-up-regulated, ENaC-mediated $Na^+$ absorption. An Ussing chamber was used to quantify amiloride-sensitive $Na^+$ transport in distal colonic mucosa. The amiloride-sensitive short circuit current ($\Delta$Isc(amil)) was used to quantify the physiological aldo action. Total and free plasma aldo levels were measured by RIA. A low sodium diet (3 weeks) was used to augment endogenous aldo. Q-PCR was used to quantify aldo target gene expression. Results: No functional differences were observed in $\Delta$Isc(amil) or aldo levels in animals on control diet. When $Na^+$ restricted, both genotypes up-regulated the $\Delta$Isc(amil). In $CBG^{+/+}$ up-regulation was 25-fold, contrasting to a much smaller degree (13-fold) in $CBG^{-/-}$ tissue. Interestingly, in $CBG^{+/+}$ mice the associated increase of aldo was only 2-fold (both free and total), whereas the $CBG^{-/-}$ mice showed a 4-fold increase in aldo. In $CBG^{-/-}$ the transcription of $\gamma$-ENaC was blunted. Summary: Despite a dramatic increase of aldo in $CBG^{-/-}$ mice on low $Na^+$ diet, the functional response in the aldo-sensitive distal colon remains markedly insufficient. Our data indicate that CBG is a necessary factor for intact aldo-responsiveness in mouse distal colon.

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**TH-PO629**

**microRNAs Expression Pattern and Regulation by Aldosterone in the Distal Nephron** Juliette Hadchouel,[1,2] Emilie Elvira-Matelot,[1,2] Christelle Soukaseum,[1,2] Celine Latouche,[2,3] Dewi Vernerey,[1,2] Nicolette E. Farman,[2,3] Frederic Jaisser,[2,3] Xavier Jeunemaitre.[1,2] *[1]Paris Cardiovascular Research Center, INSERM UMR 970, Paris, France; [2]University Paris-Descartes, Paris, France; [3]Cordeliers Research Center, INSERM UMR872, Paris, France.*

We previously showed that expression of WNK1, an important regulator of Na+ and K+ transport in the distal nephron, is controlled by miR-192, a microRNA whose expression is inhibited by aldosterone (Elvira-Matelot et al.,JASN,2010). miRs could thus participate in the regulation of renal ion transport by aldosterone.

In order to identify such miRs, we first established the pattern of expression of miRs in the nephron. Mouse Proximal Tubules (PCT/PR), Distal Convoluted Tubules (DCT), Connecting Tubules and Cortical Collecting Ducts (CNT/CCD) were microdissected and the level of expression of 518 murine miRs in each segment was quantified using TaqMan® Rodent microRNA Plates (Applied Biosystems). 357 miRs were expressed in at least one segment. Unsupervised hierarchical clustering analysis nicely separates PCT/PR from the DCT and CNT/CCD. Statistical analysis then showed that 4, 3 and 7 miRs were specifically expressed in the PCT/PR, DCT and CNT/CCD, respectively, while 16 miRs are expressed only in the distal nephron (DCT+CNT/CCD). In addition, 15 and 36 miRs were preferentially expressed in the proximal or distal nephron, respectively.

In a second step, the expression of the same miRs was quantified in the kidney of mice submitted to Na+ depletion, Na+ load or chronic aldosterone infusion. Expression of 50 miRs was modified by either one of these challenges; among those, 4 exhibited a tubular-specific expression pattern. The modification of miR expression observed was either a down-regulation, an up-regulation or a complete inhibition of an expressed miR or an induction of a non-expressed miR.

In conclusion, the proximal nephron differs from the distal nephron in terms of miR expression and miR expression is regulated by sodium intake. Computational identification of potential targets of selected miRs and in vitro validation of the miR-target interaction in mDCT cells would help unravel the roles played by miRs in the regulation of renal ion transport.

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**TH-PO630**

**Aldosterone Inhibits Repressive Histone Lysine Methyltransferases (KMT) and Recruits the Permissive Lysine-Specific Demethylase-1 (LSD1) To Activate $\alpha$-ENaC Transcription in mIMCD3 Cells** Zhiyuan Yu, Qun Kong, Bruce C. Kone. *Medicine, University of Texas-Houston Medical School, Houston, TX.*

Aldosterone increases renal tubular $Na^+$ reabsorption in large part by enhancing $\alpha$-ENaC transcription. We recently reported that a complex containing the histone H3K79 methyltransferase Dot1 and the histone deacetylase Sirt1 associates with and represses the $\alpha$-ENaC promoter in mIMCD3 cells, effects that are reversed by aldosterone (J Biol Chem 284:20917-26, 2009). In other cell types, Sirt1 promotes establishment of the repressive chromatin mark H3K9me3 through its interaction and stimulation of the KMT Suv39H1. We hypothesized that, in addition to augmenting Dot1-mediated hypermethylation of H3K79, Sirt1 facilitates repressive chromatin formation through Suv39H1-mediated hypermethylation of H3K9me3 at the $\alpha$-ENaC promoter under basal conditions. We further hypothesized that aldosterone promotes de-repression of $\alpha$-ENaC transcription by suppressing the actions of Dot1, Sirt1, and Suv39H1, and by recruiting LSD1 to mark active chromatin with H3K9me1. We performed ChIP/qPCR assays for Suv39H1 occupancy and the three H3K9 methylation states at specific subregions of the $\alpha$-ENaC promoter in mIMCD3 cells treated with vehicle or aldosterone for 2h. Under basal conditions, Suv39H1 and H3K9me3 were present in chromatin associated with the same promoter subregions as Dot1a and Sirt1. Aldosterone induced loss of Suv39H1 and H3K9me3 from chromatin at these subregions, as we had shown for Dot1 and Sirt1. Aldosterone also induced LSD1 occupation and a shift to the active chromatin mark H3K9me1 at the $\alpha$-ENaC promoter in mIMCD3 cells. Knockdown or pharmacologic inhibition of LSD1 impaired aldosterone induction of endogenous $\alpha$-ENaC mRNA expression. We conclude that basal repression of $\alpha$-ENaC transcription in mIMCD3 cells involves not only Dot1 and Sirt1 but also Suv39h1-mediated H3K9 trimethylation at the $\alpha$-ENaC promoter. Aldosterone de-represses $\alpha$-ENaC transcription by suppressing Suv39H1, Dot1, and Sirt1, and by recruiting LSD1 to demethylate H3K9 to the $\alpha$-ENaC promoter. These results identify aldosterone as the most versatile epigenetic modifier of any nuclear hormone.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO631**

**Disruption of Putative Transcriptional Regulator AF17 Impairs Na+ Retention and Lowers Blood Pressure in Mice** Wenzheng Zhang,[1,2] Hongyu Wu,[1] Le Huang,[2,] Hejab Ayub.[1] *[1]Internal Medicine, University of Texas Medical School at Houston, Houston, TX; [2]Graduate School of Biomedical Sciences, University of Texas Health Science Center at Houston, Houston, TX; [3]Department of Biochemistry and Molecular Biology, University of Texas Medical School at Houston, Houston, TX; [4]Department of Internal Medicine, Xiangya Hospital, Changsha, Hunan, China.*

We recently reported that AF17 competes with AF9 to bind the same domain of Dot1a and promotes Dot1a nuclear export, leading to H3 K79 hypomethylation, transcriptional activation of several aldosterone regulated genes including $\alpha$, $\beta$, $\gamma$ENaC and their key regulators, and elevated ENaC activity in 293T cells. To solidify and extend these observations to in vivo, we have generated and characterized the first AF17-deficient mice (AF17-/-). On a normal Na+ diet, AF17-/- mice displayed an increased H3 K79 methylation at the $\alpha$ENaC promoter, reduced mRNA and protein expression of ENaC and Sgk1 genes. Functionally, we found that these mice showed an elevated urine volume, urinary Na+ and K+ excretion, serum aldosterone and decreased serum [Na+] coupled with a lower blood pressure than those of wild type mice. These findings suggest that the elevated serum aldosterone level under normal Na+ diet is not high enough to compensate for the AF17 loss to relieve Dot1a-mediated repression of ENaC and Sgk1 gene expression. In contrast, the AF17-/--mediated renal dysfunction and decreased blood pressure were largely attenuated by three means to increase serum aldosterone levels remarkably: a low Na+ diet, a high K+ diet, or aldosterone perfusion. In conclusion, we have provided the first in vivo genetic evidence showing that AF17 functions as a positive regulator of ENaC transcription and Na+ retention through its modulation of Dot1a-catalyzed, aldosterone-sensitive H3 K79 methylation in mouse kidney.

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**TH-PO632**

**Flow Regulation of Endothelin-1 Production in the Collecting Duct** Donald E. Kohan, Brianna Lyon-Roberts, Kevin A. Strait. *Division of Nephrology, University of Utah, Salt Lake City, UT.*

Collecting duct (CD) endothelin-1 (ET-1) is an important autocrine inhibitor of CD Na reabsorption. Salt loading increases CD ET-1 production, however the mechanisms transducing this effect are poorly understood. Tubule fluid flow increases in response to Na loading, hence we studied flow modulation of CD ET-1 production. Three days of a high salt diet in mice increased acutely isolated inner medullary CD ET-1 mRNA expression.

Primary cultures of mouse IMCD detached in response to flow using a closed perfusion chamber, consequently a CD cell line (mpkCCDcl4) was examined. Flow increased ET-1 mRNA in mpkCCD cells at shear stress rates exceeding 1 dyne/cm$^2$, with the maximal effect seen between 2-10 dyne/cm$^2$. Induction of ET-1 mRNA was first evident after 1 hr of flow. Inhibition of calmodulin or dihydropyridine-sensitive calcium channels did not alter the flow response, however chelation of intracellular calcium with BAPTA largely prevented flow-stimulated ET-1 mRNA accumulation. Notably, flow increased intracellular calcium concentration as assessed by Fluo-4 fluorescence. Downregulation of PKC using overnight exposure to PMA, or PKC inhibition with calphostin C, markedly reduced flow-stimulated ET-1 mRNA levels. Flow-stimulated ET-1 synthesis was partially dependent upon sodium delivery since hypertonic NaCl perfusate increased, while absence of perfusate Na (replacing NaCl with choline Cl) decreased, flow-stimulated ET-1 mRNA. Furthermore, amiloride partially reduced the ET-1 response to flow. Initial studies do not support a role for cilia in mediating the flow response since treatment with chloral hydrate for 1 day, a procedure designed to eliminate primary cilia, did not alter flow-stimulated ET-1 synthesis. However, further studies on the role of cilia and related proteins are necessary. In summary, apical flow increases CD ET-1 biosynthesis. This effect is dependent upon intracellular calcium and PKC, and partly relates to changes in apical Na channel Na transport. These studies suggest a novel pathway for coupling alterations in extracellular fluid volume to CD ET-1 production and ultimately control of CD Na reabsorption.

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### TH-PO633

**Epithelial Na Channel Domains That Modulate the Response to Laminar Shear Stress** Tania Abi Antoun, Lindsey Tolino, Thomas R. Kleyman, Marcelo D. Carattino. *Medicine/Renal-Electrolyte, University of Pittsburgh, Pittsburgh, PA.*

The epithelial Na channel (ENaC) mediates the rate-limiting step in Na reabsorption in the distal nephron and plays a key role in volume homeostasis. Na reabsorption and K secretion in this segment of the nephron are modulated by the rate of luminal flow. Four different ENaC subunits have been identified in humans, α, β, γ, and δ. Channels composed of α, β and γ subunits respond to laminar shear stress (LSS) with an increase in open probability. Given that the δ subunit is functionally related to the α subunit while sharing only 37% of sequence identity, we investigated the response of αβδ channels to flow. Both the time course and magnitude of activation of αβδ channels by LLS were remarkably different than those of αβγ channels. ENaC subunits have similar topology with an extracellular domain (ECL) connected by two transmembrane domains (TM1 and TM2) with intracellular N- and C- termini. To identify the specific domains that are responsible for the differential response to flow, we generated a series of α-δ chimeras and studied the parameters of activation by LSS. We found that the region encompassing the TM2 and C-terminus was responsible for the differences in the magnitude and time course of channel activation by LSS.

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### TH-PO634

**Base of the Thumb Region of Epithelial Sodium Channel Modulates the Channel's Response to Shear Stress** Shujie Shi, Marcelo D. Carattino, Thomas R. Kleyman. *Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh, Pittsburgh, PA.*

The epithelial sodium channel (ENaC) has a key role in the regulation of extracellular fluid volume and blood pressure, primarily by mediating $Na^+$ uptake at the apical membrane of principal cells in the distal nephron. Studies in our laboratory and by others suggest that laminar shear stress (LSS) activates ENaC by increasing channel open probability (Po). Based on the recently resolved crystal structure of ASIC1, a member of the ENaC/degenerin family, we hypothesized that the large extracellular region is involved in the regulation of ENaC by mechanical forces. Current studies investigated whether a Tyr residue at the base of the thumb domain of all three ENaC subunits, at a location homologous to a key Trp residue in ASIC1, affects LSS-mediated ENaC gating. Three *alpha* Y418 mutations (Ala, Cys and Trp) significantly enhanced ENaC's response to LSS. Channel activation by LSS was not affected by *beta* Y356 mutations. When the Tyr in *gamma* subunit was substituted with Ala or Cys, the response to LSS was similar to the wild type channel, whereas the *gamma* Y375W mutant exhibited enhanced channel activation. Residues adjacent to Y418 in the *alpha* subunit (L414 to D420) were substituted with Trp, and LSS-mediated channel activation was examined. Each mutant (L414W, G415W, G416W, N417W, G419W and D420W) enhanced the channel's response to LSS. Taken together, our data suggest that the base of the thumb has a role in modulating channel gating in response to external mechanical forces. Our results are consistent with the hypothesis that shear stress induces movement within the periphery of the extracellular regions of ENaC subunits that are transmitted, via the base of the thumb, to the channel's gate residing in the membrane spanning domains.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO635

**TMPRSS4 Activates the Epithelial $Na^+$ Channel by Cleaving the γ Subunit in the Vicinity of the Prostasin-Dependent Cleavage Site** Christopher J. Passero, Gunhild M. Mueller, Mike M. Myerburg, Rebecca P. Hughey, Thomas R. Kleyman. *Department of Medicine, University of Pittsburgh, Pittsburgh, PA.*

The epithelial sodium channel (ENaC) facilitates sodium and fluid transport in the epithelia of the lung and kidney. Proteases modulate ENaC activity by cleaving and releasing inhibitory tracts from the α and γ subunits. Furin, a proprotein convertase residing in the trans-Golgi apparatus, cleaves the α subunit at sites flanking a 26 residue inhibitory tract, but furin only cleaves the γ subunit once. Prostasin, a GPI-anchored serine protease, activates mouse ENaC by cleaving at γRKRK186, releasing a 43 residue inhibitory tract. Prostasin cleavage and activation can be abolished by mutation of the polybasic tract γRKRK186QQQQ. TMPRSS4, a type II transmembrane serine protease, is known to activate ENaC and has previously been shown to cleave the channel at the furin-dependent site of the γ subunit. The question we addressed was whether cleavage distal to the furin site was required to activate the channel. We expressed mouse ENaC with either wild type or mutant (γRKRK186QQQQ) γ subunits in *Xenopus* oocytes with and without co-expression of TMPRSS4. Co-expression of TMPRSS4 increased ENaC currents measured by two-electrode voltage clamping by 1.9 fold. Detection of the surface pool of ENaC containing γ subunits with C-terminal V5 epitope tags identified the generation of a new 70kDa fragment similar to the previously described prostasin-induced cleavage fragment. The mutation γRKRK186QQQQ largely abolished functional activation as well as gamma subunit cleavage by TMPRSS4. The mutation γD172-182RKRK186QQQQ lacks three basic residues (K173, K175, and R177) in addition to the mutations within the polybasic prostasin cleavage site, and completely abolished functional activation by TMPRSS4. Our results show that TMPRSS4 activates furin processed mouse ENaC by cleaving the γ subunit at and/or near the prostasin-dependent cleavage site to release an inhibitory tract. As TMPRSS4 has previously been shown to cleave at the furin-dependent site, it may activate near silent channels by cleaving at sites flanking the inhibitory region.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO636

**Regulation of Epithelial Na Channels by Tissue Kallikrein** Ankit B. Patel,[1,2] Lawrence G. Palmer.[1] *[1]Department of Physiology and Biophysics, Weill Cornell Medical College, New York, NY; [2]Tri-Institutional MD-PhD Program, Weill Cornell/Rockefeller University/Sloan-Kettering Institute, New York, NY.*

Epithelial Na Channels (ENaC) are heterotrimeric proteins formed from three different subunits; αENaC, βENaC, and γENaC. The functional heterotrimer is responsible for the apical entry of Na+ in a number of different epithelia including the renal connecting tubule and cortical collecting duct. Proteolytic cleavage of αENaC and γENaC has been shown to increase channel activity by increasing the open probability (Po) of channels with low Po. A number of different serine proteases including trypsin, furin, elastase, and prostasin are implicated in activating ENaC by cleaving a part of the extracellular domain of γENaC . We tested whether tissue kallikrein, a serine protease secreted by the connecting tubule of the nephron, can also cleave ENaC leading to its activation. When Xenopus oocytes co-expressing the three rat ENaC subunits were exposed to kallikrein from porcine pancreas, there was a 3 fold increase in amiloride sensitive Na+ current within 10 minutes measured by two-electrode voltage clamp (TEVC) that was not further increased by the addition of trypsin. However, this activation was sensitive to soybean trypsin inhibitor (SBTI), suggesting serine proteases other than tissue kallikrein were responsible for ENaC activation. Subsequently, tissue kallikrein purified from rat salivary glands was found to increase ENaC mediated currents 3 fold both in the presence and absence of SBTI. In addition, biotinylation of cell surface γENaC followed by western blot showed oocytes pretreated with rat tissue kallikrein displayed an additional cleaved band of γENaC at the cell surface. Using a specific substrate to measure tissue kallikrein activity, we found the kallikrein activity in the urine of rats fed a high K+ or low Na+ diet was comparable to the kallikrein activity found to activate ENaC in vitro. This suggests tissue kallikrein may act as a physiological regulator of ENaC activity in the kidney.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO637

**Molecular Determinants of Ligand Recognition Specificity of the Novel Plasminogen Receptor, Plg-$R_{KT}$** Caitlin M. Parmer,[1] Nagyung Baik,[2] Lindsey A. Miles,[2] Robert J. Parmer.[3,4] *[1]Yale University, New Haven, CT; [2]The Scripps Research Institute, La Jolla, CA; [3]University of California, San Diego; [4]VA San Diego Healthcare System, La Jolla, CA.*

Activation of plasminogen (Plg) to plasmin by plasminogen activators is markedly promoted when Plg is bound to cells. Recent results suggest that proteolytic processing of ENaC by plasmin plays a key role in ENaC activation in nephrotic syndrome, in which increased Plg levels are present in urine. We recently identified a novel transmembrane Plg receptor, Plg-$R_{KT}$, and have observed prominent expression of Plg-$R_{KT}$ in renal tubular epithelial cells. Here, we investigated the ligand recognition specificity of Plg-$R_{KT}$ for Plg. The Plg-$R_{KT}$ peptide, E139-K147, corresponding to the 9 C-terminal amino acids, was coupled to BSA via addition of an amino terminal cysteine and coated onto microtiter wells. Human Plg was biotinylated and then incubated with the immobilized peptide and detected with streptavidin-HRP. Plg bound to the peptide in a concentration-dependent manner. In specificity controls, biotinylated-Plg binding was competed by unlabeled Plg and no biotinylated-Plg binding to the reverse peptide was detected. Plg binding was inhibited in a dose-dependent manner by the E139-K147 soluble peptide ($IC_{50}$=2 μM), but not by

a mutated peptide E139-K147A. A peptide with the proximal upstream R replaced with K (R142K) was a more effective inhibitor of biotinylated-Plg binding than the wild type peptide. Human Plg also recognized the C-termini of murine, rat, and bovine Plg-$R_{KT}$'s that contain the R142K substitution. Plg domains containing the disulfide-bonded kringle (Kr) structures (Plg has 5 Kr structures that mediate the interaction of Plg with cells) inhibited Plg binding with the following order of effectiveness: Plg>Kr1-3>Kr5>Kr4. In conclusion, the binding of Plg-$R_{KT}$ to Plg requires the presence of a C-terminal lysine and can be modulated by a proximal upstream basic residue. Furthermore, kringle domains of Plg interact with the C-terminus of Plg-$R_{KT}$. Plg-$R_{KT}$ is a structurally unique plasminogen receptor that represents a novel control point for regulating cell surface proteolysis.

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**TH-PO638**

**Regulation of ENaC Function by Oxygen** Russell F. Husted,[1] John B. Stokes.[1,2] *[1]Internal Medicine, University of Iowa, Iowa City, IA; [2]Internal Medicine, VA Medical Center, Iowa City, IA.*

The $PO_2$ of the renal interstitium changes dramatically from the surface of the cortex to the tip of the papilla and regional $PO_2$ can change under several physiological conditions. Using mpkCCDc14 cells as a model we tested the effect of changes in $PO_2$ on ENaC mediated Na transport (JNa). Incubating monolayers in 40% $O_2$ for 24 h produced a 50% increase in JNa; a 95% $O_2$ medium produced no further effect. Reducing $O_2$ to 8% reduced JNa by ~40%. The effect of $O_2$ was not evident for several hours; acute changes in $PO_2$ of the medium had no effect on JNa. Lactate production and medium pH varied inversely with $PO_2$, but changes in medium pH to mimic that produced by changes in $O_2$ produced no change in JNa. In contrast to our hypothesis, changes in $PO_2$ did not change the abundance in hypoxia inducible factors (HIF1a and HIF2a), nor did it change activation of AMP kinase. Supporting the lack of a role of these mechanisms in the oxygen effect, we found that pharmacological inhibition of HIF degradation or activation of AMP kinase did not prevent the oxygen effect on JNa. We also found that oxygen regulation was independent of corticosteroid stimulation of JNa. The effects of steroids were additive to that of oxygen and the gene expression pattern produced by steroids was different from that of oxygen. Notably, steroids increased expression of SGK1, USP2-45, and GILZ but oxygen did not; Slc2a1 (Glut1) and HO-1 were modulated by oxygen but not by steroids. Oxygen modulated the expression of the beta-ENaC subunit but not alpha- or gamma-ENaC. Surface expression of gamma-ENaC was increased by high $PO_2$ and the cleaved form was increased to an even greater extent. Incubation with aprotinin did not provide evidence that the oxygen effect was produced by an increased production of a protease. These results indicate that oxygen can have a potent role in regulating ENaC activity. The majority of its effect appears to be related to altered surface expression of ENaC. The time course of its effect suggests a mechanism involving changes in transcription.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO639**

**Role of the Cortical Actin and Cortactin in Regulation of ENaC Activity** Daria Ilatovskaya,[1,3] Alexey Karpushev,[1] Tengis S. Pavlov,[1] Vladislav Levchenko,[1] Yuri A. Negulyaev,[3] Alexander Staruschenko.[1,2] *[1]Physiology, Medical College of Wisconsin, Milwaukee, WI; [2]Kidney Disease Center, Medical College of Wisconsin, Milwaukee, WI; [3]Institute of Cytology RAS, St. Petersburg, Russian Federation.*

Sodium reabsorption via the epithelial $Na^+$ channel (ENaC) in the ASDN plays a central role in body fluid volume regulation. Coordinated regulation of ENaC activity by changes in actin cytoskeleton dynamics has been shown in several studies. However, how ENaC interacts with the actin cytoskeleton is essentially unknown. First we confirmed that destroying of the actin cytoskeleton with cytochalasin D rapidly increased ENaC activity in freshly isolated rat cortical collecting duct (CCD) and polarized mpkCCD$_{c14}$ principal cells. Furthermore, this is the first report we are aware of describing a mechanism of ENaC regulation by cortical actin-associated protein cortactin. We demonstrated that cortactin is highly expressed in kidney cortex, polarized epithelial mpkCCD$_{c14}$, M-1 and MDCK cells, and is localized to the CCD in Sprague-Dawley rat kidneys. As measured in patch-clamp experiments, co-expression of cortactin with ENaC in CHO cells decreased ENaC activity. Co-immunoprecipitation analysis revealed direct interactions between cortactin and α-ENaC in overexpressed CHO and native mpkCCD$_{c14}$ cells. Similar, fluorescence imaging and quantification techniques showed that cortactin is associated with all three ENaC subunits. Biotinylation experiments and single channel analysis revealed that cortactin decreased ENaC activity via affecting channel open probability ($P_o$). To address the question of what mechanism underlies the action of cortactin on ENaC activity, we assayed the effects of various mutants of cortactin. Only a cortactin mutant unable to bind Arp2/3 complex did not influence ENaC activity. Moreover, novel inhibitor of the Arp2/3 complex CK-0944666 precluded the effect of cortactin. Thus, these results indicate that cortical actin and cortactin are essential for ENaC activity and that Arp2/3 complex is involved in cortactin-mediated decrease of ENaC activity.

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**TH-PO640**

**Effects of CYP P450 Metabolites of Arachidonic Acid on the Epithelial Sodium Channel (ENaC)** Tengis S. Pavlov,[1] Daria Ilatovskaya,[1,2] Richard J. Roman,[3] Alexander Staruschenko.[1] *[1]Physiology, Medical College of Wisconsin, Milwaukee, WI; [2]Institute of Cytology RAS, St. Petersburg, Russian Federation; [3]Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS.*

Sodium reabsorption via ENaC in the ASDN plays a central role in body fluid volume regulation. Previous studies have indicated that arachidonic acid (AA) inhibits $Na^+$ transport in the collecting duct (CD), and that 11,12-EET inhibits ENaC activity. However the mechanism of action of EETs remains to be determined. The goal of this study was to investigate the endogenous metabolism of AA in cultured mpkCCD$_{c14}$ principal cells and the effects of each of these metabolites on ENaC activity. In addition we examined the effects of EETs and co-expression of Cyp2c8 and Cyp4a10 in CHO cells to more specifically study the mechanism of action and discriminate between actions on the channel number and channel open probability (Po). LC/MS/MS analysis in the mpkCCD$_{c14}$ cells identified the presence of 8,9-EET, 11,12-EET, 14,15-EET, 5-HETE, 12/8-HETE, and 15-HETE but not 20-HETE. Single channel analysis revealed that EETs but not HETEs acutely decrease ENaC Po in the mpkCCD$_{c14}$ cells. Interestingly that 8,9-EET and 14,15-EET similar to 11,12-EET modulate channel's activity. Neither 20-HETE nor 5-HETE, 12/8-HETE and 15-HETE had effect on ENaC activity. Co-expression of Cyp2c8 but not Cyp4a10 with all three ENaC subunits in CHO cells significantly decreased ENaC activity in whole-cell experiments. Downregulaion of ENaC activity was PKA-dependent and prevented by myristilated PKI treatment. Furthermore, biotinylation experiments and single channel analysis revealed that the long-term (4hrs) treatment with 11,12-EET decreased the number of channel in the membrane. We conclude that 11,12-EET, 8,9-EET and 14,15-EET are important for maintaining transepithelial $Na^+$ transport and ENaC activity. We hypothesize that EETs have biphasic effect on ENaC activity. Chronic treatment with 11,12-EET inhibits ENaC by decreasing the number of channels through PKA pathway, but acute treatment affect Po.

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Disclosure of Financial Relationships: nothing to disclose

**TH-PO641**

**Novel Role of Rac1/WAVE Signaling Mechanism in Regulation of the Epithelial $Na^+$ Channel (ENaC)** Alexander Staruschenko,[1,2] Alexey Karpushev,[1] Vladislav Levchenko,[1] Tengis S. Pavlov.[1] *[1]Physiology, Medical College of Wisconsin, Milwaukee, WI; [2]Kidney Disease Center, Medical College of Wisconsin, Milwaukee, WI.*

The epithelial $Na^+$ channel (ENaC) is an essential channel responsible for $Na^+$ reabsorption in the ASDN. Consequently, ENaC is a major effector impacting systemic blood volume and pressure. We have shown recently that small GTPase Rac1 increases ENaC activity, whereas Cdc42 fails to change channel activity. Here we tested whether Rac1 signaling plays a physiologic role in modulating ENaC in native tissue and polarized epithelial cells. We have found that Rac1 inhibitor NSC23766 markedly decreased ENaC activity in freshly isolated collecting ducts. Knockdown of Rac1 in native principal cells decreased ENaC-mediated sodium reabsorption and the number of channels at the apical plasma membrane. Members of the Wiskott-Aldrich syndrome protein (WASP) family play central roles in control of the actin cytoskeleton. N-WASP binds Arp2/3 in response to Cdc42, whereas WAVE proteins are effectors of Rac1 activity. N-WASP and all three isoforms of WAVE significantly increased ENaC activity when co-expressed in CHO cells. However, wiskostatin, an inhibitor of N-WASP had no effect on ENaC activity over-expressed in CHO cells and $Na^+$ reabsorption across mpkCCD$_{c14}$ monolayers. Immunoblotting demonstrated the presence of WAVE1 and WAVE2 and absence of N-WASP and WAVE3 in mpkCCD$_{c14}$ and M-1 principal cells. Immunohistochemistry analysis also revealed localization of WAVE1 but not N-WASP in the cortical collecting duct in Sprague-Dawley rat kidneys. Moreover patch clamp analysis revealed that Rac1 and WAVE1/2 are parts of the same signaling with respect to activation of ENaC. Thus, our findings suggest that Rac1 is essential for ENaC activity and regulates the channel via WAVE proteins.

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**TH-PO642**

**A Major Role for Minor Site Phosphorylation in 14-3-3-Mediated Regulation of Nedd4-2 and ENaC** Sindhu Chandran,[1] Rodrigo Alzamora,[2] Kenneth R. Hallows,[2] Vivek Bhalla.[1] *[1]Nephrology, Stanford University, Stanford, CA; [2]Renal-Electrolyte Division, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

The E3 ubiquitin ligase Nedd4-2 inhibits sodium transport through decreased cell surface expression of the epithelial sodium channel (ENaC) in the collecting duct. Several hormonally regulated kinases activate ENaC by phosphorylating Nedd4-2, thereby inducing an inhibitory interaction with 14-3-3 dimers. However, the mechanisms of 14-3-3-mediated Nedd4-2 inhibition are unclear. Our previous work suggests that phosphorylation at Nedd4-2 Ser444 (*Xenopus* numbering), the major 14-3-3 interaction site, is necessary but not sufficient for inhibition of Nedd4-2, and that phosphorylation at minor 14-3-3 interaction sites, Ser338 and Thr363, may play more important roles than previously recognized. By co-immunoprecipitation Nedd4-2 phosphorylation-deficient minor site mutants (S338A,

T363A, and S338A/T363A) bind significantly less 14-3-3e than does wild-type (WT) Nedd4-2 (by 12 ± 2%, 35 ± 17%, and 51 ± 11%, respectively). These mutations did not affect ubiquitin ligase activity by an auto-ubiquitin assay, but inhibited ENaC expression in a cell surface biotinylation assay less well than WT (by 59 ± 8%, 72 ± 5%, and 70 ± 14%, respectively). By pulse-chase analysis, we observed that S444A has a shorter half-life (1.3 h) than WT (2.7 h) or S338A (2.9 h). P446A Nedd4-2, with decreased 14-3-3 binding but comparable Ser444 phosphorylation, also has a shorter half-life than WT (2.0 h). Moreover, we found that dimerization-deficient 14-3-3ε binds Nedd4-2 and inhibits Nedd4-2 function less than WT 14-3-3ε. Although phosphorylation at the "major" site, Ser444, is important for interaction with 14-3-3 dimers, "minor" site phosphorylation at Ser338 or Thr363 by hormonally regulated kinases such as SGK1 or PKA may be the relevant molecular switch that stabilizes Nedd4-2 interaction with 14-3-3 dimers and promotes ENaC cell surface expression. We also propose that enhanced binding to 14-3-3 dimers conferred by phosphorylation at Ser444 promotes Nedd4-2 protein stability in cells, which represents a novel role for 14-3-3 proteins in ENaC regulation.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO643

**Rosiglitazone Increases Epithelial Sodium Channel Activity Via the SGK1/Nedd4-2 Pathway** Ahmed Chraibi, Stephane Renauld, Karine Tremblay, Hugo Garneau, Siham Aitbenichou. *Department of Physiology and Biophysics, Faculty of Medicine and Health Sciences, University of Sherbrooke, Sherbrooke, QC, Canada.*

Thiazolidinediones (TZDs) are peroxisome proliferator-activated receptor gamma (PPARγ) agonists used to treat type II diabetes. Recent studies have shown that TZDs can cause fluid retention and oedema by increasing sodium reabsorption in the renal collecting duct. Those side effects may be caused by the up-regulation of the epithelial Na+ channel (ENaC) and Na+/K+-ATPase. However, the mechanisms involved are not clearly understood. Our goal is to study the effects and the mechanisms involved in rosiglitazone action on the expression and function of ENaC. To do so, we performed two electrode voltage clamp studies (TEVC) in *Xenopus laevis* oocytes expressing PPARγ receptor, wild type (wt) and mutant ENaC channels. We have shown that a 48h treatment with 10 µM RGZ produced a 2-fold increase of ENaC activity and this activation is blocked by GW9662, a PPARγ antagonist. We have also generated a mutation in a potential SGK1 (serum- and glucocorticoid-regulated kinase) binding site in the αENaC subunit and expressed the mutant channel together with the PPARγ receptor in *Xenopus* oocytes. RGZ-induced activation was similar in both wt and mutant channels, suggesting that direct phosphorylation of ENaC by SGK1 is not involved in this regulation. SGK1 is also known to inhibit ENaC internalization through Nedd4-2 phosphorylation and subsequent inactivation. ENaC lacking Nedd4-2 binding motifs (Liddle's mutation) are not stimulated by RGZ. Those results suggest RGZ treatment have increased ENaC expression and activity through Nedd4-2 inhibition mediated by SGK1. In accordance with these results, RGZ increase the activity of ENaC by enhancing its cell surface expression, most probably indirectly, through the increase of SGK1 expression. Western blot analysis and confocal microscopy experiments have confirmed that the RGZ-induced ENaC activity and expression via the SGK1/Nedd4-2 pathway in our model.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO644

**Renal Phosphodiesterase-5 Inhibtion Promotes Natriuresis in Both Virgin and Pregnant Female Rats** Jennifer M. Sasser,[1] Michael H. Humphreys,[2] Christine Baylis.[1] *[1]Physiology and Functional Genomics, University of Florida, Gainesville, FL; [2]Division of Nephrology, University of California San Francisco, San Francisco, CA.*

Pregnancy is characterized by plasma volume expansion and sodium retention due to increased medullary PDE5. Here, we determined if PDE5 inhibition would induce a natriuretic response in virgin and pregnant rats. Anesthetized 16-day pregnant and virgin rats were studied at baseline and during intrarenal (IR) infusion of the PDE5 inhibitor sildenafil (SILD, 0.5 µg/min). The right kidney served as a control. IR SILD increased urine flow rate (UV) and decreased mean arterial pressure (MAP) and renal plasma flow (RPF) in virgin rats (Table), but had no effect on these parameters in pregnant rats. SILD stimulated sodium excretion (UNaV) and fractional excretion of sodium (FENa) in virgin and pregnant rats. We further investigated the regulation of renal PDE5 during pregnancy by isolating kidneys from virgin, postpartum, and pregnant rats on days 6, 12, 16, and 20 of pregnancy. No changes in PDE5 protein abundance (Western blot) were observed in the renal cortex or outer medulla at any point. On day 16, inner medullary PDE5 protein abundance was increased compared to virgin levels (2.2±0.3 vs 1.0±0.1 relative densitometric units, p=0.04). No significant increase in inner medullary PDE5 was observed during early pregnancy, and PDE5 abundance returned to virgin levels by day 20 of pregnancy and postpartum. We conclude that intrarenal PDE5 has a functional role in the regulation of sodium transport in the renal tubule in the female rat and likely plays an important role in permitting the sodium retention and plasma volume expansion required for optimal pregnancy. The role of PDE5 in tubular sodium transport in the non-pregnant state merits further investigation.

Percent change from baseline values during Sildenafil infusion

| | MAP (% of baseline) | UV (% of baseline) | GFR (% of baseline) | RPF (% of baseline) | UNaV (% of baseline) | FENa (% of baseline) |
|---|---|---|---|---|---|---|
| Virgin (n=11) | 91±1* | 153±16* | 104±11 | 86±5* | 331±55* | 327±58* |
| Pregnant (n=9) | 95±1 | 108±8 | 106±9 | 100±13 | 221±25* | 221±28* |

* p<0.05 vs non-infused right kidney

Disclosure of Financial Relationships: nothing to disclose

### TH-PO645

**TBC1D4 (AS160) Is an Aldosterone-Stabilized Protein That Increases ENaC Cell Surface Abundance** Nikolay Gresko,[1] Olivier Staub,[2] Johannes Loffing.[1] *[1]Institute of Anatomy, University of Zurich, Zurich, Switzerland; [2]Pharmacology and Toxicology, University of Lausanne, Lausanne, Vaude, Switzerland.*

Aldosterone stimulates sodium transport in the aldosterone-sensitive distal nephron (ASDN) by increasing the cell surface abundance of the epithelial sodium channel (ENaC). Small Rab-GTPases control the cell surface targeting of ENaC. Here we studied the role of the Rab-GTPase activating protein TBC1D4 (AS160) for aldosterone-dependent ENaC regulation. Immunoblotting and immmunohistochemistry revealed that TBC1D4 is highly abundant in ENaC-positive ASDN cells of mouse kidneys and in a mouse cortical collecting duct (mCCD1) cell line. In mice, dietary sodium restriction (elevating plasma aldosterone) profoundly increased renal TBC1D4 protein, but not mRNA abundances. Consistent, in mCCD cells, aldosterone (1-300 nM for 24h) stimulated the expression of TBC1D4 protein, but not mRNA in a dose-dependent manner. Pre-treatment of the mCCD cells with the translational inhibitor cycloheximide did not block the aldosterone-induced increase of TBC1D4 protein expression, while pre-treatment with proteasomal (MG132) and lysosomal (chloroquine) inhibitors diminished any difference in expression of TBC1D4 protein between control and aldosterone-treated cells. Lentiviral-based, RNAi-mediated "knock-down" of TBC1D4 in mCCD cells profoundly reduced both baseline and aldosterone-stimulated ENaC cell surface activity and abundance as evidenced by amiloride-sensitive short-circuit current measurements and cell surface biotinylation experiments, respectively. Over-expression of wild-type and mutant TBC1D4 forms in ENaC-expressing HEK293 cells confirmed that TBC1D4 increases the cell surface localization of ENaC, which likely depends on an intact TBC1D4 Rab-GTPase domain. Taken together our data suggest that TBC1D4 is an aldosterone-stabilized protein that controls cell surface activity and abundance of ENaC and hence likely contributes to the aldosterone-dependent regulation of sodium reabsorption in the ASDN.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO646

**Inhibition of ENaC by Local Purinergic Signaling as a Mechanism for Aldosterone-Escape** Elena V. Mironova,[1] Vladislav V. Bugay,[1] Timo M. Rieg,[2] Paul Insel,[2] Volker Vallon,[2] Janos Peti-Peterdi,[3] Oleh Pochynyuk,[1] James D. Stockand.[1] *[1]Physiology, UTHSCSA, San Antonio, TX; [2]Pharmacology, UCSD, La Jolla, CA; [3]Physiology, USC, Los Angeles, CA.*

We tested whether the control of ENaC by purinergic tone intrinsic to the distal nephron is involved in aldosterone-escape: the excretion of sodium during high sodium intake despite elevated mineralocorticoids. Urinary ATP concentration increases with dietary sodium intake. Physiological concentrations of ATP decrease ENaC activity in a dose-dependent manner. Increasing sodium intake increases fractional $Na^+$ excretion ($Fe_{Na}$) significantly more in wild-type than in $P2Y_2$ -/- mice, demonstrating impaired renal sodium handling in the latter. To determine the contribution of mineralocorticoid and purinergic regulation of ENaC to salt-sensitive changes in $Fe_{Na}$, we normalized ENaC activity on a high sodium diet to that on a sodium free diet. This gauges how responsive changes in ENaC activity are to changes in sodium intake. Normally, ENaC is responsive because of feedback regulation. Exogenous DOCA or deletion of the $P2Y_2$ receptor each modestly increases the resistance of the channel to changes in sodium intake; together, they markedly increase resistance. ENaC under these latter conditions cannot respond to changes in sodium intake. Due to aldosterone-escape, $Fe_{Na}$ in wild-type mice with high sodium intake is similarly elevated in either the absence or presence of high DOCA. This is not the case in $P2Y_2$ -/- mice, which have lower $Fe_{Na}$ in these conditions. Thus, control of ENaC by purinergic signaling is necessary for complete aldosterone-escape, consistent with a loss of aldosterone-escape in $P2Y_2$ -/- mice contributing to hypertension relative to normal mice in the presence of DOCA and high salt intake.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO647

**Diminished Paracrine Regulation of ENaC by Purinergic Signaling in Mice Lacking Connexin 30** Vladislav V. Bugay,[1] Janos Peti-Peterdi,[2] Elena V. Mironova,[1] James D. Stockand.[1] *[1]Physiology, UTHSCSA, San Antonio, TX; [2]Physiology and Biophysics, University of Southern California, Los Angeles, CA.*

We tested if ATP release through Connexin 30 (Cx30) is part of a local purinergic regulatory system intrinsic to the aldosterone-sensitive distal nephron (ASDN) important for proper control of sodium excretion; if changes in sodium intake influence ATP release via Cx30, and if this allows a normal ENaC response to changes in systemic sodium levels. In addition, we define the consequences of disrupting ATP regulation of ENaC in Cx30 -/- mice. Urinary ATP levels in wild-type mice increase with sodium intake, being lower and less dependent on sodium intake in Cx30 -/- mice. Loss of inhibitory ATP regulation causes ENaC activity to be greater in Cx30 -/- vs. wild-type mice, particularly with high sodium intake. These results from compromised ATP release rather than end-organ resistance: ENaC in Cx30 -/- mice responds to exogenous ATP. Thus, loss of paracrine ATP feedback regulation of ENaC in Cx30 -/- mice disrupts normal responses to changes in sodium intake. Consequently, ENaC is hyperactive in Cx30 -/- mice inappropriately lowering sodium excretion particularly during increases in sodium intake. Clamping mineralocorticoids high in Cx30 -/- mice fed a high sodium diet causes a marked decline

in renal sodium excretion. This is not the case in wild-type mice, which are capable of undergoing aldosterone-escape. It is the loss of the ability of ENaC to respond to changes in sodium levels that causes salt-sensitive hypertension in Cx30 -/- mice.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO648**

**The Complex Nature of P2 Receptor-Mediated Regulation of ENaC: Dependence on Na Concentration, Nucleotide Concentration and Exposure Time, and Tubular pH** Scott S. P. Wildman,[1] Sean G. Brown.[2] *[1]Royal Veterinary College, London, United Kingdom; [2]University of Abertay, Dundee, United Kingdom.*

ENaC controls Na reabsorption along the CD and helps determine systemic BP. We have shown that ENaC activity is inhibited by the activation of luminal $P2Y_{2\ and/or\ 4}$ receptors and $P2X_{4\ and/or\ 4/6}$ receptors when luminal concentrations of $Na^+$ are high (145 mM), and that the $P2X_{4\ and/or\ 6}$ receptors switch to being potentiators of ENaC activity when concentrations of $Na^+$ are lowered (to 50 mM) [1]. Here we address whether other luminal factors can affect P2 receptor-mediated regulation of ENaC.

Sprague Dawley rats (~200 g) were maintained on a low $Na^+$ diet (0.01% NaCl) to increase ENaC expression. Kidneys were removed, microdissected and CCD split-open to expose the apical membrane of cells for whole-cell patch clamp experiments (50 mM $[Na^+]_{ext}$).

When concentrations of P2 receptor agonists (10 µM, applied for 30 s) were high, UTP-evoked currents (i.e. $P2Y_{2\ and/or\ 4}$-mediated) reduced, and 2meSATP-evoked currents (i.e. $P2X_{4\ and/or\ 4/6}$-mediated) increased, the amplitude of subsequent ENaC-mediated currents. Lowering pH from 7.4 to 6.5 did not alter these effects. Further acidification to pH 5.5 increased the degree of inhibition by UTP and abolished the stimulatory effects of 2meSATP-evoked currents. Lowering the concentration of P2 receptor agonists (to 300 nM, applied for 30 s, pH 7.4 and 6.5) caused the inhibitory effect of UTP to be abolished. Interestingly, preincubation with UTP (300 nM, for 5 min, at pH 7.4 and 6.5) led to a significant decrease in ENaC-mediated current amplitude.

These data suggest that P2X receptor-mediated increases in ENaC activity are optimal when luminal $Na^+$ and nucleotide levels are low, and tubular fluid pH is ≥6.5. In contrast, P2Y receptor-mediated decreases in ENaC activity are optimal when luminal nucleotide levels are high and prolonged, and tubular fluid pH is 5.5. Clearly apical P2 receptor and ENaC interactions in the CCD are complex, with an interplay between P2X and P2Y receptors dependent on luminal Na concentration, the concentration of nucleotides, duration of nucleotide exposure and tubular pH.

[1] Wildman et al, (2008) J. Am. Soc. Nephrol. 19:731-42.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO649**

**Renal Sodium Retention in Cholestatic Mice Is Independent of ENaC in CCD** David Mordasini,[1] Johannes Loffing,[2] Edith Hummler,[3] Marc P. Maillard,[1] Michel Burnier,[1] Bruno Vogt.[1] *[1]Service de Néphrologie et Hypertension, CHUV, Lausanne, Vaud, Switzerland; [2]Institute of Anatomy, University of Zurich, Zurich, Switzerland; [3]Département de Pharmacologie et de Toxicologie, University of Lausanne, Lausanne, Vaud, Switzerland.*

The renal site of sodium retention in cirrhosis as well as the transporters involved and the way they are activated, although extensively studied in rats and dogs, is still a matter of debate. Our previous study using the cholestatic mouse model revealed an aldosterone independent stimulation of the basolateral Na+,K+ATPase activity exclusively in the cortical collecting duct (CCD). In order to explore the role of the apical amiloride sensitive sodium channel (ENaC) in the CCD we took advantage of existing CCD specific αENaC KO (Hoxb7 cre; scnn1aloxlox).

Control (CTL) and CCD specific αENaC KO (KO) mice underwent bile duct ligation (BDL) or sham operation. Urinary sodium and potassium excretion was measured every 3 days in metabolic studies over 3-hours period and αENaC expression analyzed by immunofluorescence.

Two to three weeks after BDL, 30% (CTL and KO) mice displayed ascites (~20 ml, BDL(+)) whether the remaining ones did not (BDL(-)). Six groups were distinguished subsequently for analysis: CTL and KO; with sham operation, BDL without ascites (BDL(-)) and BDL with ascites (BDL(+)). At the time of evident ascites the urinary Na/K ratio (mean±SEM) was as follow: CTL-sham 0.9±0.2 (n=6), CTL-BDL(-) 0.8±0.3 (n=7; ns vs CTL-sham), CTL-BDL(+) 0.1±0.1 (n=4; $p<0.05$ vs CTL-sham) and KO-sham 1.3±0.2 (n=6), KO-BDL(-) 1.2±0.3 (n=7; ns vs KO-sham), KO-BDL(+) 0.2±0.1 (n=5; $p<0.01$ vs KO-sham). The differences observed between CTL and KO groups were not significant. Macroscopically kidneys of non ascitic mice are more affected by the disease. Immunofluorescence confirmed cre-mediated deletion of αENaC in the collecting duct of floxed mice. Aside from altered ENaC abundance in CD, no other obvious changes were seen by immunofluorescence between CTL and KO mice.

In conclusion, ENaC activity in the CCD is not required for renal sodium retention in cholestatic mice. The urinary Na/K ratio reflects that aldosterone can be involved in the development of Na+ retention.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO650**

**Chronic Blockade of the Epithelial Sodium Channel (ENaC) Reduces Blood Pressure and Induces Fetal Growth Restriction in the Late Pregnant (LP) Rat** Shyama Masilamani. *Nephrology, VCU / MCV, Richmond, VA.*

A healthy pregnancy requires avid sodium retention and plasma volume expansion (PVE). However, the mechanisms of sodium retention in pregnancy are unknown. We have previously found that in vivo ENaC activity is increased in late pregnancy through a MR mediated mechanism. Further, that the increased ENaC activity maintains PV status. To access the importance of this channel in pregnancy we have chronically blocked ENaC with daily benzamil (0.7 mg/kg, increased to 1.05 mg/kg to account for plasma volume expansion in LP) SC injections from day 12-20 of pregnancy. Compared to normal LP rats, pregnant animals under chronic ENaC blockade have lower gestational body weights (LP = 403 ± 11, LP ChBz = 316 ± 22g, $p<0.05$) and growth restricted pups (LP = 5.08 ± 0.14, LP ChBz = 2.99 ± 0.76g, $p<0.05$). In addition, LP BP is reduced in rats receiving chronic benzamil compared to either virgin rats with chronic ENaC blockade (8 days) or LP animals receiving vehicle (day 12-20) (V ChBz = 102 ± 4, LP = 96 ± 3, LP ChBz = 85 ± 2 mmHg, $p<0.05$), however, heart rate is unchanged (LP = 401 ± 9, LP ChBz = 401 ± 5 BPM). Chronic ENaC blockade in LP animals retain less sodium versus normal LP rats (V = 0.10 ± 0.08, LP = 1.08 ± 0.18, LP ChBz = -0.13 ± 0.48 mmol/day, $p<0.05$). These data indicate ENaC is important for sodium retention, blood pressure regulation, and progression of a healthy pregnancy. (Support: NIH K22 award and CHRB award)

Disclosure of Financial Relationships: nothing to disclose

**TH-PO651**

**Notch3 Deficiency Is Responsible for Increased Natriuresis and Polyuria** Claire Trivin, Dominique Guerrot, Pierre M. Ronco, Nada Boulos, Christos Chatziantoniou, Jean-Claude Dussaule. *Unité 702, INSERM, Paris, France.*

INTRODUCTION: Notch3 is a transmembrane receptor predominantly expressed by vascular smooth muscle cells in mammals. We have recently shown that Notch3 is implicated in the regulation of renal vascular resistance. The mechanisms whereby the kidney regulates natriuresis and urine concentration remain incompletely understood, in spite of important clinical implications. The purpose of this study was to analyze the functional consequences of Notch3 deficiency on water and Na excretion.

METHODS: Notch3 KO mice and control WT littermates were analyzed in metabolic cages. Plasma osmolality, body weight, urine volume, osmolality and Na excretion were measured in basal, water deprivation (WD) and salt restriction conditions. An additional WD test was performed after injection of desmopressin.

RESULTS: In basal conditions, Notch3-deficiency was associated with polyuria (2.4±0.9mL/24h vs 1.4±0.5mL/24h in WT ; $p<0.01$) and reduced urine osmolality (742±185mOsm/kg vs 953±148mOsm/kg ; $p<0.01$). In WD conditions, Notch3 KO mice presented persistent increased diuresis (0.5±0.2mL/9h vs 0.2±0.1mL/9h ; $p<0.01$), reduced urine osmolality (1150±431mOsm/kg vs 1656±499mOsm/kg ; $p<0.01$), increased plasma osmolality (369±9mOsm/kg vs 358±8mOsm/kg ; $p<0.05$) and more important weight loss (-12.1±1.8% vs -10.0±2.0% ; $p<0.01$), consistent with an impaired concentration ability. Differences between KO and WT mice remained after desmopressin-WD test. Na excretion was twofold higher in Notch3 KO mice at baseline. Importantly, increased natriuresis remained during sodium-restriction regimen, with excessive weight loss, indicating primitive renal Na waste.

CONCLUSION: We demonstrate that Notch3-deficient mice present increased Na excretion associated with polyuria. These original results suggest that Notch3 may be an important actor in the regulation of natriuresis and urine concentration. Owing to the importance of these processes in renal and cardiovascular physiology, our data prompt further investigations to uncover the vascular and/or tubular mechanisms whereby Notch3 contributes to the regulation of renal excretion of Na and water.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO652**

**Renal Tubular Salt Reabsorption Is GPR91-Dependent** Anush Gevorgyan, Agnes Prokai, Arnold Sipos, Jahoon Koo, Janos Peti-Peterdi. *Physiology and Biophysics, University of Southern California, Los Angeles, CA.*

The newly identified succinate receptor GPR91 is a detector of local tissue metabolism and is highly expressed in the hypoxic distal nephron and collecting duct. GPR91 signaling involves changes in cytosolic calcium, and activation of mitogen-activated protein kinases ERK1/2 and p38. Succinate via GPR91 causes renin release, activation of the renin-angiotensin system (RAS), and elevations in blood pressure. Since the RAS and the distal nephron are important regulatory mechanisms/sites of body fluid and electrolyte homeostasis, we hypothesized that GPR91 may regulate the expression of salt reabsorbing ion transporters/channels in the nephron. GPR91 wild type (WT) and knockout (GPR91-/-) mice were fed normal or high salt (8%)-containing chow (n=3 each) for two weeks. Immunoblotting of NHE3, NCC, and alpha-ENaC was performed using whole kidney or cortical homogenates. M1 cells, a model of the mouse collecting duct were treated with 1mM succinate for 24h twice a day to assess the direct effects of succinate/GPR91 signaling. Cells treated with 5mM glucose served as controls. The expression of all three proteins, NHE3, NCC, and a-ENaC were significantly decreased in GPR91-/- mice (1.0, 9.8, 0.2, respectively, densitometry values normalized to b-actin) compared to WT (1.3, 15.5, 0.4, respectively). High salt diet significantly reduced the expression of these proteins in all groups with similar differences in WT versus GPR91-/- mice. M1 cells showed a 4-fold increase in a-ENaC levels in response to succinate compared to controls. Consistent with these data, GPR91-/- mice had lower mean arterial blood pressure (94.2±6.7 mmHg)

compared to WT (112.7±9.7 mmHg). These results suggest that GPR91 either directly or via the RAS regulates renal tubular salt reabsorption in the entire nephron and it participates in blood pressure control. GPR91-control of renal salt reabsorption may be important in diabetic and ischemic kidney disease which are associated with increased local tissue succinate levels.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO653**

**GLP1 Receptor Activation by Exendin-4, but Not the Endogenous GLP1 Receptor Tone or DPP-4 Inhibition, Induces Natriuresis and Diuresis in Awake Mice** Volker Vallon,[1] Maria Gerasimova,[1] Daniel J. Drucker,[2] Timo M. Rieg.[1] *[1]Dept of Medicine, UC San Diego and VA San Diego Healthcare System, San Diego, CA; [2]Samuel Lunenfeld Research Institute, University of Toronto, Toronto, Canada.*

The serine protease dipeptidyl peptidase-4 (DPP-4) is an ecto-enzyme that degrades glucagon-like peptide-1 (GLP1), an incretin hormone that stimulates glucose-dependent insulin release. DPP-4 inhibitors (like alogliptin; AGT) and GLP1 receptor (GLP1-R) agonists (like exendin-4, EX4) are novel antidiabetic drugs. The DPP-4/GLP1-R system is also expressed in proximal tubular brush border and may regulate $Na^+$ reabsorption. Here we assessed the role and endogenous tone of the DPP-4/GLP1-R system in renal $Na^+$ handling in awake mice. After emptying the bladder, mice were loaded by oral gavage with isotonic saline (30µl/g; ~30% of daily NaCl intake) and placed in metabolic cages for quantitative urine collection over 3h. First, the responses in urinary fluid, $Na^+$, $K^+$ and $Cl^-$ excretion were not different between wild-type (WT) and littermate gene-targeted mice lacking the GLP1-R (0.23±0.01 vs 0.21±0.01 µl/(min x g); 15±2 vs 14±2, 9±1 vs 9±1, and 23±2 vs 25±2 nmol/(min x g); n=6; NS). Second, application of AGT (10mg/kg in gavage) to WT mice did not significantly change renal excretion of fluid, $Na^+$, $K^+$ or $Cl^-$ vs vehicle (0.23±0.03 vs 0.23±0.04 µl/(min x g); 13±2 vs 15±2, 8±1 vs 9±1, and 22±3 vs 23±2 nmol/(min x g); n=6; NS). Third, application of EX4 (10µg/kg i.p.) to WT mice increased urinary excretion of fluid and $Na^+$, whereas $K^+$ was unchanged vs vehicle (0.33±0.02 vs 0.23±0.02 µl/(min x g); $P<0.001$; 20±1 vs 12±2 nmol/(min x g); $P<0.005$; 8±1 vs 7±2 nmol/(min x g); NS; n=6). This effect on fluid and $Na^+$ excretion was absent in mice lacking GLP1-R (0.16±0.03 vs 0.17±0.03 µl/(min x g); 9±1 vs 11±2 nmol/(min x g); NS; n=5). In summary, GLP1-R knockout or DPP-4 inhibition does not affect the natriuretic and diuretic response in mice being acutely gavaged with isotonic saline, indicating a negligible contribution of the endogenous DPP-4/GLP1 receptor system under these conditions. However, direct activation of the GLP1 receptor by exendin-4 induces diuresis and natriuresis without kaliuresis.

Disclosure of Financial Relationships: Consultancy: Boehringer IngelheimResearch Funding: Bristol-Myers Squibb
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**TH-PO654**

**The Role of EP4 Receptors/MKP-1/P38MAPK Signaling in the Regulation of Renal Tubular Electrolytes Transport** Noritaka Kawada,[1] Toshiki Moriyama,[1] Harumi Kitamura,[1] Yoshitsugu Takabatake,[1] Carolyn M. Ecelbarger,[2] Enyu Imai,[3] William J. Welch,[4] Christopher S. Wilcox,[4] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Division of Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Division of Endocrinology and Metabolism, Georgetown University, Washington, DC; [3]Division of Nephrology, Nagoya University, Aichi, Japan; [4]Center for Hypertension and Kidney, Diabetic and Vascular Diseases and Division of Nephrology and Hypertension, Georgetown University, Washington, DC.*

Background. cAMP has been shown to activate Amirolide sensitive epithelial sodium channel (ENaC) and Na/K-ATPase by promoting the trafficking of these proteins to the cell membrane. In contrast to the prediction that cAMP enhances the sodium absorption, the inhibition of cAMP production by EP4 blockade increases salt absorption in vivo. Therefore, we tested the role of EP4 activation on the renal tubular electrolyte transports. Results. The administration of EP4 agonist (ONO-AE1-329: EP4AG, $6x10^{-10}$mol/gram Body weight in every one hour) in rats had no effects on FENa (Vehicle, 0.036±0.008%; EP4AG, 0.029±0.008%; n=5 each; $p<0.05$), but increased urine/serum creatinine ratio (Veh, 251±22; EP4AG, 594±36; n=5 each; $p<0.05$) and reduced urinary volume (Veh, 840±170µl/2hr; EP4AG, 190±40µl/hr; n=5 each; $p<0.05$), FEK (Veh, 10.1±0.8%; EP4AG, 2.8±0.7%; n=5 each; $p<0.05$) and TTKG (Veh, 7.8±0.5; EP4AG, 3.1±1.0; n=5 each; $p<0.05$). This was accompanied by the induction of MAP kinase phosphatase1 (MKP1) mRNA in distal nephron segments. Both EP4AG ($10^{-6}$M) and adenyalte cyclase activator (Forscolin: $10^{-5}$M) in cultured mice collecting duct (M-1) cells increased the expression of MKP1 protein and mRNA and reduced the phosphorylation of p38, Akt and Sgk1 proteins. M-1 cells exposed to p38 inhibitor (BIRB796: $10^{-5}$M) reduced the expression of phosphorylated Akt and Sgk-1 protein. Conclusions. EP4AG increases water absorption, blunts aldosterone signaling, reduces pottasium excretion, and does not increase sodium absorption in renal tubular cells. Induction of MKP1 in distal nephron segments may play important roles in the renal tubular electrolyte transports.

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**TH-PO655**

**TRPV4 Mediates Shear Stress-Induced Nitric Oxide Production in Thick Ascending Limbs** Pablo D. Cabral, Jeffrey L. Garvin. *Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Hospital, Detroit, MI.*

NO inhibits sodium chloride transport in thick ascending limbs (TALs). We have shown that luminal flow stimulates nitric oxide (NO) production in this segment. Transient receptor potential vanilloid (TRPV) 4 is a Ca2+ permeable cation channel expressed in both luminal and basolateral membranes in TALs. TRPV4 is activated by mechanical stimulation such as flow and cell swelling. In endothelial cells, shear stress-induced NO production is mediated by TRPV4. We hypothesized that the shear stress component of luminal flow induces NO production in TALs and this effect is mediated by TRPV4 activation. We measured NO production in isolated, perfused rat TALs using the fluorescent dye DAF FM. The dye was excited at 488 nm and emitted fluorescence intensities were measured between 495-510 nm. The rate of increase in DAF FM fluorescence reflects NO accumulation. Increasing luminal flow from 0 to 20 nL/min stimulated NO from 21 ± 7 to 58 ± 12 arbitrary units (AU)/min ($p < 0.02$; n=7). Eliminating shear stress by pinching the distal ends of tubules closed prevented the increase in NO (2±8 vs. 8±8 AU/min (n=6). To test the role of TRPV4 we used two different inhibitors. In the presence of the TRPV antagonist ruthenium red (15 uM) increasing luminal flow did not increase NO production (from 18 ± 5 to 16 ± 9 AU/min; n = 4). When luminal flow was increased in the presence of the selective TRPV4 antagonist RN 1734 (10 uM), NO levels did not change significantly (11 ± 7 to 9 ± 2; n = 4) . We concluded that TRPV4 mediates flow-induced NO production in thick ascending limbs.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO656**

**Proximal Tubule Uptake Is Not Involved in the Generation of Urinary Albumin Fragments** Kathrin Weyer, Rikke Nielsen, Pierre J. Verroust, Erik I. Christensen, Henrik Birn. *Department of Anatomy, Aarhus University, Aarhus, Denmark.*

Urinary albumin excretion is an important diagnostic and prognostic marker of renal function. Recent data have shown that animal and human urine contains both immunoreactive albumin and additional immuno-unreactive albumin fragments. Identification of the site of formation of these albumin fragments is of importance for the understanding of their physiological significance and potential use as disease markers. The receptors megalin and cubilin play an important role in normal proximal tubule endocytic recovery of filtered albumin. It has therefore been speculated that the megalin and cubilin mediated endocytic uptake of albumin is crucial to proximal tubule degradation of albumin and that disruption of these receptors will lead to a change in the excretion of albumin fragments. Using targeted, kidney specific knockout mice of megalin and cubilin, we have examined the kidney uptake and urinary excretion of intact albumin as well as albumin fragments by size-exclusion chromatography of urine following injection of $^{125}$I-labeled mouse albumin. Consistent with previous observations, we found that most label in the urine of wildtype mice eluted as small fragments with no detectable intact, labeled albumin. Megalin and cubilin knockout mice exhibited a decreased uptake and degradation of albumin in the kidney as well as an increased excretion of intact albumin, however, no decrease in the excretion of albumin fragments was observed. This demonstrates that the generation of albumin fragments is independent of megalin and cubilin mediated endocytic uptake of albumin in the kidney proximal tubule. Although no degradation of albumin was observed by incubation with plasma or urine, small amounts of labeled albumin fragments were identified in the plasma of injected mice, suggesting that at least a fraction of the labelled albumin fragments found in the urine may result from glomerular filtration.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO657**

**Impaired Proximal Tubular and Kidney Glucose Reabsorption in Gene-Targeted Mice Lacking SGLT1** Volker Vallon,[1] Timo M. Rieg,[1] Robyn A. Cunard,[1] Hermann Koepsell.[2] *[1]Medicine, UC San Diego, San Diego, CA; [2]Institute for Anatomy and Cell Biology, University of Wuerzburg, Wuerzburg, Germany.*

The high-affinity/low-capacity sodium glucose cotransporter SGLT1 (Slc5a1) has been localized in distal parts of the proximal tubule but direct experimental in vivo evidence on its tubular site of action and quantitative contribution is lacking. Here we characterized SGLT1-deficient mice generated by gene targeting. Control wild type (WT) and *Sglt1*-/- mice were fed a glucose-free diet to prevent diarrhea in the latter. Real-time PCR confirmed knockout of *Sglt1* in the kidney of *Sglt1*-/- mice, while the renal mRNA expression of *Sglt2*, *Glut1*, *Glut2*, and *Glut5* were not significantly different compared with WT; similar SGLT2 expression was confirmed at the protein level. Body weight and hematocrit were not different between *Sglt1*-/- and WT mice independent of gender. Spontaneous urine collection in awake adult *Sglt1*-/- mice demonstrated greater urinary glucose concentrations in absolute terms and related to creatinine compared with WT (female: 18.5±1.1 vs. 1.1±0.1 mM and 11.7±0.7 vs. 0.51±0.04 mmol/mmol creatinine; male: 15.2±1.4 vs. 1.0±0.1 mM and 9.3±0.8 vs. 0.44±0.06 mmol/mmol creatinine; n=8-10; all $P<0.001$), in the absence of differences in blood glucose levels. GFR determined by FITC-inulin kinetics in awake mice was similar in *Sglt1*-/- and WT mice (11.1±0.9 vs. 11.6±0.6 µl/(min x g bw); n=9; NS). Renal clearance and free-flow micropuncture experiments under anesthesia revealed similar mean arterial blood pressure and heart rate in *Sglt1*-/- vs. WT mice (79±4 vs. 84±4 mmHg; 470±12 vs. 518±11 $min^{-1}$; n=6-7 mice; *NS*). Absolute and fractional renal excretion

of glucose were greater in *Sglt1*-/- compared to WT mice (1.63±0.31 vs. 0.18±0.04 nmol/(min x g bw) and 3.06±0.25 vs. 0.24±0.03 %; both P<0.001). Moreover, the absolute and fractional delivery of glucose to the last surface loop of proximal convoluted tubules was increased in *Sglt1*-/- compared with WT (3.8±0.4 vs. 1.8±0.3 pmol/min and 5.5±0.6 vs. 2.7±0.5 %; both P<0.001). The results demonstrate a minor but significant contribution of SGLT1 to renal glucose reabsorption in the proximal tubule and the whole kidney.

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Takeda Pharma
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**TH-PO658**

**Expression of SGLT3a in the Kidneys of Zucker Diabetic Fatty (ZDF) Rats** Niloofar Tabatabai,[1] Rajendra Kishore Kothinti,[2] Amy Blodgett.[2] *[1]Medicine, Medical College of Wisconsin, Milwaukee, WI; [2]Chemistry and Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, WI.*

Diabetes may alter normal expression of sodium-dependent glucose transporters, SGLT1 and SGLT2, in the kidney; however, effect of diabetes on expression of SGLT3 has not previously been investigated. At the amino acid sequence level, human(h) SGLT3 is most homologous to hSGLT1; however, hSGLT3 may function as a glucose stimulated sodium channel. While human has one gene encoding SGLT3, mouse(m) and rat(r) each has two genes encoding SGLT3a and SGLT3b. We previously showed that SGLT3a mRNA is expressed in mouse kidney. Here, we investigated whether SGLT3a protein is expressed in the kidneys of mouse and rat, and if its expression is modulated in diabetes. BLAST analysis showed that the amino acid sequence of rSGLT3a is 80% and 92% identical to hSGLT3 and mSGLT3a, respectively. Mouse and rat SGLT3a sequences were aligned, a common sequence was selected to synthesize peptide, which was used as antigen to raise antibody in rabbit. SGLT3a expression was examined by Western blot on kidney proteins from C57BL/6 mouse and Zucker Diabetic Fatty (ZDF) rat, *Lepr*$^{fa}$/Crl, a model of type 2 diabetes. Actin expression was used for normalization. To examine the effect of diabetes, five *fa/fa* rats at each 5, 8, 12, 15, and 19 weeks of age were used. Glucose was measured in overnight urine samples from fasted animals, and expression of SGLT3 in their kidneys was examined. Western blot analysis showed that our SGLT3a antibody reacted with single ~72 kDa protein from mouse and rat kidneys. In ZDF rats, glucose excretion was only 0.02 g/day at 5 weeks of age, but it sharply increased to 9, 16, and 20 g/day in 8, 12, and 15 weeks old diabetic rats, respectively. SGLT3a was expressed in the kidneys of ZDF rats at all ages; however, its relative expression increased by 40% from 5 to 8 weeks of age and remained elevated until 19 weeks of age. Here, we showed for the first time that SGLT3a protein is expressed in the kidneys of mouse and rat, and that its expression level was elevated in glucosuric ZDF rats. Above suggest that SGLT3a expression in the kidney may be enhanced by hyperglycemia.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO659**

**Calcium and Other Polyvalent Cations Inhibit Paracellular Sodium Conductance through the Proximal Tubule Tight Junction Protein, Claudin-2** Alan S. L. Yu,[1] Mary H. Cheng,[2] Rob D. Coalson.[2] *[1]Division of Nephrology, University of Southern California Keck School of Medicine, Los Angeles, CA; [2]Dept. of Chemistry, University of Pittsburgh, Pittsburgh, PA.*

Claudin-2 is a tight junction integral membrane protein that forms cation-selective paracellular pores in the proximal tubule epithelium. Depletion of extracellular Ca (≤ 0.1 mM) is known to cause dissociation of intercellular junctions, endocytosis of tight junction membrane proteins, and profound loss of transepithelial resistance. Here, we tested the effect of moderate changes in extracellular Ca that might be observed in vivo under physiological and pathological conditions. MDCK I cells expressing wild-type claudin-2 under a Tet-responsive inducible promoter were mounted in Ussing chambers for measurement of transepithelial conductance. Moderate increases in Ca (up to 5 mM) caused a rapid ($\tau$ ~10 s), graded and fully reversible inhibition of claudin-2 conductance, while decreases in Ca (down to 0.25 mM) similarly increased conductance. Half-maximal inhibition occurred at 5.5 mM Ca in the presence of 100 mM Na, but at 3.5 mM Ca in the presence of 50 mM Na, suggesting that the two cations interact. Indeed, Brownian dynamics simulations showed that Ca and Na compete for occupancy within the claudin pore. Mutation of D65, which we previously showed to be an intrapore Na-binding site, to asparagine, reduced calcium inhibition by half, indicating that the calcium effect is in part mediated by cation competition for occupancy at this site. Interestingly, other polyvalent cations also inhibited claudin-2 ($La^{3+}$ >> $Ba^{2+}$ = $Ca^{2+}$ > $Mg^{2+}$) suggesting that they may be useful reagents to probe claudin function. It is well known that hypercalcemia induces a profound natriuresis, presumably as a mechanism to prevent calcium nephrolithiasis, and that this occurs in part through inhibition of proximal tubule Na reabsorption (DiBona, G. F. , 1971 Am J Physiol 220, 49-53). Our findings suggest that high luminal Ca would inhibit paracellular Na reabsorption in the proximal tubule, thus providing a potential mechanism to explain this phenomenon.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO660**

**Claudins Are Differentially Controlled in the Kidney by Variations in Sodium and Water Balance** Miguel A. Lanaspa, Ana Andres-Hernando, Carlos Alberto Roncal-Jimenez, Nanxing Li, Christina Cicerchi, Weidong Wang, Tomas Berl. *University of Colorado Denver.*

Claudins are an integrative family of proteins located in the tight junctions of epithelial cells where they regulate paracellular flux of water and ions while maintaining transepithelial resistance. Claudin-2, which renders the membrane permeable to sodium, is highly expressed at isotonic conditions and markedly decreased (by 95%, p<0.01) when tonicity is increased to 550 mOsm/kg$H_2O$ by addition of NaCl to the medium. This decrease is paralleled by a robust increase in claudin-4 which renders the membrane impermeable to sodium (by 3.5-fold, p<0.01). Overexpression of claudin-4 at isotonic conditions leads to degradation of claudin-2 by the lysosome. Conversely, silencing claudin-4 maintains claudin-2 expression in hypertonic conditions reflecting that these claudins are differentially regulated by their expression in the cell during changes in either sodium or tonicity. To discriminate between the effects of high tonicity versus high sodium in claudin-4 expression, we pair-fed rats with 26 g of low sodium (LS) or high sodium (HS, 8 % NaCl) diets. Rats receiving high sodium diets were either restricted or allowed ad libitum access to water. Results: (mean ± SD)

claudin-4 expression and urinary parameters in rats fed with LS and HS diets

| | LS n=4 | HS n=4 | HS n=3 |
|---|---|---|---|
| claudin-4 fold expression | 1 | 5 ± 0.8 | 2 ± 1 |
| Water intake (ml/day) | 40 | 40 | ad libitum, > 70 |
| Urinary osmolality (mOsm/kg) | 1056 ± 150 | 2100 ± 123 | 1275 ± 185 |
| Urinary sodium concentration (meq/L) | < 40 | 415 ± 85 | 259 ± 110 |
| Total sodium excretion (mg/day) | < 10.2 | 81.1 ± 20 | 70.5 ± 20.5 |

We interpret these data to suggest that the regulation of the "tight" claudin-4 is primarily mediated by the concentration of sodium independent in changes of tonicity. This regulation may play a critical role in the control of sodium excretion by determining the sodium permeability at the terminal of collecting duct.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO661**

**The miR-21 Regulates Renal Tubular Epithelial to Mesenchymal Transition in Obstructed Nephropathy** Mingxia Xiong, Yi Fang, Yang Zhou, Ruoyun Tan, Junwei Yang. *Center of Kidney Disease, the 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Chronic kidney disease (CKD) has emerged as a huge threat to public health worldwide. Regardless of the diverse disease process, renal interstitial fibrosis is considered to be a common and irreversible pathway that eventually leads to the end stage renal diseases. Epithelial-mesenchymal transition (EMT) is a major contributor to the pathogenesis of renal fibrosis. Despite a great deal of intense study, comprehensive understanding of the pathogenesis of renal scar formation after injury remains a daunting task that poses a major obstacle toward designing effective therapeutic strategies. microRNAs (miRNAs) are a family of short non-coding RNAs, and have become one of the most fascinating topics in the studies of embryonic development, tumor metastasis, and organ fibrosis in recent years. miRNAs have the potential to play central roles in pathogenesis of kidney diseases. Here, we performed expression profiling of miRNAs in murine interstitial fibrotic kidneys induced by unilateral ureteral obstruction (UUO) and its sham-operated tissue. We found that miR-21 was up-regulated significantly in a time-dependent manner. *In vitro*, treatment of NRK-52E with TGF-β1 increased miR-21 expression. Over expression of miR-21 had similar effects as TGF- β1, whereas, miR-21 inhibitors blocked TGF-β1 induced EMT. Manipulation of the miR-21 level could influence PTEN expression. miR-21 inhibitors blocked TGF-β1 induced phosphoryltion of Akt and chemical blockade of Akt activation could completely block the phenotypic conversion of tubular epithelial cells treated with miR-21 mimic. In summary, we have discovered a mechanism for TGF-β1-mediated EMT that involves cross-talk between miR-21 and PTEN/Akt.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO662**

**MicroRNA-21 Regulates Renal Fibrosis in a Mouse Model of Unilateral Ureteral Obstruction** Abolfazl Zarjou, Anupam Agarwal, Gang Liu. *Medicine, University of Alabama at Birmingham, Birmingham, AL.*

MicroRNAs (miRNAs) are a class of non-coding small RNAs, 22 nt. in length, which bind to the 3' UTR of target genes and thereby repress translation of target genes and/or induces degradation of target gene mRNA. miRNAs play essential roles in numerous cellular and developmental processes. Aberrant expression of miRNAs is closely associated with initiation and progression of pathophysiologic processes including diabetes, cancer, and cardiovascular disease. Whereas evidence suggests that miR-21 plays a central role in cardiac and lung fibrosis, it's role in regulation of renal fibrosis is currently unknown. In this study, we found that the expression of miR-21 is significantly enhanced in the kidneys of mice undergoing unilateral ureteral obstrtruction (UUO) along with E-cadherin and α-SMA. miR-21 increased rapidly after UUO (as early as 6h) and the increase was enhanced at later time points (3 and 7 days after UUO). The enhanced expression of miR-21 was localized in proximal and distal tubule epithelial cells in UUO kidneys by in situ hybridization**.** We found that LNA modified anti-miR-21 probes effectively sequestered endogenous miR-21. Furthermore, the enhanced expression of TGF-β and α-SMA was decreased in UUO kidneys from mice that were given anti-miR-21 probes. The extent of fibrosis was also

significantly decreased with anti-miR-21 treatment. These data suggested that miR-21 plays a role in kidney fibrogenesis after UUO. These results provide novel mechanistic insights and validate miR-21 as a potential therapeutic target in renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO663**

**MicroRNA-155 Functions as a Suppressor of Endothelial to Mesenchymal Transition** Roel Bijkerk,[1] Coen van Solingen,[1] Jacques Duijs,[1] Eric P. Van der Veer,[1] Peter Dijke,[2] Hetty C. de Boer,[1] Ton J. Rabelink,[1] Marie-Jose Goumans,[2] Anton Jan Van Zonneveld.[1] *[1]Dept. of Nephrology and Einthoven Laboratory for Experimental Vascular Medicine, LUMC, Leiden, Netherlands; [2]Dept. of Molecular Cell Biology, LUMC, Leiden, Netherlands.*

Background: Endothelial to mesenchymal transition (EndoMT) is an essential process in embryonic development of the heart valves and involves dedifferentiation of endothelial cells (EC) towards a mesenchymal, pro-fibrotic phenotype. Evidence is accumulating that in renal failure, EndoMT can also occur in the adult and contribute to the loss of microvascular capillaries and the progression of renal fibrosis. MicroRNAs (miRNAs) could serve as a target to prevent EndoMT or reverse this process, as they have been shown to play a key role in cell differentiation.

Methods: We studied immortalized mouse embryonic endothelial cells (MEEC) that undergo EndoMT when stimulated with transforming growth factor-β (TGF-β). To identify differentially expressed miRNAs in EndoMT, we profiled the miRNAs in this model and confirmed differential miRNA-expression by qPCR.

Results: Upon TGF-β stimulation MEECs showed a characteristic morphological alteration towards a mesenchymal phenotype, upregulation of alpha-smooth muscle actin (α-SMA) and down regulation of EC-markers CD31 and MECA32. Using qPCR we also showed decreased expression of the EC-markers eNOS and CD31. In addition TGF-β induced the formation of stress fibers and increased extracellular matrix deposition. Furthermore, hypoxia markedly augmented TGF-β induced EndoMT.

TGF-β upregulated miR-155, which was even more elevated under hypoxic conditions. Blocking miR-155 with antagomir-155 resulted in increased mRNA-expression of its target RhoA and elevated ROS levels, indicating an EndoMT suppressive role for miR-155. Overexpression of miR-155 with synthetic miR-155 precursors resulted in full suppression of EndoMT as evidenced by a lack of change in morphology, decreased upregulation of α-SMA expression and preservation of CD31 expression.

Conclusion: Our data demonstrate a RhoA dependent protective role for miR-155 in EndoMT.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO664**

**Basigin/CD147 Promotes Renal Fibrosis after Unilateral Ureteral Obstruction** Noritoshi Kato,[1] Tomoki Kosugi,[2] Waichi Sato,[2] Takuji Ishimoto,[2] Shoichi Maruyama,[2] Yukio Yuzawa,[3] Kenji Kadomatsu,[4] Seiichi Matsuo.[2] *[1]Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; [2]Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [3]Nephrology, Fujita Health University School of Medicine, Toyoake, Japan; [4]Biochemistry, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

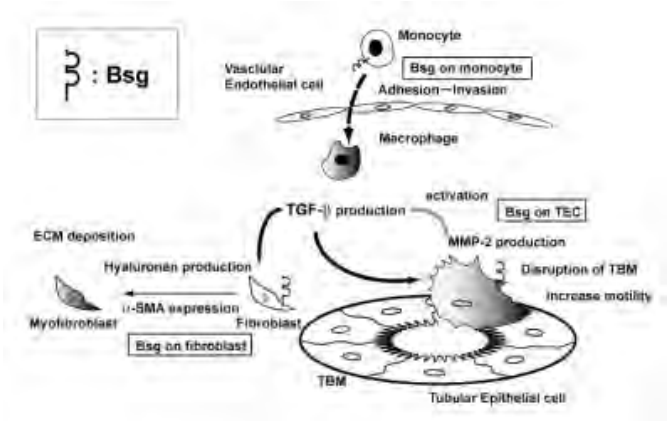
**Background**: Basigin/CD147 is a plasma membrane protein that belongs to the immunoglobulin superfamily. It is said to be a multifunctional molecule. For example, it induces matrix metalloproteinases and hyaluronan, and has a roll in inflammatory cells trafficking. Recently it was revealed that Basigin contributed to induce α-smooth muscle actin in fibroblasts. However, the relationship between Basigin and organ fibrosis has been poorly studied. Here, we investigated Basigin's roll in renal fibrosis using a unilateral ureteral obstruction model.

**Purpose**: To investigate Basigin's roll in renal fibrosis.

**Methods**: Subject Basigin wild and knockout mice to unilateral ureteral obstruction surgery.

**Results**: Basigin-deficient (*Bsg*$^{-/-}$) mice demonstrated significantly less fibrosis after surgery than *Bsg*$^{+/+}$ mice. Fewer macrophages had infiltrated in *Bsg*$^{-/-}$ kidneys. Consistent with these *in vivo* data, primary cultured tubular epithelial cells from *Bsg*$^{-/-}$ mice produced less matrix metalloproteinase and exhibited less motility upon stimulation with TGF-β. Furthermore, *Bsg*$^{-/-}$ embryonic fibroblasts produced less hyaluronan and α-smooth muscle actin after TGF-β stimulation.

**Conclusion**: Basigin has multiple rolls in renal fibrosis. We focused on Basigin's function in different cell types, monocytes, tubular epithelial cells, and fibroblasts respectively. This is the first report which demonstrates that Basigin is a key regulator of renal fibrosis. Basigin could be a candidate target molecule for the prevention of organ fibrosis.




Disclosure of Financial Relationships: nothing to disclose

**TH-PO665**

**Inhibition of Chemokine Receptor Cxcr4 Slows down Renal Tubulointerstitial Fibrosis in a Mouse Model of Unilateral Ureteral Obstruction (UUO)** Amy Yuan,[2] Yashang Lee,[1] Joseph Ueland,[1] Kevin Tang,[2] Erik Shapiro,[2] Gilbert W. Moeckel,[3] Anil K. Karihaloo.[1] *[1]Internal Medicine, Section of Nephrology, Yale University School of Medicine, New Haven, CT; [2]Biomedical Engineering, Yale University, New Haven, CT; [3]Pathology, Yale University School of Medicine, New Haven, CT.*

Renal fibrosis is the consequence of virtually every kidney disease and is characterized by the activation of myofibroblasts and matrix deposition. Multiple studies implicate macrophages, fibrocytes, pericytes and altered tubular epithelial and endothelial cell biology (via EMT) in the process of fibrosis. All these cell types express chemokine receptor Cxcr4. Presence of hypoxia during fibrosis activates Hif1α & Hif2α. Both Cxcr4 and its ligand Sdf-1 are Hif-1 targets. We thus hypothesized increased Cxcr4 and Sdf-1 expression during renal fibrosis. Increased Cxcr4 is associated with enhanced motility and EMT. Since all the cell types listed above express Cxcr4, it could potentially be a target for intervention.

We used mouse UUO model of fibrosis and a known Cxcr4 inhibitor AMD3100 (AMD). Mice underwent UUO ± AMD treatment. Fibrosis was assessed 3, 6 or 12 days post-UUO. RESULTS: UUO led to > 3-fold increase in kidney Sdf1 and Cxcr4 expression by day 3. Saline treated UUO mice developed significant fibrosis by day 6 that worsened by day 12 with major loss of parenchyma, tubular atrophy and matrix deposition. QPCR from UUO kidneys showed upregulation of TGF-β, TNF-α, CTGF, α-SMA, Fsp1, Col-I, pro-apoptotic Bax. Enhacned STAT-3 activation was detected in UUO kidneys. In contrast, AMD treated mice had 40% less fibrosis; significantly less TNF-α, α-SMA, Fsp1, and Col-I with no significant change in TGF-β. Further, reduced Bax (pro-apoptotic) and increased Bcl2 (anti-apoptotic) expression and reduced tubular atrophy, and STAT3 activation, were observed in AMD treated group. FACS and in-vivo MRI imaging, revelaed a 50% decrease in F4/80/Cxcr4+ macrophages and a 60% fewer Cxcr4/CD45/Col-I+ fibrocytes in the UUO kidneys of AMD treated mice. CONCLUSION: These results demonstrate that Cxcr4 inhibitor can slow down fibrosis and may potentially provide novel therapeutic option.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO666**

**Time Sequence of Protease Profile after the Release of Unilateral Ureteral Obstruction in Mice** Muneharu Yamada,[1] Takashi Oda,[1] Taketoshi Kushiyama,[1] Keishi Higashi,[1] Kojiro Yamamoto,[1] Toshitake Hyodo,[1] Naoki Oshima,[1] Yutaka Sakurai,[2] Soichiro Miura,[3] Hiroo Kumagai.[1] *[1]Department of Nephrology, National Defense Medical College; [2]Department of Preventive Medicine and Public Health, National Defense Medical College; [3]Department of Internal Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan.*

The role of proteases, such as plasminogen activator (PA)/plasmin cascade and matrix metalloproteases (MMPs), in the repair of renal fibrosis has yet to be elucidated. In this study we investigated the change in the profile of proteases after the release of unilateral ureteral obstruction (UUO), in order to clarify their role in the process of renal fibrosis repair. UUO was performed on the left kidney with the use of a vascular clamp and the obstruction was released by removing the clamp on day 10 after UUO. Mice were killed and kidney tissues were harvested on days 0, 3, 7, and 21 after release. The expression levels of mRNA of various molecules related with protease activity were analyzed by real time RT-PCR, and the net activities of proteases were assessed by polyacrylamide gel zymography. The mRNA expressions for u-PA, t-PA and plasminogen activator inhibitor-1 were decreased rapidly and constantly over time. On the other hand, mRNA expressions for MMP-2 and MMP-9 were significantly increased gradually from day 0 to day 7 after UUO-release, but were decreased at 21 days after release (MMP-9 mRNA: 0day 1.00 ± 0.39, 3day 1.75 ± 0.35, 7day 2.11 ± 0.44, 21day 0.35 ± 0.14). Similar to the trend of u-PA and t-PA, plasmin activity assessed by casein gel zymography was decreased rapidly and constantly over time after UUO-release (0day 1.00 ± 0.35, 3day 0.41 ± 0.15, 7day 0.29 ± 0.18, 21day 0.23 ± 0.11). On the contrary, MMP-9 activity assessed by gelatin gel zymography showed the sustained activity till day 7 after the UUO-release, and was significantly decreased at 21

days after release (0day 1.00 ± 0.22, 3day 0.91 ± 0.22, 7day 0.94 ± 0.22, 21 day 0.14 ± 0.15). These results suggested that activities of MMPs are up-regulated in the early phase of UUO-release and may contribute to the repair of renal fibrosis by degrading extracellular matrix proteins.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO667**

**MMP-2 Contribute to the Development of Interstitial Fibrosis in Rat UUO Model** Xuanyi Du,[1,2] Yukinari Masuda,[1] Shinya Nagasaka,[1] Emiko Fujita,[1] Akira Shimizu.[1] *[1]Nippon Medical School, Pathology, Bunkyo-ku, Tokyo, Japan; [2]Nephroly, Harbin Medical School, Harbin, China.*

A gelatinase matrix, known as metalloproteinase-2 (MMP-2,) can degrade a broad range of extracellular matrix components like type IV collagen . We investigated the role of MMP-2 on the development of renal interstitial fibrosis. MMP-2 knockout (KO) mice ureter was experimentally obstructed by tying ligature unilaterally and made UUO (unilateral ureteral obstruction) .Wild type (WT) and minocycline treatment (Mino) mice were also made in similar way. Minocycline, an inhibitor of MMPs, was administered at a dose of 30 mg/kg (low dose) and 150 mg/kg (high dose) twice daily intraperitoneally from the day of UUO surgery. All the mice were sacrificed at day 5 and 14 respectively after UUO. Routine pathological staining was carried out to examine the interstitial fibrosis and tubular injury and the expression of MMPs and TIMPs (Tissue inhibitor of metalloproteinase-1) in kidneys were examined. Gelatin zymography and in situ zymography were used to detect the proteolytic activity and location of MMPs and TIMPs. The expression of fibroblast phenotypes and collagen were examined by immunostaining. In WT mice at day 14 after UUO, the renal interstitial fibrosis and tubular injury score developed along with tubular epithelial cell (TEC) expressing fibroblast phenotypes, and more significantly in WT mice than in KO and Mino mice. Zymogrophy results showed both activities of MMP-2 and -9 increased in WT at day 14, without significant differences of activity of MMP-9 between WT and KO mice at day 14. In sizu zymography indicated the gelatin-degrading activity mainly localized at tubular epithelial cells in WT mice. As epithelial-to-mesenchymal transition (EMT) markers, a-SMA, heat shock proteins (HSP-47), vimentin, S100 calcium binding protein A4 (S100A4) significantly expressed in tubular epithelial cells at day 14 after UUO in WT mice than other two groups. In conclusions, MMP-2 accelerated renal fibrosis in the model of UUO possibly through enhanced the promotion of EMT. Our results shows that Inhibition of MMP-2 pathway may provide a novel therapeutic intervention aimed at attenuating progressive renal diseases.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO668**

**The Effects of Apelin on Obstructive Nephropathy** Masashi Nishida, Yasuko Okumura, Kenji Hamaoka. *Department of Pediatric Cardiology and Nephrology, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan.*

Background. Apelin is a vasoactive peptide isolated as a selective endogenous ligand of APJ receptor, which was genetically identified to have closest identity to the angiotensin II type 1 receptor. The effects of apelin/APJ system on the evolution of renal fibrinogenesis remain unclear.

Methods. The mRNA expressions for apelin and APJ receptor in the kidneys were assessed in a mouse model of unilateral ureteral obstruction (UUO). Mice were treated with either apelin or apelin + L-NAME during the early stage (day 0 to 7) after UUO, and the obstructed kidneys were assessed at day 7.

Results. Both apelin and APJ receptor mRNAs are upregulated in the UUO kidney within 7 days following ureteral ligation, with APJ receptor mRNA being increased in a time-dependent manner. Treatment with apelin resulted in an increased endothelial nitric oxide synthase (eNOS) expression, a decreased tubular apoptosis, a decreased myofibroblast accumulation as well as an attenuated renal interstitial fibrosis at day 7 after UUO, although no significant change was observed in the degree of interstitial macrophage infiltration. When mice were treated with a nitric oxide synthase (NOS) inhibitor, L-NAME, concomitantly with apelin, these effects of apelin on the obstructed kidney were all absent, and the degrees of tubular apoptosis, myofibroblast accumulation, renal fibrosis, and interstitial macrophage infiltration were all rather increased compared with the control mice.

Conclusions. Exogenous infusion of apelin ameliorates pathological changes related to renal fibrinogenesis in an early stage of mouse obstructive nephropathy, at least in part, through the NO-dependent mechanism.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO669**

**TRPV4 in Urinary Tract Obstruction** Michael J. Hiatt,[1,2] Larissa Ivanova,[1,2] Douglas G. Matsell.[1,2] *[1]Paediatrics, University of British Columbia, Vancouver, BC, Canada; [2]Child and Family Research Institute, Vancouver, BC, Canada.*

While many of the histopathological features of obstructive nephropathy have been identified using the surgically induced mouse unilateral ureteric obstruction model (UUO), the impact of obstruction on the mechanisms of collecting duct (CD) epithelial injury and repair are poorly understood. The well studied ion channel TRPV4 can be activated by physical stimuli including cellular stretch and is expressed throughout the epithelium of the distal nephron. We hypothesize that TRPV4 modulates the sensing of injury by CD epithelium and its subsequent epithelial repair.

Kidneys from mice sacrificed at 3, 6 or 9 days after surgical ligation or sham manipulation were analyzed via laser capture microdissection, qPCR, western blot, and immunohistochemistry, in vitro cell culture.

Following UUO, CD cells lose their expression of proteins integral to 1) their differentiated phenotypes (vATPase and Aquaporin-2), and 2) maintenance of epithelial integrity (E-Cadherin and beta-Catenin), in parallel with a dramatic increase of alpha-smooth muscle actin positive cells in the surrounding interstitium. In vitro, TRPV4 can be detected at the intercellular membranes of IMCD3 cells cultured on transwell supports. In vivo, TRPV4 is expressed diffusely in CDs of control and sham kidneys, but is more distinctly associated with the basolateral membrane following 3 and 6 days of obstruction. Following 9 days obstruction, expression of TRPV4 is more diffusely expressed in the cytoplasm of epithelial cells, particularly in the outer medulla. qPCR analyses indicate that while inner medullary TRPV4 expression is decreased by >2-fold over the course of the obstruction, expression in the outer medulla is increased by 2 fold at 6 days obstruction and declines toward control levels by 9 days. TRPV4 protein expression is similarly increased at 6 days.

This work identifies the expression and epithelial localization of TRPV4 in obstructive nephropathy. TRPV4 is up-regulated in the early phases of obstruction, but decreases with more prolonged obstruction and injury. These studies implicate TRPV4 in the sensing of CD epithelial injury and in the mechanisms of epithelial repair following obstruction.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO670**

**CXCL16 Mediates Recruitment of Bone Marrow-Derived Fibroblast Precursors in Renal Fibrosis** Gang Chen, Song-Chang Lin, Feixia Dong, Jie Du, William E. Mitch, Yanlin Wang. *Department of Medicine-Nephrology, Baylor College of Medicine, Houston, TX.*

Renal fibrosis is a prominent pathological feature of chronic kidney disease (CKD). Although myofibroblasts are responsible for the production and deposition of the extracellular matrix which define interstitial fibrosis in CKD, the origin of fibroblasts mediating renal fibrosis has been controversial. Recent reports have shown that circulating $CD45^+$ collagen $I^+$ fibroblast precursors termed fibrocytes are involved in the pathogenesis of renal fibrosis. However, the signaling mechanisms accounting for the recruitment of bone marrow-derived fibroblast precursors into the kidney are poorly understood. Using RT-PCR and immunohistochemistry, we found that the expression of the chemokine, CXCL16, is upregulated in the murine model of renal fibrosis induced by unilateral ureteral obstruction (UUO). CXCL16 is a small cytokine belonging to the CXC chemokine family, which exists in two forms. The soluble form generated by its cleavage at the cell surface functions as a chemoattractant to recruit cells. The transmembrane form has a transmembrane structure which functions as an adhesion molecule for CXCR6 expressing cells. Therefore, we examined if CXCL16 could regulate the recruitment of bone marrow-derived fibroblast precursors into the kidney. To test this hypothesis, wild-type (WT) and CXCL16-deficient (CXCL16-KO) mice were subjected to UUO. Flow cytometric analysis revealed that a significant number of bone marrow-derived fibroblast precursors that are dual positive for CD45 or collagen I or CD34 and collagen I accumulated in obstructed kidneys of WT mice, reaching a peak on day 5. The number of infiltrating bone marrow-derived fibroblast precursors was significantly reduced in obstructed kidneys of CXCL16-KO mice. Using real time RT-PCR, immunohistochemistry, and Western blot analysis, we found the expression of collagen type I, fibronectin, and α-SMA was upregulated in obstructed kidneys of WT mice, which was abrogated in obstructed kidneys of CXCL16-KO mice. We conclude that CXCL16 can play a pivotal role in the development of renal fibrosis by recruiting bone marrow-derived fibroblast precursors.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO671**

**Effect of Mast Cell Mediators on Fibroblast Activation: Role in Renal Fibrosis** Randi B. Silver, Arul Veerappan, Albert S. Jung, Rob Janssen, Diane Felsen, Jie Chen, Daniel Phillip Rhoades. *Physiology and Biophysics, Weill Cornell Medical College, NY, NY.*

Evidence suggests an intra-renal RAS plays a role in fibrosis. ANG II-induced TGF-β expression plays a critical role with fibroblasts (FB) the main effector cells but this mechanism has yet to be elucidated. We observe mast cells (MCs) in close proximity to FB in fibrotic regions in UUO kidneys. It is hypothesized that MC mediators (renin-ANG II and histamine) are involved in receptor-mediated FB activation. Freshly isolated rat FB were screened for ANG II AT1R and histamine H1R expression. FB were exposed to exogenous ANG II(100 nM) and histamine (1μM). Cell lysates and supernatants were assayed for TGF-β, prolyl-4-hydroxylase(P4H) and collagen. Exposure to ANG II or histamine caused significant proliferation (ANG II:2480±260 cells/mm3 and Histamine:3571±456; vs Control: 998±118, $p<0.05$) and was inhibited by the ANG II AT1R blocker, EXP3174 and pyrilamine, a H1R blocker (ANG II+EXP3174: 1554±244 cells/mm3, Histamine+pyrilamine:1414±218 $p<0.05$) respectively. Similarly, ANG II and histamine increased supernatant TGF-β (ANG II: 169.1±8.2 pg/μg, Histamine: 147.6±4.5; vs Control: 110.7±3.9, $p<0.01$), and increased P4H immunoexpression and abundance by Western blot two fold. Collagen secretion was enhanced by ANG II or histamine (22.4±0.3 and 29.2±0.9 vs. control 14.7±0.7, $p<0.001$) (ANG II+EXP3174: 15.1±1.4, Histamine+pyrilamine: 18.2±1.5). Our hypothesis was explored in MC-deficient WBB6F1-W/Wv mice(MCD) and their congenic controls WBB6F1-+/+(CC). The FB population/mm2 tissue was elevated in UUO kidneys from MCD (23.8±4.0 UUO MCD, n=3 vs 3.2±0.1 sham-operated, n=3) and CC mice (11.8±0.7 UUO CC, n=3; vs 2.0±0.1 sham-operated, n=3). Unlike the CC mice renal fibrosis was not observed in MCD mice. To determine whether MCD UUO FB were less activated than those from CC mice, UUO kidney FB were immunoscreened for H1R, AT1R, P4H

and α-SMA expression. The receptors were present in MCD and CC FB. P4H and α-SMA were more abundant in CC than MCD kidney FB. Our results show that MC mediators lead to FB proliferation and activation suggesting MCs are coupled to FB and play a role in renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO672

**Lack of Collagen XVIII/Endostatin Exacerbates Interstitial Fibrosis in a Mouse Model of Aristolochic Acid Nephropathy** Ryota Shirai,[1] Takahiro Aoki,[1] Yuki Tokui,[1] Ryota Kimura,[1] Yuki Udagawa,[1] Shiro Ueda,[1] Yoshihiko Ueda,[2] Osamu Yokosuka,[3] Makoto Ogawa,[4] Raghu Kalluri,[5] Yuki Hamano.[4] *[1]Graduate School of Pharmaceutical Sciences, Chiba University, Japan; [2]Dokkyo Medical University Koshigaya Hospital, Japan; [3]Graduate School of Medicine, Chiba University, Japan; [4]Chiba University Hospital, Japan; [5]Beth Israel Deaconess Medical Center and Harvard Medical School.*

**Objective:** Collagen XVIII (Col18) is a component of highly specialized extracellular matrix associated with basement membranes (BM) of epithelia and endothelia. Proteolytic cleavage within its C-terminal domain releases a fragment, endostatin, which has anti-angiogenesis effects. Aristolochic acid nephropathy (AAN) is a rapidly progressive tubulointerstitial nephritis that results in interstitial fibrosis and end-stage renal disease. To investigate the role of Col18/endostatin in the development of interstitial fibrosis, a mouse model of AAN was induced in Col18α1-null (KO) and wild-type (WT) mice.

**Methods:** AA sodium salt was administered into KO and WT mice from C57BL/6J background by intraperitoneal injection. On days 42 and 84, mice were sacrificed for the histological assessment.

**Results:** Upon induction of AAN in WT mice, Col18/endostatin expression was clearly upregulated within the tubular and vascular BM and interstitium. In KO mice with AAN (KO-AA) compared with WT mice with AAN (WT-AA), tubular atrophy and interstitial fibrosis were observed on day 42 and further progressed along with accumulation of collagen IV and perlecan in the interstitium on day 84. KO-AA demonstrated rupture of the tubular and vascular BM. The apoptosis and caspase-3 activation of tubular cells and interstitial endothelial cells (EC) were more observed in KO-AA on day 84. In addition, the expression of CTGF, but not TGF-β1, was upregulated exclusively in caspase-3-positive EC via activation of Smad3 and JNK signaling pathway in KO-AA compared with WT-AA on day 84.

**Conclusion:** Col18/endostatin plays an important role in preserving the integrity of tubular and vascular BM and has a protective effect against tubular damage, loss of peritubular capillaries and interstitial fibrosis in AAN.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO673

**Inhibition of Epithelial-Mesenchymal Transition (EMT) during Renal Fibrosis by an N-Type Calcium Channel Blocker, Cilnidipine** Keiichiro Mishima, Akito Maeshima, Masaaki Miya, Noriyuki Sakurai, Hidekazu Ikeuchi, Takashi Kuroiwa, Keiju Hiromura, Yoshihisa Nojima. *Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.*

By in vivo BrdU labeling, we have recently identified label-retaining cells (LRCs) with renal progenitor-like property in renal tubules of normal rat kidney. We also observed that LRCs migrated into the interstitium and transdifferentiated into myofibroblasts in a rat unilateral ureteral obstruction (UUO) model (JASN 16: 2044-51, 2005). It is known that cilnidipine, an N-type calcium channel blocker (CCB), reduces the renin activity by suppressing the peripheral sympathetic nerve activity, suggesting its renotropic actions similar to angiotensin II receptor blockers (ARBs). In this study, we examined whether cilnidipine has any effects on renal fibrosis.

After BrdU labeling for 1 week and 2 week chase periods, UUO was induced in male Wistar rats. After UUO operation, either cilnidipine or amlodipine was given orally into these rats (3 mg/kg/day each). One or two weeks later, the kidneys were removed for histological analysis. BrdU-positive cells were assessed as LRCs.

Both cilnidipine and amlodipine did not affect mean blood pressure at the dose tested. Consistent with previous study, tubular LRCs migrated into the interstitum of the UUO kidneys. The number of interstitial LRCs was significantly decreased in cilnidipine-treated group but not in amlodipine-treated group compared to that in the untreated group ($4.6 \pm 0.4$ vs. $6.2 \pm 0.7$, cilnidipine vs. none, $p<0.05$). In cilnidipine-treated group, the number of interstitial CD68-positive macrophages was significantly lower than that in amlodipine-treated or untreated group ($7.4 \pm 0.7$ vs. $13.2 \pm 0.9$, cilnidipine vs. none, $p<0.01$). In both cilnidipine-treated and amlodipine-treated groups, fibrotic area (Masson-trichrome staining), the expression of alpha-SMA, the number of interstitial PCNA-positive cells were significantly reduced compared to those in the untreated group.

These data suggest that cilnidipine has a potential to inhibit EMT. Like with ARBs, CCBs might have renoprotective effects on renal fibrosis via multiple mechanisms.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO674

**The Interaction of $\alpha_L\beta_2$ Integrin on Leukocytes and ICAM-1 on Renal Epithelial Cells Accelerates TGF-β Induced Renal Epithelial-Mesanchymal Transition by Activating ERK 1/2 Signaling Pathway** Yoshiyuki Morishita, Eiko Nakazawa, Eiji Kusano. *Nephrology, Jichi Medical University, Shimotuke, Tochigi, Japan.*

The epithelial-mesanchymal transition (EMT) induced by TGF-β has pivotal roles in the development of tubulointerstitial fibrosis. Although infiltration of leukocytes has been detected during tubulointerstitial fibrosis, their physiological roles to EMT remind to clarify. $\alpha_L\beta_2$ integrin is the predominant integrin on leukocytes and plays important roles not only adhesion and migration but also signal transduction by binding its ligands such as ICAM-1. In this study, we investigated the effect of interaction of $\alpha_L\beta_2$ integrin on peripheral blood mononuclear cells(PBMCs) and ICAM-1 on cultured human renal epithelial cells (HK-2 cells and RPTECs) to TGF-β induced EMT. Flow cytometry showed highly expressed ICAM-1 in both HK-2 cells and RPTECs. After TGF-β stimulation (0.1-10ng/ml), chemokines that activate $\alpha_L\beta_2$ integrin such as CXCL12 and CCL20 increased in both HK-2 cells and RPTECs. Then, HK-2 cells or RPTECs after stimulation with TGF-β (0.1ng/m) were co-cultured with PBMCs purified from healthy volunteers. After 24hrs co-culture, flow cytometry using anti-$\alpha_L\beta_2$ integrin mAb (TS1/22) and anti-active form $\alpha_L\beta_2$ integrin mAb (AL-57) showed active form $\alpha_L\beta_2$ integrin on PBMCs increased; however, total $\alpha_L\beta_2$ integrin did not change. Real time PCR and western blotting showed that down-regulation of E-cadherin and vimentin and up-regulation of α-smooth muscle actin and fibronectin by TGF-β stimulation in HK-2 cells and PRTECs were accelerated by co-cultured with PBMCs. These changes of EMT markers were blocked by TS 1/22 and ab20. Western blotting showed that phosphorylation of ERK 1/2 but not smad2, 3 and p38 MAP kinase increased in both HK-2 cells and RPTECs by co-cultured with PBMCs. This increased phosphorylation of ERK 1/2 was blocked by TS1/22 and ab20. In conclusion, the interaction of $\alpha_L\beta_2$ integrin on PBMCs and ICAM-1 on renal epithelial cells accelerated TGF-β induced EMT of renal epithelial cells by activating ERK 1/2 signaling pathway. These results suggested drugs that can block the interaction of $\alpha_L\beta_2$ integrin and ICAM-1 would be a potential option to treat renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO675

**Claudin-2 Expression Alters Cellular Morphogenesis and Cilia Formation** Amar B. Singh,[1] Fnu Bhawana,[1] Raymond C. Harris,[2] Punita Dhawan.[1] *[1]Surgery, Vanderbilt University Medical Center, Nashville, TN; [2]Medicine, Vanderbilt University Medical Center, Nashville, TN.*

Among claudin family members, claudin-2 is unique as its expression in polarized epithelial cells leads to a decrease in transepithelial resistance (TER). Notably, emerging studies suggest that experimental manipulations that lead to junctional remodeling also modulate claudin-2 expression. However, a causal role of claudin-2 expression in the regulation of epithelial morphogenesis remains uninvestigated. In our study, we stably knocked down claudin-2 expression in MDCK II cells (MDCKcl2KD cells) using canine claudin-2 specific shRNA. As predicted, TER in MDCKcl2KD cells increased 3-fold compared to the control cells. However, no obvious differences were observed in the morphology of confluent MDCKcl2KD cells. In contrast, MDCKcl2KD cells showed significant changes compared to the control cells when plated as single cells. In MDCKcl2KD cells, formation of focal complexes, including lamellipodia and filopodia, was markedly decreased during cell spreading ($19\pm2.2$ vs. $47\pm3.4$). This decrease in focal complexes was associated with marked decreases in the tyrosine phosphorylation of FAK and paxillin, proteins important in the regulation of cell spreading. Furthermore, cell migration and cell attachment were decreased by 60% and 40% in MDCKcl2KD cells compared to control cells. Cell scattering in response to HGF, which requires both cell spreading and migration, was significantly inhibited in claudin-2KD cells. Importantly, further investigations showed marked decreases in cilia formation in post-confluent MDCKcl2KD cells compared to the control cells. On the other hand, stable expression of claudin-2 in CHO cells, a fibroblast cell line deficient in claudin expression, resulted in cuboidal epithelial morphology, redistribution of actin along the cortical bundles and marked decreases in Rho and Rac-GTPase activity, suggesting an important role for claudin-2 expression in the regulation of the actin cytoskeleton. In summary, our data suggest an important role for claudin-2 in the regulation of actin cytoskeletal organization and possibly in the establishment of tight junctions.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO676

**Activation of the Epac-Rap Signaling Pathway Reduces Cell Detachment and Renal Failure in Ischemia-Reperfusion Injury** Geurt Stokman,[1] Yu Qin,[1] Emile De Heer,[2] Ingeborg M. Bajema,[2] Bob Water,[1] Leo Price.[1] *[1]Toxicology, LACDR, Leiden University, Leiden, Netherlands; [2]Pathology, LUMC, Leiden University, Leiden, Netherlands.*

Tubular epithelial cell detachment is an early feature of renal ischemia-reperfusion (IR) injury involving loss of cell junction and matrix adhesion. The Epac-Rap pathway is involved in the activation of cell adhesion by promoting integrin activation and formation of cell-cell contacts. Epac1 is expressed by the tubular epithelium. We investigated if selective activation of Epac using the cAMP analogue 8-pCPT-2'-O-Me-cAMP (007) could prevent ischemic renal injury using in vitro and in vivo models. Mouse proximal tubular epithelial cells were used for all in vitro experiments. Activation of Epac-Rap1 by 007 was detected using Rap1-specific pull down analysis. Hypoxia was induced by

mineral oil submersion. Cellular injury and cytoskeletal rearrangement were imaged and quantified using digital image analysis. Immunostainings for Epac1, β-catenin, ZO-1 and paxillin were performed. Knockdown for Epac1 was performed using siRNA. C57BL6 mice were subjected to bilateral ischemia for 25 minutes, 007 or saline were administered intrarenally and animals were sacrificed the next day. Plasma urea levels were determined and tissue sections were stained for β-catenin and clusterin-α.

Stimulation of cells to 007 led to Rap1 activation. Epac activation reduced monolayer disruption and loss of junction and matrix adhesion during hypoxia. Knockdown of Epac1 prevented 007-mediated cell junction stabilization. Intrarenal administration of 007 in mice increased Rap activation. Mice treated with 007 had less renal failure (plasma urea 29.09mM ± 3.014 and 20.39mM ± 1.778 for saline and 007 treated animals respectively, *P=0.02). Treatment with 007 reduced irregular β-catenin and clusterin-α expression during IR injury compared to controls demonstrating that Epac activation prevents cell junction disassembly and reduces cell stress. Our study indicates that protection of tubular epithelial cell adhesion by activation of Epac1 is a novel therapeutic strategy to prevent kidney failure following ischemia or after transplantation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO677

**Interdependence of HIF-1α and TGF-β Signaling in Normoxic and Hypoxic Renal Epithelial Cell Collagen Expression** Susan C. Hubchak,[1] Rajit K. Basu,[2] Paul T. Schumacker,[1] H. William Schnaper.[1] *[1]Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL; [2]Department of Critical Care, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

The transforming growth factor (TGF)-β pathway, acting through the Smad signaling proteins, has been shown to interact with hypoxia-inducible factor (HIF)-1α in models of hypoxic kidney injury. Previously, we showed that TGF-β1 increased HIF-1α transcriptional activity and protein expression in a human proximal tubule cell (HKC) line under normoxia (21% $O_2$) and hypoxia (1.5% $O_2$). Blockade of the Smad3 signaling pathway by TβRI kinase inhibition or transfection with the dominant-negative Smad3A construct reduced TGF-β1-stimulated HIF-1α expression but had no effect on induction of HIF-1α protein by the hypoxia mimetic, desferoxamine mesylate. Here, we describe the effect of HIF-1α on TGF-β1-Smad3 transcriptional activity and collagen expression. Transfection of HKC with a dominant-negative (DN) HIF-1α construct reduces basal Smad3 transcriptional activity 44% and TGF-β1-stimulated activity by 33% over that of empty vector so that TGF-β-stimulated activity in the presence of DN-HIF-1α was only slightly higher than control, basal activity. Similarly, DN-HIF-1α reduces basal COL1A2 reporter activity by about half and TGF-β1-stimulated activity to approximately that of basal control activity. Conversely, overexpressing HIF-1α in normoxic HKC through transfection of a non-degradable mutant increases basal COL1A2 reporter activity 5-fold and TGF-β1-stimulated activity 16-fold over that of empty vector control, significantly increasing fold induction from 2.3 to 3.0x. Finally, pretreatment of HKC with a HIF-1α transcriptional inhibitor, CAY10585, reduces both basal and TGF-β1-stimulated α1(I) collagen mRNA expression. Together, our data suggest that TGF-β1 stimulates cooperation between Smad3 and HIF-1α to promote collagen I expression.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO678

**Prostacyclin Analog ONO-1301 Ameliorates Tubulointerstitial Alterations through Induction of HGF** Tatsuyo Nasu,[1] Yohei Maeshima,[1] Daisuke Saito,[1] Hiroko Yamasaki,[1] Katsuyuki Tanabe,[1] Masaru Kinomura,[1] Hitoshi Sugiyama,[3] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama Univ., Okayama, Japan; [2]Center for Chronic Kidney Disease and Peritoneal Dialysis, Okayama Univ., Okayama, Japan.*

<Background>

Tubulointerstitial injuries are involved in the progression of CKD and predict deterioration of renal function. ONO-1301 is a novel sustained-release prostacyclin analog, possessing thromboxane A2 synthase inhibitory activity. ONO-1301 also up-regulates the production of hepatocyte growth factor (HGF) from fibroblasts. We previously observed the inhibitory effects of slow release ONO-1301 (SR-ONO) on tubulointerstitial injuries in the unilateral ureteral obstruction (UUO) model.

<Purpose>

We aimed to evaluate the potential involvement of HGF in the therapeutic efficacies of SR-ONO to ameliorate tubulointerstitial injuries in the UUO model.

<Method> Female C57/BL6J mice received a single subcutaneous injection of SR-ONO (10 mg/kg) after inducing UUO, and received intraperitoneal injections of rabbit anti-HGF antibody or normal control IgG on Day 1, 3 and 5, and were sacrificed on Day 7.

<Results>

Single injection of SR-ONO could maintain serum concentrations of ONO within therapeutic range for 7 days. SR-ONO treatment suppressed interstitial fibrosis, accumulation of type I and III collagen, monocyte/macrophage infiltration, increase in the levels of TGF-β1, phosphorylation of Smad2/3 and the number of fibroblast specific protein (FSP)1+ fibroblasts in the obstructed kidneys (OBK). The renal levels of HGF were mildly elevated in the control OBK, and SR-ONO further enhanced HGF levels. In vitro, ONO-1301 maintained the expression of E-cadherin and ZO-1 and suppressed the induction of FSP1 and α-SMA in mouse proximal tubular epithelial cells (mProx24) stimulated by TGF-β1. Injections of anti-HGF antibody reversed inhibitory effects of SR-ONO on interstitial fibrosis, accumulation of type I and III collagen and the increase of FSP1+ fibroblasts.

<Conclusion>

These results implicate the therapeutic efficacies of ONO-1301 in ameliorating tubulointerstitial injuries via inducting HGF, which may play a role in counteracting the pro-fibrotic effect of TGF-β1 and suppressing EMT.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO679

**Rat Mesenchymal Stem Cells (RMSCs) Replace Damaged for New Matrix during Remodeling Process: A 6D (Dimensional) Live Cell Model** Jiamin Teng, Elba A. Turbat-Herrera, Guillermo A. Herrera. *Pathology, Nephrocor, Tempe, AZ.*

**Background:** How mesangial matrix is renewed after injury remains poorly understood. The injured matrix is eventually replaced by new matrix with not well defined characteristics as or yet. An in-vitro platform using a 6D live cell model allows to monitor the sequential steps that must take place for mesangial repair to occur.

**Materials and methods:** Rat mesangial cells (RMCs) were cultured on Matrigel loaded glass bottomed multi-wells plates with 10% FBS/RPMI 1640 until confluence. RMCs were then made quiescent by incubating them with 0.5% FBS/RPMI for 48 hours and afterwards incubated with glomerulopathic light chains (GLCs) (10 ug/ml, 48 hours x 2) purified from the urine of renal biopsy-proven light chain deposition disease (LCDD) and AL (light chain-associated) amyloidosis added to the wells every other day. RMSCs were marked with PKH-2 fluorescence dye (green) or with Lysotracker linked to Texas Red (red) and placed later into the wells of GLCs-treated RMCs for up to 2 weeks. MCs were maintained alive and monitored with sequential photos. Fifteen different locations were monitored simultaneously and documented every 15 minutes resulting in a complete appreciation of how the injured mesangium developed and subsequent repair process with emphasis on the characteristics of the new matrix being laid down.

**Results:** The repair process followed a similar sequence of events at all monitored sites but the changes did not necessarily occurred with the same speed. Once RMSCs had cleared the damaged RMCs and affected matrix, they acquired more cytoplasm endowed with organelles normally present in MCs such as bundles of myofilaments and a large number of rough endoplasmic reticulum cisternae, while attachement plaques developed at their surface. The extracellular material that was laid down by these transformed RMSCs showed characteristics typical of the normal mesangium.

**Results:** The RMSCs repopulated the damaged mesangium and laid down mesangial matrix re-establishing mesangial homeostasis. The new matrix recapitulated normal mesangial matrix in composition and overall appearance.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO680

**Transglutaminase Inhibition Reduces Glomerulosclerosis by a Hic-5 Dependent Mechanism in Experimental Diabetic Nephropathy** Nick Hornigold,[1] Timothy Scott Johnson,[2] Andrew F. Mooney.[3] *[1]CRUK Clinical Research Centre, St James's University Hospital, Leeds, West Yorkshire, United Kingdom; [2]Sheffield Kidney Institute, University of Sheffield, Sheffield, South Yorkshire, United Kingdom; [3]Renal Unit, St James's University Hospital, Leeds, West Yorkshire, United Kingdom.*

It has recently been shown that the LIM protein, Hic-5, plays a central role in experimentally induced glomerulosclerosis (KI 2010). In vitro, Hic-5 is upregulated in mesangial cells following attachment to collagen I, and leads to increased susceptibility to apoptosis and adoption of a pro-sclerotic phenotype, including further expression of collagen I. We posited that this same feedback loop exists in vivo.

Streptozotocin-induced diabetic rats underwent unilateral nephrectomy. Some rats were also treated with the transglutaminase (TG-2) inhibitor, NTU281. Rats were sacrificed at days 30, 120 and 240. Serial measurements of proteinuria and serum creatinine were undertaken prior to sacrifice. Tissue sections were immunostained for collagen I, Hic-5 and α-smooth muscle actin (α-SMA). Glomerular expression was semi-quantitatively scored on a 0-5 scale by a blinded observer. Total glomerular cell count and number of apoptotic cells in glomeruli were counted on PAS stained sections.

Results showed that TG-2 inhibition was associated with a significant down-regulation of collagen I expression. This coincided with reduced Hic-5 expression and reduced α-SMA expression, reduced numbers of apoptotic cells and reduced proteinuria and serum creatinine. Hic-5 knock down in mesangial cells in vitro leads to reduced α-SMA expression (by Western blotting) and reduced collagen I precursor transcription (by real time PCR).

These data show that there is, in vivo, a positive feedback loop between collagen I and Hic-5. This leads to a pro-sclerotic phenotype, including increased α-SMA expression, increased collagen I precursor transcription and increased susceptibility to apoptosis. Interrupting this feedback, by NTU281treatment, leads to a major reduction in sclerotic damage to glomeruli. This has important implications for strategies to reduce progressive glomerulosclerosis.

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Disclosure of Financial Relationships: nothing to disclose

**TH-PO681**

**Full-Length Galectin-3 Mediates Polymerization of Hensin That Is Secreted by Clone C Intercalated Cells (ICs): Its Carbohydrate Recognition Domain (CRD) Is Necessary but Not Sufficient** Hu Peng, Soundarapandian Vijayakumar, George J. Schwartz. *Pediatrics, University of Rochester Medical Center, Rochester, NY.*

Hensin is a multifunctional, multidomain protein that is implicated in the regulation of epithelial differentiation and adaptation of the collecting duct to metabolic acidosis. Hensin is secreted as a monomer and then polymerized and deposited in the extracellular matrix (ECM) where it serves to differentiate ICs. To investigate the mechanism of hensin polymerization, we first examined the expression level of galectin-3 in conditioned media from clone C plated at low density (LD, 2 x $10^4$ cells/ml) versus that from cells plated at high density (HD, 5 x $10^5$ ). Galectin-3 was significantly more abundant in HD medium compared to LD medium, suggesting that galectin-3 is responsible for hensin polymerization. Opti-prep density gradient size fractionation demonstrated that LD medium only contained monomeric hensin, whereas HD medium contained oligo- and polymeric hensin. NTA-bound full-length recombinant galectin-3 (1-242 aa) pulled down hensin, and this interaction could be completely blocked by lactose (100 mM) but not by sucrose or mannitose. Incubation of LD medium with recombinant galectin-3 caused polymerization of hensin, similar to that observed in HD medium. Such galectin-3 induced polyermerization could be blocked by lactose (100 mM). The CRD (aa 110-242) alone was unable to polymerize hensin from LD medium. These data suggest that full-length galectin-3 is able to polymerize hensin in vitro. Presumably, the CRD interacts with hensin and the N-terminal domain of galectin-3 induces galectin-3 oligomerization; the oligomerization of galectin-3 brings multiple hensin monomers together, resulting in hensin polymerization.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO682**

**Role of Thioredoxin-Interacting Protein (Txnip) as a Mediator of Tubulointerstitial Fibrosis in Experimental Diabetic Nephropathy** Christina Yan Ru Tan,[1] Weier Qi,[1] Yuan Zhang,[1] Robyn G. Langham,[1,2] Darren J. Kelly.[1] *[1]Medicine, St. Vincent's Hospital, The University of Melbourne, Fitzroy, VIC, Australia; [2]Nephrology, St. Vincent's Hospital, Fitzroy, VIC, Australia.*

**Aim** To examine a potential therapeutic role for targeted Txnip inhibition in experimental diabetic nephropathy (DN) using a Txnip DNAzyme.

**Background** Txnip, an endogenous inhibitor of antioxidant thioredoxin, has been shown to be greatly upregulated by high glucose in microarray studies. It is thought to be a mediator of progressive fibrosis in DN, possibly through increased oxidative stress.

**Methods** Ren-2 rats with streptozotocin-induced diabetes were randomly assigned to receive Txnip DNAzyme or control scrambled DNAzyme delivered by osmotic minipump. Renal injury was assessed by biochemical measures of renal function and histological changes. Renal gene expression of Txnip, Collagen IV, TGFβ and Nitrotyrosine were assessed by RT-PCR, and peptide expression and localization measured by semi-quantitative immunohistochemistry.

**Results** Elevated cortical Txnip mRNA and protein expression in DN rats were shown to be significantly attenuated by Txnip DNAzyme (treated DN 1.24±0.28-fold(mRNA)/3.08 ±0.23%(protein) vs non-treated DN 2.40±0.49-fold(mRNA)/6.21±0.48%(protein), n=8-10, p<0.05). Tubulointerstitial fibrosis and oxidative stress were markedly attenuated in the tubulointerstitium of Txnip DNAzyme treated rats by 1.80- and 1.44-fold, respectively, compared to non-treated DN (n=8-10, p<0.05). Furthermore, collagen IV and TGFβ levels in Txnip DNAzyme-treated rats were significantly reduced by 2.42- and 2.03-fold respectively (n=8-10, p<0.01). The effects of Txnip inhibition were limited to the tubulo-interstitial compartment. No change was observed in the upregulation of glomerular Txnip expression in Txnip DNAzyme treated rats. Similarly, increased glomerulosclerosis, indicated by glomerulosclerotic index, noted in DN rats was not altered by Txnip DNAzyme (treated DN 1.59±0.09 vs non-treated DN 1.63±0.09, n=8-10, p>0.05).

**Conclusions** This study supports the role of Txnip as an important mediator of progressive tubulointerstitial fibrosis in DN, further highlighting Txnip as a potential new therapeutic target for DN.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO683**

**α2β1 Integrin-Mediated Signaling Induces Expression of Intercellular Adhesion Molecule-1 Expression on Human Mesangial Cells and Leukocyte Adhesion** Masahito Tamura, Narutoshi Kabashima, Ryota Serino, Tatsuya Shibata, Tetsu Miyamoto, Yumi Furuno, Junichi Nakamata, Yoko Fujimoto, Emi Hasegawa, Yutaka Otsuji. *Kidney Center, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan.*

Integrins are major adhesion receptors that regulate cytoskeletal organization, and trigger a variety of signal transduction pathways and specific gene expression. We investigated the effects of extracellular matrix (ECM) accumulation on the expression of cell adhesion molecules involved in the progression of renal diseases. Quiescent cultured human mesangial cells (MCs) abundantly expressed cell surface molecules such as α2-5 and β1 integrins, CD44, and weakly α6, αv, and β3 integrins, intercellular adhesion molecule (ICAM)-1, and Fas. Stimulation of β1 integrin using a specific monoclonal antibody induced time-dependent expression of ICAM-1 on MCs. Cell adhesion to ECM proteins, e.g., type I and III collagens with affinity for α2β1 integrin, effectively enhanced ICAM-1 expression, while type IV collagen, fibronectin, or laminin without affinity for α2β1 integrin had no effect on ICAM-1 expression. Furthermore, pretreatment of cells with anti-α2 integrin inhibitory antibody completely inhibited β1 integrin- or type I or III collagen-induced expression of ICAM-1. β1 integrin increased phosphorylation of mitogen-activated protein or extracellular signal-related kinase (MEK) 1/2 and protein kinase C (PKC), and pretreatment of cells with a MEK1/2 inhibitor, PD98059, tyrosin kinase inhibitor, genistein, or PKC inhibitor, calphostin C, suppressed levels of β1 integrin-induced ICAM-1 expression. Up-regulation of ICAM-1 expression was accompanied by increased cell adhesions between mesangial cells and leukocytes, which was completely suppressed by anti-ICAM-1 antibody pretreatment. These results indicate that stimulation of α2β1 integrin induces ICAM-1 expression through MEK1/2 and PKC, and suggest that accumulation of pathological ECM components such as type I and III collagens might play a role in the progression of glomerular diseases by enhancing ICAM-1 expression and leukocyte adhesion to mesangial cells.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO684**

**Interleukin 17 – A New Actor in Acute and Chronic Kidney Injury** Tanja Loof, Stephanie Kraemer, Hans-Hellmut Neumayer, Harm Peters. *Nephrology, Charité Universitätsmedizin Berlin, Berlin, Germany.*

**Purpose**

Overexpression of pro-fibrotic and pro-inflammatory cytokines, such as TGF-ß and IL-6, and extracellular matrix accumulation are hallmarks of acute and chronic glomerular injury. A functional interaction between TGF-ß signaling and the inflammatory cytokine IL-17 has recently been proposed. This study analyzed the expression patterns of TGF-ß and IL-17 in rat models of acute anti-Thy1 glomerulonephritis (aGN), in streptozotozin-induced diabetic nephropathy (STZ), hypertensive nephropathy (HN) and chronic anti-Thy1 glomerulosclerosis (cGs).

**Methods**

Materials were harvested at the following points: d0.5/d1 (injury), d5/d10 (matrix expansion) and d15/d20 (resolution) for aGN (induced by OX-7-injection in Wistar rats) and week 12 for STZ (i.p. streptozotocin-injected male spontaneously hypertensive-stroke-prone rats), HN (uninephrectomy, followed by a 2/3 nephectomy 2 weeks later, Wistar rats) and cGs (uninephrectomy and i.v. OX-7-injection in Wistar rats), respectively.

**Results**

Induction of aGN was characterized by marked proteinuria (d5 113±12 mg/d; 3.9-fold vs. con; p<0.001) and histological glomerular matrix accumulation (d5 +3-fold vs. con; p<0.001), in parallel with highest glomerular TGF-ß1 and IL-17 mRNA-expression (+2.25-fold; +6.50-fold vs. con; p<0.05). STZ, HN, cGs showed significantly increased proteinuria at week 12 (STZ 4.5-fold; HN; 26.0-fold; cGs 17.4-fold vs. con; all p<0.01). In the kidneys, glomerular TGF-ß1 and IL-17 protein expressions were highly up-regulated in diseased rats compared to controls (STZ: TGF-ß1 7.3-fold; IL-17 4.7-fold; HN: TGF-ß1 7.9-fold; IL-17 25.0-fold, cGs: TGF-ß1 2.5-fold; IL-17 14.2-fold; all p<0.001).

**Conclusion**

This study documents a marked up-regulation of the new pro-inflammatory cytokine IL-17 in acute anti-Thy1 glomerulonephritis. IL-17 is constitutively expressed in glomerular cells and highly induced under inflammatory conditions. In STZ, HN and cGs rats, IL-17 and TGF-ß are co-expressed by glomerular cells.The results points an new regulatory pathway between inflammation and fibrosis in the pathogenesis of acute and chronic kidney diseases.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO685**

**Inhibitors of Mitochondrial Respiration Inhibit TGF-Induced Upregulation of PAI-1 and Fibronectin Expression in Renal Cells through Upregulation of SMAD7 and TGIF** Jens Gaedeke, Hans-Hellmut Neumayer, Harm Peters. *Nephrology, Charité, Campus Mitte, Berlin, Germany.*

The anti-diabetic drugs metformin and phenformin have been shown to inhibit PAI-1 secretion in liver cells. Inhibition of mitochondrial respiratory chain and upregulation of AMP-activated kinase (AMPK) activity has been shown as one of the cellular target of these drugs. Here we analyzed the effect of established inhibitors of mitochondrial oxidative phosphorylation (OxPhos) (rotenone, oligomycin, D942) and of ciglitazone and troglitazone, also haracterized as inhibitors of mitochondrial function, on cellular signaling pathways and TGF effects in renal fibroblasts and tubular epithelial cells. Rat renal fibroblasts (NRK49F) and tubular epithelial cells (NRK52E) were treated with various inhibitors at non-toxic doses and TGF effects were analyzed by western blotting of FN and PAI-1. Signaling pathways were analyzed by western blotting. SMAD7 was analyzed by RT-PCR, TGIF expression by western blotting. D942 and oligomycin, but not rotenone activated AMPK and blocked TGF Induced PAI-1 and FN. Blockade of TGF effects was associated with an upregulation of SMAD7 mRNA in both tubular cells and renal fibroblasts and an upregulation of the transcriptional repressor TGIF protein in tubular cells. Blockade of intracellular calcium with BAPTA-AM abolished these effects.

Treatment of cells with ciglitazone and troglitazone similarly activated AMPK and blocked TGF effects, eventhough no PPARgamma was detected. Exchange of glucose in the medium for galactose (which forces the cell to use only mitochondrial OxPhos to generate ATP) leads to increased baseline AMPK activation, reduced TGF effects and increased toxicity of both ciglotazone and troglitazone, whereas high glucose had opposite effects.

Our data show that mild inhibition of mitochondrial function followed by activation of AMP-kinase leads to inhibition of TGF effects. This is associated with increased expression of the TGF-beta antagonists SMAD7 and TGIF in a calcium-dependent pathway.

Our data suggest that the functional status of mitochondria is involved in regulating TGF signalling in renal cells though modulation of SMAD7 and TGIF.

Disclosure of Financial Relationships: Honoraria: Shire.

**TH-PO686**

**Combining Renin Receptor Inhibition and Angiotensin II Blockade Results in Enhanced Antifibrotic Effect in Experimental Glomerulonephritis** Jiandong Zhang,[1,2] Wayne Border,[1] Nancy Noble,[1] Yufeng Huang.[1] *[1]Fibrosis Research Laboratory, University of Utah, Salt Lake City, UT; [2]Institute of Nephrology, Southeast University, Nanjing, Jiangsu, China.*

Recent evidence suggesting a receptor-mediated profibrotic action of renin independent of angiotensin generation and action led us to hypothesize that combining renin receptor inhibition and Ang II blockade would enhance antifibrotic effect. Using the anti-Thy-1 model of glomerulonephritis,the maximally effective dose of enalaprilate was determined and compared with the effect of silencing renin receptor. Then, both treatments at maximal doses were combined. After disease induction with OX-7, intact glomeruli from normal and nephritic rats were isolated at d4 and put in culture at 5000 gloms/2ml medium with 2.5% FBS for 48h. The supernatant was harvested for TGFß1 and FN ELISA assay and glomeruli were harvested for mRNA analysis by real time RT/PCR. Enalaprilate dose-dependently reduced markers of fibrosis, with 10-4M showing maximal effects, reducing mRNA of FN 65%, PAI-1 56% and TGFß1 49%, and production of proteins fibronectin 60%, and TGFß1 49%,. Glomerular renin mRNA and activities and Ang II levels were increased in untreated nephritic glomeruli 2-,4-and 6.8-fold, respectively vs. normal glomeruli. Administration of 10-4M enalaprilate reduced Ang II generation 65% and resulted in 2- and 10-fold further increases in diseased glomerular renin mRNA expression and activity, respectively but had no effect on glomerular renin receptor expression. Depressing the renin receptor with siRNA by 80% in cultured nephritic glomeruli reduced fibrotic markers comparably to enalapril alone but had no effect on glomerular renin expression. However, combination therapy led to a further reduction in disease markers. Notably, elevated TGFß1 and FN production were reduced 73% and 84%, respectively. These results support our hypothesis that adding renin receptor inhibition to Ang II blockade in patients may have therapeutic potential. These results also indicate that the limited effectiveness of Ang II blockade may be due, at least in part, to the elevated renin levels they induce and the receptor-mediated, profibrotic action of renin.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO687**

**Oncostatin M Inhibits Basal and TGF-β1-Induced Expression of Matricellular Proteins in Human Kidney-2 Cells** Rita Sarkozi, Christine M. Hauser, Susie-Jane Noppert, Michael Rudnicki, Andreas Kronbichler, Markus Pirklbauer, Gert J. Mayer, Herbert Schramek. *Department of Internal Medicine IV, Nephrology and Hypertension, Innsbruck Medical University, Innsbruck, Austria.*

Matricellular proteins (MP) are non-structural proteins that are secreted and sequestered in the extracellular matrix. They modulate cell function by interacting with cytokines, growth factors, receptors and extracellular matrix proteins. Microarray analysis revealed an inhibitory effect of oncostatin M (OSM) on the expression of four MP in proximal tubular human kidney-2 (HK-2) cells. As MP expression is increased in renal tubulointerstitial fibrosis, we decided to study it in HK-2 cells stimulated with the pro-fibrotic cytokine transforming growth factor-ß1 (TGF-ß1) when compared with the multifunctional cytokine OSM.

Real-time PCR analysis of HK-2 cells stimulated with 10 ng/ml TGF-ß1 revealed a time-dependent induction of connective tissue growth factor (CTGF), thrombospondin-1 (TSP1), tenascin-C (TNC), and SPARC (secreted protein, acidic and rich in cystein). In contrast, 10 ng/ml OSM did not only inhibit basal mRNA expression of all four MP after 12 h and 24 h of incubation but also almost abolished TGF-ß1-induced MP mRNA expression. This inhibitory OSM effect did not depend on the sequence of ligand administration. Simultaneous addition of TGF-ß1 and OSM blocked TGF-ß1-mediated induction of all four MP after 24 h. Identical results were obtained when OSM was added 5 min and 1 h prior or past TGF-ß1 administration. For the CTGF gene these results were verified by Western blot analysis. 24 h incubation with an inhibitor of DNA methyltransferases, 5-aza-2'-deoxycytidine (2.5 µM), increased basal and TGF-ß1-stimulated CTGF mRNA expression but hardly attenuated OSM's inhibitory effect on CTGF expression.

We conclude that TGF-ß1 is a potent stimulator of CTGF, TSP1, TNC and SPARC in HK-2 cells, while OSM inhibits basal and TGF-ß1-induced mRNA expression of these MP. Inhibition of DNA methylation further stimulates CTGF mRNA expression without major effects on the inhibitory action of OSM. Hence OSM may fulfill an anti-fibrotic function by blocking MP expression in human proximal tubular cells.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO688**

**Lymphangiogenesis Develops during Tubulo-Interstitial Fibrosis Via the TGF-β–VEGF-C Pathway in Rat Unilateral Ureteral Obstruction** Yasuhiro Suzuki,[1] Yasuhiko Ito,[1] Masashi Mizuno,[1] Akiho Sawai,[1] Hiroshi Kinashi,[1] Waichi Sato,[1] Shoichi Maruyama,[1] Enyu Imai,[1] Yoshifumi Takei,[2] Seiichi Matsuo.[1] *[1]Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Department of Biochemistry, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

**Backgroud and Purpose** We recently reported that lymphangiogenesis was observed in tubulo-interstitial lesions and was correlated with fibrosis. However, endogenous TGF-β was reported to inhibit lymphangiogenesis. In order to clarify the mechanisms of lymphangiogenesis in renal fibrosis, we studied the interaction between lymphangiogenesis and fibrosis in rat unilateral ureteral obstruction (UUO) model and in cultured cells. **Methods** We analyzed inflammation (ED1 positive macrophage), fibrosis (α-SMA, typeIII collagen), lymphangiogenesis (LYVE-1, podoplanin) and growth factors (TGF-β and VEGF-C) in UUO model by RT-PCR and immunohistochemistry. In addition, we investigated interaction between TGF-β1 and VEGF-C in cultured proximal tubular epithelial cells (HK-2), collecting tubules (M-1), macrophages (RAW264.7) and fibroblasts (NRK49F). **Results** In UUO, a strong infiltration of macrophages preceded an increase of α-SMA, typeIII collagen expression, and growth of lymphatics. VEGF-C, detected in tubules and mononuclear cells, gradually increased and peaked at Day 14. The kinetics and localization of VEGF-C were similar to those of TGF-β and the expression of these growth factors and lymphangiogenesis were linked with fibrosis rather than inflammation. Ten min after the administration of 3% Evansblue into the extended ureter, dye was excreted into the hilar lymphatics of the obstructed kidney. In vitro, VEGF-C expression was upregulated by TGF-β1 in HK-2, M-1 and RAW264.7, but not in NRK49F. Upregulation of VEGF-C was completely suppressed by TGF-β typeI receptor inhibitor. **Discussion** VEGF-C induction by TGF-β might be a key pathway that leads to lymphangiogenesis in fibrosis. Lymphatics might contribute to drain the urine from the dilated pelvis. **Conclusion** In tubulo-interstitial fibrosis of UUO, lymphangiogenesis develops in the tubulo-inerstitial fibrosis through TGF-β-VEGF-C pathway.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO689**

**Protein Kinase C Delta Inhibitor, Rottlerin, Blocks TGFβ-Stimulated Epithelial-Mesenchymal Transition in Renal Tubular Epithelial Cells** Xiaoying Liu, Constance Runyan, H. William Schnaper. *Division of Kidney Diseases, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

TGFβ signaling plays an important and complex role in renal fibrogenesis. Our laboratory has studied how non-canonical signaling pathways interact with and modulate classical Smad signaling. Previously, we defined a role for protein kinase C delta (PKCδ) modulation of Smad3 signaling in TGFβ1-induced, human mesangial cell collagen expression. Here, we investigated a role for PKCδ in renal epithelial cell fibrogenesis, examining its effect on TGFβ-stimulated phenotypic changes associated with epithelial-mesenchymal transition (EMT). We characterized EMT in our cells by increased collagen I and smooth muscle α-actin expression, and decreased E-cadherin expression. Treatment of a human kidney proximal tubular cell line (HKC) with rottlerin, a PKCδ-specific inhibitor, blocked the ability of TGFβ to induce these changes at both the RNA and protein level. Previous work from our lab has demonstrated an antagonism of EMT by Smad anchor for receptor activation (SARA), and determined that TGFβ-induced reduction in SARA expression is an important contributor to EMT progression. Here, we found that rottlerin inhibited the TGFβ-mediated decrease in SARA. However, rottlerin did not inhibit phosphorylation of Smad2 or Smad3 by TGFβ. Thus, rottlerin does not affect EMT-like changes by an effect on receptor-mediated Smad phosphorylation. Instead, our data suggest that PKCδ may play a role in TGFβ-stimulated EMT by regulating SARA expression.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO690**

**SMAD3 Knockout Mice Are Protected Against Renal Damage in a 2-Kidney, 1-Clip Model of Renovascular Hypertension** Joseph P. Grande,[1,2] Gina M. Warner,[1] Bruce Knudsen,[1] Jingfei Cheng,[1] Justin E. Juskewitch,[1] Catherine Gray,[1] Lilach O. Lerman,[2] Stephen C. Textor,[2] Karl A. Nath,[2] Karen R. Lien.[1] *[1]Laboratory Medicine & Pathology, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic, Rochester, MN.*

The two-kidney, one-clip model of renovascular hypertension is associated with interstitial fibrosis and tubular atrophy (IF/TA) in the clipped kidney and compensatory hyperplasia in the contralateral kidney. We have previously demonstrated that the development of IF/TA in the clipped kidney is associated with a marked and persistent induction of TGF-β (Cheng J, et al. Am J Physiol Renal Physiol 2009; 297(4):F1055). Based on reports that TGF-β neutralizing antibodies reduce blood pressure in salt-sensitive rats (Dahly AJ, et al. Am J Physiol Regul Integr Comp Physiol 2002; 283(3):R757), we sought to test the hypothesis that abrogation of the TGF-β signaling pathway would prevent fibrosis/atrophy in the clipped kidney. Unilateral renal artery stenosis (RAS) was induced in mice with homozygous deletion of Smad3 (KO), a critical intermediate in TGF-β signaling, and colony-matched wild type (WT) controls with a fixed 0.2 mm (I.D.) polytetrafluoroethylene tubing. In WT mice, 8 of 11 developed IF/TA >10%. In KO mice, only 4 of 20 developed IF/TA. Mean external renal artery diameter in KO mice was greater (0.256 mm) than in WT

mice (0.247 mm; p=0.0013), indicating that the protective effect of the KO genotype was not due to a smaller renal artery diameter. All 11 WT mice developed hypertension (HTN, systolic blood pressure [BP] increase >20%); 10 of 20 KO mice developed HTN. Mean maximum postoperative BP was 140±4 in WT and 117±3 mmHg in KO mice; baseline BP 93±2 in WT and 96±2 mmHg in KO mice. In both WT and KO mice, the heart:body weight ratio was increased in RAS mice compared to sham/controls (WT RAS 6.71±0.13; WT sham/control 5.34±0.24; KO RAS 9.30±0.54, KO sham/control 6.42±0.58, p=0.0002). In WT, but not KO mice, heart:body weight ratio correlated with % atrophy (Pearson $R^2$=0.5206; p=0.0076). We conclude that KO mice are protected from the development of IF/TA through a mechanism only in part related to reduction in BP.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO691

**Direct Targeting of TGF-β1 Preserves Peritoneal Membrane from Dialysis Fluid-Induced Damage** Abelardo I. Aguilera,[1] Jesus Loureiro,[1] Rafael Selgas,[4] Jose-Antonio Sanchez-Tomero,[2] Pilar Sandoval-Correa,[1] Patricia Albar-Vizcaino,[1] Mª Luisa Perez-Lozano,[1] Javier Dotor,[3] Francisco Borras-Cuesta,[3] Manuel Lopez-Cabrera.[1] *[1]Unidad de Biología Molecular, Hospital Universitario de la Princesa, Madrid, Spain; [2]Nephrology, Hospital Universitario de la Princesa, Madrid, Spain; [3]DIGNA Biotech, DIGNA Biotech, Madrid, Spain; [4]Nephrology, Hospital Universitario de la Paz, Madrid, Spain.*

Exposure to non-physiological solutions during peritoneal dialysis (PD) induces an epithelial-to-mesenchymal transition (EMT) of mesothelial cells (MCs), and this process is associated with peritoneal membrane (PM) damage. Transforming growth factor (TGF)-β1 is a well-characterized inducer of EMT and has been proposed, but not demonstrated, to be a master molecule in PD fluid-induced (PM) deterioration. Hence, we evaluated the efficacy of two TGF-β1 blocking peptides (P17 and P144) in modulating PD fluid-induced EMT of MCs and in ameliorating PM damage in a mouse PD model. Exposure of omentum-derived mesothelial cells to standard PD fluid induced a mesenchymal-like conversion of these cells, and treatment with P17 blocked the EMT process. Exposure of the mouse peritoneum to PD fluid induced fibrosis, angiogenesis, and functional impairment of the PM. PD fluid treatment also resulted in the loss of MCs monolayer and in the accumulation of trans-differentiated MCs in the compact zone (cytokeratin$^+$ cells), some of which also co-expressed "fibroblast specific protein-1" (FSP-1). Interestingly, PD fluid treatment provoked the appearance of CD31$^+$/FSP1$^+$ and CD45$^+$/FSP1$^+$ cells, indicating that activated fibroblasts may also originate from endothelial cells by endothelial to mesenchymal transition (EndMT) and from cells recruited from the bone marrow (fibrocytes). The administration of peptides P17 and P144 preserved the MCs monolayer, reduced fibrosis and angiogenesis, and improved peritoneal function. In addition, the blocking peptides markedly decreased the number of activated fibroblasts (FSP1$^+$ cells) in the compact zone, being most evident the reduction of fibroblasts derived from MCs via EMT (Cyt$^+$/FSP1$^+$). TGF-β has a central role in PD fluid-induced PM structural and functional alteration.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO692

**Secreted Klotho Protein Counteracts Renal Fibrosis through Inhibiting TGF-b1 Signaling** Shigehiro Doi,[1,2] Noriaki Yorioka,[3] Makoto Kuroo.[2] *[1]Neprology, Hiroshima-nishi Medical Center, Ohtake, Japan; [2]Pathology, University of Texas Southwestern Medical Center, Dallas, TX; [3]Advenced Nephrology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan.*

The *klotho* was identified as an "aging-suppressor" in mice. Therefore, we hypothesize that Klotho may function as a renoprotective factor. To test this hypothesis, we asked if recombinant secreted Klotho protein (rKL) might mitigate renal fibrosis induced in 129S1/SvImJ mice by unilateral ureteral obstruction (UUO). These mice were treated with rKL (0.01 or 0.02 mg/kg, every other day) or vehicle by intraperitoneal injection. On Day 3 and 7, the mice were subjected to magnetic resonance imaging (MRI) and then sacrificed to harvest kidneys for histological, mRNA, and protein analysis. Results were as follows: 1) UUO induced significant down-regulation of endogenous Klotho expression. 2) MRI and histological analysis confirmed that rKL treatment alleviated renal fibrosis induced by UUO. 3) rKL treatment suppressed expression of fibrosis markers (α-Smooth muscle actin (SMA), Vimentin, Collagen-1, Metalloproteases) and TGF-b1 target genes (Snail, Twist) in a dose dependent manner. 4) However, rKL treatment did not reduce TGF-b1 expression in UUO kidney. These observations indicate that A) decrease in endogenous Klotho expression contributes to pathogenesis of renal fibrosis and that B) rKL may not reduce TGF-b1 production but inhibit TGF-b1 signaling. In fact, we confirmed that rKL inhibited TGF-b1-induced phosphorylation of Smad2 and production of SMA and Vimentin in a rat tubular epithelial cell line (NRK52E) *in vitro*. This is a newly identified function of Klotho. Considering that injection of a neutralizing TGF-b1 antibody (1D11) alleviated renal fibrosis induced by UUO, the anti-fibrosis property of rKL may stem from its ability to inhibit TGF-b1 signaling. In fact, we confirmed that the effect of combination therapy (1D11 and rKL) on renal fibrosis was comparable to that of individual therapy (1D11 alone or rKL alone), suggesting that 1D11 and rKL acted through the same mechanism. In conclusion, rKL prevents renal fibrosis primarily through inhibiting TGF-b1 signaling.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO693

**Effect of Statin on Klotho, Anti-Aging Gene, Expression in Cyclosporine A-Treated Mice Kidney: The Possible Mechanism of Anti-Aging Effect of Statin** Ji-Hyun Song,[1] Jungyeon Ghee,[1] ShangGuo Piao,[1] Sol Kim,[2] Chul Woo Yang.[1] *[1]Transplant Research Center, The Catholic University of Korea, Seoul, Republic of Korea; [2]Department of Bioengineering, University of Pennsylvania, Philadelphia.*

**Background:** We reported that statin has a protective effect against chronic cyclosporine A (CsA)-induced nephropathy, but its effect on aging process is still undermined in kidney. This study was performed to investigate the influence of statin on aging process using KLOTHO, angi-aging, expression in experimental model of chronic CsA nephropathy.

**Methods:** Two separate studies were conducted with rats. First, dose-dependent effect of pravastatin (PRVT) on Klotho expression was evaluated (5, 10, 20 and 40 mg/kg). Second, the effect of PRVT on KLOTHO expression was evaluated with an experimental model of chronic CsA nephropathy. Mouse were put on a low-salt diet (0.01% sodium) and categorized into four groups—vehicle (olive oil; 1 ml/kg sc), CsA (30 mg/kg sc), PRVT (10 or 20mg/kg in the drinking water), and a combination of CsA and pravastatin. The drugs were injected daily for one week or four weeks. Development of nephrotoxicity caused by CsA was evaluated with renal function and tubulointerstitial fibrosis. The expression of Klotho protein and mRNA were also compared among different treatment groups. Anti-inflammatory effects of PRVT were studied by evaluating the concentrations of C-reactive protein.

**Results:** PRVT treatment increased Klotho mRNA and protein in normal mouse kidney in a dose-dependent manner. In the second study, mouse receiving PRVT treatment displayed significantly improved renal function and histopathology than those treated with CsA. CsA treatment (26.7±3.3%) significantly decreased the expression of renal Klotho protein and mRNA compared to VH (100±1.6%) or those that solely received PRVT (110.4±1.9%) treatment. However, concurrent treatment of CsA with PRVT (36.6±3.9%) reversed the increase in Klotho expression with histological improvement. This finding was more marked in long-term high dosage than short-term low dosage. **Conclusions:** Statin treatment may inhibit the acceleration of aging process by upregulating KLOTHO expression in chronic CsA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO694

**Regulation of Podocyte Function by B7-1** Chih-Chuan Yu,[1] Teruo Hidaka,[1] Dony Maiguel,[2] Hafeez Faridi,[2] Vineet Gupta,[2] Peter H. Mundel.[1] *[1]Division of Molecular Medicine, University of Miami, Miami, FL; [2]Division of Nephrology, University of Miami, Miami, FL.*

B7-1 (CD80), is a transmembrane protein of B cells and other antigen presenting cells, originally identified as a regulator of T cell activation and tolerance through binding to CD28 or CTLA-4 on T cells. We previously reported that under pathologic conditions characterized by FP effacement and proteinuria, podocytes express B7-1. The clinical significance of these results was underscored by the observation that podocyte B7-1 expression correlates with the severity of human lupus nephritis. These findings unveiled an unanticipated role for B7-1 in kidney podocytes as an inducible modifier of glomerular permselectivity, but the precise mechanisms for the regulation of B7-1 remain unknown.

Here we show that B7-1 can bind to integrin beta1 via cytoplasmic tail-tail interaction. This interaction is physiologically relevant because it suppresses beta1 activation and signaling by blocking the binding of talin to the cytoplasmic domain of beta1. Overexpression of B7-1 in wild type podocytes is sufficient to increase cell motility. Moreover, in vivo overexpression of B7-1 in mice is sufficient to suppress beta1 activation in kidney podocytes, thereby causing proteinuria. These results uncover a mechanism for the regulation of integrin activation by B7-1. This novel function of B7-1 is distinct from its role in T cell signaling: B7-1 does not act through CD28 or CTLA-4, but instead regulates cell matrix receptor function in a cell-autonomous fashion. Inhibition of B7-1 function in podocytes may be a new anti-proteinuric treatment option.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO695

**Collagen Receptor Signaling in Podocytes of COL4A3 -/- Mice with Renal Fibrosis** Diana Rubel, Gerhard A. Mueller, Oliver Gross. *Nephrology & Rheumatology, University Medical Center Goettingen, Goettingen, Germany.*

Introduction: Podocytes are the major source of the α3/4/5 (IV) chains in the glomerular basement membrane (GBM). COL4A3 -/- mice develop renal fibrosis due to the missing α3/4/5 type IV collagen chains. For that reason, we investigate the interaction between the GBM and podocytes via their collagen receptors DDR1 (discoidin domain receptor 1) and ITGA2 (Integrin α2) by comparing podocytes of wildtype mice to COL4A3-, DDR1- and ITGA2-knockout mice.

Methods: Glomeruli were isolated via perfusion with magnetic beads. TGF-β and TGF-β receptor expression was determined by quantification of PCR products in relation to housekeeping genes.

Results: After magnetic separation, cultured podocytes of the precipitated glomeruli showed a different TGF-β- and TGF-β receptor expression compared to tubular cells in the cultured supernatant . Expression of TGF-β and its receptor correlated to each other. After type I collagen stimulation, mimicking abnormal GBM composition, podocytes of wildtype and ITGA2 -/- mice did not respond with a clear rise of TGF-β production.

Further, TGF-β production was even decreased in podocytes of DDR1 -/- mice after type I collagen stimulation. In contrast, type I collagen stimulation of the tubular cells in the cultured supernatant lead to a major increase of TGF-β production.

Conclusions: Podocytes sense an incorrect GBM-composition via both collagen receptors, ITGA2 and DDR1, but do not respond on this by TGF-β overexpression. Further studies will focus on the intracellular downstream pathways in podocytes of COL4A3 -/- mice with their modified collagen receptor signal transduction.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO696

**The Uremic Retention Solute p-Cresol/p-Cresyl-Sulfate Induce Insulin Resistance in C2C12 Muscle Cells and in Mice** Laetitia Koppe,[1,2] Denis Fouque.[1,2] *[1]Service de Néphrologie, Hôpital E Herriot, Lyon, France; [2]U870 INSERM/INSA-Lyon, Régulations Métaboliques Nutrition et Diabètes, Villeurbanne, France.*

Introduction: Uremic toxins retained in uremia might contribute to increased cardiovascular risk in chronic kidney disease (CKD). CKD results in multiple metabolic disturbance (dyslipidemia, insulin resistance). Over the last years, p-cresol has been identified as one of the main uremic toxins involved in the pathogenesis of accelerated atherosclerosis in CKD. Free concentrations of p-cresol and its main metabolite p-cresyl sulfate, are independent predictors of mortality. The purpose of this study was to evaluate the putative role of p-cresol/p-cresyl sulfate in insulin resistance and metabolic complications associated with CKD.

Methods:Mouse C2C12 myotubes were stimulated with insulin in the presence or absence of p-cresol or p-cresyl sulfate (40 µg/ml) for 30 min. The glucose uptake was measured by the technique of tritiated 2-deoxy-glucose. The phosphorylation of key proteins from the insulin signaling pathway (PKB/AKT and IRS-1) was studied by Western blot. Mice were treated for 3 weeks with p-cresol (10 mg/kg intra peritoneal, twice daily) or vehicle. The insulin sensitivity was estimated by intra-peritoneal insulin tolerance test. The different compartments of white adipose tissue were dissected out, weighed and cellularity was analyzed by Coulter Counter.

Results: The p-cresol/p-cresyl sulfate inhibited insulin-stimulated glucose uptake ($p < 0.005$) and impaired serin phosphorylation of PKB/Akt ($p < 0.05$) and tyrosin phosphorylation of IRS-1, ($p<0.05$). The mice p-cresol showed a marked decrease in insulin sensitivity and exhibited a significant decrease in white adipose tissue accretion (-51%, $p< 0.05$) associated with hypercholesterolemia (27%, $p< 0.05$) and increased muscle (+46,9 % , $p <0,05$) and liver (+21%, $P <0.05$) lipid contents compared to controls.

Discussion: These results suggest that p-cresol/p-cresyl sulfate could contribute to the metabolic disturbances observed in CKD and interfere with insulin signaling pathways. The decrease in white adipose tissue accretion, high cholesterol and the ectopic lipid deposits suggest a possible role of lipotoxicity.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO697

**Differential Proteoglycan and Growth Factor Profiles in Experimental Chronic Renal Transplant Dysfunction** Kirankumar Katta,[1] Miriam Boersema,[2] Heleen Rienstra,[3] Johanna Wam Celie,[5] Gerjan Navis,[1] Jan-Luuk Hillebrands,[4] Jacob Van den Born.[1] *[1]Nephrology, UMCG; [2]Medical Biology, UMCG; [3]Cell Biology, UMCG; [4]Pathology, University Medical Center Groningen (UMCG), Netherlands; [5]Pathology, Academic Med Center, Amsterdam, Netherlands.*

Chronic transplant dysfunction (CTD) results from multifactorial progressive tissue remodelling. Proteoglycans (PG) play a role in tissue remodelling by binding and presenting adhesion molecules, chemokines and growth factors. In this study, we investigated spatial mRNA and protein expression levels of various PG and heparin-binding growth factors in tubulointerstitium (TI), glomeruli, and neointimas (NI) in rat renal allografts with CTD.

Kidneys of Dark Agouti (DA) to Wistar Furth allografts with CTD (n=5), of DA to DA isografts (n=5) and of non-transplanted DA (n=5) were used for immunohistochemistry. Glomeruli, TI and NI were isolated by laser dissection microscopy. mRNA expressions were analyzed by low density qPCR array. Proliferation assays were done with rat mesangial and human renal epithelial cells (HK2).

The following observations were done in the allografts compared to isografts and control kidneys. i) In glomeruli, TI and NI we observed uniform upregulation of *CCL2*, *TGF*-β and *Coll IV*, and downregulation of *BMP7*. ii) Matrix PG perlecan was significantly upregulated both on mRNA and protein level in glomeruli and NI, whereas syndecan-1, a cell membrane PG was upregulated in the TI and NI. iii) In glomeruli and NI, FGF2 was abundantly present on protein level, probably secondary to platelet degranulation, evidenced by CD61 (integrin beta3) positivity. HB-EGF was prominently expressed at the basal side of tubular cells. Functional significances of these findings were shown *in vitro* where we showed the FGF2-induced proliferative response of rat mesangial cells to be fully dependent on intact PG. Moreover knock-down of syndecan-1 in HK2 cells downregulates their proliferation.

These data indicate that in CTD differential PG and growth factor expression profiles contribute to tissue remodelling in different intrarenal compartments. Our results pave the way to the use of heparin-like glycomimetica to limit CTD.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO698

**Alpha1 Antitrypsin Polymers Prime Neutrophils and Are Proinflammatory** Hannah L. Morris,[1] Alice Wood,[1] Matthew David Morgan,[1] Stuart W. Smith,[1] Elena Miranda,[2] Ugo I. Ekeowa,[3] Anne Bevins,[1] Alexander J. Howie,[5] Julie M. Williams,[1] Robert A. Stockley,[4] David Lomas,[3] Caroline O. S. Savage,[1] Lorraine Harper.[1] *[1]University of Birmingham; [2]Universita' "La Sapienza"; [3]University of Cambridge; [4]University Hospitals Birmingham; [5]University College London.*

Alpha1 anti-trypsin (A1AT) is an inhibitor of serine proteases which are inflammatory mediators in ANCA vasculitis (AAV). The A1AT gene Z allele produces a protein that forms intra-hepatic and circulating polymers. It is reported to increase the risk and severity of AAV. We investigated the prevalence of Z alleles in patients with AAV and whether A1AT polymers are pro-inflammatory by priming ANCA induced neutrophil responses.

A cohort of 919 AAV patients and 1141 disease controls were screened for A1AT SNPs. Serum polymer concentrations were measured by ELISA. Neutrophils (PMN) were primed with either A1AT polymer or monomer (0.2mg/ml) and activated with either normal or ANCA containing IgG. Degranulation (MPO assay), CD62L shedding (flow cytometry) and superoxide release assays assessed activation. Immunohistochemistry (IHC) staining for polymers in the renal biopsy of a patient carrying the Z allele was performed.

Patients were more likely to have the Z but not the S allele than controls, table1. AAV patients with 1 or more Z allele had higher serum concentrations of A1AT polymer (0, 1 or 2 Z alleles 0.2, 1.3 and 7.4 mcg/ml respectively $p<0.001$). Compared to unprimed neutrophils A1AT polymer (but not monomer) priming caused CD62L shedding (MFI 132 vs 109 $p=0.046$). A1AT polymer (but not monomer) primed PMN for ANCA stimulated superoxide production (1.1 vs 3.7 nmol $SO/10^5$ PMN $p=0.013$) but not PMN degranulation. A1AT polymers were detected in glomeruli by IHC.

Table 1

| | CONTROL | PATIENT | TOTAL |
|---|---|---|---|
| M | 2149 | 1707 | 4856 |
| S | 80 | 73 | 153 |
| Z | 53 | 78 | 131 |
| TOTAL | 2282 | 1838 | 4140 |

$X=12.46$ 2 Degrees Freedom $P=0.002$

Carriage of the Z allele is increased in AAV. A1AT polymers have a direct pro-inflammatory effect in AAV by priming PMN for subsequent ANCA activation and are present at sites of inflammation.

We thank J Holle, S Wieczorek Ruhr-University and L Guillevin Paris for provision of DNA

Disclosure of Financial Relationships: nothing to disclose

## TH-PO699

**Adenosine 2a Receptor (A2aR) Regulates Dendritic Cell (DC)-Natural Killer T Cell (NKT) Immunological Synapse: A Cell-Based Strategy To Protect Kidneys from Ischemia-Reperfusion Injury (IRI)** Li Li, Liping Huang, Hong Ye, Mark D. Okusa. *Division of Nephrology, Department of Medicine, University of Virginia, Charlottesville, VA.*

Kidney resident DCs activate CD1d-restricted NKT cells to mediate damage in kidney IRI. Endogenous adenosine accumulates in the kidney following IRI and terminates an overactive immune response through A2aRs expressed on bone marrow-derived cells.We hypothesize that A2aR agonists (ATL313) protect kidneys from IRI by suppressing NKT cell activation by regulating the expression of co-stimulatory molecule on DCs and/or the production of cytokines by DCs. Both kidney pedicles were clamped in B6 mice for 28 min or 24 min (mild ischemia) and then released for 24 hrs. FACS, ELISA and plasma creatinine (PCr) measurement were performed. In vivo ATL313 (1ng/kg/min) significantly decreased migration of $Gr\text{-}1^+IFN\text{-}g^+$ neutrophils and NKT cells to the inflamed kidney compared with vehicle control IRI mice ($P<0.05$). Adoptive transfer of a-galactosylceramide loaded BMDCs (DC-aGalCer, 0.1ng/mL) significantly increased kidney mild IRI with higher PCr, more neutrophil infiltration and kidney IFN-g production compared with vehicle-loaded BMDC control ($P<0.01$); DC-aGalCer-promoted injury in WT mice was not apparent in NKT cell-deficient (CD1dKO and Ja18KO) and IFN-gKO mice ($P>0.05$). Furthermore, prior to adoptive transfer, ex vivo treatment of WT but not A2aRKO DC-aGalCer with ATL313 (1nM) (ATL313-DC-aGalCer) protected kidney from IRI and lowered PCr ($P<0.01$), neutrophil migration and plasma IFN-g secretion ($P<0.01$). In vitro studies demonstrated that prior treatment with ATL313 attenuated WT but not A2aR KO DC-aGalCer activation of NKT cells ($P<0.05$). Attenuation of NKT activation by ATL313-DC-aGalCer was not due to blocking CD1d/glycolipid trafficking. However, ATL313 significantly suppressed LPS (0.01ng/mL)-mediated DC IA, CD40 and CD86 expression, IL-6, IL-1b and IL-12p40 secretion and increased negative co-stimulatory molecules B7-H1 and B7-DC expression ($P<0.05$). We conclude that specific targeting of DC function ex vivo by A2aR activation is a unique and potent cell-based strategy to attenuate IRI by blocking DC-NKT cell immunological synapse.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO700**

**B Cell Subsets Contribute to Both Renal Injury and Renal Protection after Ischemia/Reperfusion** Joshua M. Thurman,[1] Brandon Renner,[1] Derek Strassheim,[1] Liudmila Kulik,[1] Danica Ljubanovic,[3] Magdalena Glogowska,[1] Kazue Takahashi,[2] V. Michael Holers.[1] *[1]Medicine, University of Colorado School of Medicine, Aurora, CO; [2]Developmental Immunology, Massachusetts General Hospital, Boston, MA; [3]Pathology, University Hospital Dubrava, Zagreb, Croatia.*

Ischemia/reperfusion (I/R) triggers a robust inflammatory response within the kidney. We sought to identify whether natural antibodies bind to the post-ischemic kidney and activate the complement system after I/R. We depleted peritoneal B cells in 8-10 week-old mice by hypotonic shock. Then, peritoneal B cell depleted mice or control (PBS injected) mice were subjected to 24 minutes of bilateral renal ischemia. Depletion of the B cells prevented the deposition of IgM within the glomeruli after renal I/R, although the level of circulating IgM as measured by ELISA was unaffected by this treatment. Peritoneal B cell depletion also attenuated renal injury after I/R (BUN 116 +/-13 mg/dL versus 146 +/- 4 in control mice, n=5, $P < 0.05$). We found that glomerular IgM activates the classical pathway of complement but does not cause substantial deposition of C3 within the kidney. We also subjected mice deficient in all mature B cells (μMT mice) to renal I/R and found that they sustained worse renal injury than wild-type controls (BUN 135 +/- 18 versus 80 +/- 11 in control mice, $n = 12$, $P < 0.05$). A subpopulation of splenic B cells (sometimes referred to as B regs) has been demonstrated to limit injury in autoimmune disease through the production of IL-10. Therefore, we measured the level if IL-10 in the B cell deficient and wild-type mice. The serum IL-10 level was 81 +/- 37 in wild-type mice after I/R, but was undetectable in the μMT mice ($n = 7$-8 mice per group, $P < 0.05$). Regarded together, these results indicate that natural antibody produced by peritoneal B cells binds within the glomerulus after renal I/R and contributes to functional renal injury. However, non-peritoneal B cells attenuate renal injury after I/R, possibly through the production of IL-10. Given the increasing use of B cell depleting therapies in patients with renal disease, a full understanding of the role of B cells in disease is critical.

Disclosure of Financial Relationships: Consultancy: Taligen Therapeutics, Inc.; Ownership: Taligen Therapeutics, Inc.

**TH-PO701**

**Deoxycorticosterone-Salt Induces Th17 Differentiation and Downregulation of FoxP3 in the Kidney** Juan Pedro Peña Mendez, Cristian A. Amador, Magdalena Gonzalez, Fabiola Venegas, Luis F. Michea. *ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile.*

Aldosteronism causes hypertension and an inflammatory phenotype in heart and kidney. Recent findings have implicated the Interleukin-17 (IL-17) pathway as a pathogenic mechanism in cardiovascular disease. Studies of autoimmune disease have demonstrated that regulatory T cells express the transcription factor Forkhead Box P3 (FoxP3) and antagonize Th17 differentiation. Recently, we showed the ability of aldosterone to promote autoimmune damage by enhancing Th17-mediated immunity. We hypothesized that aldosteronism-dependent hypertension induces activation of Th17 and downregulation of FoxP3. Three groups of uninephrectomized rats were studied (n=4, 8 and 16 days): Vehicle, DOCA-salt (0.5mg/0.1kg + 0.9% NaCl/0.3% KCl in drinking water), and DOCA-salt+spironolactone (50 mg/kg/d, SPIRO). The DOCA-salt treatment increased systolic blood pressure, starting at day 4 (152mmHg vs. vehicle 116 ± 7.1mmHg, $p<0.05$). Changes in blood pressure were prevented by SPIRO. The kidney of DOCA-salt rats presented increased abundance of TGF-β1 mRNA, a Th17 differentiation cytokine, at day 8; mRNA transcripts of cytokines secreted by Th17 cells (IL-17, p19 subunit of IL-23, and IL-1β) increased after 16 days of treatment (IL-17; 20 fold vs. control, IL-23; 3 fold vs. control and IL-1β; 3 fold vs. control, n=4; $p<0.05$). Protein expression of IL-17 was increased in kidney at day 16. Kidney sections showed glomerular infiltration of T CD4+ and Th17 lymphocytes (8 and 16 days). Contrasting with IL-17, the expression of FoxP3 decreased in the DOCA-salt group. All the above-mentioned changes caused by DOCA-salt were prevented by SPIRO. The analysis of peripheral blood mononuclear cells showed Th17 induction at 8 days. The upregulation of IL-17 in PBMC was also prevented by the treatment with SPIRO. Interestingly, after 16 days, we did not observe activation of IL-17 in PBMC. These results suggest that the inflammatory renal phenotype caused by DOCA-salt also implicates the downregulation of FoxP3. (Supported by CONICYT AT-24091044, FONDECYT 1090223, Millennium Nucleus on Immunology and Immunotherapy P07/088-F and Fondecyt-FONDAP 15010006).

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**TH-PO702**

**Bardoxolone Methyl (BARD) Activates the Antioxidant Keap1-Nrf2 Pathway and Increases Glutathione Levels in Cultured Cells** Ron Bumeister, Greg Miller, Rhesa D. Stidham, Pritam Kambuj, Deborah A. Ferguson, Christian Wigley. *Reata Pharmaceuticals, Inc., Irving, TX.*

Stimulation of pro-inflammatory signaling pathways by hyperglycemia and activation of the renin-angiotensin system (RAS) induces renal oxidative stress, leading to the development and progression of chronic kidney disease (CKD), which frequently progresses to end-stage renal disease. Bardoxolone methyl (BARD), the lead molecule from the Antioxidant Inflammation Modulator (AIM) drug class, is a highly potent inducer of Nrf2, a transcription factor that positively regulates a battery of antioxidant and detoxification genes. Importantly, BARD exhibits significant tissue protective effects in several models of renal damage *in vivo*. Upon activation, Nrf2 promotes transcription of a series of enzymes and transporters important for the production and maintenance of appropriate levels of glutathione, a sulfhydryl buffer that plays a central role in detoxification of various proinflammatory reactive oxygen species (ROS). To ascertain whether BARD treatment results in enhanced cellular antioxidant capacity, cell lines derived from various tissues including renal proximal tubule epithelial (NRK-52E and OK), podocyte (AB8/13), mesangial (RMC, MES13, and nHMC), and glomerular endothelial (ciGENc) cells were treated with BARD and evaluated for induction of Nrf2 and total glutathione levels. Relative transcription levels of Nrf2 target genes (including *NQO1, TXNRD1, and HO-1*) were monitored by qPCR, and total cellular glutathione was measured by chemiluminescence. In all cell lines tested, BARD induced the expression of Nrf2 target genes at concentrations as low as 10 nM. Consistent with a critical role for Nrf2 in regulating glutathione antioxidant capacity, treatment with BARD increased glutathione levels in a time- and dose-dependent manner. Significant elevations in glutathione were observed at concentrations as low as 25 nM. The results of this study strongly support the use of BARD in renal indications where oxidative stress and inflammation play a central role in the progression of the disease. A 12-month placebo-controlled, pivotal Phase 2b clinical trial in CKD patients with type 2 diabetes is underway.

Disclosure of Financial Relationships: Employer: Reata Pharmaceuticals, Inc.; Ownership: Reata Pharmaceuticals, Inc.

**TH-PO703**

**Combination Therapy with CCR2 Antagonist and Angiotensin II Type 1 Receptor Blocker Markedly Ameliorates Crescentic Glomerulonephritis** Maki Urushihara, Naro Ohashi, Kayoko Miyata, Masumi Kamiyama, Ryousuke Satou, Hiroyuki Kobori. *Department of Physiology, Tulane University Health Sciences Center, New Orleans, LA.*

Monocyte chemoattractant protein-1 (MCP-1) plays a critical role in the development of anti-glomerular basement membrane (GBM) nephritis, and inhibition of the MCP-1/CC chemokine receptor 2 (CCR2) pathway is currently used for treatment of anti-GBM nephritis. We recently showed that angiotensin (Ang) II infusion in rats activated MCP-1 and transforming growth factor-β1 (TGF-β1), which in turn induced macrophage infiltration of renal tissues. Here we examined whether combination therapy with a CCR2 antagonist (CA, RS102895) and an AngII type 1 receptor (AT1) blocker (ARB, olmesartan) ameliorated renal injury in the anti-GBM nephritis model via suppression of TGF-β1 activation by ARB, leading to more effective CCR2 inhibition. Anti-GBM nephritis rat model was introduced by single injection of anti-GBM antibody and developed progressive proteinuria and glomerular crescent formation, accompanied by increased macrophage infiltration and glomerular expression of MCP-1, angiotensinogen, AngII, AT1, TGF-β1 and type 1 collagen. Treatment with CA or ARB alone moderately ameliorated renal injury; however, combined treatment with CA and ARB dramatically prevented proteinuria and markedly reduced glomerular crescent. The combination treatment suppressed the induction of macrophage infiltration, angiotensinogen, AngII, AT1 and TGF-β1, and reversed the fibrotic change in the glomeruli. Primary cultured glomerular mesangial cells stimulated by AngII showed significant increases of MCP-1 and TGF-β1 expression. Furthermore, co-cultured model consisting glomerular mesangial cells, parietal epithelial cells, and monocyte-derived macrophage showed an increase in AngII-induced cell proliferation and soluble collagen secretion. By the treatment with ARB, these augmentations were attenuated. These data suggest that AngII enhances glomerular crescent formation of anti-GBM nephritis. Moreover, our results demonstrate that inhibition of MCP-1/CCR2 pathway with combination of ARB effectively prevents renal injury in anti-GBM nephritis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO704**

**Suppression of Adiponectin Secretion by Aberrantly Glycosylated IgA1 in Glomerular Mesangial and Endothelial Cells: A Possible Mechanism of Anti-Inflammation in IgA Nephropathy** Hitoshi Sugiyama, Tatsuyuki Inoue, Masashi Kitagawa, Hiroshi Morinaga, Keiichi Takiue, Shinji Kitamura, Yohei Maeshima, Hirofumi Makino. *Department of Medicine and Clinical Science, Okayama Univ. Grad. Sch. of Med., Okayama, Japan.*

The pathogenesis of IgA nephropathy (IgAN) is thought to be the mesangial deposition of aberrantly glycosylated IgA1 with reduced galactose and/or sialic acid. The effect of desialylated and degalactosylated (deSial/deGal) IgA1 on human mesangial cells (HMC) and human glomerular endothelial cells (hGEC) is largely unknown. We generated deSial/deGal IgA1 and investigated its effect on HMC and hGEC. DeSial/deGal IgA1 was produced by treatment to human IgA with neuraminidase and β-galactosidase. The state of deglycosylation was confirmed by the lectin bindings to *Herix aspersa* and Sambucus Nigra Agglutinin. Cultured HMC or hGEC were stimulated with native IgA or deSial/deGal IgA1 (50 μg/ml) for 48 hr and then culture supernatant was analyzed using cytokine array to detect expression levels of 507 proteins. The protein and mRNA levels were examined by ELISA and real-time PCR, respectively. Stimulation with native IgA increased the intensity of spots corresponding to adiponectin which was significantly suppressed by stimulation with deSial/deGal IgA1 in cultured HMC and hGEC. Concentration of total and high molecular weight adiponectin in culture supernatant significantly elevated after stimulation with native IgA in dose- and time-dependent manners. However, there was no significant increase in either total or high molecular weight adiponectin after incubation with deSial/deGal IgA1 up to 48 hr. Adiponectin mRNA levels were similar in HMC and hGEC after stimulation with native and deSial/deGal IgA1. The HMC and hGEC expressed mRNA of adiponectin receptor type 1 but not type 2. Our results demonstrate that adiponectin is synthesized by cultured

HMC and hGEC, and deSial/deGal IgA1 suppresses the secretion of adiponectin. The data suggest that the local suppression of the adipokine by aberrantly glycosylated IgA1 could be involved in the regulation of glomerular inflammation and sclerosis.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO705

**MMP9 Deficiency Ameliorates Nephrotoxic Serum Nephritis by Impairing Macrophage Migration** Malte A. Kluger, Gunther Zahner, Melanie Schaper, Hans-Joachim Paust, Ulf Panzer, Rolf A. Stahl. *III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

**Background:** Matrix metalloproteinase 9 (MMP9) is involved in physiologic extracellular matrix turnover and tissue homeostasis, but has also been associated with a number of inflammatory and autoimmune diseases. In this study, we assessed its functional role in a T cell-dependent model of glomerulonephritis using MMP9 deficient mice ($MMP9^{-/-}$). **Methods:** $MMP9^{-/-}$ mice were backcrossed on a C57BL/6 background (microsatellite analysis). Nephrotoxic serum nephritis (NTN) was induced in 10-12 wk-old male wildtype (WT) and $MMP9^{-/-}$ mice. Controls received nonspecific sheep IgG. Urine and blood were collected for determination of albuminuria (ELISA) and BUN at day 10. Kidneys were prepared for immunohistochemistry, RT-PCR or flow cytometry. Migratory capacity of peritonitis-elicited macrophages was assessed in a FACS-analyzed transwell chemotactic-assay. **Results:** In WT mice, induction of NTN caused a pronounced renal infiltration by monocytes and T cells followed by intense tissue injury, albuminuria and loss of renal function. These findings were attenuated in nephritic $MMP9^{-/-}$ mice. Kidney function was preserved and albuminuria was determined at significantly lower values. Histological damage was only mild. The numbers of infiltrating monocytes and T cells were dramatically reduced, though less extensive for the latter.

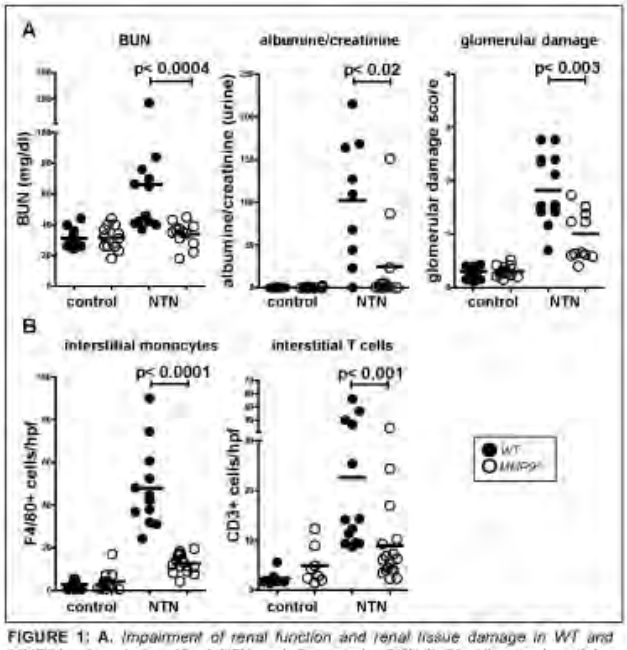



**FIGURE 1:** *A. Impairment of renal function and renal tissue damage in WT and $MMP9^{-/-}$ mice at day 10 of NTN or IgG controls. BUN* **(left)**, *albumine/creatinine* **(middle)** *and scoring of affected glomeruli in PAS-stained kidney sections* **(right)**. **B.** *Renal T cell and monocyte recruitment in WT and $MMP9^{-/-}$ mice at day 10 of NTN or IgG controls. F 4/80+ interstitial monocytes* **(left)** *and CD3+ interstitial T cells* **(right)**. *Symbols indicate individual data points, horizontal lines indicate mean values. Statistical analysis was performed using Mann-Whitney test*

These observations could be specified by flow cytometry, revealing a diminished influx of $CD45^+/F4/80^+/CD11b^+/CD11c^-$ cells into nephritic $MMP9^{-/-}$ kidneys. In a chemotactic assay, migratory capacity of $MMP9^{-/-}$ derived peritoneal macrophages, but not $T_H$ lymphocytes, was derogated when compared to WT. **Conclusions:** MMP9 deficiency ameliorates renal impairment during nephrotoxic nephritis in C57BL/6 mice by attenuating macrophage chemotaxis.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO706

**Syk Blockade Inhibits Acute Platelet and Neutrophil Mediated Glomerular Injury** Jessica Ryan,[1,2] Frank Yuanfang Ma,[1,2] John Kanellis,[1,2] Kate Blease,[3] David J. Nikolic-Paterson.[1,2] *[1]Nephrology, Monash Medical Centre, Clayton, Victoria, Australia; [2]Medicine, Monash University, Clayton, Victoria, Australia; [3]Celgene, San Diego, CA.*

Glomerular antibody deposition can induce acute renal injury via Fcγ-receptor dependent activation of platelets and neutrophils. Fcγ-receptor signalling induces cytoskeletal reorganisation and pro-inflammatory responses via the spleen tyrosine kinase (Syk). Syk inhibitors can suppress macrophage-mediated renal injury, but whether Syk is also involved in platelet/neutrophil dependent glomerular injury is unknown. This study evaluated the ability of a selective Syk inhibitor (SYKi) to inhibit the acute neutrophil-dependent phase of glomerular injury in rat anti-GBM disease. SD rats were immunized with sheep IgG and 5 days later given sheep anti-rat GBM serum. Rats were treated with SYKi (10mg/kg/qid) or vehicle (Veh) from 1hr before anti-GBM serum until being killed 3 or 24hr later. At 24hr, heterologous phase glomerular injury was evident in Veh treated disease as shown by heavy proteinuria (227±79 vs 5±1mg/day normal; P<0.001), glomerular thrombosis evident on PAS sections, prominent capillary P-selectin staining of platelets, a macrophage and T cell infiltrate, and up-regulation of glomerular mRNA levels for the pro-inflammatory markers TNF-α, iNOS and MMP-12. In contrast, SYKi treatment at 24hr prevented proteinuria (19±20mg/day; P<0.001 vs Veh), prevented glomerular thrombosis, largely abrogated glomerular P-selectin staining, reduced glomerular macrophage and T cell infiltration (by 40% and 90%, respectively), and significantly reduced the up-regulation of mRNA levels of pro-inflammatory molecules. Furthermore, immunostaining of Veh treated rats at 3hr showed Syk phosphorylation which co-localised with the transient glomerular neutrophil influx seen at this time. SYKi largely prevented this transient neutrophil influx and abrogated p-Syk staining. In conclusion, Syk signalling is required for acute antibody and neutrophil-dependent glomerular injury. These findings support the therapeutic potential for Syk inhibitors in antibody-dependent forms of kidney disease.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO707

**Inflammation and Cytokines in Polycystic Kidney Disease** Kameswaran Ravichandran, Iram Zafar, Sarah Faubel, Zhibin He, Chris Altmann, Charles L. Edelstein. *Univ of Colorado.*

Inflammation may contribute to the progression of PKD. The aims of the study were to quantify the inflammation, measure cytokines in early and late stages of PKD. 8 wk old Han: SPRD rats (Cy/+) with early PKD, 1 yr old Cy/+ rats and 4wk old cpk mice with advanced PKD were studied. Inflammation was quantified as 0=absence of infiltrates; 1= focal, mild; 2=focal moderate; 3= diffuse mild; 4=diffuse moderate or severe. Cy/+ rats were treated with rapamycin (0.2 mg/kg/weekday) from 3-52 weeks of age. Cytokines were measured using 2-bead based multiplex cytokine kits with flow-based protein detection. Inflammation score was 0.7 in ++, 2.3 in 8wk old Cy/+ (P<0.05); 1.4 in ++ , 2.8 in 1yr old Cy/+ (P<0.05) and 2.6 in Cy/+R; 1.6 in +/+ and 2.2 in cpk (NS). Furthermore, kidney myeloperoxidase (MPO) activity an indicator of polymorphonuclear leucocyte infiltration was assessed . MPO activity was 0.032 in ++, 0.11 in Cy/+(P<0.05) and 0.033 in Cy/+R(P<0.05).Cytokines are shown in the table. In 4 wk old cpk mice the following cytokines were not changed; IFN-γ, RANTES, MIP-1, IL-6, IL-12, IL-9, IL-5, IL-2, TNF-α, IL-1, IL-10. In 8 wk old Cy/+ none of the cytokines were increased. In summary, inflammation and proinflammatory cytokines are increased in the later stages of PKD. Cytokine profiles differed between cpk mice and Cy/+ rats with advanced PKD. Impressive increases in MCP-1, G-CSF and KC in cpk mice and IL-1, IL-1and IL-6 in rats were seen. Rapamycin therapy reduced IL-1, IL-1, IL-6 and decreased MPO activity in 1yr old Cy/+ rats. In conclusion, cytokine inhibition e.g IL-1Ra (Anakiren) in PKD merits further study.

Cytokines profile in PKD kidneys

| pg/mg | ++ | cpk* | |
|---|---|---|---|
| MIP-1α | 1.9 | 4.4* | |
| MCP-1 | 10.8 | 52.2* | |
| GM-CSF | 2.8 | 5.2* | |
| G-CSF | 0.4 | 98* | |
| IL-17 | 0.1 | 0.4* | |
| EOTAXIN | 37.4 | 75* | |
| IL-13 | 15.3 | 27.8* | |
| IL-4 | 0.8 | 1.5* | |
| IL-3 | 0.1 | 0.5* | |
| IL-1α | 1.9 | 4.4* | |
| KC (IL-8) | 2.8 | 14.7* | |
| pg/mg | +/+ 1 yr old | Cy/+ | Cy/+ Rapa |
| IL-1α | 0.3 | 5.4** | 1.1**** |
| IL-1β | 21.5 | 363** | 131**** |
| IL-2 | 1.6 | 4.1* | 5.1 |
| IL-6 | 4.2 | 15.2* | 7.2*** |
| IL-10 | 0 | 2.5** | 1.7 |
| *P<0.05 vs ++ | **P<0.01 vs ++ or Cy/+Rapa | ***P<0.05 vs Cy/+ | ****P<0.01 vs Cy/+ Rapa |

Disclosure of Financial Relationships: nothing to disclose

### TH-PO708

**PGE2 Promotes Cellular Recovery from Nephrotoxic Serum Nephritis (NTN) in Mice by a Direct Effect on Glomerular Cells** Nino Kvirkvelia, Maggie McMenamin, Kapil Chaudhary, Michael P. Madaio. *Medicine, Medical College of Georgia, Augusta, GA.*

Although many strategies have been shown to prevent immune-mediated nephritis, promoting recovery of established disease has been more difficult. We postulated that PGE2, a bioactive eicosanoid which exhibits multiple, tissue dependent regulatory functions to control immune-mediated inflammation, tissue repair and fibrosis, has the potential to improve the course of ongoing nephritis. To evaluate this hypothesis, the therapeutic potential and protective effect of exogenous PGE2 on established NTN in mice was evaluated along with its' cytoprotective effect on murine endothelial cells (GEC) and podocytes (POD). Initially, mice were injected with nephrotoxic serum (NTS), followed by PGE2 administration 5μg/g/d, started either on day 1, 2 or 3 after NTS dosing. Mice injected with PGE2 normalized BUN, Upr and histology. Benefit was optimal with dosing at day 1, although later treatment was effective. To determine whether the benefit was at least, in part, due to a protective effect of PGE2 on glomerular cells, the capacity of PGE2

to limit injury of cultured cells was evaluated. Thus, murine POD and GEC were pretreated with PGE2, 1-5µl/ml for 2-4h, and afterwards, NTS 5-10% was added for additional 7-12 h. The number of phosphatidyl serine and propidium iodide positive cells were assessed by FACS. PGE2 reduced NTS induced apoptosis of cultured POD and GEC up to 30%. Furthermore, when POD and GEC were subjected to NTS, and then treated with PGE2 (1-5µg/ml), cell numbers were increased (25-55%), indicating that PGE2 promoted cell regeneration after NTS induced injury. PGE2 also limited NTS induced GEC upregulation of CD47, an integrin associated protein; its' ligation in many cases leads to inhibition of cell proliferation and cell death. Altogether, the results indicate that PGE2 is candidate to further investigate its therapeutic potential to improve cellular recovery and improved function during the course of glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO709**

**Darbepoetin-α Suppresses TNF-α-Induced Endothelin-1 Production through Antioxidant Effect: Role of Sialic Acid Residues** Wonseok Yang, Su-Kil Park. *Internal Medicine, Asan Medical Center, College of Medicine, University of Ulsan, Seoul, Korea.*

Endothelin-1(ET-1) is implicated in the pathogenesis of atherosclerosis. We have previously demonstrated that, in cultured human aortic endothelial cells(HAECs), TNF-α stimulates ET-1 production through reactive oxygen species(ROS)-dependent c-Jun $NH_2$-terminal kinase(JNK) and ROS independent P38 mitogen-activated protein kinase that regulates activation of both AP-1 and NF-κB. This study was conducted to evaluate the effect of darbepoetin-α, a hypersialylated analogues of r-HuEPO, on TNF-α-induced ET-1 production in HAECs.

Darbepoetin-α (5,10, 50, 250ng/ml) attenuated TNF-a-induced ET-1 at mRNA and protein levels in ELISA and RT-PCR. This was accompanied by inhibition of TNF-a-induced DNA-binding activities of both NF-kB and AP-1 in electrophoretic mobility shift assay. Darbepoetin-α (250ng/ml) inhibited TNF-a-induced reactive oxygen species(ROS) generation which was visualized with confocal microscopy and activation of JNK, but not p38 MAPK in western blot. As with N-acetylcysteine and SP600125 (JNK inhibitor), darbepoetin-α did not inhibit either IkBa degradation or p65 nuclear translocation, but attenuated p65 phosphorylation at serine 276 in western blot. TNF-a increased phosphorylation of mitogen- and stress-activated protein kinase 1, the enzyme responsible for serine 276 phosphorylation of p65, while darbepoetin-α attenuated those response like N-acetylcysteine and SP600125. Desialation of darbepoetin-α by sialidase (5mU/ml, recombinant, *Arthrobacter ureafaciens* α2-3,6,8,9-neuraminidase expressed in *E. coli*) completely abolished its inhibitory effect on TNF-a-induced ROS generation and ET-1 mRNA expression. However, desialated darbepoetin-α still increased STAT5 in endothelial cells.

In conclusion, darbepoetin-α modulates TNF-a-induced intracellular signaling by its antioxidant effect. It suppressed TNF-a-induced ET-1 production through inhibition of ROS-dependent JNK activation. The high content of sialic acid in darbepoetin-α seems to be essential for its antioxidant effect by scavenging ROS. These results might suggest that darbepoetin-α could ameliorate the progression of atherosclerosis where ET-1 has a major role.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO710**

**Regulation of RAGE Via Paracrine Estrogens** Brooke E. Harcourt, Melinda T. Coughlan, Anna Gasser, Karly C. Sourris, Mark E. Cooper, Josephine M. Forbes. *Glycation and Diabetes Complications, BakerIDI Heart and Diabetes Research Institute, Melbourne, Victoria, Australia.*

Obesity is a major contributor to type 2 diabetic nephropathy. Additional risk factors are male gender and post-menopausal status in females. This suggests that the balance between estrone (E1) secreted from white adipose tissue and 17-ß-estradiol (E2), may be an important determinant of this disease. Previous studies have shown that the protection against cardiovascular disease afforded by higher levels of E2 in menstrual women is abrogated by obesity and that decreases in E2 levels are often associated with declining renal function in diabetes.

We examined the effects of peri-renal adipose tissue deposition on renal function in a mouse model of obesity; high fat feeding (HFF) for 16 weeks. We also studied mice with a genetic deficiency in RAGE (RAGE-/-). We identified that although all HFF mice had increases in central adiposity, obese RAGE-/- mice did not have an increase in their peri-renal fat depots compared to WT HFF mice. We also showed that in peri-renal adipose deposits RAGE-/- mice have a decreased E2/E1 ratio compared with control. Furthermore, WT HFF mice had a concomitant decline in kidney function that was not evident in obese RAGE-/- mice. Since renal cortical membrane RAGE expression was increased with HFF, we then examined modulatory factors using chromatin immuno-precipitation in renal corticies, which revealed that estrogen receptor ß (ER-ß) interacts with the RAGE promoter at binding sites for both NF-κB and Sp-1.

This study demonstrated protection against renal injury by the amelioration of local adipose tissue deposition, in this case, the peri-renal depot in HFF RAGE-/- mice, despite a lack of beneficial effects on either central obesity or glycaemic control. Importantly, it was evident that excesses of locally rather than systemically derived E1 via ligation to ER-ß, activated known pathways implicated in renal injury. Therefore, we suggest that the local paracrine environment may be of greater pathological relevance than systemic events in the development obesity related renal injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO711**

**Lipoxin A4 and a Related Synthetic Analog Suppress the Activation of Primary Dendritic Cells from Kidney and Spleen** Michelle Holland,[1] Shirley Hanley,[1] Jana Pindjakova,[1] Patrick Guiry,[3] Rhodri Ceredig,[1] Catherine Godson,[2] Matthew D. Griffin.[1] *[1]Regenerative Medicine Institute, College of Medicine, National University of Ireland Galway, Galway, Ireland; [2]Diabetes Research Centre, Conway Institute, University College Dublin, Dublin, Ireland; [3]School of Chemistry and Chemical Biology, University College Dublin, Dublin, Ireland.*

**Background and Aims:** Dendritic cells (DC) are abundant in the renal interstitium and modulate inflammatory responses to acute injury. Lipoxins (LX) are eicosanoids which aid resolution of inflammation via inhibition of neutrophils and monocyte/macrophages. LX effects on DC have been less well examined. In this study LX regulation of renal, splenic and bone marrow-derived DC was investigated. **Methods:** Primary DCs were prepared by anti-CD11c magnetic separation from collagenase-digested mouse spleen and kidney. BMDCs were prepared by 5-8 day culture of bone marrow cells with GM-CSF and IL-4. LXA4 and a synthetic LX analog (LX-analog) were added to DC cultures at final concentrations of 0.1-1000ng/ml. DC activation was induced by ligands for TLR4 (LPS), TLR2 (LTA) and CD40. DC responses and were measured by ELISA, multi-color flow cytometry and mixed lymphocyte reaction (MLR). **Results:** Pre-incubation of kidney and spleen DC with LXA4 for 6-12 hr resulted in blunting of IL-6 production in response to TLR ligands and reduced numbers of highly mature (CD80/CD86hi) DCs with maximal effect at 100ng/ml (~300nmol). LX-pre-treated splenic DC exhibited reduced T-cell stimulatory capacity in mixed lymphocyte cultures. Similar effects were observed for LX-analog at 10ng/ml. Culture of DC with LX-analog 5-10ng/ml for 6 hr was associated with strikingly increased apoptosis by AnnexinV/PI staining. Maturation of BMDCs in response to TLR ligands or CD40 ligation, assessed by surface expression of CD80, CD86, CD40 and CD54 and by IL-6 release was also potently inhibited by LX-analogue. **Conclusion:** LX and related synthetic analogs directly suppress DC innate and cognate immune functions and mediate pro-apoptotic effects at supra-physiological but therapeutically relevant concentrations *in vitro*. Inhibitory effects were observed for DC purified directly from kidney.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO712**

**Temporal and Spatial Expression of AT1 and AT2 Receptors in Pre- and Post-Natal Periods Determines Development of HIVAN in Mice with Variable Angiotensinogen (Agt) Copies** Ankita Sagar,[1] Divya Salhan,[1] Madhuri Adabala,[1] Ashwani Malhotra,[1] Praveen N. Chander,[2] Pravin C. Singhal.[1] *[1]Medicine, Long Island Jewish Medical Center, New Hyde Park, NY; [2]Pathology, New York Medical College, Valhalla, NY.*

Angiotensin (Ang) II has been demonstrated to play a role in the progression of HIVAN. In the present study, we evaluated the role of variable angiotensinogen (Agt) copies in the progression of renal lesions in Tg26 mice, a mouse model of HIVAN.

Tg26 mice with various copies of Agt (*Agt*-2, *Agt*-3, and *Agt*-4) were evaluated for expression of AT1 and AT2 receptors during embryogenesis (E13, E15, and E18) and after birth, on days 1 and 10 by immunohistochemical and *in situ* hybridization techniques, and confocal microscopy. In addition, mice were evaluated for severity of proteinuria, BUN, renal lesions, collagen deposition, blood pressure, arteriosclerosis, and renal tissue mRNA for PAI-1 at 4, 9, 12 and 16 weeks (n=5).

During embryogenesis and on days 1 and 10, renal cells showed greater expression of AT2 receptors when compared to AT1 receptors. Both tubular cells and podocytes showed temporal and spatial relationship between AT1 and AT2 receptors. Mice with 4 *Agt* copies showed lower blood pressure (BP, mean 110/80 mm Hg) at 4 and 8 wks vs. mice with two Agt copies (BP, mean 140/90 mm Hg). Four wks old mice with 4 *Agt* copies displayed attenuated expression of PAI-1 when compared to age-matched mice with 2 *Agt* copies; whereas, 16 wks old mice with 4 *Agt* copies showed 3-fold greater PAI-1 expression than mice with age-matched mice with 2 *Agt* copies ($P<0.01$). Nine weeks old *Agt*-2 mice developed renal lesions that were more severe than those seen in age-matched *Agt*-3 and *Agt*-4 mice. However, 16 wks old *Agt*-4 mice, displayed more advanced renal lesions when compared to *Agt*-2 mice.

We conclude that higher *Agt* copies in transgenic mice induce reno-protective effect during initiation of HIVAN; an effect that may be mediated through the temopro-spatial expression of AT1 and AT2 receptors during embryogenesis and post natal period. This protective effect however, dissipates at more advanced age possibly related to predominance of AT1 receptors.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO713**

**Role of PD-L1 and PD-1 Interaction in Tubular Cell-Cell HIV-1 Dynamism** Hersh Goel, Divya Salhan, Dileep Kumar, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.*

In renal biopsy studies of HIV-associated nephropathy (HIVAN), renal tubular cells have been reported to be infected by HIV-1. In addition, it has been suggested that tubular cells may be serving as a reservoir for HIV-1 infection. We previously reported that tubular cells had potential to transmit HIV-1 to T cells and facilitated replication of the transmitted HIV-1 in T cells (J. Am Soc. Nephrol. 18:780-787, 2007). In the present study, we examined whether interaction of PD-L1 (expressed by tubular cells) with its specific receptor PD-1

(expressed by normal as well as HIV-1 infected T cells) had potential to induce apoptosis/necrosis of infected T cells and thus, providing release of HIV-1 proteins and RNAs for ednocytosis by tubular cells.

Human tubular cells (HK2) were pulsed with HIV-1 (X4 HIV-$1_{92HT599}$) for two hours, followed by rescue of virus by tubular cells. One week later, HIV-1 infected or non- infected CD4+ ive T cells were incubated either with HK2 cells or 293T cells (negative for CD4 and PDL-1) for 48 hours. Subsequently, CD4+ ive T cells were assayed for proliferation and apoptosis by FACS analysis. In addition, HIV-1 proteins and RNAs were assayed in incubation media. Tubular cells probed for HIV-1 mRNA and protein expression.

The population of CD4+ ive T cells dropped significantly after interaction with HK-2 cells, and the effect was more pronounced in HIV-1-infected CD4+ ive T cells. Also, there was increased apoptosis in CD4+ ive T cells on interaction with HK2 indicating that the mechanism of depletion is via apoptosis, and is more significant in HIV-1 infected CD4+T cells. HIV-1 infected CD4+ ive T cells express higher level of PD-1, suggesting involvement of PD-1: PD-L1 pathway. Pretreatment of HK-2 cells with anti-PD-L1 antibody significantly reduced apoptosis on CD4 T cells. HK2 incubated with infected CD4 +ve cells also showed expression of HIV-1 proteins. Altogether, our results indicate that tubular cells may preferentially deplete CD4+ ive T cells especially those infected with HIV-1. Moreover, there may be bidirectional traffic of HIV-1 during interaction of tubular cells and CD4+ ive T cells.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO714

**Discrete Regulation of VEGF-A and -C in Human Proximal Renal Tubular Cells under Hypoxic and/or Inflammatory Conditions – Inhibitory Effects of Glucocorticoids on Basal and Inducible VEGF Expression** Hideki Kimura,[1] Hidehiro Sugimoto,[2] Kazuko Kamiyama,[1] Daisuke Mikami,[1] Kenji Kasuno,[1] Naoki Takahashi,[1] Haruyoshi Yoshida.[1] *[1]Div of Nephrol, Dept of General Med, Fukui Univ, Fukui, Japan; [2]Dept of Clin Lab, Fukui Univ Hosp, Fukui, Japan.*

VEGF-A and C are a main inducer of angiogenesis and lymphangiogenesis which are closely involved in renal fibrosis status after renal injury. Although proximal renal tubular cells produce the two VEGFs and probably affect reconstitution of interstitial vessel networks in the injured kidney, the production alteration and its mechanism have not been fully clarified in the renal cells under hypoxia and/or inflammation and on glucocorticoid (GC) treatments as an essential remedy for severe nephritis.

Confluent human proximal renal tubular epithelial cells (HPTECs) were treated with TGF-β (1-5 ng/ml), TNF-α (10 ng/ml), high glucose (HG; 450mg/dl) and/or GC (0.1-1 μM) for up to 48 h under normoxic or hypoxic conditions. TGF-β and hypoxia induced VEGF-A production (2-3 fold and 1.5 fold) via p38 MAPK but not ERK pathway and Src family tyrosine kinase pathway, respectively, while TNF-α and HG had no influence on the production. In contrast, TNF-α and HG increased VEGF-C production (3 fold and 1.3 fold), while TGF-β and hypoxia did not change the production. Induction of VEGF-C by TNF-α was mediated largely via p38 MAPK pathway but not ERK or Src family pathway. Dexamethasone (DXA) did not affect hypoxia-inducible factor-1α amount and activity under hypoxia, nor did hypoxia influence on DXA-activated GC response element activity. Hydrocortisone (HC) and DXA down-regulated basal VEGF-A and -C production, independently and dependently of the GC receptor, respectively, without VEGF mRNA destabilization. Finally, GC also decreased hypoxia-induced VEGF-A production and TNF-α-induced VEGF-C production.

Theses results suggest that VEGF-A and -C are discretely regulated by hypoxia, inflammatory cytokines, and HG in HPTECs and that GCs suppress hypoxia- or inflammation–stimulated expression of the two factors, potentially leading in part to impairment of orderly neovascularization and ensuing tissue restoration after renal injury.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO715

**Experimental Sepsis in Tamm-Horsfall Protein-Deficient Mice** Hajamohideen S. Raffi,[1] James M. Bates,[1] Zoltan G. Laszik,[2] Satish Kumar.[1] *[1]Medicine, University of Oklahoma HSC and VA Medical Center, Oklahoma City, OK; [2]Pathology, University of California San Francisco, San Francisco, CA.*

Tamm-Horsfall protein (THP) is the most abundant protein in normal urine. In previous studies, we generated THP gene knockout ($THP^{-/-}$) mice by homologous recombination and found them to be more susceptible to experimentally induced urinary tract infection. In this study, we examined the susceptibility of $THP^{-/-}$ mice to systemic sepsis.

Eight $THP^{+/+}$ and $THP^{-/-}$ mice were selected. Baseline urine and blood samples were collected. Sepsis was induced by the cecal ligation and puncture (CLP) method. The mice were euthanized at 24 hrs. The abdomen was opened. Urine was collected by needle aspiration from the bladder and blood by cardiac puncture. Bacteremia and bacteriuria were assessed by blood and urine cultures. Colony forming units (CFU) were counted and expressed as CFU/ml. Systemic response to sepsis was assessed by total and differential white blood cell count. Acute kidney injury was assessed by renal histology and by neutrophil gelatinase-associated lipocalin (NGAL) levels in plasma and urine. Proliferative activity in renal tubules was evaluated by quantitation of proliferating cell nuclear antigen (PCNA) expression. Renal function was assessed by serum cystatin C.

Plasma NGAL levels were lower ($THP^{+/+}$, 336 ng/ml ± 56 vs. $THP^{-/-}$, 171 ng/ml ± 23 p = 0.008) and serum cystatin C levels were higher ($THP^{+/+}$, 569 ng/ml ± 37 vs. $THP^{-/-}$, 667 ng/ml ± 36, p = 0.040) in $THP^{-/-}$ mice at baseline but similar between the two groups 24 hours after CLP. Plasma NGAL levels rose after CLP in both groups but the increase was higher in $THP^{-/-}$ mice ($THP^{+/+}$, 66 ± 93 vs. $THP^{-/-}$, 335 ± 109 p = 0.041). No significant difference were found between $THP^{-/-}$ and $THP^{+/+}$ mice in bacteremia, bacteriuria, blood white cell counts, renal histology, PCNA indices, and urine NGAL levels.

We conclude that absence of THP has minimal effect on the response to sepsis in mice.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO716

**The Role of IL-6 in Neutrophil Recruitment to Glomerular Endothelial and Epithelial Co-Cultures, a Model of the Glomerular Micro-Environment** Sahithi Josna Panchagnula,[1] Simon C. Satchell,[3] Moin Saleem,[3] Lorraine Harper,[1] Helen M. Mcgettrick,[2] Edward Rainger,[2] Samantha Tull,[1] Julie M. Williams,[1] Caroline O. S. Savage.[1] *[1]School of Immunity, Infection and Inflammation, University of Birmingham, Birmingham, United Kingdom; [2]School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, United Kingdom; [3]Academic Renal Unit, Univeristy of Bristol, Bristol, United Kingdom.*

**Background:** We have examined the hypothesis that glomerular epithelial cells (GEpCs) co-operate with glomerular endothelial cells (GEnCs) to modulate neutrophil recruitment in a simulated inflammatory environment and have investigated the mechanisms involved in the demonstrated cross-talk.

**Methods:** An *in vitro* static co-culture system was used to study neutrophil recruitment by endothelium in the presence of GEpCs following cytokine stimulation (0-100U/ml TNF-α for 4h). The conditionally immortalized human GEnCs and GEpCs were cultured on opposite sides of 3.0 μm pore transwell filters in double chamber wells allowing cell-cell interactions. Neutrophils were added to the upper chamber in contact with the GEnCs. Soluble mediators released into the cell supernatants were analysed by multiplex assay. Anti–IL-6 monoclonal antibody (5μg/ml, R&D systems) was used to block IL-6 function.

**Results:** I*n vitro* static co-cultures of TNF-treated GEnCs/GEpCs demonstrated reduced neutrophil recruitment by up to 40 % compared to monocultures of GEnCs. The reduced neutrophil recruitment was dependent on both cell-cell contact and on soluble mediator(s) release. Supernatants from co-cultures showed an 8-fold increase in soluble IL-6 concentrations compared to monocultures following analysis by multiplex assay. Function-neutralising anti–IL-6 antibody added from the initiation of co-culture, reconstituted the neutrophil recruitment to endothelium to control levels.

**Conclusions:** IL-6 can down regulate neutrophil recruitment during GEnC/GEpC co-culture indicating that it plays a negative role in local inflammatory reactions. Thus, IL-6 may be crucial in neutrophil recruitment during pathogenesis of neutrophil-mediated glomerulonephritic disease.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO717

**Targeting Keap1-Nrf2 Pathway Ameliorates Renal Inflammation and Fibrosis in Mice with Protein-Overload Proteinuria** Carlamaria Zoja,[1] Daniela Corna,[1] Monica Locatelli,[1] Chiara Corna,[1] Sara Cattaneo,[1] Colin Meyer,[3] Giuseppe Remuzzi,[1,2] Ariela Benigni.[1] *[1]Mario Negri Institute, Bergamo, Italy; [2]Ospedali Riuniti, Bergamo, Italy; [3]Reata Pharmaceuticals, Irving, TX.*

Bardoxolone methyl (RTA 402), a semi-synthetic triterpenoid, is an Antioxidant Inflammation Modulator (AIM) in clinical development for chronic kidney disease. It exerts antioxidant and antiinflammatory activity via activation of the Keap1-Nrf2 pathway, which in turn suppresses NF-kB activity. We previously showed that in experimental proteinuric nephropathies, proteinuria increases NF-kB activity. By regulating the transcription of genes encoding proinflammatory and fibrogenic molecules involved in renal injury, NF-kB is a key determinant of proteinuria-induced tubulointerstitial injury. Here we investigated the effect of a bardoxolone methyl analog RTA 405 in a murine model of protein-overload proteinuria characterized by tubulointerstitial inflammation and fibrosis. Mice (n=20) underwent uninephrectomy. Five days later they received daily i.p. injections of BSA up to 28d and were treated with vehicle or RTA 405 (100mg/kg/d in the chow). In vehicle-mice proteinuria increased within 2d after BSA, peaked at 14d (77±17 mg/d) and remained sustained at 28d (55±16 mg/d). RTA 405 significantly limited proteinuria at 14d (38±10 mg/d, $P<0.05$) and maintained it at lower level than vehicle-mice at 28d (41±17 mg/d). The presence of a non-responder mouse in the RTA 405 group precluded achievement of statistical significance at 28d. Interstitial accumulation of monocytes/macrophages was lowered by therapy (47±8 vs 70±8 cells/HPF, $P<0.01$). The antiinflammatory action of RTA 405 was accompanied by an antifibrogenic effect as indicated by significantly reduced peritubular α-SMA staining in RTA 405 compared to vehicle-treated mice (score:1.6±0.1 vs 2.2±0.1). In summary, bardoxolone methyl analog limited proteinuria, interstitial inflammation, and fibrosis in a model of proteinuric nephropathy. Studies are ongoing to assess the mediators of inflammation responsible for the protective effects. These data pave the way for clinical application of AIMs as antiinflammatory agents in progressive nephropathies.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO718**

**Inhibition of JAK/STAT Pathway Ameliorates TNF-α-Induced Adhesion Molecule Expression in Vascular Endothelial Cells** Ki Dong Lee,[1] Jung Eun Lee,[1] Duk Hoon Kim,[1] Yujin Jung,[1] Aesin Lee,[1] Mi Jeong Sung,[2] Sik Lee,[1] Kyung Pyo Kang,[1] Sung Kwang Park,[1] Won Kim.[1] *[1]Internal Medicine, Research Institute of Clinical Medicine and Diabetes Research Center, Chonbuk National University Medical School; [2]Food Function Research Center, Korea Food Research Institute.*

Vascular endothelial cells play an important role in leukocyte trafficking during inflammatory process. Pro-inflammatory cytokines activate the endothelial cells to express cell adhesion molecules. Janus kinase/signal transducers and activators of transcription (JAK/STAT) is one of the major intracellular cytokine signaling and involved in pathogenesis of renal ischemia/reperfusion injury, diabetic nephropathy. The purpose of our study is to investigate the mechanism of TNF-α-induced cell adhesion molecule by regulation of JAK/STAT pathway in the human umbilical vein endothelial cells (HUVECs).

JAK3 inhibitor, JANEX-1, decreased TNF-α-induced ICAM-1, VCAM-1 and fractalkine expression in the HUVECs. JAK3 inhibitor mediated downregulation of adhesion molecule expression was mediated through suppression of NF-κB activation and STAT3 phosphorylation. The HUVECs treated with TNF-α decreased the suppressor of cytokine signaling (SOCS)-1 and SOCS-3 expressions, however, JAK3 inhibitor attenuated these decrease. Furthermore, JAK3 inhibitor inhibited monocyte adhesion to HUVECs stimulated by TNF-α.

These results demonstrated that inhibition of JAK/STAT pathway by JANEX-1 ameliorates TNF-α-induced adhesion molecule expression in the HUVECs.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO719**

**Intrarenal Dopamine Protects Against Angiotensin II-Induced Renal Injury** Shilin Yang, Haichun Yang, Bing Yao, Xiaofeng Fan, Mingzhi Zhang, Raymond C. Harris. *Department of Medicine, Nephrolology Division, Vanderbilt University School of Medicine, Nashville, TN.*

Angiotensin II (Ang II) is a major contributor to progressive renal injury, and Ang II antagonism is a mainstay of therapy in chronic renal disease. Intrarenal dopamine can decrease intrarenal AT1 receptor expression and antagonize Ang II actions in the kidney. We have recently reported that intrarenal dopamine attenuated STZ-induced diabetic nephropathy using 129J/sv COMT KO mice, which have increased kidney dopamine levels due to deletion of the major intrarenal dopamine metabolizing enzyme. Intrarenal dopamine is primarily biosynthesized through the actions of aromatic amino acid decarboxylase (AADC) in the proximal tubule. We have generated mice with kidney dopamine deficiency through selective deletion of AADC in the proximal tubule (AADC KO). The current study investigated whether intrarenal dopamine deficiency would augment renal injury in a model of chronic Ang II infusion (1.4 mg/kg/day) using AADC KO mice on 129J/sv background. Ang II elevated blood pressure to similar levels in WT mice and AADC KO mice (205 ± 4 vs. 200 ± 5 mmHg of AADC KO). Albuminuria was similar in WT mice and AADC KO mice at baseline. Four weeks after Ang II infusion, albuminuria was significantly increased in AADC KO mice vs. WT mice (419 ± 55 vs.187 ± 38 μg/24h, P< 0.01). AADC KO mice showed more vacuoles in proximal tubular epithelial cells and more glomerulosclerosis than WT mice (0.36±0.01 vs. 0.17±0.02, p<0.05), along with more F4/80-positive macrophage infiltration, increased expression of nitrotyrosylated proteins (marker of oxidative stress), Kim-1 (a marker of renal injury) and CTGF. In summary, these results indicate that intrarenal dopamine activity serves as a major defense against Ang II-mediated renal injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO720**

**Inhibition of Nephrotoxic Nephritis in Mice by the NF-kappaB Inhibitor DHMEQ** Chen Yao,[1] Syed Muhammad Fahad Imran,[1] Eveline Piella,[1] Ulf Panzer,[1] Rolf A. Stahl,[1] Kazuo Umezawa,[2] Gunther Zahner,[1] Friedrich Thaiss.[1] *[1]III. Medical Department, Univ. Hospital, Hamburg, Germany; [2]Faculty of Science and Technology, Keio University, Yokohama, Japan.*

Experimental nephrotoxic nephritis (NTN), a Th1/Th17 cell-mediated disease induced in mice serves as a model of human rapid progressive glomerulonephritis. In this model proinflammatory cytokines activate the transcriptional factor nuclear factor-kappa B (NF-κB) in the kidney. Therefore the therapeutic effect of the NF-κB inhibitor, dehydroxy methyl epoxyquinomicin (DHMEQ), was examined.

NTN was induced by intraperitoneal injection of a sheep polyclonal anti-glomerular basement membrane antibody. DHMEQ (100 mg/kg/bw) was injected three times a week starting prior to disease induction. Clinical and histopathologic severities were determined during a 14 days observation period. Activation of NF-κB was assessed by gel shift experiments. T cells infiltrating the kidneys were quantitated by immuno-histochemistry and kidney chemokine expression determined by RT-PCR.

Kidney function significantly improved in DHMEQ treated mice at early time points (day 4) after the induction of NTN, during later time periods, however, there were no significant differences. Glomerular sclerosis score significantly (p<0.05) improved in treated mice and the number of infiltrating CD3-, Mac2- and F4/80- positive cells was significantly reduced. The reduction in infiltrating inflammatory cells correlated with a significant decrease (p<0.01) in chemokine expression as determined for CCL2, CCL5 and CXCL10. Activation of NF-κB was almost completely abolished in DHMEQ pretreated mice at early time points after NTN-induction not, however, during later time points at day 14.

In conclusion, our studies indicate that NF-κB activation during NTN can be blocked by DHMEQ. Inhibition of NF-κB activation significantly improved kidney function, glomerular morphology and renal inflammatory cell infiltration at early time points. DHMEQ inhibits localization of NF-κB in the nucleus and the inhibitory effect by DHMEQ is more potent on p50/RelA than on p50 homodimers. DHMEQ therefore might be a novel potential therapeutic applicant for patients with glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO721**

**Characterzation of TNF-Induced Inflammatory Pathways in the Glomerulus** Anela Taubitz, Volker Vielhauer. *Nephrologisches Zentrum, Medizinische Poliklinik, Ludwig-Maximilians-University, Munich, Germany.*

To investigate TNF-mediated inflammatory responses in the glomerulus we isolated glomeruli from wild-type (WT) and TNF receptor (Tnfr) 1 and 2 double-deficient mice without TNF signaling capacity. Gomeruli were challenged with murine TNF in vitro, and global gene expression levels were determined by microarray.

In TNF-stimulated WT glomeruli we found 290 differentially expressed genes, with 219 up-, and 71 down-regulated genes compared to Tnfr1,2-/- glomeruli. Functional groups of TNF-regulated genes included chemokines, cell adhesion proteins, cytokines, NF-kB regulators, apoptosis mediators, cell cycle regulators, and matrix metalloproteases. The proinflammatory chemokine Ccl2 was the most prominently regulated gene in TNF-stimulated WT glomeruli, with a 50-fold induction compared to Tnfr1,2-/-. DAVID analysis identified 136 enriched gene ontology (GO) categories in WT compared to Tnfr1,2-/- glomeruli. Enriched GO terms were highly immune related. Moreover, DAVID found 8 biological pathways enriched in WT glomeruli, including cytokine-cytokine receptor interaction, toll-like receptor signaling, cell adhesion molecules, complement pathway, and apoptosis.

Normal mouse glomeruli expressed both Tnfrs on mRNA and protein level. To determine their relative contribution to TNF-induced glomerular inflammation, we performed expression analyses on TNF-stimulated Tnfr1- and Tnfr2-deficient glomeruli. In Tnfr1-/- glomeruli 219 differentially expressed genes and 77 over-represented GO categories were identified compared to WT, being mostly identical to Tnfr1,2-/- glomeruli. In contrast, we found only 5 genes that were differentially expressed in Tnfr2-/- glomeruli. The predominant role of Tnfr1 in mediating glomerular inflammation was also suggested by compartment-specific flow cytometry of glomeruli isolated from WT and Tnfr-deficient mice after i.p. injection of TNF. Glomerular leukocytes were markedly reduced in Tnfr1-/- and Tnfr1,2-/- mice, with Tnfr2-deficiency only affecting accumulation of mononuclear phagocytes.These data demonstrate a prominent role of Tnfr1 in mediating TNF-induced inflammatory responses in the glomerulus. Only few Tnfr2-specific effects were identified.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO722**

**Increased Plasma sVCAM-1 Is Associated with Severity in IgA Nephropathy** Li Zhu,[1,2,3] Sufang Shi,[1,2,3] Lijun Liu,[1,2,3] Jicheng Lv,[1,2,3] Hong Zhang.[1,2,3] *[1]Renal Division, Peking University First Hospital; [2]Peking University Institute of Nephrology; [3]Key Laboratory of Renal Disease, Ministry of Health of China.*

**BACKGROUND**

A considerable part of IgAN patients presented with histological vasculitic/crescentic lesions in glomeruli, accompanied by distinct neutrophils and/or monocytes infiltration, indicating the activation of vascular inflammation in IgAN. sVCAM-1 was a well-proven marker for endothelial injury under inflammatory processes. In the present study, using sVCAM-1 as the marker for vascular inflammation, we investigated its association with IgAN severity, and further explored the mechanism of vascular inflammation in IgAN.

**METHODS**

In our study, 326 biopsy-proven IgAN patients were enrolled. Clinical manifestations at the time of renal biopsy, including eGFR, 24h urine protein excretion and blood pressure, were collected from clinical records. The Oxford classification was used for evaluating the pathological lesions. Plasma sVCAM-1 was measured by ELISA. In vitro, human umbilical vein endothelial cells (HUVEC) were treated with 12.5-200ug/ml IgA1. After 24 and 48 hours treatment, sVCAM-1 in culture supernatant was also measured by ELISA.

**RESULTS**

The plasma sVCAM-1 was significantly correlated with eGFR (r=-0.191, p=0.001) and 24h urine protein excretion (r=0.200, p=0.001), but not systolic blood pressure and diastolic blood pressure, in patients with IgAN. Histologically, patients with higher scores in the variable tubular atrophy/interstitial fibrosis showed significant higher sVCAM-1 (T2:T1:T0=941.98ng/ml : 699.28ng/ml : 700.33ng/ml, p=0.001), patients with higher scores in the variables mesangial hypercellularity and endocapillary hypercellularity showed relative higher sVCAM-1, although do not reach the statistic significance (M1:M0= 749.17ng/ml : 688.15ng/ml, p=0.066; E1:E0= 733.33ng/ml : 716.72ng/ml, p=0.610). In vitro, IgA1 induced the increasing sVCAM-1 in HUVEC culture supernatant in a time- and dose-dependent manner.

**CONCLUSION**

Increased plasma sVCAM-1 was associated with severe clinical and pathological findings in patients with IgAN, which might be resulted from endothelial cells under IgA1 stimulation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO723**

**Interaction of Th1 and Th17 Immune Response in Experimental Glomerulonephritis** Hans-Joachim Paust,[1] Jan-Hendrik Riedel,[1] Jan-Eric Turner,[1] Joachim Velden,[2] Oliver M. Steinmetz,[1] Hans-Willi Mittrücker,[3] Rolf A. Stahl,[1] Ulf Panzer.[1] *[1]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]Institut für Pathologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [3]Institut für Immunologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

Autoreactive CD4+ T cells have been associated with the pathogenesis of autoimmune diseases including crescentic glomerulonephritis. We have recently shown that in addition to Th1 (IFNγ) -mediated immune response IL-17, produced by Th17 cells, contributes to renal tissue damage in experimental glomerulonephritis. However, little is known to date about the time course of Th1 and Th17 cell infiltration, and their potential interactions in immunologically mediated renal disease. Therefore, we analyzed the time dependent interaction of renal and systemic Th1 and Th17 responses in a mice model of glomerulonephritis (nephrotoxic nephritis).

Th17 effector cells could already be detected at day 5 while Th1 effector cells appeared later at day 7 after nephritis induction. The number of infiltrating Th17 cells declined in contrast to numbers of Th1 cells during the course of renal inflammation implicating a time-dependent cascade for renal T cell infiltration and activity.

Systemic immune responses analyzed by antigen-stimulated splenocyte production of IL-17 or IFNγ coincided with the kinetics of renal Th1 and Th17 cell infiltration.

To study functional interactions of Th1 and Th17 immune response in vivo we transferred splenocytes from mice genetically deficient in IFNγ or IL-17 into RAG-1 deficient mice, which lack mature B and T lymphocytes and induced NTN. The renal and systemic Th17 effector cell response was remarkably elevated in mice lacking IFNγ, whereas the Th1 response was unaltered in IL-17 deficient mice. These findings suggest that the Th17 cell function might be downregulated by IFNγ during renal inflammation.

This negative feedback may be important in limiting immune-medited kidney injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO724**

**Mast Cells Mediate Acute Kidney Injury Induced by Cisplatin** Shaun Andrew Summers, Jacky Chan, Oliver M. Steinmetz, Poh-Yi Gan, A. Richard Kitching, Stephen R. Holdsworth. *Centre for Inflammatory Diseases, Monash University, Melbourne, Victoria, Australia.*

**Aim:** To define the role of Mast Cells (MCs) in experimental acute kidney injury (AKI) induced by cisplatin.

**Methods:** To determine the role of MCs in cisplatin nephrotoxicity we administered cisplatin (12mg/kg) to C57BL/6 wild-type (WT) and Wsh mice, which are deficient in MCs. AKI was assessed 4 days later. Serum blood urea nitrogen (BUN) was used as a measure of renal function, while a semi-quantative scoring system (0-4) was used to assess tubulo-interstitial histological injury. Subsequently we treated WT mice with the MC stabilizer, sodium chromoglycate, or saline control, followed by cisplatin. AKI was assessed 4 days later. Finally, we studied the effect of neutrophil depletion on AKI due to cisplatin therapy in WT mice.

**Results:** Four days after cisplatin treatment AKI was significantly attenuated in Wsh mice compared to WT controls. Serum BUN was significantly decreased (109±6 vs. 45±12mmol/l, P<0.001) in Wsh mice. Assessing tubulo-interstitial histological injury, Wsh mice were afforded significant protection (2.9±0.3 vs. 1.4±0.1, P<0.001) after cisplatin treatment. Consistent with renal injury attributable to MCs, kidney MC chymase expression was elevated in WT mice after cisplatin therapy. MC chymase expression was not detectable in Wsh mice. Prior treatment of WT mice with sodium chromoglycate followed by cisplatin, was renal protective. Sodium chromoglycate treated mice demonstrated decreased BUN (20.4±2.6 vs. 12.5±1.2mmol/l, P<0.05) and histological injury (3.1±1.2 vs. 2.1±0.1, P<0.001) compared to WT mice treated with saline and cisplatin. Owing to the fact that MCs recruit neutrophils, we depleted neutrophils prior to the administration of cisplatin. Mice which were neutrophil depleted prior to cisplatin therapy displayed decreased serum BUN (22.6±3.4 vs. 12.9±2.0mmol/l, P<0.05) and tubulo-interstitial injury (2.5±0.1 vs.1.7±0.1, P<0.001) than control treated WT mice.

**Conclusion:** MCs induce AKI after administration of cisplatin, renal injury is neutrophil mediated. Therapies aimed at inhibiting MC degranulation, using sodium chromoglycate, offer therapeutic promise in the treatment of AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO725**

**Sirolimus Inhibits Macrophage-Originated Foam Cell Formation through Activating Cholesterol Efflux Pathways** Kun Ling Ma,[1] Xiong Zhong Ruan.[2] *[1]Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China; [2]Centre for Nephrology, Royal Free and University College Medical School, Royal Free Campus, University College London, United Kingdom.*

**Objective** Studies have shown that infiltrated macrophage is one of the main cell components for the foam cell formation, which is involved in the atherosclerosis. The present study was to test if Sirolimus prevent the macrophage-derived foam cell formation under inflammatory stress.

**Methods** The primary macrophages were cultured from human peripheral blood monocytes which were isolated from buffy coats of healthy donors. The lipid accumulation in macrophages was checked by Oil Red O staining and quantitative assay of intracellular cholesterol. The gene and protein expression of cholesterol trafficking related molecules was respectively examined by real-time PCR, Western Blot, and immunohistochemical staining. Cholesterol efflux from macrophages was also measured by using [$^3$H]-labelled cholesterol.

**Results** Sirolimus inhibited lipid accumulation in macrophages and overrode cholesterol efflux reduction induced by IL-1β. Further analysis showed that Sirolimus upregulated the gene and protein of adenosine triphosphate-binding cassette transporter A1 (ABCA1), liver X receptor-a (LXRa), and peroxisome proliferators activated receptor-a (PPARa) in macrophages in the presence of IL-1b. Furthermore, Sirolimus up-regulated the expression of adenosine triphosphate-binding cassette transporter G1 (ABCG1) in macrophages. These regulatory effects of Sirolimus on cholesterol homeostasis were independent of its roles in cholesterol uptake pathways modulated by lipoprotein receptors (LDL receptor, Scavenger receptor, CD36, e.t.c).

**Conclusion** Sirolimus inhibited macrophage-derived foam cell formation by activating ABCA1 and ABCG1 mediated cholesterol efflux pathways, suggesting that Sirolimus may play important roles in the prevention of glomerulosclerotic progression.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO726**

**Triptolide Inhibits MCP-1 and ICAM-1 Synthesis Via Suppressing NF-κb in TNF-α Stimulated Mesangial Cells** Caifeng Zhu, Bin Zhu, Sheng Wei, Yongjun Wang. *Department of Nephrology, Hangzhou Hospital of Traditional Chinese Medicine (Hangzhou Guangxing Hospital), Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China.*

**Background**

TNF-α stimulated NF--κ**b** signal to induce MCP-1 and ICAM-1 secretion of mesangial cells are the major mechanism in renal injury in various renal diseases including primary and secondary autoimmune nephritis. Triptolide can inhibit renal imflammation in immunity mediated renal disease. However, its mechanism remains unclear.

**Method**

Immortalizied rat mesangial cells line was used in this study. Cells were separated into various groups: normal control cells, TNF-α(10ul/L) treated cells, TNF-α and pyrrolidine dithiocarbamate (PDTC) treated cells, triptolide(low dose, median dose and high dose) treated cells stimulated with TNF-α. MCP-1, ICAM-1, I-κb and phosphorilated IκBα were detected using enzyme-linked immunosorbent assay (ELISA) method. NF-κBp65 activation was measured with a kit adopted a new types of gel protein migration rate method (EMSA) with ELISA method in cell nucleus extractive.

**Results:**

TNF-α increased the MCP-1 and ICAM-1 excretion and activation of NF-κB in mesangical cells. TNF-α also reduced cytoplasmic I-κB. Triptolide significantly inhibited the secretion of MCP-1 and ICAM-1 in a dose-dependent manner in TNF-α treated mesangial cells. It reduced the nucleus NF-κB activation in a dose dependent manner. Triptolide also downregulated IκBα phosphorylation.

**Conclusion**

TNF-α activated IκBα to upregulate activation of NF-κB mediating MCP-1 and ICAM-1 secretion promoting the progress of renal inflammation. Triptolide inhibit IκBα phosphorylation and upregulate cytoplasmic I-κB to reduce NF-κBp65 activation, leading to an inhibition of MCP-1 and ICAM-1 secretion in TNF-α stimulated mesangial cells.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO727**

**IL-17 Expression by Tubular Epithelial Cells in Renal Transplant Recipients with Acute Rejection (AR)** A. Loverre,[1] T. Tataranni,[1] G. Castellano,[1] C. Divella,[1] P. Ditonno,[2] M. Battaglia,[2] M. Rossini,[1] Francesco Paolo Schena,[1] G. Grandaliano.[1] *[1]Nephrology, Dialysis and Transplantation Unit; [2]Urology and Transplantation Unit, Univ. of Bari, Italy.*

IL-17 is a key soluble mediator of AR, although its main cellular source in this context remains unclear. Aim of our study was to evaluate IL-17 expression in renal transplant recipients with AR.

To this purpose, we investigated IL-17 protein expression by immunohistochemistry in graft biopsies with acute antibody-mediated rejection (ABMR) (n=20), acute T-cell-mediated rejection (TCMR) (n=10), interstitial fibrosis/tubular atrophy (IFTA) (n=10) and calcineurin inhibitor-induced acute tubular damage (ATD) (n=10). IL-17 staining was mainly localized within graft infiltrating cells in TCMR (IL-17+cells: TCMR 6.4±.3; ABMR .9±.3; IFTA .01±.01; ATD .02±.01 cells+/hpf, p<.0001), whereas ABMR was characterized by a significant increase in tubular IL-17 expression (ABMR 8.0±2; TCMR 3.5±1.5; IFTA .06±.02; ATD .5±.2 IL-17pixel+/total area, p<.0001). IL-17 tubular staining was co-localized with peritubular C4d deposits. The role of complement in IL-17 induction was confirmed by the observation that C3a caused a significant increase of IL-17 protein expression in cultured proximal tubular cells at 48 hours (ELISA: C3a 13.3±4.4, basal 1.0±.2 pg/ml; p=.003. Confocal microscopy: C3a 18.2±.8, basal 6.6±1.4 IL-17+/total area) (p=.001). C3a induced in vitro an increased phosphorylation of JAK2 (250±40% vs basal, p=.02). The inhibition of this tyrosine kinase caused a significant reduction in C3a-induced IL-17 protein expression. ABMR biopsies were characterized by a marked increase of phospho-JAK-2 that co-localized with IL-17. Finally, 12 ABMRs were treated with monoclonal anti-CD3 antibodies and underwent a second biopsy within 30 days after treatment. In this group we observed a significant reduction of both IL-17 protein expression (pre 6.7±3.0; post 2.3±1.0, p=.001) and C4d deposits (pre 2.1±.3; post .6±.1 C4d+/total area).

In conclusion, we demonstrated, for the first time, that tubular cells express high levels of IL-17 in ABMR and this event might be mediated by complement activation featuring this condition.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO728**

**Monocyte and Glomerular Calprotectin in ANCA Associated Vasculitis** Ruth J. Pepper,[1] Nicholas D. Mansfield,[2] Sally Hamour,[2] Ruth M. Tarzi,[2] Charles D. Pusey,[2] H. Terence Cook,[2] Alan D. Salama.[1] *[1]Centre for Nephrology, University College London, United Kingdom; [2]Renal Section, Imperial College London, United Kingdom.*

Macrophages are critical for disease development and progression in experimental and human glomerulonephritis. Different macrophage phenotypes are recognised but no clear in vivo marker has been associated with disease. Calprotectin is abundantly expressed in neutrophils, monocytes and infiltrating macrophages and induces inflammatory responses in endothelium and phagocytes. We investigated the role of calprotectin in patients with AAV as well as in nephrotoxic nephritis.

Methods

AAV patients with active and convalescent disease, healthy controls and patients with end-stage renal disease were studied. Serum calprotectin was determined using a sandwich ELISA. Monocytes from healthy controls were stimulated with ANCA, and calprotectin expression determined by FACS.Immunohistochemistry (IHC) was performed on renal biopsies of AAV patients and kidney sections from wild-type and IL-17-deficient mice, which are protected from glomerulonephritis.

Results

Serum levels measured in acute disease (n=22), remission (n=24), HC (n=10) and ESRD (n=59). Levels significantly increased in both acute disease and in remission compared to HC and ESRD ($p<0.001$). IHC demonstrated prominent glomerular macrophage expression of calprotectin. Monocyte derived macrophages expressed the most calprotectin when stimulated with IFNg/LPS. Incubating monocytes with ANCA augmented calprotectin expression. Despite similar numbers of glomerular macrophages in WT animals compared to protected IL17-deficient animals, there were significantly fewer glomerular calprotectin positive cells in IL-17$^{-/-}$ animals.

Conclusion

Patients with AAV demonstrate elevated circulating levels of calprotectin during both active disease and remission, suggesting a persistently activated phenotype, while tissue infiltrating macrophages express calprotectin within inflamed glomeruli. IFNg/LPS or ANCA stimulation upregulated calprotectin expression in monocytes. Animal studies suggest that despite similar macrophage infiltration, calprotectin expression differentiates disease promoting macrophages from others.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO729**

**Influence of Lectin Complement Pathway Mediated by Mannose-Binding Lectin to Thickness of Glycocalyx and Expression of Syndecan-1 and Glypican-1 in High Glucose Cultured Human Renal Glomerular Endothelial Cells** Songmin Huang, Hongyu Qiu, Wenxing Fan, Wanxin Tang, Ping Fu. *Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

**Objective:** To investigate the effect of lectin complement pathway (LCP) mediated by mannose binding lectin (MBL) on thickness of the Glycocalyx and expression of Sydecan-1 and Glypican-1 in human renal glomerular endothelial cells (HRGECs) cultured in high concentration of glucose.

**Methods:** HRGECs were cultured in vitro, then randomly divided into six groups: MBL deficient normal glucose as controlled groups; MBL(8μg/ml)+normal glucose groups; MBL+mannitol groups; MBL deficient high glucose groups; MBL + high glucose groups; MBL+ high glucose + anti-MBL groups respectively. After 72 hours cultured, Confocal Laser Scanning Microscopy (CLSM) was used to observe the fully hydrated glycocalyx of HRGECs. Real time-RCR ,Fluorescence microscope and Western blot were used to detect the expression of Syndecan-1 and Glypican-1.

**Results:** Compared with controlled groups, the thickness of Glycocalyx on the surface of HRGECs in high glucose group decreased. and the mRNA and protein expression of Syndecan-1 and Glypican-1 were significantly decreased ($P < 0.01$). Immunofluorescence showed that the depositions of MBL and C3 on the surfaces of HRGECs only in MBL + high glucose groups. Compared with MBL deficient high glucose group, the thickness of Glycocalyx in MBL + high glucose groups decreased. The mRNA and protein expression of Syndecan-1 and Glypican-1 were decreased ($P < 0.01$). Compared with MBL + high glucose groups, the thickness of Glycocalyx in MBL + high glucose+ anti-MBL groups increased. The mRNA and protein expression of Syndecan-1 and Glypican-1 were increased ($P < 0.01$) .

**Conclusion:** High concentration of glucose can reduce thickness of Glycocalyx and decrease expression of core protein Sydecan-1 and Glypican-1. Exogenous MBL could promote the depositions of MBL and C3 on HRGECs in high glucose. High glucose together with MBL can downregulate the thickness of Glycocalyx and expression of Sydecan-1 and Glypican-1. The downregulated effect of MBL could be blocked by MBL-blocking antibody.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO730**

**$A_{2A}$ Adenosine Receptors Activation in Accelerated Kidney Injury in Anti-Glomerular Basement Membrane Antibody-Associated Glomerulonephritis Reveals Their Significant Contribution To Protect Kidney from Damage** Gabriela E. Garcia,[1] Luan D. Truong,[2] Carlos Alberto Roncal-Jimenez,[1] Richard J. Johnson,[1] Lili Feng.[2] *[1]Medicine, University of Colorado Denver, Aurora, CO; [2]Medicine, Baylor College of Medicine, Houston, TX.*

$A_{2A}$ adenosine receptor ($A_{2A}R$) is an endogenous inhibitor of inflammation and accelerates wound healing. We investigated whether selective activation of $A_{2A}R$ will suppress inflammation and protect the kidneys from injury in a model of increased severity of established phase of GN. To accelerate kidney damage, uninephrectomy was performed at day 21 after the injection of anti-GBM Ab. Treatment with $A_{2A}R$ agonist or vehicle was started 5 days after nephrectomy, at day 26 after GN induction, and rats were euthanized at day 32. Pharmacological activation of $A_{2A}R$ significantly blocked macrophage (Mφ) infiltration in glomeruli and interstitium. Consequently, the glomerular lesion was significantly reduced in $A_{2A}R$ agonist-treated group. Importantly, $A_{2A}R$ activation resulted in marked reduction in fibrotic crescent formation and TIN injury. At day 32 in the control group, collagen IV (Col IV) deposition was increased in glomeruli, interstitium and vessels. In contrast, Col IV expression was reduced by $A_{2A}R$ activation. Furthermore, in the control group increased α-smooth muscle actin (α-SMA) was observed in the glomeruli and tubules with E-cadherin loss. $A_{2A}R$ activation restored E-cadherin expression and reduced the appearance of α-SMA. In addition, the uninephrectomy-induced increased glomerular volume was lower in the $A_{2A}R$ agonist-treated group. In vitro studies, robust expression of profibrotic molecules, thrombospondin-1, osteopontin-1, TGF-β and TIMP-1 was observed in Mφ isolated form nephritic glomeruli. The expression of these molecules in nephritic kidney is prevented by $A_{2A}R$ activation. Notably, Mφ from nephritic glomeruli express $A_{2A}R$. $A_{2A}R$ agonist might target Mφ $A_{2A}R$ to inhibit expression of profibrotic molecules to arrest progressive GN. These results indicate that $A_{2A}R$ is crucial endogenous anti-inflammatory molecule in GN. Activation of $A_{2A}R$ represents a potential therapeutic strategy for GN during advanced kidney lesion.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO731**

**TWEAK Regulates CXCL16 Expression in Renal Tubular Cells** Maria C. Izquierdo, Ana Belen Sanz, Susana Carrasco, Marta Ruiz-Ortega, Jesus Egido, Alberto Ortiz. *Renal and Vascular Research Laboratory, Division of Nephrology, IIS Fundacion Jimenez Diaz, Madrid, Spain.*

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK, TNFSF12) is a member of the TNF superfamily that regulates the secretion of various chemokines through the engagement of canonical or non-canonical NFKB activation.

We have now explored the regulation of the expression of the cell membrane chemokine CXCL16 by TWEAK in cultured murine tubular cells and in vivo.

In renal tubular cells of proximal (MCT) and distal (NP-1) origin TWEAK up-regulated CXCL16 mRNA expression from 3 to 24 h in a NFKB-dependent manner. Flow cytometry showed an increase in cell surface bound CXCL16 after TWEAK stimulation. TWEAK further potentiated the shedding of CXCL16 from NP-1 cells and there was a trend towards increased cellular CXCL16. Confocal microscopy disclosed that TWEAK increased cytoplasmic and membrane CXCL16 in both MCT and NP-1 cells. Both cell types expressed the receptor for CXCL16, CXCR6. CXCL16 and CXCR6 were found in close association at the cell membrane.

CXCL16 modestly promoted the gene expression of chemokines (MCP-1 and RANTES) and adhesion molecules like ICAM-1 in tubular cells and collaborated with TWEAK in promoting an inflammatory response. However, CXCL16 did not modulate tubular cell proliferation or survival.

In vivo, CXCL16 plays an important role in recruitment of T cells to sites of inflammation and TWEAK induce kidney infiltration by T cells. In this regard, TWEAK increased renal CXCL16 expression and T lymphocyte infiltration in healthy murine kidneys at 4 h and this was inhibited by the NFKB inhibitor parthenolide.

We studied the relationship between TWEAK/Fn14 and kidney CXCL16 expression in acute kidney injury (AKI) induced by folic acid injection. Kidney CXCL16, TWEAK and Fn14 mRNA were upregulated at 24 and 72 h. Tubular cell CXCL16, TWEAK and FN14 protein and interstitial T cells were increased and CXCL16 localized to the basolateral tubular membrane.

In conclusion, TWEAK is a novel regulator of CXCL16 expression in tubular epithelium and CXCL16 has direct proinflammatory actions in renal tubular cells.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO732**

**Factor H-Deficiency Enhances Proteinuria and Glomerular Sclerosis in Mice upon Albumin Overload** Mauro Abbate,[1] Daniela Corna,[1] Monica Locatelli,[1] Simona Buelli,[1] Marina Noris,[1] Carlamaria Zoja,[1] Giuseppe Remuzzi.[1,2] *[1]Mario Negri Institute, Bergamo, Italy; [2]Unit of Nephrology and Dialysis, Ospedali Riuniti, Bergamo, Italy.*

Intraglomerular deposition of complement activation products is often detected in biopsies of patients with chronic proteinuric nephropathies, and evidence in experimental models indicates a determinant role of plasma-derived C3 in the progression of injury to sclerosis as a consequence of dysfunction of the glomerular filtering barrier to circulating proteins (JASN 2008, 19, 2158). The influence of control of the alternative pathway C

activation via the key regulator factor H (Cfh) on the evolution of glomerular injury was yet elusive. Here, we compared uninephrectomized Cfh-deficient vs wild type (WT) mice for their susceptibility to a promoter of proteinuria and C3 deposition, i.e., repeated i.p. injections of bovine serum albumin (BSA, n=6/group). Results showed induction of higher levels of proteinuria in Cfh-BSA mice compared to WT BSA mice (study end, d28:158±22 vs 81±33 mg/d, $p<0.05$; baseline values: 6.2 ±0.7 vs 5.7±0.7mg/d, NS). Cfh-BSA mice developed more abundant C3 deposition in glomeruli by confocal microscopy and more severe glomerular sclerosis (GS % at d28: 20±3.5% vs WT BSA 8.8±3.1%; $p<0.05$; lesion absent at baseline). More severe disease in Cfh-deficient mice appears directly related to local C-mediated injury rather than immunologic/antibody-related factors since BSA antibodies were undetectable in this model and Cfh-deficient mice failed to develop proteinuria when subjected to a strong, ad hoc BSA immunogenic protocol (cationized-BSA). We conclude that Cfh deficiency by allowing intraglomerular activation of complement amplifies deleterious consequences of abnormal passage of C3 across the glomerular capillary filter and worsens glomerular sclerosis caused by protein overload proteinuria. If applicable to humans, these findings raise the possibility that Cfh-related genes might have an impact on the course of proteinuric glomerulopathies regardless of etiology, and strengthen the potential for perspectively new approaches simultaneously targeting proteinuria and uncontrolled C activation to halt disease progression.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO733

**ac-SDKP Reduces Proinflammatory Response in PTEC during Pathogenic Albumin Load** Wo-Shing Au,[1] Gary Chan,[1] Joseph C. K. Leung,[1] Loretta Y. Y. Chan,[1] Hui Y. Lan,[2] Kar Neng Lai,[1] Sydney C. W. Tang.[1] *[1]Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China; [2]Medicine and Therapeutics and Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong, China.*

We recently demonstrated that ACE inhibitor (ACEi) but not angiotensin receptor blocker significantly ameliorated murine adriamycin nephropathy. Despite both being rennin-angiotensin system (RAS) blockers, ACEi additionally prevents the ACE-mediated breakdown of an endogenous tetrapeptide, N-acetyl-seryl-aspartyl-lysyl-proline (ac-SDKP), which possesses potent anti-inflammatory and anti-fibrotic effects in various hypertensive disease models. We, therefore, hypothesized ac-SDKP may be the candidate mediating the renoprotective effect of ACEi and investigated the renoprotective efficacy of ac-SDKP using renal proximal tubule epithelial cells (PTEC) challenged with pathogenic albumin overload and transforming growth factor beta1 (TGFβ1). Ac-SDKP (1-1000nM) dose-dependently inhibited human serum albumin (HSA) induced ERK signalling and TGFβ1 induced SMAD2/3 phosphorylation in primary human PTEC. In addition, acSDKP treatment at 100nM almost completely abolished HSA/ TGFβ1 induced IL-6 mRNA expression, and, to a lesser extent, significantly inhibited HSA/ TGFβ1 induced MCP-1 mRNA expression by 50%. To examine whether the retarded IL-6 gene induction functionally translated into reduced secretion of pro-inflammatory IL-6, we measured the level of IL-6 in culture media by ELISA. It was found that while HSA and TGFβ1 significantly induced IL-6 secretion by 5- and 3- fold respectively, acSDKP almost completely abrogated these responses. In conclusion, our *in vitro* study supports that ac-SDKP reduces proinflammatory response in PTEC during pathogenic albumin load. These results warrant further study of renoprotective efficacy offered by ac-SDKP using animal models with chronic kidney disorders.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO734

**Oncostatin M Is a Potential Early Mediator of the Renal Injury and Repair Response** Valerie A. Luyckx, Catharine Compston, Thomas F. Mueller. *Medicine, University of Alberta, Edmonton, AB, Canada.*

Transcripts associated with the acute phase response (APR) differentiate deceased and living donor kidneys at time of transplantation. Contrary to the expected classical IL6 response, Oncostatin M receptor (OSMR) was the most highly expressed IL6 family receptor. We have shown that OSM induces APR gene expression in renal and vascular cells at 24 hours. We aimed to further characterise the OSM/OSMR APR over time as well as its impact on the anti-inflammatory and repair responses.

In mouse kidney and heart transplants, kinetics of IL6 and OSM transcript expression were measured at 0, 1, 3, 6, 9, 12, 18, 24, 48, 72, 96, 120, 144 and 168 hours post-transplant. These kinetic studies showed an immediate post-transplant increase of OSM, reaching a peak fold-change of 43 after 3 hrs ($p<0.01$) and returning to baseline within the first 24 hrs. In comparison, the IL6 transcript levels increased to a lesser degree but remained higher for a longer period of time.

Kinetic studies were performed in primary vascular smooth muscle (VSMC) and human proximal tubule (PTEC) cells exposed to OSM for 0,1,3,6, 12, 24, 48, 72 and 168 hr. APR gene expression was significantly increased in response to OSM stimulation. These data show that the effect of OSM can be seen as early as 1 hour (IL6, LBP), is generally present by 6 hours (OSMR, SERPINA1, Fibrinogen, LIFR) and persist up to 7 days (OSMR, IL6R, IL6, LIFR, LIF, SERPINA1, Fibrinogen). Baseline SDF1 expression was extremely high in VSMC (6000% HPRT) vs. PTEC (0.4 % HPRT). OSM exposure significantly upregulated SDF1 in both cell types by 3 hours but the effect was longer in PTEC. OSM-mediated induction of SDF1, a stem cell mobilizer, may suggest a role for OSM/OSMR signalling in initiation of renal repair. SOCS3 (suppressor of cytokine signalling 3) expression was significantly increased (up to 20 fold) in PTEC and VSMC from 1 to 48 and 72 hours respectively. Early induction of SOCS3 is consistent with an OSM-induced anti-inflammatory response in both the renal epithelium and the vasculature.

We conclude that OSM is an early inducer of the renal APR and as such, OSM/OSMR signalling may be an important coordinator of the renal response to injury and subsequent repair.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO735

**Triggering Receptor Expressed on Myeloid Cells (TREM)-1 Regulates Monocyte Activation in Kidney Injury** Chien-Liang Chen,[1,2] Jeremy S. Duffield,[1] Ana P. Castano,[1] Shuyu Ren.[1] *[1]Renal Division, Harvard Medical School, Department of Medicine, Boston, MA; [2]Renal Division, Kaohsiung Veterans General Hospital, Department of Medicine, Kaohsiung, Taiwan.*

Using a microarray screen of differentially regulated genes in inflammatory kidney macrophage (MΦ) subpopulations we identified TREM family receptors as highly regulated. Trem-1 ligation results in potent amplification of TLR signaling in infectious diseases. Here we show that Trem-1 is highly upregulated in Ly6C high MΦs in chronic kidney injury, and functions to regulate activation of MΦs by kidney damage-associated molecular patterns (DAMPs) released by injured kidney tissue. Systemic administration of soluble TREM-Fc fusion protein by adenoviral vectors inhibits MΦ activation in vivo and regulates kidney disease progression in mice. Trem-1 is potentially an important therapeutic target to limit monocyte/ MΦ activation in sterile inflammation of the kidney.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO736

**Toll Like Receptor (TLR) Agonists Act as Adjuvants in the Induction of Experimental Autoimmune Glomerulonephritis (EAG) in CD1 Mice** Jitendra K. Gautam, Di Wu, Kline Bolton. *University of Virginia HS, Charlottesville, VA.*

EAG in Wistar Kyoto (WKY) rats can be induced by immunization with recombinant human alpha3(IV)NC1 (m732) resulting in severe glomerulonephritis, immune cell infiltration and proteinuria. Immunization is done with m732 in complete Freund's adjuvant with Mycobacterium tuberculosis h37RA (CFA). The same immunization procedure in CD1 mice results in the development of EAG. Disease progression in the mouse model is slower compared with WKY rats, but the endpoint features are similar.

CFA consists of several known and unknown TLR agonists. The present study was undertaken to examine the ability of various TLR agonists to act as adjuvants in the mouse model of EAG. TLR agonists used in this study were: PAM3CSK4 (TLR1/2), poly IC(TLR3), bacterial LPS(TLR4) and CpG oligos(TLR9). Mice sets (n = 4) were immunized with m732 and individual TLR agonists in saline. m732 with CFA was used as positive and saline as negative control. Spot urine albumin was monitored every two weeks over a 12 week period as a marker for the disease progress. Post experimental kidneys from all the animals were processed for H&E and immunofluorescence staining (total IgG and IgG isotypes) to score the disease severity. ELISA for IgG and isotypes was done on 6 and 12 week serum. Various TLR agonists showed different abilities to induce the disease over age matched controls. TLR2 and TLR3 agonists resulted in a more robust disease initiation followed by CpG. TLR4 agonist bacterial LPS on the other hand acted as a very week inducer of the disease as monitored by urine albumin analysis. Kidney isotype immunofluorescence results and 12 week serum ELISA showed statistical difference in the induction of IgG subclasses by different adjuvant sets over age matched negative control set. Group averages of H&E scores were also different when compared with control set.

These findings suggest that several individual TLR agonists are able to act as adjuvants in the induction of EAG, with variation in their ability to do so. Experiments are underway to confirm if there is a synergistic effect when various agonists are mixed and what combination works best.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO737

**Reduced Expression of ATP-Binding Cassette Transporter A1 (ABCA1) Leads to Increased Release of Interleukin-18 (IL-18) from Mononuclear THP1-Cells** Helge Höfeld,[1] Daniel Kraus,[2] Helmut Geiger,[1] Ingeborg A. Hauser,[1] Stefan Gauer.[1] *[1]Department of Nephrology, Hospital of the Johann Wolfgang Goethe-University, Frankfurt am Main, Germany; [2]Department of Nephrology, Universität Würzburg, Würzburg, Germany.*

Patients with CKD are at high risk for atherosclerotic cardiovascular complications. In experimental chronic kidney disease in $apoE^{-/-}$ mice, impaired cholesterol efflux from macrophages due to downregulation of ABCA1 and concurrent activation of nuclear factor-κB was observed. This inflammatory process may depend on ABCA1, since it seems to act anti-inflammatory by activating the JAK/STAT-pathway upon interaction with apoA-1. Our aim was to characterize the role of ABCA1 in the release of IL-18 from mononuclear cells which is closely linked to the development of atherosclerosis and may contribute to kidney allograft injury.

ABCA1 expression was inhibited by stable transfection of THP1-cells with shRNA-plasmids. THP1-cells were incubated with 200nM TPA to induce differentiation into macrophage-like cells. To initiate ABCA1-expression cells were treated with 9-cis-retinoic acid and 22-R-hydroxycholesterol (15µM each) for 24h. During the last 2h synthesis and release of IL-18 was induced by 100 ng/mL LPS and 3 mM ATP. IL-18 in the supernatant of these cells was measured by ELISA (MBL).

Inhibition of ABCA1 transporter function by 100µM glyburide reduced IL-18 in the supernatant of LPS/ATP treated THP-1 cells from 914±11pg/mL to 309±46pg/mL

(mean±SEM). In contrast, reducing ABCA1-expression by 50% via RNA-interference led to a more than ten-fold increase in IL-18 measured in the supernatant compared to THP1-cells with unimpaired ABCA1 expression (851±375pg/mL vs. 81±28.4pg/mL; mean±SEM).

These data indicate that a low expression of ABCA1 leads to increased secretion of the pro-inflammatory cytokine IL-18 from mononuclear cells and that this effect is independent of the ABCA1 lipid transporter function.

Thus our work suggests that ABCA1 has a protective effect with regards to progression of chronic kidney disease and allograft dysfunction not only by protecting vessels and the kidney from foam cell formation but also by reducing the levels of pro-inflammatory cytokines such as IL-18.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO738**

**Remission, Relapse and Renal Scarring in Experimental Autoimmune Vasculitis** Bahjat Al-Ani,[1] Hamad Al Nuaimi,[1] Stuart W. Smith,[1] Stephen P. Young,[2] Caroline O. S. Savage,[1] Mark Little.[3] *[1]Renal Institute of Birmingham, University of Birmingham, Birmingham, United Kingdom; [2]Rheumatology Research Group, University of Birmingham, Birmingham, United Kingdom; [3]Centre for Nephrology, UCL, London, United Kingdom.*

Introduction: We have previously described an animal model of acute systemic vasculitis, termed experimental autoimmune vasculitis (EAV), which is generated by immunising WKY rats with human myeloperoxidase (MPO). However, the most important clinical problems in this condition relate to vasculitis relapse and management of the consequence of irreversible organ damage.

Methods and Results: WKY rats were immunised with either MPO or human serum albumin (HSA, control) in adjuvant and sacrificed at 8 or 30 weeks, the latter group being divided into a relapse and no-relapse group. In the MPO-immunised animals, haematuria reached a maximum at 6-8 weeks (mean 3.0±0.1) and thereafter declined steadily until 26 weeks (0.6±0.3). There was a parallel decline in anti-MPO antibody level and degree of leukocyturia, but albuminuria increased slightly. Following re-challenge at this point with soluble MPO (without adjuvant) and endotoxin, haematuria (2.3±0.7) and anti-MPO antibody level increased over a period of 4 weeks. Renal scarring, as assessed by silver and picro-sirius red staining, was increased in MPO-immunised animals at 30 weeks compared to 8 weeks. No scarring was evident in control animals. We then went on to determine whether the urinary metabolite profile changed during the evolution of glomerulonephritis, scarring and relapse. Using principal components analysis of urine NMR spectra we found significant clustering in the 3 groups, indicating that active vasculitic glomerular inflammation could be distinguished by the urinary metabolome.

Conclusion: This relapsing model of anti-MPO associated vasculitis will play a role in assessing novel maintenance therapies and biomarkers of disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO739**

**Novel Role of Toll Like Receptor 3 Signal Pathway in the Pathogenesis of the RSV Nephropathy** Yuhong Tao, Zheng Wang. *Department of Pediatric, West China second University Hospital, Sichuan University, Chengdu, Sichuan, China.*

**Background:** Viral transactivation of transcription mediated by nuclear factor kappa B (NF-kB) contributes to the pathogenesis of minimal change nephritic syndrome(MCNS) triggered by respiratory syncytial virus(RSV), and the pathological change of RSV nephropathy is similar with MCNS. But the mechanism of activating NF-kB remains unclear. Toll like receptor 3(TLR3) play a bridging role in connecting virus infection and many kinds of kidney injury.

**Objective:** To investigate whether RSV evoke NF-kB activation through TLR3 signal pathway in RSV nephropathy.

**Methods:** RSV nephropathy model was established. SD rat were sacrificed at days 4,8,14,28,56 and 84 postinoculation ($RSV_4$, $RSV_8$, $RSV_{14}$,$RSV_{28}$, $RSV_{56}$ and $RSV_{84}$) after inoculation of $6\times10^6$PFU RSV. Expression of TLR3 in kidney was measured with Real time RT-PCR, immunohistochemisty and Western blot. MyD88 ,P38MAPK and NF-kB in kidney were detected through immunohistochemisty and Western blot. ELISA was used to detect the concentration of IL-6 in serum .

**Results:** After inoculation of RSV , the urinary protein increased gradually, $RSV_{14}$> $RSV_{28}$>$RSV_8$>$RSV_4$>$RSV_{56}$>$RSV_{84}$Compared with day 4,56,84 and normal group,TLR3 expression was increased significantly at day 8,14 and 28. The increased activity and nuclear translocation of NF-kB was observed at RSV 4,8 ,14 and 28. The concentration of IL-6 in serum and the protein expression of pp-P38 MAPK at RSV4, 8, 14 and 28 was significantly higher than that at RSV56 and 84, while there is no significant change in MyD88 at all time points. **Conclusion:** Toll like receptor 3 mediated activation of NF-kB through a MyD88-independent pathway in RSV nephropathy

Disclosure of Financial Relationships: nothing to disclose

**TH-PO740**

**Statin Attenuates Experimental Anti-Glomerular Basement Membrane Glomerulonephritis Together with the Augmentation of Alternatively Activated Macrophages** Emiko Fujita,[1] Akiko Mii,[2] Megumi Fukui,[2] Akira Shimizu.[1] *[1]Department of Analytic Human Pathology, Nippon Medical School, Tokyo, Japan; [2]Department of Internal Medicine (Division of Neurology, Nephrology and Rheumatology), Nippon Medical School, Tokyo, Japan.*

Macrophages(Mφ) are heterogeneous and include classically activated M1 and alternatively activated M2 Mφ, characterized by pro- and anti-inflammatory functions, respectively. Mφ that express heme oxygenase-1 (HO-1) also exhibit anti-inflammatory effects. We assessed the anti-inflammatory effects of statin in anti-glomerular basement membrane (GBM) glomerulonephritis (GN) in WKY rats, focusing on the Mφ heterogeneity and intraglomerular cytokines. Rats were treated with atorvastatin (20 mg/kg/day) or vehicle from 3 days before induction of anti-GBM GN. Control rats showed infiltration of ED1+ Mφ in the glomeruli at day 3, and developed severe crescentic GN by day 7, together with increased mRNA levels of M1 Mφ-associated cytokines, IFN-γ, TNF-α, and IL-12 ($p<0.05$). In contrast, statin reduced the level of proteinuria, reduced infiltration of ED1+ Mφ in glomeruli with suppression of MCP-1 expression, and inhibited the formation of necrotizing and crescentic lesions. The number of ED3+ activated Mφ decreased in glomeruli with down-regulation of M1 Mφ-associated cytokines. Furthermore, statin augmented ED2+ M2 Mφ with up-regulation of M2 Mφ-associated chemokine and cytokine, CCL17 and IL-10 ($p<0.01$). Statin also increased the number of IL-10-expressing HO-1+ macrophages in glomeruli. In addition, we performed in vitro study using human monocytic U937 cell line. The results indicated that the statin affect monocytes directly and inhibited the Mφ differentiation as well as mediated HO-1+ anti-inflammatory Mφ. Moreover, in vitro study using rat peritoneal Mφ, statin inhibited the development of ED3+ Mφ, and augmented ED2+ Mφ in M2-associaated cytokine environment. We conclude that statin augmented M2 and HO-1+ Mφ and inhibits glomerular inflammation in anti-GBM GN. The anti-inflammatory effects of statin are mediated through inhibition of Mφ infiltration and pro-inflammatory Mφ as well as augmentation of the anti-inflammatory Mφ.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO741**

**Effects of Bardoxolone Methyl (BARD) on Renal Protein Handling and Secondary Nephropathy** Irina Dulubova, Ron Bumeister, Rhesa D. Stidham, Pritam Kambuj, Jeffrey Laidlaw, Colin Meyer, Deborah A. Ferguson, Christian Wigley. *Reata Pharmaceuticals, Inc., Irving, TX.*

The initiating event in tubulointerstitial injury is believed to be defects in glomerular permeability that lead to increased levels of protein in the filtrate delivered to the proximal tubules. It is now understood that protein uptake into the proximal tubules and chronic overload of the protein endocytosis machinery, and not the mere presence of urinary protein, lead to interstitial inflammation and fibrosis. Elevated levels of albumin in proximal tubule cells correlate with increased ROS, which activate NF-κB signaling, leading to increased expression of proinflammatory mediators and subsequent structural damage. BARD is the lead compound from the Antioxidant Inflammation Modulator (AIM) class and is currently being studied in a Phase 2b clinical trial in patients with chronic kidney disease. BARD and other AIMs exhibit broad antioxidant and tissue protective activity and mitigate damage in several models of renal injury through induction of the Keap1-Nrf2 pathway and suppression of NF-κB. Since tubulointerstitial injury is mediated by reabsorption of protein and activation of NF-κB, the effects of BARD on this process were investigated. Expression levels of the components of the megalin/cubilin endocytic complex, NF-κB target genes, and Nrf2 target genes were measured by qPCR following BARD treatment. NF-κB activity was also assessed by western blot. Urine samples from patients were analyzed by SDS-PAGE and western blot. BARD potently induced Nrf2 target genes in multiple cell types of renal origin, suggesting broad renoprotective activity. It inhibited TNFα-mediated increases in proinflammatory targets in glomerular endothelial cells and suppressed albumin-induced proinflammatory signaling in PTECs. BARD also decreased the expression of the components of the megalin/cubilin complex in PTECs. Analysis of urine samples from patients treated with BARD revealed a pattern consistent with downregulation of the megalin/cubilin complex. These data suggest that BARD may be therapeutic in the prevention or treatment of secondary nephropathy through reduction of protein reabsorption and suppression of NF-κB activation.

Disclosure of Financial Relationships: Employer: Reata Pharmaceuticals, Inc.; Ownership: Reata Pharmaceuticals, Inc.

**TH-PO742**

**Bardoxolone Methyl (BARD) Improves Markers of Endothelial Function in Cultured Cells** Deborah A. Ferguson,[1] Christian Wigley,[1] Elke H. Heiss,[2] Verena M. Dirsch.[2] *[1]Reata Pharmaceuticals, Inc., Irving, TX; [2]University of Vienna, Vienna, Austria.*

Endothelial dysfunction is characterized by a decreased vasodilatory response caused by an insufficient amount of bioavailable nitric oxide (NO). The development of chronic kidney disease (CKD) is preceded by the appearance of endothelial dysfunction and oxidative stress markers and the levels of these markers correlate positively with disease progression. Bardoxolone methyl (BARD) is a compound from the Antioxidant Inflammation Modulator (AIM) class that potently induces activity of the antioxidant Keap1-Nrf2 pathway. To determine whether BARD improves endothelial function *in vitro*, primary human umbilical endothelial cells were treated with BARD and the levels of bioavailable NO and reactive oxygen species (ROS) were measured using fluorescent probes. Additionally, eNOS and

HO-1 protein levels were assessed by Western blot. Treatment with BARD increased the amount of bioavailable NO. This increase was accompanied by a dose-dependent decrease in ROS production and a dose-dependent increase in HO-1 protein levels. Consistent with published results for another AIM compound (*Heiss et al., JBC 284:31579-31586*), BARD treatment reduced eNOS protein levels, despite the observed increase in bioavailable NO. This Nrf2-mediated effect ensures that the stoichiometric ratio between eNOS and its cofactor, tetrahydrobiopterin, is maintained thereby preventing uncoupling and production of excess ROS. Taken together, these data demonstrate that BARD improves endothelial function *in vitro*, as measured by increased bioavailable NO and reduced oxidative stress and suggests that BARD may be effective in treating endothelial dysfunction in patients with CKD.

Disclosure of Financial Relationships: Employer: Reata Pharmaceuticals; Ownership: Reata Pharmaceuticals.

### TH-PO743

**Targeting Stat4 Transcription Factor in Severe Lupus Nephritis Reveals Stage Dependent Different Pathophysiological Effects** Julia Menke. *I. Department of Medicine, Johannes Gutenberg-University, Mainz, Rheinland-Pfalz, Germany.*

Polymorphisms in the Stat4 gene have been recently described as risk factors for systemic lupus erythematosus(SLE). Although some polymorphisms showed a strong association with auto-Abs and nephritis, the impact on pathophysiology is still unknown. We studied the impact of Stat4 on the progression of lupus nephritis in MRL-Faslpr mice in two approaches targeting Stat4: A Stat4-deficient MRL-Faslpr strain and blockade of Stat4 by delivering antisense-oligonucleotides. MRL-Faslpr mice lacking Stat4 did not show differences in survival and renal function compared to Stat4 intact MRL-Faslpr (wild type=WT) mice. However, circulating IL-18 levels were markedly elevated in Stat4 deficient compared to WT mice suggesting that IL-18 Stat4 independently might drive the progression of lupus nephritis. In the second approach, Stat4 antisense-(AS), missense-(MS) oligonucleotides or PBS were applied to MRL-Faslpr mice with advanced lupus nephritis. Interestingly, proteinuria was reduced in the AS compared to MS/PBS groups. In contrast to targeting Stat4 at gene level we found a significantly reduced leukocyte infiltration in kidneys treated with AS. However, the levels of circulating IgG subtypes did not differ between groups. In conclusion, we now report that Stat4 independent factors can drive autoimmune lupus nephritis in targeted deleted MRL-Faslpr mice, while blocking Stat4 in advanced nephritis can ameliorate disease suggesting different pathomechanisms in different stages of disease.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO744

**Mesenchymal Stromal Cells Reduces Renal Injury in a Rat Model of Renal Interstitial Fibrosis** Giulia Bedino, Chiara Balenzano, Chiara Rocca, Teresa Valsania, Elisa Gabanti, Grazia Soccio, Marilena Gregorini, Teresa Rampino, Antonio Dal Canton. *Unit of Nephrology Dilaysis and Tranplantation, IRCCS Fondazione Policlinico San Matteo and University of Pavia, Pavia, Italy.*

Mesenchymal stromal cells (MSC) are multipotent cells with anti-inflammatory effect, they have beneficial effect in acute renal damage but are not yet known in chronic kidney disease. Unilateral ureteral obstruction (UUO) is a model of interstitial fibrosis. We investigated whether MSC infusion modified histopathology of kidney in a rat model of UUO.

Methods

We studied two groups of Sprague-Dawley rats. Group A: 12 rats were undergone UUO. Group B: 12 rats were undergone UUO and injected with MSC at day 0. Animals were sacrificed at days 1, 7 and 21 after UUO and kidneys were removed for histological and morphometric analysis. IL6, TGFbeta and angiotensin II levels were measured. ED1 positive cells were evaluated, TGFbeta tissue levels by ELISA, apoptosis by TUNEL, maximal tubular diameter (MDT) was measured. Renal fibrosis was measured by Sirius Red staining.

Results

AT II increased significantly after ureteral ligation in group A on day 7, but did not increase in group B (A day 7: 7.3+/-2, day 0 0.94+/-0.32, p=0.05). IL-6 was significantly higher in group A compared to group B on day 7 (A 101.6+/-6.4, B 36.1+/-0.8, p=0.05). The number of apoptotic cells was significantly greater in group A than group B (day 7 A 1.1+/-0.1, B 0.3+/-0.07, p=0.01). On day 21 serum and tissue levels of TGFbeta were significantly higher in untreated rats compared to MSC treated rats. MTD was significantly smaller in obstructed kidneys of group B than in group A (A 81.29+/-54.3; B 48.9+/-29.13, p=0.0001). In MSC treated rats monocytes number was lower than in untreated rats (day 7, number of ED1 cells/microscopic field A 43.2+/-10.36, B 19.31+/-5.2, p=0.0001). The fibrosis was significantly less severe in group B than in group A (p=0.0001).

Conclusion

Our results demonstrate that MSC infusion in experimental model of UUO attenuates renal injury, inhibiting tubular cells apoptosis, modulating the release of inflammatory and profibrotic cytokines, reducing tubular atrophy and interstitial fibrosis.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO745

**Identification of Tubular Heparan Sulfate as a Major Docking Platform for the Alternative Complement Component Properdin in Proteinuric Renal Disease** Azadeh Zaferani,[1] Romain Vives,[4] Mohamed R. Daha,[1,3] Jelleke J. Hakvoort,[1] Gerjan Navis,[1] Harry Van Goor,[2] Pieter van der Pol,[3] Hugues Lortat-Jacob,[4] Marc Seelen,[1] Jacob Van den Born.[1] *[1]Nephrology, Univ Med Center Groningen; [2]Pathology, Univ Med Center Groningen; [3]Nephrology, Univ Med Center Leiden, Netherlands; [4]Institut de Biologie Structurale, Grenoble, France.*

**Background**: Properdin (P) binds to Proximal Tubular Epithelial Cells (PTEC) and activates the complement system via the Alternative Pathway (AP) in vitro. Cellular ligand(s) for P have not been identified yet. As P was known to interact with solid-phase heparin, we investigated whether heparan sulfate (HS) proteoglycans could be the physiological ligand of P. **Methods**: The binding of purified human P to HK-2 PTECs, and to immobilized HS has been analyzed by ELISA and Surface plasmon resonance spectroscopy (BIAcore). Functional activity of HS-bound P on HK-2 cells was investigated by FACS analysis. The binding specificity of P was determined using HS-degrading enzymes and competition assays with modified and intact HS-like polysaccharides. **Results**: Kidneys from proteinuric rats showed tubular presence of P, which was not seen in control renal tissue. In vitro, HK-2 cells did not constitutively express P. However, P binds to these cells in a dose-dependent fashion. Binding was prevented by heparitinase pretreatment of the cells, and was dose-dependently inhibited by exogenous heparin. ELISA and BIAcore showed a strong dose-dependent interaction between HS and P (KD=128 nM). Pretreatment of HS proteoglycan with heparitinase abolished this interaction in ELISA. Competition assays, using a library of HS-like polysaccharides showed that sulfation pattern, chain length and backbone composition determine interaction. Interestingly, two non-anticoagulant heparin derivatives inhibited P-HS interaction in ELISA and BIAcore. C3 binding upon P incubation on HK-2 cells was HS dependent as shown by FACS. **Conclusion**: Our data identify tubular HS as a novel docking platform for AP activation *via* P, which might play a role in proteinuric renal damage. Our study also suggests non-anticoagulant heparinoids for renoprotection in complement dependent renal diseases.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO746

**Lack of the Lectin-Like Domain of Thrombomodulin Worsens Shigatoxin (Stx)-Induced HUS in Mice** Carlamaria Zoja,[1] Monica Locatelli,[1] Chiara Pagani,[1] Edward M. Conway,[3] Giuseppe Remuzzi,[1,2] Marina Noris.[1] *[1]Mario Negri Institute for Pharmacological Research, Bergamo, Italy; [2]Unit of Nephrology and Dialysis, Azienda Ospedaliera Ospedali Riuniti di Bergamo, Italy; [3]Centre for Blood Research, University of British Columbia, Vancouver, Canada.*

Mutations of thrombomodulin (TM), a transmembrane endothelial cell glycoprotein with anticoagulant, anti-inflammatory and cytoprotective properties, have been recently implicated in the development of atypical HUS. We and others have found that in vitro TM expression was decreased in microvascular endothelial cells exposed to Stx, the causative agent of diarrhea-associated HUS (D+HUS), suggesting that impaired TM activity/levels may play a role also in D+HUS. In a murine model of Stx-HUS we studied the effect on disease course and severity of deletion of the lectin-like domain of TM that is critical for its anti-inflammatory and cytoprotective properties. Disease was induced by i.p. injection of Stx2 (200ng) plus LPS 75ug) in mice lacking the lectin-like domain ($TM^{Led/Led}$ mice) or in wild type mice ($TM^{wt/wt}$). $TM^{Led/Led}$ mice had higher ($p<0.01$) mortality after Stx2/LPS than $TM^{wt/wt}$ mice. $TM^{Led/Led}$ mice exhibited more severe thrombocytopenia and renal failure (BUN: 24h,104±6 vs 54±8 mg/dl, $p<0.01$) compared to $TM^{wt/wt}$. In $TM^{Led/Led}$ mice, intraglomerular fibrinogen deposits were detected earlier (at 3h) than in $TM^{wt/wt}$ mice (at 6h). More abundant fibrinogen deposits were also found in the brain of $TM^{Led/Led}$ mice. Lack of the lectin-like domain of TM resulted in a stronger inflammatory reaction in the kidney after Stx2/LPS, with a higher number of neutrophils infiltrating glomeruli and renal interstitium ($p<0.01$ vs WT mice). Interstitial accumulation of monocytes/macrophages was also more abundant ($p<0.05$ vs $TM^{wt/wt}$ mice). These data document that $TM^{Led/Led}$ mice exhibited earlier onset of HUS that, over the follow-up, was worse than in the $TM^{wt/wt}$ mice, suggesting that loss of TM function may increase susceptibility to the development of thrombotic microangiopathic lesions after Stx infection. These findings suggest that TM may be a therapeutic target for D+HUS.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO747

**Changing Pattern of Pediatric Nephropathy in Korea over the Last Thirty Years; a Review of Pathologic Diagnosis** Kyoung Hee Han,[1] Yun Hye Jung,[1] Hyun Kyung Lee,[1] Il-Soo Ha,[1] Hae Il Cheong,[1] Yong Choi,[2] Hee Gyung Kang.[1] *[1]Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Republic of Korea; [2]Department of Pediatrics, Inje University Haeundae Paik Hospital, Pusan, Republic of Korea.*

During the last thirty years, two major changes that might affect the pattern of pediatric nephropathy were made in Korea; mandatory hepatitis B virus (HBV) vaccination launched in 1985 and nation-wide school urinalysis screening legislated in 1998. In this study, the pathologic diagnosis during the last 30 years at our center, a tertiary-referral hospital of Korea, were studied to assess the pattern of pediatric nephropathy in Korea, .

A total of 1,769 cases (M: F 1.7:1) of pathologic diagnosis were made in pediatric native kidneys between 1980 and 2010. During the recent years since 1998 (n=786), the most common indication of renal biopsy was nephrotic syndrome (NS, in 26.5%), followed by glomerulopathy with gross hematuria (GHU, 24.4%), asymptomatic urinary abnormalities (AUA, 21.6%), and nephropathy with systemic symptoms (12.4%). On the other hand, before the legislation of school urinary screening in 1998 (n=983), NS had been the far more common indication of renal biopsy (49.7%) and AUA was uncommon (4.4%), while the others were similar.

During the recent years, the most common pathologic diagnosis was IgA Nephropathy (IgAN, 32.4%), followed by focal segmental glomerulosclerosis (FSGS, 12.7%) and minimal change disease (MCD, 10.4%). The ranks of diagnoses were different in the past; before 1998, MCD was the most common (24.6%) and IgAN (17.2%) was in the third place following FSGS (13.8%). Membranous nephropathy (MN) decreased over time from 5.2 % in the past to 2 % recently, while the proportions of other diagnoses were similar over the years.

Over the past 30 years, the pattern of pediatric nephropathy in Korea did change reflecting the changes of social system. IgAN increased after legislation of school screening, and MN decreased because the mandatory HBV vaccination eradicated HBV-associated MN, the major cause of childhood MN in Korea in the past. In addition, MCD decreased because biopsy is not performed for steroid-sensitive NS any more.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO748**

**Nationwide Epidemiological Survey of Biopsy-Proven Renal Diseases in Japan by National Hospital Organization** Kensuke Joh, Akira Sugawara. *National Hospital Organization Kyoto Medical Center, Clinical Research Center, Kyoto, Japan.*

National Hospital Organization (NHO) Kidney Network conducted nationwide registry from the 18 centers in order to elucidate epidemiological data including age, gender, laboratory data, and frequency of biopsy-proven renal diseases in Japan, because there has been no concrete data in Japanese population. The 1806 renal biopsies (male 51%, female 49%) from all native kidneys were available from 2004 to 2007. To analyze the data set, the classification of the diagnoses for each case was determined by clinical diagnoses (WHO), etiological diagnoses (A), and morphological diagnoses (B) according to Japan Renal Biopsy Registry System. Most common clinical diagnosis was chronic nephritic syndrome (CNS) (65%) followed by nephrotic syndrome (NS) (24%), rapidly progressive nephritic syndrome (RPNS) (6%), acute nephritic syndrome (ANS) (2%), and others. Most common etiological diagnosis was IgAN (37%) followed by primary GN (33%) and others. Morphological diagnosis consisted of mesangial proliferative GN (42%), minimal change disease (18%), membranous GN (MGN) (9%), Crescentic GN (7%), MPGN (6%) and others. The distribution of the diagnoses was separated by age respecting each clinical and pathological diagnoses A and B. ANS showed a peak at 10's, whereas NS consisting of minimal change NS (33%), MGN (26%), DM nephropathy (11%), and others (30%) as well as RPNS consisting of CrGN showed peak at 60's. CNS showed biphasic peaks and consisted of IgAN (10's and 50's, 49%), primary GN (19%), HSPN (0's and 70's, 5%), and hypertensive nephropathy (7%). Primary GN consisted of MCNS (41%), MGN (26%), crescentic GN (11%), and MPGN 5%. As for CKD profile, IgAN, MGN, DM nephropathy, and hypertensive nephropathy showed peaks at CKD stage 1, stage 2, stage 3, and stage 3, respectively. In conclusion, classifying data set by 3 categorical diagnoses was useful. Although renal biopsies are underwent by different policy in each country, epidemiological data of biopsy-proven renal diseases in Japan can be compared with those of foreign countries and proposed a starting point of international action against CKD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO749**

**Descriptive Epidemiology of Biopsy-Proven Glomerular Diseases in Children from the University of Florida-Shands Hospital Renal Biopsy Registry over Three Decades (1979-2009)** Ruth Indahyung, Corinne Ahmar, Kenneth E. Lamb, Stephen I.-Hong Hsu. *Division of Nephrology, Hypertension and Renal Transplantation, College of Medicine, University of Florida, Gainesville, FL.*

**Purpose**: We determined incidence of all glomerulonephritides (GNs) for children in a new renal biopsy registry for the UF-SH located in Gainesville, FL, representative of the unique racial/ethnic and age composition of the population served by our single academic medical referral center. **Methods**: Among 3,422 cases of renal biopsies performed during 1979-2009, we identified 1,517 unique cases of diagnostic biopsies including 1,331 glomerular diseases (GDs). We studied all 686 cases of GD in children age 0-19, further subcategorized into 4-year age intervals. 91% of cases resided in FL. **Results**: In our study cohort (N=686) the number of cases in each 4-year interval was similar above age 3 (20-25%) and slightly lower below age 3 (13%); M/F ratio was 1.16/1. Caucasians (58.1%) were overrepresented compared to African Americans (25.8%) or Hispanics (4.5%). The most common GNs were minimal change disease/MCD (17.5%), lupus nephritis/LN (16.4%), primary focal segmental glomerulosclerosis (12.4%) and IgA nephropathy/IgAN (10.2%). The most common primary GNs (PGNs) were MCD (26%), FSGS (18.4%) and IgAN (15.2%). The most common secondary GNs were LN (54%), post-infectious GN (9.9%) and diabetic nephropathy/DM (8.5%). The incidence declined dramatically after age 15 for MCD (22-26% vs 7%) and mesangiocapillary glomerulonephritis (10-22% vs 2%). The incidence of rose sharply after age 12 for LN (2-6% vs 31%) and DM (0% vs 8%) was observed. **Conclusion**: Unlike reports of a low incidence of IgAN (1-2%) in children by other registries in the US and India that only studied GN with nephrotic syndrome, we report the novel finding that the incidence of IgAN was as high as FSGS among PGNs. MCD remained the most common PGN. MCD and MesGN were more common in younger children age 0-15; LN and DM were more common in older children age 12-19, indicating that age can be useful in predicting the likely etiology of GN. These findings suggest differences in geographic patterns of incidence in children for the most common GNs in the US.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO750**

**Descriptive Epidemiology of Biopsy-Proven Primary Glomerular Diseases in Adults from the University of Florida-Shands Hospital (UF-SH) Renal Biopsy Registry over Three Decades (1979-2009)** Ruth Indahyung, Corinne Ahmar, Kenneth E. Lamb, Stephen I.-Hong Hsu. *Division of Nephrology, Hypertension and Renal Transplantation, College of Medicine, University of Florida, Gainesville, FL.*

**Purpose**: We determined the incidence pattern of primary glomerulonephritides (PGNs) for adults in a newly created renal biopsy registry for the UF-SH in Gainesville, FL, representing the unique racial/ethnic and age composition of the population served by our single academic medical referral center. **Methods**: Among a total of 3,422 cases of renal biopsies performed during the period of 1979-2009, we identified 1,517 unique cases of diagnostic renal biopsies. Glomerular disease was diagnosed in a total of 1,331 cases of which 638 were adults (age ≥20 years). We studied all 308 cases of PGNs in adults age 20-85. 97% of cases resided in FL. **Results**: Our study cohort (N=308) had similar numbers of young (age 20-39) vs older adults (age ≥40) (48.7 vs 50.6%); M/F ratio was 0.88/1. Caucasians (CCs, 64%) were overrepresented compared to African Americans (AAs, 22.7%) and Hispanics (HSPs, 4.9%). Among the 3 most common PGNs the incidence of IgAN (25.6%), FSGS (24.4%) and MGN (24%) was not statistically different across race except for IgAN in AAs (<1%). Incidence between age groups (young/older adults) was different for IgAN (34/17.9%) and MGN (16/32.1%) and identical for FSGS (24/23.7%). Comparison by race and age (CCs/AAs/HSPs and young/older adults) revealed significant race-age incidence patterns for IgAN (25.3/0.67/4% vs 14.1/1.3/0.64%), FSGS (10.7/12/0% vs 14.1/5.8/1.9%) and MGN (7.3/5.3/0.67% vs 19.9/8.3/1.9%). **Conclusion**: Unlike previous reports, we found that incidence associated with race alone could not predict the likelihood of a pathologic diagnosis of IgAN, FSGS or MGN. Instead, age-race incidence showed that IgAN was most common in young adult CCs. (25.3%), while MGN was most common in older adult CCs (19.9%); FSGS was similarly more common in young AAs (12%) and older CCs (14.1%). These findings suggest differences in geographic patterns of incidence for the most common PGNs in the US.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO751**

**Zero-Time Kidney Histology Predicts Early Graft Function** Helena Viana, Fernanda Carvalho, Maria Joao Galvao, Ana Rita, Fernando Nolasco. *Department Nephrology, Hospital Curry Cabral, Lisbon, Portugal.*

Our purposes are to determine the impact of histological factors observed in zero-time biopsies on early post transplant kidney allograft function. We specifically want to compare the semi-quantitative Banff Classification of zero time biopsies with quantification of % cortical area fibrosis.

Sixty three zero-time deceased donor allograft biopsies were retrospectively semi-quantitatively scored using Banff classification. By adding the individual chronic parameters a Banff Chronic Sum (BCS) Score was generated. Percentage of cortical area Picro Sirius Red (%PSR) staining was assessed and calculated with a computer program.

A negative linear regression between %PSR/ GFR at 3 year post-transplantation was established (Y=62.08 +-4.6412X; p=0.022). A significant negative correlation between arteriolar hyalinosis (rho=-0.375; p=0.005), chronic interstitial (rho=0.296; p=0.02) , chronic tubular ( rho=0.276; p=0.04) , chronic vascular (rho= -0.360;P=0.007), BCS (rho=-0.413; p=0.002) and GFR at 3 years were found. However, no correlation was found between % PSR, Ci, Ct or BCS.

In multivariate linear regression the negative predictive factors of 3 years GFR were: BCS in histological model; donor kidney age, recipient age and black race in clinical model.

The BCS seems a good and easy to perform tool, available to every pathologist, with significant predictive short-term value. The %PSR predicts short term kidney function in univariate study and involves extra-routine and expensive-time work. We think that %PSR must be regarded as a research instrument.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO752**

**Intimal Arteritis Has No Impact on Graft Survival in T Cell-Mediated Rejection** Joana Sellares, Jeff Reeve, Michael Mengel, Declan G. de Freitas, Banu Sis, Philip F. Halloran. *Department of Medicine, University of Alberta, Edmonton, AB, Canada.*

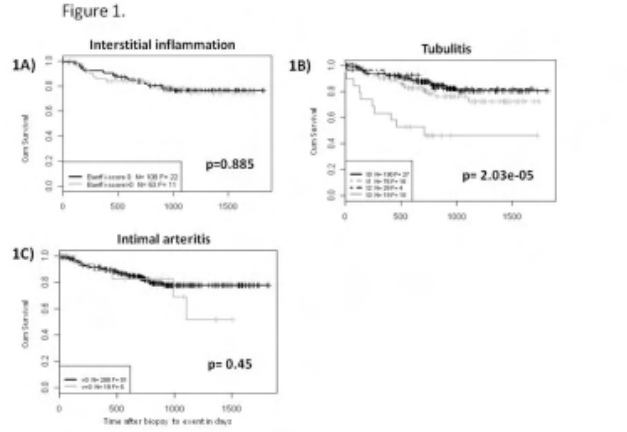
We analyzed 314 kidney transplant biopsies for cause resulting from 314 recipients, between 6 days to 32 years post-transplant. Biopsies were classified according to a modified Banff classification that included C4d-negative antibody-mediated rejection (ABMR). Death censored graft survival analysis was performed using the Kaplan–Meier method.

The current Banff i-score was not associated with graft survival (Fig.1A). Severe tubulitis correlated with worse outcomes (Fig.1B): of the 9 failures, 3 were attributed to polyoma virus nephropathy and all the 6 remaining rejection cases except for one had

donor-specific antibodies (DSA) at the time of biopsy and failure could be attributed either to ABMR, non-adherence or medical conditions. Endothelialitis was present in 19 grafts of which 11 were T cell-mediated rejection (TCMR), 7 ABMR and 1 mixed: 13 of them had v1, five v2 and one case had v3 with fibrinoid necrosis. Intimal arteritis was not associated with worse outcomes (Fig.1C); only 5 grafts failed: 3 were ABMRs and 2 TCMRs. One of these two patients was found to be non-adherent and the graft failed one year after the biopsy having DSA, and the other failed due to medical conditions months after the biopsy. Nearly 50% of the cases with v-lesions were treated with steroids alone, and almost all of them were TCMRs.

Infiltrates in non-scarred areas have no impact on graft survival: mainly represent potentially reversible conditions such as TCMR when properly treated. Severe tubulitis is associated with poor outcomes when is present in other entities than TCMR alone. Endothelialitis does not confer an additional risk when it occurs in TCMR alone regardless of the treatment received. The prognostic significance of these lesions is determined by the disease that is causing them: the lesions *per se* are non-specific.

Figure 1.




Disclosure of Financial Relationships: nothing to disclose

**TH-PO753**

**Cytometry Enhancement of Transplant Kidney Biopsies: Leukocyte Identification** Kimberly A. Muczynski, Nicolae Leca, Susan K. Anderson. *Medicine, University of Washington, Seattle, WA.*

The renal biopsy has been the gold standard for assessing transplanted kidneys. However, histology shows neither the leukocytes circulating within the kidney nor the type or activation state of leukocytes that have migrated into the tissue. Flow cytometry is a technique with the potential for providing this information.

We developed a procedure for reducing 16 gauge core renal biopsies to suspensions of cells for flow cytometric analysis without losing leukocyte epitopes (NDT, 2010) and are now characterizing leukocyte subsets and their activation state in transplant biopsies to better define the immunologic processes active in allografts. We find the following:

1) Peripheral circulating CD45+ leukocytes differ from those found within human kidneys and their activation states are also distinct. For example, granulocytes predominate in the peripheral circulation but are rarely found in normal native or transplanted kidneys.

2) Rejection is associated with a predominance of CD8+ over CD4+ T cells; this may allow rejection to be distinguished from BK nephropathy and nonspecific interstitial inflammation.

3) Renal CD3+ T cells express increased levels of HLA-DR with inflammatory conditions of rejection, BK nephropathy and nonspecific interstitial inflammation.

4) Invariant NKT cells are present in normal kidney; CD1d is expressed on renal microvascular endothelial cells and on some leukocytes.

5) NK (CD56+, CD3-) and NKT (CD56+, CD3+) cells are present in normal native and transplanted kidneys.

Flow cytometry is a valuable addition to traditional histology for evaluating immune processes in transplanted kidneys. Information provided by cytometry will likely be useful in directing immunosuppression.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO754**

**Renal Transplantation in Recipients with Positive Luminex and Negative CDC (Complement-Dependent Cytotoxicity) Crossmatch** Dong Jin Joo, Kyu Ha Huh, Yu Seun Kim, Tae-Hyun Yoo. *Transplantation Center, Yonsei University College of Medicine, Seoul, Korea.*

**Background:** The presence of preformed antibodies against HLA (human leukocyte antigen) is considered as contraindication to renal transplantation. CDC (complement-dependent cytotoxicity) crossmatch has been used to identify the donor-specific HLA antibodies. Recently, Luminex-crossmatch (LumXm, DSA; Tepnel Lifecodes, Stamford, CT) with the advantage of greater sensitivity and specificity was introduced. The aim of this study is to evaluate clinical outcomes in positive Luminex and negative CDC crossmatch (CDCXm) renal transplantation.

**Methods:** Three hundred-six renal transplants were performed from February 2008 to April 2010. Forty-four recipients had negative CDCXm with more than 20% PRA (Panel-reactive antibody). Of this 44 recipients, 17 patients showed positive LumXm defined as positive class I and/or II LumXm and 27 patients showed negative LumXm. Post-transplant outcomes of LumXm(+) was compared with those of LumXm(-) group.

**Results:** Mean follow-up duration was 11.5 ± 8.5 and 12.9 ± 7.2 months for LumXm(+) and LumXm(-) groups, respectively (p=0.582). During this period, there was no patient death and graft failure. Incidence of acute rejection which was diagnosed by biopsy or clinical evaluation was higher in LumXm(+) group (n=11, 64.7%) than in LumXm(-) group (n=5, 18.5%) (p=0.004). All biopsy-proven acute rejection (n=10) was diagnosed as cellular rejection. Mean serum creatinine of LumXm(+) and LumXm(-) group at post-ransplant 3 month was 1.18 ± 0.26 and 1.18 ± 0.23 (mg/dL), respectively (p=0.994).

**Conclusion:** Renal transplant recipients with LumXm(+) and CDCXm(-) predispose to acute rejection compared with those with LumXm(-). Short-term post-transplant graft function of LumXm(+) was comparable to LumXm(-) group.

Posttransplant Outcomes

| | LumXm(+) (n=17) | LumXm(-) (n=27) | p-value |
|---|---|---|---|
| DGF | 2 (11.8%) | 1 (3.8%) | 0.552 |
| Acute rejection | 11 (64.7%) | 5 (18.5%) | 0.002 |
| sCr-1mon (mg/dL) | 1.45±0.76 | 1.19±0.36 | 0.140 |
| sCr-3mon (mg/dL) | 1.18±0.26 | 1.18±0.23 | 0.994 |
| Graft failure | none | none | |
| Patient death | none | none | |

Disclosure of Financial Relationships: nothing to disclose

**TH-PO755**

**Urinary MCP1 Decrease Predicts Long Term Prognosis in Proliferative Lupus Nephritis** Patrícia Malafronte, Aline Resende, Cristiane Bitencourt Dias, Cilene Carlos Pinheiro, Rui Toledo Barros, Viktoria Woronik. *Nephrology, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil.*

Urinary MCP1 is related to inflammatory state and flare activity in Lupus Nephritis (LN). Its importance in renal prognosis has not been clearly established. The aim was to describe any relations of urinary MCP1 with clinical characteristics, especially renal outcomes. Twenty-three female newly diagnosed with proliferative LN were prospectively followed-up during 3.6±0.9 years. Urinary MCP1 was collected on diagnosis and after six months of treatment. Conventional laboratory tests were collected on diagnosis and on last follow-up. Patients were stratified in two groups according to renal outcome: GFR ≤ 60 mL/min/1.73m$^2$ (n=7) and GFR > 60mL/min/1.73m$^2$ (n=16). Considering treatment, all patients received prednisone and 6 pulses of cyclophosphamide (CYA) on induction. Results in Table 1.

Table1. Clinical and biochemical characteristics of patients

| | GFR > 60ml/min/1,73m2 (n=16) | GFR ≤ 60ml/min/1,73m2 (n=7) | P |
|---|---|---|---|
| Age (years) | 25.6 ± 7.5 | 29,0 ± 14.5 | ns |
| Initial GFR – MDRD (ml/min/1,73m2) | 43.0 ± 25.4 | 25.7 ± 18.4 | ns |
| Initial Proteinuria (g/dia) | 4.2 ± 2.3 | 5.5 ± 2.5 | ns |
| Systemic SLEDAI | 25.5 ± 4.8 | 24.7 ± 3.6 | ns |
| Renal SLEDAI | 13.2 ± 2.4 | 12.6 ± 1.5 | ns |
| Follow-up (years) | 3.7 ± 0.7 | 3.8 ± 1.8 | ns |
| Flare (n) | 0.4 ± 0.5 | 1.4 ± 0.5 a | 0.01 |
| Last GFR – MDRD (ml/min/1,73m2) | 98.1 ± 19.1 | 32.6 ± 16.8 | <0.0001 |
| Last Proteinuria (g/day) | 0.4 ± 0.4 | 2.8 ± 2.0 | 0.03 |
| Initial Urinary MCP1 (pg/mL) | 3133.7 ± 2165.8 | 1809.1 ± 1040.7 | ns |
| 6 months Urinary MCP1 (pg/mL) | 822.9 ± 649.9 | 1019.3 ± 554.2 | ns |
| Δ Urinary MCP1 (initial - 6 months) | 2311 ± 2237 | 790 ± 766 | 0.025 |

X ± SD a n=5

Urinary MCP1 delta values during induction treatment correlates with delta GFR on follow-up (r = -0.5, p = 0.01). Summary- Patients with proliferative LN and worse renal outcome showed lower decrease (Δ) of urinary MCP1 calculated by initial values and after 6 months of induction treatment. However, there was no correlation with absolute values of initial and 6 months urinary MCP1.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO756**

**The Morphological Evidence for the Importance of Neutrophilic to Development of Active Lesion on Lupus Nephritis (LN)** Noriko Uesugi, Michio Nagata. *Dep. of Renal Pathology, Uviversity of Tsukuba, Tsukuba, Ibafaki, Japan.*

The recent evidence of high ANCA level and lower degradation abilities for neutrophilic extracelluar traps (NET) in LN patient suggest importance of pathogenic role of neutrophil for progression of LN. Activated neutrophils release neutrophilic elastase (Ela) and myeloperoxidase (MPO) which coordinate macrophages to promote LN progression. In this regards, *in situ* interaction between neutrophils and macrophages in LN provides clue to understand pathophysiology in LN. The present study enrolled 42 renal biopsy specimens of LN (RPS/INS Class II 7, III 10, IVS 5, IVG 12 and V 8 for immunohistochemical study. Using serial sections, each glomerulus was evaluated for type of active lesions, such as crescent, endocapillary proliferation, necrosis, hyaline thrombi(HyT) and Wire loop (WL). In addition, Elas, MPO and CD68-positive cells are counted in each glomerulus. Results: Glomerular infiltration of MPO, Elas and CD68 positive cells revealed significantly higher in Class IVG and IVS than that with II, III and V(p=0.05). CD68-positive cells are highest cells. Glomeruli with necrosis, cellular crescent and endocapillary proliferation contain higher number of MPO and Elas-positive cells than WL and HyT. Extra-cellular localization

of MPO and Elas in active proliferative lesion suggests presence of NET in situ. MPO-positive cells were significantly more than that of Elas. There noted MPO and CD68- double rebelled cells in the active lesions. The number of MPO and Ela positive cells are good correlation with that of CD68 positive cells (r=0.73, 0.69, respectively).

Infiltrating cells and active lesion

| Predominant lesion | Cellular Cre | FC Cre | Necrosis | Endo | HyT | WL |
|---|---|---|---|---|---|---|
| No. of gl | 23 | 12 | 7 | 121 | 9 | 77 |
| CD68+cells /gl | 12.0±8.0 | 7.8±5.5 | 21.5±15.1 | 16.3±9.8 | 12.5±4.9 | 13.2±8.7 |
| MPO+cells /gl | 10.0±5.5a | 3.1±2.4 | 20.5±5.8b | 10.9±8.8a | 5.4±5.4 | 3.9±3.0 |
| Elas+cells /gl | 5.8±2.7 | 2.8±2.4 | 17.8±13.1c | 6.6±5.3 | 4.5±5.3 | 2.9±5.3 |

a p=0.05 vsWL, b p=0.05 vs FC cre,HyT,WL

Conclusion: Neutrophils and macrophages may involve active proliferative lesions in LN *via* production of MPO, suggesting that MPO and NET may promote tissue injury severe active LN.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO757

**Repeated Renal Biopsy in Lupus Nephritis** Jianxin Lu, Bonnie Kwan, Kai Ming Chow, Philip K. T. Li, Cheuk-Chun Szeto. *Dept of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of HongKong, HongKong, China.*

*Background* The role of sequential renal biopsies in lupus nephritis (LN) patient with regard to clinical factor remains elusive. *Methods* Clinical and pathological documents of 151 LN Patients with repeat renal biopsies (242 times) were collected from database. *Results* The percent of transformation of biopsy class from first to second biopsy is between 50-96.67%. The percent of transformation of biopsy class for III or IV, pure V and III or IV plus V is 60.47%, 47.59% and 55.56% respectively. Chronicity index is significantly different between first and second biopsy ($p<0.001$). LN patients with treatment during sequential biopsies are more likely to transform compare with patients without treatment (p=0.04). *Conclusion* In patients with lupus nephritis, there is a high frequency of transformation of biopsy class during repeat biopsy. The sequential renal biopsies have clinical meaning and can be used as a marker for the development and treatment of LN.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO758

**Renal Expression of Vasohibin-1, a Novel Endothelium-Derived Angiogenesis Inhibitor, in Patients with Renal Disorders** Norikazu Hinamoto, Yohei Maeshima, Tatsuyo Nasu, Masaru Kinomura, Shinji Kitamura, Hitoshi Sugiyama, Hirofumi Makino. *Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama-shi, Japan.*

Glomerulosclerosis and tubulointerstitial injuries are involved in the progression of renal disorders. Vasohibin-1 is an unique endogeneous angiogenesis inhibitor, initially identified in endothelial cells induced by proangiogenic factors such as VEGF-A, serving as a negative feedback regulator of angiogenesis as well as a maturation factor of neovessels. We previously reported the therapeutic efficacy of Vasohibin-1 in a mouse diabetic nephropathy model (Diabetes, 2009). The aim of the current study is to examine the renal expression of Vasohibin-1 in patients with various renal disorders and to evaluate its clinical usefulness. We examined renal expression of Vasohibin-1 by immunohistochemistry using renal biopsy sections obtained from 26 patients with renal disorders including chronic glomerulonephritis, nephrotic syndrome and diabetic nephropathy. The number of Vasohibin-1+ cells in glomeruli, cortical or medullary tubulointerstitium was determined, and correlation with clinical and histological parameters were examined. Vasohibin-1 was observed in endothelial and mesangial cells in glomerulus, and in endothelia of peritubular capillaries and inflammatory cells in tubulointerstitium. The number of Vasohibin-1+ cells in cortex correlated with the number of glomerular Vasohibin-1+ cells (r=0.78, $P<0.01$) and crescent formation (r=0.48, $P=0.03$), and tended to correlate with eGFR (r=0.40, $P=0.07$) and the number of Vasohibin-1+ cells in medulla (r=0.59, $P=0.09$). The number of Vasohibin-1+ cells in medulla correlated with crescent formation (r=0.81, $P<0.05$) and tended to correlate with proteinuria (r=0.80, $P=0.05$) and interstitial fibrosis (r=0.62, $P=0.19$). Further analysis on the renal levels of VEGF-A and microvascular density is underway. Taken together, these results suggest that endogenous Vasohibin-1 may be associated with renal function, crescentic lesion, interstitial fibrosis and proteinuria in various renal disorders, thus implicating its potential to serve as a novel renal biomarker.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO759

**Urinary Exosomal Fetuin-A Level Correlated with Urinary beta-2 Microglobulin Concentration in Patients with Various Renal Diseases** Sayaka Oshikawa,[1] Hiroko Sonoda,[1] Saki Takahashi,[1] Shigehiro Uezono,[2] Akira Ueda,[2] Naoko Yokota-Ikeda,[2] Masahiro Ikeda.[1] *[1]Veterinary Pharmacology, University of Miyazaki, Miyazaki, Japan; [2]Internal Medicine, Miyazaki Prefectural Miyazaki Hospital, Miyazaki, Japan.*

Urinary exosomes are originated from glomerular podocytes and epithelial cells lining the renal tubules, and are known to carry protein biomarkers of renal dysfunction and injury. Fetuin-A, a potent inhibitory protein of systemic calcification, in urinary exosomes has been identified as one of the biomarkers for kidney diseases such as acute kidney injury and urolithiasis. However the relationship of urinary exosomal fetuin-A level with conventional renal disease markers remains largely unknown. In this study, we examined the correlation of urinary exosomal fetuin-A level with conventional markers such as serum creatinine concentration (SCr), creatinine clearance (CCr), urinary N-acetyl-beta-D-glucosaminidase activity (NAG), and urinary beta-2-microglobulin concentration (B2M). The institutional review boards of Miyazaki Prefectural Miyazaki Hospital approved this study in accordance with Ethical Guidelines for Clinical Studies in Japan. Spot urine and blood samples were collected from 30 patients with various types of renal diseases including IgA nephropathy, membranous nephropathy, focal glomerulosclerosis, ANCA-associated glomerulonephritis, acute kidney injury, and etc. In order to isolate exosomes, urine samples were subjected to differential centrifugation, and then urinary exosomal fetuin-A was analyzed by immunoblotting. Urinary exosomal fetuin-A level in all patients did not correlate with SCr, CCr, or NAG. However exosomal fetuin-A abundance significantly correlated with urinary B2M ($r = 0.64$, $P < 0.01$). Urinary exosomal fetuin-A level in 14 patients who had higher urinary B2M (more than 200 mg/l) tended to be increased (3 fold), compared to that in 16 patients with normal range B2M. When reabsorptive ability for filtered B2M is impaired in proximal tubular cells, urinary B2M is known to be increased. Thus, together with present data, it is considered that urinary exosomal fetuin-A may be indicative of a disorder of reabsorption function of proximal tubule cells.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO760

**Urinary Levels of MMP-2, MMP-7, MMP-9, TIMP-1, and TIMP-2 in Pediatric Nephrotic Syndrome and FSGS** Kimberly Czech, Michael R. Bennett, Mark Mitsnefes, Prasad Devarajan. *Pediatric Nephrology & Hypertension, Cincinnati Children's Hospital, Cincinnati, OH.*

Most idiopathic nephrotic syndrome in children is steroid responsive and has a good long-term prognosis. About 10% of cases are steroid resistant and are diagnosed with focal segmental glomerulosclerosis (FSGS). Metalloproteinases and their inhibitors (TIMPs) are involved in tissue-remodeling. We hypothesize that urinary levels of these enzymes are increased in FSGS as compared to steroid-sensitive minimal change disease (SSNS).

**Objective**: Compare urinary levels of MMP-2, MMP-7, MMP-9, TIMP-1, and TIMP-2 in SSNS, FSGS, and healthy age-matched controls.

**Methods**: Urine and clinical data were collected from patients at Cincinnati Children's Hospital with FSGS, SSNS (relapse & remission) as well as healthy controls. Urinary measurements of MMP-2, MMP-7, MMP-9, TIMP-1, and TIMP-2 were performed using a commercially available ELISA kit.

**Results**: This study included subjects in the following three groups: biopsy-proven FSGS and steroid resistant clinical course (n=16), steroid-sensitive clinical course (n=19), and normal controls (n=15). Median levels were calculated and subjected to Kruskal-Wallis One Way ANOVA (Dunn's multiple comparison test). MMP-2, -7, -9, and TIMP-1, 2 were all significantly elevated ($P<0.05$) in FSGS as compared to SSNS and controls. When, we looked at MMP-2,-7, -9/timp1 ratios a significant decrease ($p<0.05$) was seen in FSGS compared to SSNS and controls. MMP-2,-7,-9/timp2 ratios were not different between the groups.

**Conclusion**: MMP-2,-7,-9 are significantly elevated in the urine of FSGS patients as compared to SSNS. However, inhibitory enzyme levels (TIMPs 1, 2) in the urine are also significantly increased, possibly indicating a compensatory mechanism to slow matrix remodeling enzymes. Urinary levels of MMPs and TIMPs are potential biomarkers to distinguish between FSGS and SSNS.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO761

**Correlation of NGAL Levels with RIFLE Categories in Critically Ill Patients** Lars O. Uttenthal. *BioPorto Diagnostics A/S, Gentofte, Denmark.*

NGAL levels are raised in patients with acute kidney injury (AKI), but NGAL release is not specific to the injured kidney. Urine and plasma NGAL levels were correlated with RIFLE categories of AKI in unselected critically ill patients including confounding conditions such as sepsis and cancers, and samples were used to validate a new fully automated NGAL turbidimetric assay.

Urine and EDTA-plasma levels of NGAL were monitored (daily to alternate days) in 135 consecutive patients admitted to intensive care; 3 patients were excluded because of incomplete data. NGAL was measured in all samples with the BioPorto ELISA kit. Data are reported as median (interquartile range) in ng/mL. The new turbidimetric assay uses polystyrene particles coated with mouse monoclonal antibodies and recombinant NGAL as calibrator. The assay was run on a Hitachi 917 and used to measure urine samples from 130 and plasma samples from 40 of the patients.

Of 70 patients with AKI, 15 were classified as "risk" (R), 11 as "injury" (I) and 44 as "failure" (F); 62 patients did not have AKI. Urine NGAL was significantly ($p<0.0001$, Kruskal-Wallis) higher in patients with AKI [R 377 (102-830), I 519 (321-1845) and F 2747 (559-7621)] than in patients without AKI [51 (29-213)]. Plasma NGAL was also significantly ($p<0.0001$) higher in patients with AKI [R 354 (259-511), I 546 (331-1106) and F 1062 (584-1817)] than in those without [199 (128-308)]. The turbidimetric assay showed a measuring range of 25-5000 ng/mL, within-run imprecision of 2.0% and 1.2% at 200 and 500 ng/mL, respectively, and linearity from 20 to 5000 ng/mL. No effect of antigen excess was seen up to 40,000 ng/mL. Potentially interfering substances (hemoglobin 500 g/L, bilirubin 30 g/L and emulsified lipid 5.0%) had only marginal effects (<4%) on measurements. There was no statistically significant difference between ELISA and turbidimetric results for either urine or plasma samples.

Both urine and plasma levels of NGAL reflect the degree of AKI in unselected critically ill patients despite confounding conditions and may therefore be useful for the diagnosis of renal injury and monitoring of patients in intensive care; in this process the fully automated turbidimetric NGAL test can be a useful aid.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO762**

**Epithelial-Mesenchymal and Endothelial-Mesenchymal Transdifferentiation Processes in Glomerular Diseases** Flaviu Bob,[1] Gheorghe Gluhovschi,[1] Cristina A. Gluhovschi,[1] Diana Herman,[2] Elena Potencz,[2] Ligia Petrica,[1] Silvia Velciov,[1] Gheorghe Bozdog,[1] Florica Gadalean,[1] Alin Nes.[1] *[1]Nephrology, University of Medicine and Pharmacy, Timisoara, Romania; [2]Pathology, University of Medicine and Pharmacy, Romania.*

The fibroblast, the key mediator of fibrosis, originates from resident fibroblasts, from epithelial cells through epithelial-to-mesenchymal transdifferentiation, and, as recently described, from endothelial cells through endothelial-to-mesenchymal transdifferentiation. In order to assess the process of transdifferentiation we studied the expression of markers of activated fibroblasts (alpha smooth muscle actin-SMA and vimentin-Vim), of the transforming growth factor β (TGF) and of CD34, at the level of tubular epithelial cells (TEC) and interstitial vascular endothelial cells (VEC).

We studied retrospectively 41 renal biopsies from patients with primary and secondary glomerulonephritis [M-24p,F-17p,mean age 45.5±12.9y]. Immunohistochemistry using monoclonal antibodies (SMA,Vim,CD34,TGFβ) was assessed using a semiquantitative score, that was correlated with biological and histological data (quantified using a scoring system in order to assess active-inflammatory and chronic- sclerotic/fibrotic lesions).

The presence of SMA and Vim as markers of the transdifferentiation process was found in TECs and VECs. TEC Vim expression correlated with interstitial Vim expression, (R=0.38;p=0.008), interstitial infiltrate (R=0.31;p=0.027), interstitial fibrosis(R=0.25;p=0.042), GFR(R=-0.35;p=0.016), SMA(R=-0.42;p=0.015), and TGF (R=0.25;p=0.046). VEC Vim expression showed indirect correlations with interstitial infiltrate(R=-0.32;p=0.023), activity index (R=-0.33;p=0.02), interstitial fibrosis (R=-0.34;p=0.017), chronicity index(R=-0.33;p=0.023), vascular CD34 (R=-0.5, p=0.001). VEC TGF correlated with the activity index(R=0.27;p=0.04), with vascular CD34 (R=0.28, p=0.04). We found a correlation of VEC CD34 with creatinine(R=0.27,p=0.04), GFR(R=-0.44, p=0.002), activity index (R=0.28,p=0.04), chronicity index(R=0.28, p=0.04), interstitial fibrosis(R=0.35,p=0.01).

The study reflects the complexity of the transdifferentiation process with the involvement of both TECs and VECs.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO763**

**End-Stage Renal Failure in a Young Adult: An Unusual Presentation of Late-Onset Cobalamin C Disease** Karine Hadaya,[1] Luisa Bonafé,[3] Vincent Bourquin,[1] Pierre-Yves F. Martin,[1] Olivier Boulat,[4] Matthias R. Baumgartner,[5] Brian Fowler,[6] Ilse Kern.[2] *[1]Nephrology, Geneva University Hospital, Geneva, Switzerland; [2]Pediatrics, Geneva University Hospital, Geneva, Switzerland; [3]Molecular Pediatrics, centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [4]Clinical Chemistry, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [5]Metabolism, Children's Hospital, Zurich, Switzerland; [6]Metabolism, Children's Hospital, Basel, Switzerland.*

The cblC type of methylmalonic aciduria and homocystinuria (cblC) is a rare autosomal recessive disorder leading to defective intracellular cobalamin metabolism. Late-onset disease in young adults has been reported in less than 20 cases, characterised by neuro-psychiatric and/or thrombo-embolic manifestations and a more favourable outcome than in early-onset cases.

In this report, we describe the clinical, biochemical and molecular findings in a 23-year- old man who was initially misdiagnosed as essential malignant hypertension with haemolytic uremic syndrome (HUS). He progressed to end-stage renal failure 15 days after admission. Thereafter, the finding of hyperhomocysteinemia prompted further studies in fibroblasts leading to the diagnosis of the cblC disorder. Sequencing of the *MMACHC* gene showed the homozygous missense mutation c.565C>A. Hyperhomocysteinemia decreased rapidly under intensive treatment with i.v. hydroxycobalamin, oral betaine, folate and carnitine, allowing the patient to undergo a living related renal transplantation. Eighteen months post-transplant, biochemical parameters had improved dramatically and kidney function is normal.

Renal complications such as thrombotic microangiopathy, proximal renal tubular acidosis, and chronic renal failure have been described mainly in early-onset CblC disease. To our knowledge, this is the first case of end-stage renal failure with HUS in a young adult, presumably secondary to thrombotic microangiopathy, although this could not be confirmed since hypertensive crisis precluded a diagnostic kidney biopsy.

cblC disease should be included in the differential diagnosis of malignant hypertension and/or HUS in adults, as cblC disease, although rare, is a treatable disorder.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO764**

**The Injurious Role of IL-33 in Cisplatin-Induced Acute Kidney Injury** Ali Akcay, Quocan Nguyen, Kultigin Turkmen, Zhibin He, Sarah Faubel, Alkesh Jani, Charles L. Edelstein. *Univ of Colorado Denver.*

Caspase-3 is a mediator of cisplatin-induced apoptosis and necrosis of endothelial cells in kidney and induces release of uncleaved (active) IL-33 from necrotic endothelial cells. IL-33 signals via the ST2 receptor on CD4 T cells resulting in the production of pro-inflammatory cytokines. The aim of this study was to investigate the potentially injurious role of IL-33 in cisplatin-induced acute kidney injury (Cis-AKI). Mice were injected with cisplatin 25 mg/kg. There was tubule apoptosis (day 1), necrosis (day 2) and elevated serum creatinine (SCr) (day 3). IL-33 was measured by ELISA and the uncleaved and the cleaved forms on immunoblot. Serum IL-33 (pg/mL) was 58.7 in vehicle-treated (V) and 370.9 on day 2 (P<0.05). IL-33 (pg/mg) in kidney was 280 in V and 380 on day 1 (P<0.01). On immunoblot there was a 3-fold increase in both uncleaved and cleaved IL-33 on days 1 in nuclear and whole kidney extracts (P<0.01). On IF staining of kidney, IL-33 was localized in the endothelium. Cultured microvascular endothelial cells exposed to 10 and 50 μM Cis showed a 4-fold increase in uncleaved and a 3-fold increase in cleaved IL-33 in the medium on immunoblot that was prevented by the pancaspase inhibitor QVD-OPH (50 μM) establishing that active IL-33 is released from injured endothelial cells. To determine the injurious role of IL-33, mice were either injected with recombinant IL-33 (rIL33) or sST2, a fusion protein that can neutralize IL-33 activity by acting as a decoy receptor. SCr (mg/dL) was 0.6 in mice injected with low-dose Cis (15 mg/kg)+V and 1.7 in mice injected with low-dose Cis+rIL-33 (P<0.001). SCr was 2.2 in Cis+V and 1.0 in Cis+sST2 (P<0.01). To demonstrate the effect of IL-33 on CD4 T cells, low-dose Cis+rIL-33 was injected into the CD4 T cell -/- mice. SCr was 1.9 in WT+Cis+IL-33 and 0.6 in CD4 -/- +Cis+IL-33 (P<0.01). In summary, IL-33 is abundantly present in endothelial cells in the kidney. Cis induces a systemic IL-33 response that precedes AKI. Injection of rIL33 worsens Cis-AKI in WT but not CD4 T cell -/- mice. Inhibition of IL-33 protects against Cis-AKI. In conclusion, IL-33 may have an important role in the pathogenesis of Cis-AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO765**

**Engulfment of Apoptotic Cells by Kidney Injury Molecule-1 Is Mediated by Tyrosine Phosphorylation and Src Kinase Signaling** Lakshman Gunaratnam,[1,2] Joseph V. Bonventre,[3] Xizhong Zhang.[1,2] *[1]Medicine and Microbiology & Immunology, Schulich School of Medicine and Dentistry and The University of Western Ontario, London, ON, Canada; [2]Lawson Health Research Institute, London Health Sciences Centre, London, ON, Canada; [3]Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.*

Apoptosis or programmed cell death is a mechanism of eliminating damaged, harmful or useless cells and is crucial for maintaining tissue homeostasis in multicellular organisms. Kidney injury molecule-1 (KIM-1) has been recently identified as a scavenger receptor that transforms renal proximal tubular epithelial cells (PRTECs) into semi-professional phagocytes for clearance of apoptotic cells resulting from acute kidney injury. KIM-1 specifically recognizes phsophatidylserine (PtdSer) and oxidized lipids, both classical "eat-me" signals displayed on the surface of cells undergoing programmed cell death. The purpose of this study was to decipher how KIM-1 transduces signals downstream of PtdSer recognition to mediate engulfment of apoptotic cells. First we show that two tyrosine residues within the cytoplasmic tail of KIM-1 are tyrosine phosphorylated when KIM-1-expressing cells are treated with a protein-tyrosine phosphatase inhibitor (pervanadate). In addition, overexpression of active or wild type Src kinase in PRTECs was sufficient to trigger phosphorylation of KIM-1 while inhibition of Src activity blocked endogenous KIM-1 phosphorylaiton in PRTECs. We also show that Src activity is essential for KIM-1-mediated oxidized LDL-uptake and phagocytosis of apoptotic cells. Finally, expression of phosphorylation-defective double tyrosine mutant KIM-1 in PRTECs dramatically inhibited oxidized LDL-uptake and phagocytosis of apoptotic cells compared to wild type KIM-1 expressing cells. We propose that ligand-dependent KIM-1 activation initiates Src-dependent KIM-1 phosphorylation that is required for the phagocytic function of KIM-1 in PRTECs. Hence, KIM-1 is a functional receptor with an extracellular domain that recognizes "eat-me" signals, and an intracellular domain that regulates phagocytosis through tyrosine phosphorylation-Src-mediated signaling.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO766**

**HuR Inhibits Apoptosis in Proximal Tubule Cells by Activating PI3K/Akt Signaling Via Grb10** Mamata Singh, Beth S. Lee. *Physiology and Cell Biology, The Ohio State University College of Medicine, Columbus, OH.*

HuR is an RNA-binding protein with multiple post-transcriptional effects on gene expression. Under cellular stress, HuR translocates from the nucleus to the cytoplasm, where it binds and stabilizes a subset of mRNAs bearing adenine and uridine rich sequences in their 3' untranslated regions. We previously demonstrated an anti-apoptotic role for HuR in ATP-depleted proximal tubule cells, suggestive of its protective function in native proximal tubules against ischemic stress. We also demonstrated specific upregulation of HuR expression in ATP-depleted cultured proximal tubule cells and in native rat proximal tubules subjected to ischemia-reperfusion injury. We have since found HuR to be protective against both the intrinsic and extrinsic apoptotic pathways, with its greatest effect in the intrinsic pathway. Here we used PCR array analysis to determine how suppression of HuR levels might affect expression of mRNAs within apoptotic pathways. Control- or siRNA-

treated proximal tubule cells were ATP-depleted and subjected to PCR array analysis. mRNAs with at least a 2-fold increase or decrease in response to HuR knockdown were taken into consideration.

The major effect of HuR knockdown in proximal tubule cells was suppression of anti-apoptotic mRNAs such as those encoding Akt and members of the Bcl-2 family. We found that HuR plays a central role in PI3K/Akt signaling, as not only did knockdown of HuR suppress many members of this pathway, but inhibition of PI3K also resulted in suppression of HuR levels, suggesting a role for HuR in a positive feedback loop. In particular, Grb10, an adaptor protein with anti-apoptotic activities downstream of PI3K/Akt, was found to be strongly suppressed by HuR knockdown. Further, Grb10 was found to contain a consensus HuR binding site in its 3' untranslated region. Therefore, these data demonstrate a central role for HuR in cell survival as both an activator of PI3K/Akt signaling and as a downstream target of PI3K/Akt-mediated gene regulation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO767

**Lung Inflammation after Acute Kidney Injury (AKI) Is Dependent on Alveolar Macrophages (MØ)** Chris Altmann, Ana Andres-Hernando, Nilesh Ahuja, Zhibin He, Sarah Faubel. *Renal, University of Colorado Denver.*

We have shown that systemic depletion of Mø in CD11b Tg mice improves AKI-mediated lung injury, characterized by a decrease in lung CXCL1 (KC) and lung MPO activity (a marker of neutrophil infiltration). The role of **alveolar** Mø in AKI-mediated lung injury is unknown. To determine the role of alveolar Mø in AKI-mediated lung injury, we administered intratracheal (IT) liposome encapsulated clodronate (LEC) to deplete alveolar Mø, but not systemic Mø. Sham operation (Sham) or 22 min of renal pedicle clamping (AKI) were performed in IT vehicle-treated (Veh) or IT LEC-treated C57BL6 mice. As determined by flow cytometry for Mø markers F4/80 and CD11c in the bronchoalveolar lavage fluid (BALf), IT LEC injection resulted in a significant decrease in alveolar Mø compared to IT Veh (29% vs 71%, $P < 0.01$, $N = 5$). 4 hours post-procedure, lung MPO activity and lung CXCL1 were reduced in Mø depleted mice (Table 1). SCr and serum IL-6 were the same in AKI with and without Mø depletion. Interestingly, pulmonary capillary leak, as measured by lung Evans blue dye (EBD) and BALf protein, was *worse* after alveolar Mø depletion in AKI, suggesting a protective role of alveolar Mø in maintaining the pulmonary capillary membrane. These data emphasize that alveolar Mø play an important role in mediating lung inflammation after AKI.

Table 1

| | Sham + Veh | Sham + IT LEC (Alveolar MØ depleted) | AKI + Veh | AKI + IT LEC (Alveolar MØ depleted) |
|---|---|---|---|---|
| Serum creatinine (mg/dL) | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.9 ± 0.2 † | 1.0 ± 0.2 † |
| Serum IL-6 (pg/mL) | 153 ± 77 | 152 ± 62 | 941 ± 550 † | 1094 ± 120 † |
| Lung CXCL1 (pg/mg) | 293 ± 66 | 248 ± 76 | 601 ± 176 | 443 ± 103 * |
| Lung MPO (Δ/min/mg) | 0.6 ± 0.1 | 0.7 ± 0.1 | 1.0 ± 0.2 | 0.8 ± 0.2 * |
| BALf protein (ug/uL) | 118 ± 22 | 212 ± 89 * | 106 ± 32 | 174 ± 76 * |
| Lung EBD (ug EBD/g lung weight) | | | 141 ± 22 | 223 ± 91 * |
| Total BALf macrophages | 344,000 ± 62,000 | 144,000 ± 56,000 * | 259,000 ± 50,000 | 148,000 ± 71,000 * |
| | | | | |
| | | | | * P < 0.05 vs Veh |
| | | | | † P < 0.01 vs Sham |

Disclosure of Financial Relationships: nothing to disclose

## TH-PO768

**Lack of Splenic IL-10 Production Exacerbates Lung Injury after Ischemic Acute Kidney Injury (AKI) in Mice** Ana Andres-Hernando, Chris Altmann, Nilesh Ahuja, Zhibin He, Takuji Ishimoto, Sarah Faubel. *University of Colorado Denver.*

In this study, we examined the early pro-inflammatory response of AKI and the development of lung injury in a mouse model of ischemic AKI. Kidney, spleen and liver mRNA for IL-1β, TNF-α, CXCL1, IL-6 were determined at baseline, 2, 4 and 6 hours by QPCR after sham operation (S) or ischemic AKI (22 minutes of bilateral renal pedicle clamping). In the kidney, IL-6, CXCL1, and IL-1β were increased by 2 hours after AKI (25, 28 and 8 fold increase vs. S). In the liver, IL-6 and IL-1β were increased by 2 hours after AKI (11 and 5 fold increase vs. S) and CXCL1 was increased 4 hours after AKI (6 fold increase vs. S). In the spleen, CXCL1 was increased at 2 hours (9.5 fold increase vs. S), IL-6 by 4 hours (100 fold increase vs. S), and IL-1β and TNF-α increased by 6 hours (24 and 8 fold increase vs. S). Since splenic pro-inflammatory cytokines were increased, we hypothesized that splenectomy (Splnx) would protect against AKI-mediated lung injury. On the contrary, Splnx with AKI resulted in increased serum IL-6 and worse lung injury than AKI alone. Since Splnx exacerbated the lung injury in the setting of AKI, we hypothesized that the spleen might produce a beneficial factor after AKI. We examined splenic production of the anti-inflammatory cytokine IL-10, and found that IL-10 production in the spleen was highly up-regulated 2 hours post AKI (5 fold increase vs. S), while IL-10 did not increase in the kidney. To confirm that IL-10 down-regulates the pro-inflammatory response of AKI, IL-10 was administered to splenectomized mice with AKI which reduced serum IL-6 (2599±437 pg/mL) vs. vehicle (1052±281 pg/mL) and was associated with a reduction in lung CXCL1 and lung MPO activity as well as improved lung histology. Our data demonstrate that a counter anti-inflammatory response by extra-renal IL-10 production is necessary to limit the pro-inflammatory response of AKI. In patients, AKI in the setting of conditions which limit the anti-inflammatory response may lead to acute lung injury.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO769

**Protective Role of Autophagy in Oxidant and ER Stress in Renal Tubular Cells** Sarika Kadam, Cheng Yang, Christian Herzog, Gur P. Kaushal. *Division of Nephrology, University of Arkansas for Medical Sciences,CAVHS, Little Rock, AR.*

Autophagy is a dynamic process of degradation of damaged cytoplasmic organelles and macromolecules in lysosomes to produce free amino and fatty acids that are recycled for energy production and protein synthesis. Autophagy aids cell survival during stress. Since acute kidney injury is associated with cellular stress we hypothesize that induction of autophagy during oxidative and endoplasmic reticulum (ER) stress will be protective to renal tubular epithelial cells.

Methods and Results: $H_2O_2$ and tBHP (tertiary-butyl hydroperoxide) markedly induced autophagy in LLC-PK1 cells as detected by (1) punctate staining of autophagosomes following GFP-LC3 plasmid transfection, (2) Induction of the autophagy related proteins like Atg5 & Beclin1, (3) conversion of LC3-I to lipidated LC3-II.

Overexpression of Atg5 and Beclin1 protected LLC-PK1 cells from $H_2O_2$ and tBHP induced cell death. (Data: cell death at 1 h 200μM $H_2O_2$ = 17%, $H_2O_2$+ overexpressed Atg5 = 9%, $H_2O_2$+ overexpressed Beclin1=13%. SiRNA inhibition of autophagy: 200μM $H_2O_2$=17%, $H_2O_2$+si RNA beclin1=50%, $H_2O_2$+ siRNA atg5 =98% cell death. Cell death with autophagy inhibitors at 1 h: $H_2O_2$ =37%, $H_2O_2$+ wortmannin =54%, $H_2O_2$+chloroquine =46 %). LDH and MTT assays were used for cell death determination.

Autophagy inhibition did not increase caspase 3/7 at 1, 2 and 4 h after $H_2O_2$ treatment.

We examined induction of autophagy and its role in ER stress by tunicamycin in LLC-PK1 cells. Autophagy was induced by 2μg/ml tunicamycin treatment for 16 h. (Data:(1) cell death at 1h, 5 h $H_2O_2$=73%; 81%, pretreated tunicamycin +$H_2O_2$ =28%; 13% (2) cell death at 1 h 40μM tBHP was 27% but tunicamycin pretreatment decreased cell death to 4.54%). All results are the average of at least 4 times repeated experiments.

Conclusions: (1) Autophagy is induced during $H_2O_2$ and tBHP injury in LLC-PK1 cells. (2) Autophagy provided cytoprotection from the oxidant stress. (3) Autophagy inhibition caused caspase-independent cell death. (4) Tunicamycin-induced ER stress induced autophagy and protected LLC-PK1 cells from oxidant stress.

Induction of autophagy may serve as an intervention in preventing AKI.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO770

**Monocyte Chemoattractant Protein-1 (MCP-1) and Associated Gene Activating Histone Marks: Tools for Studying Experimental and Clinical Acute Kidney Injury** Raj P. Munshi,[3] Edward Siew,[2] Jonathan Himmelfarb,[1] T. Alp Ikizler,[2] Richard A. Zager.[1] *[1]Medicine, University of washington, Seattle, WA; [2]Medicine, Vanderbilt University, Nashville, TN; [3]Pediatrics, Seattle Children's Hospital Medical Center, Seattle, WA.*

Monocyte chemoattractant protein 1 (MCP-1) is a mediator of acute ischemic and toxic kidney injury (AKI). This study assessed whether urinary MCP-1 concentrations, and markers of MCP-1 gene activity (mRNA, MCP-1 gene-associated histone changes), have potential utility as AKI biomarkers with implied mechanistic significance. Kidney and urine samples were obtained from mice with intra-renal (maleate), pre-renal (endotoxemia), or post renal (ureteral obstruction) AKI. Independent effects of uremia (mice with bilateral ureteral transection) were also assessed. Selected samples were assayed for MCP-1, MCP-1 mRNA, and for an activating histone mark (H3K4m3) at urinary fragments of the MCP-1 gene. Results were contrasted to those obtained for NGAL, used as a comparator 'AKI biomarker' gene. To assess potential clinical relevance, comparable assays were performed on urines from critically ill ICU patients with (n,10) or without (n,10) severe AKI (ultimate need for dialysis). Patients were matched by age, sex, ethnicity, sepsis status and closest APACHE II scores. Maleate induced early (4 hr), marked (3-9x) increases in urine MCP-1 protein and mRNA (≥ NGAL). Endotoxemia and ureteral obstruction induced comparable NGAL and MCP-1 up-regulation. Conversely, uremia induced the NGAL, but not the MCP-1 gene (suggesting potentially greater MCP-1 specificity). Clinical assessments confirmed MCP-1's utility (complete separation of urine MCP-1 concentrations in AKI+ vs. AKI- populations). Increased MCP-1 gene activation in AKI+ patients was supported by increased urine levels of MCP-1 mRNA and of H3K4m3 at the MCP-1 gene. Conclusions: 1) coordinated assessments of urinary MCP-1 protein / mRNA have potential value as AKI biomarkers; and 2) the results of the urinary H3K4m3 assessments provide the first 'proof of concept' that quantifying histone alterations at specific genes can provide pathogenic insights in patients with kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO771**

**Are TNF-α, IL-6 and IL-10 Genetic Polymorphisms Risk Factors to Acute Kidney Injury (AKI) in ICU Patients?** Maria Dalboni, Marie Beata Redublo Quinto, Roberto Narciso, Julio Cesar Monte, Marcelino Durao, Oscar Pavao Dos Santos, Caren Cristina Grabulosa, Miguel Cendoroglo, Marcelo Costa Batista. *Medicine/Nephrology, Universidade Federal de São Paulo, São Paulo, Brazil.*

Introduction: Patients in ICU with SIRS have a high risk to development acute renal failure (AKI) and death. Genetic polymorphism studies of cytokines represent a perspective to the understanding of AKI in ICU settings.

Aim: To investigate whether genetic polymorphism of –308 TNF-α, -174 G>C IL-6 and 1082 G>A IL-10 gene could to predispose ICU patients with SIRS to development AKI and death.

Methods: In a prospective nested case-control study, we enrolled 797 ICU patients. The study group included those development AKI (n = 221/27.7%)) and the control group included those without AKI (n = 576/72.3%) according to AKIN criteria. We measured lipid profiles, CRP, albumin and accessed genetic polymorphism to TNF-α, IL-6 e IL-10.

Results: AKI patients presented a higher APACHE and CRP levels and lower cholesterol HDL and LDL levels than non-AKI ($p<0.01$). AKI patients who died had a higher APACHE and lower albumin and HDL compared to non AKI. In univariate analysis, we observed that frequency of GG genotype TNF-α (high producer) was 29% in AKI vs. 11% in non-AKI ($p<0.01$). To IL-6 the frequency of GG genotype (high producer) was 43% in AKI vs. 19% in non-AKI ($p<0.01$). However, in multivariate analysis this difference did not remain significant after adjustment for risk factors associated with AKI and death. The IL-10 genotype most frequent was GCC ACC (intermediate producer) in 42% in AKI and 17% non-AKI patients ($p = 0.003$). In this case, the difference remained significant after applying multivariate analysis.

Conclusion: Low levels of lipid profile and albumin in patients with AKI was associated with a high mortality rate. Although the high producer of TNF-α and IL-6 polymorphism among AKI patients may lead to a pro-inflammatory state, these polymorphisms did not predict AKI or death in our population. On the other hand, the GCC ACC IL-10 variant remained significant predictor for AKI occurrence, suggesting that these polymorphisms might be an independent risk factor for this complication.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO772**

**Regulation of Autophagy by Caspase-Dependent Cleavage of ATG5 and Beclin1 in Cisplatin Injury to Renal Tubular Epithelial Cells** Christian Herzog, Cheng Yang, Gur P. Kaushal. *Medicine, University of Arkansas for Medical Sciences & CAVHS, Little Rock, AR.*

Autophagy is a catabolic process within cells for the purpose of cellular regeneration. Normally autophagy plays a pro-survival role however excessive or unchecked autophagy leads to autophagic cell death. We have recently demonstrated that autophagy as well as key autophagy proteins LC3, Beclin1 and Atg5 were induced in cisplatin injury to LLC-PK1 cells during the pre-apoptotic lag phase and this induction of autophagy suppressed apoptosis and played an adaptive role (AJP-RP 294:777). Our present study shows that in LLC-PK1 cells Atg5 and Beclin1 are cleaved after treatment with cisplatin (CP) at a time when cisplatin-induced caspase activation maximally occurs. The pan-caspase inhibitor ZVAD-fmk prevented cisplatin-induced cleavage of Atg5 and Beclin1, suggesting that caspases are involved in the breakdown of key autophagy proteins. Neither caspase-3 inhibitor DEVD-fmk nor calpain-I/-II inhibitors ALLM or ALLN prevented the cleavage, suggesting that caspases other than caspase-3 are involved in the cleavage. ZVAD-fmk treatment prevented cisplatin-induced caspase activation (-97.4%) as well as subsequent apoptosis and enhanced autophagy as revealed by conversion of LC3-I to lipidated LC3-II. However, ZVAD-fmk did not completely prevent cisplatin-induced cell death but resulted in cisplatin-induced cytotoxicity as shown by MTT assay (%viability after 16h CP: 70.9±3.2, CP+ZVAD-fmk: 85.4±5.8) and LDH-release (% cytotoxicity after 16h CP: 9.1±0.7, CP+ZVAD-fmk: 22.9±2.2). The autophagy inhibitor 3-methyl adenine significantly reduced ZVAD-fmk-induced cytotoxicity in cisplatin-treated cells, suggesting a switch from apoptosis to autophagic cell death. These findings reveal that inhibition of caspase activity by ZVAD-fmk prevented cleavage of Atg5 and Beclin1 that regulated autophagy and switched cisplatin-induced apoptosis to autophagic cell death. In conclusion, our studies demonstrate that in cisplatin injury to LLC-PK1 cells, autophagy is regulated by breakdown products of key autophagic proteins Beclin1 and Atg5 and that inhibition of cisplatin-induced apoptosis results in autophagic cell death.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO773**

**Glycoprotein nmb (Gpnmb) Is Highly Upregulated Macrophage Protein in Ischemia/Reperfusion Injured Kidneys and Shed into Urine** Letian Zhou,[1] Takaharu Ichimura,[1] Michael A. Ferguson,[2] Sushrut S. Waikar,[1] Joseph V. Bonventre.[1] *[1]Renal Division, Brigham and Women's Hospital, Boston, MA; [2]Nephrology, Children's Hospital Boston, Boston, MA.*

Gpnmb is a glycosylated transmembrane protein which is expressed in various types of cells, including macrophages (MΦs) and dendritic cells (DCs). Gpnmb modulates inflammation in MΦs and activation of T lymphocytes. Gpnmb is expressed at high levels in post-ischemic kidney, but the function of Gpnmb in injury and repair has not been elucidated. We investigated the expression and function of Gpnmb in postischemic kidney in vivo and in bone marrow-derived macrophages (BMMΦs) in vitro. Gpnmb was highly expressed in MΦs in the outer medulla in rat and mouse kidneys after ischemia/reperfusion injury. Gpnmb positive MΦs were detected in the interstitium at 48 hours and were subsequently located in the kidney tubule lumina at 96 hours after ischemic injury. Flow cytometry of isolated CD45 positive cells from the post-ischemic kidney revealed surface Gpnmb on MΦs but also on CD3+T lymphocytes which do not express endogenous Gpnmb. In vitro, a majority of primary cultured mouse large multinucleated and small mononucleated BMMΦs expressed Gpnmb. Lipopolysaccharide activation strongly induced Gpnmb secretion into the conditioned media from BMMΦs and RAW264.7 mouse monocytic leukemia cells. Since Gpnmb can bind to the surface of leukocytes, we determined whether Gpnmb-Fc bound to BMMΦs and DCs. Gpnmb-Fc strongly and specifically bound to the surfaces of BMMΦs and DCs, and was quickly endocytosed moving to lysosomes.

The robust secretion of Gpnmb suggests that Gpnmb can be a biomarker of inflammation, produced by inflammatory MΦs in injured kidneys. Using Western blot analysis and ELISA, we detected that Gpnmb protein was markedly increased in urine after murine ischemic kidney injury, as early as 6hr after reperfusion, as well as in humans with kidney disease.

These data suggest that Gpnmb may play a role in the modulation of MΦs, DCs and T lymphocytes activation during injury and repair. Furthermore, Gpnmb may serve as a biomarker of inflammation associated with acute kidney injury in humans and rodents.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO774**

**Autophagy Plays Cytoprotective Role in Cisplatin Nephrotoxicity In Vitro and In Vivo** Cheng Yang,[1] Christian Herzog,[1] Sarika Kadam,[1] Gur P. Kaushal.[1,2] *[1]Medicine, UAMS, Little Rcok, AR; [2]Medicine, CAVHS, Little Rock, AR.*

Autophagy is a physiologically regulated and evolutionarily conserved process in which cellular organelles and macromolecules sequestered in a double-membraned autophagosomes are delivered to lysosomes for degradation. The degraded cellular components are recycled to meet energy demands of the cell. We have previously reported autophagy is induced in response to cisplatin (CP) and suppressed apoptosis *in vitro* and played an adaptive role in CP injury to renal tubular epithelial cells (RTEC). (AJP Renal Physiol. 2008 294(4):F777-87). To further investigate the role of autophagy in CP nephrotoxicity *in vivo*, we examined the expression of autophagic proteins and formation of autophagosomes during the progression of CP nephrotoxicity in a mouse model and in RTEC *in vitro*. The key autophagy proteins LC3, Atg5, Atg12, and beclin-1 were induced after 1 day of CP administration and their expression was markedly increased at 2, 3, and 4 days. In addition, the formation of lipidated LC3 (LC3-II) from LC3-I was significantly increased in the kidneys at 2, 3, and 4 days following CP administration. The autophagosomes were maximally detected in the kidney cortex at 3 day after CP administration. The punctate fluorescent staining of autophagosomes was identified after administration of adeno-GFP-LC3 to the kidneys and by immunostaining with specific antibody to LC3. Overexpression beclin-1 and Atg5 by transfection with pmCherry-ATG5 and pcDNA3-Beclin1 in RTEC prevented CP induced cell death as revealed by LDH release and caspase assays. Since chloroquine inhibits the fusion of autophagosomes with lysosomes to prevent the efflux of autophagy process, we examined the effect of chloroquine in renal injury during CP nephrotoxicity. Administration of chloroquine increased kidney dysfunction as revealed by BUN and creatinine and kidney histology. These findings suggest that during the progression of CP nephrotoxicity, autophagy is maximally induced at the injury-repair junction and plays a cytoprotective role. Thus, the autophagy pathway may serve as an important therapeutic intervention for preventing or reducing CP-induced acute kidney injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO775**

**Chemical Anoxia (CA) Activates 5' Adenosine Monophosphate-Activated Protein Kinase (AMPK) Which Ameliorates Apoptosis of Renal Tubular Cells (RTCs) by Stimulating the Akt Pathway** Wilfred Lieberthal,[1] Leiqing Zhang,[1] Vimal Patel,[2] Jerrold S. Levine.[2] *[1]Medicine, Stony Brook Medical Center, New York, NY; [2]Medicine, University of Chicago at Illinois, Chicago, IL.*

AMPK is a ubiquitous kinase that is activated by any form of cell stress that reduces ATP levels. AMPK is a heterodimer comprised of a catalytic (α-) and two (β and γ) regulatory subunits. ATP depletion activates AMPK by inducing phosphorylation of its catalytic subunit . There is evidence that activation of AMPK ameliorates ischemic injury in brain, heart and liver. However, its role in determining the renal response to acute injury is not known. We have evaluated the role of AMPK in modulating the fate of cultured renal tubular cells (RTC) exposed to chemical anoxia (CA) by "knocking-down" expression of the catalytic domain of AMPK using short-hairpin RNA (shRNA).

There are two isoforms of the catalytic subunit of AMPK (α-1 and α-2 ) each encoded by a separate gene. We show that while both isoforms are expressed in substantial amounts in heart and liver, the α2 isoform represents most (>85%) of the total catalytic domain expressed in RTCs. We also show that shRNA directed against the α2-subunit, knocks down the expression of this isoform by >80%. CA induced by 2-deoxyglucose (DOG) reduces ATP levels, increases phosphorylation (activation) of AMPK, and induces apoptosis of RTCs. Knocking down expression of the α2 subunit of AMPK during CA reduces the level of total and activated AMPK (by >80%) and markedly exacerbates apoptosis of RTCs without altering cellular ATP levels. Interestingly, in addition to activating AMPK, CA also activates Akt. Furthermore, knocking down the expression of the α2 subunit of AMPK inhibits Akt activation, suggesting that AMPK acts upstream in the activation of Akt by CA.

Conclusions: i) The α2-subunit is the predominant catalytic isoform of AMPK expressed in RTCs; ii) AMPK plays an important role in reducing apoptosis of RTCs subjected to CA; iii) AMPK's protective effect is independent of changes in cellular ATP levels; iv) reduction of apoptosis by AMPK may be mediated, at least in part, by activation of Akt by AMPK.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO776

**Kidney Biopsies in Patients with Cardiorenal Syndrome Awaiting Heart Transplant** Priya Deshpande,[1] James M. Pullman,[2] Ladan Golestaneh.[1] *[1]Medicine, Division of Nephrology, Montefiore Medical Center, Bronx, NY; [2]Pathology, Montefiore Medical Center, Bronx, NY.*

Background: Cardiorenal syndrome is defined as the renal manifestations of hemodynamic failure; renal parenchymal disease is not a part of this definition.

Purpose: We characterized the renal biopsies of patients awaiting heart transplantation and diagnosed with cardiorenal syndrome.

Methods: Nine heart transplant candidates underwent kidney biopsies from January 2007 to Februrary 2010 at Montefiore Medical Center. The biopsies were done to evaluate the presence of parenchymal disease in patients with CKD III or worse. Proteinuria was not a factor in the decision to do the biopsy. The renal pathology regarding the appearance of the glomeruli, tubules, interstitium and blood vessels were assessed.

Results: All 9 patients were male, 7 of whom had ischemic cardiomyopathy and 2 with non-ischemic cardiomyopathy. None of the patients had significant proteinuria or sonographic abnormalities in kidney size. The average eGFR was 33.6 mL/min. Five patients had evidence of expansion of the mesangial matrix, 4 of whom had glomerulomegaly. Five patients also showed mild to severe atrophy of the tubules, and 2 patients had acute tubular necrosis. Six patients had mild to moderate interstitial fibrosis. Two patients had hyaline changes in the blood vessels, and 3 patients had mild or focal arteriolosclerosis.

| Patient | Cardiac disease | Comorbidities | Cr | eGFR (mL/min) | Proteinuria | Ultrasound | Renal pathology |
|---|---|---|---|---|---|---|---|
| 1 m-ale | Ischemic CM | atrial fibrillation<br>hypertension<br>PVD | 2 | 34 | 0.3 | R 12.7, L 13.2 | glomeruli: mabtic moderately expanded<br>tubules: moderate atrophy<br>interstitium: moderate fibrosis<br>blood vessels: hyaline change |
| 2 m-ale | Non-ischemic dilated | hypertension<br>diabetes<br>MGUS | 2.1 | 35 | n/a | R 11.3, L 11.5 | glomeruli: glomerulomegaly<br>tubules: mild atrophy<br>interstitium: mild fibrosis<br>blood vessels: mild arteriolosclerosis |
| 3 m-ale | Ischemic CM | diabetes<br>hyperlipidemia<br>atrial fibrillation | 2 | 35 | 0.18 | R 13.2, L 13.5 | glomeruli: ↑m-esangium, glomerulomegaly<br>tubules: moderate atrophy<br>interstitium: moderate fibrosis<br>blood vessels: focal hyaline change |
| 4 m-ale | Ischemic CM | diabetes<br>hypertension<br>atrial fibrillation | 1.9 | 36 | 0.5 | R 10.8, L 11.1 | glomeruli: some enlarged glomeruli<br>tubules: mild atrophy<br>interstitium: mild atrophy<br>blood vessels: focal arteriolosclerosis |
| 5 m-ale | Ischemic CM | hypertension<br>atrial fibrillation<br>hyperlipidemia | 2.2 | 35 | none | R 14.1, L 14.8 | glomeruli: glomerular enlargement, ↑ m-esangium<br>tubules: no atrophy<br>interstitium: no fibrosis<br>blood vessels: no inflammatory lesions |
| 6 m-ale | Ischemic CM | hypertension<br>diabetes | 2.4 | 29 | 0.36 | R 12.9, L 12.9 | glomeruli: glomerulosclerosis; ↑ mesangial matrix<br>tubules: severe atrophy<br>interstitium: moderate to severe fibrosis<br>blood vessels: arteriolar hyaline changes |
| 7 m-ale | Ischemic CM | none | 1.8 | 38 | none | R 9.6, L 10.3 | glomeruli: n/a<br>tubules: n/a<br>interstitium: mild fibrosis<br>blood vessels: n/a |
| 8 m-ale | Non-ischemic Dilated CM | atrial fibrillation<br>hypertension | 5.1 | 13 | n/a | R 12.2, L 10.6 | medulla only<br>luminal necrotic cells consistent with ATN |
| 9 m-ale | Ischemic CM | Hypertension | 1.6 | 47 | 0.05 | R 9.7, L 10.4 | glomeruli: normal<br>tubules: normal<br>interstitium: normal<br>blood vessels: mild arteriosclerosis |

Conclusion: In this cohort of heart transplant candidates with cardiorenal syndrome but without evidence of significant proteinuria, renal biopsies showed some consistent parenchymal abnormalities, including tubular atrophy, interstitial fibrosis, glomerulomegaly and mesangial expansion. These findings are contrary to the common belief that cardiorenal syndrome is solely secondary to hemodynamic perturbations without parenchymal renal disease.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO777

**Rapid Restoration of ATP by SS-31, an Inhibitor of Mitochondrial Permeability Transition, Prevents Tubular Cytoskeletal Rearrangement in Renal Ischemia-Reperfusion Injury** Hazel H. Szeto, Shaoyi Liu, Yi Soong, Surya V. Seshan. *Pharmacology and Pathology, Weill Cornell Medical College, New York, NY.*

*Purpose*. ATP depletion during renal ischemia induces rapid rearrangement of b1-integrin, E-cadherin, and $Na^+K^+$-ATPase, leading to acute kidney injury. SS-31, an inhibitor of mitochondrial permeability transition (MPT), was shown to accelerate the recovery of ATP during early reperfusion and prevented tubular necrosis. Here we examined the effects of SS-31 on tubular cell architecture during early reperfusion after prolonged ischemia.

*Methods.* Sprague Dawley rats were subjected to bilateral renal ischemia for 45 min followed by reperfusion. SS-31 (2 mg/kg) or saline was given s.c. 30 min before ischemia and prior to reperfusion. Kidneys were harvested after 5, 20, 60 min or 24 h reperfusion for mitochondrial studies, ATP content, histopathology and immunohistochemistry of E-cadherin, b1-integrin and $Na^+K^+$-ATPase. Blood and urine were collected at 24 h for renal function.

*Results*. The early recovery of ATP provided by SS-31 preserved brush border and b1-integrin and prevented cell detachment. Preservation of E-cadherin on the lateral membrane and $Na^+K^+$-ATPase on the basal membrane led to reduction in serum creatinine and fractional $Na^+$ excretion at 24 h (***P<0.001).

Effects of SS-31 on early cytoskeletal changes

| Parameter | 5 min | | 20 min | | 60 min | |
|---|---|---|---|---|---|---|
| | saline | SS-31 | saline | SS-31 | saline | SS-31 |
| Brush border | disrupted | present | some loss | present | dramatic loss | some loss |
| β1-integrin | some loss | present | loss | present | dramatic loss | present |
| Cell detachment | no | no | no | no | yes | no |
| E-Cadherin | present | present | present | present | redistribution | present |
| Na+K+-ATPase | present | present | present | present | redistribution | present |
| Histopathology | some swollen cells | some swollen cells | swollen cells | few swollen cells | sloughed cells | few swollen cells |

*Conclusion*. These results indicate that prevention of MPT during renal ischemia results in rapid restoration of ATP upon reperfusion that is critical for maintaining tubular epithelial cytoskeleton and polarity, and minimizes tubular necrosis and renal dysfunction. SS-31 is a promising drug candidate for acute kidney injury.

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## TH-PO778

**Generation and Characterization of Site-Specific Heme Oxygenase-1 Overexpressing Mice** Subhashini Bolisetty,[1,2,3] Amie Traylor,[1,3] Daniel Mcfalls,[3] Anupam Agarwal.[1,2,3] *[1]Medicine, University of Alabama at Birmingham, Birmingham, AL; [2]Cell Biology, University of Alabama at Birmingham, Birmingham, AL; [3]Division of Nephrology, University of Alabama at Birmingham, Birmingham, AL.*

Heme Oxygenase-1 (HO-1), an anti-oxidant enzyme is induced during oxidative stress and is protective against cell death. HO-1 catalyzes the degradation of heme, resulting in the liberation of biliverdin, iron and carbon monoxide. HO-1 deficient mice are highly sensitive to acute kidney injury secondary to ischemia, rhabdomyolysis and nephrotoxins and prior induction of HO-1 is protective. It has been suggested that generalized HO-1 overexpression may be harmful due to excess generation of its by-products. The purpose of this study was to generate cre-lox inducible site-specific overexpression of HO-1 in mice. To this end, we generated a floxed expression vector (chicken ß-actin CMV hybrid promoter (CBA)–lox-lac z-stop signal cassette-lox-HO-1). This vector expresses only the lac Z gene and not HO-1. However, in the presence of cre recombinase, the lox sites recombine to release the lac Z-stop signal cassette, resulting in HO-1 overexpression. Transgenic mice harboring the CBA lox HO-1 construct were generated and the presence of the transgene was verified by PCR, Southern blot analysis. These mice were first characterized for the expression of lac Z in various organs by staining for β-galactosidase. The proximal tubules are the primary target of damage in acute kidney injury and are also the site where maximal HO-1 induction occurs. Therefore, CBA lox HO-1 mice were bred with phosphoenolpyruvate carboxykinase cre mice (PEPCK cre mice, obtained from Dr.Volker Haase). Successful cre recombination was confirmed in kidneys of double transgenic mice by positive immunostaining for HO-1 in renal proximal tubules using lotus lectin as a marker for proximal tubules. Proximal tubular cells isolated from double transgenic mice also showed increased HO-1 expression compared to the PEPCK-cre mice. These mice will be a valuable tool for further in vivo studies to test the effects of site specific HO-1 expression in renal proximal tubules in models of acute kidney injury.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO779

**Hibernation Affects Kidney Function and Morphology** Alkesh Jani, Jessica Martin, Elaine Epperson, Kultigin Turkmen, Kameswaran Ravichandran, Arijana Pacic, Danica Ljubanovic, Sandy Martin, Charles L. Edelstein. *U of Colorado.*

Prolonged cold ischemia (CI)/warm reperfusion (WR) are important causes of DGF that manifest as apoptosis, necrosis and brush border injury (BBI). The 13-lined ground squirrel is a hibernating mammal that cycles through alternating phases of extreme CI for several days during torpor (T) followed by re-warming during interbout arousal (IBA) each winter. Hibernation is a natural model of repeated prolonged CI followed by WR. We hypothesized that hibernating squirrel kidneys are protected from apoptosis, necrosis and BBI. Methods Radiotelemeters were implanted in 1-2 yr old squirrels, and CBT was monitored constantly. Kidneys from summer control animals (S), Torpid (T), and IBA animals were assessed. Tubules displaying apoptosis, necrosis and BBI were counted. Results There were no necrotic and very few apoptotic cells at all stages. BBI was significantly worse and more severe in IBA (table 1).

Table 1

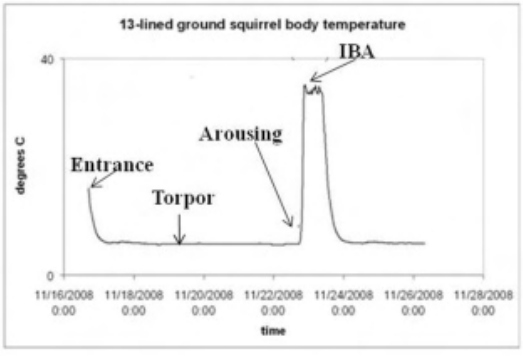
| Stage | Apoptotic cells/10 hpf | Necrotic cells/10 hpf | BBI/100 tubules | Severity of BBI |
|---|---|---|---|---|
| Summer (S) | 2 | 0 | 44±6 | 1+ |
| Torpor (T) | 1 | 0 | 0 | 0 |
| IBA | 1* | 0 | 74±3** | 3+ |

n = 3; * p = NS vs S, T ; **p<0.05 vs S, LT

To assess the functional significance of BBI we measured serum creatinine (SCr) and urine osmolality (Uosm) at various stages (Table 2) SCr peaked during the Arousing phase (the transition phase between Torpor and IBA), following 1-2 weeks of CI, but then normalized during IBA suggesting a return of renal function.

| Stage | Duration | CBT (°C) | sCr | Urine osm |
|---|---|---|---|---|
| Summer (S) | 5 mos | 37 | 0.3 | 2172± 364 |
| Entrance (E) | 25 hrs | 37→4 | 0.3 | 558±94 |
| Torpor (T) | 5-20 days | 0-4 | 0.4 | 840±115 |
| Arousing | 2 hrs | 4→37 | 0.7* | 491±87 |
| IBA | 12 hrs | 37 | 0.3 | 1491± 157 |

* $p < 0.001$ vs. S, E, T, IBA



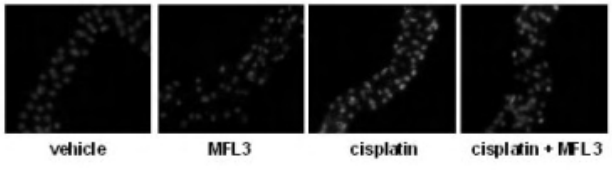

Uosm peaked in Summer animals, was lowest in T animals and approached Summer values during IBA. Conclusion Despite extreme CI followed by repeated WR, hibernating kidneys are completely protected from apoptosis and necrosis. With WR during IBA, BBI was significantly worse but remarkably, SCr rapidly normalized and the ability to concentrate urine returned.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO780**

**Fas Ligand Mediates Fratricide of Tubular Cells In Vivo** Andreas Linkermann,[1] Nina Himmerkus,[2] Ulrich Kunzendorf,[1] Stefan Krautwald.[1] *[1]Nephrology and Hypertension, Christian-Albrechts University, Kiel, Germany; [2]Institute for Physiology, Christian-Albrechts University, Kiel, Germany.*

The apoptosis inducing death factor Fas Ligand (FasL) is best characterized for its function in T-cells and NK-cells regarding the clearance of virally infected cells and the termination of an immune response by activation-induced cell death, a process referred to as fratricide. Recent reports have suggested a physiologically relevant expression of FasL in the kidney. We treated SCID/beige mice with the FasL-neutralizing monoclonal antibody MFL3 that completely restored survival after otherwise lethal CIN, suggesting a functionally relevant source of FasL in renal homeostasis besides immune cells. In order to analyze whether kidney cells, like T-cells, die in a FasL-dependent manner after cisplatin-stimulation, we isolated segments of the thick ascending limb (TALs) from mouse kidneys and determined FasL-mediated apoptosis, which could be blocked with MFL3 in the complete absence of immune cells.



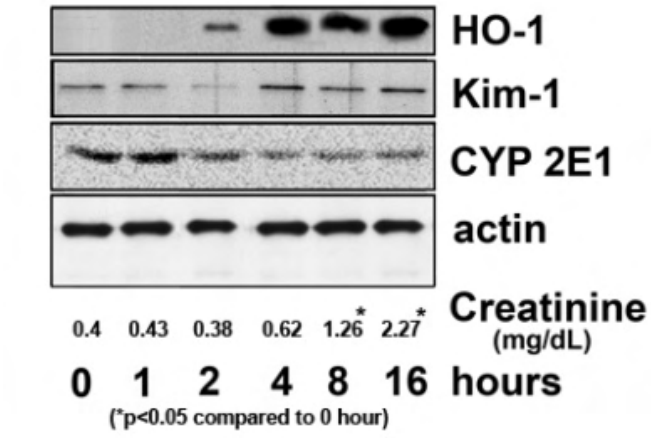

Furthermore, cisplatin-stimulated primary tubular cells induced apoptosis in TALs freshly isolated from GFP-transgenic mice. This renal-cell fratricide could be blocked by MFL3. We conclude that CIN is mediated though FasL, which is functionally expressed on fratricide-inducing tubular cells. Our observations reveal an additional mechanism that significantly contributes to organ failure besides the infiltration of FasL-bearing immune cells into the kidney.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO781**

**Cytochrome P450 (CYP) 2E1 Mediates Rhabdomyolysis-Induced Acute Kidney Injury** Radhakrishna Baliga, Niu Tian, Istvan Arany. *Pediatrics, University of Mississippi Med Ctr, Jackson, MS.*

Catalytic or "free" iron released from heme proteins following rhabdomyolysis plays an important role in ROS mediated acute kidney injury (AKI ) . The source of this catalytic iron is currently not known even though myoglobin has been implicated. CYPs are a group of heme proteins that can function as oxidases, degrade the heme protein and promote the release of "free" iron. We have previously demonstrated a marked increase in catalytic iron accompanied by a significant decrease in the CYP content in the kidneys in this model of injury ( Kid Int: 49:362, 1996 ). CYP 2E1 has been identified to the rat renal tubular cells however its role in rhabdomyolysis induced AKI has not been previously examined. Rats injected with 50% glycerol ( 8ml/Kg/IM ) following overnite fast had marked increase in myoglobin in the kidney and plasma as early as 15min with no significant change in the liver. The onset of AKI as demonstrated by the upregulation of the kidney injury molecule-1 (KIM-1 ) occurred at 4h following glycerol injection and prior to significant deterioration in renal function.




This clearly indicates that despite very early and markedly elevated levels of myoglobin in the kidney the onset of AKI occurs only following the breakdown of the CYP 2E1 heme protein and the induction of heme oxygenase (HO-1)- an indicator of oxidative stress . Administration of Chlormethiazole ( CMZ ) a specific inhibitor of CYP2E1 transcription, completely inhibited the CYP 2E1 induction of the mRNA and the enzyme levels, and provided significant functional protection (SCr: Gly 1.6 vs Gly+CMZ 0.8 mg/dl, $P < 0.05$) against glycerol induced- AKI. Our results thus suggest an important role of CYP 2E1 as a source of catalytic iron in rhabdomyolysis induced oxidant injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO782**

**Up-Regulated Sirt1 in Renal Ischemia/Reperfusion Injury Causes Autophagy and Inhibits Apoptosis and Protects Against Oxidative Stress in Renal Tubules** Yoshiko Shimamura. *Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Kohashu, Oko-cho, Nankoku, Japan.*

Autophagy has been shown to contribute to the elimination of accumulated misfolded proteins. In the previous study we reported autophagy occurred in the rat AKI model. One genetic pathway that mediates cell response to ROS stress comprises the sirtuin family. Sirt1 deacetylates many target proteins, which protect against ROS stress and plays an essential part in mediating the survival of many types of the cells.

To clarify the significance of Sirt1 pathway in AKI, we used a rat AKI model *in vivo* and cultured renal tubular cells (NRK-52E cells) as an *in vitro* model. After clamping renal artery for 1 h, kidney homogenate at 1~72 h after reperfusion was extracted. Sirt1 expression and deacetylates activity is increased at 12-24h. To understand the regulation of Sirt1, we transfected Sirt1-promoter-luciferase construct to NRK-52E cells. $H_2O_2$ significantly increase promoter activity and protein expression of Sirt1.Overexpression of Sirt1 and resveratrol protect $H_2O_2$-induced apoptosis . Furthermore, to examine Sirt1 causes autophagy or not, we established NRK-52E cells which stably transfected with a fusion protein between GFPand light chain 3 as a marker of autophagy. Using this cell line, we detect autophagy as a GFP-positive-autophagosome. Overexpression of Sirt1 caused autophagy and the number of autophagosome markedly increased by resveratrol.

In conclusion, Sirt1 is transcriptionally up-regulated in hypoxic condition . Sirt1 constitutes a determinant of renal tubular cell apoptosis and autophagy. The current study therefore unravels both physiological and pathological significance of Sirt1 in ROS-dependent cell autophagy and apoptosis of renal tubular cells in vivo and in vitro.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO783**

**Src Family Kinases Regulate Renal Epithelial Dedifferentiation through Activation of EGFR/PI3K Signaling** Shougang Zhuang. *Department of Medicine, Brown University School of Medicine, Rhode Island Hospital, Providence, RI.*

Dedifferentiation, a process by which differentiated cells become mesenchymal-like proliferating cells, is the first step in renal epithelium repair and occurs *in vivo* after acute kidney injury and *in vitro* in primary culture. However, the underlying mechanism remains poorly understood. We studied the signaling events that mediate dedifferentiation of proximal renal tubullar cells (RPTC) in primary culture. RPTC dedifferentiation characterized by increased expression of vimentin concurrent with decreased expression of cytokeratin-18 was observed at 24 h after the initial *plating of* freshly *isolated* proximal *tubules* and persisted for 72 h. At 96 h, RPTC started to redifferentiate as revealed by reciprocal expression of cytokeratin-18 and vimentin and completed at 120 h. Phosphorylation levels of Src, epidermal growth factor receptor (EGFR), AKT [a target of phosphoinositide-3-kinase (PI3K)] and ERK1/2 were increased in the early time course of culture (<72 h). Inhibition

of Src family kinases (SFKs) with PP1 blocked EGFR, AKT and ERK1/2 phosphorylation, as well as RPTC dedifferentiation. Inhibition of EGFR with AG1478 also blocked AKT and ERK1/2 phosphorylation and RPTC dedifferentiation. Although inactivation of the PI3K/AKT pathway with LY294002 inhibited RPTC dedifferentiation, blocking the ERK1/2 pathway with U0126 did not show such an effect. Moreover, inhibition of SFKs, EGFR, PI3K/AKT, but not ERK1/2 pathways, abrogated RPTC outgrowth. SFK inhibition also decreased RPTC proliferation and migration. These findings demonstrate a critical role of SFKs in mediating RPTC dedifferentiation through activation of the EGFR/PI3K signaling pathway.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO784**

**p53 Target Siva Regulates Apoptosis in Ischemic Mice Kidneys** Babu J. Padanilam, Kurinji Singaravelu. *Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE.*

The role of p53 in inducing apoptosis following AKI is well established; however, the molecular mechanisms remain largely unknown. We report here that the p53 pro-apoptotic target Siva and its receptor CD27, a member of TNFR family, are upregulated following renal IRI. Inhibition of Siva using antisense oligonucleotides conferred functional and morphological protection, and prevented apoptosis post-renal IRI in mice. Renal IRI in CD27-deficient mice displayed functional protection, attenuated caspase-8 activation and partial inhibition of apoptosis, suggesting an incomplete role for CD27 in Siva-mediated apoptosis. In vitro, hypoxia induced Siva expression in LLCPK1 cells in a p53-dependent manner and Siva inhibition prevented apoptosis. In Siva transfected LLCPK1 cells, Siva is persistently expressed in the nucleus at 3h onwards and its translocation to mitochondria and the plasma membrane occurred at 6h. Moreover, Siva overexpression induced mitochondrial permeability, cytochrome c release, caspase-8 and -9 activation, translocation of apoptosis-inducing factor (AIF) to the nucleus, and apoptosis. Inhibition of Siva in ischemic kidneys prevented mitochondrial release of cytochrome c and AIF. These data indicate that Siva function is pivotal in regulating apoptosis in the pathology of renal IRI. Targeting Siva may offer a potential therapeutic strategy for renal IRI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO785**

**The Mismatch Repair Protein MSH2 Is a Novel Regulator of ATR during Cisplatin-Induced DNA Damage Response and Apoptosis in Kidney Cells** Navjotsingh P. Pabla,[1] Zheng Dong.[1] *[1]Department of Cellular Biology and Anatomy, Medical College of Georgia and Charlie Norwood VA Medical Center, GA; [2]; [3]GA.*

p53 is activated during cisplatin treatment of renal cells and tissues and contributes to cisplatin-induced nephrotoxicity or acute kidney injury (AKI). Recent work has further shown that p53 activation in cisplatin-AKI involves ATR (ATM- and Rad3-related) and Chk2 (checkpoint kinases-2), two important DNA damage response protein kinases. ATR is activated early during cisplatin treatment, leading to Chk2 activation and subsequent phosphorylation and activation of p53, resulting in the expression of pro-apoptotic genes such as PUMA-a and subsequent apoptosis. Despite these findings, the early molecular events responsible for ATR activation are not known. In this study, we used the Blue-native PAGE technique to reveal several proteins that interact with ATR. Tandem Mass Spectrometry further identified one of the ATR-interacting proteins to be MSH2, a DNA mismatch repair protein. ATR/MSH2 interaction was drastically induced during cisplatin treatment of renal tubular cells. Importantly, cisplatin-induced ATR activation or nuclear foci formation was diminished in MSH2-knockout mouse embryonic fibroblasts and MSH2-knockdown kidney cells. Consistently, in these cells cisplatin-induced Chk2 activation, p53 induction and PUMA-a accumulation were all suppressed, so was apoptosis. On the other hand, MSH2 deficiency did not affect cisplatin-induced translocation of the 9-1-1 (Rad9-Hus1-Rad-1) protein complex, the canonical system for ATR activation. Thus we have identified a novel mechanism of ATR activation via MSH2, which plays a key role in cisplatin-induced DNA damage response and apoptosis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO786**

**Distinct Pathophysiologic Mechanism of Septic Acute Kidney Injury: Role of Tregs and the Effect of Mesenchymal Stem Cells** So-Young Lee,[1] Sang-Kyung Jo,[2] Kichul Yoon,[2] Myung-Gyu Kim,[2] Won-Yong Cho.[2] *[1]Nephrology, Eulji Medical Center, Seoul, Republic of Korea; [2]Nephrology, Korea University Hospital, Seoul, Republic of Korea.*

Introduction : Although mechanisms of sepsis on acute kidney injury (AKI) has been known to be associated with intense renal vasoconstriction with tissue inflammation, precise pathophysiologic mechanisms leading to AKI is unknown. Recent reports suggested that excessive immune suppressive status (CARS) following SIRS in sepsis might be responsible for transient organ dysfunction. The purpose of this study was to examine the mechanisms of septic AKI by providing on-site quantitative comparison between septic vs ischemia/reperfusion (I/R) AKI and also examine the effect of mesenchymal stem cells (MSCs) on septic AKI.

Methods : At 24 h after cecal ligation puncture, biochemical, histologic kidney injury, kidney and systemic inflammation were assessed and effect of MSCs on these parameters were also compared.

Results : Despite comparable level of functional impairment in both groups, degree of systemic and kidney inflammation and histologic kidney injury showed marked differences. While tubular necrosis, predominant in I/R kidneys was hardly observed in septic kidneys, the amount of apoptosis and caspase 3 activation showed positive correlation with plasma creatinine in septic AKI. Serial systemic cytokine profile indicated the possible conversion from SIRS to CARS and this was also associated with scant tissue inflammation in septic AKI. Percentage of immunosuppressive Tregs increased in spleen of septic mice and depletion of Treg abrogated kidney dysfunction. Administration of MSCs that has known to have immune-modulatory effect protected kidney dysfunction in septic AKI. The beneficial effect of MSCs in septic AKI was associated with paradoxical increase in kidney inflammation and decrease in percentage of Treg.

Conclusion : These results suggest that septic AKI is mediated via distinct pathophysiologic mechanisms. Apoptosis might substantially contribute to kidney dysfunction and excessive immune suppressive status induced by sepsis is thought to be responsible for transient functional impairment in septic AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO787**

**Renal Gene Expression Profiling Demonstrates a Local Inflammatory Response Induced by Cardiopulmonary Bypass in Rat** Hjalmar R. Bouma, Iryna V. Samarska, Maria Schenk, Leo E. Deelman, Robert H. Henning. *Clinical Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands.*

**Background:** Cardiopulmonary bypass (CPB) is a commonly used technique in cardiac surgery. However, CPB is associated with acute kidney injury (AKI). Also, a temporary peri-operative decrease of renal function negatively influences long-term survival. To obtain more insight into the pathogenesis of impaired renal function following CPB, we performed a microarray analysis of kidney gene expression in rat.

**Methods:** Rats underwent CPB or Sham procedure and were sacrificed at 60 min, 1 and 5 days following the procedure. Microarray analysis was used to determine expression changes in the kidney following CPB and Sham.

**Results:** Expression of 421 genes was significantly altered in CPB as compared to Sham, of which 407 genes in the acute phase (60 min) following CPB. Gene-ontology analysis revealed 28 of these genes involved in inflammatory responses, with major activation of mitogen activated protein-kinase (MAP-kinase) signaling pathways. Potent inducers identified constitute of the interleukin-6 cytokine family that consists of interleukin-6 (IL-6) and oncostatin M (OSM), which signal through the gp130-cytokinereceptor. Downstream signaling leads to production of chemokines, adhesion molecules and molecules involved in coagulative pathways. Production of chemokines and adhesion molecules stimulate early influx of monocytes, neutrophils and lymphocytes, thereby augmenting the renal inflammatory response.

**Conclusions:** The signaling pathways identified may represent pharmacological targets to limit CPB induced renal injury.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO788**

**HuR: A Candidate Post-Transcriptional Regulator of Immune Responses during Acute Kidney Injury** Rudolf Pullmann,[1] Gang Jee Ko,[1] Bernard S. Marasa,[2] Magdalena Juhaszova,[2] Yanfei Huang,[1] Karl L. Womer,[1] Hamid Rabb.[1] *[1]Division of Nephrology, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Biomedical Research Center, NIA, Baltimore, MD.*

Immune responses are important in the pathogenesis of renal ischemia reperfusion injury (IRI). Post-transcriptional regulation of gene expression is a powerful adaptive mechanism in immune activation. RNA-binding protein HuR functions as a positive regulator of mRNA stability, and/or translation, regulating immune genes such as IL-2, -4, and TNF-α.

We hypothesized that HuR level changes in T cells can underlie immune responses during AKI.

We isolated T cells from various locations [spleen, kidneys, kidney draining lymph nodes (LN)] in C57BL6 mice subjected to severe bilateral IRI, and studied the expression of HuR and its subcellular distribution.

As demonstrated by immunohistochemistry in splenic T cells, IRI caused translocation of HuR from nucleus to cytoplasm starting 4h post-IRI (1.8-fold elevation), persisting until 24h (2.1-fold elevation) and returning to baseline within 48h. By western blotting, total HuR protein levels remained unaffected within 24h in every evaluated T cell population. Using real-time PCR, there was 1.6-fold increase of HuR mRNA in splenic T cells, and 4.4-fold increase of HuR mRNA in kidney isolated T cells 24h post-IRI. T cells from kidney draining LN showed no change in HuR mRNA 24h post-IRI. In comparison to T cells, whole kidney levels of HuR at 4 and 24h after IRI remained unchanged at both mRNA and protein levels. Total HuR levels were also measured up to 4 weeks in mice subjected to moderate (30 min) of unilateral ischemia. At 10 days post-IRI, the total HuR levels in splenic T cells were 70% of the baseline, and returned to baseline by 4 weeks.

HuR translocation into cytoplasm of T cells during IRI suggests its role in regulating immune response genes. HuR mRNA elevation can be explained by increased transcription of HuR or increased HuR mRNA stability as HuR binds and stabilizes its own mRNA. Furthermore, decreased total HuR protein levels in T cells 10 days after IRI could contribute to the increased susceptibility to infection during AKI.

Disclosure of Financial Relationships: nothing to disclose

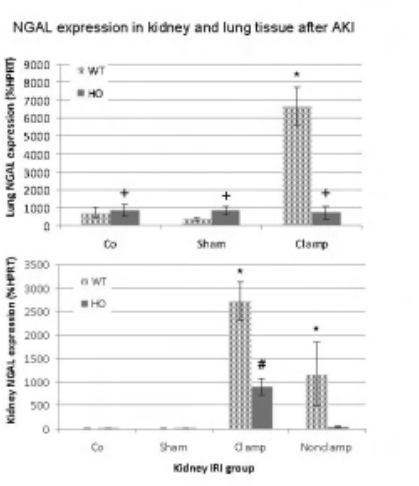
**TH-PO789**

**Oncostatin M Receptor Is a Major Gateway of the Local and Systemic Inflammatory Response after Acute Kidney Injury** Barbara Pedrycz, Catharine Compston, Lin-Fu Zhu, Colin C. Anderson, Valerie A. Luyckx, Thomas F. Mueller. *Medicine, Nephrology and Immunology, Edmonton, AB, Canada.*

Oncostatin M - Oncostatin M receptor (Osm-Osmr) signalling induces a strong renal acute phase response as shown in human kidney implant biposies and cell lines. This study investigates in more detail the local and systemic response to ischemia-reperfusion injury (IRI) using wild type (WT) and Osmr deficient (HO) mice [Blood 2003; 102:3154].

Acute kidney injury (AKI) was induced by clamping of the pedicle of one kidney for 45 mins followed by 24 hrs of reperfusion. Transcript levels of Osm, Osmr, Il6, Il6r, Lif, Lifr, Serpina3, and Ngal were measured in clamped, nonclamped contralateral, sham operated and untouched control kidneys and lungs of mice from each kidney experimental group by real-time RT-PCR.

The local inflammatory/injury response was significantly higher in the kidneys of the WT compared to the Osmr deficient mice after clamping. The significantly lower Ngal levels in the HO vs. WT clamped kidneys may suggest some protection against the IRI in Osmr deficient mice (# p<0.0001).




In addition both inflammatory and injury response (Ngal expression) in the lungs and contralateral nonclamped kidneys was significantly increased only in WT mice (* p<0.001). This suggests absence of distal effects of local inflammation in Osmr deficient mice.

In conclusion Oncostatin M receptor is a major gateway for amplification of the local inflammatory and injury response as well as a likely important mediator of injury and inflammation in distal organs.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO790**

**The Role of Chloride Intracellular Channel 4 (CLIC4) in Acute Tubular Injury** Christina R. Kahl, John C. Edwards. *Department of Medicine, UNC Kidney Center, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Chloride intracellular channel 4 (CLIC4) is highly expressed in both proximal tubule epithelial cells and peritubular capillaries, and previously published studies indicate CLIC4 may act as both an ion channel and an intracellular signaling molecule involved in apoptosis and TGFβ signaling pathways. The aim of this study is to determine if CLIC4 modulates the response to acute tubular injury in a mouse model system. *Clic4-/-* mice are viable and have normal renal development histologically. The folic acid nephrotoxic model of acute kidney injury results in rapid tubular injury and apoptosis followed by a recovery phase. Cohorts of *Clic4-/-* and wild type mice of similar age were injected with folic acid. The degree of acute renal injury was assessed by plasma BUN and histology. Male *Clic4-/-* mice demonstrated statistically significant lower BUN values than wild type mice at baseline (p<0.05).

Renal Injury After Injection

| Type of mouse | Number of mice | BUN baseline | BUN day 2 | Percent with BUN≥50 |
|---|---|---|---|---|
| Wild type male | 22 | 36 | 114 | 68 |
| *Clic4-/-* male | 20 | 25 | 74 | 30 |
| Wild type female | 17 | 28 | 208 | 76 |
| *Clic4-/-* female | 30 | 24 | 238 | 73 |

Following treatment, 68% of wild type male mice develop renal injury (which was defined as BUN≥50 2 days after injection) versus only 30% of male *Clic4-/-* mice (p<0.05), and there is a trend towards lower BUN values in *Clic4-/-* males. For female mice, 76% of wild type mice and 73% of *Clic4-/-* mice develop injury with similar average BUN values at day 2. Surprisingly, about 40% of *Clic4-/-* female mice died (or were sacrificed due to severe clinical illness) several days after folic acid injection compared to no deaths among wild type or *Clic4-/-* males. These results demonstrate a significant dichotomy in acute renal injury in male versus female *Clic4-/-* mice, providing a useful model to study differences in acute tubular injury between males and females. Consistent with the hypothesis that CLIC4 may regulate apoptotic and TGFβ pathways, male *Clic4-/-* mice demonstrate significant protection from acute tubular injury and allow an opportunity to elucidate functions of CLIC4 using an *in vivo* system.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO791**

**Activation of NALP3 Inflammasome in Cisplatin-Induced Acute Kidney Injury** Ali Akcay, Quocan Nguyen, Kultigin Turkmen, Dong Won Lee, Zhibin He, Alkesh Jani, Sarah Faubel, Charles L. Edelstein. *Univ of Colorado Denver.*

We have demonstrated that caspase-1 activity is increased in the kidney and caspase-1-deficient mice are protected against cisplatin-induced acute kidney injury (Cis-AKI). The "inflammasome" is a cytosolic protein complex composed of NALPs, ASC-CARD and caspase-5 that are required for the activation of caspase-1 and "leaderless proteins" such as the proinflammatory cytokine, IL-1α and the proapoptotic protein, BID. The aim of the study was to determine whether the inflammasome is activated in Cis-AKI. Wild-type mice were injected with cisplatin 25 mg/kg and sacrificed on days 1, 2 and 3. BUN and serum creatinine were elevated on day 3. On quantitative PCR of whole kidney, levels of NALP3 mRNA expression were increased on day 3 *vs.* on day 1 and vehicle-treated (Veh) (P<0.001, 0.75 *vs.* 0.1 fold change) and on day 3 *vs.* day 2 (P<0.01, 0.75 *vs.* 0.25 fold change). There was no increase in NALP1 mRNA levels (P>0.05). On immunoblots, NALP3 (106 kDa) was present in the freshly isolated proximal tubules, but not in cisplatin-treated endothelial cells or LPS-treated macrophages. On immunoblot of whole kidney, there were a 2-fold increase in ASC-CARD (22 kDa) (p<0.05) and a 3-fold increase in caspase-5 (47 kDa) after cisplatin on days 2 and 3 (P<0.05 *vs.* Veh). Caspase-5 activity measured by the fluorescent substrate WEHD-AMC was 11.2 in Veh and 29.2 in Cis-AKI (p<0.01). IL-1α (pg/mg) was 0.1 in Veh, 2.4 in Cis-AKI (P<0.001 *vs.* Veh) and 0.1 in caspase-1 -/- Cis-AKI (P<0.001 *vs.* Cis-AKI). Immunoblot of whole kidney showed a 2-fold increase increase in parent BID (22 kDa) and a 2-fold increase in caspase-1 cleaved BID (15 kDa) on day 3 (P<0.05 *vs.* Veh). In summary, the NALP3 inflammasome is activated in Cis-AKI as evidenced by increased caspase-1, caspase-5, ASC-CARD, IL-1α and BID. In conclusion, the exact role of the inflammasome in Cis-AKI merits further studies.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO792**

**Oxidative and Inflammatory Renal Injury Signaling Pathways Differ between the Sexes in Tubulointerstitial Fibrosis** Hong Ji,[1] Wei Zheng,[1] Jun Liu,[1] Xie Wu,[1] Janet J. Zhu,[1] Thurston Sandberg,[1] Kathryn Sandberg.[1] *[1]Medicine, Georgetown University, Washington, DC; [2]Washington, DC; [3]Washington, DC.*

Renal disease progression is faster in men compared to women in a number of nondiabetic renal diseases and this sex difference is also observed in numerous experimental animal models of chronic and acute renal disease including the unilateral ureteral obstruction (UUO) model of interstitial fibrosis. To study the underlying mechanisms of this sex difference, we investigated the mRNA expression of oxidative stress and inflammatory markers in male (M) and female (F) mice subjected to UUO. UUO increased the expression of the $p40^{phox}$ NADPH oxidase regulatory subunit to a 2.1-fold (p<0.01) greater extent in the male than in the female kidney one week after UUO [(AU): M-Sham, 0.20±0.02; M-UUO, 6.81±0.77; F-Sham, 0.21±0.04; F-UUO, 3.27±0.03]. Similar findings were observed for other key NADPH oxidase regulatory subunits; $p67^{phox}$ and $gp91^{phox}$ were increased by a 3.1-fold and 2.3-fold greater extent in the male than in the female kidney, respectively. Compared to sham surgery, monocyte chemotactic protein-1 (MCP-1) increased to a 4.5-fold (p<0.02) greater extent in the male compared to the female kidney [MCP-1 (AU): M-Sham, 0.22±0.01; M-UUO, 12.4±1.3; F-Sham, 0.19±0.03; F-UUO, 2.38±0.61]. Similar findings were observed for Th2 cytokines; the UUO-induced increase was greater in the male compared to the female kidney [IL-4 (UUO/Sham): M, 5.2-fold vs F, 2.6-fold, p<0.03][IL-10 (UUO/Sham): M, 8.1-fold vs F, 2.3-fold, p<0.02]. In contrast, the Th1 cytokines increased to the same extent in both male and female kidneys after UUO (data not shown). These data suggest that NADPH oxidase signaling pathways and Th2 but not Th1 signaling cascades are amplified in the male kidney compared to the female and that these sex differences in signal transduction underlie why the progression of renal interstitial fibrosis is greater in the male compared to the female mouse kidney. Supported by NIA R01 AG019291.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO793**

**Expression of Calcineurin and Regulator of Calcineurin 1 (RCAN1) in the Rat Kidney Following Lipopolysaccharide Administration** Jae-Youn Choi, So Young Lee, Jin Kim, Jung Ho Cha. *Anatomy, The Catholic University of Korea, College of Medicine, Seoul, Republic of Korea.*

RCAN1 (calcipressin 1, DSCR1, or MCIP1), an inbibitor of calcineurin, has been known to be a stress responsive protein that can be induced by multiple stresses. It is likely that RCAN1 may also play roles in a variety of organ including the kidney. However, no study about renal RCAN1 has been reported. In this study, we investigated the expression patterns of RCAN1 and calcineurin in the rat kidney after injection of lipopolysacchride (LPS). Male SD rats were subjected to a LPS injection (20 mg/kg bw, ip) and sacrificed at 8h, 1, 3, 7d after injection. Immunohistochemistry was perfomed using polyclonal anti-RCAN1 and anti-calcineurin Aα, Aβ and Aγ antibodies. In normal kidney, RCAN1 was expressed moderlately in S3 portion of proximal tubule (PT) compared with totally negative in S1 & S2

portions of PT, weakly in distal tubule (DT), cortical collecting duct (CCD), outer and inner medullary collecting duct (OMCD & IMCD), thick ascending limb (TAL), juxtaglomerular cell (JGC) and interlobular arteries (IR). RCAN1 was localized mainly in the cytoplasm, but in a few of cells, nuclear immunostaings were deteced. After LPS administration, RCAN1 was upregulated progressively to 1d, and RCAN1 immunoreactivity had returned to normal control levels by 7d. In 1d kidney, RCAN1 expression was very strong in S3 portion of PT, moderately strong in DT and CCD, and moderate in TAL, MCD, JGC and IR. After LPS administration, nuclear immunostaings were also increased progressive to 3d, especially in inner medulla, about half of IMCD cells showed nuclear staing. Calcineurin Aα and Aβ was expressed strongly in JGC, and thin limbs of inner medulla, and weakly in IMCD in normal kidney. After LPS administration, calcineurin Aα and Aβ was strongly induced in IR and some cells of CCD, OMCD and TAL. No calcineurin Aγ expression was detected both in the normal and LPS-treated kidney. Taken together, these results suggest that RCAN1 may play a role in LPS-induced renal hemodynamic change by regulating calcineurin activity both in IR and JGCs, and also perform some calcineurin-independent function especially in S3 segment of PT.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO794**

**Autophagy Induction and Alterations of Multiple Signaling Pathways in Response to Hypoxic and Ischemic Renal Injury** Man Jiang, Zheng Dong. *Department of Cellular Biology and Anatomy, Medical College of Georgia and Charlie Norwood VA Medical Center, Augusta, GA.*

Recent work has demonstrated autophagy and its protective role in hypoxic and ischemic renal injury. However, it remains unclear how autophagy is induced and regulated under these pathological conditions. The current study examined the signaling pathways that may contribute to the regulation of autophagy during hypoxia (1% $O_2$ in full culture medium) and anoxia-reoxygenation (<0.1% $O_2$ in glucose-free buffer followed by recovery in full culture medium with 21% $O_2$) in renal proximal tubular cells. In the hypoxia model, autophagy was induced within 6 hours of hypoxia and significantly increased after 12-24 hours. HIF-1α was induced at 1 hour of hypoxia, reached the peak at 3 hours and decreased thereafter. Bnip3, a BH3-only protein that has been suggested to regulate mitophagy, was induced in a HIF1-dependent manner and its up-regulation was paralleled with autophagy progression during hypoxia. In contrast, AMPK was not activated until 12-24 hours. Akt was transiently phosphorylated at 3-6 hours of hypoxia, while mTOR was inactivated at 12-24 hours. The changes of AMPK, Akt and mTOR showed temporal correlation with autophagy during the late time points of hypoxia incubation. In the anoxia-reoxygenation model, anoxia alone induced LC3-II accumulation but not formation of GFP-LC3 puncta. During the reperfusion phase, autophagy was induced undoubtedly, indicated by both GFP-LC3 punctuated cells and a time-dependent LC3-II turnover. HIF-1α was slightly upregulated during anoxia, whereas both Akt and mTOR were inactivated. MAP kinases, including ERK1/2, JNK1/2/3 and p38, were activated during reoxygenation. N-acetylcysteine significantly suppressed autophagy during anoxia-reoxygenation, suggesting a role for oxidative stress in autophagy in this model.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO795**

**Cdk2-Dependent Phosphorylation of p21 Regulates Cdk2 Role in Cisplatin (CP) Toxicity** Rawad Hodeify, Adel Tarcsafalvi, Judit Megyesi, Robert L. Safirstein, Peter M. Price. *UAMS, Little Rock, AR.*

CP cytotoxicity is dependent on Cdk2 activity *in vivo* and *vitro*. We are studying the substrates phosphorylated by this kinase after cells are exposed to cisplatin. Cdk-cyclins can form stable complexes with their substrates that can subsequently be phosphorylated *in vitro*. Cdk2 was IP'ed from mouse proximal tubule (TKPTS) cells before and after CP exposure, and bound proteins were phosphorylated by Cdk2. We found that a 18 kDa protein was not phosphorylated before CP exposure and that its phosphorylation increased starting twelve hours after CP, which coincided with the time when Cdk2 inhibition no longer protected from CP cytotoxicity.

Mass spectrometry identifed the 18 kDa protein as $p21^{WAF1/CIP1}$. This protein was identified as a Cdk inhibitor induced by CP, and whose induction prior to CP exposure protected from cytotoxicity by inhibiting Cdk2. Analysis showed it was phosphorylated by Cdk2 at serine 78, a site not previously identified. This site lies within the region of p21 that stearically blocks the ATP binding site of the Cdk and prevents Cdk catalytic activity. We therefore hypothesized that phosphorylation of this residue could affect the role of endogenous induced p21 as a Cdk inhibitor. To investigate the effect of serine 78 phosphorylation on p21 activity, we replaced serine 78 with aspartic acid, creating the phosphomimic $p21^{S78D}$. Mutant $p21^{S78D}$ was an inefficient inhibitor of Cdk2 and we subsequently found that it was inefficient at protecting TKPTS cells from cisplatin-induced cell death. Previous studies showed that both Cdk2 and p21 are induced after cisplatin exposure, and that p21 is a Cdk2 inhibitor that can prevent CP cytotoxicity. However, even though p21 is induced after CP exposure, it only reduces rather than protects from cytotoxicity. We now report that induced p21 is phosphorylated by Cdk2 at Ser78. This phosphorylation reduces the activity of p21 as a Cdk inhibitor and significantly reduces its ability to protect from CP cytotoxicity.

We conclude that phosphorylation of p21 by Cdk2 limits the effectiveness of p21 to inhibit Cdk2, which is the mechanism for continued CP cytotoxicity even after the induction of a protective protein.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO796**

**Serum from Rats with Acute Kidney Injury Appears To Stimulate the Metabolic Activity and Reduce the Proliferation of Proximal Tubular and Mesenchymal Stem Cells** Jon D. Ahlstrom,[1] Jake C. Jones,[1] Florian E. Toegel,[2] Zhuma Hu,[1] Ping Zhang,[1] Christof Westenfelder.[1,3] *[1]Medicine, University of Utah and VA Medical Centers, Salt Lake City, UT; [2]Medicine, Cornell College of Medicine, New York, NY; [3]Physiology, University of Utah, Salt Lake City, UT.*

Acute kidney injury (AKI) is a common and largely treatment-resistant syndrome in which complex mechanisms mediate injury and repair. To investigate how an AKI-induced "uremic" milieu affects the biology of tubular cells (principal target of AKI) or Mesenchymal Stem Cells (MSC; renoprotective), we cultured normal rat proximal tubular cells and MSCs in 10% rat serum collected at 24 hrs post ischemia/reperfusion AKI (SCr 4-5 mg/dL) and in 10% serum from shams. Incubation of both cell types with 10% AKI serum resulted in significantly increased metabolic activity when assessed by the conversion of resazurin to resorufin (CellTiter-Blue/Alamarblue cell viability assay). As metabolic activity is generally proportional to cell numbers, this suggested that AKI serum might be highly mitogenic for cultured cells. Paradoxically, when direct cell counts were performed there were significantly fewer cells in the AKI serum- than in sham serum-treated wells (at 48-72 hours). Hence, even though there were fewer cells in the post-AKI serum wells, the post-AKI serum treated cells had higher cellular metabolism as assessed by CellTiter-Blue. A possible explanation for these responses may be that uremic serum damages cell membranes and reduces mitogenesis, associated with membrane leakiness, which can result in enhanced LDH release. The former would result in reduced cell numbers, while the latter would increase their apparent metabolic rate, due to LDH mediated conversion of resazurin to resorufin. Conclusion: if this explanation is confirmed, this would require caution with the use of this viability assay in settings where cellular LDH is released. More importantly, our data warrant investigation of the mechanisms whereby AKI serum exerts this cytotoxicity, an adverse effect that may further aggravate the overall magnitude of cell injury and also affect stem cell-based interventions in AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO797**

**Levels of Angiotensin II (AII) Decrease by Lipopolissacaride (LPS) from E. coli and Occurs through p-Akt in Immortalized Human Mesangial Cells (hMC) in Culture** Nayda P. Abreu,[1] Edgar Maquigussa,[1] Renata Cristina Tassetano,[1] Jessica S. Garcia,[1] Elizabeth B. Oliveira-Sales,[2] Guilherme Albertoni,[1] Fernanda Teixeira Borges,[1] Nestor Schor.[1] *[1]Medicine, UNIFESP, Sao Paulo, Brazil; [2]Cardiovascular Physiology, UNIFESP, Sao Paulo, Brazil.*

Acute Kidney Injury(AKI)is associated with sepsis. AII plays a role in maintaining blood pressure in sepsis, but few is known about it signaling pathway on hMC. The aim of this study was to evaluate the effect of LPS in activating the Akt/NFkB signaling and also tested the hypothesis that changes in levels of AII is modulated by Akt/NFkB. hMC were cultivated in DMEM exposed to LPS(100ug/ml)for sepsis induction during 30min, 1h, 5h and 24 hours. mRNA expression of p65(NFkB member)were estimated by RT-PCR, AII quantification by ELISA,calcium concentration $[Ca^{(2+)}]i$ using fura-2, nitric oxide(NO) by Griess, apoptosis/necrosis by flow citometry and Western blot for p-Akt molecule. After 24h of LPS, mRNA expression levels of p65 were increased compared to untreated hMC(3.3±0.6vs1.0±0.09 Arbitrary Units,$p<0.05$). Moreover, in LPS-treated hMC the onset of p-Akt was seen after 30min. Interestingly AII decreased after LPS30min(0.14±0.01ng/ml) and 1h(0.07±0.01ng/ml)compared to untreated control(0.08±0.008ng/ml,$p<0.05$). Under Akt inhibitor(LY294002) AngII level of same groups, did not decrease. Additionally,$[Ca^{(2+)}]i$ decreased with LPS for 1h(188.5±18.2% basal/peak) compared to control (265.0±18.4%basal/peak, $p<0.05$) but not decrease under LY inhibitor. Contrarily, NO rise after 5h(0.18±0.03uM/mg of protein) and 24h(0.17±0.02uM/mg of protein)in LPS treatment compared with untreated cells(0.07±0.01uM/mg,$p<0.05$). Apoptosis was increased after LPS 5h(32.6 ± 8.3vs20.3 ± 4.3 %,$p<0.05$), while necrosis rise after LPS 1h(46.0±13.2 vs 8.8±2.3%,$p<0.05$)compared with control. These data suggest LPS exposure decrease the production of AII through the activation of Akt signaling pathway and possibly NFkB. The action of LPS on hMC involves an early stage(up to 5 hours)with inhibition of AII and decreases in intracellular calcium mediated by Akt and a late phase(over 5h) which involves rise in NO and apoptosis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO798**

**Inhibition of Renin Angiotensin System Decreases TLR 4 in Kidney Injured by Ischemia-Reperfusion** Ki Ryang Na,[1] Jin Young Jeong,[1] Ji Yoon Jung,[1] Dong-Suk Chang,[1] Dae Eun Choi,[1] Yoon-Kyung Chang,[2] Kang Wook Lee,[1] Young Tai Shin,[1] Sarah Chung.[1] *[1]Internal Medicine, Chungnam National University Hospital, Daejeon, Korea; [2]Internal Medicine, Daejeon St Mary's Hospital, Daejeon, Korea.*

**INTRODUCTION:** It is well known that renin angiotensin system (RAS) plays important role in ischemia reperfusion (IR) injury of kidney. It was revealed IR renal injury activates innate immunity through the engagement of Toll-like-receptors (TLR) by endogenous ligands, and suppression of TLR attenuate IR renal injury. Although it was reported angiotensin II activate innate immunity recently, there are little study of relationship between RAS and TLR signaling in IR injured kidney. We examined whether RAS inhibition by AT1 receptor blocker modulates the renal expression of TRL4 and its ligands and attenuates renal injury induced by IR of kidney.

**METHODS:** We use male Spargue-Dawley rats which were divided into 4 groups; sham group, losartan treated sham group, control IR group, and losartan treated IR group. Losartan (40mg/kg) was injected intraperitoneally 60 minutes before IR injury. Duration of renal ischemia is 30min. We sacrificed rats 24hr after reperfusion. We evaluated BUN, serum creatinine, and the renal mRNA expression levels of TLR4, HMGB1, MCP-1, TNF-α, and IL-6 by real-time RT-PCR. We also evaluated renal inflammation by H&E stain and ED1 immunohistochemistry.

**RESULTS:** The levels of BUN and serum creatinine (s-Cr) in control IR rats were significantly increased compared to sham group (all. $p$ <0.05). The levels of BUN and s-Cr of losartan treated IR group were significantly lower than those of control IR group (all, $p$ <0.05).Losartan treated IR group showed significantly lower levels of renal TLR4 and HMGB1 mRNA expression than those of control IR group (all, $p$<0.05). Losartan decreased significantly renal mRNA expressions of TNF-α, MCP-1, and IL-6 (all, $p$ <0.05). Losartan also reduced significantly infiltration of the ED1 positive cells in IR injured kidneys ($p$ <0.05).

**CONCLUSIONS:** Losartan attenuated the renal inflammation induced by renal IR rats and at least in part, suppression of TLR4 may be involved in this mechanism.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO799

**Glomerular Endothelial Cells Express the Proinflammatory Cytokine Interleukin-17 in Acute Glomerular Disease** Tanja Loof, Stephanie Kraemer, Hans-Hellmut Neumayer, Harm Peters. *Depart. of Nephrology, Charité Universitätsmedizin Berlin, Berlin, Germany.*

**Purpose**

Overexpression of pro-fibrotic and pro-inflammatory cytokines and extracellular matrix accumulation are hallmarks of acute glomerular injury. A connection between TGF-ß signaling and the inflammatory cytokine IL-17, as well IL-17 expression by renal cells in kidney diseases has recently been proposed. The present study investigated IL-17 expression in different glomerular cells in a rat model of acute anti-Thy1 glomerulonephritis (aGN).

**Methods**

aGN was induced in male Wistar rats by i.v. injection of anti-Thy1 OX-7 antibody. Tissues were harvested at the day 5 (matrix expansion), PBS-injected animals served as controls (con). Immunofluorescence was performed for cell marker (OX-7: mesangial cells, PECAM: endothelial cells, synaptopodin: podocytes) and IL-17. For *in vitro* experiments NRK 52E cells were used.

**Results**

Induction of aGN was characterized by marked proteinuria (d5 113±12 mg/d; 3.9-fold vs. con; p<0.001) and histological glomerular-matrix accumulation (d5 +3-fold vs. con; p<0.001), in parallel with highest TGF-ß1 and IL-17 mRNA expression (+2.25-fold; +6.50-fold vs. con; p<0.05). Immunofluorescence staining with synaptopodin and OX-7 showed no colocalization with IL-17, whereas PECAM was highly colocalized with IL-17. In vitro, IL-17 was secreted by NRK 52E at basal levels, up-regulated after exposure to 25 mM glucose (+7-fold) and reversed in the presence of a specific TGF-ß receptor blocker (SB 431542). Stimulation of cells with TGF-ß1 or IL-6 amplified IL-17 expression by 2.0-fold. Co-administration of TGF-ß and IL-6 led to highly synergistically enhanced IL-17 secretion by more than 4000-fold.

**Conclusion**

This study documents that the glomerular expression of IL-17 cytokine is localized into glomerular endothelial cells in acute, anti-Thy1 glomerulonephritis, thus under pro-fibrotic and pro-inflammatory conditions. Downregulation of IL-17 expression by TGF-ß receptor antagonism in vitro points a crucial regulatory interaction with TGF-ß signalling.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO800

**Urotensin II Protects Rat Renal Tubular Cell from Gentamicin-Induced Apoptosis through Prostacyclin-Mediated Pathway** Yung-Ho Hsu,[1] Tso Hsiao Chen,[2] Cheng-Hsien Chen.[1,2] *[1]Division of Nephrology, Taipei Medical University-Shuang Ho Hospital, Taipei, Taiwan; [2]Division of Nephrology, Taipei Medical University-Wan Fang Medical Center, Taipei, Taiwan.*

Urotensin II (UII) is a cyclic vasoactive peptide expressed in multiple organs. Kidney appears to be a major source of both circulating and urinary UII. Studies investigated effects of UII on renal function yielded conflicting results. Whether UII has a causative or protective influence on renal disease remains to be determined. In this study, we studied the protective effect and mechanism of UII on gentamicin-induced apoptosis in rat renal tubular cells (NRK-52E). We found gentamicin induced UII generation in these cells within 24 hours, but this UII induction did not influence gentamicin-induced apoptosis. Pretreatment of UII reduced the quantity of cleaved caspase-3 and increased Bc*L*-$x_L$ expression, resulting in protecting NRK-52E cells from gentamicin-induced apoptosis. This anti-apoptotic effect of UII was inhibited by the pretreatment of UII antagonist, urantide. UII was also found to decrease $PGE_2$ and increase the $PGI_2$ generation in NRK-52E cells. Because $PGI_2$ is a potential ligand for IP receptor and peroxisome proliferator-activated receptors alpha and delta (PPARa and PPARd), we blocked those receptors by the neutralization of IP receptor and siRNA transfection of PPARa and d to investigate the $PGI_2$ signaling pathways involved in UII protective mechanism. We found PPARa blockage reduced the anti-apoptotic effect of UII more potently. Our results reveal that UII protects rat renal tubular cells from gentamicin-induced apoptosis through $PGI_2$ and PPARa-signaling pathways.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO801

**Acute Kidney Injury after Syngeneic Orthotopic Liver Transplantation in Rats** Nan Chen, Jun Wang, Zhiyong Du, Wen Zhang, Chenghong Peng. *Department of Nephrology, Ruijin Hospital, Shanghai, China.*

**Objective:** Orthotopic liver transplantation is the optimal treatment for end stage liver disease and acute kidney injury (AKI) is a frequent complication after liver transplantation. The prognosis of AKI post-liver transplantation is poor because mechanisms are incompletely characterized, in part due to the lack of experimental research. In this research we studied AKI post-liver transplantation in the syngeneic orthotopic liver transplantation (SOLT) rat model and attempted to investigate the mechanism of AKI post-liver transplantation.

**Methods:** Sprague-Dawley rats were separated to sham-operated group and SOLT group. SOLT was performed according to the Kamada and Calne's cuff technique. Sham-operated rats were subjected to laporotomy and identical liver manipulations without the vascular occlusion. The animals were sacrificed 24h after surgery.

**Results:** SOLT rats developed typical histological changes of acute tubular injury including loss of brush border, flattening of the tubular epithelium, medullary congestion and hemorrhage. In addition, marked rarefaction of renal peritubular capillary was also observed in SOLT rats. Macrophages infiltration and upregulation of macrophage-derived cytokine like interleukin-6, interleukin-1β, and tumor necrosis factor-α were obvious in the renal tissue of SOLT rats. There was a downregulation of angiogenic factor like vascular endothelial growth factor and an upregulation of antiangiogenic factors like thrombospondin-1 and matrix metalloproteinase 9 in the renal tissue of SOLT rats compared with sham-operated rats.

**Conclusions:** Our results show that AKI develops rapidly in rats post-liver transplantation and is characterized by renal tubular injury, peritubular capillary rarefaction, and inflammatory changes. The rat model of AKI post SOLT may be useful in delineating the mechanisms and discovering potential therapies for AKI post-liver transplantation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO802

**Regulation of Megalin and Cubilin during Lipopolysacchride-Induced Acute Renal Failure in the Mouse** Klaus Höcherl. *Institute of Physiology, University of Regensburg, Regensburg, Germany.*

Hypoalbuminemia is a common finding in septic patients and is associated with higher morbidity and mortality rates. Because severe sepsis is often accompanied by acute renal failure (ARF) with renal tubular dysfunction, an impairment of proximal tubular endocytosis of glomerular-filtered albumin may result in the development of albuminuria during sepsis. Since the multiligand receptors megalin and cubilin are of major importance for proximal tubular reabsorption of albumin, we investigated in the present study the regulation of these receptors during severe experimental inflammation.

Male C57BL/6J mice (n=6 per group) were injected with lipopolysaccharide (LPS) and the time- and dose-dependent effects on the expression of megalin and cubilin were determined. In addition, plasma and urinary levels of albumin were determined by ELISA.

Treatment with LPS (10 mg/kg; i.p.) for 12 hours caused a decrease in plasma albumin levels from 41 ±2 mg/ml to 22 ±5 mg/ml which was paralleled by a 3.1-fold increase in the urinary excretion of albumin without alterations in hepatic albumin expression. Injection of LPS caused a time-dependent decrease in cubilin mRNA abundance to 68, 51 and 44 % of control values after 3, 6 and 12 h, respectively. Further, LPS dose-dependently decreased cubilin mRNA expression to about 74, 57 and 42% of control values 12h after the injection of 1, 3 or 10 mg/kg of LPS, respectively. Furthermore, injection of LPS caused a time-dependent decrease in megalin mRNA abundance to about 84, 60 and 34 % of control levels after 3, 6 and 12 h, respectively. Moreover, LPS dose-dependently decreased megalin mRNA expression to about 67, 51 and 39% of control values 12h after the injection of 1, 3 or 10 mg/kg of LPS, respectively. In addition, we found a decrease in megalin protein expression 12h after the injection of LPS. Megalin immunoreactivity was clearly decreased in the apical membrane of proximal tubules in mice treated with LPS.

Taken together, these data indicate that the expression of megalin and cubilin is decreased during experimental endotoxemia and that this downregulation may contribute in part to the development of albuminuria and also to hypoalbuminemia during sepsis.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO803

**Lowering Dietary Phosphorus (Pi) Results in an Improvement of Blood Pressure, an Increase in Both Urinary Na Losses and Fractional Excretion of Na (FENA), and a Reduction in Blood Levels of Parathyroid Hypertensive Factor (PHF) in Dahl Salt-Sensitive Hypertensive Rats (SSR)** Larry A. Slomowitz,[1] Christina G. Benishin,[2] Brian E. Peerce.[3] *[1]Duophos LLC, Vancouver, BC, Canada; [2]Department of Physiology, University of Alberta, Edmonton, AB, Canada; [3]Dpeartment of Neuroscience and Cell Biology, UTMB, Galveston, TX.*

Given that interventions therapeutic for progressive renal failure are usually also effective for hypertension, we studied the response to moderate Pi restriction in SSR. After an equilibration phase when rats were fed normal rat chow, two groups of SSR were pair-fed high-salt diets (9%). Group1 (n=6) received high Pi diets (0.7%), Group2 (n=12) received moderate Pi-restricted diets (0.2%). A second set of studies employed moderate salt-restricted diets (Group3- 4%NaCl + 0.7%Pi, n=6; Group 4- 4%NaCl + 0.2%Pi, n=6). Blood pressure between Groups 1&2 began to diverge after 4 wks of Pi restriction, and by

6 wks, Group1 developed hypertension as expected (sys 193±7, dia 123±8, MAP 147±8 mmHg), while Group2 did not (sys 147±6, dia 100±6, MAP 115±4 mmHg, all significant p≤0.025). Rats given 4%NaCl needed more time to become hypertensive, but benefited from Pi restriction: After 12 wks, Group3-sys 168±5, dia 132±5, MAP 144±4 mmHg; Group4-sys 143±5, dia 102±6, MAP 129±6 mmHg (all signficant p≤ 0.05). After 6 wks, blood and urine were collected from Groups 1&2. With Pi restriction, urinary sodium losses and FENA jumped 36% and 43%, respectively (p≤0.05). Group 1 versus 2 blood levels of PTH, Vit D3, FGF-23, aldosterone, and PRA were not significantly different. PHF blood levels fell with Pi restriction (Group1- 1.9±0.15, Group2- 1.05±0.25 U/kg body wt, p≤0.05).

The results indicate moderate Pi restriction is linked to blood pressure and sodium balance in SSR. The nexus between a function ascribed to distal tubule elements (FENA) and a proximal tubule function (Pi balance) is novel and unexplained. The data support the proposed role of PHF in the developement of hypertension in SSR. PHF blood levels correlated with Pi intake in SSR, suggesting that a regulatory loop might be present. Studies to determine the role of dietary Pi in human hypertension are needed.

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### TH-PO804

**Dietary Phosphate Restriction Ameliorates Endothelial Function in Adenine-Induced Chronic Kidney Disease Model Rats** Vu Van Tan, Eriko Watari, Yutaka Taketani, Tomoyo Kitamura, Asuka Shiota, Ayako Tanimura, Hironori Yamamoto, Eiji Takeda. *Department of Clinical Nutrition, University of Tokushima, Tokushima, Japan.*

Chronic kidney disease (CKD) causes the dysregulation of systemic mineral metabolism including hyperphosphatemia that is a risk factor for cardiovascular disease (CVD) by leading both endothelial dysfunction and vascular calcification. Control of serum phosphate (P) level by dietary P restriction or P binders is considered to be beneficial to prevent CVD in CKD patients, but it has been unclear whether keeping lower serum P level can ameliorate endothelial function. In this study we investigated whether low-P diet can improve endothelial function in adenine-induced CKD rats model. In first, 7 week-old Sprague-Dawly rats were divided into two groups: control group rats were fed with control-P diet containing 1% P, and CKD group rats were fed with adenine diet containing 0.75% adenine and 1% P for three weeks to induce CKD. Then, CKD group rats were divided into two groups: one group rats were received control-P diet (CKD-CP) and the other group were received low-P diet containing 0.2% P (CKD-LP). After sixteen days, aortic rings were prepared for evaluation of acetylcholine-induced vasodilation by using isometric transducer, and blood samples were also collected. Serum P and creatinine levels were 17.0±0.85 and 2.09±0.12 mg/dl in CKD group (n=6), and 5.50±0.25 and 0.64±0.05 mg/dl in control (n=6), respectively. Acetylcholine-dependent vasodilation in CKD rats was significantly deteriorated compared with control group rats. After sixteen days treatment with low-P diet (CKD-LP), serum P level was significantly lower (3.24±0.46 vs 10.6±1.79 mg/dl, p<0.01) despite a high creatinine level (2.71±0.48 mg/dl). Acetylcholine-dependent vasodilation in CKD-LP rats was improved compared with CKD-CP rats (85.1±2.64% vs 60.1±3.25%, respectively, p<0.05). This improvement was thought to be due to a decrease in serum P level by dietary P restriction. In conclusion, dietary P restriction would be a beneficial way to ameliorate endothelial function, and useful to reduce a CVD risk under CKD condition.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO805

**All-Trans Retinoic Acid Maintains the Blood Phosphate Levels through the Positive and Negative Regulation of Type II Sodium-Dependent Phosphate Co-Transporter Family Genes in Kidney and Intestine** Masashi Masuda,[1] Hironori Yamamoto,[1] Mina Kozai,[1] Yuichiro Takei,[1] Otoki Nakahashi,[1] Shoko Ikeda,[1] Ayako Otani,[1] Yutaka Taketani,[1] Ken-Ichi Miyamoto,[2] Eiji Takeda.[1] *[1]Dept. Clinical Nutrition, Inst. Healthbioscience, Univ. Tokushima, Tokushima, Japan; [2]Dept. Molecular Nutrition, Inst. Healthbioscience, Univ. Tokushima, Tokushima, Japan.*

It has been known that the type II sodium-dependent phosphate co-transporter (Npt2) family (Npt2a, Npt2b and Npt2c) play a critical role for the Pi absorption in kidney and intestine. Vitamin A metabolites are potent regulators of a variety of physiological processes including development, cell differentiation and proliferation, reproduction and bone formation. All-trans retinoic acid (ATRA) and 9-cis retinoic acid have been recognized as important signaling molecules and physiological ligands for the retinoic acid receptors and the retinoid X receptors, which act as ligand-activated transcription factors controlling the expression of a number of target genes. Recently, we published that vitamin A-deficient (VAD) rats increased the levels of urine Pi but not plasma Pi, and renal expression of Npt2a and Npt2c genes are transcriptionally up-regulated by ATRA and its receptors (Biochemical J. 2010). In this study, we addressed the effect of VAD diet and ATRA administration on the Pi absorption and Npt2b gene expression in small intestine. VAD rats decreased fecal Pi excretion and increased intestinal Pi uptake activity. Western blots and quantitative RT-PCR analysis revealed that Npt2b protein and its mRNA expression were up-regulated by VAD. In contrast, the administration of ATRA to VAD rats significantly suppressed the Pi uptake activity and the Npt2b gene expression in intestine. These results suggest that ATRA inhibit the intestinal Npt2b gene expression. In summary, ATRA maintains the blood Pi levels through the positive and negative regulation of Npt2 family genes in kidney and intestine.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO806

**The Identification of histidine-712 as a Critical Residue for Constitutive TRPV5 Internalization** Theun de Groot, Sjoerd Verkaart, Rene J. Bindels, Joost G. Hoenderop. *Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

The epithelial Ca2+ channel transient receptor potential vanilloid 5 (TRPV5) constitutes the luminal entry gate for active Ca2+ reabsorption in the distal part of the nephron. As TRPV5 is the rate-limiting factor in active Ca2+ reabsorption, understanding channel regulation is essential to comprehend renal Ca2+ handling. Molecular regulation of TRPV5 by calciotropic hormones, associated proteins and other factors is highly concentrated in the carboxy (C)-terminus. To fully identify the role of the C-terminus in TRPV5 function, we generated channels harboring C-terminal deletions. Fura-2 analysis was employed to measure the intracellular Ca2+ concentration ([Ca2+]i) in TRPV5-expressing human embryonic kidney (HEK) 293 cells. TRPV5 Ca2+ and Na+ currents were determined by electrophysiology, while cell surface biotinylation was implemented to investigate TRPV5 plasma membrane expression and internalization. Analysis of the [Ca2+]i of TRPV5 deletion mutants demonstrated that removal of amino acid histidine-712 (H712) elevated TRPV5 activity. Substitution of the positively charged H712 for a negative (H712D) or neutral (H712N) amino acid also stimulated TRPV5 activity. This critical role of H712 was confirmed by patch clamp analysis, which demonstrated increased Na+ and Ca2+ currents for TRPV5-H712D. Cell surface biotinylation studies revealed an enhanced cell surface presence of TRPV5-H712D as compared to wild-type (WT) TRPV5. This elevated plasma membrane expression was not due to the increased [Ca2+]i, as a Ca2+-impermeable pore mutant TRPV5-H712D-D542A, which does exhibit Na+ currents, was also more abundant at the cell surface then TRPV5-WT-D542A. An internalization assay revealed a delayed cell surface retrieval for TRPV5-H712D. Experiments using sucrose and filipin indicate that caveolae-mediated endocytosis of TRPV5-H712D is affected, rather than clathrin-mediated endocytosis of TRPV5. These results demonstrate that residue H712 mediates TRPV5 internalization and thereby controls Ca2+ reabsorption, as few constitutively open TRPV5 channels at the cell surface allow a massive Ca2+ influx.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO807

**Insulin Increases TRPM6 Activity by Raising Cell Surface Expression** Anil V. Nair, Rene J. Bindels, Joost G. Hoenderop. *Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Diabetes type 2 (DM2) is associated with insulin resistance and renal $Mg^{2+}$ wasting, but the molecular mechanism of this defect is unknown. DM2-related hypomagnesemia has been linked to coronary artery diseases, hypertension, diabetic retinopathy, nephropathy, neuropathy, abnormal platelet activity, and foot ulcerations. To investigate the molecular mechanism behind renal $Mg^{2+}$ wasting associated with DM2, we measured the effect of insulin on the activity of renal epithelial $Mg^{2+}$ channel, the transient receptor potential M6 (TRPM6). We performed whole-cell patch clamp recordings of TRPM6 expressed in human embryonic kidney 293T (HEK293T) cells. Stimulation of the insulin receptor (IR) increased the current through TRPM6 expressing cells in a dose dependent manner ($EC_{50}$ = 0.102 nM) but not through the non-transfected cells. To address whether the stimulatory action of insulin was specific to IRs, we added a high-affinity peptide antagonist of the IR, the S961, which abolished the stimulatory effect of insulin on TRPM6-mediated current. The effect of insulin on TRPM6 was abrogated by the application of phosphoinositide 3 kinase (PI3K) (a downstream effector of insulin signaling cascade) inhibitors wortmannin and LY294002. Activation of Rac1, another effector of PI3K, increased the TRPM6-mediated current. In contrast, co-expression of TRPM6 with the dominant negative Rac1 mutant (Rac1-T17N) failed to show increase in current by insulin stimulation. Total internal reflection fluorescence microscopy showed an increased trafficking of TRPM6-containing vesicles towards the plasma membrane upon insulin application. In summary, insulin mediated stimulation of TRPM6 current which occurs via PI3K and Rac1 signaling, thereby increasing the amount of plasma membrane availability of TRPM6 from the endomembranes. These results show a possible regulatory mechanism for transepithelial $Mg^{2+}$ transport in the kidney and consequently whole-body $Mg^{2+}$ homeostasis mediated by insulin signaling. The impaired insulin signaling due to insulin resistance and subsequent inability to retain $Mg^{2+}$ through TRPM6 in kidney may explain hypomagnesemia in DM2 patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO808

**Interaction between TRPM6 and EGF in a Rat Model of Cyclosporine Nephrotoxicity** Kristien J. Ledeganck,[1] Caroline Aj Horvath,[2] Inge Brouns,[3] Rita M. A. Van den Bossche,[4] Benedicte Y. De Winter,[1] Gert A. Verpooten.[1] *[1]Labo Experimental Medicine and Pediatrics; [2]AMBIOR; [3]Labo Cell Biology and Histology; [4]Labo of Pharmacology, University of Antwerp, Belgium.*

Hypomagnesemia is a well known side effect of cyclosporine (CsA) treatment in humans due to renal Mg loss but the underlying mechanisms are still unclear. Recently, it was shown that epidermal growth factor (EGF) stimulates Mg reabsorption in the distal convoluted tubule via TRPM6. We studied the renal expression of TRPM6, TRPM7 and EGF in a rat model of CsA nephrotoxicity, the effect of EGF administration on the Mg homeostasis and the expression of TRPM6, TRPM7 and EGF. A daily dose of 15 mg/kg CsA was given subcutaneously to male Wistar rats during 4 weeks. Two groups received a daily dose of 150 µg/kg hEGF subcutaneously during 4 weeks. Real-time RT-PCR analyses were performed to determine the renal mRNA expression levels of TRPM6, TRPM7, EGF, TGF- and PAI-1. The CsA treatment resulted in an increased plasma creatinine (0.45±0.05

mg/dL in the CsA-treated groups vs 0.26±0.07 mg/dL in the controls), and an increase in fractional excretion of Mg (12.02±3.32 % in the CsA-treated group vs 8.9±4.02% in the controls). Microscopically cortical areas of tubular vacuolisation, tubular dilatation and juxtaglomerular apparatus hyperplasia were noticed. RT-PCR analysis showed that CsA significantly decreased the renal mRNA expression of TRPM6 (Normalized ratio (NR): 1.25±0.32 vs 1.48 0,24 in controls), TRPM7 (NR: 1.35±0.4 vs 1.62±0.22 in controls) and EGF (NR: 0.51±0.39 vs 1.74±0.45 in controls). In control rats treated with EGF an increase in renal expression of TRPM6 (NR: 1.77±0.25) and EGF (NR: 1.95±0.28) was demonstrated together with a decrease in fractional excretion of Mg (5.49±2.34% vs 8.9±4.02% in controls). On the other hand there was no effect of EGF administration on the renal expression of TRPM6 (NR: 0.99±0.13), TRPM7 (0.99±0.23) and EGF (0.49±0.14) in CsA-treated rats. CsA-induced renal Mg loss may be the result of the downregulation of TRPM6. These findings further support the hypothesis that EGF plays an important role in the renal expression/activation of TRPM6.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO809

**Genome-Wide Association Study of Serum Calcium Levels Reveal Common Variants in the Calcium Sensing Receptor Gene Associated with Features of Familial Hypocalciuric Hypercalcemia** Anna Kottgen,[1,9] Conall M. O'Seaghdha,[2] Qiong Yang,[3] Nicole Glazer,[4] Tennille S. Leak,[5] Abbas Dehghan,[6] Albert Vernon Smith,[7] Vilmundur Gudnason,[7] Yongmei Liu,[8] Wen Hong Linda Kao,[9] Bryan R. Kestenbaum,[4] Caroline S. Fox.[2] *[1]Freiburg University Hospital, Germany; [2]NHLBI's Framingham Heart Study; [3]Boston University; [4]University of Washington Seattle; [5]University of Pittsburgh; [6]Erasmus MC, Rotterdam, Netherlands; [7]Icelandic Heart Association, Iceland; [8]Wake Forest University; [9]Johns Hopkins University.*

Serum calcium levels are tightly regulated and highly heritable, but the role of common genetic variation influencing serum calcium concentrations in the physiologic range is largely unknown.

We performed genome-wide association analyses (GWAS) in population-based studies participating in the CHARGE Consortium to uncover common genetic variation associated with serum calcium levels. GWAS of serum calcium concentrations was performed in 20,611 individuals of European ancestry for ~2.5 million genotyped and imputed single-nucleotide polymorphism (SNPs).

The SNP with the lowest p-value was intronic rs17251221 (p=2x10^-22, minor allele frequency 14%) in the calcium sensing receptor gene (CASR). This SNP was associated with higher serum calcium levels (0.06 mg/dl per copy of the minor G allele). The G allele of rs17251221 was also associated with higher serum magnesium levels (p=1x10^-3), lower serum phosphate levels (p=3x10^-7) and lower bone mineral density at the lumbar spine (p=0.038). No additional genomic loci contained SNPs associated at genome-wide significance (p<5x10^-8).

These associations resemble clinical characteristics of patients with familial hypocalciuric hypercalcemia, an autosomal-dominant Mendelian disease arising from rare mutations in the CASR gene. Common genetic variation in the CASR gene is associated with similar but milder features in the general population, and suggests that the causal variant results in reduced CASR function.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO810

**Decline of Renal Klotho Expression Is Associated with Hypercalciuria in Patients with Diabetes** Osamu Asai,[#1] Kimihiko Nakatani,[#1] Hirokazu Sakan,[#1] Ken-Ichi Samejima,[#1] Shuhei Yoshimoto,[#1] Tomohiro Tanaka,[#2] Masayuki Iwano,[#1] Akihiro Imura,[#2] Yo-Ichi Nabeshima,[#2] Yoshihiko Saito.[#1] *[1]First Department of Internal Medicine, Nara Medical University, Kashihara, Nara, Japan; [2]Department of Pathology and Tumor Biology, Kyoto University Graduate School of Medicine, Kyoto, Japan.*

The Klotho(KL) is expressed predominantly in renal distal convoluted tubules, where transepithelial $Ca^{2+}$ reabsorption is actively regulated. Hypercalciuria has been documented in human and experimental diabetic nephropathy(DN), but its detailed mechanism is still unclear. In this study, we studied the association of renal KL expression levels with hypercalciuria in human DN and STZ-diabetic model mice. We evaluated fractional excretion of Ca (FeCa) for DN(n=74), and also quantitatively evaluated KL mRNA expression for 31 renal frozen biopsy specimens with DN by real-time PCR. We also studied for IgA nephropathy(IgAGN)(n=90), and minor glomerular abnormalities(MCD)(n=26), as control. Patients with DN showed significantly lower levels of renal KL expression and higher levels of FeCa, than those with IgAGN and MCD. Moreover, FeCa was significantly correlated with renal KL mRNA expression levels, and multiple regression analyses showed FeCa was significantly and negatively associated with renal KL expression levels as independent variables in order of importance. In STZ-induced diabetic mice, renal KL mRNA levels were significantly decreased and urinary Ca excretions increased at 8 weeks after the induction ofdiabetes, compared with control mice. To confirm whether the reduction of renal KL expression was associated with hypercalciuria in DN, we induced diabetes in $KL^{+/-}$ mice and wild-type mice by STZ injection. When diabetes was induced, $KL^{+/-}$ mice showed the significant increase of urinary Ca excretions, compared with wild-type mice, at 2 weeks after the induction of diabetes. In conclusion, remarkable reduction of renal KL expression levels may lead to hypercalciuria in DN.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO811

**Major Facilitator Superfamily (MFS) Transporters Mediate Cellular Effects of Phosphate in Drosophila S2R+ Cells** Clemens Bergwitz,[1] Charles Derobertis,[1] Sumi Sinha,[1] Hway Helen Chen,[1] Harald Jueppner,[1] Matthew D. Rasmussen,[3] Norbert Perrimon.[2] *[1]Endocrine Unit/Dept. of Pediatrics, Massachusetts General Hospital, Boston, MA; [2]Dept. of Genetics, Harvard Medical School/Howard Hughes Medical Institute, Boston, MA; [3]Computer Science and Artificial Intelligence Lab, MIT, Cambridge, MA.*

Pho84 is a proton-dependent phosphate co-transporter, which permits yeast to sense the environmental phosphate concentration. It belongs to the major facilitator superfamily (MFS) of transporters, which includes 77 members in yeast, 219 members in Drosophila, and 229 members in humans. Database searches based on protein sequence conservation alone have been unable to identify the pho84 ortholog in higher species. We postulated that activation of MAPK by phosphate and phosphate-transport after expression of candidates in Xenopus oocytes can be used to functionally evaluate members of the MFS to identify pho84 orthologs, which are involved in metazoan phosphate sensing.

We used BLAST followed by Bayes phylogenetic analysis to identify 29 fly orthologs that are most closely related to pho84 and human SLC17A1-9, an anion transporter subfamily with members known to mediate phosphate transport. Some of these transporters are expressed in the fly hemocyte cell line S2R+. Individual RNAi knockdown identified three fly SLC17A orthologs, which inhibited phosphate-induced activation of MAPK in S2R+ cells, while knockdown of the Drosophila Pit1 ortholog was less effective. We next injected cRNA encoding each individual fly transporter into Xenopus oocytes and identified one transporter, which mediates uptake of [33P]-orthophosphate in a sodium-dependent fashion. Phosphate conductance by this fly transporter was blocked by phosphonoformic acid and appears to be independent of protons.

Our findings suggest that MFS transporters mediate cellular effects of phosphate in fly S2R+ cells. The transmembranous sodium-gradient rather than a proton-gradient may be used to permit import of phosphate into fly cells against it's electrochemical gradient. Multiple MFS members may be involved in phosphate-induced activation of MAPK in fly.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO812

**Regulation of Renal Cortical $Ca^{2+}$ Transporters and Tight Junction Proteins in Rats on High-Salt Diet** Midori Sasaki Yatabe,[1,3] Junichi Yatabe,[1,2] Hironobu Sanada,[2] Kimura Junko,[3] Tsuyoshi Watanabe.[1] *[1]Dept. of Nephrology, Hypertension, Diabetology, Endocrinology and Metabolism, Fukushima Medical University, Fukushima, Japan; [2]Division of Health Science Research, Fukushima Welfare Federation of Agricultural Cooperatives, Fukushima, Japan; [3]Dept. of Pharmacology, Fukushima Medical University, Fukushima, Japan.*

Introduction: Excess dietary salt intake increases urinary $Ca^{2+}$ excretion. However, this mechanism and chronic effects of salt on calcium-transporting molecules and paracellular pore/barrier proteins are not clear.

Methods: Wistar-Kyoto rats (8-week old) were fed either 0.3% or 8% NaCl diet for 8 weeks with regular urine collections. Kidneys were collected at the end of the study.

Results: Urinary $Ca^{2+}$ excretion increased with high-salt diet (0.3% vs 8% NaCl, 0.70±0.05 vs. 4.56±0.27 mg/day, n=15, p<0.001). Renal cortical $Na^{+}/Ca^{2+}$ exchanger 1 (NCX1) mRNA (100±9% vs. 120±15%, n=15, p<0.05) and protein (100±6% vs. 126±6%, n=15, p<0.05) expressions were higher in the high-salt group. Apical $Ca^{2+}$ channel, TRPV5 (mRNA; 100±7% vs. 158±18%, protein; 100±8% vs. 143±10%, both n=15, p<0.05), and intracellular $Ca^{2+}$ shuttle, calbindin-D28k (mRNA: 100±9% vs. 148±20%, n=14-16, p<0.05), also increased with high-salt diet. Basolateral $Ca^{2+}$ pump, PMCA1b, expression was not altered. Renal cortical claudin 7 and 8 mRNA levels were similar between the groups. However, claudin 2 mRNA level was greater in the high-salt than the control group (100±12 vs. 140±8, n13-14, p<0.05).

Conclusion: Renal cortical claudin 2, TRPV5, calbindin-D28k, and NCX1 increased with chronic high-salt diet. Claudin 2 is a proximal tubule tight junction protein that forms cation pore. TRPV5, calbindin-D28k and NCX1 in the distal tubule facilitate $Ca^{2+}$ reabsorption. Although the functions of these proteins may be altered by excess salt intake, extrapolating from their basal functions suggests that these changes may be adaptive to minimize urinary $Ca^{2+}$ loss. Further study is necessary to clarify the mechanism and consequences of these up-regulations in salt-induced calciuria, which may be involved in the pathophysiology of osteoporosis, renal calculi and hypertension.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO813

**Role of NHERF PDZ Proteins in the Differential Regulation of the Proximal Tubule NaPi Transporters** Hector Giral-Arnal,[1] Luca Lanzano,[2] Yupanqui A. Caldas,[1] Judith Blaine,[1] Kayo Okamura,[1] Enrico Gratton,[2] Moshe Levi.[1] *[1]Renal Division, U of Colorado Denver, Aurora, CO; [2]LFD, U of California Irvine, Irvine, CA.*

The Na-dependent phosphate (NaPi) transporters NaPi-2a and NaPi-2c play a major role in the renal reabsorption of Pi. The functional need of several transporters accomplishing the same role is still not clear. However, the fact that the transporters show differential regulation under dietary and hormonal stimuli suggests different roles in Pi reabsorption. The pathways controlling this differential regulation are still unknown. One of the candidates involved in this regulation is the NHERF family of PDZ proteins. We

propose that differences in the molecular interaction with these PDZ proteins are related with the differential adaptation of the NaPi transporters. To test if NHERF proteins play a different role in the regulation of NaPi transporters we studied the NHERF-3 (PDZK1) KO mouse. NHERF-3 KO adapted to low Pi diets showed an increased expression of NaPi-2a protein in the apical membrane of proximal tubules, but an impaired upregulation of NaPi-2c protein levels. Similar results were obtained when analyzing the total renal homogenates. These results suggest that NHERF-3 has an important role in the stabilization of NaPi-2c in the apical membrane. We studied the specific protein-protein interactions of NaPi-2a and NaPi-2c with NHERF-1 and NHERF-3 by Fluorescence Lifetime Imaging (FLIM)-FRET technique. FRET efficiency measurements showed a stronger interaction of NHERF-1 with NaPi-2a than with NaPi-2c. However, both NaPi transporters showed similar FRET efficiencies with NHERF-3. Interestingly, when the cells where adapted to several Pi concentrations mimicking the in vivo model, a gradual increase in the FRET efficiency was observed under lower Pi concentrations in the pairs NaPi-2c/NHERF-3 and NaPi-2a/NHERF-1. These results suggest that differential affinity of the NaPi transporters for NHERF-1 and -3 could partially explain their differential regulation and stability in the apical membrane. In this regard, direct interaction between NaPi-2c and NHERF-3 seems to play an important role in the physiological regulation of NaPi-2c.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO814**

**Phosphate Transporters in Human Urine: Window into the Molecular Physiology and Pathophysiology of Renal Phosphate Handling** Ion Alexandru Bobulescu, Anthony S. Nguyen, Orson W. Moe. *Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX.*

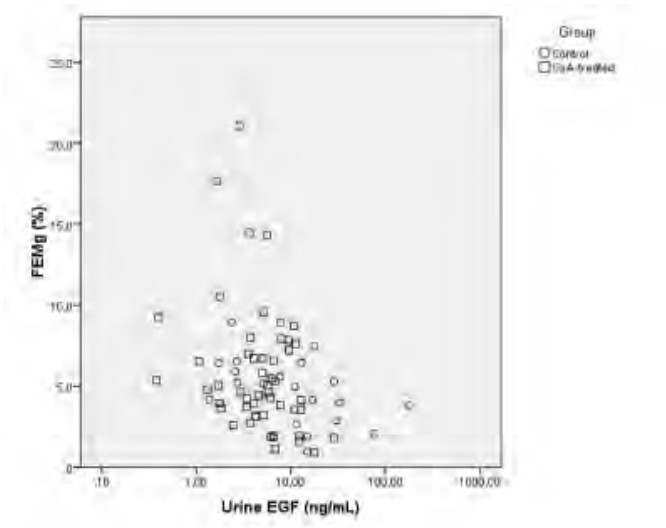
The kidney is key for total body phosphate (PO4) homeostasis. Filtered PO4 is reabsorbed in the proximal tubule via 3 sodium-coupled transporters (NaPi-2a, NaPi-2c, and Pit-2) with different substrate affinities, stoichiometries, pH-dependence, and regulatory characteristics. Numerous physiologic and pathophysiologic conditions involve regulation or dysregulation of renal PO4 transporters. To fully understand PO4 handling, and to pave the way for novel diagnostic tools and better treatment options for PO4 disorders, it is crucial to study PO4 transporters individually. Rodent studies are informative but insufficient, since gene inactivation studies have shown that the relative contribution of individual transporters to PO4 homeostasis is significantly different in mice compared to humans. Clinical evaluation of individual PO4 transporters in humans has been impossible to date. We developed a simple and reliable assay to measure PO4 transporters in sediment-free human urine, based on the Wessel-Flugge protein precipitation method. This assay is much simpler, faster, and less expensive than urinary exosomal preparations. We conducted several studies to validate this assay: 1) In rats given sodium phosphate gavage or dietary PO4 manipulation (0.1%, 0.6% and 1.2% PO4 diets), we correlated changes in PO4 transporter levels in the urine and in renal brush border membrane vesicles. 2) In healthy human volunteers given a PO4 load or a PO4 binder, urinary NaPi-2a excretion reflected the expected changes in proximal tubule brush border NaPi-2a. 3) In a patient with tumor-induced osteomalacia, both urinary NaPi-2a and NaPi-2c were low, and increased after surgical removal of the disease-causing tumor, concomitant with decreased renal PO4 wasting. Taken together, these findings indicate that the levels of urinary PO4 transport proteins provide an invaluable window into the molecular physiology and pathophysiology of renal PO4 handling. This will take clinical pathophysiology to a new level, where the roles of individual PO4 transporters can be studied in addition to plasma and urinary chemistry.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO815**

**Decreased Urinary EGF Excretion Is Associated with Renal Mg Leak in Transplant Patients Treated with Cyclosporine** Kristien J. Ledeganck,[1] Annelies Van den Driessche,[2] Jean-Louis Bosmans,[1,2] Marie M. Couttenye,[1,2] Benedicte Y. De Winter,[1] Gert A. Verpooten.[1,2] *[1]Experimental Medicine and Pediatrics, Univ Antwerp; [2]Dept of Nephrology, Antwerp Univ Hosp, Edegem, Antwerpen, Belgium.*

Hypomagnesemia is a well known side effect of cyclosporine (CsA) treatment in humans due to renal Mg loss but the underlying mechanisms remain unclear. It was recently shown that epidermal growth factor (EGF) stimulates Mg reabsorption in the distal convoluted tubule via TRPM6. We investigated the urinary excretion of EGF in patients treated with CsA and its relation to the fractional excretion of Mg. 25 kidney transplant patients treated with CsA and 11 controls were recruited at our hospital. Two or more blood and urine samples were taken from each patient with a time interval of at least 1 month. Urinary EGF was measured using an EGF human Elisa kit. Mean eGFR was comparable in both CsA-treated patients (44±27 mL/min/1.73m²) and controls (44±16 mL/min/1.73m²). Serum Mg was decreased in the CsA-treated group (1.87±0.24 mg/dL) compared to the control group (1.94±0.21 mg/dL) although this difference is not significant (p=0.27). The fractional excretion of Mg was increased in the CsA-treated group (5.9±4.1%) compared to the controls (4.8±2.2), although not significantly (p=0.25). Urinary excretion of EGF was significantly decreased in the CsA-treated patients (6.2±5.0 ng/dL) compared to the controls (22.1±38.4 ng/dL, p=0.01). Analysis using a Generalized Linear Model showed that, in both groups, log urinary EGF concentration was inversely correlated with FEMg (p=0.04).




A 5-fold decrease in urinary EGF excretion was found in CsA-treated patients. The decrease in EGF excretion was correlated with an increased fractional excretion of Mg. These findings support the hypothesis that EGF plays an important role in de renal excretion of Mg and confirm the results of animal studies in humans.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO816**

**Urinary Calcium Excretion Is Controlled by the Circadian Gene Clock** Vlasta Zavadova,[1] Svetlana Nikolaeva,[1] Dmitri Firsov,[1] Olivier Bonny.[1,2] *[1]Department of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland; [2]Service of Nephrology and Hypertension, Lausanne University Hospital, Lausanne, Switzerland.*

Circadian rhythms have been described for plasma calcium, parathormone (PTH) and for urinary calcium under constant conditions, and may play a role in kidney stone formation and osteoporosis. The mechanisms underlying these rhythms remain however largely unknown. Entrainment by systemic cues or regulation by local molecular clock have been proposed. Here, we aimed (i) at describing the physiological circadian rhythms for calcium in the mouse, (ii) at measuring the diurnal variations in mRNA levels of the main renal calcium transporters and (iii) at assessing the role of the molecular clock in the regulation of calciuria. We first studied the variations in plasma calcium, PTH, and in calciuria every 4 hours for 24h in C57BL/6 mice. Circadian variations of, respectively, 2.5, 40 and 20% over the 24h mean has been observed under light/dark cycles and under constant darkness. Second, the renal mRNA levels for the main partners involved in calcium reabsorption have been assessed by qPCR every 4 hours for 24h. TRPV5 and NCX1 mRNA levels were varying by 17 and 12% over the 24 hours mean, respectively, while calbindin-D28K and PMCA did not change significantly. NCX1 protein variations were confirmed by Western Blot analysis. Third, we studied the effect of the *clock* gene on calciuria. In metabolic cages, *clock*-deficient mice displayed higher urine calcium excretion (34.7% higher in light/dark conditions and 46.2% in constant darkness) and exhibited a disturbed circadian rhythm compared to their wildtype littermates. In *clock*-deficient mice, plasma calcium and PTH levels were not significantly different from wildtype mice. Overall, we characterized in detail the plasma calcium, PTH and calciuria circadian rhythms in the mouse and we showed that TRPV5 and NCX1 renal mRNAs expression were also varying over 24h. In addition, our data suggest that the *clock* gene is involved in the regulation of calciuria, as clock-deficient mice are hypercalciuric. In order to define the cause of hypercalciuria, kidney-specific disruption of the molecular clock is awaited.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO817**

**Renal Extracellular Ca2+-Sensing Receptor CaSR Regulates Calcium Homeostasis Independently of Parathyroid Hormone** Alexandre Loupy,[1] Bharath Wootla,[1] Suresh Krishna Ramakrishnan,[1] Kamel Laghmani,[1] Patrick Bruneval,[2] Chantal Mandet,[2] Pascal Houillier.[1] *[1]INSERM U872; [2]INSERM U872.*

CaSR plays a crucial role in Ca2+ homeostasis by tuning parathyroid hormone (PTH) secretion. However, the homeostatic importance of extraparathyroid CaSR is less understood. CaSR is significantly expressed in the thick ascending limb where its role remains uncertain. The aim of the present study is to determine if CaSR is able to control Ca homeostasis independently of PTH, and how CaSR acts on the renal tubule.

In thyroparathyroidectomized, PTH-supplemented rats (1U/hr), the CaSR antagonist NPS2143 (1mg daily) elicited a significant decrease in urinary Ca excretion on the first day of treatment (from 2.2±0.1 to 0.8±0.02 mM/mM creatinine, p<0.01), creating a positive Ca balance. Blood ionized calcium concentration was increased by 29% at day-2, 30% at day-4 and finally 35% at day-7 of treatment in the NPS-treated rats as compared to controls (p<0.03 in all cases). Besides its effects on Ca, treatment with NPS2143 did not alter glomerular

filtration rate, blood electrolytes concentrations, urine output and urinary electrolytes excretions. No change occurred in the control group throughout the experiment.

In in vitro microperfused cortical TAL, the peritubular addition of 1 μM NPS2143 reversibly increased by 50 % net Ca2+ reabsorption and transepithelial permeability to calcium, as compared to control period ($p<0.03$), without any modification in NaCl transport or transepithelial voltage.

Taken together, our results demonstrate that inhibiting the extraparathyroid CaSR increases renal tubular Ca absorption through an increase in the paracellular pathway permeability to Ca in the TAL, and blood Ca2+ concentration, independently of changes in PTH secretion. Renal CaSR may act as an additional player besides PTH in the regulation of extracellular calcium metabolism.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO818**

**Differential Regulation of the Sodium Phosphate Cotransporters NAPI2A and NAPI2C at the Apical Membrane by Parathyroid Hormone** Judith Blaine, Kayo Okamura, Hector Giral-Arnal, Omid Masihzadeh, Yupanqui A. Caldas, Moshe Levi. *Medicine, University of Colorado Denver, Aurora, CO.*

Parathyroid hormone (PTH) plays a critical role in the regulation of phosphate homeostasis which is crucial for cellular energetics and signaling. In the kidney, PTH regulates phosphate reabsorption via the sodium phosphate cotransporters NaPi2a and NaPi2c which are found on the brush border membrane (BBM) of renal proximal tubule cells. The kinetics of regulation of the cotransporters by PTH, however, are markedly different. Whereas NaPi2a is removed from BBM microvilli within an hour of PTH treatment the same process takes 4 to 6 hours for NaPi2c. The reasons for this difference are not known. We have previously shown that the unconventional myosin motor myosin VI is required for PTH-induced removal of NaPi2a from BBM microvilli using total internal reflection fluorescence microscopy (an excellent technique for examining membrane events). Here we demonstrate that myosin VI is also required for PTH-induced removal of NaPi2c from microvilli. In addition, we use fluorescence correlation spectroscopy to show that at baseline the diffusion coefficients for NaPi2a and NaPi2c within the BBM are the same. After addition of PTH, however, NaPi2a has an early increase in its diffusion coefficient which is not seen for NaPi2c. Thus NaPi2a and NaPi2c appear to be released from membrane tethers on different timescales after PTH treatment which can partially account for their differential response to PTH.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO819**

**Characterization of the Specific Effects of Renal Tubular Calcium-Sensing Receptor (CaR)** Renaud De la Faille, Alexandre Loupy, Pascal Houillier. *centre de Recherche des Cordeliers, Université Pierre et Marie Curie, INSERM UMRS 872, Paris, France.*

CaR regulates the secretion of parathyroid hormone (PTH) and calcitonin in response to changes in extracellular calcium concentration. In the kidney, it inhibits calcium reabsorption in thick ascending limb of Henle's loop. Direct effects of CaR activation on the renal handling of other ions and water remain unclear.

Objectives: to define the specific role of renal tubular CaR using specific allosteric agonists of CaR, AMG073 and NPS R568, and a non-specific agonist of CaR, namely calcium.

Methods: Thyro-parathyroïdectomized (TPTX), PTH-supplemented (1 U/hr) male Sprague-Dawley rats were orally treated with AMG073, NPS R568, high (3 %)-calcium diet or vehicle for 1 week. All rats were studied in metabolic cages. Time-courses of serum calcium, and urinary calcium, phosphate, magnesium, sodium, potassium, ammonium and water excretions were recorded. The renal expression of CaR, Bumetanide Sensitive Cotransporter of Henle's loop (BSC1), and aquaporin 2 (AQP2) was studied by immunoblot on membrane fractions of cortex and outer medulla.

Results: In TPTX, PTH-supplemented rats, treatment with AMG073 or NPS R568 transiently increased urinary calcium excretion on the first day of treatment and elicited a stable decrease in serum calcium concentration by the second day of treatment ; no significant change in urine volume, or other electrolyte excretions was observed. Conversely, high-calcium diet induced hypercalcemia, hypercalciuria, hypermagnesiuria, polyuria, transient natriuresis and metabolic acidosis. After 1 week of study, expressions of CaR, BSC1 and AQP2 were unchanged in the group treated with CaR agonists, but were decreased in the high-calcium diet group.

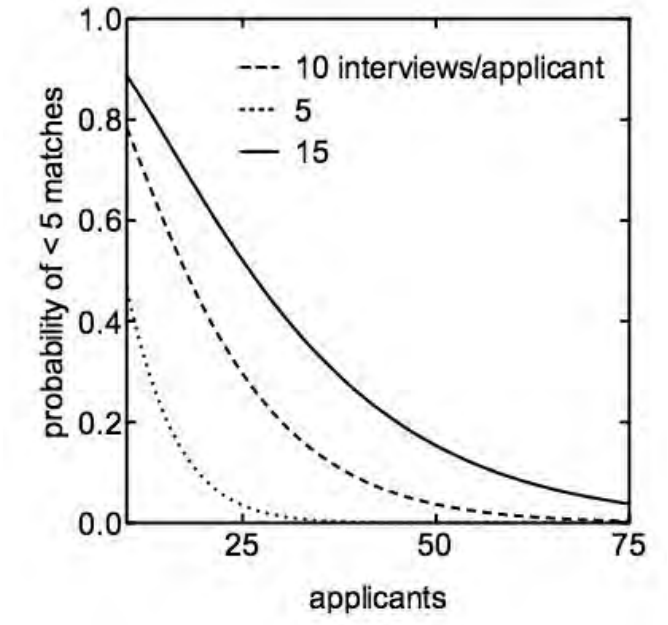
Conclusion: CaR activation decreases renal tubular calcium reabsorption by an effect independent on PTH. Moreover, CaR activation not fully explains all the tubular effects of hypercalcemia, particularly polyuria and natriuresis.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO820**

**How Many Applicants Should a Fellowship Program Interview: A Conservative and Probabilistic Approach** Brent Wagner,[1] Julie A. Harris,[1] Casey Cotant.[2] *[1]Department of Medicine, University of Texas Health Science Center, San Antonio, TX; [2]Nephrology, Wilford Hall Medical Center, Lackland Air Force Base, TX.*

Background: The NRMP was established to provide a fair recruiting process for both programs and applicants to positions in graduate medical education. Failure of a training program to fill all available positions leads to a "scramble". Common advice is to interview 10 applicants for every training slot, but there is a paucity of literature that supports this. Our purpose was to use a computer simulator to calculate the minimal number of applicants per training slot that are required for ranking. Methods: A simulation of a match was created using Excel. Hypothetical results based on varying the number of interviews (n) per applicant were generated. The probability of matching (P(m)) was estimated as 1/(n). The $P(m) < 5$ positions was calculated as the sum of the probabilities of filling 0, 1, 2, 3, or 4 positions. Results: When plotting fellowship slots against the number of ranked applicants, the mean P(m) follows a linear relationship with a slope of 1/(n). When extrapolated, the probability of not matching is inversely related to n.




For example, if the n is 10, a program with 5 available positions will need to rank 27 and 47 applicants for a 75% and 95% chance, respectively, to fill all slots. Conclusion: There is a balance between the labor-intensive investment in interviewing a large number of applicants with the goal of a high-probability successful match. The practical application of this simulator is to define how many applicants it is reasonable to interview in order to minimize the risk of not filling a fellowship slot. Our data support the anecdotal recommendation to interview at least 10 applicants for every fellowship position.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO821**

**"The Amazing Base": A Novel Use of Technology and Game Theory in Undergraduate Medical Education** Maury N. Pinsk,[1] Brettany Johnson,[2] Robert Hayward.[2] *[1]Pediatric Nephrology, University of Alberta, Edmonton, AB, Canada; [2]Centre for Health Evidence, University of Alberta, Edmotnon, AB, Canada.*

Introduction: Acid base physiology is one of the more challenging concepts that first year medical students learn. To assist with learning, we introduced an application of Game Theory utilizing HOMER, an online learning environment developed in the Faculty of Medicine. Methods: Building on the premise of the reality show "The Amazing Race", students were organized into small groups of 10 students. At the beginning of the workshop, the group logged into HOMER and started "The Amazing Base", a series of four stations where students either answer a mathematical or knowledge-based question relating to acid base physiology, or perform a task related to acid base concepts learned in lecture. Responses to each station were entered into HOMER and scored in real time. Group progress through the stations was logged online with time stamps as they moved through each station. The workshop culminated with an in-class review of the problem set answers and awarding of a prize to the group finishing with the most correct answers. Results: 87 students (60%) responded to post-workshop questionnaires. As the workshop was voluntary, self-reported attendance was 66%. 60% of respondents stated they found the workshop useful and assisted their learning. Of the attendees, 64% felt they agreed or strongly agreed that the workshop was excellent. Specific strengths of the workshop included aspects of teamwork, the introduction of a fun, creative way to learn, and the usefulness of supplementing a didactic lecture. Weaknesses included poor attendance by some groups and frustration when choosing tasks rather than calculations. Tracked progress of the groups detected unprofessional behavior in one group who claimed they had finished the race without evidence of data entry. Conclusion: "The Amazing Base" is a novel way to reinforce concepts of acid base physiology using Game Theory. The method is well received by first year medical students, provides opportunity for communication and collaboration between students, encouraged pre-workshop preparation, and has the ability to identify issues of professionalism.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO822**

**Factors Associated with Kidney Transplant Education: Results of a National Survey** Kamna Balhara, Lauren M. Kucirka, Bernard G. Jaar, Dorry L. Segev. *Johns Hopkins University School of Medicine, Baltimore, MD.*

**Background:** Patient education about kidney transplantation (KT) plays an important role in determining access to KT. While education modalities differ, counseling time provided by the patient's nephrologist may be pivotal to patient decision-making regarding KT. The goal of this study was to assess factors affecting duration and format of KT education provided by nephrologists.

**Methods:** An anonymous online survey was completed by 471 nephrologists across the US. Associations with length of KT education were examined using generalized linear models.

**Results:** 96% of respondents stated the nephrologist is most responsible for advocating for KT to eligible patients, and 95% felt reimbursement was needed for time spent on KT education. 77% reported that the ideal time to spend was over 20 minutes, but only 43% reported actually spending over 20 minutes. 82% reported that this discrepancy was due to lack of time; 28% cited lack of reimbursement, and 11% cited lack of patient interest. Spending >20 minutes on KT education (table) was associated with greater depth of counseling (covering more KT topics) and having one-on-one conversations. Those who reported that >50% of their patients were African-American had a 33% lower rate of spending >20 minutes on KT education.

**Conclusion**: In this national survey, we found significant associations between provider characteristics, the demographics of a provider's practice, and time spent on KT education. A better understanding of the mechanisms underlying these differences is needed to reduce potential discrepancies in quality of education provided to different patient populations.

**Factors Associated with Spending >20 minutes on Transplant Education**

| Factor | Relative Rate | P-value |
|---|---|---|
| ***Provider characteristics*** | | |
| Increasing age | 1.16 (1.02-1.32) | 0.02 |
| Increasing years in practice | 1.11 (1.03-1.19) | 0.006 |
| Non-profit center | 1.42 (1.05-1.92) | 0.02 |
| ***Patient characteristics*** | | |
| >50% African-American patients | 0.67 (0.48-0.92) | 0.01 |
| >50% female patients | 0.86 (0.58-1.28) | 0.5 |
| ***Format and depth of education*** | | |
| Greater depth of counseling | 1.39 (1.25-1.56) | <0.001 |
| One-on-one conversation with patient | 1.31 (1.06-1.61) | 0.01 |
| Discuss transplant repeatedly with reluctant eligible patients | 1.30 (1.12-1.51) | 0.001 |

Disclosure of Financial Relationships: nothing to disclose

**TH-PO823**

**The Relationship between Health-Related Quality of Life with EuroQol (EQ-5D) and Renal Prognosis in Chronic Kidney Disease Patients in Japan** Reiko Tajima, Hirayasu Kai, Chie Saito, Shuichi Tsuruoka, Kunihiro Yamagata. *Pathophysiology of Renal Disease, University of Tsukuba, Tsukuba, Ibaraki, Japan.*

**Background**: Chronic kidney disease (CKD) is a health-related quality of life (HRQOL) deteriorating disease which is not only a public health but also socioeconomic problem. Interest in developing cost-effective interventions to control CKD has increased. The aim of this study was to measure HRQOL for cost-effectiveness analysis using EQ-5D in patients with CKD. The relationships between the measured HRQOL and progression of CKD stage/ the incidence of cardiovascular disease (CVD) were also analyzed.

**Methods**: EQ-5D, a generic preference-based instrument, was administered to 475 CKD outpatients at Tsukuba University Hospital between November and December 2008.

Data on sex, age, creatinine, hemoglobin, serum albumin, and presence of CVD were obtained from the patients' records, and followed one year after HRQOL measurement.

**Results**: Measured quality-adjustment weights by the CKD stage were 0.940 (95% C.I.: 0.915-0.965), 0.918 (0.896-0.940), 0.883 (0.857-0.909), 0.839 (0.794-0.884), and 0.798 (0.757-0.839) for stages 1 to 5, respectively. The decrease in weight was significant by ANOVA (P<0.0001), and the weight for all stages was 0.885 (0.871-0.898). During the one-year observation period, CKD stage progression was observed in 22.3% of CKD stage 1, 6.8 % of stage2, 8.6% of stage 3 and 22.8% of stage 4, and 41.8% of stage 5 patients (end-stage renal failure). Incidence of CVD was 1.4, 0.7, 1.4, 2.9 and 6.0% for stages 1-5, respectively. Average weight of the subjects who showed progression of CKD stages (0.895) was significantly lower than those of stable renal function (0.834).

**Conclusions**: HRQOL decreases with progression of the CKD stage and decreased HRQOL also affect the prognosis in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO824**

**Taking Time To Think: Does Scheme-Inductive Reasoning Improve Diagnostic Performance in Nephrology Physicians and Trainees?** Adam J. Bass,[1] Colin C. Geddes,[2] Kevin McLaughlin.[1] *[1]Nephrology, University of Calgary; [2]Nephrology, Glasgow University.*

Erroneous information processing contributes to most cases of medical error. As the most frequent processing error is premature closure, cognitive strategies have been developed to reduce the risk of this. In this study we hypothesized that analytic information processing would improve diagnostic performance compared to automatic processing (or pattern recognition) alone. We also predicted that scheme-inductive reasoning, by virtue of superior organization and efficiency, would be a more effective analytic processing strategy than hypothetico-deductive reasoning. Our participants were physicians at 2 academic centres: the University of Calgary and Glasgow University, and we randomly allocated them to receive one of two booklets containing ten identical challenging nephrology cases that differed only in the reasoning instructions given for each case. Participants were given an initial case vignette without imaging or laboratory investigations, and were asked to provide an initial diagnosis. They were subsequently directed to use either scheme-inductive reasoning or hypothetico-deductive reasoning to generate differential diagnoses. Finally, they were given additional information and then asked to decide upon their final diagnosis. Nine practicing nephrologists and six nephrology fellows completed the cases. We found that when participants used any form of analytic information processing their diagnostic success improved significantly – from 34.7% to 67.3% (p<0.0001). There was no difference between the nephrology staff and fellows in diagnostic success and there was no difference between the two centres. There was also no difference in diagnostic performance between using scheme-inductive reasoning and hypothetical-deductive reasoning. In conclusion, analytic information processing, irrespective of the type of processing, improves diagnostic performance in Nephrologists.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO825**

**Life after Renal Fellowship Survey** Deepti D. Torri, Kellie R. Calderon, Hitesh H. Shah, Kenar D. Jhaveri. *Nephrology, North Shore/LIJ Health System & Hofstra Medical School, NY.*

**Introduction:** The outlook for nephrology jobs has been one of the weakest in the past 25 years. If one looks at the NEJM classified advertising pages, you notice a 50% decrease in nephrology positions across the health care market. In order to assess how the graduating renal fellows are doing this year we conducted a simple survey which consisted of 9 questions. The survey was created online using survey monkey website. The link was placed in popular nephrology fellow blogging sites. The survey was anonymous.

**Methods:** The fellows were asked about their region of training in the US; if they were a US citizen or green card holder; if they are an American based medical university graduate, what their dream job is; do they have a job position secured for July 2010; if yes, what type of job; and finally if they enjoyed doing nephrology.

**Results:** 30% of the graduating renal fellows responded to our survey. 34.5% of the graduating fellows did not have job yet and 8.8% of the fellows are doing extra training in nephrology to buy time to look for job of interest. When the question was asked regarding "dream job", only 3.4% chose to be hospitalist but in fact 13% of them will be hospitalist after completing the training. 18.6% of the fellows said that "They were not glad that they chose nephrology as a career". There are several factors this year that are playing a role in decrease job opportunities for graduating nephrology fellows like economical recession, housing market collapse and passing of health care reform.

In summary, even though nephrology positions are out there, this year and probably for the next year to come, there is a high possibility that one may not find pure Nephrology jobs and many will have to take other options such as hospitalists and internal medicine. There is also high possibility that one may not find the location of practice where they primarily desire. As a profession, we have to better promote our field and not have close to 20% of fellows feel that they made a wrong decision.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO826**

**Educational Tools in Nephrology: Creative Writing** Kellie R. Calderon, Rajiv S. Vij, Kenar D. Jhaveri. *Nephrology, North Shore/LIJ & Hofstra Medical School, NY.*

**Purpose:** To enhance traditional renal medical education with creative writing exercises.

**Introduction:** American medical students have traditionally matriculated through a four-year medical education system with a clear distinction between the basic science and clinical years that has seen little change since the Flexner report of 1910. Medical students and residents are often overwhelmed and find little time for reading as they enter the latter portion of their training. As an adjunctive effort, our institution has been experimenting with creative writing exercises to encourage reading in an entertaining way.

**Methods:** Twenty-eight medical residents completed a 5 question pre-test on tubulo-glomerular feedback. They were then randomly assigned to one of two groups of 14 house-staff each to receive either a basic review article on the renin-angiotensin system or a fictional short story that described the same system from the point of view of the renin enzyme entitled "The Call of Renin." The next day, the same five questions were administered to the house-staff. Eleven house-staff in each group completed the post-test. Statistical Analysis was done using GraphPad statistical software.

**Results:** There was no statistical difference in the pre test scores between the two groups (p=0.69). There was a statistically significant difference between pre and post creative reading scores with p value of 0.027 and t 2.38. Also there was a statistically significant difference between pre and post basic review article scores with p value of 0.019 with t 2.58. There was no difference between the post test scores of both groups (p= 0.71). Of the house-staff who received the creative writing exercise, 82% (9 of 11) reported reading any amount of the material, compared to only 45% (5 of 11) of house-staff who received the basic review article ( p=0.09, Fischer Exact test). Of the responders who read the creative writing exercise, 78% felt it was a helpful supplemental teaching tool.

**Conclusions:** Our study shows that when used by residents, the two teaching materials had similar effect. The benefit of the creative writing exercise however, was the 37% increase in the number of house-staff who read the material.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO827

**Patient Knowledge Is Associated with Systolic Blood Pressure in Patients with CKD** Julie A. Wright, T. Alp Ikizler, Kerri L. Cavanaugh. *Division of Nephrology and Hypertension, Vanderbilt University Medical Center, Nashville, TN.*

Data are scarce concerning disease specific knowledge and outcomes in patients with chronic kidney disease (CKD). We examined associations between patient hypertension knowledge and blood pressure measurements.

Established adult patients with non-dialysis dependent CKD (Stages 1-5), seen at least once previously in Nephrology Clinic, were enrolled from April to October 2009. Patients self reported hypertension (HTN). Knowledge questions included "Can high blood pressure hurt the kidney (Yes/No)?", and target blood pressure goal, "On average, your blood pressure should be (160/90, 150/100, 170/80, Lower than 130/80)?" with "lower than 130/80" considered correct. Blood pressure measurements (mmHg) were performed by trained personnel using automated sphygmomanometers. Awareness of CKD diagnosis was also assessed.

Of 401 participants, 86% reported having HTN. The mean (SD) age was 58 (16) years, 55% were male, 82% were Caucasian, and 79% had CKD Stage 3-5. Twenty-nine percent were aware of CKD diagnosis, 97% knew hypertension may harm the kidney, and 91% identified the correct blood pressure goal. The systolic blood pressure (SBP) range was 75 - 217 mmHg, with a mean (SD) of 136 (22) mmHg. Higher SBP was associated with older age (rho = 0.20, p<0.01), male sex [male, mean (SD) 138 (22) vs. female 133 (23) mmHg; p=0.04], not knowing HTN can hurt the kidney [No 152 (29) vs. Yes 136 (22) mmHg; p=0.03], lack of knowledge of blood pressure target [Incorrect 145 (25) vs. Correct 135 (22) mmHg; p=0.02], and lack of awareness of CKD diagnosis [Unaware 140 (23) vs. Aware 134 (22) mmHg; p=0.04]. A model adjusted for age, sex, race, income, stage of CKD, HTN knowledge, number of physician visits, and awareness of CKD diagnosis, revealed that only age (β = 0.30, p<0.01) and knowledge of blood pressure goal (β = -10.6, p=0.02) were independently associated with SBP.

Patients with established CKD, who report more hypertension-specific knowledge, had lower systolic blood pressures. Efforts to increase patients' knowledge, particularly about blood pressure goals, may have an important role in optimizing hypertension management in patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO828

**Assessing Blood Pressure Control in Diabetic Patients in an Internal Medicine Residency Continuity Clinic** Michael Grant Selby,[1] Jason Post,[1] Suzanne Norby.[2] *[1]Internal Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology & Hypertension, Mayo Clinic, Rochester, MN.*

**Background:** Optimal BP control in diabetics can slow the progression of diabetic nephropathy, and the State of MN has established BP <130/80 as a reportable quality measure in diabetics. To fulfill our program's requirement that each internal medicine (IM) resident participate in a quality improvement project, BP control rates for diabetic patients in an IM resident continuity clinic (CC) were measured in order to design a subsequent intervention to improve BP control rates. **Methods:** The number of diabetics with hypertension (HTN) assigned to 23 resident patient panels in this CC was determined by reviewing an electronically generated list of assigned patients. Medical records of 184 diabetics were audited, and patients were classified as having controlled, uncontrolled, and/or unidentified HTN based on most recent clinic BP measurement. Also, visit notes were reviewed to ascertain whether uncontrolled HTN had been addressed in the care plan. Next, each resident was provided with feedback on HTN control rates in diabetics in the form of a bar graph illustrating individual and anonymous aggregate data, allowing comparison of his/her own patients' control rate with those of other residents. **Results:** Overall, 32% (range 9.1-80%) of diabetic patients assigned to IM resident patient panels had uncontrolled HTN, and 5.4% (range 0-40%) had unidentified HTN. Of those with uncontrolled HTN, only 10% (range 0-100%) had HTN addressed in care plans. Although each patient was assigned to an IM resident panel, 22% (0-75%) had not been seen by the primary resident. **Conclusion:** Most residents identify uncontrolled HTN in diabetics but do not document a HTN treatment plan. Opportunities exist to improve HTN control in diabetics in this IM resident CC practice overall. The ability to determine HTN control rates for individual IM residents based on patient panel assignments could be limited since patients may not have been seen by the primary resident. Another audit of these parameters could be completed in the future to determine if HTN control rates in diabetics improve in this IM resident CC practice following performance feedback.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO829

**Efficacy of Clinical Audit To Improve Blood Pressure Control in Hemodialysis Patients** Pasquale Esposito,[1] Attilio Di Benedetto,[3] Carmine Tinelli,[2] Daniele Marcelli,[4] Antonio Dal Canton.[1] *[1]Nephrology, Dialysis and Transplantation Unit, Fondazione IRCCS Policlinico "San Matteo", Pavia, Italy; [2]Unit of Biometry and Clinical Epidemiology, Fondazione IRCCS Policlinico "San Matteo"; [3]NephroCare Medical Direction Italia, Italy; [4]NephroCare Coordination EMEA- LA, Germany, Germany.*

Introduction

High prevalence of hypertension in hemodialysis patients(HD)is related to cardiovascular disease risk.Various therapeutic approaches have been proposed to ameliorate blood pressure(BP)in HD patients.We evaluated the effects of a clinical audit in BP management.

Patients and methods

We studied 71 patients undergoing on-line HDF from NephroCare Clinics.For each patient we recorded,on an individual chart,predialysis BP,demographic factors,comorbidities,dry weight,interdialytic weight gain,drug therapy,patient compliance,hematocrit and epoetin therapy.Moreover,we also considered KT/V and dialysate conductance and temperature. These data were collected from the last 6 HD sessions performed before the audit notification(PRE) at the audit(AUDIT) and 1(POST 1) and 6(POST 6) months after the audit. We assumed a value of ≤ 140/90 mmHg as BP target.On the basis of BP value we suggested individual strategies to improve BP management,such as reduction of dry weight,increase of dialysis dose and/or changes in antihypertensive treatments.

Results

There was a significant improvement of BP control after the audit, associated to a reduced interdialytic weight gain in presence of a concomitant reduction of the use of antihypertensive drugs.No significant differences in dialytic parameters were found.

Table 1

| | PRE | AUDIT | POST 1 | POST 6 |
|---|---|---|---|---|
| Systolic BP>140 mmHg(pts %) | 39.4 | 43.7 | 31.2 | 25.4 * |
| Dry weight(kg) | 65.6±12.2 | 66.5± 12.2 | 65.6 ± 12.8 | 65.4 ± 13.3 |
| Interdialytic weight gain>5% dry weight(pts %) | 16.4 | 13.0 | 12.5 | 8.7 * |
| Pts on antihypertensive treatment(%) | 52.24 | 57.14 | 48.48 | 45.61 |

* p<0,05vsPRE

Conclusions

Clinical audits are a part of the quality improvement process.Through the comparison between clinical outcome and established standards, they lead to implement changes in clinical practice.Our study shows the efficacy of a clinical audit to promote improvements in BP management in HD patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO830

**Survey of Statistical Methods Used in the Clinical Nephrology Literature for Teaching Purposes** Eleanor D. Lederer,[1,2] Snehal S. Patel,[2] Ryan L. Haulk,[2] Michael Brier.[1,2] *[1]Medicine, Robley Rex VA Medical Center, Louisville, KY; [2]Medicine, University of Louisville, Louisville, KY.*

In order to evaluate the published clinical literature, post graduate trainees should receive training in statistical methods; however, this endeavor is not adequately documented. To test the hypothesis that instruction in interpretation of clinical literature is not adequate in nephrology training programs, we surveyed nephrology training programs on statistics education and tabulated statistical methods in the 2008 issues of the Journal of the American Association of Nephrology (JASN), Clinical JASN, Kidney International (KI) and the American Journal of Kidney Disease (AJKD). We found the following number of articles: JASN, 211; CJASN, 156; KI, 86; AJKD, 149. The tests with the highest frequencies were: crosstabulation 35.8%, t-test 32.3%, nonparametric t-test 26.2%, survival analysis 17.6%, nonparametric related tests 15.1%, paired t-test 10.5%, logistic regression 13.6%, anova 12.8%, correlation analysis 7.6%, linear regression 6.1%, and nonlinear regression 5.2%. All other methods were less than 2% (multivariate anova, anova with repeated measures, generalized linear regression, ROC). Certain tests were more prevalent in specific journals. Chi-square & survival analysis (CJASN, AJKD), anova (JASN), correlation & linear regression (AJKD) and logistic regression (KI, AJKD) (p<0.001). The survey results from 69 Training Program Directors indicated that statistics are primarily taught through informal discussion (78.3%) by someone experienced in statistical methods (78.8%). However, fellows are not formally tested (91.3%) and most directors were only somewhat confident in the fellows ability to assess the clinical literature (65.2%). We conclude that fellowship programs would benefit from development of a standardized curriculum to teach and evaluate trainees in critical literature analysis, including the statistical methods employed. The significant journal dependent differences in use of applied statistical techniques suggest that fellows should read and critique manuscripts from multiple journals in order to attain competency in a variety of statistical methods.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO831**

**Patient Educational Programme (PEP) in Subjects with Unplanned Haemodialysis Treatment May Increase the Number of Home Dialysis Therapies** Edyta Golembiewska,[1] Joanna Kabat-Koperska,[1] Kazimierz Ciechanowski,[1] Ewa Suchowierska,[2] Michal Mysliwiec,[2] Jozef Penar,[3] Marian Klinger,[3] Jacek Lange,[4] Danuta Deckert.[4] *[1]Nephrology, Transplantology, Medical Univ., Szczecin, Poland; [2]Nephrology and Transplantology with Dialysis Unit, Medical Univ., Bialystok, Poland; [3]Nephrology and Transplantation, Univ. of Medicine, Wroclaw, Poland; [4]Baxter HealthCare.*

Purpose: Approximately 50%of patients with end-stage renal disease (ESRD) start renal replacement therapy (RRT) as an acute,life-saving treatment.Unplanned start of dialysis treatment using temporary access is associated with increased morbidity, mortality and number of hospitalizations.In some centers, after routine implementation of haemodialysis treatment,the method of peritoneal dialysis can be introduced to patients.The purpose of the study was to assess how many patients (%) would choose peritoneal dialysis as a method of chronic treatment.

Results:Median age of the patients was 64.5 years (range 21 to 80 years. There were 11 females and 9 males. The causes of ESRD were as follows: diabetes (8patients), hypertension (5 patients), glomerulonephritis (3patients),other (5 patients). 11 patients started haemodialysis without prior nephrological care. In 6 patients nephrological care lasted below 6 months, in remaining three lasted from 1 to 10 years. Median (range) values of chosen parameters in patients were as follows: urea 190 mg/dL (82-449), albumin 33 g/L (18-40), calcium 8.3 mg/dL (5.9-10.1),phosphorus 5.6 mg/dL (4.3-11.7),CRP 16.35 mg/L (2.0-54.3).

Median eGFR was 10.2 mL/min (range 3.2-25).

No haemodialysis complications were observed. 6 of 20 patients (30%)chose peritoneal dialysis as the mode of chronic RRT.In one patient, dysfunction of Tenckhoff catheter required catheter replacement.No other complications of PD have been observed.

Conclusions:Patient Educational Programme implemented in subjects starting unplanned haemodialysis therapy can increase the number of patients choosing peritoneal dialysis therapy.This can bring potential benefit especially to patients with residual diuresis or awaiting kidney transplantation.Home therapy may also better meet patient's expectations as to quality of life.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO832**

**Prevalence and Utility of "Fellows' Survival Guides" in Post-Graduate Nephrology Training Programs** Alejandro Diez. *Division of Nephrology, Indiana University, Indianapolis, IN.*

Purpose:

Informal discussions amongst nephrology fellows training in the United States reveal that many programs have locally developed versions of a "Nephrology Fellows' Survival Guide". In general, these "fellow survival guides" are compilations of practical tidbits of information, brief "how-to" instructions, and succinct clinical protocols which can be accessed easily and quickly by a trainee in the course of daily practice. Even though many nephrology fellows attest to the value of these guides, no formal data exists as to their prevalence and perceived utility.

Methods:

A web-based survey was designed and implemented on "Survey Monkey", a commercial web-based survey platform. Questions were designed to determine the prevalence, content and perceived utility of these guides. Invitations to complete the survey were e-mailed to nephrology training program directors in the United States via the American Society of Nephrology (ASN) "listserv". Results were collected using 'Survey Monkey's" proprietary software and downloaded to a Microsoft Excel file for further analysis.

Results:

Responses were received from 34 nephrology training programs (approximately 25% of all programs). 55% of the respondents had a locally compiled guide/manual distributed specifically to nephrology trainees. The most common focus of these manuals was administrative (ACGME rules, division policies, fellow responsibilities, etc.) followed by clinical (protocols and guidelines for renal replacement therapy, transplantation, diagnosis and treatment, etc.). Over 75% of respondents rated their guides as "useful" or "very useful", having a positive impact on trainee education (79%) and efficiency (71%).

Conclusions:

While common, "Fellows' Survival Guides" are not ubiquitous. Because these guides have evolved according to institution-specific needs and experiences there is great variability in focus and content. Most programs with these guides recognize their utility and positive impact when used as an adjuvant tool in post-graduate nephrology training.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO833**

**Nephrology as a Source for Ethical Teaching in Medicine. Lessons from Dialysis and Transplantation** Giorgina B. Piccoli, Valentina Consiglio, Rossella Attini. *SS Nephrology, ASOU San Luigi, Torino, Italy.*

**Background.** The history of dialysis is deeply rooted in the history of bioethics; the possibility to live with the help of a machine the initial problem of the limited resources for "artificial kidney" allocation (the Seattle Committee) obliged not only the medical community, but also the laymen to face ethical problems never previously foreseen. Organ shortage for transplantation poses again similar open questions.

**Aim.** Teaching ethical problems in the context of the Nephrology courses may both enhance student interest on our discipline and offer a wide range of practical examples.

**Methods**. In the context of the Medical School (75-100 students/year), ethical problems were posed, via semistructured questionnaires, on different occasions between the 1st and the 4th year.

**Results**. Over 300 questionnaires were gathered in the last 4 years. Three main questions were analysed: would you start dialysis in a patient with a life expectancy not exceeding 6 months; organ shortage for transplantation obliges to choose between choosing younger and fit patients versus high risk patients; a dialysis patient asks on the possibility to buy a kidney abroad. What would you answer?

In the first case, answers were almost evenly divided into not starting dialysis, starting it and uncertain. A similar patter was observed as for choosing younger or sicker patients for transplantation and uncertain. The majority of the students (54%) would allow buying a kidney graft in a foreign Country provided that this is not forbidden by the local laws. Once again, the prevalence of uncertain answers was high (28-32% in the different years). The questionnaire was rated as interesting by over 90% of the students, each year.

**In conclusion,** Nephrology offers a wide range of suggestions for the ethical discussion that should be included in the conventional, often strictly technical, Academic teaching.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO834**

**Clinical Impact of Educational Interventions on the Recognition and Treatment of Chronic Kidney Disease in a General Medicine Clinic** Margaret MacDougall, Sriram Narsipur. *Medicine, SUNY Upstate Medical University, Syracuse, NY.*

Under-recognition of chronic kidney disease (CKD) remains a problem in a general medicine population despite attempts at education. We attempted to better define the magnitude of the problem by reviewing charts of patients seen in the Internal Medicine Clinic for primary care to assess recognition and treatment of CKD, Stage 3 or more. At least 30 charts were reviewed in 2005, 2007 and 2010. Patients were excluded if they were co-managed by Nephrology, did not have follow-up visits within the time frames assessed, or were seen by their primary care physician less than 3 times. Data collection included the recognition of CKD, monitoring of phosphorus, intact parathyroid hormone (PTH), and iron studies.

The 2005 chart review indicated that 17% of included patients were properly identified as CKD, Stage 3 or 4. Only 20% of patients had a serum phosphorus and 10% had a PTH checked and 27% had iron studies. Data and results were reviewed with the Internal Medicine Residents and a flow chart of the KDOQI recommendations for metabolic bone monitoring was developed and provided to care-givers. Wall charts were also posted in general medicine clinics for quick reference.

In the 2007 chart audit, the identification of CKD increased to 19% and 58% now had a phosphorus checked but PTH was checked in only 9%. Iron studies were done in 35%. Following the 2007 audit, a focused CKD lecture was given to the housestaff and an audio/slide tape presentation was prepared for review. Wall charts remained visible but individual distribution of the flow chart did not occur yearly.

In the 2010 chart audit, recognition of CKD decreased to 17%. Only 13% of patients had a phosphorus checked and only 7% had a PTH checked. Iron studies increased to 50% of patients.

We conclude that, despite aggressive education and re-enforcement efforts with the internal medicine housestaff, it is apparent that major issues related to CKD are not being recognized and addressed in a general medicine clinic. In an environment with an ever-increasing CKD population, it is clear that attempts to improve recognition and management must improve and novel strategies need to be developed.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO835**

**Assessing Patients' Knowledge on Chronic Kidney Disease and Interest in Renal Pharmacy Services** Priscilla P. How,[1,2] Junice Yi Siu Ng,[1] Srinivas Subramanian,[2] Vathsala Anantharaman.[2] *[1]Pharmacy, National University of Singapore, Singapore, Singapore; [2]Medicine (Nephrology), National University Health System, Singapore, Singapore.*

Chronic Kidney Disease (CKD) patients have multiple medical problems and complications. As a result, they may have inadequate knowledge of their disease, be prescribed with several medications and are at increased risk for drug-related problems. Pharmacist-run clinics have been shown to reduce healthcare costs and improve outcomes. However, pharmacist-provided services are not common in outpatient CKD clinics in Singapore. This study was conducted to assess CKD patients' knowledge of their disease and their interest in services provided by a renal pharmacist.

This cross-sectional survey study was conducted in an outpatient CKD clinic in a tertiary hospital in Singapore. Patients ≥21 years old with stage 1-5 CKD were included. Information on patient demographics, patients understanding of CKD and their medications, as well as interest in using renal pharmacy services were collected. These services include collaborative management of CKD and its complications with nephrologists, laboratory monitoring, renal dosing of medications, medication review, education and counseling.

Of the 155 patients interviewed, 61(39.4%) and 19(12.7%) knew the cause and stage of their CKD, respectively, and 74(47.7%) think CKD can be cured. A total of 101(65.2%) patients expressed interest in renal pharmacy services. Most (>90%) patients were aware of the indications and dosing instructions of their medications. However, 114(74.5%) patients did not know their side effects and many (23-56%) had wrong perceptions of their

medications e.g. branded medications are better than generics; development of immunity, tolerance and dependence on their medications; and doubling medication dosage if they missed a dose.

Our patients showed suboptimal understanding of CKD and their medications. Majority were interested in renal pharmacy services. More effort is needed to educate patients of their disease and medications. Pharmacists can play an important role in CKD education, treatment monitoring, as well as medication review and counseling, to improve care and outcomes of these patients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO836

**The Impact of Structured Group Education on Blood Pressure (BP) Control in People with Early Kidney Disease: An Exploratory Randomised Controlled Trial (RCT)** Susan Carr,[1] Jo Byrne,[1] Margaret A. Stone,[2] Azhar Farooqi,[3] Kamlesh Khunti.[2] *[1]Department of Nephrology, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom; [2]Department of Health Sciences, University of Leicester, Leicester, United Kingdom; [3]East Leicester Medical Practice, Leicester, United Kingdom.*

**Objective:** We conducted an exploratory RCT to assess feasibilty and evaluate the implementation of structured group education to improve BP control in people with early kidney disease.

**Methods:** Patients with sustained hypertension were recruited from nephrology clinics and randomly assigned to either:

- A control group (n=41) that received routine clinical management of hypertension.
- An intervention group (n=40) that received routine care plus structured group education involving facilitated discussion with support to engage and empower patients to improve concordance with treatment and advice.

The primary outcome measure was the proportion of participants in each group achieving recommended BP targets at 12 months. Other outcomes included other cardiovascular risk factors, self-efficacy levels, knowledge of cardiovascular risks, well-being and patient satisfaction with intervention.

**Results:** The intervention was positively received with 100% of participants rating it as enjoyable. However, analysis revealed no significant differences for the other outcomes. The findings of the study showed difficulties with recruitment (only 30% of all patients approached for inclusion) and retention of participants in the intervention group (only 37.5% attended a planned educational session). Lack of interest (44%) and time (48%) were the main reasons cited for not participating. Non-attendance in the intervention group was significantly more likely in older participants (p=0.039) and in those with lower levels of self-efficacy (p=0.034).

**Conclusion:** Although the intervention was well received, the findings suggest that delivering and evaluating an effective group education intervention to improve BP control is difficult to achieve. People are initially reluctant to be involved and do not perceive education as an essential part of their care. Novel ways of delivering education that appeal to patients are required.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO837

**Innovative Teaching Tools in Nephrology** Rajiv S. Vij, Kellie R. Calderon, Kenar D. Jhaveri. *Nephrology, North Shore LIJ, Hofstra Medical School & the Raggio Institute, Great Neck, NY.*

**Purpose:** To create innovative teaching tools in Nephrology.

**Introduction:**Teaching any subject with vast spectrum of knowledge is a difficult task. At our institution, we developed many innovative ways of delivering the knowledge of nephrology and allowing it to be a creative and fun experience.

**Methods:**We have developed following tools that are unique and useful in delivering the same message that a traditional lecture would give.

1. Nephrology Crosswords. These are designed by a fellow and faculty member keeping a specific topic of nephrology in mind (peritoneal dialysis, natremias). Active learning is seen and fellows are searching for answers to the clues and trying to solve the puzzle. It provides a nice review of the topic for them that can supplement the traditional lecture format.

2. Role Playing: Fellow and resident driven teaching tool. For example, each participant plays the role of an assigned immunosuppresive agent, with a brief introduction followed by discussion and debates ( staying, of course, in character). This tool is extrapolated further to involve disease states, devices and techniques depending on the topic of discussion.

3. Nephrology Blogs : We have created a blog nephronpower.com in 2009 with goal to further medical education by publishing interesting nephrology cases, journal clubs, quizzes and current news in Nephrology. Contributors to the blog include fellows, academic and private nephrologists from USA and around the world. Nephron Power gets around 60 site visits per day with average 106 page views per day from all over the world.

**Results**:We also conducted an online survey comparing the current traditional teaching methods with above mentioned innovative teaching tools. Survey was open to all students, residents, fellows, nephrologists and other physicians. More than 80% of the people said that incorporating these innovative teaching tools will increase the interest and increase recruitment to the field of Nephrology. Only 50% thought that the current teaching methods were adequate.

**Conclusions:** In addition to traditional teaching methods, there is a clear need and role of creative teaching tools in nephrology.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO838

**Development and Usability Evaluation of a Web-Based Simulation for Teaching Electrolyte and Acid-Base Disorders** Mogamat Razeen Davids,[1] Usuf M. E. Chikte,[3] Mitchell L. Halperin.[2] *[1]Stellenbosch University, Cape Town, South Africa; [2]St Michael's Hospital and University of Toronto, Toronto, ON, Canada; [3]Stellenbosch University, Cape Town, South Africa.*

**Introduction**

We developed a Flash® application to help students learn about electrolyte and acid-base disorders. The subject presents a heavy intrinsic cognitive load, and reducing extraneous load by improving interface design could lead to gains in learning efficiency. This study reports on the development and usability evaluation of our application.

**Methods**

Flash® was used to develop the simulation, with ActionScript™ controlling it by calculating the effects of user-selected therapies on parameters like plasma Na concentration and brain volume, then displaying appropriate messages and animations based on the results of the treatment.

User testing: Morae® software recorded the interaction of participants with the application. Participants also completed a questionnaire including the System Usability Scale (SUS). Usability inspection: Experts conducted a heuristic evaluation (HE). Problems were cataloged and categorized, and their detection by different methods compared.

**Results**

Questionnaire: SUS score was 78.4 ± 13.8. Participants rated the content as scientifically sound (15/16), liked the "clinical detective story" approach (14/16), and would recommend the application to others (15/16).

Morae: Major problems included a "hidden" Lab Data panel. The treatment simulation was completed easily by only 3/15 participants. Most unsuccessfully tried to apply multiple treatments, and had problems using a slider control, resulting in dosages of zero.

Heuristic evaluation detected 22 errors.

The slider control problem was not detected by HE or questionnaire. Most problems with case accuracy were detected via questionnaire and Morae, and problems related to user feedback by HE.

**Conclusion**

We have created an engaging e-learning resource to help students develop expertise in this area. The usability evaluation will guide improvements. Combining evaluation methods ensures that most serious usability problems will be addressed, to maximize the potential for effective learning.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO839

**Do Medical Trainees Receive Adequate Training in the Management of Acute Kidney Injury?** Mansoor N. Ali, Andrew J. P. Lewington. *Renal Medicine, Leeds Teaching Hospitals NHS Trust, Leeds, Yorkshire, United Kingdom.*

**Background:** The recent National Confidential Enquiry into Patient Outcome and Death (NCEPOD) adding insult to injury acute kidney injury (AKI) study identified that only 50% of patients with AKI received good care. One of the issues raised surrounded a lack of medical training on the management of AKI.

**Aim:** To better understand whether medical trainees perceive that they receive adequate training in the management of AKI.

**Method**: A simple questionnaire was used to explore medical trainees understanding of the management of AKI. They completed the questionnaire prior to receiving a lecture on the management of AKI. The questionnaire was completed by 8 final year medical students, 22 interns and 22 residents in a large UK teaching hospital.

**Results:**

Only 6 of the trainees correctly cited the RIFLE/AKIN definition for AKI. The remaining trainees either gave no definition (24) or came up with their own unique definitions (22).

All trainees had managed patients with AKI; most of whom were cared for in the acute admissions wards.

Only 1 trainee felt confident enough to manage AKI, the remaining felt unconfident and were uncertain as to when to refer patients to nephrology.

Medical trainees indicated that there were deficiencies in training at both undergraduate and postgraduate levels in basic skills such as volume assessment and intravenous fluid management.

**Conclusion:** As a result of the questionnaire teaching programmes on the management of AKI are being developed for undergraduate and postgraduate trainees. The first UK wide e-learning module funded by the government will concentrate on AKI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO840**

**Does Excessive Intake of Sodium Contributes to the Interdialytic Weight Gain?** Bárbara Margareth Menardi Biavo,[1] Clarissa B. B. Uezima,[2] Camila Machado de Barros,[1] Elzo R. Junior,[1] Elvino Barros,[3] João Paulo L. B. Martins,[2] Carmen B. Tzanno-Martins.[2] *[1]Home Dialysis Center, São Paulo, São Paulo, Brazil; [2]Integrated Center of Nephrology, Guarulhos, São Paulo, Brazil; [3]Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.*

**OBJECTIVE**

The aim of this study was to assess interdialytic weight gain and sodium intake of patients undergoing hemodialysis in a private clinic in São Paulo.

**METHODS**

Cross-sectional study with 97 patients [39 (40.2%) women and 58 (59.8%) males] undergoing chronic hemodialysis. We evaluated the clinical data (kt/V), measured pre-dialysis weight, post-dialysis weight (dry weight) and height in order to calculate interdialytic weight gain and BMI (body mass index) classified according to WHO-1998. In order to assess dietary intakes of sodium, we used three 24-hour recalls, two related to food on days when patients weren't undergoing hemodialysis and another on the day of hemodialysis.

**RESULTS**

Among the 97 study participants, 16 (15.5%) had weight gain above the recommended (> 5% dry weight).

As for sodium intake, comparing the groups that gained less than 5% and more than 5% of dry weight between dialyses, both showed an average sodium consumption of 3000.1 mg and 2975.31 mg respectively, values higher than recommended. Recommended values range from 1000 to 2300 mg per day, according to blood pressure, edema and interdialytic weight gain.

There was no difference in sodium intake in relation to nutritional status. Both eutrophic and overweight patients had similar sodium intake. Kt/V of both groups had similar values (1.18±0.20 - group with adequate weight gain; and 1.20±0.21 - group with weight gain above recommended).

**CONCLUSION**

Sodium consumption was above recommended levels, which did not influence the interdialytic weight gain in the studied population, since most of it had weight gain within normal limits. Still, it is of utmost importance to focus on following a low sodium diet, since sodium excess can aggravate hypertension, a condition present in most of our patients, if not the cause of their CKD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO841**

**Pre-Emptive Live Donor Kidney Transplants Have Increased by Improving Access to Information** Wendy Brown, Jen C. McDermott, Marina Loucaidou, Tom Cairns. *Imperial College Kidney & Transplant Institute, London, United Kingdom.*

Transplantation is considered the optimum treatment of choice for patients with end stage renal disease. Pre-emptive living donor transplantation is increasing and has benefits over those transplanted post dialysis. However, despite written information; invitations to seminars; discussions with the medical team, coordinators and nurses, living donor transplant rates were static.

Aim: To improve access to information to enable people to make an informed decision regarding living donor transplantation.

Methods: Feedback from patients was reviewed and the structure and content of the information seminars was changed. The seminars became more frequent and less process focused. More patient volunteers and staff were enlisted and seminars now run on Saturdays. The agenda includes factual information e.g. long-term outcome results for donors and recipients by medical experts. Structured small group sessions were introduced where transplant and donor volunteers share their experience with the patients and potential donor group.

Outcome: These changes resulted in a massive increase in attendance at the seminars from 85 attendees in 2007 to 392 attendees in 2009. The proportion of pre-emptive living donor transplants carried out has also significantly increased from 21/81 (25%) in 2006 to 35/79 (44%) in 2009 (p=0.020). The 2 monthly information seminars in 2009 generated 72 self referrals to commence transplant work up.

Conclusion: Attention to the written and verbal feedback from patients has resulted in a programme that is more appropriate, meaningful and now meets patients' needs; Saturday seminars have captured a working audience and the participation by many transplant and donor volunteers has provided valid reassurance and honest explanations of the process. This has resulted in better information and experience for patients; *"thank you for making it easier to understand and therefore [allowing me] to make the right decision"*, and an increase in pre-emptive living donor transplant rates.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO842**

**The Impact of Reporting Estimated Glomerular Filtration Rate** Abdallah Sassine Geara, Nadine Azzi, Pratima Ghimire, Marie Abdallah, Claude Bassil, Ayesha S. Siddiqui, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

**Purpose of study:** Our objective is to determine if reporting eGFR while resulting blood tests was beneficial as far as: Physician awareness for CKD, dosage of antibiotic, avoidance of nephrotoxic medications and hydration for IV contrast.

**Methods:** Our laboratory started reporting calculated eGFR starting November 2005. We reviewed 2400 medical records, 1200 before and 1200 after this date. We excluded all non-medical admissions, patients evaluated by a nephrologist for known CKD, ESRD, AKI or nephrolithiasis. We analysed the subgroup of patients with normal Cr (<1.5mg/dl) and a calculated eGFR < 60 ml/min.

**Results:** Most of our patients were elderly female in both groups. We did not notice any significant increase in the number of patients evaluated by or referred to a nephrologist. The prescription of aminoglycosides and NSAIDs did not change significantly. The dose adjustment for antibiotics was in the same range pre- and post-reporting eGFR. We did not notice significant change in the percentage of hydration prior to IV contrast administration.

table 1

| | Pre-reporting GFR | Post-reporting GFR | p-value |
|---|---|---|---|
| Total number | 475 | 680 | |
| CKD stage 3 and normal Creatinine | 132 (27.79%) | 159 (23.38%) | |
| Mean age | 76.05 | 78.2 | |
| Mean GFR (MDRD) | 48.9 | 49.1 | |
| Patients evaluated | 12 (9.09%) | 16 (10.06%) | 0.9362 |
| Renal consult | 5 (3.79%) | 6 (3.77%) | 0.7642 |
| Renal ultrasound | 6 (4.55%) | 9 (5.66%) | 0.8729 |
| Urinalysis | 12 (9.09%) | 16 (10.06%) | 0.9362 |
| Renal referral | 3 (2.27%) | 4 (2.52%) | 0.8057 |
| NSAIDS (in-hospital) | 5 (3.79%) | 12 (7.55%) | 0.2670 |
| NSAIDS (out-hospital) | 2 (1.52%) | 2 (1.26%) | 0.7520 |
| Aminoglycoside | 2 (1.52%) | 5 (3.14%) | 0.6073 |
| Hydration for Radiocontrasts | 2/6 (33.33%) | 12/21 (57.14%) | 0.5714 |
| Antibiotic adjusted | 8/21 (38.1%) | 19/49 (38.78%) | 0.8305 |

**Conclusions:** Reporting eGFR did not translate into an improvement of medical management for the patients with CKD stage 3 and creatinine within the normal reference range. Our results underlie the need to improve the teaching of medical residents and physicians concerning the limitations of creatinine.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO843**

**Searching for Medical Information Online: A Survey of Canadian Nephrologists** Salimah Z. Shariff,[1] Shayna Amrita Devi Bejaimal,[1] Jessica M. Sontrop,[2] Matthew A. Weir,[1] Arthur Iansavichus,[1] Amit X. Garg.[1,2,3] *[1]Department of Medicine, University of Western Ontario, London, ON, Canada; [2]Department of Epidemiology and Biostatistics, University of Western Ontario, London, ON, Canada; [3]Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, ON, Canada.*

**Objectives:** Physicians often search for information to answer questions that arise in patient care. We evaluated how nephrologists use online information sources for this purpose.

**Methods:** From the years 2008 to 2010, we asked a random sample of nephrologists practicing in Canada to complete a survey of their online search practices. We queried respondents on their searching preferences, practices and use of nine online information sources.

**Results:** Respondents (n=115; 75% response rate) comprised both academic (59%) and community-based (41%) nephrologists. The average age of respondents was 48 years having practiced nephrology an average of 15 years. Nephrologists used a variety of online sources for information on patient treatment including UpToDate (92%), PubMed (89%), Google (76%) and Ovid MEDLINE (55%). Community-based nephrologists were more likely to consult UpToDate first (91%), while academic nephrologists were divided between UpToDate (58%) and PubMed (41%). When searching bibliographic resources such as PubMed, 80% of nephrologists scan a maximum of 40 citations (the equivalent of two search pages in PubMed). Searching practices did not differ by age, sex or years of practice.

**Conclusions:** Nephrologists routinely use a variety of online resources to search for information for patient care. These include bibliographic databases, general search engines and specialized medical resources.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO844**

**Hydroxyl Radical Production Is Increased in Combind Exposure of Iron and TNF-α in Human Mesothelial Cells** Rie Kitamura, Masayoshi Nanami, Takanori Nagai, Aritoshi Kida, Yoshinaga Otaki, Yukiko Hasuike, Takahiro Kuragano, Hiroshi Nonoguchi, Takeshi Nakanishi. *Hyogo College of Medicine, Internal Medicine, Division of Kidney and Dialysis, Nishinoiya, Japan.*

The development of PMC damage could be the significant cause of encapsulating peritoneal sclerosis in continuous ambulatory peritoneal dialysis patients. We have previously demonstrated that TNF-α caused the misregulation of iron transport proteins and accelerated the oxidative stress in human umbilical endothelial cells.[1)] In the present study

we investigated the expression of iron import proteins (transferrin receptor; TfR and divalent metal transporter 1; DMT1) and iron export protein (ferroportin 1; FP1), and the production of radicals in human mesothelial cell lines (MeT-5A) in the presence of TNF-α.

**Methods:** MeT-5A cells were exposed to TNF-α (10-50 μM) for 24h. M-RNA levels of these iron transport proteins were quantitied using RT-PCR.Western blot analysis was also used for the expression of these proteins. A salicylate trap methods was used for the determination of hydroxyl radical production during iron challenge (transferrtn(TF: 50μM) or ferrous sulfate($FeSO_4$: 50μM)).

**Results:** In MeT-5A, TNF-α significantly increased the expression of mRNA levels of TfR and DMT1, but decreased that of FP1.Western blot analysis showed the similar effect as the result of RT-PCR. Only the exposure of TNF-α and TF or $FeSO_4$ to these cells significantly increased hydroxyl radical production.

**Conclusion:** In MeT-5A cells, TNF-α affect the iron transport proteins, which might contribute to the increase in iron and accelerate the production of hydroxyl radical.

[1)] Nanami et al. Arterioscler Thromb Vasc Biol. 2005

Disclosure of Financial Relationships: nothing to disclose

## TH-PO845

**A Two-Hit Approach in the Development of an Experimental Peritoneal Sclerosis Model** Anniek Vlijm, Denise Sampimon, Raymond T. Krediet. *Medicine, Division of Nephrology, Academic Medical Center, Amsterdam, Netherlands.*

Background Models of encapsulating peritoneal sclerosis (EPS) are often based on local administration of chemical irritants. Our aim was to develop a clinically relevant "two-hit" model with incorporation of renal failure and exposure to conventional dialysis solutions.

Methods Thirty-six male Wistar rats underwent a catheter implantation and a 70% nephrectomy. They were randomly divided into 3 peritoneal infusion groups. The experimental group was exposed to a 3.86% glucose-based conventional dialysis solution for 8 weeks and then received a second hit of intraperitoneal blood administration. Two weeks later the rats were sacrificed. Two control groups were exposed to the conventional dialysis solution alone or to a buffer without glucose for 8 weeks. All animals underwent a peritoneal function test at the end of the experiment. The number of peritoneal adhesions was counted at autopsy, and omental tissue was obtained for morphometrics.

Results The rats that received blood as a second hit had developed numerous intraperitoneal adhesions as seen in EPS, but without cocoon formation. Microscopically no differences were present in fibrosis scores and vessel counts between the three groups. Also peritoneal function parameters were similar in all groups.

Conclusion The short infusion period could be the reason that we did not find differences between the groups, with the exception of a large amount of intraperitoneal adhesions in the experimental group. Modifications of the described rat model are required to develop a clinically relevant EPS model. Besides renal failure and long-term exposure to bioincompatible peritoneal dialysis solutions, a different second hit or several additional hits could be incorporated in an experimental model of EPS.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO846

**PI3K/Akt Mediate the Epithelial-Mesenchymal Transition (EMT) of Human Peritoneal Mesothelial Cells (HPMCs) during Peritoneal Dialysis In Vitro and In Vivo** Fu-You Liu,[1] Xiang Peng,[1] Youming Peng,[1] Yinghong Liu,[1] Hong Liu,[1] Yashpal S. Kanwar,[2] Lin Sun,[1] Li Xiao.[1] *[1]Department of Nephrology,The Second XiangYa Hospital, Kidney Institute of Central South University, Changsha, Hunan, China; [2]Departments of Pathology and Medicine, FSM, Northwestern University, Chicago, IL.*

To verify the phosphatidyl inositol 3 kinase(PI3K)/Akt regulate the EMT formation of HPMCs during peritoneal dialysis (PD) in vitro and in vivo. We constructed a mice model of PD with mesothelial cells undergo EMT by intraperitoneally injection of 1.5ml of 4.25% peritoneal dialysis solution per day for 30 days (n=18), and EMT HPMCs cell model with 5ng/ml TGF-beta1 treatment as well. The levels of Akt phosphorylation, total Akt and EMT related protein Zo-1, Vimentin were assessed by real-time PCR, Western blot, cell Immunofluorescence. A obvious up-regulation of Akt phosphorylation was seen, which was positively associated with Morphological changes of EMT and its related gene expression in this model compared to control (0.9% NS intraperitoneally injection per day for 30 days, n=18). A similar result was seen in TGF-beta1 induced HPMCs EMT cell model. However, pre-treatment of LY294002 (PI3K/Akt inhibitor) or domain-negative Akt plasmid, decreased Akt phosphorylation and reversed the EMT of HPMCs induced by TGF-beta. Furthermore, the Smad3, phosphorylated-Smad2/3 and smuf2 expressions were up-regulated significantly after stimulated by TGF-beta1, while, these effects was also dramatically inhibited with pre-treatment of LY294002 or domain-negtive Akt plasmid as detected by Western blot assess and cell confocal image. These data suggested that PI3K/Akt plays a crucial role in EMT transformation of HPMCs during PD, which partially through cross-talk with TGF-beta1/Smad pathway and Smurf2, a SMAD specific E3 ubiquitin protein ligase.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO847

**Therapeutic Effects of Factor Xa Inhibitor and Saireito on Rat Peritoneal Fibrosis** Akihisa Sugimoto,[1] Takahiko Ono,[2] Kozue Kato,[1] Tatsuya Morimoto.[1] *[1]Division of Molecular Medicine, University of Shizuoka School of Pharmaceutical Sciences, Shizuoka, Japan; [2]Department of Nephrology, Shimada Municipal Hospital, Shimada, Japan.*

Fibrin deposition and angiogenesis in the peritoneum were frequently observed in peritoneal fibrosis induced with long-term peritoneal dialysis. We previously reported that factor Xa inhibitor fondaparinux ameliorated rat peritoneal fibrosis through regulation of angiogenesis (Perit Dial Int 29: 340-351, 2009). In another study, herbal Kampo prescription saireito ameliorated extracellular matrix accumulation in experimental rat glomerulonephritis, partly through anti-oxidative effect. Therefore, we conducted an experimental study to examine the suppressive effects of combination therapy of a factor Xa inhibitor with saireito in rat peritoneal fibrosis. Vehicle of Wistar rats (n = 8) were intraperitoneally injected with chlorhexidine gluconate (CG) everyday. Treatment groups were the fondaparinux group (subcutaneous administration of 0.025 mg/kg/day fondaparinux everyday, n = 8), saireito group (oral 500 mg/kg/day everyday in drinking water, n = 8), and both combination group (n = 8). Twenty-eight days after the injection, the rats were sacrificed and peritoneal specimens were examined by immunohistochemical analyses. Treatments with fondaparinux alone, saireito alone, and combination of them significantly suppressed the increase in macrophage infiltration by 38% ($p < 0.01$), 59% ($p < 0.05$), and 24% ($p < 0.01$) of vehicle group, respectively. Furthermore, these treatments significantly suppressed the increase in CD31-positive vessels number by 64% ($p < 0.001$), 59% ($p < 0.001$), and 42% ($p < 0.001$) of vehicle group, respectively. Although vascular endothelial growth factor (VEGF) mRNA expression was not sufficiently reduced by fondaparinux alone or saireito alone, the combination therapy suppressed the expression significantly ($p < 0.05$). Accordingly, synergistic effect was observed in the combination therapy. Present data suggest that the factor Xa inhibitor and saireito may induce suppressive effects on peritoneal fibrosis in different manners, respectively. Combination of these treatments may effectively regulate peritoneal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO848

**Physiological Levels of Endostatin, a Fragment of Collagen XVIII α1 Chain, Attenuate Peritoneal Sclerosis in Mice** Ryota Shirai,[1] Yuki Tokui,[1] Ryota Kimura,[1] Osamu Yokosuka,[2] Makoto Ogawa,[3] Raghu Kalluri,[4] Yuki Hamano.[3] *[1]Drug Information and Communication, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan; [2]Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University, Chiba, Japan; [3]Nephrology, Chiba University Hospital, Chiba, Japan; [4]Matrix Biology, Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.*

**Objective:** Disruption of angiogenesis balance to favor enhanced angiogenesis represents a key step in a number of pathological disorders. Although an emphasis has been placed on the increase of angiogenesis stimulators on the disruption of the balance, the potential role of the physiological levels of endogenous inhibitors of angiogenesis is poorly understood. Peritoneal sclerosis is a major complication of peritoneal dialysis therapy and the progression is related to enhanced angiogenesis. To address the role of endogenous angiogenesis inhibitors in peritoneal sclerosis, the two independent models were induced in mice deficient in endostatin, an endogenous angiogenesis inhibitor.

**Methods:** Peritoneal sclerosis was induced by intraperitoneal injection of either chlorhexidine gluconate (CG) for 2 weeks or 4.25% glucose peritoneal dialysis fluid (PDF) for 2 months. The development of new blood vessels and fibrosis in parietal peritoneum was compared between endostain-deficient (KO) and wild-type (WT) mice.

**Results:** Upon induction of peritoneal sclerosis in WT mice, endogenous endostatin expression was clearly upregulated within the blood vessels. Intraperitoneal injection of either CG or PDF into KO mice compared with WT mice resulted in significant thickening of the submesothelial area, increase in the number of blood vessels and accumulation of extracellular matrices in the peritoneum. Consistent with these results, the expression of profibrotic TGF-β1 and thrombospondin (TSP)-1, an endogenous activator of latent TGF-β and an endogenous angiogenesis inhibitor, was upregulated in the peritoneum of KO mice compared with WT mice.

**Conclusion:** Physiological levels of endostatin, a fragment of collagen XVIII, can serve as a suppressor of angiogenesis and fibrosis in peritoneal sclerosis.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO849

**(-)-Epigallocatechin Gallate Suppresses the Progression of Peritoneal Fibrosis in Methylglyoxal Induced Peritoneal Fibrosis Model in Mice** Mineaki Kitamura,[1] Akira Furusu,[1] Yoko Obata,[1] Tomoya Nishino,[1,2] Yoshitaka Hishikawa,[2] Takehiko Koji,[2] Shigeru Kohno.[1] *[1]Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan; [2]Department of Histology and Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.*

**Introduction** Angiogenesis and inflammation caused by the glucose degradation products (GDPs) play a role in peritoneal fibrosis on peritoneal dialysis (PD). Methylglyoxal (MGO) is one of GDPs and enhances the formation of advanced glycation end products (AGEs). AGEs bind to the receptor for AGEs (RAGE) and activate nuclear factor-kB (NF-kB) which regulates angiogenesis and inflammation. Recent studies indicated

that (-)-Epigallocatechin gallate (EGCG), a tea polyphenol, has an inhibitory effect on angiogenesis and inflammation. However, its effect on peritoneal fibrosis remains unknown. In the present study, we examined whether EGCG suppressed the progression of peritoneal fibrosis of mice.

**Methods** C57/BL6 mice were divided into three groups, MGO, MGO + EGCG, and PD group. PD fluid with or without MGO were injected intraperitoneally and EGCG was injected subcutaneously for 3 weeks. Morphologic peritoneal changes were assessed by Masson-Trichrome staining. The expression of CD31, vascular endothelial cell growth factor (VEGF), monocyte chemotactic protein-1 (MCP-1), F4/80 and RAGE were examined by immunohistochemistry. Southwestern histochemistry was used for the detection of activated NF-kB.

**Results** In MGO group, peritoneal tissues showed marked thickening of the submesothelial zone, and the numbers of VEGF, MCP-1, RAGE expressing cells, F4/80 positive macrophages and CD31 positive vessels were significantly increased compared to PD group. The expression level of NF-kB was increased as well. On the contrary, these changes were significantly suppressed in MGO + EGCG group.

**Discussion** These results suggested that EGCG could attenuate peritoneal fibrosis through the suppression of angiogenesis and inflammation.

**Conclusion** We conclude that EGCG might be one of the candidates for a novel therapeutic agent in preventing peritoneal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO850

**The Role of 3,4-dideoxyglucosone-3-ene in Peritoneal Fibrosis** Hideki Yokoi,[1] Masato Kasahara,[1] Kiyoshi Mori,[1] Takashige Kuwabara,[1] Ryo Yamada,[2] Shinji Namoto,[2] Takashi Yamamoto,[2] Nana Seki,[2] Taku Yamaguchi,[2] Nozomi Souma,[2] Akira Sugawara,[1] Masashi Mukoyama,[1] Kazuwa Nakao.[1] *[1]Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]JMS Co., Ltd., Hiroshima, Japan.*

Peritoneal dialysis solution contains high concentrations of glucose and glucose degradation products (GDPs). Several GDPs have been identified including glyoxal, methylglyoxal, formaldehyde, 3-deoxyglucosone (3-DG), 3,4-dideoxyglucosone-3-ene (3,4-DGE) and 5-hydroxymethyl furaldehyde (5-HMF). 3,4-DGE is recently identified as the most reactive and toxic GDP in peritoneal dialysis (PD) fluids. 3,4-DGE has been shown to induce mesothelial cell death and lose cell viability *in vitro*. However, the role of 3,4-DGE in peritoneal fibrosis *in vivo* was unclear. In this study, we administered 0.1% chlorhexidine gluconate (CG) three times a week for peritoneal injury in male C57BL/6J mice. Control mice received PBS. After peritoneal injury, 145 μM 3,4-DGE dissolved in PBS was given intraperitoneally five times a week for 4 weeks. Control mice were given PBS without 3,4-DGE. There were four groups; initial PBS and following PBS without 3,4-DGE (PBS+3,4-DGE(-)), initial PBS plus following 3,4-DGE in PBS (PBS+3,4-DGE(+)), initial CG plus PBS without 3,4-DGE(CG+3,4-DGE(-)), and CG plus 3,4-DGE in PBS (CG+3,4-DGE(+)). The thickness of peritoneal membrane was significantly increased in CG+3,4-DGE(+) group. TGF-β1, CTGF, fibronectin, COL1A1, VEGF and α-SMA mRNA expressions were upregulated, suggesting that 3,4-DGE is related to peritoneal fibrosis. NADPH oxidase NOX4 was also increased in CG+3,4-DGE(+) mice. We next identified increased apoptotic cells in submesothelial compact zone. Immunohistochemical study showed that type I collagen, fibronectin and α-SMA staining was increased in CG+3,4-DGE(+) mice and that macrophage infiltration was augmented by the analysis of F4/80 immunostaining. These results indicate that 3,4-DGE can increase peritoneal injury when peritoneal membrane was already damaged, by augmenting macrophage infiltration and extracellular matrix deposition.

Disclosure of Financial Relationships: nothing to disclose

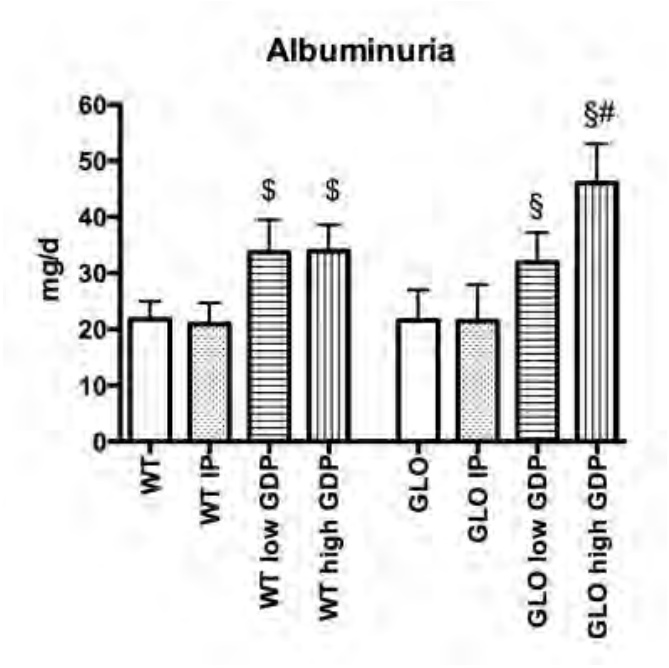
## TH-PO851

**Pronounced Kidney Damage in a Peritoneal Dialysis Model of Glyoxalase I Deficient Mice** Lars Kihm, Sandra Muller-Krebs, Svenja Horlacher, Martin G. Zeier, Vedat Schwenger. *Nephrology, University of Heidelberg, Germany.*

**Background**: Morbidity and mortality of peritoneal dialysis (PD) patients are significantly influenced by residual renal function. Glucose degradation products (GDP) were suggested as contributing factors for deterioration of residual renal function in PD patients. Glyoxalase I plays a critical role in the detoxification of GDP and may be protective in PD.

**Methods:** We compared the effects of peritoneal dialysis fluid (PDF) with different amounts of GDP on morphological changes of the kidney in 48 wild type (WT) and 48 Glyoxalase I -deficient (GLO-) mice. PDF (1 ml) were instilled twice daily over a period of 12 weeks. Groups with 12 animals each received either no manipulation (sham), sham instillation (sham i.p.), and high and low GDP-containing PDF. After 12 weeks albuminuria was measured and kidney damage was quantitated by morphometric analysis and immunohistochemical stainings using an established score was evaluated.

**Results:** After a long-term exposure to GDP-containing PDF Glyoxalase I deficient mice compared to WT mice showed a marked increase of markers of fibrosis (i.e., peritoneal expression of TGFβ1: WT plus high GDP 6.11±0.26 vs. GLO- plus high GDP 7.43±0.40; $p<0.01$) and inflammation (i.e., CD3+ T-cells, IL6 expression). A GDP-dependent increase of renal AGE formation occurred in WT and glyoxalase I deficient mice compared to WT mice when treated with PDF Glyoxalase I deficient mice revealed a significantly increased expression of AGE and the receptor for AGE (i.e. glomerular expression of AGE: WT plus high GDP 3.12±0.35 vs. GLO- plus high GDP 4.53±0.41 $p<0.001$). Albuminuria was highest in Glyoxalase I deficient mice treated with PDF containing high dose GDP.




**Conclusions:** Our findings suggest a pivotal role for Glyoxalase I in GDP associated systemic toxicity in a peritoneal dialysis model in mice.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO852

**MicroRNA Expression Pattern in Human Peritoneal Mesothelial Cells (HPMCs) of CAPD Patients, and Effect of Mir-129-5p and Mir-589 in TGF-beta1 Induced Epithelial-Mesenchymal Transition of HPMCs** Li Xiao,[1] Ke Zhang,[1] Xun Zhou,[1] Lin Sun,[1,2] Guanghui Ling,[1] Youming Peng,[1] Yinghong Liu,[1] Yashpal S. Kanwar,[2] Fu-You Liu.[1] *[1]Department of Nephrology, Second Xiangya Hospital of Central South University, Changsha, China; [2]Department of Pathology and Medicine, Northwestern University Medical School, Chicago.*

We investigated the expression pattern of microRNAs(miRNAs)in human peritoneal mesothelial cells (HPMCs) derived from CAPD patients, and in particular, the role of Mir-129-5p and Mir-589 in the epithelial-mesenchymal transition (EMT) induced by TGF-beta1. Total miRNA was isolated from HPMCs of dialysate effluents of CAPD patients and subjected to miRNA profiling using miRNA array. Compared to baseline (CAPD at 0 day, N=20), expression of 33 microRNA was up-regulated (CAPD at 1 year, N=20); while that of 58 microRNA expression was down-regulated. Similar results were observed in human mesothelial cells treated with TGF- beta1. Among various microRNAs, miR-129-5p and miR-589 expression decreased 2-3 folds as measured by QPCR. The expression of E-cadherin, Claudin1, Vimentin, ZO-1 and FN was assessed by QPCR, Western blot, and Immunofluorescence and Confocal microscopy. Decreased expression of miR-129-5p and mi-R589 correlated with EMT phenotypic changes. The binding of miR-129-5p and miR-589 to 3'UTR regions of transcription factor SIP1 and ets-1 lead to the positive/negative regulation of the genes conducive for the process of EMT. In in vitro experiments, upregulation of SIP1 and ets1 correlated with the expression of EMT related genes in cells treated with TGF-beta1. The expression of these transcription factors was reversed by the pre-treatment of precursor miR-129-5p or mir-589, and this associated with the reversal of EMT. These data suggest that miR-129-5p or mir-589 modulate the EMT partly via post-transcriptional repression of SIP1 and ets1, the latter could serve as novel targets to ameliorate EMT-fibrosis of the peritoneal wall in PD patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO853

**Target Related Therapy in Encapsulating Peritoneal Sclerosis – Nuclear Receptors and the Role of Tamoxifen** Niko Braun,[1] Peter Fritz,[2] Martin Kimmel,[1] Christoph Ulmer,[4] Dagmar Biegger,[3] Mark Dominik Alscher.[1] *[1]Division of Internal Medicine and Nephrology, Department of Internal Medicine, Robert-Bosch Hospital, Stuttgart, Germany; [2]Division of Pathology, Department of Diagnostic Medicine, Robert-Bosch Hospital, Stuttgart, Germany; [3]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmcology, University of Tubingen, Stuttgart, Germany; [4]Department of Surgery, Robert-Bosch Hospital, Stuttgart, Germany.*

Objective: Encapsulating peritoneal sclerosis (EPS) is a rare but life threatening complication of peritoneal dialysis (PD). The optimal management is not clear. Aim of this study was to look for potential targets for medical therapy. We investigated the expression of progesterone- (PR), androgen- (AR), vitamin D- (VDR) and glucocorticoid-receptor

(GCR) in the human peritoneum. Estrogen-receptor (ER), matrixmetalloproteinase 9 (MMP9) and transforming growth factor-ß1 (TGF-ß1) were investigated in the context of a potential target for therapy with tamoxifen.

Methods: 72 peritoneal biopsies were investigated, including 22 EPS patients, 11 PD patients, 15 uremic patients and 24 controls (hernia repair). Histologic characteristics were investigated and an EPS-score was evaluated. For immunophenotyping we used antibodies raised against VDR, GCR, ER, PR, AR, MMP9 and TGF-ß1.

Results: EPS-score > 6 was highly indicative for the diagnosis EPS. In contrast to ER, PR and AR, VDR and GCR are common in all investigated cells of the peritoneum (98.6% and 87.3%). The distribution of the nuclear receptors is not specific for one group. There is no overexpression of MMP9 and TGF-ß1 in PD or EPS.

Conclusion: We found possible targets in the peritoneum of patients with uremia, EPS or on PD. Glucocorticoids, vitamin D, ACE-inhibitors or ARBs could be suitable therapies. Clinical evidence for the widespread use of tamoxifen is lacking. The mechanism of action is cloudy, receptors in the peritoneum are lacking and data about the discussed pathways (MMP9, TGF-ß) are inconsistent.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO854

**Paricalcitol Induces VDR Expression and Reduces Glucose Profibrotic Effect in Human Mesothelial Cells** Vittoria Esposito, Massimo Torreggiani, Fabrizio Grosjean, Clara Migotto, Giuseppe Sileno, Noemi Maggi, Nicoletta Serpieri, Ciro Esposito, Antonio Dal Canton. *Nephrology, IRCCS San Matteo, Univ. of Pavia, Pavia, Italy.*

Glucose induces peritoneal membrane denudation with loss of mesothelial cells (MC), thickening and sclerosis probably through an excessive production of TGF-b by MC. Paricalcitol (PCT), binding to VDR receptor, can act as antifibrotic agent reducing TGF-b1 expression. We evaluated the effect of PCT on human peritoneal mesothelial cell (HPMC) cultures exposed to glucose. HPMC were extracted from peritoneal effluent, cultured and morphologically characterized. VDR expression was confirmed by RT- PCR, Western blot and immunofluorescence. Cells were treated with: A) glucose 2.3%; B) glucose 2.3% + PCT 0.1 uM; C) glucose 4.25%; D) glucose 4.25% + PCT 0.1 uM; E) mannitol 4.25%; F) mannitol 4.25% + PCT 0.1 uM; G) PCT 0.1 uM. Cells cultured in medium containing FCS 1% were used as controls (H). High glucose induced a significant detachment of cells ($2.1x10^5 \pm 8.1 \times 10^4$ vs $0.4 \times 10^5 \pm 1.6 \times 10^4$ vs $0.6 \times 10^5 \pm 2.5 \times 10^4$ vs $0.6 \times 10^5 \pm 1.6 \times 10^4$ groups C, A, E, H, respectively, $p<0.05$) and this result was not influenced by PCT treatment ($2.5 \times 10^5 \pm 1.1 \times 10^4$ vs $6.6 \times 10^5 \pm 4.2 \times 10^4$ vs $0.8 \times 10^5 \pm 3.2 \times 10^4$ vs $0.7 \times 10^5 \pm 3.0 \times 10^4$ D, B, F, G, respectively). HPMC expressed VDR and paricalcitol increased its expression. Nonetheless, the highest concentration of glucose was able to suppress VDR expression and this effect was not counteracted by PCT. Metalloproteinase 2 (MMP2) activity, measured by zymography , was reduced by glucose in a dose-dependent manner but was not affected by mannitol; this effect was partially prevented by PCT. TGF-b expression was increased by glucose, independently of the dose, and by mannitol. PCT reduced TGF-b1 expression only in the highest glucose concentration group ($0.285 \pm 0.053$ vs $0.217 \pm 0.085$, C and D respectively, NS). In conclusion HPMC express VDR. VDR is increased by paricalcitol and reduced by high glucose. Moreover, PCT blunts the suppression of MMP2 induced by glucose and prevents TGF-b1 increase. Paricalcitol maintains an adequate ECM turnover and prevents peritoneal dialysis associated sclerotic changes.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO855

**Pathological Diagnosis and Staging of Encapsulating Peritoneal Sclerosis (EPS) in PD Patients** Kazuho Honda,[1] Chieko Hamada,[2] Masaaki Nakayama,[3] Masanobu Miyazaki,[4] Takashi Harada,[5] Hideaki Oda.[1] *[1]Pathology, Tokyo Women's Medical University, Tokyo, Japan; [2]Nephrology, Juntendo Medical University, Tokyo, Japan; [3]Dyalisis and Chronic Kidney Disease, Tohoku University Graduate School of Medicine, Sendai, Japan; [4]Miyazaki Clinic, Nagasaki, Japan; [5]Sakuramachi Hospital, Nagasaki, Japan.*

A unique pathological hallmark of encapsulating peritoneal sclerosis (EPS) is a newly-formed membrane on visceral peritoneum which leads to encapsulation of entire abdominal organs. Using the peritoneal biopsy materials obtained at dissecting surgery or catheter removal, we confirmed the presence of encapsulating membrane histologically in 23 cases of PD patients (EPS group, n=23). The encapsulating membrane showed various phases of the pathological consequence, from an early exudative change of fibrin deposition, leading to fibroblast proliferation and fibrosis, and finally resulting in adhesion and scar formation on the peritoneal membrane. Immunostaining of fibrin and podoplanin was useful to evaluate the stage of encapsulating membrane. Fibrin was detected in an early exudative phase (stage I, n=6), whereas podoplanin was detected in the proliferating interstitial cells in stage I and subsequent proliferative phase (stage II, n=9). Neither fibrin nor podoplanin was detected in the late fibrotic phase (stage III, n=8). The extents of peritoneal sclerosis were evaluated by our reported method (Clin J Am Soc Nephrol. 2008; 3:720-8) and compared with those of the duration-matched PD peritoneum without EPS or peritonitis (control group, n=41). The average thickness of submesothelial compact zone was increased in EPS group than in control group ($645.5 \pm 358.8$ vs. $377.7 \pm 164.6$, $p=0.0016$). The lumen/ vessel ratio of post-capillary venule was significantly lower in EPS group than in control group ($0.096 \pm 0.18$ vs. $0.39 \pm 0.29$, $p<0.0001$). These results suggest that (1) the pathological diagnosis of EPS requires confirming a newly-formed encapsulation membrane, (2) fibrin deposition and podoplanin expression of peritoneal interstitial cells are key phenomena to evaluate the stage of EPS, and (3) advanced peritoneal sclerosis is associated with the pathogenesis of EPS.

Disclosure of Financial Relationships: nothing to disclose

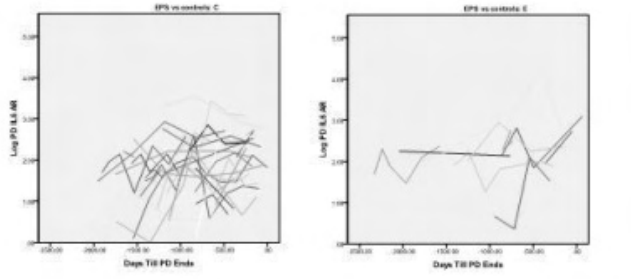
## TH-PO856

**IL-6 in Peritoneal Dialysate Prior to Encapsulating Peritoneal Sclerosis: Results from the GLOBAL Fluid Study** Mark Lambie,[1] James A. Chess,[2] Simon J. Davies,[1] Nicholas Topley.[2] *[1]Keele University, United Kingdom; [2]Cardiff University, United Kingdom.*

Encapsulating peritoneal sclerosis (EPS) is an inflammatory and fibrotic complication of peritoneal dialysis with significant mortality. Recent evidence suggests changes in the peritoneal membrane several years before EPS so identification of these changes may allow EPS avoidance. One study with limited numbers found a tendency for IL-6 to be elevated in subsequent EPS cases. We sought to confirm this in a larger study.

We identified 13 definite EPS cases from the Global Fluid Study, a prospective, multicentre, international study of incident and prevalent PD patients collecting repeat dialysate and plasma samples from routine PET's every 6 months. The cases were matched on PD duration, age, gender, centre, number of samples and diabetic status with 37 controls who had finished PD, and all samples were analysed by ECL (MSD Pro-inflammatory 4plex) for IFN-γ, TNF-α, IL-1β and IL-6. The results were analysed by linear mixed models built iteratively, adding fixed effects then random effects, using AIC and BIC for model assessment.

No differences between groups in matching criteria were found. Spaghetti plots for dialysate IL-6 appearance rates (AR) suggested a small difference in levels between groups.



A linear mixed model for dialysate IL-6 AR's found EPS status to be a significant association, p=.039, with estimates of 267 and 131 pg/min for cases and controls when stopping PD with an average D/P Cr. UF capacity and gender did not improve the models, but IL-6 AR's increased the closer the sample was taken to the end of PD, p=.038. Other cytokines were not significantly associated with EPS.

These results suggest that peritoneal inflammation, represented by dialysate IL-6, is a necessary precursor to EPS.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO857

**Early Changes in Local and Systemic Inflammation in Peritoneal Dialysis: Results from the GLOBAL Study** Mark Lambie,[1] Nicholas Topley,[2] Simon J. Davies.[1] *[1]Keele University; [2]Caridff University, .*

Solute transport increases in the first month of peritoneal dialysis, and after 3 years but between then there is inconsistency. Solute transport is consistently associated with dialysate IL-6 levels, linked with inflammation. We hypothesised there is local inflammation in the first month followed by a suppression over the next 5 months. We undertook an analysis of the Global Fluid Study for supportive evidence of this. Samples were taken every 6 months, so cross-sectional rather than longitudinal analysis was used.

697 patients with samples in the first 6 months from 10 centres in the UK, Canada and Korea had plasma and 4-hour dialysate samples assayed by ECL using a commercial kit (MSD Pro-Inflammatory Multiplex I).

Loess regression of cytokines with time on PD suggests D/P Cr and plasma IL-6, TNF-α and IFN-γ rose during the first month on PD, falling over the following 2 months, whilst dialysate IL-6, IL-1β and TNF-α rose over the first 3 months and fell over the next 3 months. Bivariate correlations for duration of PD with cytokines are shown in the table.

| Weeks of PD | | IL-6 | TNF-α | IFN-γ | IL-1β | D/P Cr |
|---|---|---|---|---|---|---|
| | | Dialysate | Dialysate | Dialysate | Dialysate | |
| 0 to 12 | Spearman's rho | 0.180 | 0.095 | | 0.100 | |
| | p value | <0.001 | 0.024 | NS | 0.016 | |
| 12 to 24 | Spearman's rho | -0.280 | -0.324 | | -0.265 | |
| | p value | 0.003 | 0.001 | NS | 0.006 | |
| | | Plasma | Plasma | Plasma | Plasma | |
| 0 to 4 | Spearman's rho | 0.242 | 0.225 | 0.122 | | 0.220 |
| | p value | <0.001 | <0.001 | 0.035 | NS | <0.001 |
| 4 to 12 | Spearman's rho | -0.145 | -0.120 | | | -0.130 |
| | p value | 0.016 | 0.042 | NS | NS | 0.028 |

Dialysate IL-6 was present at concentrations a median of 2.91 times higher than plasma (IQR 1.05-7.05), suggesting diffusion of IL-6 from local to systemic occurs.

These results, subject to confirmation in a longitudinal study, suggest that starting PD is associated with an increase in local inflammation over 3 months, with a subsequent increase in systemic inflammation and solute transport, but this effect is suppressed after 4 weeks.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO858**

**Systemic Endothelial Albumin Leak Is Associated with Markers of Platelet Activation Independent of Systemic Inflammation in Peritoneal Dialysis Patients** Zanzhe Yu, Boon Kay Tan, Derek L. Mattey, Simon J. Davies. *University Hospital of North Staffordshire, Stoke-on-Trent, United Kingdom.*

Background: Endothelial dysfunction is a feature of renal failure as evidenced by an increased transcapillary albumin escape rate (TERalb). Our purpose was to explore associations between TERalb, systemic inflammation and markers of endothelial injury.

Methods: 41 prevalent PD patients were studied. TERalb was measured from the disappearance rate of radiolabelled albumin. 17 plasma biomarkers, including pro-inflammatory cytokines, endothelial biomarkers and MMPs, were measured by Luminex suspension array multiplex assays. Hierarchical clustering (HCA) and principle component analysis (PCA) were used to identify patient groups with common patterns which were then compared for TERalb and clinical characteristics.

Results: The mean TERalb was 13.7±8.9(%/h), higher than in normal populations (typically 5%). HCA identified three patient groups according to their biomarker results that converged with 3 of 7 distinct factors identified through PCA. (see table) Group 1 was most inflamed, had intermediate TERalb and high Factor 1 (TNF-α, VCAM-1, IFN-ϒ, IL-6, inversely with MMP-3) and Factor 3 (MMP-8, MMP-9 and E-selectin). Group 2 was non-inflamed and had opposite associations to Group 1 with Factors 1 and 3. Group 3 had the highest TERalb, was less inflamed but had the highest Factor 5 (markers of platelet activation, CD40L, P-selectin, MMP-1 and inversely with ICAM-1).

Conclusions: Endothelial barrier function is decreased in PD patients. This is not associated solely with systemic inflammation, but is also seen in relatively non-inflamed patients with markers of platelet activation.

| | Group1(n=14) | Group 2(n=9) | Group3(n=17) |
|---|---|---|---|
| TERalb (%/h) | 13.88±9.23 | 9.12±5.29a | 17.29±9.11 |
| CRP(mg/L) | 14.3(4.8-21)b | 1.5(0.6-4) | 4.6(0.8-9.5) |
| Factor 1 | 0.60±0.95 | -1.00±1.02c | 0.09±0.71 |
| Factor 3 | 0.83±0.92b | -0.33±0.69 | -0.10±1.23 |
| Factor 5 | -0.43±0.86 | -0.39±0.74 | 0.97±0.98d |

a, Group 2 vs 3, P<0.05 b, Group 1 vs 2, P<0.01; Group 1 vs 3, P<0.05 c, Group 2 vs 1 and 3, P<0.01 d, Group 3 vs 1 and 2, P<0.01

Disclosure of Financial Relationships: nothing to disclose

**TH-PO859**

**Inflammation Limits the Beneficial Response to Nutritional Supplements in Continuous Ambulatory Peritoneal Dialysis** Alma R. J. Romero,[1] Fabiola Martin del Campo,[1] Laura Cortes Sanabria,[1] Leonardo Pazarin,[2] Alfonso M. Cueto-Manzano.[1] *[1]UIMER, Hospital Especialidades, IMSS, Guadalajara, Jalisco, Mexico; [2]HGR 46, IMSS, Guadalajara, Jalisco, Mexico.*

Inflammation may limit the beneficial effect of nutritional supplements on CAPD patients.

Aim: to compare the effect of systemic inflammation on nutritional response of CAPD patients receiving an oral egg albumin-based supplement.

Twenty-one malnourished patients were randomly selected. All patients received nutritional counselling and oral egg albumin-based supplement during 6 months. Patients were classified according to baseline serum C-reactive protein: with inflammation (≥5 mg/L) and without inflammation (<5 mg/L). All patients had monthly clinical and biochemical evaluations, and dialysis adequacy and nutritional evaluations every 3 months.

Main results are shown in the table.

| Variable | With inflammation (N 6) | | Without inflammation (N 15) | |
|---|---|---|---|---|
| | Baseline | Final | Baseline | Final |
| Time on dialysis (months) | 16 (10-62) | | 12 (4-23) | |
| Previous peritonitis (N) | 0 (0 – 5.7) | | 0 (0 – 1) | |
| Serum albumin (g/dL) | 2.9 ± 0.7 | 2.8 ± 0.8 | 3.1 ± 0.5 | 3.3 ± 0.9 |
| RRF (ml/min) | 0.46 (0.1-2.1) | 0.34 (0-1.4) | 0.86 (0.3-1.8) | 0.21 (0-1.6) |
| Energy intake (Kcal/kg) | 18.3 (14-26) | 25 (15-34) | 24.4 (21-28) | 27.5 (21-44) |
| Protein intake (g/Kg) | 0.81 (0.7-1) | 1.24 (0.6-1.6) | 1.1 (0.8-1.3) | 1.38 (0.7-2.1) |
| Subscapular skinfold (mm) | 16.0 (13-22) | 14.5 (9-19) | 12.5 (8-19) | 12.5 (8-17) |
| Midarm muscle area (cm²) | 22.8 (17-29) | 23.2 (16-33) | 24.9 (21-28) | 28.8 (24-34) |
| Nutritional status (%) | | | | |
| Normal | 0% | 33% | 0% | 33% |
| Mild malnutrition | 50% | 17% | 67% | 67% |
| Moderate/severe malnutrition | 50% | 50% | 34% | 0%£ |

£p<0.05 vs same evaluation in patients with inflammation; RRF: residual renal function

Conclusion: A higher systemic inflammation tended to be associated with a worse baseline nutritional status in CAPD patients, and limited the positive effect on anthropometric variables and subjective global assessment compared with patients without inflammation, even though all patients were receiving oral nutritional supplements.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO860**

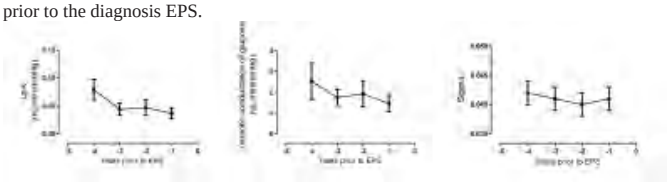
**Longitudinal Analysis of Fluid Transport and Their Determinants in PD Patients Who Develop Encapsulating Peritoneal Sclerosis** Denise Sampimon, Raymond T. Krediet. *Medecine - Division Nephrology, Academic Medical Center, Amsterdam, Netherlands.*

**Background:** Encapsulating peritoneal sclerosis (EPS) is a severe complication of long-term peritoneal dialysis (PD). Almost all patients with EPS have severe decreased fluid transport (ultrafiltration failure). Fluid transport is determined by several factors such as the ultrafiltration coefficient (LpA) and the glucose induced osmotic conductance (GOC). The latter is the product of LpA and the reflection coefficient (sigma). Decreased GOC is an important cause of ultrafiltration failure in long-term PD patients. It remains unclear whether a decreased GOC causes ultrafiltration failure in patients who develop EPS.

The **aim** of this study was to longitudinally analyze the determinants of fluid transport during the time course of the development of EPS.

**Methods:** Twelve PD patients who developed EPS were included for this study. The last 4 peritoneal function tests available were analyzed. The peritoneal function was assessed annually with 3.86% glucose dwell/4 hours with the addition of a volume marker. Sigma was estimated from pore size modeling and LpA from Starlings equation. The determinants of fluid transport were analyzed with a linear mixed model test.

**Results:** During the last 4 years prior to EPS the net ultrafiltration showed a linear decrease. This trend was determined by both a decrease in LpA and a decrease in GOC. Sigma showed an initial decrease however a slight increase was present in the last year prior to the diagnosis EPS.



**Conclusion:** A decrease of fluid transport prior to the development of EPS was present. The increase of sigma one year prior to EPS may be due to thickening of the interstitium or interstitial fibrosis. A follow-up study should compare these results with long-term PD patients with ultrafiltration failure.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO861**

**Neutral Solution Suppresses Peritoneal Sclerosis but Promotes Angiogenesis in PD Peritoneum** Kunio Kawanishi,[1] Kazuho Honda,[2] Misao Tsukada,[1] Kosaku Nitta.[1] *[1]Department of Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan; [2]Department of Pathology, Tokyo Women's Medical University.*

Objective: The present study was performed to explore the effects of neutral peritoneal dialysis (PD) solutions on peritoneal pathology in comparison with acidic, especially on the extent of peritoneal sclerosis and angiogenesis. Methods: Twenty-four patients who had been treated with either acidic solution (n=17) or neutral solution (n=7) were enrolled and each patient was undertaken the parietal peritoneal biopsy at the time of PD catheter removal. We evaluated the extent of peritoneal sclerosis by the previously reported method (CJSN.2008;3:720-8);measuring the thickness of submesothelial compact zone (SMC) and the ratio of lumen/vessel diameter (L/V ratio) of post-capillary venule at 5 different points, respectively. To evaluate the extent of angiogenesis, we underwent CD31 immunostaining and counted the number of blood vessels per unit area(mm²) in the 5 randomly-selected fields (x200) with an image analyzer. The blood vessels were classified by their short axis diameter into the 3 levels; <15μm:capillary (CAP), 15-50μm:post-capillary venule (PCV), >50μm:small artery and vein (SAV).Results: The age (acidic:46.4±4.8 vs. neutral:42.5±7.6) and PD duration (acidic:65.4±5.7 vs. neutral:53.6±8.9 months) were not different. The average thickness of SMC (μm) was lower in the neutral group (292.5±73.7) than acidic (496.1±40.9) (p=0.029). In addition, the average L/V ratio was significantly higher in the neutral group (0.86±0.06) than acidic(0.44±0.04) (p<0.0001). On the other hand, the number of CAP(count/mm²) was significantly higher in the neutral group (107.8±11.8) than acidic (45.9±7.6) (p=0.0002). The number of PCV tended to be higher in the neutral group but not significantly different (10.9±2.1 vs. 6.2±1.4, p=0.0735). The number of SAV was not different(neutral:1.1±0.7 vs. acidic:1.3±0.5, p=0.8276).Conclusions: Neutral solution suppresses the extent of peritoneal sclerosis but promotes capillary angiogenesis in PD peritoneum, suggesting the altered physiological effect by neutral solution can modify the peritoneal morphology and function in the recent PD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO862**

**The Effect of Peritoneal Dialysis Prescription in Peritoneal Methothelial Cells** Hideki Yamahara,[1] Takanobu Imada,[1] Sanae Kikuchi,[1] Hiroya Masaki,[2] Mitsushige Nishikawa,[1] Toshiji Iwasaka.[1] *[1]Department of Medicine II, Kansai Medical University, Moriguchi, Osaka, Japan; [2]Department of Laboratory Medicine and Clinical Sciences, Kansai Medical University, Moriguchi, Osaka, Japan.*

**Background:** Continuous ambulatory peritoneal dialysis (CAPD) is an important renal replacement therapy. However, chronic exposure to peritoneal dialysate causes progressive mesothelial cell injury. Recent studies report that an increase in the mesothelial cell surface area indicates degradation of peritoneal membrane. Furthermore, these studies report that within 72 months PD period patients with more than 350 μm² of mean surface area of peritoneal mesothlial cell have risk of sclerosing encapsulating peritonitis (SEP). **Aim:** We investigated the effect of peritoneal dialysis prescription in the surface area of peritoneal mesothelial cell in the CAPD effluent. **Method:** Total of 16 CAPD patients was analyzed. We performed a peritoneal equilibration test and examined a mean surface area of peritoneal mesothelial cell. **Results:** The mean PD period was 23.3±18.7 months. The dose of total small-solute clearance (weekly Kt/V urea)(2.41±0.664) was associated with the surface area of peritoneal mesothelial cell (333±30.1 μm²)(r=0.41, p=0.017). According to linear regression analysis, more than 3.16 weekly Kt/V urea corresponds to

more than 350 μm² of mean surface area of mesothelial cell. **Conclusion:** The main finding of this study is that high dose dialysis prescription is clearly associated with degradation of peritoneal membrane. These results suggest that patients who need high dose dialysis prescription have risk of SEP. Thus, as for the patient whose weekly Kt/v urea is more than 3.16, discontinuation of CAPD is desirable.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO863

**Superior Biocompatibility of Icodextrin by Maintaining the Wound Healing Process of Peritoneal Mesothelial Cells** Masahito Tamura, Tetsu Miyamoto, Narutoshi Kabashima, Ryota Serino, Tatsuya Shibata, Yumi Furuno, Junichi Nakamata, Yoko Fujimoto, Emi Hasegawa, Yutaka Otsuji. *Kidney Center, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan.*

Exposure to peritoneal dialysis fluid (PDF) causes structural alterations of the peritoneal membrane such as loss of mesothelial cell monolayer. These alterations of the mesothelium are associated with membrane dysfunction and progressive peritoneal fibrosis. Although icodextrin has been proven to have improved biocompatibility compared to the glucose-containing conventional PDF, effects of icodextrin on peritoneal wound healing have not been elucidated. We investigated the effects of icodextrin on wound healing of peritoneal mesothelial cells and determined the mechanisms involved. We examined the effects of icodextrin on the regeneration process of the peritoneal mesothelial cell monolayer by in vitro wound healing assay in cultured rat peritoneal mesothelial cells (RPMC) treated with icodextrin-containing PDF. Cell migration over fibronectin was inhibited in conventional acidic, lactate buffered glucose-containing PDF in concentration-dependent manner, however, icodextrin-containing PDF had no significant inhibitory effects. To evaluate the sole effect of icodextrin, remesothelialization was also examined in RPMC exposed to icodextrin powder-dissolved culture medium without PDF. Culture medium containing 2.5% glucose inhibited remesothelialization, however, 7.5% icodextrin-dissolved culture medium had no inhibitory effects. Glucose suppressed tyrosine phosphorylation of FAK, a key mediator in the integrin-mediated cell adhesion signaling, formation of focal adhesions, and cell spreading, while icodextrin had no effects on any of these mesothelial cell functions. Our results demonstrated that icodextrin had no deteriorative effects on the wound healing of peritoneal mesothelial cells by preserving integrin-mediated cell adhesion, and suggest that icodextrin-containing PDF may be more biocompatible than conventional glucose-containing PDF.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO864

**Clinical and Biochemical Profile of a Low-Glucose Degradation Product Peritoneal Dialysis Fluid: A Four-Year Study** Kar Neng Lai, M. F. Lam, Joseph C. K. Leung, Loretta Y. Y. Chan, Sydney C. W. Tang. *Medicine, University of Hong Kong, Hong Kong, Hong Kong.*

Although peritoneal dialysis (PD) is a widely accepted form of renal replacement therapy, concerns remain regarding the bioincompatible nature of standard peritoneal dialysis fluid (PDF). Short-term studies of new biocompatible low glucose degradation products (GDP) peritoneal dialysis fluid reveal divergent results in patient survival, and peritoneal integrity.

We recruited 125 patients on maintenance PD for at least 12 months. They were previously assigned to receive either conventional or low-GDP PDF at random. Two groups of patients were matched for gender, age, duration of dialysis and incidence of cardiovascular disease or diabetes. Serum samples and overnight effluent dialysate were simultaneously collected and assayed for different cytokines, chemokines, adipokines and cardiac biomarkers.

After an average duration of CAPD treatment of 2.3 years, both groups had comparable weekly creatinine clearance, peritoneal clearance, total Kt/V or peritoneal Kt/V. However, patients receiving low-GDP PDF had better residual renal function with higher urine output. Compared with new patients receiving the first dialysis, higher concentrations of TNF-a, TGF-b, HGF, MIF, IL8, IL6, CRP and leptin comparable to serum level were found in effluent dialysate in both groups of patients on long-term PD. There was also decreased concentration of adiponectin in the effluent. The abnormally raised leptin and reduced adiponectin levels in serum and effluent were partially corrected in patients on low-GDP PDF when compared with the conventional PDF group. The effluent concentration of interleukin 8 was also significantly lower in those using low-GDP PDF. The survival rate and incidence of cardiovascular complications did not differ between two groups of patients after maintenance PD for an average of 3.6 years.

It appears that low-GDP PDF results in an improvement in local peritoneal homeostasis and may potentially have a positive impact on long-term survival and cardiovascular complication by reducing the chronic inflammatory status in the peritoneum.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO865

**Adapted APD, Varying Sequentially Dwell-Time Exchanges Short and Thereafter Longer, and Fill Volume Exchanges Small and Thereafter Larger: Impact on Dialysis Efficacy** Michel Fischbach,[1] Belkacem Issad,[2] Cécile Courivaud,[4] Vincent Dubois,[3] Redouane Taamma.[3] *[1]Dialysis Children's Unit, University, Strasbourg, France; [2]Nephrology, Pitie, Paris; [3]FMC, Paris, France; [4]Neprology, University, Besançon, France.*

Varying the duration of dwell-time or fill volume could modify peritoneal dialysis efficiency. Nevertheless APD is classically given as a recurrent repetition of exchanges each of them having the same dwell-time and the same fill volume, that is conventional APD (APD-C). We propose a new, adapted APD (APD-A) by first using a short dwell time with a small fill volume to favour ultrafiltration (UF) and subsequently using a longer dwell time and a larger fill volume, to promote blood purification. The study was a prospective, cross over, multicenter trial to investigate the impact on overnight UF and weekly peritoneal Kt/$V_{urea}$, weekly peritoneal creatinine clearance per 1.73 m² BSA ($K_{creat}$), phosphate/sodium dialytic removal (PDR, SDR: mmol/session). Normalization to the absorbed glucose estimated the metabolic cost. Blood pressures were recorded. 25 patients were selected in the study, 6 withdrawned, 2 at enrolment, 1 at day 75 (transplantation), 2 at day 30 (catheter dysfunction), 1 for drainage alarms.

The same amount of dialysate (same cost), balance lactate 1.5%glucose, and the same duration of overnight APD were prescribed.

Tolerance was good. APD-A compared to APD-C realized a significant enhancement of Kt/$V_{urea}$, of $K_{creat}$, and of PDR. The glucose metabolic cost for urea, creatinine and PDR was significantly lower in APD-A compared to APD-C. The UF increased during APD-A versus APD-C. The SDR was significantly higher with APD-A compared to APD-C, 35.23±52.00 mmol/session and 18.35±48.68 mmol/session ($p<0.01$). The meanBP (day 45) were significantly lower for the APD-A periods compared to the APD-C periods.

Our study supported that varying the dwell-times short and thereafter longer, and the fill volumes small and thereafter larger as described (APD-A) could improve dialysis efficiency for UF, Kt/v urea, Kcreat, phosphate dialytic removal and sodium dialytic removal. These results where achieved without more costs, even with reduced glucose metabolic cost.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO866

**Automated Wearable Artificial Kidney (AWAK): Tidal Peritoneal Dialysis (TPD)-Based Large Volume Dialysate Exchange (LVDE) Using Low Reserve and Tidal Volume** Marjorie Wai Yin Foo,[1] Martin Roberts,[2-4] David B. Lee,[2-4] Siti Noor Huda,[1] Christian G. Bluchel,[5] Kok-Seng Wong.[1] *[1]Renal Medicine, Singapore General Hospital, Singapore; [2]VAGLA, Los Angeles, CA; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]AWAK Technologies, Singapore; [5]Temasek Engineering School, Temasek Polytechnic, Singapore.*

AWAK provides round-the-clock LVDE for steady-state metabolic and fluid regulation and for maximizing toxin clearance. We studied LVDE using TPD with low reserve (R) and tidal (T) volume for minimizing both the weight of AWAK and patient discomfort. We also examined the possibility that rapid dialysate exchanges could enhance ultrafiltration (UF) in high average transporters (HA). Five-hour TPD was conducted using Baxter HomeChoice system and 1.5% pH-neutralized, dextrose dialysate at LVDE (4L/hr). Eight R/T (in mL) combinations with T+R ≤ 1,500 mL, were studied in each of 8 established PD patients (2 low average transporters (LA) and 6 HA). Clearances are tabulated in mL/min and UF in mL/5 h. R500T500 is not included.

| | R250T250 | R250T500 | R250T1000 | R500T250 | R500T1000 | R1000T250 | R1000T500 |
|---|---|---|---|---|---|---|---|
| CUN HA/LA | 12.9/11,9 | 15.5/14.6 | 15.6/15.3 | 13.7/13.3 | 16.1/14.4 | 13.6/14.1 | 17.1/17.0 |
| UF HA/LA | 382/725 | 482/766 | 418/448 | 386/579 | 461/773 | 463/736 | 427/811 |
| Kt/V HA/LA | 3.4/4.2 | 4.1/5.1 | 4.5/5.4 | 3.6/4.7 | 4.3/5.4 | 3.7/5.5 | 5.9/6.5 |

Clearance of urea nitrogen (CUN) ranged from 12.9 to 17.1 which, based on AWAK providing 24/7 dialysis, extrapolates to a weekly Kt/V of 3.4 to 6.5. UF ranged from 382 to 811, which extrapolates to a daily volume of 1.8 to 3.9 L. Compared to LA, HA exhibited higher CUN and lower UF. Patients reported no discomfort and no inflow or drainage problems were encountered. We conclude the use of TPD with low R and T and high dialysate flow in AWAK can achieve high solute clearance and adequate UF even in HA without the need for higher than 1.5% glucose dialysate. Functional optimization of AWAK can be further achieved by using R+T of less than 0.75 L during the waking hours and up to 1.5 L through the sleeping hours.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO867

**Peritoneal and Renal Phosphate Handling in PD Patients** Ana Paula Bernardo,[2] Sebastián Azorín,[1] Gloria Del Peso,[1] M. Auxiliadora Bajo,[1] Marta Ossorio,[1] Amaia Ros Abando,[1] Rafael Selgas.[1] *[1]Nephrology Department, H. Universitario La Paz, Madrid, Spain; [2]Nephrology Department, ULS Castelo Branco, Castelo Branco, Portugal.*

**Introduction and aims:** Phosphate control has shown a major impact on morbidity in dialysis patients. The aim of this study was to evaluate factors associated with hyperphosphatemia (HyPh), to determine the effect of peritoneal characteristics and PD modality on phosphate clearance (PPhCl) and to establish its impact on survival. **Patients and methods:** 264 patients were evaluated at months 3 and 12 (age 51±16 years, 61% on CAPD). PPhCl was calculated from 24h peritoneal effluent samples. Residual renal function (RRF), phosphate(Ph) and creatinine(Cr) dialysate/plasma(D/P) were calculated at a 4h, 3.86%

PET.**Results:**The prevalence of HyPh (>5.5mg/dl) was 27% at baseline and 30% at 1year (1y).On multivariate analysis at baseline, RRF was independently associated with HyPh; at 1y RRF, age and CCPD were associated with HyPh.In anuric patients at 1y, D/PPh was the only independent factor for HyPh.D/PPh correlated with D/PCr at baseline and 1y (r=0.81, both;p<0.0001).PPhCl-1y correlated better with D/PPh-1y(r=0.48;p<0.0001) than with D/PCr-1y(r=0.46;p<0.0001).At 1y we classified patients as low(Lo),low-average(LA),high-average(HA) or high(H) transporters (mean±SD of D/PPh).16% patients were classified as H (D/PPh≥0.68),31% as HA (D/PPh 0.57-0.68),35% as LA(D/PPh 0.47-0.57) and 17% as Lo transporters(D/PPh ≤0.47).Patients with a Lo transport status had lower PPhCl than LA, HA and H transporters(31±14vs34±8vs39±1vs47±13L/wk respectively,p<0.0001).Among H and HA transporters, PPhCl were comparable in both PD modalities.In comparison to CCPD, CAPD was associated with increased PPhCl among LA(36±8vs32±7L/wk,p=0.005) and Lo transporters(34±15vs24±9L/wk,p=0.016).No significant differences on survival concerning HyPh, D/PPh or PPhCl were demonstrated.**Conclusion:**RRF contributes decisively to Ph control in PD patients.In anuric patients, membrane transport determines Ph control.Peritoneal clearance is determined by membrane transport category and PD modality, suggesting that PD regimes with longer dwell times may increase phosphate removal among lower transporters.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO868

**Two-in-One Protocol: Simultaneous Free Water Transport Quantification and Standardized Small Solute Rate PET Categorization** Ana Paula Bernardo,[3] Anabela S. Rodrigues,[1] António Cabrita,[1] M. Auxiliadora Bajo,[2] Gloria Del Peso,[2] Rafael Selgas.[2] *[1]Nephrology Department, Centro Hospitalar do Porto, Portugal; [2]Nephrology Department, H. Universitario La Paz, Madrid, Spain; [3]Nephrology Department, ULS Castelo Branco, Portugal.*

**Introduction and aims:**Reduced free water transport (FWT) may contribute to the ultrafiltration failure (UFF) frequently seen in long-term peritoneal dialysis (PD) patients. Our aim was to simultaneously evaluate small solute transport by using a standard 4h dwell PET, to more accurately assess FWT in a group of prevalent PD patients and to further characterize them according to UFF.**Patients and Methods:**We performed a 4h, 3.86% PET with additional measurement of UF at 60 minutes (60') in 66 PD patients (age 50±16 years, 61% female, on PD for 26±23 months).We calculated FWT and %FWT;small pore fluid transport (SPUF);Dialysate(D)/Plasma(P) ratio of sodium (Na) and the dip in D/PNa at 60',120' and 240'. FWT determination was improved by the introduction of a correction algorithm (FWTc).**Results:**The following mean values were obtained:FWT 172±77ml,FWTc 211±79 ml, %FWT 38±0.1,SPUF 301±137 ml,UF60 473±163ml,UF240 673±233ml,D/PNa60 0.89±0.04, dipD/PNa60 0.03±0.04. D/PNa at 60',120' and 240' correlated well with FWT (r=-0.71; r=-0,69; r=-0.62, respectively, p<0.0001).UF240 correlated better with FWT and FWTc than with any other indirect measurement of Na sieving (r=0.37, p=0.002 and r=0.41, p=0.001, respectively).Twelve patients (18%) had UFF (UF240' <400ml).These patients had significant higher values of D/PCr(0.80vs0.72, p=0.0015), D/PNa60(0.92vs0.89, p=0.009), D/PNa120(0.93vs0.89, p=0.008), D/PNa240(0.94vs0.91, p=0.009) and lower values of FWT(113vs184, p=0.001) and FWTc (148vs225,p<0.0001), compared with the normal UF group.Seven UFF patients (58%) were on PD for more than two years, and among these higher compromise of FWT disproportional to small solute transport rate was shown: 5 had FWT<40% and 3 fast transport status. **Conclusion:** Measurement of FWT is feasible by its simultaneous quantification during a modified 3.86% glucose PET and is an important parameter to detect additional causes of UFF besides increased effective capillary surface.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO869

**Effect of Fill Volume (FV) on Accelerated Peritoneal Equilibration Examination Time (APEX)** Kassem Safa,[1] Salim K. Mujais,[2] Ali K. Abu-Alfa.[3] *[1]Hospital of St Raphael; [2]Astellas Pharma Global Development; [3]Yale School of Medicine.*

**A**im is to examine effect of increasing FV on APEX using standard PET with 2.5% dextrose dialysate (DD) and to evaluate APEX in different peritoneal membrane transport categories. Verger introduced APEX using 4.25% DD to estimate ultrafiltration (UF) time and predict peritoneal permeability.

**Methods**

19 adult patients on PD for > 3 months underwent PET with 3 different randomly assigned FV (2, 2.5 & 3 L) of 2.5% DD, 1 week apart. Dialysate was obtained hourly between 0 and 4 hrs and blood at 2 hrs. Study was approved by Yale Human Investigation Committee. APEX was determined for each patient by plotting urea D/P and dextrose dissipation Dt/D0 as y-variables, and projecting their intersection on time x-axis (fig 1). Minitab®15 was used for data analysis.

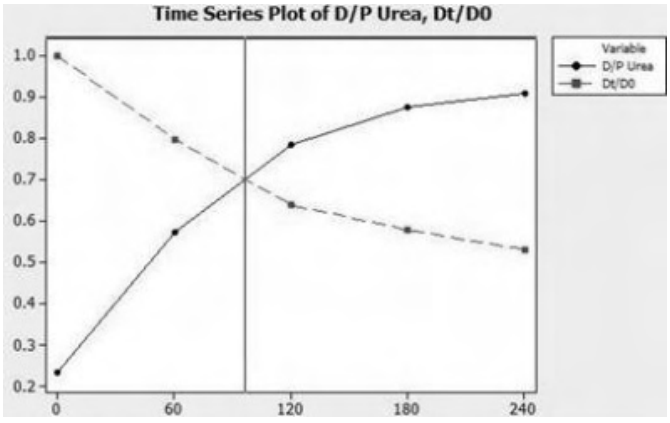



Fig 1. APEX determination

**Results**

11 men and 8 women (APD/CAPD 15/4) were enrolled with membrane types characterized as high (H:3), high average (HA:8), low average (LA:7) and low (L:1). Mean age was 43.1±13.4 yrs and BMI 26.72±5.59 Kg/m². Mean APEXT increased from 95.21±19.92, 110.52±20.54 to 132.36±29.58 min with increase in FV from 2, 2.5 to 3L. (p≤0.001, ANOVA and paired t-test)

Analysis grouping H with HA and L with LA membrane types showed significant increase in APEX as FV increased in both subgroups (p<0.05, ANOVA and paired t-test). Although APEX was consistently longer for L/LA vs. H/HA types, statistical significance was only noted for 2.5L (t-test). Changes in APEX based on interaction between progressive increase in FV and transport status were not statistically significant.

**Conclusion**

APEX responds in biologically plausible fashion to changes in FV and differentiates adequately between peritoneal transport types. It can be a useful summation index that can be used in select patients to determine optimal dwell times on APD appropriate to membrane transport type.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO870

**Peritoneal Clearance of N-Terminal Telopeptide X (NTx)** Ramin Tolouian,[1] Sean Connery,[1] Shiva Mansourkhani,[1] Natalia Vega,[1] Ajay Gupta.[2] *[1]Nephrology and Hypertension, Texas Tech University Health Sciences Center, El Paso, TX; [2]Rockwell Medical, Wixom, MI.*

**Introduction**: Peritoneal Equilibrium Test (PET) has been considered the gold standard for measuring small molecule clearance such as urea & creatinine in peritoneal dialysis. Increased middle molecule retention may increase cardiovascular mortality. NTx is a bone marker with molecular weight of 1700 Dalton (Da) considered a middle molecule.

The purpose of this study is to show that NTx is cleared by peritoneal dialysis and thus assess the PET based on middle molecule clearance & potentially assess mineral bone disease simultaneously.

**Methods:** During routine PET, we measured NTx in dialysate at 0, 2 and 4-hour time points & in serum at 2 hours. NTx was measured by enzyme-linked immunosorbent assay (ELISA) according to manufacturer instructions (Wampole Lab, Osteomark; Seattle, WA). NTx is reported in nanomoles Bone Collagen Equivalent (nM BCE).

**Results:** 30 subjects undergoing PET were recruited. Mean Age = 56 yrs, mean length of time on peritoneal dialysis = 2 yrs, 63% male, 97% Hispanic, 67% diabetic. Dialysate/Plasma Ratio (D/P) for Cr, Urea, and NTx; 0.68 ±0.11, 0.91 ±0.05, 2.53 ±1.06 respectively (mean ±SEM). Results of diffusion of molecules measured over time are presented below;

**Diffusion Across Peritoneal Membrane**

| **PD fluid** | | | | **Serum** |
|---|---|---|---|---|
| | **0 hr** | **2 hr** | **4 hr** | **2 hr** |
| **NTx** (nm BCE) | 84.4 ±56.7 | 138.4 ±55.4 | 164.8 ±64.4 | 68.1 ±15.3 |
| % Change | | 270% (140-406%) | 340% (167-514%) | |
| **Cr** (mg/dl) | 0.97 ±0.51 | 4.3 ±1.75 | 5.7 ±2.48 | 8.4 ±3.6 |
| % Change | | 480% (396-548%) | 650% (530-736%) | |
| **Urea** (mg/dl) | 6.4 ±3.13 | 38.4 ±10.9 | 48.5 ±13.97 | 53.3 ±15.2 |
| % Change | | 713% (568-804%) | 897% (720-998%) | |

Mean ±SD or (95% CI)

**Discussion:** The kinetics of NTx transfer across the peritoneal membrane during a PD exchange demonstrated a slower transfer rate of NTx, compared to urea and creatinine, is consistent with a molecular weight of 1700 Da for NTx vs.(Cr= 113 Da, Urea = 60 Da).

This study demonstrates the feasibility of the use of NTx as a marker of middle molecule clearance to study peritoneal membrane characteristics and simultaneously assess mineral bone disease.

Disclosure of Financial Relationships: Honoraria: Involve in the CME activity. (Astella)- Novartis.

**TH-PO871**

**The Role of NGAL in Peritoneal Dialysis Patients** Ilenia Filippi,[1] Francesca K. Martino,[1] Massimo De Cal,[2] Ching Yan Goh,[1] Pierluigi Di Loreto,[1] Maria Pia Rodighiero,[1] Carlo Crepaldi,[1] Claudio Ronco.[1] *[1]Nephrology, S Bortolo Hospital; [2]Medical Surgical Sciences, University Padua.*

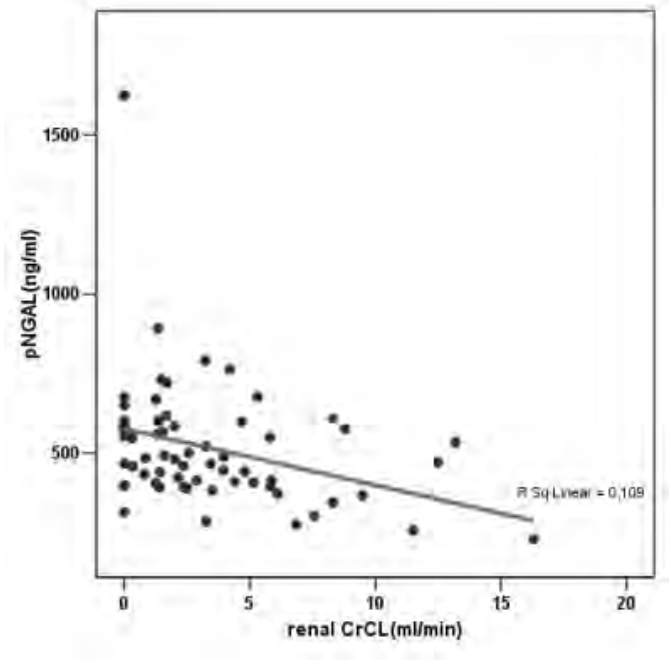
**BACKGROUND:** NGAL is a promising biomarker of renal function. Residual renal function (RRF) is the most important prognostic factor in peritoneal dialysis patients (PD pts) and NGAL may be useful in their management. In this study, we aim to access NGAL expression in PD pts and evaluate factors that affect its level.

**METHOD:** We performed a cross-sectional study of 69 pts, who were on PD for ≥3 months. We evaluated their hydration status by bioimpedence, RRF [urine output (UO), renal CrCL, renal Urea CL], dialysis adequacy (weekly CrCL, KT/V), serum NGAL and other biochemical parameters (Albumin, Hb, CRP, Ferritin). All statistical analyses were performed on SPSS version 16.

**RESULTS:** 56.5% of pts were male, 26% were diabetes, 84% had hypertension. Median (Mdn) age was 61years (IQR46-71); mdn duration of RRT 30months (IQR11-51); mdn creatinine 8.7mg/dl (IQR6.7-11.3); mdn UO 800cc/day (IQR400-1200); mdn renal CrCL 2.3ml/min (IQR0.8-4.9); mdn weekly CrCL 67.7L/w (IQR54.9-86.7); mdn KT/V 1.9 (IQR1.76-2.19); mdn Urea CL 1.7ml/min (IQR0.55-2.8); mdn CRP 1.1ng/ml (IQR0.1-1.27); mdn extracellular water (ECW) 17.9L (IQR14.8-20.8); mdn intracellular water (ICW) 18.9L (IQR15.6-22.6).

Mdn NGAL level was 487.5ng/ml (IQR406.5-596.2). NGAL was inversely correlated with UO (ρ=-0.31 p=0.01), renal CrCL (ρ=-0.358 p=0.004), weekly CrCL (ρ=-0.396 p=0.001) and KT/V (ρ=-0.26 p=0.031). However, when we analyzed the correlation between NGAL and weekly CrCL as well as KT/V after correction for RRF, such correlation were lost. There were no correlation between NGAL and CRP, Ferritin, Albumin, ECW and ICW (p>0.05).

**CONCLUSION:** In this analysis, NGAL is significantly correlated with RRF but not with dialysis adequacy, inflammation and hydration in PD pts. Its level may be useful in PD pts management.




Disclosure of Financial Relationships: nothing to disclose

**TH-PO872**

**Automated Peritoneal Dialysis (APD) Therapy Prescriptions for Enhancing Fluid and Sodium Removal** Steven Guest, Alp Akonur, James A. Sloand, J. Ken Leypoldt, Ying Lo. *Renal Division, Baxter Healthcare Corporation, McGaw Park, IL.*

Remaining edema-free is a challenge for many APD patients, especially those with fast (or high) transport membrane characteristics. Although increased use of dialysates containing high glucose (G) concentrations may improve volume control, the frequent use of such solutions is undesirable. We used the three-pore kinetic model to evaluate for anuric high and high-average transport patients 3 alternative therapy prescriptions for the day exchange, namely 1) use of a short daytime dwell (5 hrs) with a dry period (Therapy 1); 2) use of a midday exchange (Therapy 2); and 3) use of icodextrin-containing dialysate during a daytime dwell of 14 hrs (Therapy 3). The control therapy used the standard G-containing daytime dwell of 14 hrs. The nighttime prescription was identical for each therapy (10 L over 10 hrs), and all G-containing dialysates contained 2.27% G. Net ultrafiltration (UF), sodium removal (NaR), total carbohydrate absorption (CHO) and weekly urea Kt/V were computed and compared over a 24-hour period (see table). Use of a short daytime dwell with a dry period resulted in substantially higher UF and NaR without significant changes in CHO or urea Kt/V compared with control therapy. Use of a midday exchange does not improve upon UF and NaR compared with a short daytime dwell with a dry period; however, it provides higher Kt/V at the expense of higher G absorption. UF and NaR are highest using a long icodextrin-containing daytime dwell. Compared to standard (control) APD regimens with G, using either a short daytime dwell with a dry period or icodextrin reduces G absorption while enhancing UF and NaR.

Computer Simulated Parameters for APD Prescriptions

| Transport Type | Therapy | UF (L/day) | NaR (meq/day) | CHO (g/day) | Kt/V (per week) |
|---|---|---|---|---|---|
| High | Control | 0.46 | 23 | 177 | 1.80 |
| High | 1 | 0.90 | 74 | 172 | 1.87 |
| High | 2 | 0.83 | 65 | 215 | 2.21 |
| High | 3 | 1.44 | 152 | 173 | 1.97 |
| High-average | Control | 0.73 | 47 | 150 | 1.70 |
| High-average | 1 | 1.13 | 90 | 142 | 1.75 |
| High-average | 2 | 1.21 | 94 | 184 | 2.11 |
| High-average | 3 | 1.62 | 165 | 139 | 1.85 |

Disclosure of Financial Relationships: Employer: Baxter Healthcare, Renal Division; Ownership: Ownership interest in Baxter Healthcare.

**TH-PO873**

**Sodium Balance in an Automated Peritoneal Dialysis (APD) Population** Radu R. Raducu,[1] Aura Cernii,[1] Fredric O. Finkelstein.[1,2] *[1]Department of Nephrology, Hospital of Saint Raphael, New Haven, CT; [2]Yale-New Haven Hospital, CT.*

***Objective:*** The study was designed to evaluate the sodium balance in 61 patients with ESRD maintained on APD.

***Design:*** Longitudinal cohort study based on patient records and data files.

***Setting:*** One outpatient PD unit in New Haven, CT.

***Patients:*** A total of 61 patients who were on APD over a 1 year period (2009-2010).

***Main Outcome Measures:*** For each patient, the following parameters were evaluated: total volume of infused dialysate, total volume of drained dialysate, plasma sodium, dialysate sodium, amount of ultrafiltration, and amount of sodium removed by dialysis.

***Results:*** 61 patients were studied. The patients had mean KT/V of 2 (dialysis and residual). Mean plasma sodium was 139.3 mEq/l. Mean dialysate (drained) [sodium] was 129.7 mEq/l (range of 123-133), compared to an infused dialysate [sodium] of 132 . Mean ultrafiltration was 1.044+/-0.724 L/ 24 hours (mean+/- SD) . Mean sodium removal by peritoneal dialysis was 98.08+/-91.1 mEq daily . The mean decrease in [sodium] in the PD solution was 2.22+/-3.5 mEq/l. The correlation coefficient between amount of ultrafiltration and amount of sodium removed by PD was 0.81. The correlation coefficient between the decrease of [sodium] in the peritoneal fluid and amount of sodium removed by PD or the amount of ultrafiltration was 0.25 and 0.32, respectively.

***Conclusions:*** The data suggest that there is a drop in peritoneal dialysis solution sodium concentration during APD, likely related to water transport via peritoneal membrane aquaporin channels. This sodium sieving reduces the net sodium removal with APD. Decreasing the sodium concentration of peritoneal dialysis fluid during APD (currently set at 132 meq/l) might increase the sodium gradient and sodium clearance in APD patients. This might have important clinical advantages given the association of sodium balance with hypertension and cardiovascular events in ESRD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO874**

**Assessment of Volume Status and Its Relationship with RisK of Inflammation and Nutrition in Korean PD Patients (ASKK Study)** Young Youl Hyun,[1] Young Sun Kang,[1] Jinjoo Cha,[1] Young Soo Kim,[2] Kook-Hwan Oh,[3] Yang Wook Kim,[4] Yeong Hoon Kim,[4] Nam Ho Kim,[5] Dae R. Cha.[1] *[1]Nephrology, Korea University, Ansan, Republic of Korea; [2]Nephrology, Catholic University, Republic of Korea; [3]Nephroloy, Seoul National University, Republic of Korea; [4]Nephrology, Inje University, Republic of Korea; [5]Nephrology, Chunnam University, Republic of Korea.*

Volume overload is thought to be associated with microinflammation, endothelial dysfunction, malnutrition and cardiac dysfunction. Adequate volume status is important to prevent worse outcomes in CKD patients. We evaluated the markers of inflammation and malnutrition in peritoneal dialysis(PD) patients according to the objective volume status, and the relationship of volume overload with cardiac dysfunction. Two hundred peritoneal dialysis patients from 5 nephrology center were included in the study. Using body composition monitor, total body water, overhydration, intracellular water(ICW), extracellular water(ECW), lean tissue mass(LTM), adipose tissue mass(ATM), body cell mass(BCM) were mesured. Demographic and laboratory data of inflammatory markers of CRP, TNFα, MCP-1, IL-6 and VEGF, Fractalkine were collected. The patients with excess ECF volume (more than 2.5L) showed lower LTM index, lower Hb, lower calcium, lower protein, lower albumin, lower cholesterol level, higher MCP-1, higher TNF-α, higher glucose and higher antero-posterior calcification scores than that in normohydrated and mild overhydrated patients. Overhydration was positively correlated with the values of MCP-1, VEGF, glucose, ALP, height, systolic blood pressure and negatively correlated with LTM, albumin, Hb level. ECW/ICW ratio was also positively correlated with MCP-1, height, weight discrepancy between patients weight and ideal dry body weight, and negatively with LTM index, ATM index, albumin, Hb. Overhydration was an independent risk factor of LVH, but did not increase the risk of vascular calcification. Conclusively in PD patients, fluid overload was associated with microinflammation, malnutrition, and possibly cardiac dysfunction. Adequate volume monitoring is a cornerstone to improve outcome in PD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO875**

**Bioimpedance Assisted Fluid Management Optimises Blood Pressure Control without Effect on Residual Renal Function** Stanley Fan, Sinead E. Burke. *Renal Unit, Royal London Hospital, United Kingdom.*

Purpose of the study:

Fluid overload predicts hypertension and cardiac dysfunction in dialysis patients. We used the Body Composition Monitor (BCM, Fresenius Medical Care) to screen PD patients and manage their fluid balance

Methods:

Patients were screened to identify severely overhydrated patients (OH > 2.L). These patients were reveiwed monthly, advised on sodium and fluid restriction and their PD regimens were adjusted to optimise ultrafiltration (UF). Residual renal function (RRF) and fat tissue mass were monitored. Achievement of euvolaemia was considered to be a BCM measurement of +/-1.1L.

Results:

Of the 200 PD patients screened, 19 patients (mean age 64.0yrs, Male 90%, CAPD 63%, mean PD vintage 17.5 months) had an OH >2L. 5 could not be followed up because of early PD technique failure or death from CCF. Mean follow up of patients was 8.4 months. Baseline overhydration was +2.4L (Table 1). All patients achieved euvolaemia (OH of +0.4L) after approx 3 months and 70% maintained euvolaemia during the remaining follow up period.

BP remained the same despite a significant reduction of antihypertensive medications use. Mean PD glucose use increased from 410 (38) kCal to 416 (45) kCal but there was no change to fat tissue mass. Residual renal function was also unchanged throughout the study period.

Change in BCM overhydration, MAP, antihypertensive use and RRF

| | BCM | MAP | No of anti-HT | RRF (24hr Vol) | RRF (weekly kt/V) | RRF (CrCl, L/wk) |
|---|---|---|---|---|---|---|
| Baseline | +2.4 (0.3) | 100 (6) | 1.1 (0.2) | 598 | 0.72 | 39.0 |
| Follow up | 0.4 (0.2)* | 95 (6) | 0.6 (0.3)* | 607 | 0.57 | 37.0 |

* denotes p<0.005 from baseline

Conclusion:

Bioimpedance identified patients that have benefited from optimisation of fluid balance. It permitted a significant reduction in antihypertensive medication without impact on BP control. Despite higher glucose usage to achieve euvolaemia, there has been no increase to fat tissue mass observed. Importantly, residual renal function has not been compromised in these patients.

We suggest that the BCM is a useful tool and achieving euvolaemia does not compromise residual renal function in PD patients.

Disclosure of Financial Relationships: Research Funding: The Department is in receipt of educational and research grants from Baxter Healthcare and Fresenius Medical Care; Honoraria: Travel expenses and speaker honoraria paid by Baxter Healthcare and Fresenius Medical Care.

**TH-PO876**

**Is N-Terminal Probrain Natriuretic Peptide a Marker of Volume Overload in Peritoneal Dialysis Patients?** Jenny Papakrivopoulou, Andrew Davenport. *Center for Nephrology, University College London Medical School, London, United Kingdom.*

Recent reports have suggested that peritoneal dialysis (PD) patients are volume overloaded.

We audited the usefulness of N-terminal probrain natriuretic peptide (NTproBNP) in determing volume overload in 80 ambulant PD patients, 45.7% male, 24.7% diabetic, mean age 57.1±1.7 yr, 73.4% treated by peritoneal cyclers, 82.7% icodextrin and 55.6% used one or more 2.27% glucose exchanges, mean dialysis vintage 32.9±3.3 months, mean urine output 1050±88.7 ml/day. Mean systolic blood pressure 137.9±2.8 and diastolic blood pressure 79.8±1.5 mmHg, 58% prescribed antihypertensives and 47.4% diuretics. Mean logNTproBNP 2.46±0.07 pmol/l.

Patients underwent chest X ray, 2dimensional transthoracic echocardiography, asssessment of peritoneal dialysis adequacy and transport status, and volume using 8 contact electrode multifrequency bioimpedance.

Simple correlations were found between logNTproBNP and cardiac size (left ventricular end systolic volume, diameter, and end diastolic diameter), right ventricular systolic pressure, residual renal function (urine output, urine sodium, urine Kt/V and creatinine clearance) volume (extracellular water to total body water (ECW/TBW)), 24 hour peritoneal ultrafiltration volume, icodextrin usage, serum albumin and prescription of antihypertensive medications, but not blood pressure measurements, cardiothoracic ratio, sex, age, diabetes, CRP, peritoneal dialysis clearances, transport status or dialysis prescription.

On logistical regression analysis only 4 factors remained independent predictors of logNTproBNP; liters creatinine cleared per week (F20.8, β -0.006, 95%CL 0 to-0.008, p<0.001), number of blood pressure medications prescribed (F8.04, β0.132, 95%CL 0.39 to-0.226, p=0.007), volume status ECW/TBW (F7.43, β13.75, 95%CL 3.62 to 23.9, p=0.009) and left ventricular end systolic diameter (F5.84, β0.186, 95%CL0.019 to0.032, p=0.019).

In peritoneal dialysis patients BNP increases with volume overload (raised ECW/TBW) but is also affected by residual renal function, hypertension and left ventricular size.Thus a single point BNP estimation alone may not be a strong indictor of volume status in PD patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO877**

**Hydration Status, Cardiac Function and Brain Natriuretic Peptide in Peritoneal Dialysis** Gianpaolo Amici,[1] Adriana Caberlotto,[1] Paolo Cogliati,[2] Carmelo Cascone.[1] *[1]Nephrology and Dialysis, Regional Hospital, Treviso, Italy; [2]Medical Department, Fresenius Medical Care Italy, Palazzo Pignano, Italy.*

Hydration status affects cardiac function in peritoneal dialysis (PD) where obtaining the necessary ultrafiltration can give inflammatory stress to the biologic membrane. In this clinical contest blood and instrumental indices of heart function can be integrated by the multifrequency bioimpedance analysis giving relevant quantitative data. A cross-sectional study was performed on 27 prevalent PD patients, 19 APD and 8 CAPD, 17 males and 10 females, aged 64±16 years, on dialysis for 2.8±2.6 years, 5 patients used icodextrin and 6 were diabetics. In all patients body composition analysis was performed using BCM-FMC spectroscopy estimating overhydration status (OH, L), Total Body Water (L), Extra-Cellular Water (L), Intra-Cellular Water (L), Lean Tissue Mass (LTM kg), Fat Tissue Mass (FTM kg) and echocardiography with Ejection Fraction % and LVMI (g/sqm, Devereux-Penn). N-terminal pro-brain natriuretic peptide (NT-ProBNP) pg/mL (n.v. 0-125) was assayed in all patients. Statistical analysis was performed by JMP SAS software. Median NT-ProBNP showed high values 5398 pg/mL with a wide dispersion (range 266-35000) so in the following analyses has been transformed (Log), EF % was 65.7±8.7, LVMI 176±60 g/mq, SBP 156±27 and DBP 95±18 mmHg, OH 1.8±1.6 L, TBW 35±7 L, ECW 17±3 L, ICW 18±4 L, LTM 37±11 kg, FTM 23±9 kg. LogBNP showed linear correlations with residual diuresis (r-0.576 p=0.003), GFR (r-0.627 p=0.002) total weekly CLCR (r-0.588 p=0.004); with EF% (r=-0.603 p=0.003), LVMI (r=0.458 p=0.032) and spectroscopy data: OH (r=0.528 p=0.007), ICW (r=-431 p=0.032). Applying a multivariate regression model LogBNP is explained (rsq=0.834) by diuresis (F=38.1, p<0.001), LVMI (F=26.9, p<0.001), age (F=5.6, p=0.006) and OH (F=4.0, p=0.016). The complex of evaluated indices shows a relationship between residual renal function, hydration status and cardiac function in PD patients suggesting a diagnostic scheme. NT-ProBNP assay and spectrometry analysis are of primary interest for the follow-up of the patient integrating more complex cardiovascular exams and giving clinically relevant data.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO878**

**Left Atrial Volume Index (LAVI) and Left Ventricular End Diastolic Volume Index (LVEDVI) May Not Reflect Overhydration in Peritoneal Dialysis Patients with Hypertensive Cardiac Injury** Boon Kay Tan,[1] Frauke Wenzelburger,[1] Biju John,[1] Yu Ting Tan,[2] John E. Sanderson,[2] Simon J. Davies.[1] *[1]Institute for Science and Technology in Medicine, Keele University, Stoke on Trent, Staffordshire, United Kingdom; [2]Cardiovascular Medicine, University of Birmingham, Birmingham, West Midlands, United Kingdom.*

Background:

Left atrial volume (LAV) and left ventricular end diastolic volume (LVEDV) are often considered measures of fluid status. We want to establish whether LAV and LVEDV are more dilated in PD patients compared to non CKD patients with a similar history of hypertension.

Method:

30 stable PD patients (15 male, mean age 60±17) and 30 non CKD hypertensive patients (12 male, mean age 67±6) were studied. They were matched for gender, mean BP and duration of hypertension. Left ventricular mass index (LVM),LVEDV and LAV were determined using standard full Doppler-2D-echocardiography with M-Mode and calculated according to the guidelines of the American society of echocardiography and adjusted for body surface area (BSA). Plasma volume (PVc) was measured using 125I-albumin and corrected for BSA (in PD patients only); extracellular (ECW) to total body water (TBW) ratio were determined by Bioimpedance analysis (Xitron Hydra) in PD patients and predicted using Lindley and Lopot equation in non CKD patients.

Result:

While PVc was found to be within the normal range in > 85% of PD patients, ECW:TBW was significantly higher compared to non CKD patients. (0.478±0.356 v 0.458±0.104, p=0.005). LVMI was greater in PD patients than in non CKD patients (111.72 ± 29.54 g/m2 v 89.54 ± 31.48 g/m2, p<0.05) but LAVI and LVEDVI were comparable in both groups of patients (31.86 ± 12.67 ml/m2 v 29.94 ± 10.54 ml/m2 and 43.48 ± 15.52 ml/m2 v 40.80 ± 9.02 ml/m2, respectively. p=ns). LAVI correlated with LVMI only in PD but not in non CKD patients (r=0.44, p<0.05).

Conclusion:

Both LAVI and LVEDVI may not be reliable surrogate parameters of current fluid excess in PD patients with established hypertensive heart disease. The correlation between LAVI and LVMI seen in PD patients may suggest more diastolic dysfunction and reduced left ventricular compliance in PD patients compared to non CKD hypertensive patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO879**

**Impact of Body Mass Index (BMI) on Early and Late Mortality and Technique Failure in Peritoneal Dialysis (PD)** Kelvin Leung, Manish M. Sood, Paul Komenda, Mauro Verrelli, Claudio Rigatto. *Medicine, University of Manitoba, Winnipeg, MB, Canada.*

Increasing BMI is associated with higher mortality in the general population. In PD patients, however, the relationship between BMI and outcomes is unclear. Some studies demonstrate lower, and others higher mortality with high BMI. These variable results

may reflect either a time dependent relationship (e.g. different impact on early and late mortality in PD) or a J-curve phenomenon. To test these hypotheses we analyzed data from a prospective database of all 727 incident patients initiating PD between 1997 and 2007 in the province of Manitoba, Canada. Separate proportional hazards models were constructed to model the impact of BMI on early (<12 months) and late (>12 months) mortality and technique failure, with adjustment for the following confounders: age, sex, race, cause of end stage renal disease, residual renal function, peritoneal KT/V, peritoneal transport, and comorbid medical conditions). The average BMI was 27.1 (S.D. 5.8). Most patients (64%) were overweight (BMI>25) with 31% obese (BMI>30). Total mortality and technique failure during the follow up period was 45.9% and 47.2% respectively. Increasing BMI was associated with lower adjusted early mortality (hazard ratio [HR] 0.762, 95% CI: 0.584-0.996, P<0.05) and lower rate of adjusted early mortality or technique failure (HR 0.741, 95% CI 0.601-0.914, P<0.01). No association was apparent between BMI and late mortality, or BMI and late technique failure. A J-curve phenomenon could not be demonstrated when BMI was analyzed as quartiles or as a quadratic polynomial. In summary, low BMI was consistently associated with high early mortality, but no association was apparent between BMI and late mortality. These findings support the concept that the association of BMI and outcomes may differ in the early versus late period after starting PD.

Disclosure of Financial Relationships: nothing to disclose

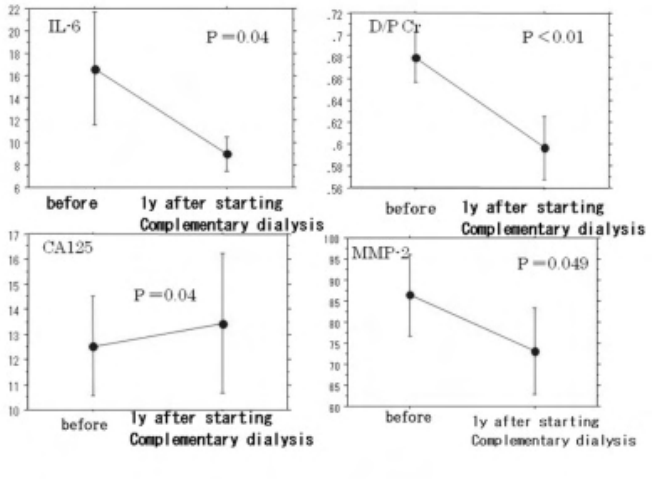
## TH-PO880

**Complementary Therapy Might Be Effective To Preserve Peritoneal Function** Nanae Matsuo, Keitaro Yokoyama, Yukio Maruyama, Yoshimi Ueda, Rinako Iida, Yudo Tanno, Kazushige Hanaoka, Hiroyasu Yamamoto, Tatsuo Hosoya. *Division of Kidney and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan.*

Complementary therapy, which comprises 6 days of PD combined with 1 HD session per week, is the treatment of choice for PD patients who cannot control body fluid status and/or cannot achieve adequate solute removal by PD alone. We previously reported several benefits of complementary therapy including improvement of hydration status, correction of inadequate solute removal and improvement of anemia (Clini Neph in press). Oppositely, there is a possibility to increase peritoneal damage resulting the prolonged PD duration as to complementary therapy. The aim of this study was to evaluate the influences of complementary dialysis on peritoneal damage.

In this prospective cohort study, we recruited twenty patients with complementary therapy. Markers of peritoneal damage as well as fast peritoneal equilibration test (PET) were compared before and 1year after starting complementary therapy.

D/P-cre decreased significantly 1 year after starting complemantary therapy. Level of MMP-2 in PET drainage also decreased significantly. In addition, level of CA125 in PET drainage increased significantly, and it might reflect the improvement of peritoneal mesothelial cells viability. Furthermore, serum C-reactive protein and fibrinogen decreased significantly. The mechanism of the preservation of peritoneal function by complementary therapy was quite obscure. However, the reduction of glucose exposure form PD solution on PD free day (performed HD day), together with adequate dialysis after starting complementary therapy, might be lead patients to improve in inflammation and peritoneal function. We concluded that complementary therapy might be effective to preserve peritoneal function.




Disclosure of Financial Relationships: nothing to disclose

## TH-PO881

**Icodextrin Blocks Progression of Cardiac Hypertrophy and Arterial Valve Calcification in Incident Peritoneal Dialysis Patients** Takeyuki Hiramatsu, Shinji Furuta, Yoshiyasu Iida. *Department of Nephrology, Konan Kosei Hospital, Konan, Aichi, Japan.*

Background and aim : We reported use of icodextrin ameliorates cardiovascular (CV) parameters in peritoneal dialysis (PD) patients. One of remaining questions would be when icodextrin to be started for better CV management. Therefore, we retrospectively compared the change in CV parameters, according to when they started icodextrin after the start of PD therapy.Methods and patients : A total of 45 incident PD patients with available 2-year data was analyzed retrospectively. Group A (n=7); started icodextrin within 1 month after the start of PD, Group B (n=10); started icodextrin between 1 month to 2 years on PD , Group C (n=28); never used icodextrin for 2 years after the start of PD. The criteria to start icodextrin were overt edema and ultrafiltration volume less than 500mL/day. Results : At baseline, brain natriuretic peptide (BNP) and left ventricular mass index (LVMI) in Group B were significantly higher than those in Group C. Aortic valve calcification and plaque score in cervical artery in Group A were significantly worse than those in Group C. Thus, some cardiovascular parameters in group A and B were worse than those in Group C at baseline. However, the ameliorations in those parameters after 2 years were observed in both Group A and B, whereas those in Group C deteriorated as shown in the Table 1.

Changes in CV parameters in each group:values at 2 years minus baseline

| parameter | Group A | Group B | Group C |
|---|---|---|---|
| BNP (pg/mL) | -281(0.0047) | -526(<0.001) | 188 |
| LVMI (g/m2) | -47.9 (<0.001) | -78.2(<0.001) | 28.4 |
| Aortic valve calcification grade | -1.29 (<0.001) | -0.70 (<0.001) | 0.79 |
| Intima Media Thickness (mm) | -0.20(0.001) | -0.04(0.088) | 0.11 |
| Plaque score | -2.97 (<0.001) | -0.56(0.037) | 1.85 |

(p value vs Group C)

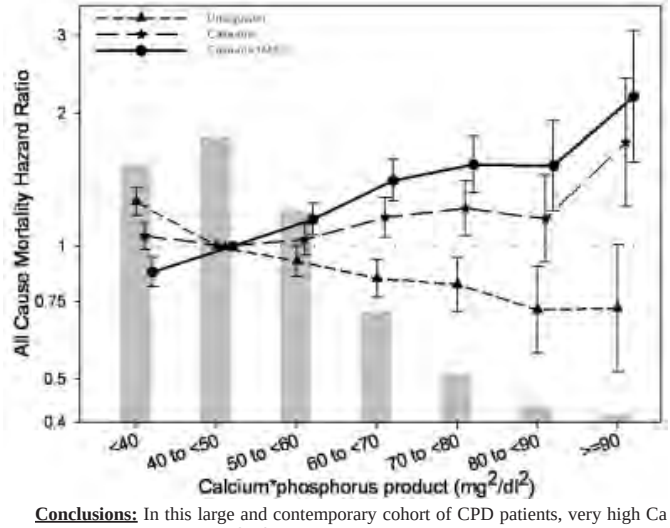
There was no association between the change in CV parameters and month of icodextrin started.Conclusion : Icodextrin can be started whenever it is necessary even from the start of PD for the sake of better CV management.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO882

**Association of Serum Calcium-Phosphorus Product and 6-Year Mortality in Chronic Peritoneal Dialysis Patients** Uyen Duong,[1] Rajnish Mehrotra,[2] Csaba P. Kovesdy,[3] Lilia R. Lukowsky,[1] Allen R. Nissenson,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Salem VA Medical Center, Salem, VA; [4]Davita, Lakewood, CO.*

**Background:** Previous studies have showed that higher serum calcium (Ca) and phosphorus (P) levels and their product (Ca x Phos) were incrementally associated with increased death risk in hemodialysis patients. We hypothesized a similar mortality predictability of Ca x Phos product in chronic peritoneal dialysis (CPD) patients. **Methods:** We examined a large contemporary cohort of CPD patients who underwent CPD treatment for at least 90 days in any DaVita dialysis clinic from July 2001 through June 2006, with additional survival follow up until June 2007. **Results:** We identified 12,269 CPD patients whose serum minerals were measured during the 5 year cohort. They were 54±16 year old and included 47% women, 23% African Americans and 13% Hispanics. Ca x Phos product was categorized into 7 a priori selected groups of <40 $mg^2/dl^2$ to ≥ 90 $mg^2/dl^2$ and 5 groups of 10 $mg^2/dl^2$ increments in-between (reference: 40-<50 $mg^2/dl^2$). A Ca x Phos product greater than 90 $mg^2/dl^2$ was associated with 2-times increased death risk; Death hazard ratio (95% CI) was 2.1(1.5-2.9) (See Figure).




**Conclusions:** In this large and contemporary cohort of CPD patients, very high Ca x Phos product, esp >≥ 90 $mg^2/dl^2$, is associated with increased death risk. Examining interventions to reduce Ca x Phos product are warranted.

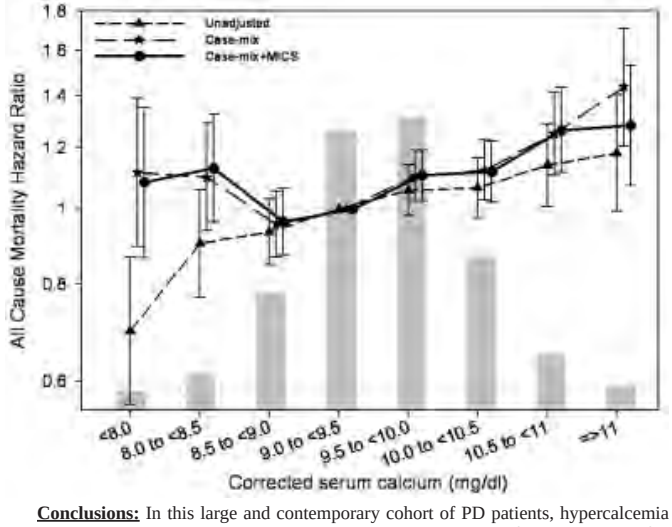
Disclosure of Financial Relationships: nothing to disclose

**TH-PO883**

**Serum Calcium and Mortality in Peritoneal Dialysis Patients** Uyen Duong,[1] Rajnish Mehrotra,[2] Csaba P. Kovesdy,[3] Lilia R. Lukowsky,[1] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Salem VA Medical Center, Salem, VA.*

**Background:** Hypercalcemia independently increases death risk in hemodialysis (HD) patients. However, it is not clear if such associations apply to peritoneal dialysis (PD) patients.

**Methods:** We examined a large contemporary cohort of all PD patients who underwent dialysis treatment for at least 90 days in DaVita dialysis from July 2001 through June 2006 and followed up to June 2007. All serum calcium values measured within a 3-month calendar quarters were averaged into one single value. Associations with mortality were examined in Cox models. **Results:** We identified 12,173 PD patients who had calcium measure during their base calendar quarter; they were 54.4±16.4 years old and included 47% women, 23% African Americans and 13% Hispanics. Albumin adjusted calcium was categorized into 8 a priori selected groups of <8.0mg/dl to ≥ 11.0 mg/dl and 6 groups of 0.5 mg/dl increments in-between. Taking calcium 9.0-9.5 mg/dl as the reference, calcium levels greater than 9.5 mg/dl was associated with increase death risk after adjusting for demographics, co-morbid conditions and additional malnutrition-inflammation complex syndrome (MICS). Death hazard ratio (HR) and 95% CI for calcium in 9.5-<10 mg/dl, 10.0-<10.5mg/dl, 10.5-<11.0 and ≥11.0 mg/dl were 1.10(1.02-1.19); 1.11 (1.02-1.22); 1.27 (1.12-1.44) and 1.28(1.07-1.53), respectively. (See Figure).



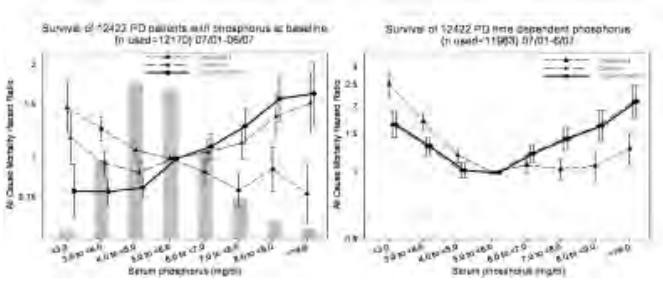

**Conclusions:** In this large and contemporary cohort of PD patients, hypercalcemia appears independently associated with increased death risk, after controlling for demographic and laboratory data. In particular, serum calcium >9.5 mg/dL is associated with mortality.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO884**

**Serum Phosphorus and 6-Year Mortality in Chronic Peritoneal Dialysis (CPD) Patients** Uyen Duong,[1] John J. Sim,[1] Rajnish Mehrotra,[2] Csaba P. Kovesdy,[3] Allen R. Nissenson,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Salem VA Medical Center, Salem, VA; [4]Davita, Lakewood, CO.*

**Background:** Both very low and high serum phosphorus (phos) levels are related to mortality in maintenance hemodialysis patients (pts). The mortality predictability of phos in chronic peritoneal dialysis (CPD) pts may be different. **Methods:** We examined a large contemporary cohort of over 12,000 CPD pts who underwent peritoneal dialysis treatment for at least 90 days in DaVita dialysis clinics from July 2001 through June 2006 and were followed up to June 2007. Baseline and time-dependent Cox models were examined. **Results:** We identified 12,170 CPD pts whose serum phos levels were measured; they were 54.4±16.4 year old and included 47% women, 23% African Americans and 13% Hispanics. Phos was categorized into 8 a priori selected groups of <3.0, ≥9.0 and 6 groups of 1 mg/dL increment in-between. Taking phos 5.0-6.0 mg/dl as reference, phos greater than 7.0 was associated with increased death risk after adjusting for demographics, co-morbid conditions and surrogates of malnutrition-inflammation complex syndrome (MICS). Death hazard ratios (and 95% CIs) in baseline model (Left Figure) for phos in 7.0-<8.0 mg/dl, 8.0-<9.0 mg/dl, ≥9.0 mg/dl were 1.3(1.1-1.5); 1.6(1.3-1.9) and 1.6(1.3-2.0), respectively, whereas lower baseline phos levels (<5.0mg/dl) appeared protective: 4.0-<5.0 mg/dl, 3.0-<4.0 mg/dl, <3.0 mg/dl were 0.79 (0.65-0.96); 0.78 (0.71-0.86); 0.81 (0.75-0.87), respectively. Time-dependent model (Right Figure) showed a U-shaped association.



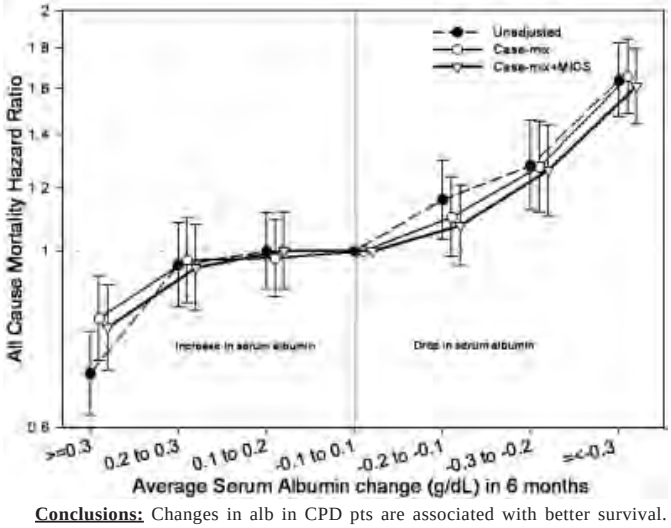

**Conclusions:** In this large and contemporary cohort of CPD pts, hyperphosphatemia (>7.0mg/dl) appears an incremental death risk.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO885**

**Changes in Serum Albumin over 6 Months Are Associated with Significant Changes in Survival in Peritoneal Dialysis Patients** Uyen Duong,[1] Rajnish Mehrotra,[2] Csaba P. Kovesdy,[3] John J. Sim,[5] Lilia R. Lukowsky,[1] Allen R. Nissenson,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Salem VA Medical Center, Salem, VA; [4]DaVita, Lakewood, CO; [5]Kaiser Permanente, Los Angeles, CA.*

**Background:** Higher serum albumin (alb) is by far the strongest predictor of greater survival compared to other outcome predictors in dialysis patients (pts). It is not known whether changes in alb over time are associated with survival in chronic peritoneal dialysis (CPD) pts independent of baseline alb and other covariates. **Methods:** We examined a large cohort of all CPD pts who underwent peritoneal dialysis treatment for at least 90 days in any DaVita dialysis clinic from July 2001 through June 2006 and followed their survival up to June 2007. Associations with all-cause mortality were examined in Cox models. Albumin change within -0.1 and +0.1g/dL over 6 month were considered stable (reference group). Pts whose alb changed beyond +/-0.1 g/dl were subdivided into decrements or increments of 0.1 g/dL.**Results:** We identified 8,568 CPD pts who survived and had alb measured at baseline and the next 6 months. They were 54±16 years old and included 47% women, 22% African Americans and 15% Hispanics. After adjusting for baseline alb, demographics and surrogates of malnutrition-inflammation-cachexia syndrome, patients with fall in alb between 0.2 and 0.3 g/dl and greater than 0.3 g/dl had all-cause death hazard ratio (HR) and 95% confidence interval (95% CI) of 1.3(1.1-1.4) and 1.6(1.4-1.8), respectively, whereas a rise in alb beyond 0.3 g/dl exhibited an HR of 0.8(0.7-0.9) (Figure).




**Conclusions:** Changes in alb in CPD pts are associated with better survival. Interventional studies to modulate serum albumin are indicated.

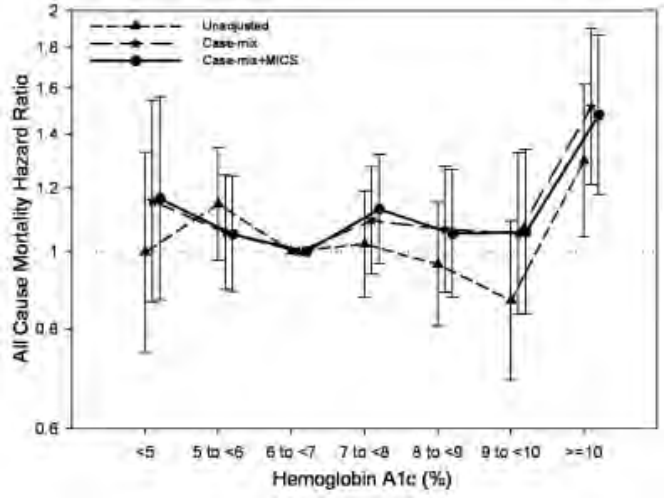
Disclosure of Financial Relationships: nothing to disclose

**TH-PO886**

**Hemoglobin A1c and 6-Year Survival in 2,798 Chronic Peritoneal Dialysis Patients with Diabetes Mellitus** Jong C. Park,[1] Rajnish Mehrotra,[2] Uyen Duong,[1] John J. Sim,[4] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Salem VA Medical Center, Salem, CA; [4]Kaiser Pamanente, Los Angeles, CA.*

**Background:** There is mixed data about the mortality predictability hemoglobin A1c in diabetic hemodialysis patients. In chronic peritoneal dialysis (CPD) patients, the association may be confounded by glucose loading in PD fluid. **Methods:** We examined a large cohort of diabetic CPD patients who underwent peritoneal dialysis treatment for at least 90 days

in any DaVita dialysis clinic from July 2001 through June 2006 and followed up to June 2007. **Results:** We identified 2,798 diabetic CPD patients who had A1c measure during their base calendar quarter; they were 58±12.8 years old and included 44% women, 20% African Americans and 16% Hispanics. A1c was then categorized into 7 a priori selected groups of <5%, >=10% and 1% increments in-between. A U-shaped trend with death hazard ratios (HR) was noted. Taking A1c 6-6.9% as the reference, A1c>=10% had a 5-year death HR (and 95% confidence interval [CI]) of 1.3 (1.1-1.6), 1.5 (1.2-1.9) and 1.5 (1.2-1.9) representing the unadjusted, case-mix (gender, age, race, ethnicity, dialysis vintage, type of insurance, 10 comorbid conditions, smoking residual renal function and Kt/V) adjusted and additional malnutrition-inflammation complex syndrome (MICS) (BMI, serum albumin, ferritin, creatinine, phosphorus, calcium, bicarbonate, TIBC, WBC, and lymphocyte percentage and blood hemoglobin) adjusted models, respectively (see figure).




**Conclusions:** In this large national cohort of diabetic CPD patients, A1c≥10% appears associated with 50% increased mortality compared to A1c 6-7%. Clinical trials to examine the benefit of glycemic control in CPD patients are indicated.

Disclosure of Financial Relationships: nothing to disclose

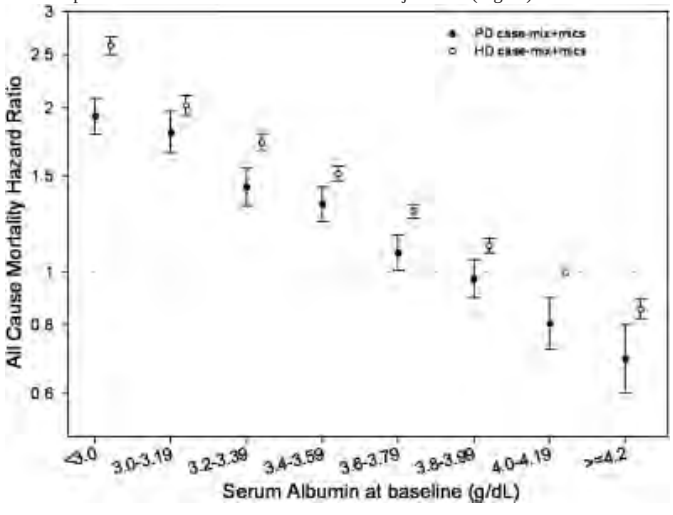
## TH-PO887

**Comparing Mortality-Predictability of Serum Albumin Levels between Hemodialysis and Peritoneal Dialysis Patients** Rajnish Mehrotra, John J. Sim, Uyen Duong, Lilia R. Lukowsky, Allen R. Nissenson, Csaba P. Kovesdy, Kamyar Kalantar-Zadeh.

**Background:** Serum albumin is an independent and strong predictor of mortality in both hemodialysis (HD) and peritoneal dialysis (HD) patients. It is not known if this mortality predictability is different between HD and PD patients.

**Methods:** We examined a large and contemporary cohort of all HD and PD patients who underwent dialysis treatment for at least 90 days in any DaVita dialysis clinic from July 2001 through June 2006 with survival follow-up till June 2007. Associations with all-cause mortality were examined in Cox models.

**Results:** We identified 120,592 HD and 12269 PD patients who had serum albumin measurements during their base calendar quarter; they were 62±16 and 54±16 years old and included 45% and 47% women and 32% and 22.6% African Americans, respectively. HD and PD patients were then divided into 8 albumin increments of albumin <3g/dL and ≥ 4.2g/dL and 0.2 g/dL intervals in-between. Taking HD patients with albumin 4.0-<4.2g/dL as a reference, we found that in any given albumin groups, PD patients had better survival than HD patients even after extensive multivariate adjustment (Figure).




**Conclusions:** In this large nationally representative cohort of HD and PD patients higher serum albumin was associated with greater survival and PD patients appeared to have superior survival to HD patients given same serum albumin level.

Disclosure of Financial Relationships: Research Funding: Baxter Health Care, Genzyme, Amgen; Honoraria: Baxter Health Care, Shire, Mitsubishi; Scientific Advisor: Novashunt.

## TH-PO888

**The Impact of Dialysis Modality on Incidence of 2009 Pandemic H1N1 Influenza in ESRD Patients** Jang-Hee Cho,[1,4] Young-Deuk Yoon,[1,4] Ja-Yong Park,[1,4] Eun-Joo Song,[1,4] Ji-Young Choi,[1,4] Se-Hee Yoon,[1,4] Sun-Hee Park,[1,4] Chan-Duck Kim,[1,4] Jong-Won Park,[2,4] Jun-Young Do,[2,4] Duk Hyun Lee,[3,4] Sung-Ho Kim,[3,4] Yong-Lim Kim.[1,4] *[1]Nephrology, Kyungpook National University School of Medicine; [2]Nephrology, Yeungnam University Hospital; [3]Nephrology, Daegu Fatima Hospital; [4]Clinical Research Center for End Stage Renal Disease in Korea.*

**Background.** This study evaluated the effect of dialysis modality on the incidence of 2009 pandemic H1N1 influenza in ESRD patients and identified the characteristics of dialysis patients with pandemic H1N1 influenza.

**Methods.** We defined the dialysis population as dialysis patients regularly visiting three general hospitals from Sep 2009 to Dec 2009. Patient demographics and comorbidities were evaluated, with analysis of comorbidity based on the Davies comorbidity score. We surveyed clinical data derived from interviews and chart reviews for all laboratory-confirmed patients.

**Results.** As of Dec. 31, 2009, 862 dialysis patients were recruited for the dialysis population and 18 cases of pandemic H1N1 influenza were confirmed from three general hospitals. H1N1 incidence was lower in the peritoneal dialysis (PD) group (4/525, 0.8%) than in the hemodialysis group (14/337, 4.2%, $P = 0.001$). The PD group was younger (53.6 ± 14.1 vs 57.3 ± 13.0, $P < 0.001$) and had lower Davies comorbidity scores ($P < 0.001$). Patients with pandemic H1N1 influenza were younger compared to the general dialysis population (46.6 ± 14.0 vs 55.1 ± 13.8, $P = 0.010$). Fifty-six percent had more than one comorbidity and the most common symptoms were fever and cough (77.8%). All patients were treated with oseltamivir except one patient with zanamivir. 6 patients (33.3%) were admitted and the median hospitalization length was 15 days (range 2-51). The mean age (58.5 ± 10.3 vs 39.9 ± 11.5, $P = 0.004$), Davies score ($P = 0.016$), and proportion of pneumonia (50.0% vs 0.0%, $P = 0.025$) among inpatients with pandemic H1N1 influenza were higher compared to outpatients.

**Conclusion.** Incidence of pandemic H1N1 influenza in dialysis patients was lower in the PD group. Hospitalization rate in dialysis patients was associated with older age, comorbidity, and presence of pneumonia

Disclosure of Financial Relationships: nothing to disclose

## TH-PO889

**Regional Variations in Length of Time Treated with Peritoneal Dialysis (PD) in United States** Rajnish Mehrotra,[1] Kenneth Story,[2] Steven Guest,[2] Michelle Fedunyszyn.[2] *[1]LA Biomed at Harbor-UCLA, CA; [2]Baxter HealthCare, IL.*

**Background**: The length of time a patient is treated with PD (PD time on therapy, PD-TOT) can be prolonged by reducing mortality rate and transfer to HD. Thus, PD-TOT is potentially an important measure for continuous quality improvement. We tested the null hypothesis that there is no relationship between facility neighborhood characteristics and geographic location on PD-TOT, censored for renal transplantation, among new U.S. PD patients using Baxter supplies from 2004 to 2009.

**Methods**: Survival analyses were used to estimate the association of period prevalent facility census, dialysis facility zip code level neighborhood characteristics (per capita income, % household units occupied by owner, % residents > 25 yrs with high school diploma, % with college degree, % Blacks) and geographic location (End-Stage Renal Disease Network) with PD-TOT.

**Results**: Of the 62,948 eligible subjects, zip code information was available for 58,700 subjects (55% men, known diabetic, 35%). The median PD-TOT for this cohort was 24 months. Except for a small effect size of per-capita income, neither the facility neighborhood characteristics nor the period prevalent patient census were predictive of PD-TOT. PD-TOT was significantly longer for patients in Networks 14, 15, 17, and 18 but shorter for patients in Network 11 (AHR, 1.09 (1.03, 1.15)). The longest PD-TOT was for patients in Network 18 (33 months) and shortest for patients in Network 11 (20 months). The probability of < 90 d PD-TOT was lower for patients treated in Networks 13, 15, 17, and 18.

**Conclusions**: To our knowledge, this is the first study to describe that PD-TOT varies considerably by geographic location in the United States and this difference is not explained by differences in either the socio-economic characteristics of facility neighborhood, or period prevalent facility census. Investigating the reasons underlying regional differences may help identify best-demonstrated practices that could prolong PD-TOT and reduce morbidity of PD patients.

Disclosure of Financial Relationships: Research Funding: Baxter Health Care, Genzyme, Amgen; Honoraria: Baxter Health Care, Shire, Mitsubishi; Scientific Advisor: Novashunt.

**TH-PO890**

**Association of Facility Census and Location with Transfer of Peritoneal Dialysis (PD) Patients to Hemodialysis (HD)** Rajnish Mehrotra,[1] Kenneth Story,[2] Steven Guest,[2] Michelle Fedunyszyn.[2] *[1]LA Biomed Res Inst at Harbor-UCLA, CA; [2]Baxter Health Care, IL.*

In most recent cohorts, there is no difference in the five-year adjusted survival of HD and PD patients. However, the risk of HD transfer remains high for PD patients. We tested the relationship between facility neighborhood characteristics and geographic location on risk of HD transfer among new PD patients using Baxter supplies from 2004 to 2009 (n=58,700). Survival analyses were used to estimate the association of period prevalent facility census, dialysis facility zip code level neighborhood characteristics (per capita income, % household units occupied by owner, % residents > 25 yrs with high school diploma, % with college degree) and geographic location (end-stage renal disease network) with time to HD transfer. Logistic regression was used to identify predictors of early transfers (within first 90 d). At a median follow-up of 45 months, 29% transferred to HD; 6% of all transfers were early. The overall transfer risk was significantly lower in facilities with 5-15 patients (adjusted hazards ratio (AHR) (95% confidence interval), 0.93 (0.89, 0.97)). However, there was no relationship between facility census and early transfer. There was no association between facility neighborhood characteristics and HD transfer, including early transfer. The overall risk for HD transfer was significantly lower in Networks 1, 5, 9, 10, 14, 17, and 18 but higher for patients in Network 16 (AHR, 1.10 (1.01, 1.19)). The risk for early transfer was lower for patients treated in Network 18 (adjusted odds ratio (AOR), 0.84 (0.72, 0.98)) but higher in Network 16 (AOR, 1.25 (1.06, 1.48)). To conclude, our study suggests that the relationship between risk for HD transfer and facility census may not be linear. To our knowledge, this is the first study to describe that the risk for HD transfer varies considerably by geographic location in the United States and this difference is not explained by differences in socio-economic characteristics of facility neighborhood. Investigating the reasons underlying regional differences may help identify best-demonstrated practices that could reduce transfers of PD patients to HD.

Disclosure of Financial Relationships: Research Funding: Baxter Health Care, Genzyme, Amgen; Honoraria: Baxter Health Care, Shire, Mitsubishi; Scientific Advisor: Novashunt.

**TH-PO891**

**Determinants of Peritoneal Dialysis (PD) Uptake: Physician Bias or Renal Clinic Practice?** Jay Hingwala,[1] Jeffrey Edward Diamond,[2] Paul Klassen,[3] Manish M. Sood,[1] Claudio Rigatto,[1] Mauro Verrelli,[1] Joe A. Bueti,[1] Paul Komenda.[1] *[1]Medicine, University of Manitoba, Winnipeg, MB, Canada; [2]TRLabs, Winnipeg, MB, Canada; [3]InfoMagnetics Technologies Corporation (IMT), Winnipeg, MB, Canada.*

***Background*:** ESRD rates are rapidly increasing, placing great burdens on healthcare resources. PD as an initial dialysis modality has shown more cost effective than Hemodialysis with at least equal survival. There are relatively few contraindications limiting its use. Significant variation in PD penetration patterns exist all over the world, suggesting a complex interplay of patient, social, economical, & political factors. In Canada, the prevalence rates for PD range from 14-35%, with significant variation within the same healthcare regions. Our study attempts to isolate the effect of differing practice patterns on initiation of PD as a renal replacement therapy in Manitoba (MB).

***Methods*:** We performed a retrospective review of the MB Renal Program Database from 2004–2010 comprising over 1081 records of patients starting dialysis. We calculated rates of PD starts by individual nephrologist associated with each patient. The majority were followed in a multidisciplinary clinic. We used a propensity matched genetic algorithm to adjust the impact of nephrologist for a wide array of demographic & comorbidity factors that could influence PD suitability.

***Results*:** The overall PD prevalence in MB ranged from 18-21% annually. The rate of PD starts by individual nephrologist associated with a patient ranged from 0.16–0.46 with a mean of 0.33 ($p<0.01$). Individual nephrologist remained a significant predictor of PD uptake after propensity matching. Patients with Polycystic Kidney Disease ($p<0.02$), glomerulonephritis ($p<0.001$), younger age ($p<0.01$) & of Caucasian race ($p=0.019$) were more likely to try PD, while a history of pulmonary edema ($p<0.002$) was a negative predictor.

***Conclusion*:** In a province with universally homogenous funding models, reimbursement schemes & clinic structures, it appears that individual nephrologist bias may be a significant modifiable factor in patient uptake of PD. To grow this modality further, health policy schemes should address this with improved education & clinic processes.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO892**

**Australian Nephrologist Survey Strongly Supports Home Therapies: Both HD and PD** John W. M. Agar, Carmel M. Hawley. *Home Dialysis Advisory Group, Kidney Health Australia, Melbourne, Victoria, Australia.*

**Background:** Australia remains significantly supportive of home-based dialysis therapies (HBDT). At 31.12.08, although 32% of all national dialysis was by HBDT (peritoneal dialysis (PD) 22% and home haemodialysis (HHD) 10%), a marked regional variation was noted in the 32 largest dialysis services, with HHD in particular varying 0% to >25%.

**Objective:** To survey Australian Heads of Units (HoU) and practicing nephrologists (PNx) regarding their attitudes to HBDT versus facility-based HD.

**Methods:** An online survey of all HoU and PNx was conducted in April 2009. 76 questions covered: unit demographics, medico/nursing staffing and respondent experience in HBDT (both PD and HHD), the current availability of HBDT training in units and unit training practices, the adequacy of facilities and support systems, the separate attitudes of respondents to recommending PD and/or HHD, and what perceived barriers might exist to inhibit any increase if the uptake of HBDT.

**Results:** 71 of ~300 known Australian nephrologists replied, representing 35% of all HoU and 16% of all PNx. Most Australian units were represented in the responses with most offering or accessing all modalities of care. In-centre HD was offered by 99% and satellite HD by 93%. Training and support was available and used for HPD (98%) and HHD (92%). 92% offered vascular access surgery and 90% Tenchkoff catheter insertion. 97% had onsite nephrology (bed mean = 15±9). 88% of all responses agreed longer-hour, more frequent HD confered an outcome advantage for HD. 93% believed this was best accomplished by HHD. PD was not considered inferior and 34% of PNx reported practicing a 'PD first' policy where possible. Social work, water, equipment maintenance and industry support was thought adequate but psychiatric support, respite care, home visits, dietitian access and concerns re patient-centric financial disadvantage for HHD were thought to be the major short-comings.

**Conclusion:** Despite long-hour HD and a further expansion of HBDT being strongly supported, HBDT is not currently proportionately expanding in Australia. This survey identified several perceived barriers to HBDT and where opportunities to expand HBDT utilisation exist.

Disclosure of Financial Relationships: Consultancy: Medical Advisory Board: Renal Solutions Inc. Warrendale, PA; Honoraria: Travel Assistance, Amgen Australia Travel Assistance, Fresenius Australia.

**TH-PO893**

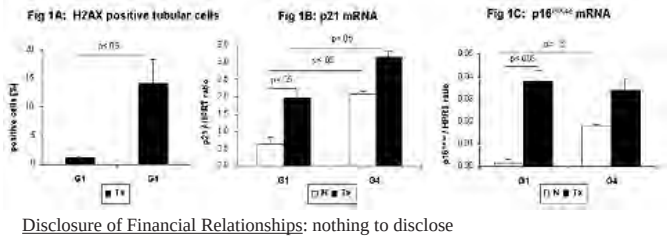
**Telomerase Knockout Mice – A Model for Renal Transplants with Limited Regenerative Capacity** Christoph Jacobi,[1] Andrea Weißbrodt,[1] Song Rong,[2] Verena Broecker,[3] Chunfang C. Wang,[4] Faikah Gueler,[2] Anette Melk.[1] *[1]Department of Pediatric Nephrology, Hannover Medical School, Hannover, Germany; [2]Department of Nephrology, Hannover Medical School, Hannover, Germany; [3]Department of Pathology, Hannover Medical School, Hannover, Germany; [4]Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Objectives: Cellular senescence results in reduced regenerative ability. Telomerase (Terc) KO mice show critically short telomeres in late generations. The outcome of Terc KO kidneys in a syngeneic life-supporting transplantation (tx) model is evaluated.

Methods: Early (G1,n=9) and late (G4,n=5) generation male Terc KO kidneys were transplanted into female WT recipients with nephrectomy of both recipient kidneys (d0+d4) and sacrificed after 6 wks.

Results: Survival after transplantation was not different between the groups (G1: 8/9, G4: 5/5). G4 transplants showed significantly more interstitial fibrosis (IF) (G4: .42, G1: .22 % of tubulo-interstitial area; $p<.05$) and a tendency to more glomerulosclerosis. Markers for telomere-dependent senescence were significantly higher in G4 transplants: more H2AX positive cells (fig.1A) and higher p21 mRNA expression (fig.1B). Interestingly, $p16^{INK4a}$ mRNA expression, a marker for telomere-independent, stress-induced senescence, even though it was higher in G4 kidneys prior to tx, was not different after tx between groups (fig.1C).

Conclusions: Mild transplantation stress induced telomere-dependent and -independent senescence. This was more pronounced in G4 mice leading to increased IF, reflecting the situation of old donor transplants. The increases in $p16^{INK4a}$ seen also for G1 mice suggest that senescence can be triggered even in ideal donors. As this tx model enables us to mimic the human situation, it should be used to explore factors that reduce regeneration even in optimal transplants.




Disclosure of Financial Relationships: nothing to disclose

**TH-PO894**

**A Novel Clinically Relevant Strategy To Abrogate Autoimmunity and Regulate Alloimmunity** Andrea Vergani,[1] Mohammed Javeed Ansari,[2] Mohamed H. Sayegh,[1] Paolo Fiorina.[1] *[1]Childrens' Hospital-Harvard Medical School, Boston; [2]Northwestern Memorial Hospital, Chicago.*

OBJECTIVES—To investigate a new clinically relevant immunoregulatory strategy based on treatment with murine Thymoglobulin (mATG) and CTLA4-Ig in NOD mice in order to prevent allo- and autoimmune activation using a stringent model of islet transplantation and diabetes reversal.

RESEARCH DESIGN AND METHODS— Using allogeneic islet transplantation models as well as NOD mice with recent onset type 1 diabetes, we addressed the therapeutic

efficacy and immunomodulatory mechanisms associated with a new immunoregulatory protocol based on prolonged low-dose mATG plus CTLA4-Ig.

RESULTS— BALB/c islets transplanted into hyperglycemic NOD mice under prolonged mATG+CTLA4-Ig treatment showed a pronounced delay in allograft rejection compared with untreated mice (mean survival time: 54 vs. 8 days, $p<0.0001$). Immunological analysis of mice receiving transplants revealed a complete abrogation of autoimmune responses and severe downregulation of alloimmunity in response to treatment. The striking effect on autoimmunity was confirmed by 100% diabetes reversal in newly hyperglycemic NOD mice and 100% indefinite survival of syngeneic islet transplantation (NOD.SCID into NOD mice).

CONCLUSIONS— The capacity to regulate alloimmunity and to abrogate the autoimmune response in NOD mice in different settings confirmed that prolonged mATG+CTLA4-Ig treatment is a clinically relevant strategy to translate to humans with type 1 diabetes.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO895

**Characterization of Regulatory and Effector T Cells in Renal Transplant Recipients after Induction with Thymoglobulin vs. Basiliximab: An Interim Report** Sacha A. De Serres, Bechara G. Mfarrej, Monica Grafals, Ciara N. Magee, Adam Hetland, Christine Dyer, Anil Chandraker, Nader Najafian. *Transplantation Research Center, Brigham & Women's Hospital, Harvard Medical School, Boston, MA.*

ATG (Thymoglobulin) at low, non-depleting doses, promotes expansion and *de novo* generation of human regulatory T cells (Tregs) *in vitro*. We hypothesized that ATG given at standard induction doses may also expand Tregs *in vivo* in kidney transplant recipients.

22 subjects enrolled in this ongoing, single-center, prospective, longitudinal observational study. Subjects received induction with ATG (1.5 mg/kg/day x 5 days, n=11) or basiliximab (20 mg on days 0 and 4, n=11) and were maintained on Prograf and Cellcept. Blood was collected before (n=22) and at 3 (n=21), 6 (n=19) and 12 (n=7) months after transplant. $CD4^+$ T cell subsets including $25^+Foxp3^+$ (Tregs), $45RO^+$ (memory) and $25^{hi}45RA^+31^+$ (recent thymic emigrant nTregs) were monitored by flow cytometry. Statistical analysis of absolute cell counts and ratios were performed using mixed models.

ATG led to profound depletion of $CD4^+$ (pretransplant, 3, 6, 12mo: 0.60, 0.12, 0.15, 0.20 $x10^3/\mu L$; $p<0.01$), Tregs (0.026, 0.008, 0.009, 0.009; $p<0.01$) and memory T cells (0.43, 0.09, 0.12, 0.10; $p<0.01$). In contrast, basiliximab did not cause significant depletion of $CD4^+$ (0.50, 0.49, 0.48, 0.47; $p=0.01$ *vs* ATG) or memory T cells (0.37, 0.30, 0.27, 0.25; $p=0.02$ *vs* ATG), but intriguingly led to reduction in the Treg population (0.023, 0.011, 0.014, 0.013; $p=0.22$ *vs* ATG). Thus, the percentage of Tregs among $CD4^+$ cells increased in the ATG (4.3, 6.8, 6.8, 8.0%), but decreased in the basiliximab group (4.7, 2.8, 2.9, 2.5; $p<0.04$ *vs* ATG). Over time, there was a trend towards a higher ratio of Tregs:Teffector cells with ATG (0.06, 0.10, 0.10, 0.21) compared to basiliximab (0.06, 0.05, 0.05, 0.07; $p=0.08$). There was no evidence of an increase in the thymic emigrant proportion within Tregs in either group.

These observations suggest that induction with ATG, but not basiliximab, leads to an increase in Tregs:Teffector ratio, mainly by relative Treg sparing. It further suggests that thymic output of nTregs is not affected by these drugs.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO896

**Prolonged Allograft Survival with Partial Deletion of T-bet in a Model of Chronic Rejection** Bara Sarraj,[1] Vishnupriya Samarendra,[1] Guodong Chen,[1] Omar A. Shah,[1] Zheng Jenny Zhang,[1] Mohamed H. Sayegh,[2] Mohammed Javeed Ansari.[1] *[1]Comprehensive Transplant Center, Northwestern University Feinberg School of Medicine, Chicago; [2]Brigham and Women's Hospital, Boston.*

INTRODUCTION: T-bet, which affects T helper cell differentiation, plays an important role in inhibiting proinflammatory Th17 type immune response while positively regulating Th1 immunity in a dose-sensitive manner. Reduced T-bet expression might afford protection from both Th1/Th17 mediated allograft rejection and vasculopathy, allowing long-term allograft survival and tolerance.

METHODS: We investigated graft survival and alloimmune responses in T-bet+/- recipients, with appropriate controls in an established MHC class-II mismatched (bm12 into B6) model of chronic cardiac allograft rejection, 14 days post-transplantation by multi-parameter flow-cytometry (MFC), cytokine bead array (CBA) and allograft histology.

RESULTS: Partial deletion of T-bet gene reversed the Th17 phenotype in T-bet-/- recipients and graft survival was significantly prolonged in T-bet+/- compared to WT and T-bet-/- (83%, 66%, 0% respectively; $p<0.05$). Leukocyte infiltration 14 days post-transplantation in grafts from WT, T-bet+/- and T-bet-/- recipients were of lymphocytic, mixed lymphocytic/granulocytic and predominantly granulocytic in nature, respectively. CD8 T cell infiltration and IL-17 expression in grafts is reduced in T-bet+/- compared to WT and T-bet-/-. In vitro, T-bet+/- splenocytes produced higher levels of IL-10 compared to WT and T-bet-/- (4804.3, 1834.9, 2475.3pg/ml respectively; $p<0.05$). There was a corresponding increase in IL-10 producing CD4 T cells in the grafts (5.3, 1.19, 2.14% respectively).

CONCLUSION: In a model of chronic cardiac allograft rejection, reduced, but not absent, T-bet expression promotes longer allograft survival by modulating the proinflammatory cytokine IL-17 by CD8 T cells and regulatory cytokine IL-10 production by CD4 T cells. These data suggest that strategies targeting both Th1/Th17 cytokines may prevent chronic rejection and promote tolerance.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO897

**ESRD-Related Premature Ageing of T Cells Significantly Decreases the Risk for Acute Rejection after Renal Transplantation** Michiel G. H. Betjes, Elly L. E. A. De Wit, Willem Weimar, Nicolle H. R. Litjens. *Dept of Nephrology, Erasmus Medical Center, Rotterdam, Netherlands.*

**Background:** ESRD is associated with an impaired T cell immunity which may be related to excessive premature ageing of circulating T cells. In this study the hypothesis was tested that ESRD-related premature ageing decreases the risk for acute rejection after renal transplantation.

**Patients and methods:** Prior to transplantation the circulating CD4pos and CD8pos T cells of 188 ESRD patients were analyzed for their differentiation profile using the combination of markers;CD4, CD8, CD45RO, CCR7 and CD28 . Healthy individuals matched for age, socio-economic status and CMV-seropositivity were included as controls. The basic immune suppressive drug regimen consisted of MMF, tacrolimus and prednisone. After transplantation, the patients were followed for 1-2 years.

**Results:** Forty-eight acute rejections were observed of which 95% occurred in the first year after transplantation. Overall, patients showed a significant decreased number of circulating naïve T cells and an increased terminal differentiation of memory T cells both in the CD4pos and CD8pos compartment, compatible with the notion of ESRD-related premature ageing. In many aspects, premature ageing of T cells was less or absent in rejectors. Rejectors as compared to non-rejectors, had a significantly higher number of HLA-mismatches with the donor kidney, a higher number of circulating CD4 T cells, and a lower percentage of highly differentiated CD4 and CD8 memory T cells negative for CD28. In a multivariate analysis, only HLA mismatches and the percentage of highly differentiated CD8 T cells (known as Temra cells) were significantly related to acute rejection. Patients in the lowest tertile of Temra cells had a 4-fold increased risk for acute rejection compared to patients in the highest tertile (11% versus 43 %, $p<0.01$).

**Conclusion:** ESRD-related premature ageing, specifically of the CD8pos T cells, yields significant protection against acute rejection after renal transplantation. Pre-transplantation assessment of T cell ageing offers a promising new concept for patient-tailored immune suppressive regimens.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO898

**Apoptotic Endothelial Cell Microenvironment Reprograms Macrophages into Reparative Cells** Jean-Francois Cailhier, Stephanie Lepage, Marie-Joelle Brissette. *Medicine, CRCHUM, Centre Hospitalier de l'Université de Montréal, Institut du Cancer de Montréal, Montreal, QC, Canada.*

Chronic transplant vasculopathy (CTV) is an important cause of interstitial fibrosis and tubular atrophy in the renal allograft. It is characterized by the presence of apoptotic endothelial cells (EC), which generate a microenvironment that leads to the typical myointimal proliferation found in CTV. Macrophages (Mφ) play an important role in CTV. However, the exact nature of the reprogramming induced by this microenvironment on Mφ phenotype and potential mediators responsible are ill defined.

Human umbilical vein and murine EC were serum-starved for 4 h as a model of apoptotic EC [apoptotic serum-starved conditioned medium (SSC)]. Non-apoptotic SSC was generated where apoptosis was prevented by caspase-3 inhibition prior to starvation (caspase-3 inhibitor, SSC-DEVD vs SSC-DMSO control). A proteomic approache was used to identify a secretome specific to apoptotic EC. To evaluate reprogramming, we stimulated different leukocyte subsets. Monocytes were harvested from healthy donors by Ficoll gradient and immunomagnetic selection and matured for 5-7 days to generate monocyte-derived Mφ (HMφ). HMφ and murine bone marrow-derived Mφ (MMφ) were stimulated for 24h with SSC. Cells were harvested and supernatants kept for ELISA analysis to determine cytokine/chemokine production.

We identified that milk fat globule-epidermal growth factor 8 (MFG-E8) was secreted during apoptosis. Since MFG-E8 is important in Mφ biology, we characterized it further. Immunoblotting of apoptotic EC revealed that intracellular MFG-E8 decreased overtime whilst its secretion was increased in SSC. Caspase-3 inhibition prevented MFG-E8 release. MMφ produced more TGF-β and VEGF and less MIP-2 when stimulated with SSC-DMSO compared to SSC-DEVD. HMφ generated less IL-6, IL-8 and MCP-1 when exposed to SSC-DMSO.

This study constitutes the first description of the apoptotic-dependent release of MFG-E8 by EC. It also demonstrates the importance of the apoptotic microenvironment in the reprogramming of a regulatory, pro-fibrotic Mφ phenotype. These results suggest an important contribution of Mφ in the CTV-associated fibrosis.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO899

**Partial Deletion of T-bet Favorably Modifies the Alloimmune Response in Acute Allograft Rejection** Bara Sarraj,[1] Vishnupriya Samarendra,[1] Guodong Chen,[1] Omar A. Shah,[1] Zheng Jenny Zhang,[1] Mohamed H. Sayegh,[2] Mohammed Javeed Ansari.[1] *[1]Comprehensive Transplant Center, Northwestern University Feinberg School of Medicine, Chicago; [2]Brigham and Women's Hospital, Boston.*

INTRODUCTION: T-bet, a master regulator of Th1 immune response, plays an important role in inhibiting proinflammatory Th17 immune response. As T-bet regulates Th1 response in a dose-dependent manner, reduced T-bet expression might afford protection from both Th1/Th17 mediated allograft rejection, allowing long-term allograft survival and tolerance.

METHODS: We investigated the alloimmune responses in T-bet+/- recipients in an established full MHC-mismatched (BALB/c into B6) model of acute cardiac allograft rejection, 7 days post-transplantation by multi-parameter flow-cytometry (MFC), cytokine bead array (CBA) and allograft histology.

RESULTS: Leukocyte infiltration in grafts in WT, T-bet+/- and T-bet-/- recipients were of lymphocytic, mixed lymphocytic/granulocytic and predominantly granulocytic nature, respectively. Serum TNF-a was lower in T-bet+/- and WT compared to T-bet-/- (3.2, 3.5, 10.7pg/ml; $p<0.05$). CBA analysis following in vitro allostimulation revealed, proportional increase in IL-17 and decrease in IFN-g with T-bet gene zygosity. IL-10 was preserved in T-bet+/- and diminished in T-bet-/- compared to WT (24.3, 6.9, 22.9pg/ml; $p < 0.05$). Interestingly, MCP-1, the ligand for CCR2/CCR4 important in Th17 cell and Th17-induced monocyte recruitment, was reduced in T-bet+/- recipients compared to WT and T-bet-/- (22.6, 102.9, 65.9pg/ml; $p<0.05$). MFC analysis showed a decrease in CD4 memory generation and IL-17 production by these cells in the spleen and reduced graft-infiltration of IL-17 producing CD4 and CD8 memory cells in T-bet+/- compared to WT or T-bet-/-, whereas IL-10 and Foxp3 expression in CD4 cells was preserved in T-bet+/- recipients.

CONCLUSION: Reduced expression of T-bet has multiple beneficial effects on the acute alloimmune response: reduction in IL-17 and MCP-1, restoration of IL-10 and Foxp3 expression together may contribute to immune regulation leading to reduced memory cell generation and infiltration of grafts and facilitate longer graft survival in T-bet+/- recipients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO900

**T Regulatory Cells (CD4+CD25+FOXP3+) Function in Transplant Recipients with Chronic Allograft Dysfunction** Raj K. Sharma, Sharad Mittal, Amit Gupta, Sita Naik. *Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, UP, India.*

We studied the role of CD4+CD25+FOXP3 T regulatory cells (Tregs in renal transplant patients with chronic graft dysfunction. Patients with Gr-I chronic graft dysfunction (n=20, Serum Creatinine; range 2.0 to 4.23mg/dl), Gr-II patients with normal graft function (n=20, Serum Creatinine; 0.8 to 1.5mg/dl) for >2.5 years, Gr-III healthy volunteers (n=10) were enrolled. Of 20 patients with chronic allograft dysfunction, 10 patients had evidence of immune mediated chronic rejection, post RT, 1-6 yrs and 10 had non immunological graft dysfunction (intestinal fibrosis or tubular atrophy ). CD4+CD25+FOXP3+ Tregs were enumerated by flow cytometry. CD4+CD25+ T cells (Tregs) and CD4+CD25- cells (Teffector) from PBMC of renal patients were isolated. Teff cells were stimulated with anti CD3 for 3 days and cocultured in the presense and absence of Tregs at ratios (Tregs:Teff) of 1:1 and 2:1. Proliferation was measured using the thymidine (H3) uptake. Suppression was calculated using the following formula: % suppression = [(Teff proliferation without Tregs-Teff proliferation with the Tregs)/Teff proliferation without Tregs)*100]. The median suppressive capacity of CD4+CD25+ Tregs of patients with chronic graft dysfunction (n=20) was 20.4% (range 0 to 51.29) at 1:1 ratio and 8.95% (0 to 28.5) at 2:1 ratio. This was significantly less than in patients with normal graft function at 1:1 ratio (median 66.38%; range 30.1 to 95.35; $p<0.05$) and at 2:1 ratio (23.3%; 0 to 59; $p<0.05$). Frequency of Tregs in patients with chronic allograft dysfunction (median 7.54%; range 2.61 to 15.45) and patients with normal graft function (8.21%; 3.25 to 19.1) was similar but higher than healthy control (5.53%; 3.79 to 10.79; $P<0.05$). We conclude that functionally Tregs in patients with chronic allograft dysfunction were less suppressive as compared to patients with normal graft function, However, expansion of Tregs was similar in both group of renal transplant patients with chronic graft dysfunction. Expanded Tregs with suppressive potential may be functionally important for the maintenance of the good graft function in renal transplant recipients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO901

**CXCR3+ and CCR5+ Tregs Positively Correlate with Renal Allograft Function in Patients Receiving a Tacrolimus Based Immunosuppression** Andre Hoerning,[1] Sebastian Köhler,[2] Jun Cao,[2] Jian Fu,[2] David M. Briscoe,[3] Thorsten Feldkamp,[2] Andreas Kribben,[2] Peter F. Hoyer,[1] Oliver Witzke.[2] *[1]University Hospital Essen, Pediatric II, Nephrology and Gastroenterology, University Duisburg-Essen, Essen, NRW, Germany; [2]University Hospital Essen, Nephrology, University Duisburg-Essen, Essen, NRW, Germany; [3]Transplantation Research Center, Harvard Medical School, Boston, MA.*

Background: Several chemokine ligands are expressed in allografts at early and late times post transplantation, and previous rodent studies have demonstrated that the chemokine receptors CCR5 and CCR7 play a major role in Treg cell recruitment. Here, we investigated whether the expression of the chemokine receptors CXCR3, CCR5 and CCR7 in CD4+FOXP3+ Tregs that are circulating in human peripheral blood of renal transplant patients correlate with allograft function (MDRD) up to 12 months after Tx.

Methods: Four color FACS analysis of PBMCs of 30 renal transplant recipients receiving a calcineurin inhibitor based immunosuppression for CD4, CD39, FOXP3 and CXCR3 or CCR5 or CCR7 was performed. Mean age in the studied group was 51 years (±13), mean time after Tx was 4.4 months (±3), mean cold ischemia time was 14 hrs (±6.7) and mean MDRD was 45 ml/min/1.73m2 (±11). Correlation analysis was performed with Pearson's test.

Results: The percentage of circulating CD4+FOXP3+ (6.1 ± 2.2%, n=30) or CD4+CD39+FOXP3+(1.8 ± 0.8%, n=25) Tregs did not correlate with MDRD. Among CD4+FOXP3+ cells mean expression of CXCR3 was 42 ± 7.8% (n=30), of CCR5 23 ± 9.2% (n=30) and of CCR7 20 ± 7.8% (n=30), respectively. Also, among CD4+CD39+FoxP3+ cells expression of CXCR3, CCR5 or CCR7 showed no correlation to MDRD. Subgroup analysis revealed that FOXP3+ Tregs that co-express CXCR3 or CCR5 ($r=0.72$, $p<0.01$ and $r=0.68$, $p=0.02$) positively correlated with MDRD in patients receiving tacrolimus (n=12), but not CsA (n=18). For CCR7 in both groups no correlation was found.

Conclusion: These results suggest a previously unrecognized graft-protective role of both CXCR3+FOXP3+ and CCR5+FOXP3+ Tregs circulating in the periphery of renal transplant recipients receiving a tacrolimus based immunosuppression.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO902

**Infiltrating Macrophages in Renal Allograft Glomerulitis Are Association with Peritubular Capillaries C4D Deposition** Juan Jin, Pingping Wu, Huiping Wang, Qiang He, Jianghua Chen. *Kidney Disease Center, The First Affiliated Hospital of Medical College, Zhejiang University, Hangzhou, Zhejiang, China.*

The aim of our study was to determine the amount and composition of immune cells within glomeruli and its relationship with peritubular capillaries (PTC) C4d deposition. Fifty-one biopsy specimens with acute rejection and glomerulitis were immunostained for macrophages, T cells and C4d. Gromeruli macrophages and T cells were counted and mean numbers of them per glomerulus were determined.

C4d staining was present in 26 biopsy specimens (C4d-positive [$C4d^+$] group) and negative in 25 specimens ($C4d^-$ group)**.** The total number of infiltrating cells in glomerulitis in $C4d^+$ group significantly higher than in $C4d^-$ group (17.79±7.70 vs 8.17±3.80, $P<0.0001$). Although the $C4d^+$ group showed a significantly higher mean number of macrophages per glomerulus (13.73±7.03) than in $C4d^-$ group (2.57±1.22, $P <0.0001$), the $C4d^-$ group showed a higher mean number of T cells per glomerulus (5.60±2.81) than in $C4d^+$ group (4.05±2.60, $P=0.023$). Comparing cell counts in diffuse $C4d^+$ and focal $C4d^+$ groups, a significant difference of absolute numbers of intracapillary cells could be observed in glomeruli ( 23.25±4.71 vs 14.49±6.86, $p=0.01$). The mean number of macrophages per glomerulus in diffuse $C4d^+$ group (19.62±4.97) was significantly greater than that of the focal $C4d^+$ group (9.11±4.48, $P<0.0001$). while mean T cells per glomerulus was less in cases of diffuse $C4d^+$ (3.64±1.50) than in focal $C4d^+$ glomerulitis (4.38±3.24). The differences however, did not achieve statistical significance ($p=0.74$). The total number of infiltrating cells in glomerulitis has association with PTC C4d deposition, the infiltrating cells were predominantly macrophages in $C4d^+$ group, especially in diffuse $C4d^+$ group, significantly higher than in the $C4d^-$ group. whereas, the infiltrating cells were predominantly T cells in $C4d^-$ group, greater than in $C4d^+$ group.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO903

**Bone Marrow Derived Immature Dendritic Cells Transfected with Adenovirus Carrying IDO Gene Suppress T Cell Proliferation in Allogeneic Reaction In Vitro** Diana Vavrincova-Yaghi,[1] Maria Sandovici,[1] Marc Seelen,[2] Peter Vavrinec,[1] Harry Van Goor,[3] Robert H. Henning,[1] Leo E. Deelman.[1] *[1]Clinical Pharmacology-Experimental Pharmacology, University Medical Center Groningen, Groningen, Netherlands; [2]Internal Medicine, University Medical Center Groningen, Groningen, Netherlands; [3]Pathology and Laboratory Medicine, University Medical Center Groningen, Groningen, Netherlands.*

Background: Kidney transplantation is the treatment of choice for patients with end-stage renal disease. Because current immunosuppressive therapy is accompanied by serious side-effects, new therapies are investigated. Indoleamine 2,3-dioxygenase (IDO) is involved in maternal tolerance and prevents rejection of allografts, including the kidney. However, the mechanisms still remain unclear. Immature dendritic cells (DC), characterized by low expression of MHC II and costimulatory-molecules, play pivotal role in the induction of tolerance. The aim of this study is to investigate, whether immature dendritic cells transfected with RGD modified adenovirus carrying IDO gene (RGD-AdTIDO) are able to suppress T cell proliferation in vitro.

Methods: Immature DC, obtained from bone marrows from Brown Norway (BN) rats, were transfected with RGD-AdTIDO at day 9 after harvesting. At day 11, cells were used as stimulators in mixed leukocyte reaction (MLR) with naive Lewis rat lymphocytes as responders. DC transfected with RGD modified adenovirus carrying gene for GFP (RGD-AdTL) and mature DC were used as a control. To assess phenotype of DCs, fluorescence-activated cell sorting (FACS) was performed. The proliferation of T cells was quantified by CyQUANT Proliferation Assay kit.

Results: FACS of RGD-AdTIDO transfected DC revealed significantly decreased expression of CD11c, CD86 and MHC II molecules ($p<0.05$), compared to mature DC. In co-culture with Lewis T cells, RGD-AdTIDO transfected immature DC significantly suppressed the proliferation, compared to RGD-AdTL transfected DC and mature DC ($p<0.05$).

Conclusion: Our results show that immature DCs expressing the IDO gene effectively inhibited T cell proliferation in alloreaction, indicating a therapeutic potential for IDO in preventing transplant allograft rejection.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO904**

**The Prevalence and Clinical Significance of HLA-Cw and HLA-DP Antibodies in Sensitized Patients on the Kidney Transplant Waiting List**
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Antibodies to HLA-Cw and HLA-DP have not been studied routinely in the management of kidney transplant recipients. We have studied anti-HLA antibodies of 778 patients on the kidney transplant waiting list by Luminex PRA (Panel Reactive Antibody). While 580 patients had 0% PRA, the remaining 198 patients had PRA > 0%. The specificity of antibodies to HLA-A, B, Cw, DR, DQ, and DP were determined by Luminex single antigen beads and the strength of the antibodies by median fluorescence intensity values (MFI). Of the 198 patients, 116 were female, 82 male, 90 African-American, median age of 51 (range, 20-79), median calculated PRA 45% (range, 2-100%) and median time on the waiting list was 763 days (range, 13-8,328). While 18 patients did not have any history of sensitization, 56% had blood transfusion, 41% pregnancy and 38% previous organ transplantation. 113 patients had more than one sensitizing event and 67 had only one sensitizing event: prior transplantation (32), blood transfusion (21), and pregnancy (14).The frequency of anti-HLA antibodies to HLA-Cw (55%) and HLA-DP (35%) were lower than HLA-A (77%), -B (86%), -DR 9 (68%) and DQ (71%) ($p < 0.01$). Median MFI values of the strongest antibodies were also lower in HLA-Cw (5,547) and HLA-DP (2,752) compared to HLA-A (11,318), -B (11,497), -DR (9,033), and –DQ (9,187) ($p < 0.01$). Unacceptable antigens were reported to UNOS if the MFI values were over 5,000. The percentage of unacceptable antigens was lower in HLA-DP (33%) and HLA-Cw (53%) compared to HLA-A (76%), -B (78%), -DR (67%) and DQ (70%) ($p < 0.01$). While the risk factors for the development of anti-HLA-A and HLA-DR antibodies were blood transfusion, pregnancy and previous transplantation, only a history of previous transplantation was a risk factor for HLA-Cw and HLA-DP, but not blood transfusion or pregnancy. Our results suggest that anti-HLA-Cw and HLA-DP are lower in both prevalence and intensity compared to anti-HLA-A, B, DR, and DQ, and that prior organ transplantation is the main sensitizing event.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO905**

**The Association between CYP3A4,CYP3A5,ABCB1 Genetic Polymorphisms and Kidney Grafe Rejection, CNI Nephrotoxicity**
Chuan-Ming Hao. *Huashan Hospital, Fudan University.*

**Background** CNI(calcineurin inhibitor) is a substrate of both cytochrome P450 3A (CYP3A) and P-glycoprotein (P-gp),some of the single nucleotide polymorphisms (SNPs) in these genes are associated with interindividual variations in CNI pharmacokinetics.We studied the influence of these SNPs on the incidence of acute cellular rejection(ACR),CNI nephrotoxicity,and anti-HLA antibody and anti-MICA antibody positive,which are associated with chronic rejection. **Methods** A total of 208 renal transplant recipients receiving CNI were genotyped for ABCB1 (C1236T,G2677T/A, and C3435T), CYP3A4*18B,and CYP3A5*3 by direct sequencing method,and presence of anti-HLA antibody and anti-MICA antibody of 94 renal transplant recipients were determined by Luminex microbeads technology. Retrospective case control study was utilized to identify the association between these SNPs and CNI-related outcomes.**Results** The patients with a CYP3A4*18B/*18B genotype were found to have a higher incidence of ACR compared with those with CYP3A4*1/*1(P=0.016),as well as between the subjects with the CYP3A4*18B/5*1 AA-AA genotype and those with GG-GG genotype(P=0.046).The positive of anti-MICA antibody was higher in patients with a CYP3A4*18B/*18B or CYP3A5*1/*1 genotype compared with those with CYP3A4*1/*18B or CYP3A5*1/*3(P=0.012;P=0.002);but the positive of anti-HLA antibody and CNI nephrotoxicity were comparable in genotype groups. **Conclusions** We conclude that CYP3A4*18B/*18B and CYP3A5*1/*1 genotype predict increased risk of ACR and anti-MICA antibody positive.But the recipient's CYP3A4*18B, CYP3A5*3 and ABCB1 genotype are not the risk factor of CNI nephrotoxicity.Genetic evaluation may help to identify patients at risk and to modulate CNI therapy.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO906**

**Tolerogenic Antigen Presenting Cells (APCs) Are Responsible for Induction and Maintenance of Tolerance by ECDI-Fixed Donor Splenocytes**
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**Background** Previously we showed that infusion of BALB/c splenocytes chemically fixed with ethylcarbodiimide (ECDI) induces indefinite protection of islet allografts in diabetic C57BL/6 mice. **Material and Methods** Diabetic B6 mice were transplanted with BALB/c islets on day 0. 1x108 ECDI-treated donor splenocytes were injected on day -7 and +1. **Results** Splenectomized mice that were treated with ECDI-fixed splenocytes rejected their grafts, suggesting that the spleen is obligatory for tolerance induction. Injection of LPS activated recipient DCs pulsed with donor lysates abrogates tolerance induction. In tolerized mice, TCR transgenic TEa CD4 T cells showed initial vigorous proliferation in the spleen in response to alloantigen-loaded APCs followed by profound depletion in numbers. The remaining cells were sequestered in the spleen. In contrast, in non-tolerized hosts these cells traffic to the dLNs following significant proliferation, and persisted in numbers. During tolerance maintenance, mice nephrectomized and re-grafted with islets from the same donor were able to retain the second graft if re-transplanted immediately or up to 30 days post nephrectomy, but not later. Skin transplants from the same donor but not third party were able to break established tolerance, as were LPS-activated donor DCs. T cells from tolerized hosts were able to reject same donor islets in RAG-/- hosts. **Conclusions** Depletion and sequestration of alloreactive T cells in response to alloantigen-loaded APCs in the spleen is a mechanism for tolerance induction. Tolerance maintenance requires persistence of alloantigen and can be overcome by activated APCs. Collectively these data supports a role of tolerogenic APCs for controlling allo-specific T cells in this regimen.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO907**

**Temporally Mediated Abrogation of T Cell Receptor Signals Alters CD8 Memory T Cell Differentiation**
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Memory T cells are a barrier to successful solid organ transplantation. The need for persistent signaling through the T cell receptor (TCR) during memory T cell differentiation is unclear. In contrast to signals generated by MHC plus antigenic peptide that result in an immune response to pathogen, the "tonic" TCR-generated signals are weaker and generated by interaction with MHC:self-peptide. The SH2-domain-containing phosphoprotein of 76 kilodaltons (SLP-76) adaptor protein is critical for proximal TCR-generated signaling. To assess CD8 memory T cell generation, we ablate TCR signals through conditional deletion of SLP-76 in antigen-specific memory T cells. Specifically, mice were infected with lymphocytic choriomeningitis virus (LCMV) Armstrong strain, SLP-76 was deleted ten or thirty days following infection. LCMV-specific CD8 T cells are identified using H-2Db:GP33 tetramer staining and deletion is monitored using the ROSA26R-EYFP Cre reporter. Deletion during the contraction phase of the immune response shifts the response to one that favors memory precursor formation based on cell surface expression of KLRG-1 and IL7Ra. By delaying deletion until thirty days after infection, we allow normal generation of the CD8 memory T cell compartment. Abrogation of tonic TCR signals during the memory phase does not alter the ability to form an antigen-specific central memory compartment. Interestingly, the shift from predominantly effector memory to central memory occurs more rapidly than in control mice. Taken together, these data indicate that normal CD8 memory T cell differentiation requires tonic TCR signals throughout both the contraction and memory phases of an immune response and have applicability in development of vaccines and transplant immunosuppression.

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**TH-PO908**

**Renoprotective Effects of Green Tea Extract on Renin Angiotensin Aldosterone System in Chronic Cyclosporine Treated Rats**
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**Purpose:** Cyclosporine A (CsA) is a potent and effective immunosuppressive agent, but use is frequently accompanied by severe nephrotoxicity. Renin angiotensin aldosterone system (RAAS) activation plays an important role in CsA nephropathy. For the aims we tested whether the administration of green tea extract (GTE) prevents the development of CsA-induced nephrotoxicity and found its mechanisms. **Method**: The rats were treated for 21 days and divided into 4 groups (n=6/group): control group (0.9% saline injection), CsA group (30 mg/Kg/day by intraperitoneal injection), CsA-GTE group (CsA plus GTE 100mg/Kg/day subcutaneous injection), GTE group (GTE alone). **Results:** There were significant increased serum blood urea nitrogen and creatinine in CsA group compared with that of control group and significantly improved in CsA-GTE group compared with that of CsA group ($p<0.01$). Biochemical analysis showed that the plasma renin activity and serum concentration of aldosterone were significantly increased in CsA group compared with control group and significantly decreased in CsA-GTE group compared with CsA group ($p<0.05$). Total amount of renin protein expression was significantly higher in the CsA group than in the control group and was lower in the CsA-GTE group than that of CsA group ($p<0.05$). In the histologic examination, there were proximal tubular necroses and mild interstitial inflammation in the kidneys of rats in CsA group but no significant pathologic changes in CsA-GTE group. **Conclusion:** CsA-treatment increases the plasma renin activity and intrarenal renin levels and induces the nephrotoxicity. The protective effects of GTE on CsA-induced structural and functional alternations of the kidney may be blockage of RAAS.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO909**

**The Association of IL-2/IL-2Rβ Genetic Polymorphisms with Acute Renal Allograft Rejection in Korean Population** Seok Ju Park,[1] Hyun Seung Lee,[1] Sunwoo Kang,[1] Yang Wook Kim,[2] Tae Hee Kim,[1] Miseon Kang,[3] Sang Seop Lee,[4] Yeong Hoon Kim.[1] *[1]Internal Medicine, Busan Paik Hospital, Busan, Republic of Korea; [2]Internal Medicine, Haeundae Paik hospital, Busan, Republic of Korea; [3]Pathology, Busan Paik Hospital, Busan, Republic of Korea; [4]Phamacology and Phamacogenomics Research Center, Busan Paik Hospital, Busan, Republic of Korea.*

Background: Advancement of immunosuppressive therapy has improved the allograft survival in patients undergone transplantation surgery. However, acute rejection (AR) of transplanted allograft in certain subgroup of patients still remains a major obstacle to overcome. The occurrence of acute rejection shows inter-individual variation and genetic make-up of patients may contribute to higher risk of AR. Since interleukin 2 (IL-2) plays a key role in immune modulation and target pathway to immunosuppressive therapy, the effect of genetic polymorphisms of IL-2 and IL-2 receptor beta (IL-2Rβ), two key molecules of IL-2 signal pathway, on renal AR risk was investigated in 337 Korean kidney transplant recipients.

Methods: Common haplotype tagging SNPs in IL-2 and IL-2Rβ genes were genotyped by polymerase chain reaction followed by restriction fragment length polymorphism in 61 AR patients and 276 control renal allograft recipients.

Results: All of four tagging SNPs of IL-2 and IL-2Rβ genes showed Hardy-Weinburg equilibrium in both AR and control groups. The occurrence of AR was significantly associated with the presence of allelic variation of IL-2Rβ gene ($P$=0.016). Genetic variations of IL-2 gene also showed tendency of AR association, but could not reach statistical significance. When haplotype distribution was tested for the association, both IL-2 and IL-2Rβ genes showed significant association with AR risk.

Conclusion: Our results demonstrate that genetic variations of IL-2Rβ and IL-2 genes are associated with the development of acute rejection. Though further confirmative evidence will be required, our data suggest that genotyping of these genes may help predict AR risk in kidney transplantation patients.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO910**

**Remote Ischemic Preconditioning Using the Hind Limb Protects Against Renal Ischemia Reperfusion Injury** Kimberley E. Wever,[1,2] M. C. Warle,[1,3] Frank Adtg Wagener,[1,2] Rosalinde Masereeuw,[1,2] Gerard Rongen,[1,2] J. Adam Van der Vliet.[1,3] *[1]Radboud University Medical Centre; [2]Dept. of Pharmacology & Toxicology; [3]Dept. of Surgery.*

Remote ischemic preconditioning (RIPC) is a strategy to protect a target organ against ischemia/reperfusion injury (IRI) by inducing brief ischemia/reperfusion (I/R) in a remote organ. RIPC of the kidney by brief I/R of a limb would be a safe, cheap and non-invasive method to prevent renal IRI in e.g. transplantation and aortic surgery. We investigated the optimal remote ischemic stimulus in the hind limb to protect the kidney against IRI. Anesthetized rats underwent either no RIPC, unilateral RIPC (one limb) or bilateral RIPC (both limbs). Limbs were occluded using a 1.9cm blood pressure cuff. The preconditioning stimulus was either continuous (12'/12' I/R), or fractionated (3 times 4'/4' I/R). After the last reperfusion period, we induced 25' ischemia in the right kidney and the left kidney was nephrectomized. Renal damage was assessed after 48h of reperfusion. Plasma creatinin and urea were improved in both bilateral RIPC groups and in the fractionated unilateral group. Kidney injury molecule-1 (KIM-1) mRNA was reduced in the unilateral continuous and bilateral fractionated RIPC groups. Renal damage, as scored in histological sections of the cortex, was reduced 3 out of 4 RIPC groups. Notably, the persisting residual injury provides opportunities to further enhance RIPC pharmacologically. In conclusion, we found that RIPC using the hind limb as remote organ reduces renal IRI, making it a promising tool to prevent renal damage. Bilateral RIPC appeared to be more effective than unilateral RIPC, and fractionated RIPC appeared to be more effective than continuous RIPC.

| | sham | No RIPC | Unilateral 12'/12' | Bilateral 12'/12' | Unilateral 3x 4'/4' | Bilateral 3x 4'/4' |
|---|---|---|---|---|---|---|
| Plasma creatinine | 42±5 | 320±108 | 316±124 | 215±81** | 221±110 | 181±121** |
| Plasma urea | 9±2 | 52±15 | 47±15 | 37±11** | 37±14** | 31±16** |
| KIM-1 mRNA | 2±5 | 687±197 | 513±32* | 574±206 | 549±209 | 407±117** |
| Renal damage score | 0.0±0.03 | 3.9±0.5 | 2.4±1.3** | 3.1±0.8* | 3.2±0.9 | 2.1±1.5** |

Data are means±SD; all values are p<0.01 vs sham; *p<0.05, **p<0.01 vs No RIPC.

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Disclosure of Financial Relationships: nothing to disclose

**TH-PO911**

**Cold Preservation of Kidneys from Hibernating Squirrels** Alkesh Jani, Jessica Martin, Elaine Epperson, Kultigin Turkmen, Arijana Pacic, Danica Ljubanovic, Sandy Martin, Charles L. Edelstein. *U of Colorado.*

We have shown that cold ischemia (CI) results in increased caspase-3 activity, tubular apoptosis, and brush border injury (BBI) in mouse kidneys. The 13-lined ground squirrel (GS) is a hibernating mammal that undergoes alternating periods of *torpor* when core body temperature (CBT) is very low at 4C for 7-18 days, followed by re-warming during *interbout arousal* (IBA) when CBT rises to 37C for 12 hrs. These cycles occur >15 times each winter. Since IBA is preceded by a prolonged period of CI during torpor, we hypothesized that kidneys from IBA animals would better tolerate *ex vivo* CI than kidneys from non-hibernating summer ground squirrels.Methods Radiotelemeters were implanted in 1-2 yr old GS, and CBT was remotely monitored. The kidneys of 1-2 year old summer and IBA GS were perfused with cold University of Wisconsin (UW) solution. The right kidney was used as a control and the left kidney was stored *ex vivo* in UW for 72 hours at 4C. Tubules with BBI and apoptotic cells/hpf were counted. Caspase-3/7 activity was measured using fluorescent substrates.Results(Table): Caspase-3/7 activity was significantly greater in IBA vs summer kidneys after 72 hrs of *ex vivo* CI. Neither the number of apoptotic cells/hpf nor the BBI score differed significantly between IBA and summer kidneys at 72 hrs of ex *vivo* CI. The levels of apoptosis and BBI in all animals were extremely low. Conclusion Squirrel kidneys have markedly less apoptosis and BBI on cold preservation compared to what we have previously reported in mouse kidneys. Both summer and IBA animals were protected from *ex vivo* apoptosis and BBI. IBA animals had significantly greater caspase-3/7 activity after 48 hrs of CI but this did not result in more apoptosis or BBI, suggesting resistance to caspase-mediated injury. These results indicate that the ability to tolerate severe, prolonged CI is present year-round, as opposed to being specifically induced during hibernation.

| | Apoptosis (cells/hpf) | | BBI score | | Caspase-3 activity (nmol/min/mg) | |
|---|---|---|---|---|---|---|
| | 0 hr CI | 72 hr CI | 0 hr CI | 72 hr CI | 0 hr CI | 72 hr CI |
| Summer kidney | 0.2 | 0.2* | 1 | 1* | 14±1 | 28±3* |
| IBA kidney | 0.1 | 0* | 1 | 1* | 24±3 | 52±3** |

CI=cold ischemia; *p=NS vs 0 hr; **p<0.005 vs 0 hr IBA kidney and 72 hr CI summer kidney; n=3 in all groups

Disclosure of Financial Relationships: nothing to disclose

**TH-PO912**

**Preconditioning with Immunosuppressive Drugs to Donors in Renal Transplant Attenuates Ischemia Reperfusion Injury and Downregulates Local Immflamatory Markers** Federico Cicora,[1,] Diego Guerrieri,[1,2] Natalia Lausada,[1] Pablo Stringa,[1] Paola Cicora,[1] Daniela Vasquez,[1] Gustavo Palti,[1] Pedro Gonzalez,[1] Eduardo Chuluyan,[2] Clemente Raimondi.[1] *[1]Fundacion para la Investigación y Asistencia de la Enfermedad Renal, La Plata, Argentina; [2]Departamento de Farmacología, Facultad de Medicina, UBA, Buenos Aires, Argentina.*

Isogenic renal transplant (IRT) in rats is the most widely used model for the study of non-immunological damage factors. Our aim was to determine if the administration of immunosuppressive drugs to donors modify the IRI process. **Materials and methods**: IRT was performed in 18 rats. Donors were untreated (Group 1) or treated with 2 mg/kg Rapamycin (Group 2) or 0,3 mg/kg Tacrolimus (Group 3), 6-12 hs before the rats were sacrificed. Then, donor left kidney was removed washed and stored (3 hs, 4ºC). Grafts were implanted after a bilateral nephrectomy of the receptors. A Sham Group (n=6) was also assessed. Plasma urea and creatinine determinations are shown as the difference before and after the transplant. Histological samples were analyzed for tubular injury and apoptosis. TNF-a, IL-6, IL-17, IL-21 receptor and C3 *in situ* expression were measured by immunohistochemistry. **Results:** A decrease in plasma urea (G1=2,2±0,06; G2=1,9±0,06; G3=1,5±0,06 mg/dl) and creatinine (G1=4,7±0,55; G2=2,1±0,04; G3=2,0±0,13) were observed for treated rats (G1vsG2 p<0,05; G1vsG3 p<0,01 for urea and creatinine). Less damage was also observed among the control and treated groups (G1vsG2 p<0.01 and G1vsG3 p<0,05). Remarkably, less apoptotic nuclei were observed in G2 (22±1.8) and G3 (60±13) compared to G1 (140±10); G1vsG2 p<0.01 and G1vsG3 p<0.05. The cortex and medulla tubular epithelium expression of pro-inflammatory markers C3, IL-6, TNF-a and IL-21 receptor but not IL-17 was increased in G1 compared to G2 or G3. **Conclusion:** administration of Rapamycin or Tacrolimus to donors attenuates the IRI process in kidney transplant. We suggest that isogenic renal transplant is a pro-inflammatory process and induces a potent IRI. The decrease expression of IL-6 and IL-21 receptor in situ, both related to Th17 differentiation, might generate a hostile environment to Th17 differentiation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO913**

**Association of Specific HLA Alleles with the Risk of High PRA and Acute Rejection in Kidney Allograft Recipients** Nilgun Kacak,[1] Krzysztof Kiryluk,[1] Eric K. Ho,[2] Nicole Suciu-Foca,[2] David J. Cohen,[1] Ali G. Gharavi.[1] *[1]Medicine, Div. of Nephrology, Columbia University, New York; [2]Pathology and Cell Biology, Div. of Immunogenetics, Columbia University, New York.*

Objective: To determine the impact of HLA antigens on the Panel Reactive Antibody (PRA) levels and the risk of biopsy proven acute rejection in kidney allograft recipients.

Methods: We analyzed 705 kidney allograft recipients including 336 (47.7%) Caucasians, 200 (28.4%) Hispanics, 115 (16.3%) Blacks, and 40 (5.7%) Asians transplanted at our medical center. HLA alleles were determined by tissue typing. We analyzed the association of HLA types with PRA levels stratified by ethnicity and adjusted for known risk factors (prior pregnancies, transfusions, and previous transplants). We also analyzed the association of HLA alleles with the risk of acute rejection adjusted for HLA mismatch, high PRA titers, pregnancies, transfusions, prior transplants, and ethnicity. Multivariate analyses were performed using logistic regression (SPSS v.17.0).

Results: The cohort characteristics included: mean age 46 (range 5-84); male-female ratio 1.5, average follow-up time 6.6 (+/-5.8) years. During the follow-up period, 214 (30.4%) of patients were diagnosed with acute rejection by biopsy. Pre-transplant PRA levels were high (>30) in 79 (11.2%) patients, with a notable trend for greater frequency in Blacks (16.5% vs. 10.5% in non-Blacks, p=0.066). After multivariate adjustment, HLA alleles that were associated with high PRA included HLA-A66 (p=0.007) and HLA-DR9

(p=0.0004). The HLA-A66 association was strongest in Whites (p=0.018), while HLA-DR9 in Blacks (p=0.009). In the analysis of acute rejection, we detected an association with increased risk for HLA-DR3 (OR=1.9, 95%CI: 1.04-3.7, p=0.039), B52 (OR=2.3, 95%CI: 1.05-5.1, p=0.036), and B81 (OR=5.33, 95%CI: 1.03-27.6, p=0.046). In addition, we replicated a previously reported protective effect of HLA-DR1 on rejection (OR=0.55, 95%CI: 0.33-0.92, p=0.023).

Conclusions: Distinct HLA alleles influence the risk of high PRA and acute rejection independently of the known risk factors. These data may help to identify individuals at high risk of developing elevated PRA or acute rejection.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO914**

**Long Term Gene Therapy with Thrombospondin 2 Is Anti-Inflammatory but Increases Capillary and Lymphatic Vessel Rarefaction during Chronic Allograft Nephropathy in the Rat** Christoph Daniel,[1] Regina Vogelbacher,[1] Andrea Stief,[1] Christian Hugo.[2] *[1]Nephrology and Hypertension, University of Erlangen-Nürnberg, Erlangen, Germany; [2]Nephrology, University of Dresden, Dresden, Germany.*

We recently identified Thrombospondin-2 (TSP-2) as a regulator of matrix remodelling and inflammation in experimental kidney disease by using TSP-2 null mice or TSP-2 overexpression. In this study we investigated if long term TSP-2 overexpression is also capable to ameliorate the progression of a chronic kidney disease using the chronic allograft nephropathy (CAN) F344-Lewis model in the rat.

Two weeks after renal transpantation rats were transfected with either a TSP-2 overexpressing plasmid (n=8) or a luciferase-expressing plasmid as control (n=8). Rats were monitored for renal function and histological changes in the graft using immunohistochemistry 30 weeks after transplantation. Surprisingly, only in the TSP-2 treated group 2 rats died before the end of the experiment. Renal function tended to be worsened in the TSP-2 group compared to the luciferase-treated controls. In addition, glomerular sclerosis (score:1,9±0,6 vs. 1,3±0,3) and tubular interstitial injury (score: 2,2±0,7 vs. 1,2±0,3) as well as cortical fibronectin deposition (score: 2,2±0,4 vs. 1,5±0,2) was significantly increased in the TSP-2 treated kidneys. Long term TSP-2 therapy impaired repair of renal endothelium, as demonstrated by significant higher glomerular (% pos. area: 7,5±2,3 vs. 18,6±7,5) and peritubular (score: 49,6±4,3 vs. 62,4±4,5) endothelial rarefaction in the transplanted kidneys from TSP-2 treated rats compared to controls. Furthermore, number of lymphatic vessels, as assessed by podoplanin staining, was significantly reduced in TSP-2 treated rats compared to luciferase-treated controls.

In contrast, renal inflammation was significantly ameliorated in the TSP-2 treated group, as assessed by counting macrophages, CD8 positive T-cells and CD45R positive B-cells reaching reduction rates from 31% up to 68%. In conclusion, despite its anti-inflammatory effects, TSP-2 gene therapy did not ameliorate but rather worsened experimental CAN most likely via increased capillary and lymphatic vessel rarefaction.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO915**

**Loss of Peritubular Capillaries Induced by Renal Ischemia Reperfusion Injury Is Associated with a Dysbalance of Angiopoietins and a Loss of Pericytes** Meriem Khairoun,[1] Pieter van der Pol,[1] Hetty C. de Boer,[1,2] Kyra A. Gelderman,[1] Nicole Schlagwein,[1] Dorottya De Vries,[3] Ellen Lievers,[1] Cees van Kooten,[1] Ton J. Rabelink,[1,2] Anton Jan Van Zonneveld,[1,2] Marlies Reinders.[1] *[1]Nephrology, LUMC; [2]Einthoven Laboratory for Experimental Vascular Research, LUMC; [3]Surgery, LUMC, Leiden, Netherlands.*

Introduction: Peritubular capillaries (PTCs) are highly susceptible to ischemia reperfusion injury (IRI) after renal transplantation. This microvascular injury contributes to graft dysfunction and ultimately graft loss. Pericytes play a critical role in the stabilization and proliferation of endothelial cells (ECs). This process is mediated by angioregulatory factors, including the anti-inflammatory factor Angiopoietin-1 (Ang-1) produced by pericytes and the proinflammatory factor Angiopoietin-2 (Ang-2) produced by ECs. Here, we investigated the impact of IRI on pericyte and angiopoietin expression.

Methods: Male Lewis rats (n=6 per group) were subjected to 45 minutes of unilateral renal ischemia followed by removal of the contralateral kidney and sacrificed at 2, 5, 24, 48 and 72 hr after IRI. Leukocyte infiltration (CD45), EC integrity (RECA-1), pericytes (NG2), Ang-1, and Ang-2 were measured and quantified using immunohistochemistry. Sham operated rats and contralateral kidneys served as control.

Results: Our study shows a significant loss of PTCs after IRI in the cortex after 24 hr ($p< 0.05$) and an increase of infiltrating leucocytes after 48 hr ($p< 0.05$). Furthermore, we found a decrease in pericytes at 5 hr, reaching significance at 48 and 72 hr after IRI ($p< 0.05$). This was accompanied by a reduction in Ang-1 after 24 hr and remained decreased up to 72 hr ($p< 0.001$). In contrast, Ang-2 increased after 24 hr ($p< 0.01$) and remained elevated up to 48 hr ($p< 0.05$) after IRI.

Conclusions: Our results demonstrate that decrease in PTCs induced by renal IRI is associated with a dysbalance of angiopoietins and a loss of pericytes. Since pericytes and angiopoietins are considered as important hallmarks of microvascular integrity, strategies to counteract microvascular destabilization after IRI may well improve long term graft function.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO916**

**Pharmacodynamic Monitoring of mTOR-Inhibitor Based Immunosuppression in a Cell Culture Model: Establishment of a Novel ELISA Method for Quantitative Assessment of phospho-p70 S6K** Bertram Hartmann,[1] Xuemei He,[1] Frieder Keller,[1] Michael Fischereder,[2] Holger Schmid,[2] Markus Guba.[3] *[1]Medical Department I, Section of Nephrology, Ulm University, Ulm, Germany; [2]Medical Department I, Section of Nephrology, Munich University Hospital, Campus Grosshadern, Munich, Germany; [3]Department of Surgery, Munich University Hospital, Campus Grosshadern, Munich, Germany.*

Background

Rapamycin is a critical dose drug with a low therapeutic index. Current pharmacokinetic monitoring may not correlate with the pharmacological effects of rapamycin on immune cells. Semiquantitative Western blot analysis has been used to investigate the immunosuppressive properties of rapamycin by assessing phosphorylation status at the Thr 389 site of the p70 S6 kinase (phospho-p70 S6K) a downstream effector of mTOR. A pharmacodynamic approach by quantitative assessment of phospho-p70 S6K via ELISA-technique might provide a novel and clinically feasible way to directly analyze the effects of rapamycin.

Methods

Phosphorylation status of p70 S6K was analysed in a cell culture model (Jurkat cells and PBMCs from buffy coats) before and after stimulation with the phorbol-ester PMA. Phospho-p70 S6K expression was measured by (i) semiquantitative Western blot analysis, recognizing both isoforms of S6 kinase1 (p70 S6K and p85 S6K) and (ii) a newly established ELISA based assay.

Results

Average levels of phospho-p70 S6K were increased after PMA-stimulation in Jurkat cells (unstimulated 4.18 vs. stimulated 7.65 U/100 µg protein) and in PBMCs (3.09 vs. 12.39 U/100 µg protein). We found an obviously dose-dependent down-regulation of phospho-p70 S6K induced by rapamycin in PMA-stimulated PBMCs ($n = 20$, $P< 0.05$), which was not statistically significant in unstimulated PBMCs.

Conclusions

Here we successfully established a novel ELISA-based method, which allowed reproducible quantitative measurement of phospho-p70 S6K in an in vitro model. Large scale studies have now to demonstrate, that this ELISA assay is capable to optimize immunosuppressive therapy.

Disclosure of Financial Relationships: Research Funding: financial support by Wyeth for one research project.

**TH-PO917**

**Human Polyomavirus BK-Encoded microRNA Suppresses p53 and p21 Expressions and Maintains Host Cell Survival** Ya-Chung Tian, I.-Jung Li, Cheng-Hao Weng, Hsin-Hsu Wu, Yung-Chang Chen, Cheng-Chieh Hung, Chih-Wei Yang. *Nephrology, Lin-Kou Chang-Gung Memorial Hospital, Taiwan.*

**Background** Polyomavirus BK (BKV) infection has been identified as an important cause of early renal transplant graft failure. Following primary infection, BKV remain latent in host cells through regulating host cell cycles. Various viral microRNAs have been shown to be capable of controlling post-transcription gene expression in host cells. The aim of this study was to determine whether BKV microRNA could regulate host cell cycle.

**Method** A fragment of BKV genome, including candidate encoded microRNA (miR-B1) and its flanking regions, was cloned into expression vectors. Following transfection of renal proximal tubular cells, HK-2, with miR-B1 for 24 hr, real-time PCR using stem-loop primers was utilized to assess miR-B1 expression.

**Results** Transfection of HK-2 cells with pre-miR-B1 expression vector successfully produced mature miRNAs assessed by real-time PCR. p53 transcripts contain a match to miR-B1. We generated a luciferase-reporter containing entire p53 3'-UTR. Dual luciferase assay revealed that miR-B1 suppressed luciferase activity of this p53-3' UTR reporter in comparison with the control vector. In addition, miR-B1 overexpression reduced endogenous p53 protein level measured by Western blot analysis in a dose-dependent manner. Similarly, miR-B1 also suppressed p21 protein expression. These suggest that miR-B1 can suppress p53 and p21 expression, therefore potentially driving cell cycle progression. Furthermore, administration of cisplatin (5 mM) led to an increase in p53 protein expression. Overexpression of miR-B1 suppressed cisplatin-induced p53 and p21 expression. Finally, miR-B1 overexpression also attenuated cisplatin-mediated inhibition of cell proliferation determined by BrdU immunostaining.

**Conclusion** Our findings showed that the BKV-encoded miRNA, miR-B1, suppressed p53 and p21 protein expressions and attenuated cisplatin-mediated inhibition of cell proliferation. Since p53 can promote apoptosis, the inhibition of host p53 production may prevent host cells from apoptosis, thus benefiting viral survival and providing a long latency.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO918**

**Surrogates, Predictors and Outcomes in Renal Transplantation (*Sports*) Study – Rationale and Design** Adekunle Bamidele Adesina, Christopher Edwards, Andrew J. Williams, Jonathan Bailey. *Renal Unit, Morriston Hospital, Swansea, United Kingdom.*

**Rationale**

The optimal immunosuppressive therapy of posttransplant patients requires a delicate balance of prevention of rejection whilst minimising complications. Clinicians rely on monitoring of clinical signs, drug lvels, and routine laboratory parameters to guide treatment decisions. A combination of immune parameters may allow early detection and adequate prediction of likelihood competing long-term outcomes including rejection, tolerance, infection and malignancy.

**Purpose of study:** To determine a reliable and valid composite index of immune status manging renal transplant patients.

**Methods:** Systematic review of published studies of asessment of immune status in relation to renal transplant outcomes, followed by statistical analysis to determine the most valid and reliable predictive assays, and then conduct a prospective trial of a candidate multiparameter composite index of immunosuppression.

**Results** Our on-going review of the literature identified forty-three (43) published studies in which posttransplant immune status was assessed as a means of profiling recipients' likelihood of developing either rejection, tolerance, infections or malignancy in the post transplant period. Seventeen (17) different cellular/cytokine and antibody-based immune assays were found, of which only one cellular assay, which is rather non-transplant-specific has been approved by the FDA for routine use in posttransplant immune monitoring.

**Conclusion**: The growing body of research data on parameters for monitoring posttransplant immune status indicate clinician interest in more relaible and scientifically valid tools for tailoring immunosuppression. Although several urine and serological assays are predictive of transplant outcomes of interest, only one has been formally approved for routine use. We are now computing a novel composite assay of postransplant immune status based on both humoral and cellular parameters that are scientifically sound surrogates and predictive of transplant outcomes. We are also designing a prospective study to assess the feasibility, validity and clinical utility of the composite index of post transplant immune status.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO919**

**HLA-DR$^+$-Regulatory T Cells Are Involved in the Induction of Preterm Labor during Pregnancy and in the Induction of Organ Rejection after Transplantation** Matthias Schaier,[1] Nicole Seissler,[1] Friederike Hug,[1] Luis Eduardo Becker,[1] Martin G. Zeier,[1] Andrea Steinborn.[2] *[1]Department of Nephrology, University of Heidelberg, Heidelberg, Germany; [2]Department of Obstetrics and Gynecology, University of Heidelberg, Heidelberg, Germany.*

Regulatory T cells (Tregs) were shown to suppress alloimmune responses after organ transplantation and are known to be of substantial importance for the induction of maternal tolerance towards the fetal antigens during pregnancy.

We assessed the percentage of CD4$^+$CD127$^{low+/-}$CD25$^+$-Tregs and a FoxP3$^+$DR$^+$-Treg subset in healthy volunteers, in healthy and preterm-laboring pregnant women and in kidney transplant patients with stable transplant function or acute rejection. In addition, the suppressive activity of CD4$^+$CD127$^{low+/-}$CD25$^+$-Tregs was tested by co-culture suppression assays.

In comparison to healthy volunteers, the total number of CD4$^+$CD127$^{low+/-}$CD25$^+$-Tregs was significantly decreased, both during the normal course of pregnancy and after successful kidney transplantation. It was decreased in neither preterm-laboring women nor in kidney recipients with acute rejection. In analogy, the percentage of the FoxP3$^+$DR$^+$-Treg subpopulation decreased during the normal course of pregnancy and was also not diminished in preterm-laboring women or in transplant patients showing acute rejection. However, the HLA-DR expression of the FoxP3$^+$DR$^+$-Treg cells was significantly reduced, both in preterm-laboring women and in kidney transplant patients with acute rejection. This weak expression of HLA-DR ex vivo points to a reduced suppressive potency of the FoxP3$^+$DR$^+$-Treg subset under these conditions. Interestingly, the suppressive activity of the CD4$^+$CD127$^{low+/-}$CD25$^+$-Tregs, isolated from the same preterm-laboring women and transplant patients with acute rejection, was significantly decreased.

Hence, our results propose that the FoxP3$^+$DR$^+$-Treg subset may essentially contribute to the suppressive activity of the total CD4$^+$CD127$^{low+/-}$CD25$^+$-Treg cell pool and that the immunologic mechanisms leading to preterm labor may be similar to those leading to acute rejection after organ transplantation.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO920**

**Bortezomib for Desensitization of Patients in Awaiting List for Deceased Donor Kidney Transplant: Experience in Mexico City** Sergio A. Leyva Campillo, Lluvia A. Marino-Vazquez, Luis E. Morales-Buenrostro. *Nephrology, INNSZ, Mexico City, DF, Mexico.*

Introduction:

In our experience for acute humoral rejection, the use of 4 doses of bortezomib in days 1, 4, 7, and 10 (1.3 mg/m2 BSA each) plus 3 plasmapheresis (PP) sessions produced both: a good clinical response and a reduction in DSA. In this report, we showed our experience using this treatment for desensitization.

Patients and Methods:

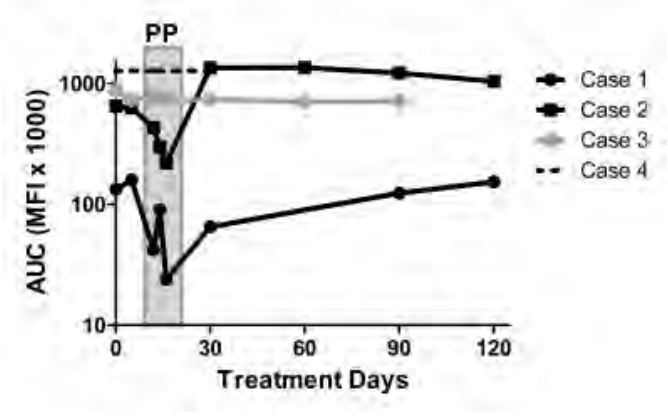
Four adult patients in the awaiting list for deceased donor kidney transplant underwent desensitization protocol with Bortezomib (1.3mg/m2 BS, days 1,4,7,10) + Plasmapheresis (PP) with or without rituximab in 3 patients and only bortezomib in 1 case. Pre and posttreatment sera samples were tested by Luminex Single Antigen assay, and the change in mean fluorescence intensity (MFI) for all specificities was analyzed with Area Under Curve (AUC) to examine the changes in antibody levels and to evaluate the efficacy of the treatments. Patients with pretreatment antibodies of MFI great than 10,000 at neat were further tested at 1:8 and 1:32 dilutions.

Results:

In 2 of the 4 patients, antibody reduction from pre to post treatment was observed but only after PP, but returned to baseline level early after treatment (figure). In those non-responders, a further dilutions analysis showed a transient reduction of antibody levels after PP.

Conclusions:

In highly sensitized patients, the use of Bortezomib for desensitization is not as effective as it was for acute humoral rejection. It has been suggested that long living plasma cells (probably the main cells in our cases) are not susceptible to Bortezomib. This result must be confirmed with larger studies. Testing the sera at dilution may aid in a more accurate assessment of antibody removal in those patients.




Disclosure of Financial Relationships: nothing to disclose

**TH-PO921**

**Effect of Proteasome Inhibition by Bortezomib on HLA-Antibody Titers and Specificity in Sensitized Patients Awaiting Renal Allograft Transplantation** Martina Guthoff,[1] Barbara Schmid-Horch,[2] Katja C. Weisel,[3] Nils Heyne.[1] *[1]Dept. of Diabetes and Endocrinology, Angiology, Nephrology and Clinical Chemistry, University of Tuebingen, Tuebingen, Germany; [2]Center of Clinical Transfusion Medicine, University of Tuebingen, Tuebingen, Germany; [3]Dept. of Hematology, Oncology and Immunology, University of Tuebingen, Tuebingen, Germany.*

Sensitization to human leukocyte antigen (HLA) prolongs waiting list time and reduces allograft survival in organ transplantation. Current antibody depleting strategies include B-cell directed therapy and extracorporeal techniques. Considerable interest has arisen from initial reports using the proteasome inhibitor bortezomib in antibody mediated rejection, in addition to standard therapy. Potential benefits include direct targeting of the antibody producing plasma cell and memory B-cells with alteration of donor specificity. We report bortezomib preconditioning in sensitized patients awaiting transplantation, providing first clinical data on dynamics and donor specificity of preformed HLA antibodies in response to bortezomib alone.

**Methods**: Two highly sensitized patients awaiting third kidney transplantation were treated with bortezomib (1.3 mg/kg bwt., days 1, 4, 8, 11). Time-course and levels of HLA antibody titers, as well as specificity to previous transplant antigens were monitored weekly by luminex.

**Results**: Bortezomib was well tolerated without side effects. In all patients, changes in IgG levels were small and no sustained reduction in HLA class I or II antibody titers was observed following bortezomib preconditioning over more than 100 days of follow-up. No differences among antibody responses to donor specific and non-donor specific antigens were oberserved.

**Conclusion**: Bortezomib preconditioning alone does not result in sustained reduction of HLA antibody titres in sensitized patients. Also, no change in specificity of antibody production was observed over a period of time clearly exceeding half-life of preformed IgG HLA antibodies. Hence, in a pretransplant setting, combination immunosuppressive strategies are required to obtain benefit from proteasome inhibition.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO922**

**Outcome after Renal Transplantation in Systemic Amyloidosis** Jennifer H. Pinney,[1,2] Helen J. Lachmann,[1] Prayman Sattianayagam,[1] Simon Gibbs,[1] Ashutosh D. Wechalekar,[1] Carol J. Whelan,[1] Philip Hawkins,[1] Julian D. Gillmore.[1] *[1]National Amyloidosis Centre, Division of Medicine, UCL Medical School, Royal Free Campus, London, United Kingdom; [2]Centre for Nephrology, Division of Medicine, UCL Medical School, Royal Free Campus, London, United Kingdom.*

Renal transplantation (RTx) is contentious in patients with systemic amyloidosis because of the progressive and multi-system nature of the disease and the risk of amyloid recurrence in the graft.

We report outcome following isolated RTx in 98 patients with various forms of systemic amyloidosis evaluated at the UK National Amyloidosis Centre (NAC) between 1978 and 2009. Patient and graft survival were estimated by Kaplan-Meier analysis.

Twenty-eight patients with AL amyloidosis followed for a median of 58 months from RTx, 39 with AA amyloidosis followed for a median of 62 months, 15 with fibrinogen Aa-chain amyloidosis (AFib) followed for a median of 63 months, 13 with apolipoprotein AI amyloidosis (AApoAI) followed for a median of 111 months and 3 with lysozyme amyloidosis (ALys) followed for 14, 26, and 83 months respectively, received 28, 41, 16, 15 and 3 RTx respectively (total 103). Median age at RTx in AL, AA, AFib, AApoAI, and ALys was 61, 36, 60, 45, and 46 years respectively. One and 10 year patient survival in AL amyloidosis was 93% and 47%, in AA amyloidosis was 92% and 84%, in AFib was 100% and 48%, in AApoAI was 92% at one year with no further deaths at 10 years, and no patient with ALys had died following transplantation at the time of censor. One and 10 year renal allograft survival in AL amyloidosis was 100% and 93%, in AA amyloidosis was 94% and 89%, in AFib was 81% and 27%, in AApoAI was 100% and 71%, and in ALys was 100% with no ALys patient followed for 10 years from RTx. Recurrence of amyloid was evident in 7 AL, 12 AA, 3 AFib, 2 AApoAI and no ALys patients but only caused 6 grafts (3 AA and 3 AFib) to fail, the earliest after 70 months.

This data supports RTx in selected patients with AL and AA amyloidosis. Pre-emptive RTx should be considered in all patients with hereditary systemic amyloidosis who progress to end stage renal failure.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO923**

**Transplant Nephrectomy: Association with Survival, Retransplantation, and Sensitization** Sejan B. Patel, Stephen R. Smith. *Division of Nephrology, Duke University Medical Center, Durham, NC.*

Patients with renal allograft failure make up an increasing fraction of patients initiating dialysis. The management of the allograft after failure remains controversial. We examined the impact of transplant nephrectomy on survival, retransplantation, and sensitization.

We performed a retrospective study of subjects with allograft failure returning to dialysis between 1994 and 2009 in our institution. The primary outcome was time to all cause death. The secondary outcome was time to retransplantation and second allograft failure.

Of 248 subjects with allograft failure, 103 subjects (42%) had a nephrectomy. Subjects with nephrectomy were younger and had fewer comorbidities (age 43±11 vs. 49±13 years, cerebrovascular disease 2% vs. 10%, coronary artery disease 10% vs. 26%, diabetes mellitus 25% vs. 43%, $p<0.01$) than those with no nephrectomy. There was no difference in PRA before transplant. There were 113 deaths over a mean period of 3.9±3.6 years after allograft failure associated with 5-year survival of 70% with nephrectomy vs. 45% with no nephrectomy ($p<0.001$). Cox regression adjusting for demographics, comorbidities, deceased donor transplant, allograft failure > 12 months, and PRA > 30% showed that nephrectomy was associated with a 60% hazard reduction for all cause death (HR 0.41, 95%CI 0.24-0.70, $p<0.001$). There were 14 subjects with nephrectomy and 12 subjects with no nephrectomy who were retransplanted. Of those with nephrectomy, the PRA increased from 10±25% before the first transplant to 56±40% before the second transplant ($p<0.01$). Of those with no nephrectomy, there was no difference in PRA before the first and second transplant. There was no difference in time to retransplantation and no difference in allograft survival after the second transplant.

Transplant nephrectomy was associated with better survival but greater sensitization, although there was no difference in time to retransplantation or second allograft failure.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO924**

**Immunosuppression Protocols and Cardiovascular Risk Factor Monitoring Techniques at Midwest Pediatric Nephrology Consortium Member Centers** Raquel Mitchell, Donald J. Weaver. *Pediatrics, Levine Children's Hospital at Carolinas Medical Center, Charlotte, NC.*

**Introduction:** One reported risk factor for the continued progression of cardiovascular disease in pediatric renal transplant recipients is steroid use. The goal of the current study is to obtain information on trends of steroid utilization and methods for monitoring cardiovascular risk factors in the pediatric renal transplant recipients.

**Methods:** Member centers of the MWPNC were surveyed on immunosuppressive protocols and methods of cardiovascular risk factor monitoring.

**Results:** At the 11 centers that responded, 120 transplants were performed annually and over 500 transplant recipients were followed. Eighteen percent of surveyed centers utilized protocols completely steroid-free. Forty-six percent had used rapid discontinuation of steroids, 55% used a steroid avoidance regimen, and 36% used a steroid minimization protocol. As an induction regimen, four centers used thymoglobulin with anti-interleukin 2 therapy, three used thymoglobulin alone, three reported using basiliximab alone, and one used alemtuzumab. For maintenance therapy, 64% of hospitals used MMF, TAC, and steroids whereas 27% used MMF/TAC only. Finally, 9% used MMF/TAC/CYC. No centers were using sirolimus. Seven centers obtained annual echocardiograms; two centers only performed echocardiograms on patients who were hypertensive. Only one center used ambulatory blood pressure monitoring routinely. Hemoglobin A1c levels and glucose tolerance testing were not performed routinely. More than 80% of centers obtained annual lipid measurements. Primary indications for use of steroid-free immunosuppression were living-related donor transplant and first renal transplant.

**Conclusions:** The results of this survey reflect the variability in renal transplant protocols utilized by North American centers. This study highlights the importance of developing a consensus protocol for managing the pediatric renal transplant patient population to better assess long-term outcomes. Moreover, standardized protocols for cardiovascular risk surveillance are imperative because of the significant morbidity and mortality inherent in this patient population.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO925**

**Donor Pair Effect on Post Transplant Kidney Outcome and Function** Carol A. Traynor, Frank J. O Brien, Colm Magee, Peter J. Conlon. *Nephrology, Beaumont Hospital, Dublin, Ireland.*

**Background**

Donor characteristics such as age and cause of death are known to contribute to the incidence of both delayed graft function (DGF) and graft survival. We aim to assess the genetic effect of paired donor kidneys transplanted into different recipients on DGF and kidney function at 1 & 5 years post transplant.

**Methods**

We conducted a retrospective analysis of deceased donor kidneys transplanted between Jan 1 1992 and Dec 31 2008. Recipients all underwent transplant at a single centre. We excluded unpaired single kidneys, nephron dosing, en bloc kidneys and SPK recipients. DGF was defined as the need for dialysis in the first week after kidney transplantation. We estimated attributable risk (AR) to compare the relative proportion of the risk for DGF experienced by recipients when DGF occurred in the recipient of the contralateral kidney. We also examined the relationship within pairs for serum creatinine at 1 year and at 5 years post transplant using Spearmans Correlation.

**Range**

652 recipient pairs were analysed. The incidence of delayed graft function was 11%.

When DGF occurred in one recipient, the attributable risk for DGF in the recipient of the contralateral kidney was 0.322 (95% CI, 0.237 to 0.425), allowing for adjustment for confounding variables including donor age and sex, donor cause of death, HLA mismatch, recipient age and sex, PRA and CIT. The odds ratio of DGF occurring if the contralateral kidney had DGF was 5.986 (95% CI, 3.186 to 11.247).

Spearmans correlation between the kidney pairs for creatinine at year 1 and year 5 post renal transplant was 0.29 at year 1 and 0.29 at year 5($p<0.001$).

**Conclusions**

There is a significant degree of correlation within pairs of kidneys transplanted from the same donor for the occurrence of delayed graft function and also for serum creatinine at 1 year and 5 year post transplant. We conclude that a hidden genetic and environmental effect of the donor has a significant effect on post transplant outcomes. Important work needs to be done to establish the genes that affect these outcomes.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO926**

**Evaluation of Native Kidney Function after Simultaneous Liver-Kidney Transplant (SLK)** Jean M. Francis,[1] Matthew R. Palmer,[2] Michael P. Curry,[2] Didier A. Mandelbrot.[2] *[1]Renal section, Boston University, Boston, MA; [2]Renal Section, Beth Israel Deaconess Medical Center, Boston, MA.*

There is controversy over which patients with kidney disease that are candidates for liver transplantation should undergo liver transplant alone versus SLK. Identifying predictors of native kidney function recovery after SLK requires an accurate measure of the relative function of all three kidneys in patients with SLK. We report important modifications of standard Mag 3 renal scans that allow us to accurately measure the relative function of the transplant kidney and native kidneys in 13 SLK recipients.

The distance of a transplanted kidney from the renal scan camera can be substantially different from that of native kidneys. We developed a technique to correct for attenuation of the counts of all three kidneys based on their distance from the camera. Attenuation correction increased the measured renal function of native kidneys by up to 29%, demonstrating the importance of this procedure for detecting recovery of native kidney function.

The table shows clinical characteristics of the 13 SLK patients and the relative function of each kidney.

| Pt | Age/sex | eGFR baseline/peak | AKI duration (days) | Dialysis | Corrected % function | | | Met UNOS-proposed criteria for SLK |
|---|---|---|---|---|---|---|---|---|
| | | | | | L | R | Tx | |
| 1 | M/55 | >60/25 | 25 | Yes | 13% | 16% | 71% | No |
| 2 | M/52 | >60/9 | 7 | No | 6% | 7% | 87% | No |
| 3 | M/72 | >60/11 | >90 | No | 4% | 4% | 92% | Yes |
| 4 | F/51 | >60/6 | 15 | Yes | 15% | 11% | 74% | No |
| 5 | M/56 | 39/19 | 30 | No | 23% | 22% | 55% | No |
| 6 | M/61 | 44/9 | >90 | Yes | 4% | 6% | 90% | Yes |
| 7 | F/48 | 17/17 | >90 | No | 1% | 4% | 95% | Yes |
| 8 | M/60 | >60/15 | >90 | No | 24% | 24% | 52% | Yes |
| 9 | M/62 | >60/6 | 65 | Yes | 33% | 19% | 48% | Yes |
| 10 | M/51 | >60/15 | 30 | Yes | 9% | 5% | 86% | No |
| 11 | F/37 | >60/8 | >90 | Yes | 25% | 40% | 35% | Yes |
| 12 | F/63 | ESRD | >90 | Yes | 31% | 17% | 52% | Yes |
| 13 | M/46 | 33/12 | 62 | No | 19% | 5% | 76% | Yes |

Eight Patients (3, 6, 7, 8, 9, 11, 12 and13) met the UNOS-proposed criteria for SLK, but four of these (8, 9, 11, and 12) nevertheless had significant (>40% function) native kidney function. Five patients (1, 2, 4, 5, and10) did not meet the UNOS-proposed criteria for SLK, yet only one of these (5) had native kidney function recovery.

We report that modifications of the Mag 3 renal scan improve the measurement of the relative function of all three kidneys after SLK. The criteria proposed by UNOS to determine that SLK is indicated, and thus that native kidney recovery is not expected, are not always accurate. Further study of factors associated with native kidney recovery after SLK is required.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO927

**Serum Angiopoetin-2 Concentrations Correlate with Renal Resistance Index and Transplant Function in Renal Allograft Recipients** Ute Eisenberger,[1] Alexander Lukasz,[2] Ivo Peter Bergmann,[1] Elisabeth Weber-Bach,[1] Jan T. Kielstein,[2] Felix J. Frey,[1] Philipp Kümpers.[2,3] *[1]Nephrology and Hypertension, University Hospital Bern, Bern, Switzerland; [2]Nephrology, Medical School Hannover, Hannover, Germany; [3]General Internal Medicine, Nephrology and Rheumatology, University Hospital Münster, Münster, Germany.*

**Introduction:** Renal allograft dysfunction and mortality are associated with the Renal Resistance Index (RI) as an indicator for impaired intrarenal hemodynamics. Angiopoietin-2 (Ang-2), a circulating antagonistic ligand of the endothelial-specific Tie2 receptor, has been identified as non-redundant primer for endothelial activation. We tested the hypothesis whether Ang-2 serum concentrations are associated with renal transplant function, arterial stiffness and RI in renal allograft recipients.

**Methods**. We performed a prospective single centre cohort study of 200 renal allograft recipient (mean time after transplantation 7.0 ± 6.2yrs). At study inclusion, Ang-2 serum concentrations were measured by an in-house immuno-luminometric assay and RI was determined in segmental arteries of the allograft by colour-coded duplex ultrasound. Current mean follow-up time is 3.3 ± 0.5 yrs.

**Results**: Mean (SD) patient age at inclusion was 53 ± 13 years, eGFR 61± 20 ml/min, serum albumin 3.9 ± 0.3g/dl, cholesterol 206 ± 42 mg/dl, pulse pressure 52 ± 17 mmHg, RI 0.71 ± 0.07 and Ang-2 serum concentrations 2.8 ± 1.7ng/ml. Ang-2 values are correlated with renal resistance index (r=0.32; p<0.001), $eGFR_{Nankivell}$ (r=-0.29, p<0.001), recipient age (r=0.27; p<0.005), serum albumin (r=-0.26;p<0.01); C-reactive protein (r=0.26; p<0.01), Framingham risk score (r=0.22; p<0.05) and mean arterial pressure (r=0.23; p<0.05), but not with donor age, arterial stiffness measured by pulse pressure, total cholesterol, HbA1c or PTH. After multivariate adjustment in a regression analysis, Ang-2 remained an independent predictor of RI (standard coefficient 0.15; p<0.01).

**Conclusion:** In addition to established cardiovascular risk factors, circulating Ang-2 appears to be a relevant determinant of renal resistance index and allograft function and deserves consideration in prospective outcome trials in renal transplantation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO928

**Poor Outcomes Associated with Neutropenia after Kidney Transplantation: Analysis of USRDS** Frank P. Hurst,[1] Stephen W. Olson,[1] Robert Nee,[1] Lawrence Agodoa,[3] Rahul Jindal,[2] Kevin C. Abbott.[1] *[1]Nephrology, Walter Reed AMC, Washington, DC; [2]Organ Transplant, Walter Reed AMC, Washington, DC; [3]NIDDK, NIH, Bethesda, MD.*

**Background:** Post-transplant neutropenia (PTN) is relatively common after kidney transplantation and frequently leads to reduction in immunosuppressive agents, which can be associated with acute rejection. Granulocyte colony-stimulating factors (GCSF) have been used to treat PTN, although outcomes associated with the use of this medication in this population are unknown.

**Methods:** In a retrospective cohort of 41,705 adult Medicare primary patients transplanted from January 2001 to June 2006 and followed through December 2007, we assessed Medicare claims for neutropenia and GCSF use, respectively. Outcomes included allograft loss and death.

**Results:** There were 6043 (14.5%) patients with claims for PTN. Neutropenia occurred at a mean of 0.58 ± 0.65 years after transplantation. Factors associated with PTN included female gender, Caucasian ethnicity, ischemic heart disease, donor CMV positive, deceased donor, expanded criteria donor, delayed graft function, elevated PRA level, increased HLA mismatch, and later year of transplant. Thymoglobulin induction and maintenance tacrolimus and mycophenolate were also associated. PTN was less frequent among patients with CHF, recipient CMV positivity, and IL-2 induction. PTN was associated with increased risk of subsequent allograft loss [AHR 1.59 (95% CI 1.43-1.76)] and death [AHR 1.74 (95% CI 1.59-1.90)]. Of the 6043 patients with PTN, 740 (12.2%) received GCSF. While there was no effect on allograft loss, patients who received GCSF had a lower risk of death on unadjusted analysis.

**Conclusions:** Neutropenia after renal transplantation is common and is associated with an increased risk of allograft loss and death. GCSF was used in 12% of cases and did not increase risk of allograft loss. Strategies to avoid neutropenia and greater use of GCSF may be indicated to prevent graft loss and death.

The views expressed in this abstract are those of the authors and do not reflect the official policy of the National Institutes of Health, the Department of Defense, or the United States government.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO929

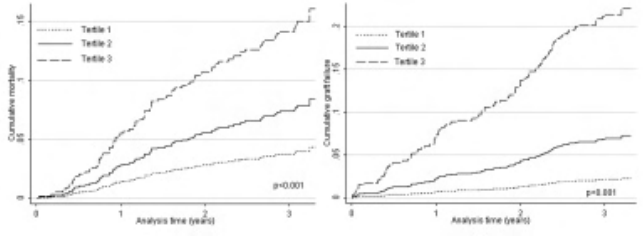
**Serum FGF-23 Levels Predict Mortality and Allograft Loss in Kidney Transplant Recipients** Istvan Mucsi,[1] Miklos Z. Molnar,[1,2] Maria Eszter Czira,[1] Anna Rudas,[1] Akos Ujszaszi,[1] Istvan Kiss,[3] Adam Remport,[3] Laszlo Rosivall,[1] Csaba P. Kovesdy,[4] Myles S. Wolf.[5] *[1]Semmelweis University, Budapest, Hungary; [2]Harbor-UCLA Medical Center, Torrance, CA; [3]Szent Imre Hospital, Budapest, Hungary; [4]Salem VA Medical Center, Salem, VA; [5]University of Miami Miller School of Medicine, Miami, FL.*

Background: Significant associations have been demonstrated between increased serum FGF-23 levels and adverse clinical outcomes. No published studies examined the association between FGF-23 and outcomes in kidney transplant (Tx) recipients.

Methods: Stable prevalent Tx recipients (n= 984; mean age 51±13 years, 57% males, mean eGFR 51±21 ml/min/1.73m2, median Tx vintage 72 months [interquartile range 74 mo] and 21% diabetics) were enrolled. Serum FGF-23 was measured using a C-terminal enzyme-linked immunosorbent assay. To assess if serum FGF-23 predicts the risk of all-cause mortality and of death censored graft loss we used semiparametric competing risks regression analysis.

Results: During the 37 months follow-up, 87 subjects died and 101 patients returned to dialysis. Both mortality and death-censored allograft loss was significantly higher in patients in the higher tertiles of baseline FGF-23 (Figure A and B). Ln-transformed serum FGF-23 was independently associated with mortality (HR associated with 1 log-unit higher FGF23 = 1.78; 95% CI: 1.39-2.28) and of death censored graft loss (1.60; 95% CI: 1.26-2.03) in multivariable-adjusted models. In the multivariable models, the highest tertile of FGF-23 was independently associated with a 2.29 (95% CI 1.15, 4.55) HR of mortality, and a 2.77 (95% CI 1.29, 5.92) HR of death-censored allograft loss.

Conclusions: High FGF-23 levels are independently associated with increased risk of mortality and death-censored graft loss in prevalent kidney transplant recipients.




Disclosure of Financial Relationships: nothing to disclose

## TH-PO930

**Single Nucleotide Polymorphisms and Decline in Renal Function Post-Transplant** Ajay K. Israni,[1] John H. Holmes,[2] Robert Leduc,[3] David P. Schladt,[3] Pamala A. Jacobson,[4] William S. Oetting,[4] Arthur J. Matas.[5] *[1]Medicine, Hennepin County Medical Center, University of Minnesota (MN), Minneapolis, MN; [2]Epidemiology, CCEB, University of Pennsylvania, Philadelphia, PA; [3]Biostatistics, University of MN, Minneapolis, MN; [4]Pharmacy, University of MN, Minneapolis, MN; [5]Surgery, University of MN, Minneapolis, MN.*

**Background**: We examined the association between single nucleotide polymorphisms (SNPs) and chronic graft dysfunction (CGD) in a multicenter, prospective cohort of kidney transplant (Tx) recipients from six international transplant centers in Canada and the United States. In order to determine SNPs associated with CGD after accounting for a strong Tx center effect, we used knowledge discovery methods.

**Methods:** Our cohort consisted of 966 patients receiving kidney Tx between 2006-2008. Genotyping was conducted for 2,723 SNPs. CGD was defined as a persistent 25% decline in eGFR from a baseline established at 3 months post-transplant. We used a correlational feature selection algorithm from the Weka data mining software suite called best-first procedure to select SNPs associated with CGD at each center separately. We then used a rule discovery tool named "JRip" which returns a set of "IF-THEN" rules, where the outcome is CGD. The "IF" side is comprised of one or more SNPs from the best-first procedure, such as if SNP x and SNP y are present, then CGD=Yes.

**Results:** The mean eGFR at baseline was 56±37 ml/min and 188 (19%) of the subjects experienced CGD at a mean time of 17± 8 months post-tx. Among subjects with a biopsy, the following distribution of biopsy scores: 28 % ci≥2, 28 % ct≥ 2, 27 % I ≥2, 24% T≥2, 11% CV≥2, 5% CG≥2, 5% g≥2, 2% AH≥2 and 2% V≥2.

There were 39 SNPs that were present in the 16 rules associated with CGD. The SNPs in these rules were in pharmacogenomic genes such as ABCC1, ABCC2, SLCO1B3, GSTA4, CYP1A1, cyclophilin A, and immune related genes such as IL12A, NFKB1 and PPARA.

**Conclusions:** Novel methods were used to determine SNPs associated with CGD after accounting for transplant center influences. These findings will be validated in another cohort of over 2,000 kidney transplants.

Disclosure of Financial Relationships: Research Funding: Research grants >$10,000 from Roche, BMS, Genzyme and Amgen.

## TH-PO931

**Pre-Transplant Peripheral Blood Myeloid Dendritic Cell Levels Associated with Infection and Death Following Kidney Transplantation** Qianmei Sun,[1] Pamela R. Patton,[2] Yanfei Huang,[1] Cassie B. Zhang,[1] Dorry L. Segev,[1] Karl L. Womer.[1] *[1]Johns Hopkins University School of Medicine, Baltimore, MD; [2]University of Florida, Gainesville, FL.*

Background: Dendritic cells are potent antigen presenting cells important for immunity and tolerance. We previously demonstrated a significant reduction of pre-transplant peripheral blood dendritic cell (PBDC) levels in renal transplant patients with evidence of post-transplant BKV reactivation, suggesting PBDC deficiency identifies a group of patients particularly susceptible to post-transplant infections. In the current report, we determined the association of PBDCs with other outcomes following kidney transplantation.

Methods: Pre-transplant peripheral blood myeloid and plasmacytoid DC levels (mDC and pDC, respectively) were measured by FACS in 79 patients with ESRD undergoing kidney transplantation and reported as absolute number of cells/mL blood. Post-transplant outcomes data were collected, including infection, rejection, graft loss and patient death for a minimum follow-up of 4.5 years. PBDC levels were compared across strata of outcomes by t-tests. Associations between PBDC levels and survival were assessed by Cox proportional hazards models.

Results: There was a trend towards lower DC levels in patients with any post-transplant infection compared to those without, with significance in the mDC subset (mean mDC level 8294.5 vs 10832.5 cells/mL; p=0.04). Significance in this DC subset was also observed when the outcome was limited to CMV infection (6930.4 vs 9673.2; p=0.05). DC levels were not associated with development of rejection. However, there was a strong association of mDC level with death (5758.1 vs 9678.6; p=0.02); for every quartile decrease in mDC level, the risk of death doubled (hazard ratio 2.12; 95% CI 1.16-3.89; p=0.01).

Conclusions: Pre-transplant peripheral blood mDC deficiency is associated with post-transplant infection and death in renal transplant patients. PBDC level monitoring may prove to be a useful clinical tool that allows individualization of immunosuppression and prediction of adverse outcomes.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO932

**Renal Allograft Loss during Transfer of Care to Adult Healthcare Services in Canadian Pediatric Renal Transplant Patients** Susan M. Samuel,[1] Brenda Hemmelgarn,[1] Marcello Tonelli,[2] Alberto Nettel-Aguirre,[1] Andrea Soo,[1] Camillia G. Clark,[1] R. Todd Alexander,[2] Bethany J. Foster.[3] *[1]University of Calgary, Calgary, AB, Canada; [2]University of Alberta, Edmonton, AB, Canada; [3]McGill University, Montreal, QC, Canada.*

**Rationale:** Among pediatric renal transplant patients, transition to adult-oriented programs usually occurs during adolescence, around age 18. We sought to determine if a *'transition period'*, defined as a 3-yr time interval centered around the first visit at an adult oriented care center, was associated with increased risk of renal allograft loss in this population. **Methods:** We used population-based data from a national organ failure registry. Patients (ages 0-18) undergoing kidney transplantation in a Canadian pediatric centre between 1992 and 2007 were included and followed until death, loss to follow-up or Dec 31, 2007. Primary outcome was time to first allograft loss. We defined *'transition period'* as the interval 1.5 years before and 1.5 years after first recorded visit to adult care; results were adjusted for gender, donor type, age at transplant, race. **Results:** We included 460 pediatric transplant patients of whom 227(49.3%) received living donor transplants. In total, 98 (21.3%) patients experienced first graft loss by study end. Median time to first graft loss was 4.87 (IQR 1.87-7.66) years. Compared with experience outside the transition period, the adjusted hazard ratio (HR) for first renal allograft loss within the transition period was 2.38 (95 % CI 1.53-3.71). Black race was associated with increased risk of graft loss compared to Caucasians (adjusted HR 2.90; 95% CI 1.15-7.32). Age at first renal transplant of 10-15y or 15-18y was also associated with increased risk of allograft loss compared with 0-10y (adjusted HRs 10-15y: 2.61(95% CI 1.49-4.55), 15-18y: 2.38(95% CI 1.35-4.19)). **Conclusion:** We found increased risk of renal allograft loss during the *'transition period'* defined as 1.5 years before and after the date of transfer to adult care. Adolescent age (10-18y) at transplant and Black race were associated with increased risk of first graft loss. Further studies are needed to determine causes of graft loss during the transition period.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO933

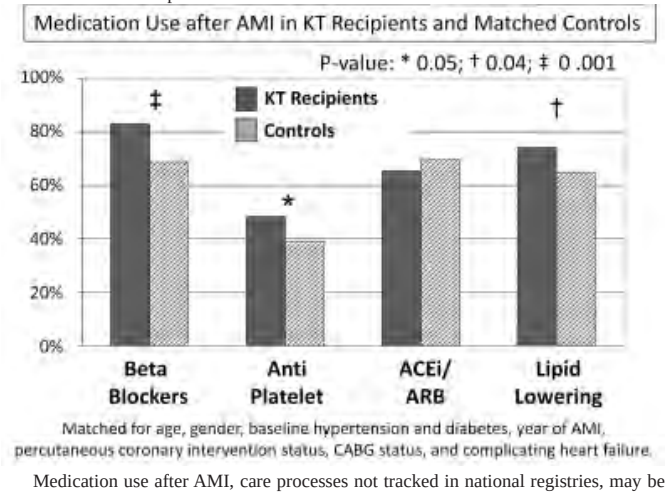
**Novel Approaches to Quantifying Cardioprotective Medication Use in High-Risk Transplant Recipients** Krista L. Lentine,[1] Kevin C. Abbott,[2] Daniel C. Brennan.[3] *[1]Saint Louis University; [2]Walter Reed; [3]Washington University.*

Understanding medication use among kidney transplant (KT) recipients may identify opportunities for care process improvement

We examined a novel database linking OPTN data for KT recipients to billing claims (2003-2006) of a private health insurer. KT recipients with posttransplant acute myocardial infarction (AMI) were identified by diagnosis codes on claims (n=209). General beneficiaries with AMI were identified by the same algorithm. The prevalence of beta-blocker (BB), antiplatelet, ACE inhibitor/angiotensin-2 receptor blocker (ACEi/ARB) and lipid-lowering agent use within 60d after AMI was ascertained by pharmacy claims.

In multiple logistic regression with adjustment for demographic and clinical factors (including peri-AMI revascularization and heart failure), KT status was associated with increased likelihood of BB (aOR 2.50, 95% CI 1.70–3.68) and lipid-lowering medication (aOR 1.53, 95% CI 1.08–2.15) use after AMI. The likelihood of ACEi/ARB and anti-platelet use after AMI did not differ significantly in KT vs general patients.

In a separate analysis, we also matched KT recipients 1-to-1 with general beneficiaries on the vector of factors associated with medication use after AMI in the sample. Compared to matched controls, KT recipients with AMI more commonly received BB (83.0% vs 69%, P=0.001) and lipid-lowering agents (74% vs 65%, P=0.04), and showed a non-significant trend towards more common receipt of anti-platelets (48% vs 39.0%, P=0.05). The frequency of ACEi/ARB prescriptions after AMI did not differ significantly by KT status in matched comparison.




Medication use after AMI, care processes not tracked in national registries, may be monitored in administrative data. While KT status does not appear to be a barrier, there may be room for optimizing potentially cardioprotective medication use after AMI in KT patients.

Disclosure of Financial Relationships: Research Funding: Novartis Pharma.

## TH-PO934

**Impact of Etiology of Acute Kidney Injury (AKI) on Outcomes Following Liver Transplantation: Acute Tubular Necrosis (ATN) Versus Hepatorenal Syndrome (HRS)** Mitra K. Nadim, Christopher A. Tokin, Wei Ye, Rick Selby. *University of Southern California.*

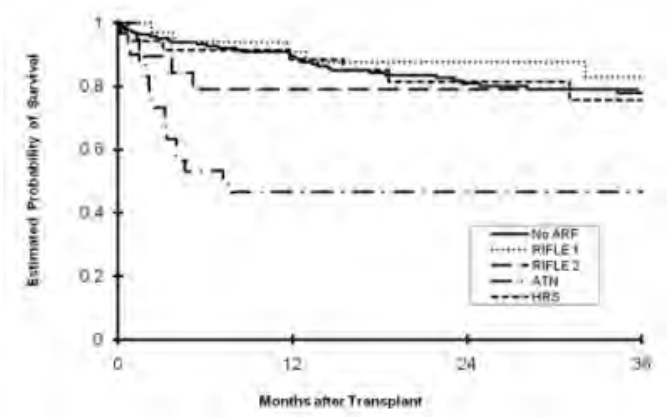
Background:AKI at the time of liver transplantation (LT) has been associated with increased morbidity & mortality. Previous studies have focused mainly on severity of renal dysfunction or duration of dialysis pre-LT on post transplant outcomes. The purpose of this study was to determine the impact of etiology of AKI (ATN vs HRS), on post LT outcomes.

Methods:We reviewed 293 patients who underwent LT following the MELD-based allocation. Patients were stratified according to the severity of AKI at the time of LT based on RIFLE classification: Risk, Injury, Failure. The "Failure" group was subdivided based on etiology of AKI as either HRS (defined by IAC criteria) or ATN.

Results: 1 and 3 year patient survival following LT were the lowest in patients with ATN (p<0.001). Multivariate analysis revealed that the presence of ATN was the ONLY variable associated with higher mortality at 1 year after LT (HR 2.97;CI 0.84-10.54;P< 0.025). Complete renal recovery at 1 year was: 71% (Risk), 47% (Injury), 20% (ATN) and 71% (HRS). Duration of pretansplant dialysis in the HRS & ATN groups was similar.

Patient Characteristics & Outcomes

| | No AKI (n=165) | Risk (n=34) | Injury (n=19) | Failure (n=65) | | p-value |
|---|---|---|---|---|---|---|
| Patient Characteristics at Time of Transplant | | | | ATN (n=30) | HRS (n=35) | |
| MELD-median | 22 | 28 | 29 | 40 | 40 | <0.001 |
| In ICU-% | 3 | 5 | 21 | 80 | 49 | <0.001 |
| SOFA score-median | 4 | 6 | 8 | 14 | 12 | <0.001 |
| On Dialysis-% | | | | 73 | 86 | 0.21 |
| Patient Survival-% | | | | | | <0.001 |
| 1 year | 89 | 91 | 79 | 47 | 89 | |
| 3 year | 78 | 80 | 79 | 47 | 77 | |
| Incidence of CKD | | | | | | <0.001 |
| 1 year | 11 | 13 | 21 | 53 | 12 | |
| 3 year | 22 | 19 | 37 | 59 | 23 | |




Conclusions: Our study is the first to show that AKI etiology, rather than severity or duration of dialysis, has the greatest impact on patient and renal outcomes following LT.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO935

**The Association between Inflammation and Serum Erythropoietin Level in Kidney Transplanted Recipients** Miklos Z. Molnar,[1,2,3] Maria Eszter Czira,[2] Anna Rudas,[2] Akos Ujszaszi,[2] Marta Novak,[2,5] Laszlo Rosivall,[1,4] Istvan Mucsi.[2,4] *[1]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary; [2]Institute of Behavioral Sciences, Semmelweis University, Budapest, Hungary; [3]Harold Simmons Center for Chronic Disease Research & Epidemiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, UCLA, Torrance, CA; [4]Hungarian Academy of Sciences and Semmelweis University Research Group for Pediatrics and Nephrology, Budapest, Hungary; [5]University of Toronto, Toronto, ON, Canada.*

**Background:** The presence of iron deficiency and inflammation are associated with erythropoietin (EPO) resistance in patients on maintenance dialysis and with post-transplant anemia in kidney transplant (Tx) recipients. These factors may contribute to resistance to endogen erythropoietin in Tx patients resulting in higher serum EPO levels. To assess this hypothesis we analyzed the cross-sectional association between serum EPO versus markers of inflammation and iron deficiency in Tx patients.

**Methods:** We collected socio-demographic parameters, medical and transplant history and laboratory data from a prevalent cohort of 891 Tx patients not treated with erythropoiesis stimulating agents. A solid-phase, chemiluminescent immunometric assay was used to measure baseline serum erythropoietin (EPO).

**Results:** Mean age was 51±13 years, 60% of the patients were males, 21% had diabetes. The median of serum EPO was 10.8 U/l (IQR=7.4 U/l). The age and eGFR adjusted serum EPO level was significantly correlated with serum IL-6 (rho=0.229) and CRP (rho=0.128) and also with the serum soluble transferrin receptor concentration (rho=0.308) and the percentage of hypochromic reticulocytes (rho=0.228), p<0.001 for all. In a linear regression model serum IL-6, the percentage of hypochromic reticulocytes and serum soluble transferrin receptor were significantly associated with the log-transformed serum EPO after adjusting several clinical, socio-demographic and laboratory co-variables.

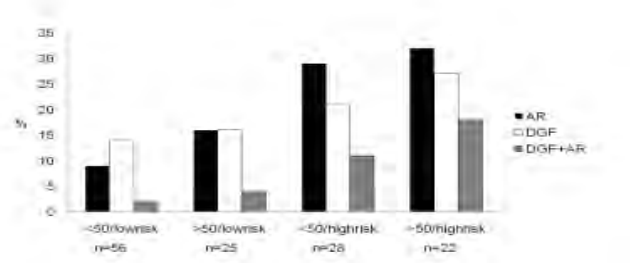
**Conclusions**: Iron deficiency and inflammation are independently associated with serum EPO levels in kidney transplanted patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO936

**Cellular or Humoral Presensitization Increases the Risk of Delayed Graft Function and Acute Rejection in Recipients of Kidneys from Older Donors** Donald E. Hricik,[1] Joshua J. Augustine,[1] Aparna Padiyar,[1] Peter S. Heeger.[2] *[1]Medicine, University Hospitals Case Medical Center, Cleveland, OH; [2]Medicine, Mt. Sinai School of Medicine, New York, NY.*

Outcomes of kidney transplantation are influenced by donor age but may be modifed by recipient alloreactivity. We hypothesized that patients at high immune risk, defined by pretransplant donor specific ELISPOT for interferon gamma ≥ 25/300k cells and/or PRA>80%, may have particularly bad outcomes after receiving kidneys from older donors. We reviewed outcomes of 131 deceased donor recipients in whom pretransplant values for both ELISPOT and PRA were available. 52 patients were enrolled in an NIH-sponsored CTOT study. Based on donor age <or ≥ 50 yrs, we categorized patients into 4 groups (see x axis of figure). Population characteristics: recipient age 48.3±11 yrs, 62% male, 53% African American, HLA mismatches 4.0±1.9. High risk patients included 46 with high ELISPOT alone, 7 with high PRA alone, and 3 with both. The incidence of delayed graft function (DGF), acute rejection (AR) and AR after DGF (DGF+AR) are shown in the figure.




AR occurred in 32% of the ≥50/highrisk group vs 9% in the <50/lowrisk group (p<0.001). DGF occurred in 27% of the ≥50/highrisk group vs 14% in the <50/lowrisk group (p=0.014). Logistic regression showed that the combination of high risk status and donor age ≥50 increased the risk of AR (odds ratio 1.7, p=0.007), and DGF+AR (odds ratio 2.1, p=0.036) independent of recipient age, gender, ethnicity, and HLA mismatch. The results show that cellular or humoral presensitization magnify the adverse effects of advanced donor age. Caution should be used in offering kidneys from older donors to patients with cellular or humoral presensitization. In the absence of presensitization, recipients of kidneys from older donors have outcomes similar to those with younger donors.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO937

**Association of Body Mass Index and Abdominal Circumference with Mortality in Kidney Transplant Recipients** Csaba P. Kovesdy,[1,2] Maria Eszter Czira,[3] Anna Rudas,[3] Akos Ujszaszi,[3] Laszlo Rosivall,[3] Marta Novak,[3] Kamyar Kalantar-Zadeh,[4] Miklos Z. Molnar,[3,4] Istvan Mucsi.[3] *[1]Salem VA Medical Center, Salem, VA; [2]University of Virginia, Charlottesville, VA; [3]Semmelweis University, Budapest, Hungary; [4]Harbor-UCLA, Torrance, CA.*

Higher body mass index (BMI) is associated with lower mortality in patients with CKD and ESRD, but it is unclear if this is a result of higher muscle mass or higher fat mass, both of which could be reflected by elevated BMI. The impact of BMI and body composition on survival is unclear in kidney transplant (KT) recipients.

We examined the association of BMI and abdominal circumference (AC) with all-cause mortality in 993 KT recipients. Associations were examined in Cox models with adjustment for AC (in the case of BMI) and BMI (in the case of AC) and for age, gender, transplant vintage, delayed graft function, diabetes, Charlson index, smoking, blood pressure, eGFR, albumin and CRP.

In unadjusted analyses neither BMI nor AC was associated with mortality (Table). However, higher BMI was associated with lower mortality after adjustment for AC, and higher AC was associated with higher mortality after adjustment for BMI (Table). These associations remained significant after additional adjustments (Table).

In KT higher BMI and AC displayed opposite associations with mortality. Since AC-adjusted BMI could be considered a marker of muscle mass and BMI-adjusted AC a marker of fat mass, these associations may reflect a protective effect of muscularity but a detrimental effect of adiposity. The impact of strategies aimed at lowering elevated BMI prior to and after transplantation should be critically assessed based on their effects on body composition.

All-cause mortality associated with a one standard deviation higher BMI and AC

| | Unadjusted | Adjusted for AC (BMI only) and for BMI (AC only) | Multivariable adjusted |
|---|---|---|---|
| BMI (5 kg/m2 higher) | 0.84 (0.67, 1.06) p=0.15 | 0.38 (0.25, 0.59) p<0.001 | 0.55 (0.36, 0.84) p=0.005 |
| Abdominal circumference (15 cm higher) | 1.14 (0.91, 1.41) p=0.25 | 2.47 (1.63, 3.73) p<0.001 | 1.65 (1.04, 2.60) p=0.042 |

HR, 95%CI

Disclosure of Financial Relationships: Consultancy: GenzymeResearch Funding: Abbott, Genzyme, Shire; Honoraria: Genzyme, Novartis, Shire.

**TH-PO938**

**Initial Comparisons of Performing Routine Surveillance Protocol Biopsies vs No Surveillance Biopsies: Analysis of the Mycophenolic Acid Observational Renal Transplant (MORE) Registry** Ali Olyaei,[1] Laurence Chan,[2] Anne Wiland,[3] Kevin M. Mccague,[3] Richard W. Carson.[4] *[1]Oregon State University/ OHSU, Portland, OR; [2]University of Colorado, Denver, CO; [3]Novartis, East Hanover, NJ; [4]Providence Sacred Heart Medical Center, Spokane, WA.*

Surveillance Biopsies (BXs) have been recommended to detect early subclinical rejection and calcineurin inhibitor (CNI) toxicity. Early treatment of rejection or immunosuppressive (IS) modifications may alter the natural history of chronic allograft injury (CAI) in renal transplant recipients (RTRs). The objective of this analysis was to evaluate the utility of surveillance renal BXs in detecting early rejection, CNI toxicity and other complications. **Methods:** Using data reported to the MORE Registry, we analyzed whether surveillance BXs are associated with a greater detection of biopsy-proven acute rejection (BPAR), early graft failure, improved serum creatinine (SCr), CAI and CNI toxicities at 6 months post-transplant. Standard-of-care was determined by local practice at 40 US sites. The outcomes of 722 RTRs were reviewed (mean age 51 yrs, 63% male). Log-rank tests were used to compare graft survival (GS), patient survival (PS) and cumulative incidence of first BPAR. **Results:** Preliminary data were analyzed {213 patients underwent surveillance BXs, 509 did not (control group)}. There were no significant differences in GS (98.1/99.0%, p=0.70), PS (99.5/99.3%, p=0.23), BPAR (7.6%/5.9%, p=0.33), mean SCr (1.47/1.49 mg/dL, p=0.83) or GFR by MDRD (58.8/56.8 mL/min, p=0.25) in the BX group vs. control group. CAI or CNI toxicities were noted in 1.4/0.8% and 0.9/2.0% (NS) of surveillance BX group vs. the control group. The incidences of BK nephropathy, GI toxicities, CV complications, tacrolimus levels, mycophenolate and prednisone dosage were similar between groups. There were more reported infections (17.8/11.7%, p=0.03) and CMV (7.0/3.1%, p=0.03) in the surveillance BX group. **Conclusion:** Early rates of BPAR, CAI and CNI toxicity were similar between the two groups. Newer IS regimens have reduced the overall incidence of clinical rejection, potentially resulting in reduced benefit of early post-transplant surveillance BXs in RTRs.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO939**

**Enteric-Coated Mycophenolate Sodium (EC-MPS) Versus Mycophenolate Mofetil (MMF) in African American (AA) Renal Transplant Recipients from the Mycophenolic Acid Observational Renal Transplant (MORE) Registry** Mohanram Narayanan,[1] Oleh G. Pankewycz,[2] Mohamed A. El-Ghoroury,[3] Anne Wiland,[4] Kevin M. Mccague,[4] John A. Daller.[5] *[1]Scott and White Clinic, Temple, TX; [2]Buffalo General Hospital, Buffalo, NY; [3]St. Clair Specialty Physicians, Detroit, MI; [4]Novartis, East Hanover, NJ; [5]Temple University, Philadelphia, PA.*

**Introduction:** The MORE Registry, a prospective study of adult de novo renal transplant recipients (RTRs) receiving mycophenolic acid (MPA) therapy, is designed to determine effectiveness, tolerability and safety of EC-MPS vs. MMF regimens. **Methods:** Based on practices at 40 US sites, outcomes analyzed included: graft survival (GS), patient survival (PS), first biopsy-proven acute rejection (BPAR), adverse event (AE) rates, serum creatinine (SCr) and proportion of RTRs maintained on at least full recommended MPA dose (1.44/2.0 g/day, EC-MPS/MMF). A total of 165 AA (114 EC-MPS/51 MMF) and 521 non-AA (352 EC-MPS/169 MMF) tacrolimus-treated RTRs were included. **Results:** At 1, 3, 6 and 12 months, more AA EC-MPS RTRs received full MPA dose (EC-MPS/MMF: 83.3/ 74.5%, p=0.20; 67.6/ 66.0%, p=0.85; 62.4/ 43.6%, p=0.05; 50.0/45.5%, p=0.68). This was also observed in the non-AA patients (EC-MPS/ MMF: 79.1/69.9%, p=0.02; 73.6/57.8%, p<0.01; 54.8/46.3%, p=0.13; 48.1/42.1%, p=0.34). Comparable 6-month effectiveness, tolerability and safety outcomes were achieved in both groups. In the AA RTRs, there were less frequent GI AEs in the EC-MPS group (58/75%, p=0.05). Comparing EC-MPS/MMF in the AA RTRs, there was similar GS (99.1/100%, p=0.18), PS (100/98.0%, p=0.07), BPAR (12.9/12.3%, p=0.61), mean SCr (1.58/1.94 mg/dL, p= 0.19) and non-GI AEs by organ system, infections or neoplasia. Comparing AA to non-AA RTRs regardless of MPA type, both BPAR (12.7/4.5%, p<0.01) and SCr (1.70/1.41 mg/dL, p<0.01) were higher whereas GS (99.4/98.8%) and PS (99.3/99.4%) were similar. **Conclusion:** More AA and non-AA RTRs treated with EC-MPS were maintained on full doses of MPA. Despite this, AA RTRs exhibited higher BPAR and mean SCrs than non-AAs which may impact clinical outcomes at later timepoints in this study.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO940**

**Predictors of Compensatory Hyperfiltration Years after Kidney Donation** Marc L. Weber,[1] Aleksandra Kukla,[1] Richard S. Spong,[1] Erin Leister,[1] Arthur J. Matas,[2] Hassan N. Ibrahim.[1] *[1]Medicine - Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN; [2]Surgery, University of Minnesota, Minneapolis, MN.*

Without compensatory hyperfiltration, the best post-donation GFR would be half of the pre-donation value. GFR values increase in the months after donation. There is little data regarding change in GFR many years after donation. We studied a representative sample of 255 kidney donors from a pool of 3698 donors 12.2±9.2 years after donation to determine the pattern and predictors of GFR change overtime. Characteristics of these donors, GFR estimated using the re-expressed MDRD formula and other formulas are shown in table. 70% of post-donation $eGFR_{MDRD}$ values were less than 85% of pre-donation $eGFR_{MDRD}$ and the average decline in $eGFR_{MDRD}$ was 21 mL/min/1.73m². Nearly 20% of patients had a post-donation $eGFR_{MDRD}$ within 15% of their pre-donation $eGFR_{MDRD}$ and about 10% of kidney donors actually had a post-donation $eGFR_{MDRD}$ greater than their pre-donation $eGFR_{MDRD}$.

| | |
|---|---|
| n | 255 |
| Female | 61.6% |
| White | 98.8% |
| Age | 40.9±11.0 |
| BMI | 26.0±4.2 Kg/m² |
| Creatinine (pre-donation) | 0.90±0.22 mg/dL |
| Creatinine $clearance_{CG}$ (pre-donation) | 88.5±23.1 mL/min/1.73m² |
| $eGFR_{CKD-EPI}$ (pre-donation) | 99.1±20.0 |
| Mean $eGFR_{MDRD}$ (pre-donation) | 92.9±234 mL/min/1.73m² |
| Mean $eGFR_{MDRD}$ (post-donation) | 71.6±14.9 mL/min/1.73m² |
| Change in $eGFR_{MDRD}$ (pre & post-donation) | -21.8±21.6 mL/min |
| $eGFR_{MDRD}$ (post-donation outcomes) | Post-donation eGFR < 85% of two kidney eGFR: 70.2% |
| | Post-donation eGFR within 15% of two kidney eGFR: 19.8% |
| | Post-donation eGFR 15% of two kidney eGFR: 9.9%≥ |

Pre-donation higher GFR, higher BMI, and the development of hypertension post-donation were associated with a lower compensatory increase in GFR. A more robust increase in post-donation GFR is predicted with a pre-donation BMI less than 30 Kg/m². This data demonstrates that nearly a third of kidney donors attain a 12-year post-donation eGFR that is near to or greater than their pre-donation two kidney eGFR and a lean body habitus at donation was associated with higher post-donation GFR.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO941**

**Moderate Alcohol Consumption Is Associated with Low Prevalence of Post-Transplant Diabetes and Reduced Risk for Mortality in Renal Transplant Recipients** Dorien M. Zelle,[1] Eva Corpeleijn,[2] Ronald Stolk,[2] Jaap Homan vd Heide,[1] Willem Van Son,[1] Gerjan Navis,[1] Stephan J. L. Bakker.[1] *[1]Kidney Centre, University Medical Center Groningen, Netherlands; [2]Epidimiology, University Medical Center Groningen, Netherlands.*

Renal transplant recipients (RTR) are often advised to refrain from alcohol because of use of immunosuppressive drugs. While in the general population moderate alcohol consumption is associated with reduced risk for diabetes and mortality, it is unknown if the same holds true for RTR. Therefore we investigated the association of alcohol consumption with post transplant diabetes (PTD) and mortality in RTR.

RTR were investigated between 2001 and 2003. Alcohol consumption was assessed by self-report. Mortality was recorded until May 2009.

A total of 600 RTR (age 51±12 years, 55% men) participated at a median time of 5.9 years post-transplant. Of these RTR, 288(48%) were abstainers, 94(16%) sporadic users, 210(35%) had moderate intake and 8(1%) were heavy drinkers. Total prevalence of PTD was 78(12%). Moderate alcohol consumption was associated with low prevalence of PTD (OR= 0.33 [0.2-0.6], P<0.002). During median follow-up for 7.0 years, 33(15.7%) subjects died in the group of moderate alcohol consumption, while 75(26.0%), 23(24.5%), and 2(25%) died in the groups of abstainers, sporadic and heavy drinkers resp. (P=0.01).


In univariate Cox-regression analyses moderate alcohol intake was associated with reduced risk for mortality (HR=0.56 [0.4-0.8],P=0.005). Adjustment for potential confounders, including diabetes and smoking did not materially change this association.

Moderate alcohol consumption is associated with low prevalence of PTD and reduced risk for mortality in RTR. In contrast with common advices, drinking moderate amounts of alcohol appears to be protective against diabetes and mortality in RTR, similar to the general population.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO942**

**Luminex-Based Desensitization Protocols: Short Term Outcomes** Arjang Djamali, Brenda L. Muth, David Lorentzen, John D. Pirsch, Anthony M. D'Alessandro, Hans Sollinger. *Medicine and Surgery, UW Madison School of Medicine and Public Health, Madison, WI.*

We have demonstrated that pretransplant donor specific antibody (DSA) levels > 100 mean fluorescent intensity (MFI) by Single Antigen Bead Luminex Assay (One Lambda) are associated with antibody mediated rejection (AMR). There is no information on whether

Luminex-based preconditioning protocols are effective desensitization strategies. We have developed protocols based on MFI levels in patients with a negative CDC crossmatch.

| Protocol number | Transplant Type | MFI | Induction | Pheresis + IVIG |
|---|---|---|---|---|
| P1 | Live | 100-500 | Basiliximab | - |
| P2 | Live | 500-1000 | Basiliximab | 2 pre 2 after |
| P3 | Live | 1000-3000 | Basiliximab | 4 pre 2 after |
| P4 | Deceased | 100-500 | Basiliximab | - |
| P5 | Deceased | 500-1000 | Thymoglobin | - |
| P6 | Deceased | 1000-3000 | Thmyglobin | 1 pre 3 after |

Live donor recipients are treated with TAC and MPA for 1 week pretransplant. All patients receive maintenance immunosuppression with TAC, MPA and steroids. Between January 2009 and December 2010 we performed 249 kidney transplants, of those 48 (23%) were sensitized across the HLA barrier (P1-P6) and required desensitization (mean MFI values against HLA 1 and 2 were 1,100 and 900 MFI and peak PRA 23%). Median age in sensitized and non-sensitized groups was 48.5 vs 52 years (p=ns). Most patients were male (56% vs 65%, p=ns). There were more retransplants (50% vs 18%, p<0.001) and live donor transplants (56% vs 30%, p<0.001) in the sensitized group. The incidence of AMR (25% vs 12.5%, p=0.008) and acute cellular rejection (23% vs 14%, p=0.02) was significantly higher in the sensitized group. However, mixed rejection (8%), graft loss (0 vs 6) or patient death (0 vs 3) were not significantly different between sensitized and control groups. Furthermore, 6 months (1.5 vs 1.4 mg/dL) and last (1.3 vs 1.4 mg/dL) serum creatinine levels were similar between the two groups. In conclusion Luminex based preconditioning protocols may be used in sensitized patients. However, long-term data are required to assess the efficacy of these protocols.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO943

**Basiliximab Is Associated with New Onset Diabetes Mellitus (NODAT) after Transplantation** Willy Aasebo,[1] Hallvard Holdaas.[2] *[1]Medical Department, Akershus University Hospital, Lorenskog, Norway; [2]Medical Department, Oslo University Hospital, Rikshospitalet, Oslo, Norway.*

**Background.** The interleukin-2 antibody basiliximab suppresses proliferation of T cells resulting in an impairment of regulatory T-cell function. Regulatory T-cells are associated with autoimmune diseases, diabetes mellitus included. Even though NODAT resembles diabetes type-2 more than type-1 we hypothesized that basiliximab might be associated with NODAT after transplantation.

**Methods**. In a retrospective study we included all kidney recipients >18 years of age without diabetes mellitus transplanted in 2005, 2006 or 2007 (n = 485). Our immunosuppressive protocol consisted of steroids, mycophenolate and cyclosporine/tacrolimus. Basiliximab was introduced as induction therapy in 2007, and were given to 264 recipients. The recipients transplanted in 2005 and 2006 not receiving induction therapy served as a control group. At week 10 post transplant an oral glucose tolerance test was performed (75 mg Dextrose). Diagnosis of diabetes mellitus was defined as a fasting plasma glucose ≥7.0 mmol/l or two hours plasma glucose ≥11.1 mmol/l.

**Results.** In the basiliximab-group 52/264 (19.7%) developed NODAT, versus 24/221 (10.9%) in the group with no induction therapy (p = 0.0068). Analysis from a subgroup of recipient without rejection, hence with comparable total steroid load, showed NODAT in 34/184 (18.5%) in the basiliximab group and 6/123 (4.9%) in the no induction group (p <0.001)

In multivariate logistic regression odds ratio for NODAT was 2.3 (95% CI: 1.3-4.2) (p = 0.056) in the basiliximab group compared to the group with no induction, after adjusting for age, CMV-infection, donor type (LD/DD), rejection and BMI.

**Conclusion**. Basiliximab use is associated with an increased risk for developing in NODAT short-time after transplantation.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO944

**The Clinical Outcome after ABO Incompatible Kidney Transplantation in Patients with High Baseline Antibody Titer** Byung Ha Chung, Sun Ryoung Choi, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Department of Internal Medicine, Seoul St. Mary's Hospital, Seoul, Republic of Korea.*

**Purpose**: The aim of this study is to investigate the clinical outcome of ABO incompatible kidney transplantation (ABO icKT) in patients with high baseline anti-ABO antibody titer.

**Methods**: This retrospective study was done about 12 cases of ABO icKT performed in our center. High titer was defined when baseline anti-ABO antibody titer was over 1:256. We used preparation composed of rituximab, plasmapheresis (PP) and intravenous immune-globulin (IVIG).

**Results**: Six patients belonged to high titer group and another six were low titer group. The highest titer was 1:1024. Mean follow-up period was 22.3±21.3 weeks in high titer group and 27.7±12.3 weeks in low titer group. More session of pre-transplant (9.3±3.0 vs. 6.0±1.38, respectively) and post-transplant PP/IVIG (2.3±2.9 vs. 0) was required in high titer group compared to low titer group. Protocol biopsy was done in five patients and C4d was diffusely positive in four cases (80%) without any evidence of antibody mediated rejection. There has been no episode of antibody mediated rejection during follow up period in both groups. There were total three cases of infectious complication (two viral infections in high titer and one pyoknee in low titer group) and all of them were cured by anti-viral or antibiotics. There were two cases of post-operative bleeding, one in high titer and one in low titer group. The patient from high titer group suffered nephrectomy because of uncontrolled bleeding. All patients show immediate graft function in early post-transplant period. Six patients have been observed for longer than six months (two patients from high titer group and another four were low titer group) and the serum creatinine level was 1.18±0.24 mg/dL in high titer group and 1.07±0.28 mg/dL in low titer group at six months after KT. .

**Conclusion**: ABO icKT can be carried out even in patients with high baseline antibody titer without increased risk for humoral rejection. However, we should be careful about post-operative bleeding in those patients because increased number of pre and post-transplant PP/IVIG can increase bleeding tendency.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO945

**Plasma EPO Is an Independent Predictor of Cardiovascular and Total Mortality in Renal Transplant Recipients** Steef Jasper Sinkeler, Jaap Homan vd Heide, Gerjan Navis, Stephan J. L. Bakker. *Nephrology, University Medical Center Groningen, Groningen, Netherlands.*

Cardiovascular disease (CVD) is the main cause of mortality in renal transplant recipients (RTR). Classical risk factors are involved, but only partly explain the excess risk. We hypothesized high EPO levels – a combined marker for inflammation, angiogenesis and hypoxia – might be a novel risk factor for CVD in RTR.

606 RTR from our center (age 51±12 years; 45 % female; creatinine clearance (CrCl) 57±20 mL/min/1.73 m²) were included at median 6 [IQR 3-11] years after Tx. All patients on exogenous EPO were excluded (n=18). Endogenous EPO levels were measured by Roche enzymatic assay.

Median EPO level was 17.4 [11.9–24.4] IU/L. Baseline EPO values were positively associated with waist circumference (r=0.08, p=0.04), CVD history (r=0.13, p=0.001), CRP (r=0.10, p=0.01), MCV (r=0.10, p=0.02), CrCl (r=-0.13, p=0.001) and proteinuria (r=0.10, p=0.02) and inversely with RAAS blockade (r=-0.15, p<0.001), Hb (r=-0.16, p<0.001) and borderline with ferritin (r=-0.07, p=0.09). During follow-up for 7 [6-7] years, 134 RTR died, of which 70 from CV causes. High EPO levels (per 10 IU/L) predicted both CV (HR 1.19 (95%CI 1.11–1.28), P=0.009) and total mortality (HR 1.15 (1.08 - 1.23), P<0.001).

These were independent of age, gender, CVD history, Hb, CRP, ferritin, CrCl, use of RAAS inhibitors, proteinuria and Framingham risk factors (smoking, diabetes, total and HDL- cholesterol and systolic blood pressure) in a multivariate Cox-regression analysis, with HR (per 10 IU/L EPO) 1.15 (1.04–1.28), P=0.009 for CV mortality and 1.11 (1.01–1.21), P=0.03 for total mortality.

In conclusion, EPO levels predict total and CV mortality in RTR, independent of potential confounders, including inflammation, renal function and levels of hemoglobin. This suggests other mechanisms may be involved. Further dissecting the determinants of EPO levels in RTR may provide new possibilities for reducing CV mortality in this population.

Disclosure of Financial Relationships: nothing to disclose

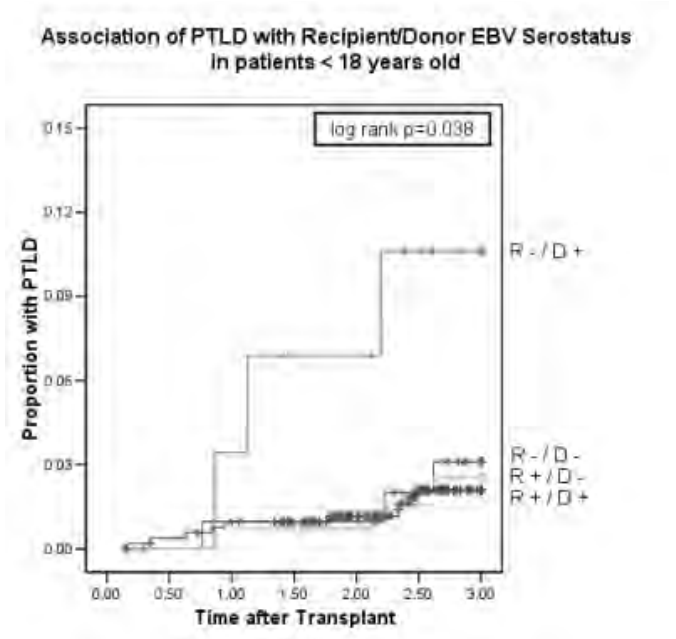
### TH-PO946

**Risk Factors and Prognosis of Posttransplant Lymphoproliferative Disorders after Renal Transplantation: Analysis of USRDS** Robert Nee,[1] Frank P. Hurst,[1] Lawrence Agodoa,[3] Rahul Jindal,[2] Kevin C. Abbott.[1] *[1]Nephrology, Walter Reed Army Medical Center, Washington, DC; [2]Organ Transplant, Walter Reed Army Medical Center, Washington, DC; [3]NIDDK, National Institutes of Health, Bethesda, MD.*

**Background:** Post-transplant lymphoproliferative disorders (PTLD) represent a well-recognized complication of renal transplantation. Known risk factors include Epstein-Barr virus (EBV) seronegativity and over-immunosuppression, with others to be further characterized.

**Methods:** In a retrospective cohort of 53,719 Medicare primary patients transplanted from January 2001 to June 2006 and followed through December 2007, we assessed Medicare claims for PTLD.

**Results:** There were 719 (1.3%) patients with claims for PTLD. Caucasian ethnicity, recipient history of malignancy, and rejection within the first year post-transplant were associated with an increased risk of PTLD. Sirolimus and tacrolimus were also associated with a higher risk. PTLD is associated with an increased risk of death [AHR 3.84 (95% CI 3.27-4.50); p < 0.001]. EBV serostatus was missing for 10.9% of recipients and 74.3% of donors. Given this limitation, paired recipient (R) negative and donor (D) positive EBV serostatus was not associated with PTLD in this cohort. However, in an unadjusted analysis of patients 18 years or younger, the EBV R-/D+ combination was associated with a higher risk for PTLD (p = 0.038).




**Conclusion:** Our results from recent USRDS data further support the higher mortality risk and delineate risk factors for PTLD. The EBV R-/D+ combination was associated with a higher risk for PTLD in younger patients.

The views expressed in this abstract are those of the authors and do not reflect the official policy of the National Institutes of Health, the Department of Defense, or the United States government.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO947

**Previous Renal Support and Classic Risk Factors for Renal Disease Are Predictors for Chronic Renal Replacement Therapy (RRT) after Orthotopic Liver Transplantation (OLT)** Maria C. C. Andreoli, Maria P. V. Coelho, Nadia K. Guimaraes, Miguel A. Goes, Adriano L. Ammirati, Thais N. Matsui, Ilson Jorge Iizuka, Fabiana Dias Carneiro, Jose Ben-Hur Ferraz-Neto, Bento Santos. *Centro de Diálise Einstein, Hospital Israelita Albert Einstein, Sao Paulo, Brazil.*

In the MELD (Model for End-Stage Liver Disease) era of organ allocation, RRT has been very common in OLT patient and the probability of kidney function recovery is essential for transplant program management. In this study we evaluated a sample of stable post-Intensive Care hemodialysis (HD) patients from a group of 297 adults who were submitted to OLT in an urban tertiary medical center from June 1, 2005, to December 31, 2009. The aim was to evaluate the average time of renal function recovery (out of need of RRT) in OLT patients on post intensive care HD and determine risk factors for chronic HD support during a 1-year follow-up period. Patients were censored at recovery of kidney function, death on HD or end of the follow-up period. The Cox proportional hazards model was used to compare the relative risk (RR) of remained or not in HD after 1 year. We evaluated data of 83 patients (50±14 yo, 64% male, 22% pre-OLT diabetes mellitus[DM], 31% HCV- disease, MELD 27.5±11.8, pre-OLT serum creatinine 1.5±1,4mg/dL, 17% acute re-OLT, 37% pre-OLT RRT, 28% pre-OLT proteinuria). During the study period, 70 (84%) were removed from dialysis; of these, 6 (7%) remained on HD for more than 90 days until renal function recovery and the longest period was 184 days. Nine (11%) patients died on HD and only 4 (5%) patients were on HD after 1 year. The median of recovery time was 28 days (from 6 to 184d). Classic risk factors for renal disease like age, DM and pre-OLT RRT were significant predictors of chronic RRT. Of note, the liver re-transplant did not have any impact in renal function recovery rate. In the multivariate analysis, the most important prognostic factor for chronic RRT was the presence of pre-OLT RRT (RR= 1.89, 95%IC= 1.15 to 3.13, p= 0,013). In conclusion, kidney transplantation after or concomitant to OLT must be judiciously done, especially in OLT patients who were not submitted to pre-OLT RRT.

Disclosure of Financial Relationships: nothing to disclose

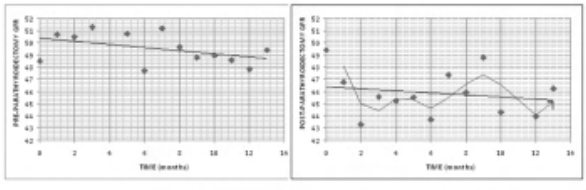
### TH-PO948

**Impact of Post Kidney Transplant Parathyroidectomy on Early Graft Function** Samir Parikh, Anil K. Agarwal. *Nephrology, The Ohio State University Medical Center, Columbus, OH.*

INTRODUCTION: The impact of parathyroidectomy (PTx) on kidney transplant (KTx) function is unclear. While worsening graft function in presence of uncontrolled hyperparathyroidism is proposed to be an indication for PTx, the procedure itself reportedly affects graft function. METHODS: We conducted a retrospective analysis of 3000 KTx patients from 1988 to 2008. 33 from this group underwent PTx secondary to uncontrolled hyperparathyroidism. All patients were monitored for change in e-GFR for one year pre and post PTx, graft loss, and patient death. RESULTS: The demographics are listed in Table. Following PTx mean GFR declined significantly in first 3 months with a mean GFR slope of -3.0611/month compared with mean GFR slope of -0.12853/month pre-PTx (p= 0.001). By 1 year, the mean GFR significantly improved with overall mean GFR slope of -0.08515/ month compared with mean GFR slope pre-PTx, a difference of -0.0438/month. (p=0.001). During median follow up of 58.6 months, 6(18 %) patients lost their graft with mean time of 35 months. No graft loss occurred during first year and causes of graft loss were rejection(2), reflux pyelonephritis(1) and chronic allograft nephropathy(3). 3 patients died in one year after parathyroidectomy secondary to MI, surgical complication and sepsis.

Demographics and Clinical Characteristics

| Patient Population | Number |
|---|---|
| Age (Years) (mean ± S.D) | 49.27 |
| Male Sex | 5:3 |
| Caucasian Race | 81.81% |
| BMI(mean) | 29.13 |
| DM | 36% |
| HTN | 97% |
| PRA > 10% | 15% |
| Non Primary Transplant | 18% |
| Time to parathyroidectomy from KTx | 45 months |
| Subtotal vs. total Parathyroidectomy | 32:1 |
| PTH- Baseline | 629.27 |
| PTH- 1 week | 85.05 |
| Ca2+ -Baseline | 10.03 |
| Ca2+ - 1 week | 7.86 |
| GFR – Baseline | 47.146 |
| Median time to follow up | 58.6 months |



CONCLUSION: Post PTx, GFR may decline transiently in the first few months but subsequently improve to a level significantly superior to pre-PTx values.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO949

**Providers' Reports of Discussion of Transplant Options at the Time of Dialysis Initiation** Kirsten L. Johansen,[1,3] Rebecca H. Zhang,[2,3] Yijian Huang,[2,3] Nancy G. Kutner.[2,3] *[1]UCSF; [2]Emory University; [3]USRDS Rehab/QOL SSC.*

In 2005, the ESRD Medical Evidence Report Form was altered to include information about whether transplant options were discussed and reasons for not discussing these options. We used these data to determine the rates of transplant discussion among incident dialysis pts, reasons for non-discussion, and the association of non-discussion with delays in wait listing. Pts aged ≥18 who started dialysis from 2005-2008 were included in the analysis. Pts who had been wait listed or had received a previous transplant (n=9,350) were excluded. Pts were stratified into age categories: 18-34, 35-49, 50-64, 65-79, 80+. 317,434 pts (age 63±15, 56% male, 30% Black) were included. Overall, 70% of pts were informed about transplant options, but rates varied by age, ranging from 84% of pts age 18 to 34 to 53% of pts 80+. Reasons for not being informed by age are shown in the Table.

Reasons for not being informed of transplant option (among those not informed)*

| Reason | 18≤Age<35 | 35≤Age<50 | 50≤Age<65 | 65≤Age<80 | Age≥80 |
|---|---|---|---|---|---|
| Pt has not been assessed, % | 70.3 | 68.3 | 62.4 | 40.0 | 20.6 |
| Unsuitable due to age, % | 0.2 | 0.1 | 1.0 | 26.3 | 59.2 |
| Medically unfit, % | 13.1 | 19.0 | 26.2 | 36.6 | 34.1 |
| Psychologically unfit, % | 2.2 | 3.6 | 3.4 | 2.6 | 2.8 |
| Pt declines information, % | 0.6 | 1.0 | 1.2 | 1.8 | 1.5 |
| Other, % | 16.7 | 11.5 | 9.7 | 5.9 | 3.5 |

*Column % total greater than 100% because more than one reason was permitted. All reasons differed across age groups, p<0.0001.

Of those who were not informed about transplant options, 43.9% had not been assessed (13.3% of total cohort). Not being assessed was the most common reason cited for not informing pts of transplant options in the younger age groups. Those who had not had transplant discussed had lower rates of and longer time to transplant wait listing (HR of 0.70 (p<0.0001) for not being informed because of not assessed and HR 0.30 (p<0.0001) for any other reason in Cox regression analysis). While a smaller proportion of young pts have not had transplant discussed, more of them are simply not assessed, which is associated with delayed wait listing.

Disclosure of Financial Relationships: Research Funding: Amgen, Abbott Laboratories; Scientific Advisor: Amgen Nephrology Advisory Board.

**TH-PO950**

**Higher Mean Tacrolimus Levels at Day 2 to 5 Post Transplant Are Associated with Reduced Rates of Acute Cellular Rejection in the Era of Routine Basiliximab Induction** James Lineen, Wael F. Hussein, Frank J. O Brien, Amin Bahabri, Paul J. Phelan, Colm Magee, Peter J. Conlon. *Nephrology and Transplantation, Beaumont Hospital, Dublin, Ireland.*

**Introduction:** In a previous study by our group, higher mean tacrolimus levels between day 2 to 5 post renal transplantation were independently associated with reduced rates of acute cellular rejection (ACR) in the first 3 months post transplant. In this study we aimed to establish whether this beneficial effect persisted following the introduction of routine anti- CD25 induction therapy.

**Methods:** Three hundred and twenty two consecutive single organ cadaveric kidney transplant recipients were retrospectively stratified into 4 groups based on quartiles of mean tacrolimus levels at D2-5 post op. In addition to standard immunosupression with tacrolimus, mycophenolate mofetil and corticosteroids, all patients received basiliximab induction. Patients were followed for 3 months. Acute cellular rejection and delayed graft function were defined by standard criteria.

**Results:** Results are presented in the table 1: Subsequent multivariate analysis showed that the reduction in ACR was statistically significant (OR 0.63, p 0.033). Increased Delayed Graft Function (DGF) was also statistically significant on multivariate analysis (OR 1.63, p 0.008).

Table 1:

| Variable | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | p Value |
|---|---|---|---|---|---|
| TAC | 11 | 16 | 22 | 29 | 0.001 |
| Age | 42.5 | 44.1 | 50.7 | 55.2 | 0.001 |
| Donor Age | 39.5 | 37.2 | 44.8 | 46.7 | 0.001 |
| Time on Dialysis | 2.4 | 2.7 | 3.8 | 3.0 | 0.016 |
| CIT | 15.4 | 16.5 | 15.3 | 16.3 | 0.348 |
| HLA Mismatches | 3.5 | 3.2 | 3.5 | 3.5 | 0.338 |
| PRA group 1/2/3 | 47/17/36 | 41/32/27 | 54/19/27 | 47/23/30 | 0.258 |
| ACR (%) | 13 | 12 | 4 | 6 | 0.107 |
| DGF (%) | 4 | 12 | 18 | 26 | 0.001 |

TAC- mean tacrolimus level; CIT- cold ischaemia time; PRA group-low/medium/high; results expressed as mean unless otherwise stated

**Conclusions:** Higher mean tacrolimus levels at Day 2-5 post transplant are associated with reduced ACR at 3 months at the expense of increased DGF.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO951**

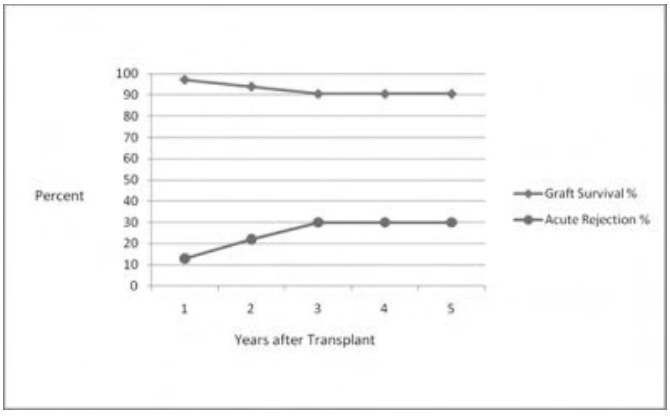
**Five Year Experience with Sirolimus-Based Immunosuppression in Pediatric Renal Transplantation** Leonard C. Hymes, Barry L. Warshaw. *Emory University and Children's Healthcare of Atlanta, Atlanta, GA.*

From 2003-2008, we employed a protocol for replacing calcineurin inhibitors (CNI) with sirolimus (SRL) in a cohort of low-risk pediatric renal transplant recipients. We report graft survival, acute rejection episodes, renal function and adverse events in children converted to SRL.

**Methods:** All patients initially received basiliximab induction, tacrolimus (TAC), mycophenolate and prednisone. Criteria for conversion to SRL included first renal transplants without clinical acute rejection (AR) or histologic AR on 3 month surveillance biopsies. Exclusion criteria included BK virus nephropathy, history of nephrotic syndrome, or multiple organ transplants.

**Results:** From Dec 2003 to Dec 2008, 51 patients met the criteria among 117 first renal transplants and received SRL for 45 months (mean) after withdrawing TAC. Actuarial graft survival was 91% at 5 years (Figure). AR had occurred in 13% by the first transplant year after conversion to SRL, 30% by 3 years, and none thereafter (Figure). Estimated GFR data showed preservation of renal function from the time of starting SRL to 36 month post-transplant (71 ± 18 ml/min vs. 72 ± 18 ml/min, p =.76, n= 22). Complications during SRL included aphthous ulcers (30%), BK viremia (20%), EBV (13%), CMV (3%), proteinuria (7%), elevated cholesterol (7%), IDDM (2%), leukopenia (2%), thrombocytopenia (2%), erectile dysfunction (2%) and lymph edema (2%). PTLD did not occur. SRL was discontinued in 20% within 1 year, predominantly for apthous ulcers.

**Conclusions:** Our experience from Dec 2003-2008 demonstrates excellent graft outcome and stable renal function utilizing a SRL-based, CNI-free regimen. Aphthous ulcers and BK viremia were the most prevalent adverse events.




Disclosure of Financial Relationships: nothing to disclose

**TH-PO952**

**Renal Transplantation in Patients with Sarcoidosis: A French Multicenter Study** Jessie Aouizerate,[1] Marie Matignon,[1] Nassim Kamar,[2] Eric Thervet,[3] Bruno Moulin,[4] Emmanuel Villar,[5] Vincent Audard,[1] Philippe Lang,[1] Philippe Grimbert.[1] *[1]Nephrology and Transplantation Department, Henri Mondor Hospital, AP-HP, Creteil, France; [2]Nephrology and Transplantation Department, Rangueil Hospital, Toulouse, France; [3]Transplantation Department, Necker Hospital, Paris, France; [4]Nephrology and Transplantation Department, Strasbourg Hospital, Paris, France; [5]Nephrology and Transplantation Department, Hospices Civils de Lyon, Lyon, France.*

*Objectives*: The outcome of renal transplantation on patients with sarcoidosis is not well known. A few case reports have described recurrence of sarcoidosis after transplant. We report for the first time results and outcome of renal transplantation in a serie of patients with sarcoidosis.

*Patients*: Eighteen patients with sarcoidosis who underwent renal transplantation were identified retrospectively in 8 french renal transplantation departments.

*Results*: Initial renal disease was related to sarcoidosis in 10 patients. At the end of the follow-up (median 42 months (range 18-196)), patient and death-censored graft survival were 94.4% and the mean GFR was 60 (+/- 25) ml/min/1.73m². Five patients (27%) experimented recurrence of sarcoidosis including extra renal involvement in two patients and renal involvement in three patients. Median GFR was lower in the group of patients with renal recurrence: 31 ml/min/1.73 m² ($p<0.05$). Recurrence occurred shortly after transplantation (median period: 13 months (range 7-192)). Risk factors for recurrence included primary renal disease related to sarcoidosis and a shorter delay between the last episode of sarcoidosis and renal transplantation.

*Conclusions*: Our results indicate that renal transplantation may be carried out safely in transplant candidates with sarcoidosis. Recurrence is not rare and is likely to impact on graft outcome and these results justify a clinical and histological monitoring mainly during the early post-transplant period.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO953**

**Association between Circulating Angiopoietin-2 Levels and Serum Phosphorus in Kidney Transplant Recipients** Istvan Mucsi,[1] Philipp Kümpers,[2] Maria Eszter Czira,[1] Anna Rudas,[1] Akos Ujszaszi,[1] Mario Schiffer,[3] Jan T. Kielstein,[3] Miklos Z. Molnar.[1,4] *[1]Semmelweis University, Budapest, Hungary; [2]University Hospital Münster, Münster, Germany; [3]Hannover Medical School, Hannover, Germany; [4]Harbor-UCLA Medical Center, Torrance, CA.*

Introduction: Hyperphosphatemia is an important risk factor for cardiovascular disease (CVD) in patients with chronic kidney disease (CKD). Recent data suggest a link between phosphate (PO4) load and endothelial dysfunction. Angiopoietin 2 (Angpt2) impairs endothelial function. Serum Angpt2 increases with progression of CKD and correlates with arteriosclerotic burden in dialysis patients. Here we examined whether circulating Angpt2 levels are associated with serum PO4 in renal transplant recipients (Tx).

Methods: Data from 258 Tx patients (age 54+12, mean eGFR 42+21 ml/min/1.73m2) from a single transplant center were analyzed. Serum Angpt2 was measured by immuno-luminometric assay. Correlation analysis and multivariable regression analysis was used to assess the independent association between serum PO4 and serum Angpt2 levels. Variables with skewed distribution were natural log-transformed.

Results: We observed a moderate positive correlation between serum Angpt2 versus serum PO4 ($r=0.337$, $p<0.001$). In addition, serum Angpt2 was negatively correlated with eGFR ($r=-0.330$, $p<0.001$) and serum albumin ($r=-0.327$, $p<0.001$) but positively correlated with serum CRP ($r=0.246$, $p<0.001$). The correlation between serum Angpt2 versus serum PO4 has remained significant after adjusting for age, gender and eGFR. Furthermore, in a multivariable linear regression model serum PO4 (beta=0.180, p=0.006) was independently associated with serum Angpt2 after statistical adjustment for age, gender, eGFR, comorbidity, serum albumin, CRP and LDL-cholesterin and use of calcineurin inhibitors and steroids.

Conclusion: Serum PO4 is independently associated with Angpt2 in kidney transplant recipients. These findings may be consistent with a hypothesis postulating serum PO4 as a novel risk factor for endothelial dysfunction and may demonstrate a novel mechanism to explain the relationship between higher serum PO4 level and cardiovascular risk.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO954

**Dietary Sodium Intake as Risk Factor for High Blood Pressure in Renal Transplant Recipients** Else van den Berg,[1,2] Marielle Francis Engberink,[1,3] Johanna M. Geleijnse,[1,3] Jaap Homan vd Heide,[2] Gerjan Navis,[1,2] Stephan J. L. Bakker.[1,2] *[1]Top Institute Food and Nutrition, Wageningen, Netherlands; [2]Nephrology, University Medical Center Groningen, Netherlands; [3]Division of Human Nutrition, Wageningen University, Netherlands.*

Hypertension is ubiquitous after renal transplantation and a risk factor for graft failure and mortality. Blood pressure (BP) control is of major importance in renal transplant recipients (RTR). Sodium ($Na^+$) intake is a determinant of BP in the general population, and even more so in renal patients. DASH guidelines recommend a maximum salt intake of 6g/24h (100mmol $Na^+$/24h). The association between $Na^+$ intake and BP in RTR has not been well-established. Therefore, we investigated the association of $Na^+$ intake with BP in a large single center RTR cohort.

Outpatient RTR (n=499, age 53±13 year (mean±SD) 58% male, 83% on antihypertensive drugs, number of these drugs 2.0±0.9) who were at least one year post-transplantation were included. $Na^+$ intake was assessed from 24h urine, and adequacy of collection checked from 24h creatinine excretion. The morning after completion of urine collection, BP was measured for 15 minutes (half-sitting) with an automatic device (Dinamap®). Values of three last measurements were averaged.

Systolic and diastolic BP were 137±18 and 83±11 mmHg respectively. $Na^+$ excretion was 156±61 mmol/24h (range 19-374); 85% had a $Na^+$ excretion above 100mmol/24h. Both systolic and diastolic BP were positively associated with 24h urinary $Na^+$ excretion (B=0.048 mmHg per mmol/24h, P=0.006 and B=0.032 mmHg per mmol/24h, P=0.002 resp.) independent of age, sex, body surface area, use of antihypertensive drugs and renal function.

Thus, $Na^+$ intake is an important determinant of BP in RTR. The majority of RTR has a $Na^+$ intake above current guidelines. Translation of these cross-sectional data to a reduction of $Na^+$ intake by 100 mmol/d in RTR would theoretically reduce systolic and diastolic BP by 4.8 and 3.2 mmHg respectively, which exceeds similar calculations for the general population (2.4 mmHg per 100 mmol/day). Better control of $Na^+$ intake in RTR may help to prevent occurrence of graft failure and mortality due to hypertension among RTR.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO955

**Aortic Pulse Wave Velocity as a Predictor of Long-Term Renal Allograft Function** Joerg Seckinger,[1] Bernd Krumme,[2] Anna K. Schlenker-Bø,[1] Claudia Sommerer,[1] Martin G. Zeier,[1] Vedat Schwenger.[1] *[1]Department of Nephrology, University Hospital Heidelberg, Heidelberg, Germany; [2]Department of Nephrology, Deutsche Klinik fuer Diagnostik, Wiesbaden, Germany.*

**Purpose:** Aortic pulse wave velocity (PWV) is an established marker of atherosclerotic vascular damage and cardiovascular risk. We examined the role of PWV as a predictor of long-term renal allograft function.

**Methods:** In a retrospective analysis the records of 147 renal transplant recipients with a history of PWV measurements were studied. All patients were treated with calcineurin-inhibitors as the primary immunosuppressive drug. Exclusion criteria were: measurement of PWV >5 yrs. post transplantation (TX) (n=76), preemptive TX (n=9), switching of the primary immunosuppressive drug (n=11). The remaining 51 patients were distributed into groups A (PWV>=9 $ms^{-1}$; n=25) and B (PWV<9 $ms^{-1}$; n=26).

**Results:** There were no significant differences between the two groups concerning age distribution (49.6±11.5 vs. 45.3±13.1 yrs., p=0.11), time on dialysis (4.5±2.9 vs. 5.6±3.9 yrs., p=0.16), time of initial PWV assessment (2.2±1.8 vs. 2.4±1.9 yrs. post TX, p=0.41), renal transplant function (MDRD-clearance 55.0±19.6 vs. 64.6±20.1, p=0.06; s-creatinine 1.5±0.64 vs. 1.3±0.38 mg/dl, p=0.09), renal resistance indices (RI 0.65 vs. 0.64, p=0.49) or pulse pressure (PP) (52.8±12.6 vs. 47.7±10.9 mmHg, p=0.06). The concomitant immunosuppressive therapy and overall acute rejection rate was comparable in both groups. The follow-up period after PWV measurement was 5.0±1.3 and 5.2±1.1 yrs., respectively (p=0.16). While PP (46.7±23.4 vs. 47.3±15.1 mmHg, p=0.38) and RI (0.69 vs. 0.68, p=0.35) showed no significant differences at the time of the follow-up, the allograft function in group A (elevated PWV) was significantly reduced (MDRD-clearance 41.4±20.2 vs. 58.8±24.7, p=0.005; s-creatinine 2.1±1.6 vs. 1.6±1.3 mg/dl, p=0.03).

**Conclusion:** In renal transplant recipients an elevated aortic pulse wave velocity of >=9 $ms^{-1}$ was associated with a significantly impaired long-term allograft function. Routine assessment of PWV in renal transplant recipients may help to improve the long-term outcome by identifying patients at risk for chronic allograft dysfunction.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO956

**Prediction of Cardiovascular Disease during Pre-Transplant Evaluation** Nimrit Goraya, Mohanram Narayanan, Luis A. Concepcion. *Nephrology, Texas A & M, Scott and White Hospital, Temple, TX.*

Cardiovascular disease (CAD) is a major contributor to overall mortality in renal transplant recipients. Stress echocardiography (SE) is generally the most commonly employed tool for pre-transplant evaluation of diabetic ESRD patients. Out of a total of 368 patients who underwent pre-transplant evaluation at our transplant clinic from year 2000-2009, all our diabetic patients (DM) (n= 89) were screened with cardiac catheterization (CC) irrespective of their SE findings and data compared to that of non-diabetics (NDM) (N=29) who underwent CC. Of the combined 118 patients who underwent CC, some of the variables tested are included in the table.

Variables of all patients who underwent cardiac catheterization

| | mean | standard deviation |
|---|---|---|
| age years | 57.2 | 10.7 |
| BMI | 29 | 5.6 |
| Total Cholesterol | 160 | 45.47 |
| LDL | 79.9 | 30.27 |
| time to cath from dialysis (months) | 37 | 118 |
| time to transplant from dialysis onset (months) | 55 | 143 |
| Number of stents | 0.31 | 0.7 |

No difference in demographic variables was noted between DM and NDM for age, sex, race, BMI, cholesterol and EF with the exception of patients with CAD requiring CABG who had higher cholesterol (p =0.07) and lower EF (p=0.031). Additionally, EF > or < than 50% was not a predictor of CAD in DM or NDM patients (p=0.632) and SE abnormality was not a predictor of CAD in DM (p=0.76). Comparison of demographic and echocardiographic variables according to gender and race, did not reveal any statistically significant difference in gender but African-Americans (AA) were more likely to have a higher BMI ( p=0.049) and lower EF (p=0.021) as compared to Caucasians. Our data shows that use of conventional SE to predict CAD in diabetics may not be an accurate diagnostic tool and cardiac catheterization should be considered in all diabetic patients undergoing pre-transplant evaluation.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO957

**Early and Late-Term Outcomes of Preemptive Simultaneous Pancreas-Kidney and Living Donor Kidney Transplantation** Edmund Huang, Hung-Tien Kuo, Sean Michael Masao Okumura, Suphamai Bunnapradist. *Medicine, UCLA David Geffen School of Medicine, Los Angeles, CA.*

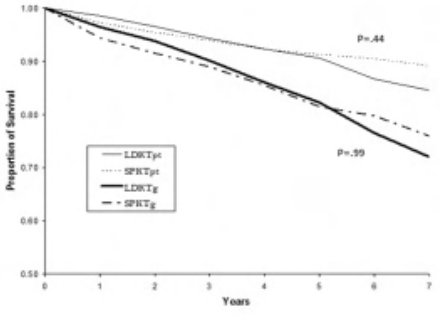
Introduction: In order to minimize dialysis time, type I diabetics (DM) with end-stage renal disease are often recommended for living donor kidney transplantation (LDKT) if a suitable donor is available rather than wait for a simultaneous pancreas-kidney transplant (SPKT). However, it is unclear how outcomes of LDKT compare with SPKT when pre-transplant dialysis can be avoided.

Methods: Using Organ Procurement and Transplantation Network/United Network of Organ Sharing data as of November 27, 2009, all preemptive type I DM recipients of a LDKT (N=1353) and SPKT (N=1429) from 2000-2009 were selected. The Kaplan-Meier product limit method was used to generate patient and kidney graft survival curves. Cox regression models were used to compare patient and kidney graft survival of LDKT and SPKT recipients.

Results: LDKT recipients were older and more likely to be obese and have cardiovascular disease compared to SPKT. Median age of donors was higher for LDKT than SPKT (42 vs. 23, P<0.001). SPKT recipients had greater median waiting time than LDKT (211 vs. 134 days, P<0.001). Although there was no difference in overall patient survival over 7 years of follow-up (log-rank P=0.44), survival was lower for SPKT compared to LDKT until approximately 3.5 years after transplant. No difference in kidney graft survival was observed over the study period (log-rank P=0.99), although after approximately 5 years there was a trend towards decreased graft survival for LDKT.

Conclusion: A trend towards greater long-term patient and graft survival was observed with preemptive SPKT recipients compared to LDKT, however this came at the expense of higher mortality earlier after transplantation. These findings should be considered when counseling type I DM candidates about transplantation options.

Figure 1. Unadjusted patient (pt) and graft (g) survival according to transplant type.




| Year | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LDKTpt | 1353 | 1103 | 930 | 761 | 593 | 438 | 300 | 185 |
| SPKTpt | 1429 | 1128 | 921 | 708 | 537 | 392 | 290 | 202 |
| LDKTg | 1353 | 1083 | 914 | 741 | 575 | 427 | 293 | 178 |
| SPKTg | 1429 | 1097 | 896 | 686 | 516 | 374 | 279 | 194 |

Disclosure of Financial Relationships: nothing to disclose

## TH-PO958

**Efficacy of Two Different Plasma Volume Expansion/Vasoconstriction Regimens Compared to Tips in Patients with Hepatorenal Syndrome Type-2 Waiting for Liver Transplantation – A Prospective Randomized Trial** G. Junge,[1] Lioba Schewior.[2] *[1]University Hospital Charite Berlin; [2]University Hospital UKE Hamburg.*

**Background**

Hepatorenal Syndrome (HRS) is a reversible renal failure which occurs in patients with advanced liver disease and portal hypertension and is characterized by a marked decrease in GFR and RPF in absence of other identifiable causes. Vasodilatation theory is currently the most accepted hypothesis to explain the pathogenesis. In decompensated cirrhotics, probability of developing HRS is 8-20%/yr and increases to 40% at 5 yrs. Ideal treatment is LTx. However, there is an urgent need for effective alternative treatments to increase survival chances for pts until LTx can be performed. Interventions that have shown some promise are vasoconstrictors in splanchnic circulation and TIPS. Main objective was to compare efficacy of two different regimens (albumin/terlipressin (IIa) resp HES/terlipressin (IIb) both w/wo midodrine) versus TIPS (IIc) whereas eGRF was considered as primary efficacy endpoint.

**Patients/Method**

Dx of HRS was based on criteria, as proposed by International Ascites Club. Only patients with ESLD on the waiting list for LTx were eligible for enrolment. Patients were assigned to 3 treatment arms (IIa,b,c) and randomized w/wo midodrine. Volume/vasoconstrictor treatment lasted for 10d, mitodrine was continued; follow up for 90d.

**Results**

| | pts no | age | Gender [f:m] | CHILD | MELD |
|---|---|---|---|---|---|
| II a | 20 | 47 | 9/11 | 10 ±2 | 26 ±4 |
| II b | 15 | 48 | 8/7 | 10 ±2 | 27 ±4 |
| II c | 10 | 52 | 4/6 | 9 ±1 | 23 ±3 |

| | Crea [mg/dL] | eGFR [mL/min] | UVol [mL/24h] | Urine Na [mmol/L] | MAP [mmHg] | COP [mmHg] |
|---|---|---|---|---|---|---|
| II a | 2.8 ±0.4 | 24.0 ±5.6 | 585 ±72 | 12.2 ±3.1 | 93 ±5 | 16.0 ±0.9 |
| II b | 3.1 ±0.5 | 21.0 ±4.8 | 590 ±105 | 13.3 ±3.4 | 94 ±5 | 15.8 ±0.9 |
| II c | 2.7 ±0.4 | 25.0 ±6.9 | 680 ±113 | 17.5 ±3.4 | 94 ±6 | 16.4 ±0.6 |

**Discussion**

Combination of volume expansion/vasoconstriction effectively improved eGFR (urine output, MAP and COP) in patients with HRS Typ-2. Use of albumin shows no advantage compared to (cost effective) HES. Although a marked improvement was observed during iv-treatment, renal function deteriorated upon treatment withdrawal whereas patients with continued midodrine showed sustained treatment response.

Disclosure of Financial Relationships: Other Relationship: Medical Scientific Expert, Novartis Pharma AG Basel/CH

Research association, Charite Berlin/GER.

## TH-PO959

**Routine Doppler Sonography in Kidney Graft Recipients: Should We Do It?** Ana Cortesao Costa, Alice Santana, José Guerra, Antonio Gomes da Costa. *Nephrology and Kidney Transplantation, CHLN-HSM, Lisbon, Portugal.*

Renal vein thrombosis is an infrequent complication after renal transplantation, occurring in less than 5% of cases. The spectrum of signs and symptoms is broad and ranges from anuria and sudden onset of pain in the graft to the absence of any symptoms. It is a serious complication that often leads to renal graft loss but when the diagnosis is made early allows for therapeutic intervention that can save the organ. The Doppler ultrasonography is a noninvasive method with high specificity and sensitivity for diagnosis. In our unit Doppler sonography is carried out routinely on all kidney graft recipients in the period after transplantation and has made the early diagnosis of partial renal vein thrombosis in 7 patients. In 4/7 patients the diagnosis was made at 1 month after transplantation and in the remaining at 2, 3 and 5 months. Only one patient had symptoms (edema in the ipsilateral leg), in the other six cases there were no signs or symptoms and the diagnosis was made on routine Doppler. In all cases, anticoagulant therapy was instituted, initially with low molecular weight heparin and subsequently with oral anticoagulation with warfarin, which was maintained for a minimum period of six months. Full patency of the vein was restored in all cases. There were no identifiable risk factors for venous thrombosis in these patients. The average age of the donor was 51 +/-7 years, and recipient age 41 +/-11 years. The etiology of CKD was ADPKD in 2 cases, GNC in 2, diabetic nephropathy in 2 and unknown in the remaining. The duration of dialysis before transplantation was highly variable between 7 and 192 (mean 87 +/- 67) months. Of the seven patients only one had delayed graft function and there were no episodes of acute rejection. In conclusion, the performance of Doppler routinely in our patients allowed early diagnosis of treatable vascular complications. More studies are needed to establish the frequency with which these tests should be performed after renal transplantation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO960

**Preservation of Renal Function in Kidney Tranplant Recipients Exposed to Epoetin Therapy** Ingeborg A. Hauser, Andeep Singh Pannu, Aida Asbe-Vollkopf, Stefan Gauer, Helge Höfeld, Ernst H. Scheuermann, Helmut Geiger. *Department of Nephrology, J. W. Goethe University, University Hospital, Frankfurt/Main, Germany.*

Post-transplant anemia (PTA) in kidney transplant recipients (KTR) occurs in 20-40% caused by comorbidities, drug therapy and declining epoetin secretion. In this single center retrospective study the clinical course of 207 anemic KTR (120 male) receiving epoetin (EPO)-therapy for at least 6 months (m) between 1999 and 2009 was analyzed.

Demographic, donor-and recipient related clinical data were collected. Information on concomitant medication, rejection episodes, hematological parameters, cardiovascular events and GFR were gathered from 12 m pre-EPO-treatment to 24 m post-EPO-initiation.

Mean age at transplantation (Tx) was 47.0±15.2 y, at initiation of EPO therapy 52.4±14.0 y. The median time between discharge after Tx and EPO start was 7.4 m. EPO α (22.2%), -b (42.5%), darbepoetin (34.7%) and C.E.R.A. (1.4%) were used. Mean Hb levels were 10.5±1.2 (at discharge after Tx), 12.8±1.6 (1 y post-Tx), 10.4±1.2 (EPO start), 12.3±1.3 (12 m EPO) and 12.2±1.4 g/dL (24 m EPO). Mean serum creatinine was 1.8±0.7 (1 y post-Tx), 2.1±0.8 (EPO start), and 2.2±1.1 mg/dL (24 m EPO). Mean GFR (MDRD) was 44.3±18.4 (at discharge), 40.8±15.8 (1 y post-Tx), 39.9±13.5 (1 y pre-EPO), 35.3±13.8 (EPO start), 35.7±14.7 (12 m EPO) and 34.7±14.8 ml/min (24 m EPO). The mean yearly loss of GFR pre-EPO therapy was 5.7±14.6 ml/min (median 2.80), undergoing a significant decrease to 0.85±6.30 ml/min (median 0.80) after EPO start ($p<0.01$). During 24 m of EPO therapy,18/207 (9.2%) recipients encountered central (3), cardiac (15) or peripheral (6) cardiovascular events. 4 KTR died, 1 experienced graft loss during the 24 m observation period.

In this observational study a renoprotective effect of EPO, initiated due to anemia, is demonstrated. Across a heterogeneous range of patients, encompassing over a decade, and initiated at various time points post Tx, the decline in renal function was significantly reduced in the presence of EPO therapy without severe side effects. Future prospective studies are needed to examine this renoprotective effect of EPO therapy in KTR.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO961

**Use of Rituximab for Membranous Nephropathy in Renal Transplant Allografts** Alejandro Diez, Dennis P. Mishler, Tim E. Taber, Asif A. Sharfuddin. *Division of Nephrology, Indiana University, Indianapolis, IN.*

Purpose:

The treatment of membranous nephropathy (MN), a leading cause of nephritic syndrome, remains controversial. MN in the setting of a renal transplant, whether arising de novo or as recurrence of primary disease, is particularly challenging to treat given the paucity of available data. Rituximab, an anti-CD20 monoclonal antigen, may prove to be a therapeutic option in patients already receiving calcineurin inhibitors.

Methods:

A single-center retrospective review of biopsy proven cases of MN occurring in renal transplants was performed. Cases in which rituximab was used as a treatment modality were identified. Changes in proteinuria and serum creatinine were used as markers for response to treatment and allograft function.

Results:

Four cases of idiopathic MN treated with rituximab were identified. Three of the four cases were recurrence of the primary disease. In all cases maintenance immunosuppression at the time of diagnosis consisted of tacrolimus and mycophenolate mofetil. Rituximab was dosed at 375 mg/m², with either two or four doses given. No adverse effects were noted. Three patients had significant decreases in proteinuria lasting greater than six months.

Conclusions:

Rituximab appears to be a viable treatment option in renal transplant patients with MN. Studies with larger cohorts and longer follow-up periods will aid in the validation of this therapeutic approach.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO962

**A Prospective, Randomized, Single Center Trial of Calcineurin Inhibitor (CNI) Avoidance in Kidney Transplantation: 5-Year Results** Heidi M. Schaefer, Irene D. Feurer, J. Harold Helderman, David Shaffer. *Vanderbilt University, Nashville, TN.*

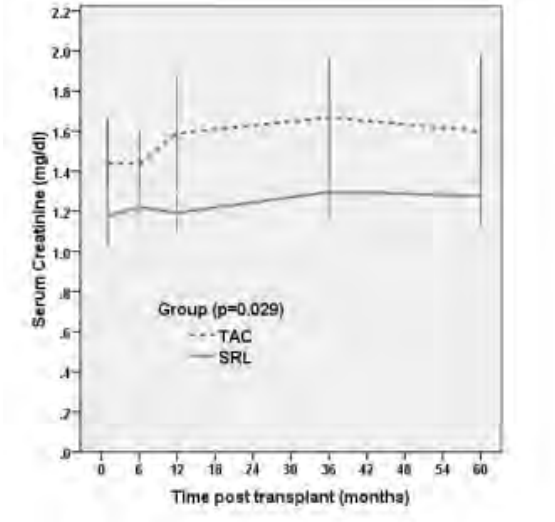
**Purpose:** Calcineurin inhibitor (CNI)-associated nephrotoxicity may exacerbate chronic allograft nephropathy (CAN) and reduce long-term graft survival. We report 5-year results comparing sirolimus (SRL) and tacrolimus (TAC) in kidney transplantation.

**Methods:** We randomized 60 recipients (31 SRL, 29 TAC) of primary cadaver or non-HLA identical kidney transplants to either SRL 5 mg QD beginning POD#3 (target level 8-12 ng/ml) or TAC 0.075 mg/kg BID POD#1 (target level 8-12 ng/ml). All received Thymoglobulin induction, mycophenolate mofetil 1 gm BID, and prednisone tapered to 5-10 mg QD by 3 mos.

**Results:** Baseline characteristics were similar between groups. Mean f/u was 1626 d for SRL and 1625 d for TAC.

At 5 yrs, there was no difference in patient survival (87% vs 89%, p=0.432) or graft survival (80% vs 75%, p=0.992) in SRL vs TAC treated pts. The incidence of biopsy proven rejection (16% vs 10%) was comparable between groups.

Linear mixed models were used to test the effect of drug on renal function over time. Serum creatinine was significantly lower in SRL-treated recipients over the 5-yr period (p=0.029).




14 pts (45%) in SRL group discontinued SRL a mean of 854 days (range 233-1467) post transplant, including 5 for late wound complications. The remainder discontinued SRL due to rejection (3), anemia (2), proteinuria (2), pulmonary toxicity (1), and diarrhea (1). In contrast, only 4 pts (14%) in TAC group discontinued TAC due to BK virus (2), CAN (1) and neurotoxicity (1).

**Conclusion:** Although complete CNI avoidance with SRL maintenance resulted in improved serum creatinine and comparable patient and graft survival vs. TAC over 5 yrs, long-term use of SRL was poorly tolerated requiring discontinuation of study medication in a significant number of patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO963

**Post-Transplantation Encapsulating Peritoneal Sclerosis Contributes Significantly to Mortality after Kidney Transplantation** Mario R. Korte,[1] Sayed Meelad Habib,[2] Hester Lingsma,[3] Willem Weimar,[2] Michiel G. H. Betjes.[2] *[1]Department of Internal Medicine, Albert Schweitzer Hospital, Dordrecht, Netherlands; [2]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands; [3]Department of Public Health, Erasmus Medical Center, Rotterdam, Netherlands.*

Encapsulating peritoneal sclerosis (EPS) is a severe complication of peritoneal dialysis (PD). EPS occurs frequently after kidney transplantation, known as post-transplantation EPS. In general, EPS has a high mortality, but the impact of post-transplantation EPS on patient survival after kidney transplantation is unknown.

From January[1st] 1996 until July[1st] 2007 fifty post-transplantation EPS patients were identified from the Dutch multicenter EPS study. In the partcipating centers overall 1254 PD patients were transplanted. The medical records of all transplanted PD patients were analyzed using the data from the Dutch renal registry (RENINE) and the Dutch transplantation foundation (NTS). EPS patients were younger at last transplantation than non-EPS patients (38.2 ± 14.6 vs. 46.1 ± 14.7, p<0.0001). Pre-transplant dialysis duration was longer in EPS patients (66.8 ± 37.1 vs. 34.7 ± 25.5 months, p=0.004) and EPS patients had more transplantations (1.56 ± 0.8 vs. 1.2 ± 0.5, p<0.0001).

Two-hundred-nine (16.7%) patients died after transplantation, of which twenty-five EPS patients. The majority of EPS patients died within one year after the diagnosis (median time of 10.05 ± 18.8 months). After infection (23%) and cardiovascular disease (21.1%), EPS (12%) was the third known cause of death after transplantation.

Ten-years survival of transplanted PD patients with EPS was worse compared to non-EPS patients, p-value < 0.0001. EPS, age at last transplantation, dialysis duration and transplant failure were associated with increased risk for death after kidney transplantation. Post-transplantation EPS contributes significantly to overall mortality after transplantation and lowers the survival of transplanted PD patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO964

**Does Donor Neutrophil Gelatinase-Associated Lipocalin (NGAL) Predict Post Transplant Allograft Function?** G. Junge,[1] Lioba Schewior.[2] *[1]University Hospital Charite Berlin; [2]University Hospital UKE Hamburg.*

**Background:**

The donor pool for KTx has plateaued worldwide. Expansion of the pool by including LR-donations and donors who previously were not accepted is the only way to increase the numbers. Data on specific parameters to predict success/failure of KTx are limited. It has been suggested that NGAL may serve as an early marker for renal injury, but have not as yet been investigated in kidney donors (KD). Therefore, it was the objective to evaluate NGAL in KD as a predictor of early allograft function after KTx.

**Method:**

We prospectively evaluated NGAL (urine, serum) in healthy volunteers (n=30) to compare results with (1) BDOD before organ procurement (n=58), (2) LR kidney donors (n=15), (3) KTx recipients who received an allograft from a BDOD (n=58) and (4) KTx recipients who received an allograft from LR donor (n=15). In addition, we followed the function of associated kidneys in correspondent transplant recipients that were classified into 2 groups depending on allograft function after KTx: IF vs DGF. The primary objective was to evaluate the predictive value of NGAL for post transplant allograft function. Secondary objectives were: (1) To compare NGAL levels in BDOD and CKD stage V patients on HD to healthy volunteers, (2) to evaluate NGAL evolution post (LR-)KTx by visit, etc.

**Results:**

We were able to show that urine (and serum) NGAL levels in corresponding KD correlate with post transplant allograft function where higher NGAL levels where predictive for DGF.

| | Initial allograft function | | DGF | | |
|---|---|---|---|---|---|
| | mean | SD | mean | SD | p=0.05 |
| SCR [mg/dL] | 1.7 | 0.53 | 2.0 | 0.62 | 0.078 |
| BUN [mg/dL] | 66.6 | 27.22 | 54.1 | 31.41 | 0.089 |
| CysC [mg/L] | 0.88 | 0.17 | 1.23 | 0.25 | <0.05[1] |
| eGFR [ml/min] | 79.5 | 12.33 | 63.6 | 12.88 | <0.05[1] |
| sNGAL [ng/mL] | 1242.3 | 502.38 | 1136.1 | 557.48 | 0.454 |
| uNGAL [ng/mL] | 81.9 | 17.42 | 153.8 | 29.41 | <0.05[1] |

**Conclusion:**

In summary, our data indicates that NGAL represents a novel, sensitive and non-invasive urinary (and serum) biomarker predictive for primary graft function after KTx. Even in cases where our classical diagnostic parameters do not allow further differentiation of potential KD (marginal donors), NGAL seems to remain a stable and significant indicator.

Disclosure of Financial Relationships: Other Relationship: Medical Scientific Expert, Novartis Pharma AG Basel/CH

Research association, Charite Berlin/GER.

## TH-PO965

**Pretransplant Course and Risk of Kidney Transplant Failure in IgA Nephropathy Patients** Rune Bjoerneklett,[1] Bjørn Egil Vikse,[1] Hilde K. Smerud,[4] Torbjorn Leivestad,[2] Bjarne M. Iversen.[1] *[1]Medicine, Haukeland University Hospital, Bergen, Norway; [2]Institute of Immunology, Oslo University Hospital Rikshospitalet, Oslo, Norway; [3]Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway; [4]Smerud Medical Researche International AS, Oslo, Norway.*

**Background:** Few studies have investigated whether the presentation and course of IgA nephropathy (IgAN) prior to end-stage renal disease (ESRD) are risk factors for graft loss after kidney transplantation. The outcome with living genetically related (LRD) compared with unrelated donor (UD) grafts in transplanted IgAN patients is also not well studied.

**Material and Methods:** Patients diagnosed with IgAN between 1988 and 2006 (registered in the Norwegian Kidney Biopsy Registry) who later received a kidney transplant (registered in the Norwegian Renal Registry) were included. The cohort was followed up

regarding death censored graft loss through 2008. Graft survival with a rapid progressive (RP) vs. a slow progressive (SP) course of IgAN (annual GFR loss of more or less than 30ml/min/1.73m2) were studied.

**Results:** Duration of follow-up with the first kidney transplant was 6.9±4.4 (range 0.1-19) years. There were 14 graft losses. Graft loss rate was 1.9/100 patient years.

Patients with RP had a higher graft loss rate compared to SP patients (6.3 vs. 1.3/100 patient years p<0.001).

Recipients with LRD organs had the same graft loss rate as UD recipients (1.8 vs. 2.0/100 patient years p=0.68) but were more often rapid progressors than UD recipients (18 vs. 4%, p=0.01).

In SP patients, LRD recipients had a lower graft loss rate than UD recipients, (0.3 vs. 2.1/100 patient years, p=0.055).

**Conclusions:** A rapid pretransplant course is a strong risk factor for transplant failure in IgAN patients. In SP patients, LRD may have a better graft survival than UD organs.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO966

**Successful Renal Transplantation in Muckle-Wells Syndrome Treated with Anti-IL1β-Monoclonal Antibody** Birgit Kortus-Götze, Joachim Hoyer. *Division of Nephrology, University of Marburg, Germany.*

Background

The Muckle-Wells syndrome (MWS) belongs to the group of rare hereditary cryopyrin-associated periodic syndromes (CAPS) and causes recurrent fever attacks, myalgia, arthralgia, urticarial rash, headache, sensorineural deafness and a severe fatigue syndrome. The development of systemic AA amyloidosis and especially renal amyloidosis is a severe complication of the Muckle-Wells syndrome. Only little is known about the feasibility of renal transplantation in Muckle-Wells syndrome patients and its impact on MWS activity.

Methods

The anti-IL1ß-monoclonal antibody canakinumab has been recently introduced as a specific treatment option in patient with Muckle-Wells syndrome with normal and impaired renal function. Here we report on a 32-year old woman with Muckle-Wells syndrome and biopsy proven systemic AA amyloidosis and end stage renal disease treated with canakinumab subcutaneously in a dosage of 150 mg every eight weeks who underwent renal transplantation.

Results

In the pre-transplantation period the patient was stable with low activity markers (CRP<5mg/l, SAA<6.4mg/l) under therapy with canakinumab. The immunological challenge after renal transplantation and the triple immunosuppressive therapy (CyA, MMF, prednisone) had no impact on activity of Muckle-Wells syndrome. The patient had no flares of Muckle-Wells syndrome under an excellent graft function (creatinine 1.2 mg/dl) and therapy with canakinumab after renal transplantation. No severe infections or adverse events were observed under canakinumab in combination with immunosuppressive therapy.

Conclusions

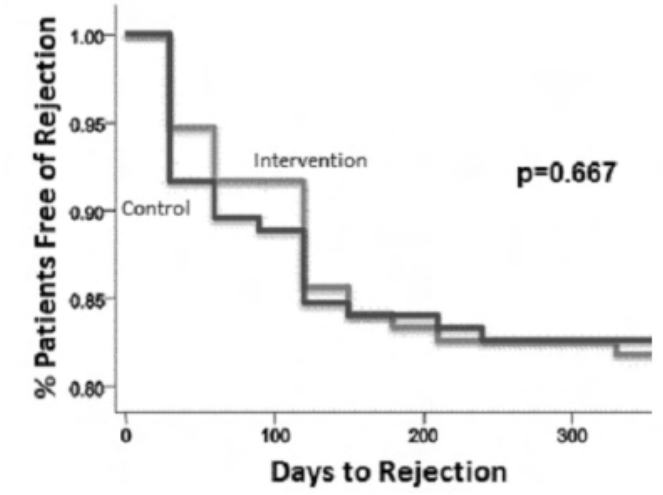
To our best knowledge, we describe the first case of a patient with Muckle-Wells syndrome and end stage renal disease successfully treated with canakinumab before and after renal transplantation. In our opinion, treatment with the anti-IL1ß-monoclonal antibody canakinumab in patients with Muckle-Wells syndrome and renal transplantation is feasible, safe and without severe side effects.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO967

**Rejection Risk of Tacrolimus Minimization Assessed by Surveillance Biopsies in Renal Transplantation** Peter C. Chang,[1] Ali Olyaei,[1,2] Eric D. Langewisch,[1] Jagdeep Obhrai,[1] Anuja Mittalhenkle,[1] Douglas J. Norman.[1] *[1]Oregon Health & Science University; [2]Oregon State University.*

**Introduction:** There is much interest in renal transplant immunosuppressive protocols that minimize nephrotoxicity. At our institution, we adopted tacrolimus (FK) minimization in 2008. With surveillance biopsies at 3 and 12 months along with ones for cause, we were able to evaluate the risk of acute rejection. **Methods:** All patients received thymoglobulin or daclizumab as induction based on immunologic risk then maintained on FK, mycophenolate and prednisone. We compared 137 adult single organ kidney transplant recipients with FK target of 5-10ng/ml to a historical group of 132 recipients with FK target of 10-15ng/ml for three months followed by 5-10ng/ml. Primary endpoint of interest is biopsy proven acute rejection (BPAR) within the first year; secondary endpoints were serum creatinine at 3 and 12 months, development of opportunistic infections and calcineurin inhibitor (CNI) toxicity on biopsy. Independent t-test was used to compare continuous variables while chi-square test was used for categorical variables; time to first BPAR was analyzed using the Kaplan-Meier method. **Results:** Immunosuppression did not differ except for FK levels which were 11.5±1.0ng/ml vs 8.0±0.9ng/ml in the first three months and 8.3±1.2ng/ml vs 7.7±0.9ng/ml thereafter between the control and intervention arms. Time to first BPAR was not different:




There were also no differences among our secondary endpoints:

| | Control | Intervention | p-value |
|---|---|---|---|
| 3m Cr* | 1.34 | 1.37 | 0.72 |
| 12m Cr* | 1.32 | 1.31 | 0.88 |
| CNI Toxicity | 15/95 | 22/86 | 0.32 |
| BK | 15/131 | 16/110 | 0.92 |
| CMV | 10/128 | 13/111 | 0.12 |

*mean

**Conclusion:** With the use of surveillance biopsies, we found no increased risk of BPAR with de novo low dose FK. Our findings support the safety of adopting CNI minimization protocols in renal transplantation.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO968

**Bimodal Relationship between Urinary Uromodulin and Renal Allograft Failure** Anna Reznichenko,[1] Marcory Van Dijk,[2] Marc Seelen,[1] Jaap Homan vd Heide,[1] Gerjan Navis,[1] Stephan J. L. Bakker.[1] *[1]Nephrology, Univ Med Centre Groningen; [2]Pathology, Univ Med Centre Groningen, Netherlands.*

Background

Urinary uromodulin excretion (UE) predicts progressive renal function decline in native kidneys. We investigated whether UE predicts renal function loss (graft failure, GF) in renal transplant recipients (RTR).

Methods

Outpatient RTR (n=606; age 51.5±12.1 yrs, 55% male; median [IQR] 6.0 [2.6-11.4] yrs post-transplant) with a functioning graft >1 yr were included. Uromodulin urinary concentration was measured by ELISA at baseline. In a subset (n=59) biopsies were scored according to Banff classification, and interstitial fibrosis/tubular atrophy (IFTA) assessed semi-quantitatively. Control values for UE were obtained in healthy volunteers (n=20), in which interindividual variation of UE was 22% (intra-day) and 60% (day-to-day), intraindividual – 89% (intra-day) and 60% (day-to-day).

Results

During the follow-up of 5.3 [4.5-5.7] yrs 42 (6.9%) RTR developed GF and 95 (15.7%) RTR died. UE was 20.3 [14.0-39.4] mg/24h in RTR versus 5.3 [2.2-10.3] mg/24h in controls (p<0.0001).

There was a curvilinear association between UE and death-censored GF, with 5.5%; 11.9% and 3.5% cases of GF in subsequent tertiles for UE, respectively (p=0.002). On multivariate Cox regression analysis hazard ratios for GF for the 1st and 3rd tertiles were 0.37 [95% CI 0.17-0.80] (p=0.01) and 0.18 [0.07-0.44] (p=0.0002) respectively, in the model adjusted for serum creatinine and proteinuria. Histopathologically IFTA was prevalent in the middle tertile of UE, whereas in case of morphologically preserved, non-atrophic tubuli both lower and higher levels of UE were observed. In line with worse morphology and graft survival, renal function by creatinine clearance was significantly lower in the second tertile of UE (p<0.05).

Conclusions

UE is associated with GF in a non-linear, bimodal fashion with reduced risk at lower and higher values. Non-linear association holds also between UE and allograft histopathology in terms of IFTA. Described association pattern is unusual for biomarkers. Dissection of the disparate mechanisms of GF prediction by UE might provide new clues for its role in progressive renal disease.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO969**

**Corticosteroid Withdrawal in Renal Transplant Recipients: An Analysis of the Mycophenolic Acid Observational Renal Transplant (MORE) Registry** Kimi Ueda Stevenson,[1] Anne Wiland,[2] Kevin M. Mccague,[2] V. Ram Peddi.[1] [1]*California Pacific Medical Center, San Francisco, CA;* [2]*Novartis, East Hanover, NJ.*

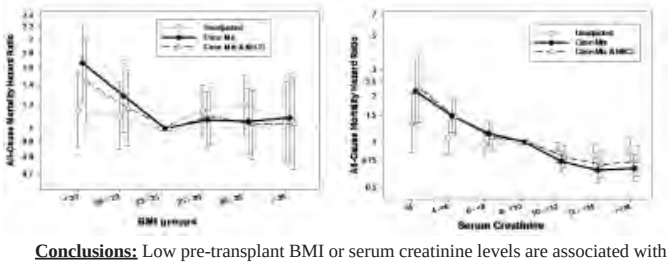
**Introduction:** Corticosteroid withdrawal (CSW) protocols are desired by renal transplant recipients (RTRs) given the potential for reduction of corticosteroid adverse effects (AEs). **Methods:** Using data from the MORE registry, a prospective, observational study of adult RTRs receiving mycophenolate (MPA) where standard-of-care is determined by 40 US sites, 6-month CSW protocol outcomes were analyzed. CSW was defined as withdrawal of steroids by 3-months post-transplant. A total of 645 tacrolimus-treated RTRs (267 CSW, 378 CS) were analyzed. **Results:** There were no differences in patient demographics (mean age 51.4 yrs, 63% male, 25% African American, 42.6% living donor RTRs). Nearly 100% of the RTRs received at least one induction agent (CS/CSW: 57.1/66.7% thymoglobulin; 4.5/20.2% alemtuzumab; 28.0/11.2% basiliximab; 12.7/0.8% daclizumab). Tacrolimus trough levels at months 1, 3, 6 and 12 were similar between groups (CS/CSW: 10.5/9.9 ng/mL; 9.4/9.0 ng/mL; 8.1/7.8 ng/mL; 7.5/7.5 ng/mL). Biopsy-proven acute rejections (BPAR) were low (7% CS vs. 5% CSW, p=0.21). Interim results at 1, 3, 6 and 12 months showed that more of the CS patients were maintained on at least full dose of MPA (CS/CSW: 85.0/68.6%, p=<0.01; 77.0/55.6%, p=<0.01; 63.6/38.1%, p=<0.01; 54.1/37.1%, p=<0.01). There was a statistically significant difference in graft survival (100 vs. 99.5%, p=<0.01) favoring the CSW group. Patient survival was similar. The CS RTRs had a higher mean serum creatinine (CS 1.52 vs.CSW 1.41mg/dL, p=0.05) but there were no differences in reported infections (including CMV and BK), bone disease, cardiovascular events, diabetes, malignancies or GI events. There was a significant difference in hematological AEs in the CSW RTRs (57.7% vs. 26.2%, p=<0.01). **Conclusions:** CS allowed for better tolerance of full dose MPA, however, there was no difference in BPAR. More RTRs in the CSW group received depleting antibody induction (86.9% vs. 61.6%). Graft survival and SCr were better in the CSW group in the short-term, however, longer follow-up is needed to assess long-term outcomes of CSW.

Disclosure of Financial Relationships: Research Funding: Novartis Pharmaceutical Corporation; Honoraria: Astellas.

**TH-PO970**

**Associations of Pre-Transplant Obesity and Muscle Mass with Mortality in Renal Transplant Recipients** Miklos Z. Molnar,[1,2] Elani Streja,[1] Elani Streja,[1] Csaba P. Kovesdy,[3] Suphamai Bunnapradist,[4] Jennie Jing,[1] Allen R. Nissenson,[5] Istvan Mucsi,[2] Kamyar Kalantar-Zadeh.[1] [1]*Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA;* [2]*Semmelweis University, Budapest, Hungary;* [3]*Salem VA Medical Center, Salem, VA;* [4]*Division of Transplantation, UCLA, Los Angeles, CA;* [5]*DaVita Inc, Lakewood, CO.*

**Background:** The association between pre-transplant body composition and post-transplant graft and patient survival in renal transplant recipients is not clear. We hypothesized that higher estimated muscle mass, represented by pre-transplant serum creatinine level, and larger body size, represented by higher BMI, are associated with better post-transplant outcomes. **Methods:** Linking the 5-year patient data of a large dialysis organization (DaVita) to the Scientific Registry of Transplant Recipients, we identified 10,090 maintenance hemodialysis patients who underwent their first kidney transplantation during the 7/2001-6/2007 period. **Results:** Renal transplant recipients were 49±13 years old and included 49% women and 45% diabetics. Increased mortality and worse combined patient and graft survival were associated with pre-transplant BMI<20 kg/m$^2$, whereas higher BMI values (>25 kg/m$^2$) were not associated with increased mortality or graft loss. There was a 2.3-fold increased risk of combined death and graft loss with the pre-transplant serum creatinine <4 mg/dL (p=0.002), whereas creatinine >14 mg/dL exhibited 26% greater graft and patient survival (p=0.01, reference: creatinine 8 to <10 mg/dL).



**Conclusions:** Low pre-transplant BMI or serum creatinine levels are associated with the worst post-transplant outcomes, whereas highest pre-transplant serum creatinine, a surrogate of larger muscle mass, is associated with the best post-transplant graft and patient survival.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO971**

**Impact of Donor and Recipient Body Mass Index Incompatability on Graft Function after Kidney Transplantion** Catherine Morgan, Rhonda Rosychuck, Stephen Newman, Sita Gourishankar. *University of Alberta, Edmonton, AB, Canada.*

Size compatibility between renal graft and recipient may have an impact on graft outcomes. Matching donor to recipient based on measures of graft mass relative to recipient size adds complexity to organ allocation and has the potential to change an individual's access to an organ. Our aim was to evaluate the relationship between donor and recipient size compatibility and graft outcome, using donor to recipient body mass index ratio (D/R BMI) as a predictor. We studied 538 first, non-expanded criteria donor, kidney transplants at the University of Alberta with graft survival past 6 months. Multiple linear regression was used to determine the association between D/R BMI and estimated glomerular filtration rate (eGFR) at 1, 3 and 5 years posttrasplant as well as annualized change in GFR. Cockcroft-Gault adjusted to body surface was used to determined eGFR and annualized change in GFR was determined by application of linear regression methods to serial posttransplant eGFR measurements. D/R BMI predicted eGFR at 1 year; eGFR increased as D/R BMI decreased (p=0.004 ). D/R BMI was also a significant predictor of eGFR at 3 and 5 years, an effect which was attenuated after adjusting for eGFR at 1 year. In subsequent regression which included recipient BMI category (by World Health Organization definition), the significance of D/R BMI effect disappeared. Associations were not modified by donor source (living / deceased) and there was no synergistic effect between D/R BMI and factors previously reported to influence graft function. There was little change in mean eGFR at different times after transplant and we found no association between D/R BMI and annualized change in GFR. Coupled with our finding that D/R BMI is associated with eGFR at 1 year but not 3 or 5 years after adjusting for early graft function suggests that any effect on graft survival is likely determined in the early post transplant period. The finding that the association between D/R BMI and eGFR is attenuated by including recipient BMI category in additional multivariate models indicates that recipient BMI may underlie the observed relationship between D/R BMI and graft function.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO972**

**Association of Immunosuppressive Regimen with the Development of Native Kidney and Allograft Cysts after Renal Transplantation** Matthew K. Abramowitz, Pooja Malhotra, Maria C. Bermudez, Maria Coco, Graciela De Boccardo, Enver Akalin, Daniel G. Glicklich. *Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY.*

Background: Acquired cystic kidney disease (ACKD) is a recognized risk factor for the development of renal cell carcinoma. We sought to identify risk factors for ACKD in patients after renal transplantation.

Methods: A retrospective chart review was performed of adult renal transplants at Montefiore Medical Center from 1996 – 2007. ACKD was identified by routine screening ultrasound. Patients were excluded if sonograms at baseline and a minimum of 1 year post-transplantation were not available. Logistic regression models were created to examine the association of covariates with ACKD in patients without baseline cystic disease.

Results: 472 of 887 patients met inclusion criteria. The mean age was 48 years, 43% were women, 56% were white, 34% were black, 57% underwent cadaveric transplantation, 42% had pre-transplant dialysis, 65% were treated with tacrolimus, 21% with cyclosporine, 28% with rapamycin, and 50% with mycophenolate, and median baseline serum creatinine was 1.9 mg/dL (interquartile range (IQR) 1.3 – 3.5). Overall, 1812 sonograms were performed over a median 5.2 years (IQR 3.7 – 8.1). Baseline cysts were present in the native kidney and allograft in 138 (29.2%) and 27 (5.7%) patients, respectively. Of those without baseline ACKD, 132 of 334 (39.5%) developed native kidney cysts and 51 of 445 (11.5%) developed allograft cysts during follow-up. After adjustment for the aforementioned characteristics and etiology of ESRD, only age (odds ratio (OR) per 10 year higher age, 1.43; 95% CI 1.08 – 1.88) and rapamycin use (OR 0.51, 95% CI 0.25 – 1.03) were associated with native ACKD. Covariates associated with allograft ACKD included cyclosporine use (OR 3.47 versus tacrolimus, 95% CI 1.32 – 9.10) and pre-transplant dialysis (OR 2.55, 95% CI 1.08 – 6.06).

Conclusions: ACKD is common after renal transplantation. Potential risk factors include older age, pre-transplant dialysis, and cyclosporine use, while rapamycin may have a protective effect. Further studies are needed to examine the effect of immunosuppression on ACKD.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO973**

**Hyperparathyroidism Bone Health and Aortic Calcification in Renal Transplant Recipients** Sinead Kinsella,[1] Joe P. Coyle,[2] Abina Harrington,[1] Michael G. Molloy,[3] Conor Bogue,[2] Joseph A. Eustace.[1] [1]*Renal Medicine;* [2]*Radiology;* [3]*Rheumatology, Cork University Hospital, Ireland.*

**Introduction:**The relationship between post transplant hyperparathyroidism (HPT) decreased bone mineral density (BMD) and vascular calcification is unclear.

**Methods:**This is a prospective study quantifying these relationships in 1st renal allograft recipients (eGFR >30ml/min).Patients undergo DXA scan, markers of bone turnover,plain XRay of the L1-L4 spine to quantify aortic calcification score (AoCS) using a standard Framingham method (Range 0-24) and pulse wave velocity (PWV) measurement.Results expressed as mean (SD).Correlation by Spearman Rank unless otherwise stated

**Results:**Study population (n=89):61% male, age 46.6(13)yrs, dialysis vintage 2.5 (1.9) yrs, median transplant duration 3.6 yrs.MDRD eGFR was 53(16)ml/min.

Median (IQR) iPTH was 98ng/ml(72–142). 79% had PTH >65ng/ml. Median PTH was similar in 8 patients with vs. those without prior subtotal parathyroidectomy (94 vs. 98ng/ml). T-score at lumbosacral spine, neck of femur and forearm was -0.1(1.6), -0.7(1.3) and -1.1(1.4) respectively.22% had osteoporosis.Forearm DXA was associated with wrist fracture independent of age and gender, adjusted OR(aOR)0.50(0.29, 0.87). PTH positively correlated with bone specific alkaline phosphatase (r=0.62, p<0.001,Pearsons), tartrate resistant acid phosphatase 5b (r=0.48,p=0.001, Pearsons),urinary N-Telopeptide(r=0.65,p<0.001) and ionised calcium (r=0.37,p=0.001).15% of patients had subnormal phosphate and there was a significant negative correlation between PTH and phosphate(r=-0.45, p<0.001) 84% of patients were Vitamin D deficient (<50nmol/L), mean 25-hydroxyvitamin D: 37(13) nmol/L. 68% had aortic calcification, 8.2% had a score ≥10. AoCS correlated with PWV(r=0.24, p=0.046). PWV and AoCS were associated with history of atherosclerotic cardiovascular disease on univariate analysis but only PWV was independent of age(aOR 1.3; p=0.01).Neither PWV nor AoCS were independently associated with PTH or markers of bone turnover.

**Conclusion:**Post transplant HPT is common and is associated with decreased BMD, increased markers of bone turnover and prior wrist fracture but not with Aortic calcification or PWV

Disclosure of Financial Relationships: nothing to disclose

## TH-PO974

**Evaluation of the Performance of Estimated GFR To Estimate the Evolution of Measured GFR over Time in Kidney Transplant Patients** Olivier Moranne,[1] Nicolas Maillard,[2] Christopher R. Mariat.[2] *[1]Service de Néphrologie et Département de Santé Publique, CHU Nice, Nice, France; [2]Service de Néphrologie et Laboratoire d'Explorations Fonctionnelles, CHU Saint Etienne, Saint Etienne, France.*

Analysis of estimated GFR to estimate the evolution of mGFR was done in native kidney but remains largely unknown in kidney transplant patients. Objective of the study was to evaluate the performance of estimated GFR with MDRD, Cockroft and Gault formula and 1/Serum creatinin (SC) to estimate the evolution of measured GFR (mGFR) in kidney transplant patients.

In one Nephrology department, since 1996 all patients have had a mGFR at 1 year and every 5 years with urine inuline clearance. All consecutive patients with at least 3 mGFR were selected for a modelisation of the evolution of GFR over time. A generalized linear mixed model effect was used to estimate the mean baseline GFR and slope for each method. Agreement and precision between mGFR and eGFR over time was determined with calculation of bias (differences in mean slope), intraclass correlation (IC).

Two hundred and two patients were studied with 688 mGFR whom 15%, 79% and 6% have had 3, 4, 5 measures respectively. At baseline, mean age was 45 years, 75% were men, mean mGFR were 50 mL/min/1.73m2 and a first transplant in 88%. Estimation of mean baseline GFR (mL/min/1.73m2) and slope by year were as follows: mGFR[CI95%]: **48.4** [46-51] **- 0.24** [-0.46;-0.02]; MDRD: **49.0** [46-51] **- 0.15** [-0.32; 0.05]; CG: **30.0** [29-31] **– 0.27** [-0.39; 0.17]; 1/SC: **64.0** [62-67] **– 0.05** [-0.2; 0.25]. The mean bias between eGFR and mGFR for the slope was +0.11 ± 0.41; – 0.04 ± 0.4 and + 0.30 ± 0.47 ml/min/1.73m2/ year respectively for MDRD, CG and 1/SC. The intraclass correlation for the slope were 0.63, 0.65 and 0.55 respectively for MDRD, CG and 1/SC.

In this study with MDRD, the bias to estimate the mean mGFR at baseline was moderate but the slope was underestimated by 40%. The CG formula underestimates the mean baseline GFR and over estimates the slope. The 1/SC over estimates mean baseline GFR and underestimate the slope.

In clinical research mGFR has to be used. Failing to dispose of mGFR, MDRD equation could be used knowing that it will underestimate the slope by 40%.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO975

**Transplantation after Encapsulating Peritoneal Sclerosis (EPS)** David Makanjuola,[1] Hersharan Sran,[1] Peter A. Andrews.[1] *[1]Nephrology, St. Helier Hospital, Surrey, United Kingdom; [2]Nephrology, St. Helier Hospital, Surrey, United Kingdom; [3]Nephrology, St. Helier Hospital, Surrey, United Kingdom.*

Background

EPS is a rare but serious disease, often associated with a very poor prognosis. Risk factors associated with its development include longer duration of peritoneal dialysis, high transport membrane characteristics, and frequent episodes of peritonitis.

Several reports have described the development of EPS after renal transplantation, and have proposed a link between cessation of PD, and the use of calcineurin inhibitors with the development of acute EPS.

We describe two patients whose details are described in the table below. One patient underwent peritoniectomy and enterolysis prior to transplantation, while the other was managed medically alone. Both required total parenteral nutrition, but improved sufficiently to undergo transplantation, and are surviving 41 and 11 months post-transplantation respectively with functioning grafts.

Demographics and current status

| | Patient 1 | Patient 2 |
|---|---|---|
| Age at diagnosis (years) | 47 | 40 |
| Gender | M | F |
| Cause of ESRD | FSGS | MPGN |
| Duration on Peritoneal Dialysis | 15 months | 60 months |
| Surgery for EPS | No | Yes |
| Pre-transplant immunosuppression | Tamoxifen, Prednisolone, Azathioprine | Tamoxifen, Prednisolone, Azathioprine |
| Nutrition | TPN | TPN |
| Time from EPS diagnosis to transplantation | 21 months | 20 months |
| Type of transplant | Living related | Cadaveric |
| Post-transplant immunosuppression | Prednisolone, Tacrolimus, Mycophenolate Mofetil | Prednisolone, Tacrolimus, Mycophenolate Mofetil |
| Transplant complications | Nil | Delayed graft function for 2 months, Acute rejection at 2 weeks & 3months |
| Current creatinine | 109 µmol/l | 189 µmol/l |
| Graft survival to date | 41 months | 12 months |

Conclusion

Our experience suggests that aggressive medical and surgical management of EPS can be successfully pursued with the goal of eventual organ transplantation and that the results are acceptable in highly selected patients.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO976

**The Assessment of Expected Versus Observed Post-Transplant Function by Integrating Kidney Donor and Transplant Recipient Characteristics Improves Outcome Prediction** Shuai (Stone) Li, Gordan Broderick, Kelly L. Craig, Valerie A. Luyckx, Thomas F. Mueller. *Medicine, University of Alberta, Edmonton, AB, Canada.*

Transplant recipient (R) metabolic demand and kidney donor (D) supply are the major determinants of the expected post-transplant function. However, clinically only R based serum creatinine or glomerular filtration rate are used to assess transplant function. Our study tried to integrate R and D factors to compare expected to observed post-transplant kidney function.

First we calculated the adaptive capacity (AC) of single native kidneys after donation in living donors (n=28). In average the GFR in the single remaining kidney increased by 35% post-donation ($R^2$=0.66, p<0.0001). This AC was independent of D age, gender, and weight. Next we identified the lowest observed s-crea in living (n=77, s-crea 108 ± 25 umol/L) and deceased (n=58, s-crea 110 ±30 umol/L) donor kidney recipients in the first post-transplant year. Then we calculated metabolic demand and supply in the R and D (incl. R/D age, weight, height, gender and D s-crea) and adjusted for an AC of 35%. The resulting formula of optimum expected transplant function allowed for a comparison to the observed s-crea. Despite similar observed s-crea, CG and MDRD values at 1, 2 and 3 yrs post-transplant between deceased and living donor transplants the calculation of expected vs observed s-creas differed significantly in the long-term (p<0.05). The overall regression formula showed a significant correlation and 30% prediction accuracy of 81% in LD and 65% in DD recipients. Weight differences b/w R and D were the major cause for discrepancies in expected vs observed s-crea levels. High R weight and low D weight lead to a higher than expected GFR, i.e. hyperfiltration, exceeding the usual 35% AC.

The integration of D and R characteristics in the assessment of kidney function might permit a more robust evaluation of post-transplant organ performance and identify kidneys that do not perform as they should.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO977

**Impact of ESAs, Blood Transfusions, and Iron Supplementation on Patient and Graft Survival in Anemic Kidney Transplant Recipients** Rajiv J. Gandhi, Aleksandra Kukla, Arthur J. Matas, Hassan N. Ibrahim. *Department of Medicine, Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN.*

Post-transplantation anemia is associated with reduced patient and graft survival. Moreover, the use of erythropoiesis stimulating agents (ESAs) in kidney transplant recipients is linked to increased mortality at high (≥ 12.5 g/dL) hemoglobin (Hb). This data is primarily from centers with smaller numbers of patients. Herein, we describe the prevalence of anemia and the use of ESAs, transfusions, and iron supplementation in 1520 recipients transplanted from 2000 to 2010 and assess the adjusted risk of graft and patient loss associated with anemia and its treatment.

Results: Fifty-four percent of all recipients had anemia (Hb < 12 g/dL). Women, non-whites, those with previous transplants, and those exposed to pre-transplant dialysis were more likely to be anemic. In anemic recipients not receiving iron, 22.5% received ESAs alone, 37.5% received transfusions alone, 31.4% received both, and 8.9% received neither. In those on iron, corresponding percentages were 20.8, 18.1, 41.6, and 19.5. Results of a multivariate risk analysis of graft and patient loss adjusted for demographics, comorbidities, and laboratory values are shown in the table.

Predictors of Graft Loss and Recipient Death

| Predictor | | Graft Loss* | Recipient Death |
|---|---|---|---|
| Untreated anemia | | HR 3.0(95% CI 1.8-4.8),p<.01 | 1.6(0.9-3.2),0.13 |
| Treated anemia | | | |
| | pRBC | 2.0(1.4-2.9),<.01 | 1.8(1.2-2.8),<.01 |
| | ESA | 1.0(0.6-1.8),0.88 | 0.7(0.3-1.6),0.39 |
| | pRBC+ESA | 3.5(2.4-4.9),<.01 | 1.9(1.1-3.0),0.01 |
| | Iron | 0.9(0.3-3.0),0.92 | 0.9(0.2-3.7),0.89 |
| | Iron+pRBC | 0.5(0.1-3.7),0.50 | 0.8(0.1-5.6),0.80 |
| | Iron+ESA | 2.8(1.3-6.2),<.01 | 2.2(0.8-6.1),0.13 |
| | Iron+ESA+pRBC | 3.4(2.0-5.9),<.01 | 3.6(1.9-6.8),<.01 |

*Death censored graft loss followed a similar pattern

Conclusion: Anemia after transplantation is common, as are transfusions. Transfusions are associated with adverse patient and graft outcomes. These results indicate no adverse consequences of ESA use in this population.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO978

**Serum Asymmetric Dimethylarginine (ADMA) Levels Are Associated with Serum Tumor Necrosis alpha and Hypoalbuminemia in Prevalent Kidney Transplant Recipients** Jan T. Kielstein,[1] Miklos Z. Molnar,[2,3] Mario Schiffer,[1] Maria Eszter Czira,[2] Anna Rudas,[2] Akos Ujszaszi,[2] Stefanie M. Bode-Böger,[4] Philipp Kümpers,[5] Adam Remport,[6] Istvan Mucsi.[2] *[1]Hannover Medical School, Hannover, Germany; [2]Semmelweis University, Budapest, Hungary; [3]Harbor-UCLA Medical Center, Torrance, CA; [4]Otto-von-Guericke University, Magdeburg, Germany; [5]University Hospital Münster, Münster, Germany; [6]Szent Imre Hospital, Budapest, Hungary.*

Background: Asymmetric dimethylarginine (ADMA) has been implicated in inflammation through induction of proinflammatory genes. We analyzed the association between serum ADMA, C-reactive protein (CRP), serum interleukin 6 (IL6) and tumor necrosis factor-alpha (TNF-alpha) levels in kidney transplant recipients (Tx).

Methods: Data from 258 prevalent Tx patients (age 54+12, mean eGFR 42+21 ml/min/1.73m2) from a single transplant center were analyzed. ADMA was determined using liquid chromatography-mass spectrometry. Serum IL-6 and TNF-alpha levels were measured with ELISA. Correlation analysis and multivariable regression analysis was used to assess the independent association between inflammatory markers and serum ADMA. Variables with skewed distribution were natural log-transformed.

Results: Serum ADMA was negatively correlated with eGFR (r=-0.407, p<0.001) and serum albumin (r=-0.248, p<0.001), positively correlated with IL6 (r=0.165, p=0.007) and TNF-alpha (r=0.322, p<0.001) but not with serum CRP (r=0.105, p=0.081). The correlation between serum IL6 has become non-significant after adjusting for age, gender and eGFR but the correlation between TNF-alpha and ADMA remained significant. In a multivariable linear regression model ln-TNF-alpha (beta=0.205, p<0.001) but not ln-IL6 or ln-CRP were independently associated with serum ADMA after adjustment for age, gender, eGFR, comorbidity, serum albumin and LDL-cholesterin.

Conclusion: Serum ADMA is independently associated with TNF-alpha but not with IL6 in kidney transplant recipients. Also, ADMA increases in the state of malnutrition. Hence, ADMA may be an important player of the malnutrition, inflammation atherosclerosis (MIA) syndrome in patients with impaired kidney function.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO979

**Recurrence of Primary Glomerular Disease in Second and Subsequent Kidney Transplants** Claire Kennedy, Frank J. O Brien, Ayanfeoluwa Obilana, Peter J. Conlon. *Department of Nephrology, Beaumont Hospital, Dublin, Ireland.*

Introduction

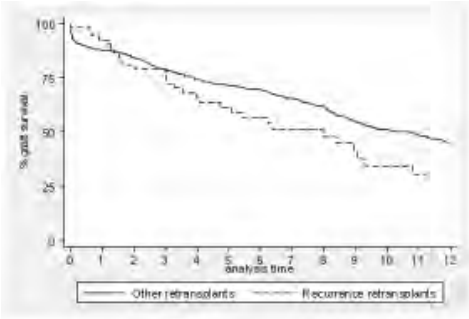
Primary glomerular diseases such as IgA Nephropathy, membrano-proliferative glomerulonephritis (MPGN) and primary focal segmental glomerular sclerosis (FSGS) are known to recur in kidney transplants and can lead to graft loss. The rate of recurrence in second kidney transplants, following failure of the first due to recurrence, is largely unknown.

Methods

A retrospective review of the Irish transplant database from 1982 – 2009 was performed. Patients were included for analysis if they had two or more kidney transplants and their first graft failed due to recurrence of the primary disease.

Results

40 patients had a deceased donor re-transplant following first graft loss to recurrence. Overall, the median graft survival of such re-transplants was 10.5 years in the case of IgA Nephropathy, 8 years in the case of MPGN, and 6 years in the case of FSGS. The median graft survival was 10.5 years for all other re-transplants (p=0.0915).




We identifed several factors which were predictive of recurrence and time to recurrence.

Conclusion

Glomerular disease does not necessarily recur in second grafts following loss of the first graft to recurrence. Graft survival following recurrence in re-transplants is comparable to survival in all other re-transplants.

Patients whose first kidney transplant fails due to recurrent glomerular disease should not be precluded from receiving a second transplant.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO980

**In Pancreas after Kidney Transplant Recipients (PAK), the Pre-Pancreas Level of Kidney Allograft Function Is Associated with Kidney Allograft Failure** Sarah A. Browne, Jagbir Gill, James Dong, Caren L. Rose, John S. Gill. *UBC Division of Nephrology, St Pauls Hospital, Vancouver, BC, Canada.*

There is limited information to guide the selection of diabetic kidney transplant recipients for subsequent pancreas transplantation. In clinical practice, the level of kidney allograft function is relied upon to guide selection of PAK candidates.

Methods:In this analysis of n = 3030 diabetic kidney transplant recipients in the UNOS/OPTN database between 1989- 2007 who subsequently received a pancreas transplant we determined the independent association of last available GFR prior to pancreas transplantation with all-cause kidney allograft failure after the pancreas transplant.

Results: The median GFR prior to pancreas transplantation was 57ml/min (95% CI 29-93ml/min). The loss of GFR in kidney allograft recipients prior to pancreas transplant was negligible (mean GFR slope -0.07 ml/min/year, 95% CI -0.42 – 0.28). The table shows that patients with a higher pre-pancreas transplant GFR had a lower risk of all-cause kidney allograft failure after pancreas transplantation.

| Factors | Risk of kidney allograft failure |
|---|---|
| | HR (95%CI) |
| **eGFR pre-Pancreas transplant (ml/min)** | Ref <45ml/min |
| 45-60 | 0.67(0.56-0.79) |
| 61-90 | 0.58(0.48-0.69) |
| >90 | 0.49(0.33-0.74) |

1)Cox Mulitivariate Regression adjusted for patient age, gender,race, year of kidney transplantation, dialysis exposure prior to kidney transplantation, kidney donor source, and time between kidney and pancreas transplantation. 2)GFR estimated using 4 variable MDRD equation and last available creatinine prior to pancreas transplantation. GFR slope(ml/min/1.73m$^2$/year) determined using mixed effects repeated measures model

Conclusion: In this cohort of kidney allograft recipients who were selected to receive a pancreas based on their stability of kidney allograft function, the level of kidney allograft function prior to pancreas transplantation was still associated with all-cause kidney allograft failure after the pancreas transplant.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO981

**Allograft Nephrectomy: A Nine Year Experience in One Center** Samir Baroudi,[1] Nicholas J. Mayer,[1] Omar S. Abu-Romeh,[1] Mihoko Yoshino,[2] Kiyoko Oshima,[2] Bahar Bastani.[1] *[1]Division of Nephrology, Saint Louis University Health Sciences Centre, Saint Louis, MO; [2]Division of Nephropathology, Saint Louis Universiy Health Sciences Centre, Saint Louis, MO.*

**Objectives:** There is controversy on the role of transplant nephrectomy in the management of patients with failed kidney allografts. Herein, we reviewed our experience with allograft nephrectomy.

**Methods:** This retrospective observational study included patients with allograft nephrectomy performed from January 2001 through December 2009 in Saint Louis University Hospital. Clinical, laboratory and pathological data were collected. Indications for nephrectomy and the associated complications were analyzed.

**Results:** We identified 98 patients, with 100 allograft nephrectomies. The mean (±SD) duration of dialysis prior to nephrectomy was 8±15 months with 73% of patients being on low dose steroids at the time of nephrectomy. Causes of graft failure were: chronic allograft nephropathy 82%, acute rejection 4% , acute tubular necrosis 4%, vascular thrombosis 3%, and recurrent primary disease 3%. Indications for transplant nephrectomy were: hematuria 28%, local tenderness 26%, re-transplant 19%, fever 15%, and graft infection 5%. Post operative blood transfusion was required in 54% of nephrectomies. Complications were reported in 26% (n=26):10 hematoma/bleeding, 5 wound infections, 4 pneumonia, 3 sepsis, 2 intra-abdominal infection, 1 varicella zoster and 1 death (1%). The mean duration

of hospitalization was 8.9±10.6 days. The most common pathologies in the allograft nephrectomy were: vascular (arterial rejection, transplant arteriopathy or thrombosis) in 96% followed by ischemia/necrosis in 48% and hemorrhage in 14%.

**Conclusion:** Mortality and morbidity from allograft nephrectomy remains high in contemporary experience. Medical management of the failed allograft, unless serious indications for surgery (i.e., allograft infection, thrombosis/infarction, bleeding), might be a preferred approach.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO982

**Clinical Significance of Early-Onset Hyperuricemia in Renal Transplant Recipients** Byung Ha Chung, In O. Sun, Hoon Suk Park, Jayoung Lee, Sun Ryoung Choi, Seokhui Kang, Bumsoon Choi, Cheolwhee Park, Yong-Soo Kim, Chul Woo Yang. *Department of Internal Medicine, Seoul St. Mary's Hospital, Seoul, Republic of Korea.*

***AIMS***: It is undetermined whether the effect of uric acid on graft outcome is independent of graft dysfunction. This study was designed to explore whether early-onset hyperuricemia has clinical significance regardless of graft function.

***Methods***: This study was done based on retrospective chart review. We calculated time-average uric acid (TA-uric acid) and estimated glomerular filtration rate (TA-eGFR) from the value at 3, 6, 9 month after transplantation. Hyperuricemia was defined when TA-uric acid is more than 7.0 mg/dL in male and 6.0 mg/dL in female. Reduced graft function was defined when TA-eGFR is less than 60 mL/min/1.73m$^2$. One year eGFR, cardiovascular complications during follow-up and long-term graft survival were assessed according to uric acid level and graft function.

***Results***: Three hundred and fifty one patients were included. Two hundred and two patients belonged to hyperuricemia and One hundred forty eight patients were normouricemia group. Acute rejection episode within one year of transplant, delayed graft function and deceased donor increased the risk for development of hyperuricemia. Hyperuricemia increased the risk for cardiovascular complications (HR=2.8, 95% CI: 1.1 – 7.1, *P*=0.02) but reduced graft function did not. In hyperuricemia group, 5,10 year graft survival was significantly lower than in normouricemia group (89, 81% vs. 96, 92% respectively, *P*=0.02) In reduced graft function group, those were also lower than in normal graft function group (89, 81% vs. 96, 93% respectively, *P*=0.02). In multivariate analysis, both of hyperuricemia (HR = 1.3, 95% CI: 1.0–1.6, *P* = 0.04) and reduced graft function (HR = 0.97, 95% CI: 0.95–1.0, *P* = 0.03) significantly increased the risk for graft failure and the presence of both factors gave the highest risk for graft failure (HR = 4.4, 95% CI: 2.0–9.7, *P* = 0.000).

***Conclusion***: Early-onset hyperuricemia is a significant predictor for cardiovascular complication and graft survival independent of graft function.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO983

**Assessing Renal Graft Function in the First Week Post-Transplant by Comparing Different Clearance Estimation Equations to Renal Scintigram Using DTPA-99Tc** Nuno Figueiredo, Susana Machado, Rui Alves, Fernando Macário. *Nephrology, Hospitais Universidade Coimbra, Coimbra, Portugal.*

Introduction: The surveillance of glomerular filtration rate(GFR) is very important in management of kidney transplant recipients.

Methods: From January to October of 2009 a renal scintigram was performed in the first week post-transplant in 63 patients and GFR was determined by DTPA-99Tc. We then assessed the performance of 3 different estimation equations as compared to DTPA-99Tc GFR namely: modification of diet in renal disease (MDRD), Nankivell and Cockcroft-Gault methods.

Results: Sample characterized by 63 kidney transplanted patients, with a mean age of 50,55 ± 13,46 years, 97,1% were Caucasian and 2,9% African, 55,7% were masculine and 44,3% feminine. The mean donor age was 52,48 ± 16 years, 62 transplants were from deceased donors, one from a living donor and 54,3% were from extended criteria donors. The mean cold ischemia time was 16,75 ± 4,9 hours. The mean HLA match was 2(28,6%), with a minimum of 0 in 8,6% and a maximum of 5 in 4,3%. About 55,6% received induction (36,5% Thymoglobulin and 19,1% Basiliximab). All had a calcineurin inhibitor (61,9% FK and 38,1% cyclosporine). The mean DTPA-99Tc GFR was 56,7 ± 29,1 ml/min/1,73M2. The mean differences observed comparing to DTPA-99Tc GFR were: 11,9±17,2 ml/min/1,73M2 for MDRD equation, 8,18 ± 17,4 ml/min/1,73M2 for Cockcroft-Gault equation and 35 ± 17,7 for Nankivell equation, all with a p<0,01. When we assessed the correlation between the three equations compared DTPA-99Tc GFR, all were statistically significant (p<0,01) and the highest Pearson correlation value was found for Nankivell equation(R=0,976).

Conclusion: The overall performance of the Nankivell equation to estimate the GFR in the 1st week post-transplant was superior to the other studied formulas.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO984

**End Stage Renal Disease in Lung Transplant Recipients** Ritu Khanna, Holly J. Kramer, Charles Alex, David Holt, John Milner, Susan H. Hou. *Department of Medicine, Division of Nephrology and Hypertension, Loyola University Medical Center, Maywood, IL.*

BACKGROUND: Improved outcomes in recipients of nonrenal solid organ transplants have resulted in an increased frequency of chronic kidney disease. The goal of this study was to determine the incidence of ESRD in our lung transplant population, the time to renal replacement therapy (RRT), and the difference in survival between patients who underwent dialysis vs. renal transplantation. METHODS: We reviewed the records of 627 recipients who underwent lung transplantation between January 1991 and April 2009 and were followed at our institution. Data were reviewed through June 2010. We excluded patients dialysed as part of a terminal illness. RESULTS: Of 481 patients who survived to >1 year, 37 needed RRT. Three patients needed dialysis from the time of lung transplant and died within 18 months. Thirty four patients required RRT later in their course. For 19 patients, time to dialysis varied from 17 to 168 months with a mean of 73.7 months. Of 22 dialysis patients, 3 are alive at 18 to 72 months. Mean survival for those who died was 23.4 months. A total of 15 patients received a kidney transplant (10 living donor, 3 deceased donor, 2 unknown). Eight were preemptive and 3 were done after a short period of dialysis (7 to 22 months). Data for the remaining 4 are unknown. The time to renal transplantation ranged from 27 to 141 months post lung transplant with a mean of 96.4 months. Eleven patients are currently alive with a mean survival of 56.7 months. Two were lost to follow up and 2 are deceased. Patient and renal graft survival was 100% at 1 year and 90.9% at 3 years (and 87.5% at 5 years) compared to dialysis patient survival of 64.7% at 1 year and 30.7% at 3 years (p<0.02). Five percent of 5 year lung transplant survivors and 33.3% of 10 year survivors needed chronic RRT. CONCLUSIONS: ESRD is a common complication of lung transplant seen in approximately one third of 10 year survivors. Survival in lung transplant patients treated with dialysis was significantly worse than in renal transplant recipients. Short term graft survival is comparable to graft survival in other renal transplant recipients.

Disclosure of Financial Relationships: nothing to disclose

### TH-PO985

**Risk Factors for Severity of Acute Rejection on Kidney Allograft Biopsies** Ajay K. Israni,[1] Robert Leduc,[2] David P. Schladt,[2] Pamala A. Jacobson,[3] William S. Oetting,[3] Arthur J. Matas.[4] *[1]Medicine, Hennepin County Medical Center, University of Minnesota (MN), Minneapolis, MN; [2]Biostatistics, University of MN, Minneapolis, MN; [3]Pharmacy, University of MN, Minneapolis, MN; [4]Surgery, University of MN, Minneapolis, MN.*

**Introduction**: We examined the clinical risk factors associated with severity of acute rejection (AR) in a multicenter, prospective cohort of kidney transplant (Tx) recipients from six transplant centers in Canada and the United States.

**Methods:** Our study consisted of patients receiving kidney Tx between 2006-2008 from 6 transplant centers. We had 155 recipients with clinical AR and another 216 with biopsies for cause which did not show AR and the remaining 620 with no biopsies and no clinical AR. In order to assess severity of AR, we divided the study population into 3 groups: t-score ≥2, t-score ≤1 and no biopsy group. We then used a multinomial logistical regression model to determine risk factors associated with severity of tubulitis. Since the t-scores were strongly correlated with the i-scores, we did not repeat the same analysis for i-score.

**Results:** 966 kidney transplant recipients were genotyped. Their average age was 49 ± 14 years, 17% were African Americans (AA). The 155 AR biopsies showed 70% cell-mediated AR, 18% antibody mediated and 10% with features of both and 2% other. The following t-scores for those recipients with an AR were observed: 51% t-score ≥2, 34% with t=1 and 15% with t=0. The following t-scores for those recipients without AR were observed: 2% t-score≥2, 11% with t=1 and 87% with t=0.

During the median follow-up period of 22 months post-tx, the rate of return to dialysis or retransplantation was 10% in the t ≥2 group, 5% in the t ≤1 and 1% in the no biopsy group. The factors that were associated with increasing severity of tubulitis were: *transplant center (p<0.0001), younger recipient age (p=0.002), male gender (p=0.007), PRA greater than 10% (p=0.007), increasing number of HLA mismatches (p=0.007) and donor age (p<0.0001).*

**Conclusions:** Clinical factors as well as the transplant center were significantly associated with severity of tubulitis.

Disclosure of Financial Relationships: Research Funding: Research grants >$10,000 from Roche, BMS, Genzyme and Amgen.

### TH-PO986

**Presence of Calcium Oxalate Deposits in Protocol Transplant Biopsies Does Not Affect Kidney Allograft Prognosis** Manish Nepal,[1] Ping L. Zhang,[2] Anil Kotru,[1] Patrick Dorion,[1] Michael F. Schultz,[1] Joseph V. Bonventre.[3] *[1]Nephrology, Transplant and Pathology, Geisinger Medical Center, Danville, PA; [2]Pathology, William Beaumont Hospital, Michigan, PA; [3]Renal Division, Brigham and Women's Hospital, Boston, MA.*

Presence of renal parenchymal calcium oxalate (CaOx) deposits can be confirmed in renal biopsy by examining the birefringence of CaOx under polarized light microscopy. Deposition of such crystals in renal tubular cells has been described with short term adverse

effect like acute tubular injury and long term effects such as nephrocalcinosis and poor allograft survival. This study was designed to identify CaOx in protocol renal biopsies and evaluate an association between CaOx deposition and prognosis.

We evaluated 232 renal transplant recipients between Jan 2002 to July 2006 at Geisinger Medical Center of whom 68 patients had undergone protocol renal biopsies. Protocol biopsies performed mainly at 4 weeks, 8 weeks, 12 weeks and one year post transplant were analyzed. Mean creatinine at biopsy was 1.4 mg/dl for CaOx group and 1.6 for the non-oxalate group. Patients did not show evidence of rejection or acute kidney injury. Serum tacrolimus levels were within normal limits. Hematoxylin and eosin stained sections were (N = 68) examined under polarized light microscopy under 40 x magnification. CaOx crystals containing tubule cross sections were identified. 22/68 (32.3%) patients had CaOx deposits in the allograft biopsy. These patients had no history of hyperoxaluria or evidence of oxalate calculi. Biomarkers of injury including kidney injury molecule-1 (KIM-1) and NGAL were not detectable in renal tubules and C4d staining was also negative in peritubular capillaries. Mean follow-up time at outcome analysis was 66 months for the CaOx group (n = 22) and 68 months for the non-oxalate group (n = 46). Patient survival and graft survival were 86% and 77% for the CaOx group and 84% and 80% for the non-oxalate group.

We conclude that CaOx deposits in the allograft renal biopsy without clinical evidence of graft malfunction may represent an incidental finding and should not be taken as a prognostic marker of long term allograft function.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO987

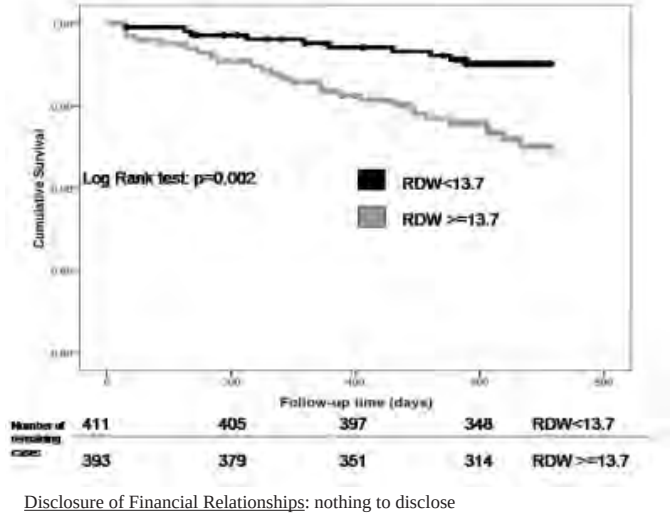
**The Red Cell Distribution Width Is Associated with Mortality in Kidney Transplanted Recipients** Miklos Z. Molnar,[1,2,3] Maria Eszter Czira,[2] Anna Rudas,[2] Akos Ujszaszi,[2] Istvan Kiss,[4] Adam Remport,[4] Marta Novak,[2] Laszlo Rosivall,[1,5] Istvan Mucsi.[2,5] *[1]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary; [2]Institute of Behavioral Sciences, Semmelweis University, Budapest, Hungary; [3]Harold Simmons Center for Chronic Disease Research & Epidemiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, UCLA, Torrance, CA; [4]Dept. of Nephrology, Szent Imre Hospital, Budapest, Hungary; [5]Hungarian Academy of Sciences and Semmelweis University Research Group for Pediatrics and Nephrology, Budapest, Hungary.*

**Background:** Red Cell Distribution Width (RDW) is associated with inflammation, ineffective erythropoiesis, iron deficiency and impaired renal function in patients with heart failure. It is also associated with increased morbidity and mortality risk. No published data is available about the association between mortality and RDW in kidney transplanted patients.

**Methods:** We collected socio-demographic parameters, medical and transplant history and laboratory data from 804 prevalent kidney transplant recipients. RDW was measured as part of a standard complete blood count measurement. To assess if RDW predicts with all-cause mortality risk we used Cox proportional hazards regression.

**Results:** During the 22 month follow-up, 37 subjects died. Mean age was 51±13 years, 56% of the patients were males, 21% were diabetics. Mortality was significantly higher in patients with high vs low (above vs below median) RDW at baseline. RDW was a significant predictor of mortality both in the univariate model ($HR_{1\ increase}$ = 1.594; 95% CI: 1.353-1.877) and also in a multivariate model ($HR_{1\ increase}$ = 1.451; 95% CI: 1.139-1.848) adjusting for several co-variables (age, gender, eGFR, Charlson Comorbidity Index, CRP and albumin).

**Conclusions**: RDW independently predicts mortality in prevalent kidney transplant recipients.




Disclosure of Financial Relationships: nothing to disclose

## TH-PO988

**Effect of Early Steroid Withdrawal (ESW) in a Mexican Kidney Transplants Recipients (KTR) Cohort, Treated with Tacrolimus (TAC) and Mycophenolate Mophetil (MMF)** Jorge Andrade-Sierra,[1,3] Enrique Rojas-Campos,[1] Ernesto Cardona,[3] Abel Puentes Camacho,[2] Luis Alberto Evangelista Carrillo,[2] Trinidad Orlando Lugo Lopez,[2] Benjamin Gomez-Navarro,[2] Mario Sandoval Sandoval.[2] *[1]Medical Research Unit in Renal Diseases, IMSS, Guadalajara, Jalisco, Mexico; [2]Nephrology and Organ Transplant, IMSS, Guadalajara, Jalisco, Mexico; [3]Physiology, University of Guadalajara, Guadalajara, Jalisco, Mexico.*

**Introduction:** Acute rejection (AR), is a risk in ESW. **Objective:** Compare the effect of ESW (<1week), on AR, metabolic profile, frequency of TAC toxicity. **Methods:** Retrospective cohort; 61 KTR, with PRA<20%, between Dec/2005-Mar/2010; 31 (ESW-G) 30 (CG), TAC, MMF, PDN (CG), as maintenance; ESW-G, MPD reduction from 250 (day1) to 0mg (day 5). Evaluations at baseline, 3, 6, 12 mo. Age, BP, time of RT, HLA matching, serum glucose, triglycerides, cholesterol, lipoproteins; antihypertensive and lipid lowering treatment, and eGFR by MDRD, in all evaluations graft biopsies were done to detect AR/Tox **Statistical analysis:** Comparisons between ESW/CG, were done with student´s *t* and $c^2$ tests. **Results:** Main results in Table.

Comparisons between ESW vs control group.

| | ESW Group (31) | | Control Group (30) | |
|---|---|---|---|---|
| | Baseline | Follow-up | Baseline | Follow-up |
| Receptor Age (years) | 25±9 | | 25±10 | |
| Time at follow-up (months) | 21±20 | | 31±22 | |
| HLA antigens matching | 3.6±1.7 | | 3.2±1.6 | |
| PRA (%) Class I | 1.8±2.4 | | 3.9±5.5 | |
| PRA (%) Class II | 6.9±8.2 | | 7.4±9.3 | |
| Acute rejection (%) | | 10% | | 28% |
| TAC toxicity (%) | | 38% | | 14%* |
| SBP mmHg | 132±16 | 112±9 | 147±21* | 121±15* |
| DBP mmHg | 82±10 | 71±7 | 90±15* | 81±7* |
| Lipid lowering treatment % | 10% | 20% | 10% | 27% |
| Antihypertensive treatment % | 71% | 13% | 83% | 60%* |
| Glucose (mg/dl) | 100±37 | 88±12 | 90±10 | 93±14 |
| Triglycerides (mg/dl) | 151±53 | 135±40 | 192±112 | 174±60* |
| Cholesterol (mg/dl) | 154±33 | 147±35 | 190±64* | 183±45* |
| HDL (mg/dl) | 40±13 | 50±14 | 40±14 | 44±17 |
| LDL (mg/dl) | 87±23 | 88±27 | 103±30* | 101±21* |
| eGFR ml/min | 8±4 | 81±25 | 10±15 | 65±23* |

*p<0.05 vs same evaluation ESW-group.

**Conclusions:** ESW-G had lower BP and less antihypertensive use; CG had higher total/LDL cholesterol and triglycerides vs ESW-G. A trend to lower AR in ESW-G and significant higher TAC tox in ESW-G.

Disclosure of Financial Relationships: nothing to disclose

## TH-PO989

**Protocol Biopsies (PB) Preserves Renal Allograft Function in Patients with Acute Calcineurin-Inhibitor Toxicity (CIT): A Single Center Experience** Adriana Banda Lopez,[1] Enrique Rojas-Campos,[2] Maria Guadalupe Salcedo Ceja,[1] Paul Garcia Cobian,[1] Benjamin Gomez-Navarro,[1] Francisco Ramos Solano.[3] *[1]Nephrology and Organ Trasplant Unit, CMNO, IMSS, Guadalajara, Jalisco, Mexico; [2]Medical Research Unit in Renal Diseases, CMNO, IMSS, Guadalajara, Jalisco, Mexico; [3]Unit of Pathology, CMNO, IMSS, Guadalajara, Jalisco, Mexico.*

**Objective**: Evaluate the effect of acute CIT on eGFR at 1 year in RTRs.

**Methods:** 166 RTRs between Jan-2006 to Nov-2008, with diagnostic of acute CIT, followed for 1 year. Serum Cr, glucoses, eGFR, Proteinuria, Blood pressure, at 0,3,6, 12 months were registered. Cohort was divided into: PB (69 RTRs) and biopsies for indication IB (97 RTRs).

**Outcomes**. 119(72%) were man, 144(87%) with unknown cause of ESRD. 156(94%) had living related donor. Table 1 shows clinical and biochemical comparisons. Fig.1 shows the evolution of eGFR between those with CIT and added AR.

Table 1. Features baseline and follow up

| Variable | PB 42% | IB 58 % | p value |
|---|---|---|---|
| Time since TR(mo) | 6.1±5.5 | 11.3±20.2 | 0.02 |
| CrS(mg/dL) | 1.1±0.3 | 1.7±0.6 | <0.0001 |
| Glucose | 99±33 | 100±36 | 0.88 |
| TAS(mmHg) | 126±10 | 129±12 | 0.07 |
| TAD(mmHg) | 79±6 | 81±8 | 0.05 |
| *eGFR baseline | 78.0±19.0 | 56.8±15.8 | <0.0001 |
| +Proteinuria baseline | 0.34±0.12 | 0.16±0.59 | 0.05 |
| *eGFR 6m | 78.0±18.0 | 66.2±16.0 | <0.0001 |
| +Proteinuria 6m | 0.18±0.51 | 0.91±3.62 | 0.06 |
| *eGFR 12m | 82.0±17.0 | 65.8±15.6 | <0.0001 |
| +Proteinuria 12m | 0.12±0.28 | 0.53±1.40 | 0.01 |

*ml/min/1.73m2, +g/24hs. Registers the clinical and biochemical variables during the follow up.

**Conclusions**. PB patients had better graft function compared to IB, CIT does not affect GFR, however when AR is added eGFR decreases significantly, with no longer recovery.

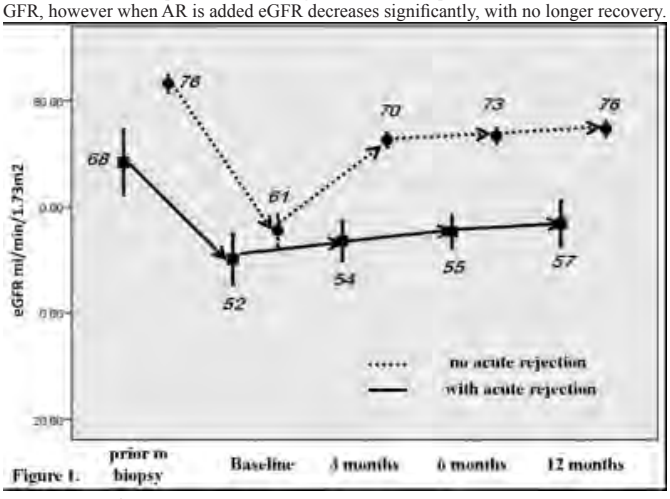


Figure 1.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO990**

**Cause of Death in Kidney Transplant Patients – Single Center Study** Seung Yeup Han, Mi Hyun Jang, Choong-Hwan Kwak, Eun-Ah Hwang, Sung Bae Park, Hyun Chul Kim. *Internal Medicine, Keimyung University Dongsan Medical Center, Daegu, Korea.*

**Purpose** : Graft survival rate in kidney transplantation (KT) has improved over the last two decades, however patients death has been major cause of graft loss. This study performed to identify cause of death and changes according to time.

**Method** : We reviewed retrospectively medical data of patients received KT in Keimyung university Dongsan medical center from Nov. 1982 to Aug. 2009. Patients death included death with functioning graft (DWF) and death less than 3months after graft loss.

**Results** : There were 837 cases of KT in 769 patients. During average follow up duration 81±73.5 months, 72(9.4%) patients died. There were 63(87.5%) DWF. The mean age of dead patients was 39.4±11.1 years(16~59), and there was a male predominance(1.8:1). The causes of patients death were infections including sepsis constituted the largest group(30.6%). The second common cause was malignancy (22.2%), and then cardiovascular disease (11.1%). The causes of patients death were grouped for ananlysis in three time interval: less than 1year, 1year to 5years, and after 5years KT. Infection was the most common cause of death less than 1year (35.7%) and cardiovascular disease was second cause(28.6%). In 1year to 5years after transplantation, also infection was the most common cause(35.5%). After 5years transplantation, malignancy was the most common casuse(42.3%). The major causes of infection consisted of cytomegalovirus(22.7%), bacterial sepsis(22.7%), tuberculosis(18.2%), bacterial pneumonia(13.6%), meningitis(9.1%), fungus(9.1%). Major causes of malignancy were post-transplant lymphoproliferative diseases(25%), lung cancer(18.8%), renal cell carcinoma(12.5%), hepatoma(12.5%). Survival rate of transplant patients at 10 years was 88.3%. Graft survival was 51.5% when DWF was considered as a graft failure, but DWF was considered as censored data, the graft survivial rate increased to 57%. **Conclusion** : Infection was the most common cause of death less than 5years, and malignancy was the most common cause after 5years. DWF was major cause of graft loss, so early detection and treatment of cancer or infection should be paid to transplant patients.

Disclosure of Financial Relationships: nothing to disclose

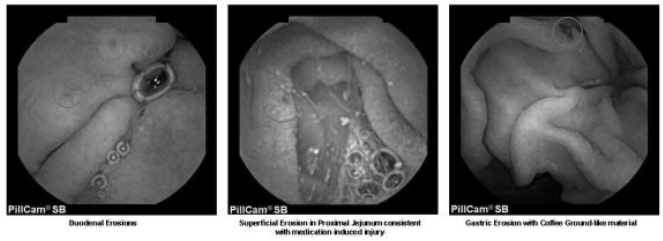
**TH-PO991**

**Endoscopic Observations of Gastrointestinal Symptoms and Lesions in Patients Converted from Mycophenolate Mofetil to Enteric-Coated Mycophenolate Sodium** Suphamai Bunnapradist, Alan H. Wilkinson, Phuong-Thu T. Pham, Edmund Huang, Gabriel M. Danovitch. *UCLA Medical Center, Los Angeles, CA.*

**Introduction:** Mycophenolic acid toxicity is associated with gastrointestinal (GI) complications in renal transplant. Our goal is to evaluate GI mucosal changes in patients receiving mycophenolate mofetil (MMF) using small bowel capsule endoscopy (SBCE), and to determine if equimolar conversion to enteric-coated mycophenolate sodium (EC-MPS) may alleviate GI side effects as demonstrated by SBCE and the Gastrointestinal Symptom Rating Scale (GSRS).

**Methods:** Adult kidney only recipients transplanted more than 30 days presenting with GI symptoms underwent SBCE and completed a GSRS questionnaire, a 15 question self-assessment to rate specific GI symptoms using a 7 tier scoring scale. All were receiving tacrolimus, corticosteroids and MMF (500mg-1000mg bid). MMF was converted an equimolar dose of EC-MPS. At day 30, another SBCE and GSRS questionnaire were repeated.

**Results:** Eleven subjects completed the study (39-68 y/o, 3-8 months post-transplant), and 10 of them reported improvement of symptoms. The average GSRS at baseline and 30-days post-conversion was 3.16 and 1.79, respectively ($p < 0.01$). Baseline SBCE confirmed that 9 subjects had small bowel pathology, including: deunuded mucosa, ulcerations, lymphangiectasia, and erythema. Other findings included gastritis and colonic ulcers. At 30-days post-conversion, there was no clear correlation between resolution of symptoms and improvement in SBCE findings.




**Conclusion:** Small bowel mucosal pathology was common in kidney recipients with GI symptoms on MMF treatment. Conversion to EC-MPS for 30-days significantly improved GSRS by an average of 1.37 (clinically meaningful difference). A repeat SBCE at longer follow-up time post-conversion will be essential to evaluate whether mucosal injury may eventually improve.

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**TH-PO992**

**Conversion to Sirolimus (SRL) of Pediatric Renal Transplant (TX) Patients: A Single Center Experience** Marta L. Monteverde, Juan Ibañez, Alicia Chaparro, Mario Diaz, Amalia F. Turconi. *Nephrology Unit, Hospital JP Garraham, Buenos Aires, Argentina.*

We reviewed 92 children (age at TX 10, 5 ± 4, 1 y) switched from cyclosporine (86/92) to SRL after 106 ± 96 months after TX; in 57, from a cadaver donor. Conversion was due to: Chronic allograft nephropathy (CAN), 69%, Ebstein Barr virus related diseases, 12%, and thrombotic microangiopathy, 6.5%. Median time of follow up: 75 months. All patients were on ACE inhibitors. Results: In the whole group, eGFR (Schwartz formula) and proteinuria increased significantly at 3 months and then did not change. In patients with CAN grade I, eGFR increased at 3 months and remained increased up to 36 month; proteinuria did not change. In patients with CAN grade II eGFR did not change in 36 months; proteinuria increased at 3 months and then remained unchanged. Patients who increased eGFR >15 ml/min had an initial eGFR > 50 ml/min. At baseline 47% (n= 43) of patients needed another antihypertensive drug to achieve normal blood pressure; at month 3, 12% (n= 11; $p<0.01$). Two deaths occurred: one from relapse of an EBV- related B lymphoma, the other, in an accident. Actuarial patient survival was 100% at 1 year, and 96, 5% at 3, 5, 7 y 10 years. Graft survival was 96% at 1 year 90 % at 3 and 5 y; 75% at 7 y and 70% at 10; 10/92 patients lost the graft (10, 8%) due to: CAN, N=9; recurrent FSGS, N=1. SRL was stopped in 15/92 patients (16%) due to nephrotic proteinuria with or without renal failure. eGFR < 30 ml/ min at conversion and SRL withdrawal were predictors of graft loss ($p=0.001$). There were 2 de novo neoplasias: PTLD and multiple leiomiomatosis. There were 2 biopsy proven, steroid sensitive acute rejections. 51% patients had 1 or more episodes of infection (50% during the first 3 months): Low respiratory tract infection, 29%, diarrhea,15%, Herpes Zoster,13%, chicken pox, 9%; TBC in 2 patients and no late CMV infections. Non-infectious adverse events (64% in the first 3 months) were: diarrhea, 62%, oral ulcers, 15%, acne, 7%, severe anemia, 7%. We conclude that conversion to SRL can be done safely in children with good graft survival and tolerable side effects, but improvement of renal function depends on baseline CAN grade and eGFR.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO993**

**Is Voiding Cystourethrography Necessary for Pediatric Renal Transplant Candidates as Pre-Transplant Evaluation?** Yun Hye Jung,[1] Kyoung Hee Han,[1] Hyun Kyung Lee,[1] Il-Soo Ha,[1] Hae Il Cheong,[1] Yong Choi,[2] Hee Gyung Kang.[1] *[1]Pediatrics, Seoul National University Children's Hospital, Seoul, Republic of Korea; [2]Pediatrics, Inje University Haeundae Paik Hospital, Busan, Republic of Korea.*

For adult renal transplant candidates, a voiding cystourethrography (VCUG) is no longer considered to be necessary as urologic evaluation unless the patient has a history of genitourinary abnormalities. However for children, it is still considered necessary despite its invasiveness and the risk of post-VCUG urinary tract infection (UTI). Thus, to investigate the necessity of pre-transplant VCUG for children, we reviewed 122 children with non-urologic causes of end-stage renal disease (ESRD, M:F 75:47) who underwent renal transplantation at our center between 1983 and 2010.

Primary diagnoses leading to ESRD were glomerular disease (n=60), cystic kidney disease (n=18), tubulointerstitial disease (n=7), others (n=8) and unknown (n=29). VCUG revealed vesicoureteral reflux (VUR) in 33 cases (26.8%, Gr I -III in 31, and IV in 1). Although UTI did not complicate the procedure of VCUG in these patients, two of them experienced extravasation of the contrast through their unused bladder.

During the follow-up of 7.95 years (range 0.16 - 26.92), 7 patients caught UTI 5.2 months (range 0.7-121.8 months) after transplantation, with the post-transplant UTI incidence of 0.71 /100 patient–year. Five of the 7 patients with post-transplant UTI had VUR, Gr. I (n=2) or II (n=3). On risk factor analysis, abnormal finding of VCUG was the only significant risk factor for post-transplant UTI.

In our study, VCUG as pre-transplant urologic evaluation revealed VUR in significant number of the patients. On the other hand, while the procedure of VCUG itself may cause UTI, the incidence of UTI for our pediatric kidney transplant recipients (5.7%) was not higher than that for general population of childhood (reported as up to 8%). Thus, despite the significance of low-grade VUR as a risk factor for post-transplant UTI shown in this study, we suggest that the procedure of VCUG is not cost-effective, if not unnecessary, in the pediatric population for the purpose of identifying patients at risk of post-transplant UTI.

Disclosure of Financial Relationships: nothing to disclose

**TH-PO994**

**Characteristics & Outcome of Pediatric Atypical Hemolytic Uremic Syndrome (aHUS) at a Tertiary Care Center** Melissa A. Cadnapaphornchai,[1] Danielle Soranno,[1] Douglas M. Ford,[1] Gary M. Lum,[1] Frederick Karrer.[2] *[1]Pediatrics, University of Colorado Denver, Aurora, CO; [2]Pediatric Surgery, University of Colorado Denver, Aurora, CO.*

**Background:** aHUS is a rare condition affecting 3.3 per million children. It is associated with high risk of chronic renal failure (CRF)/end-stage renal disease (ESRD) and recurrent aHUS following isolated renal transplantation. **Purpose**: To evaluate the epidemiology and outcomes in children with aHUS at a pediatric tertiary care center. **Methods:** We reviewed the medical records of children ≤18 years of age with aHUS with respect to clinical course and outcome. **Results:** 9 children with aHUS were identified at our institution from 1993-2008. 66% were male. The mean ± SD age at presentation was 4.6 ± 3.7 yrs (0.3-11.2 yrs). Two subjects had documented influenza infection. All subjects required dialysis during initial hospitalization. Of these, five (56%) showed no evidence of renal recovery. Of four subjects who recovered renal function, two demonstrated recurrent aHUS leading to ESRD and one had residual CRF leading years later to ESRD. Extensive genetic evaluation demonstrated 4 subjects with factor H mutation. One subject was found to have low serum factor I but full evaluation was not obtained prior to death from unrelated causes. Five subjects received plasma infusion and/or plasmapheresis, including one with factor H deficiency; only one showed improvement in aHUS. Nine renal transplants were performed in six children, including one with factor H deficiency who underwent isolated kidney transplant lost to recurrent aHUS and then combined liver-kidney transplant with death from complications of liver transplant. One child with a factor H mutation thrombosed two renal transplants but then achieved successful transplantation with anticoagulation. A child with the same factor H mutation underwent renal transplantation without incident. Successful renal transplantation was achieved in 56%. Recurrent aHUS was noted in 1 of 8 isolated renal transplants. **Conclusion:** aHUS may be more common than previously reported. Studies are needed to identify factors favoring successful isolated renal transplant.

Disclosure of Financial Relationships: nothing to disclose

**F-PO995**

**Renal Proximal Tubule Specific GSK3 Deletion Protects from Acute Neprotoxic Injury in Miceβ** Reena Rao,[1] Raymond C. Harris.[1] *[1]Nephrology, Vanderbilt University, Nashville, TN; [2]Nephrology, Vanderbilt University, Nashville, TN.*

Glycogen synthase kinase 3 (GSK3) is a serine/threonine protein kinase that plays an important role in cell survival, proliferation and epithelial to mesenchymal transition. In the current study, we examined whether GSK3 plays an essential role in injury and recovery of renal proximal tubules after nephrotoxic acute kidney injury (AKI). We used renal proximal tubule specific GSK3 knockout mice (KO) generated by breeding GSK3 floxed mice with gamma GT-CRE mice. To examine if GSK3 gene deletion affected renal injury or repair, the mice were treated with a single subcutaneous dose of 8.14mg/Kg of HgCl2 and their renal structure and function were monitored for 8 days. Treatment with HgCl2 reduced survival rate by 40% in the WT group but only 10% in the KO group. By day 2 of treatment, the blood urea nitrogen (BUN) levels peaked in KO mice, increasing by 4-fold, and then decreased sharply, returning to baseline levels by day 4. However, in WT mice, BUN levels increased by 8-fold and its decrease was delayed, remaining above baseline even by day 8. Histological examination revealed tubular dilatation, cellular necrosis and loss of defined brush border membrane in both WT and KO kidneys. However, injury levels were markedly reduced in KO mice. This was supported by observations that renal expression of proapoptotic caspase 3 and BAX levels were lower in KO mice compared to WT. Renal tubular repair and regeneration was also accelerated in the KO mice, as indicated by morphological observations as well as increased levels of cell proliferative markers, Ki-67 and BRDU staining. GSK3 is generally constitutively active in cells. In WT mice, AKI induced by HgCl2 treatment led to a time dependent decrease in GSK activity, as indicated by an increase in the inactive phospho-GSK3 levels as well as increase in levels of -catenin, a substrate of GSK3. These results indicate that in the KO mice, HgCl2-induced proximal tubule injury is reduced and cellular repair is accelerated compared to WT mice. This study suggests that modulation of GSK3ß activity is crucial for HgCl2-induced acute nephrotoxic injury and repair in mice.

Disclosure of Financial Relationships: nothing to disclose

**F-PO996**

**Obestatin Improves Renal Function and Ameliorates Renal Ischemia/Reperfusion Injury in Rats Via a Neutrophil-Dependent Mechanism** Mehmet Koc,[1] Zarife Ozdemir,[2] Gulsun Memi,[2] Naziye Ozkan,[3] Sule Cetinel,[3] Berrak Yegen.[2] *[1]Division of Nephrology; [2]Department of Physiology; [3]Department of Histology, Marmara University Medical Faculty, Istanbul, Turkey.*

Obestatin (OBS)is a newly described hormone derived from the same gene with ghrelin, shown to have antiinflammatory properties. Recently, OBS was also reported to protect cardiac and intestinal cells against ischemia-reperfusion (I/R) injury and apoptosis. In order to investigate the potential protective effects of OBS in renal I/R injury, male Sprague Dawley rats underwent right nephrectomy and I/R was induced by placing a microvascular clamp across left renal artery for 60 minutes, while the control group had no clamp placement (n=8). Immediately after clamp placement, rats were injected intraperitoneally with either saline (n=9) or 10 µg/kg (n=6) or 30 µg/kg (n=5) or 100 µg/kg (n=6) OBS. Following a 24-h reperfusion period, serum and kidney samples were obtained for the assessment of histopathological changes and the determination of malondialdehyde (MDA), glutathione (GSH) levels and myeloperoxidase (MPO) activity, an index of tissue neutrophil infiltration. In saline-treated I/R group, serum creatinine level was increased significantly as compared to sham-operated group ($p<0.05$), while OBS at all doses prevented I/R-induced increase in creatinine levels ($p<0.05$). Renal MPO activity in the saline-treated group was significantly increased ($p<0.05$), but only 10 µg/kg dose of OBS abolished I/R-induced neutrophil infiltration. MDA or GSH levels were not different among experimental groups. Histological analysis revealed severe injury in the kidney samples of saline-treated rats, characterized by congestion, hyalinization and hemorrhage in the cortex and medulla along with tubular necrosis. However, in the OBS-treated (10 and 30 µg/kg) I/R groups the cortical and medullary injuries were ameliorated and the given scores were reduced ($p<0.05$). The present data demonstrate that OBS ameliorates renal I/R-injury by its possible anti-inflammatory properties, which appear to involve the suppression of neutrophil accumulation. Obestatin merits further investigation for its supportive use in ischemic renal injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO997**

**Knock-Down of HGF and VEGF Production Reduces the Renoprotective Effects of Adipose-Derived Stromal Cells in the Acute Kidney Injury** Takayuki Katsuno,[1] Hansu Kim,[1] Kazuhiro Furuhashi,[1] Yosuke Saka,[1] Takenori Ozaki,[1] Tokunori Yamamoto,[2] Waichi Sato,[1] Naotake Tsuboi,[1] Enyu Imai,[1] Yasuhiko Ito,[1] Seiichi Matsuo,[1] Shoichi Maruyama.[1] *[1]Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; [2]Urology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.*

**Introduction:** We have previously reported that human adipose-derived stromal cells (ASC) cultured in low (2%) serum media (hLASC) secreted higher levels of HGF and VEGF than in high (20%) serum media (hHASC) and markedly promoted kidney repair in a rat AKI model (ASN Renal Week 2009 Katsuno). The aim of this study is to further analyze the therapeutic effects of hLASC and to investigate the mechanisms involved.

**Methods:** AKI was induced by folic acid in nude rats at one week after heminephrectomy. Simultaneously, hLASC, hHASC or control media were given into the renal subcapsular space. Macrophage infiltration and rat HGF and VEGF in the renal cortex were assessed on day 2. Staining for αSMA and Collagen III was evaluated on day 14. In the hLASC tracking study, frozen sections were stained with anti human specific nuclear antigen. In vitro, siRNA targeted to human HGF and VEGF were transduced into hLASC respectively. Knock-down efficiency was determined by the protein levels in the supernatant. HGF knock-down hLASC, VEGF knock-down hLASC, hLASC with negative control siRNA or control media were administrated in the same AKI model, and renal function analysis was assessed.

**Results:** Administration of hLASC attenuated macrophage infiltration and interstitial fibrosis. The levels of rat HGF in the renal cortex were significantly higher in the hLASC group. Transplanted hLASC stayed alive in the subcapsular space for at least 14 days. In vitro, greater than 80% knock-down of HGF and VEGF was recognized for 96 hours after transfection. In vivo, knock-down of either HGF or VEGF in hLASC significantly reduced renoprotective effects.

**Conclusion:** We identified that hLASC modify the early inflammation and subsequent regenerative process in the AKI. Our study demonstrated that HGF, as well as VEGF, is an important mediator in the renoprotective effects of hLASC.

Disclosure of Financial Relationships: nothing to disclose

**F-PO998**

**Renal Protection by Bile Acids in Experimental Models of Acute Kidney Injury** Kajohnsak Noppakun,[1, 2] Shunan Li,[1, 2] Lawrence Wang,[1] Sandeep Gupta.[1, 2] *[1]Stem Cell Institute; [2]Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis.*

**Background:** Presently there are no treatments available for amelioration and prevention of acute kidney injury (AKI). Tauroursodeoxycholic acid (TUDCA) acid and ursodeoxycholic acid (UDCA) are bile acids with anti-apoptotic and pro-survival properties that are protective in animal models of acute tissue injury.

**Objective:** To determine and elucidate mechanisms of renal protection by TUDCA/UDCA in animal and cell culture models of AKI.

**Methods:** *In vivo*: Three rats each in the experimental and control groups were given daily IP injection of TUDCA (400 mg/kg/d) or vehicle from day -3 until day 6; day 0 being the day of bilateral renal artery clamping for 45 minutes. Kidney function was determined by daily estimation of blood urea nitrogen. Kidneys were harvested 5 days following AKI. Apoptotic cells in kidney sections were detected by TUNEL assay. Histological damage was scored . *In vitro*: Rat proximal tubular cells were grown in medium containing either 150 µM solution of TUDCA/UDCA or vehicle. Cells were subsequently treated with 20 µM cisplatin for 20 hours. Early apoptotic event was determined by Annexin V assay and late apoptotic event was determined by Western blot analysis for activated Poly ADP ribose polymerase (PARP) and caspase-3 enzyme activity. Mitochondrial pathway of apoptosis was assessed by translocation of cytochrome C from the mitochondria to the cytosol by Western blot analysis.

**Results:** *In vivo*: Rats treated with TUDCA had significantly less severe injury, and faster recovery of renal function. There were significantly less apoptotic cells in the TUDCA group at the cortico-medullary junction and medulla, and less severe histological damage in the deep cortex. *In vitro*: TUDCA/UDCA significantly inhibited early apoptotic events and activation of PARP. Caspase-3 activity was significantly reduced after treatment with UDCA. The translocation of cytochrome C to the cytosol following cisplatin induced injury was blocked by TUDCA.

**Conclusions:** TUDCA/UDCA can ameliorate rat experimental AKI both *in vivo* and *in vitro* and has high translational potential for use in treating clinical AKI.

Disclosure of Financial Relationships: nothing to disclose

**F-PO999**

**Relaxin Ameliorates Ischemia Reperfusion Induced Acute Kidney Injury** Takuya Yoshida,[1] Naoki Ikegaya,[2] Hiromachi Kumagai,[1] Tatsuo Yamamoto,[3] Akira Hishida.[4] *[1]Dep. of Clin. Nutrition, University of Shizuoka, Shizuoka, Japan; [2]Med. Care Center, Shizuoka Univ., Shizuoka, Japan; [3]Dep. of Health and Nutrition Sciences, Hamamatsu Univ., Hamamatsu, Jamaica; [4]First Dept. of Med., Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan.*

Relaxin (RLX), the pregnancy hormone, has anti-apoptotic, anti-oxidative and anti-inflammatory properties. We have previously reported protective effects of RLX on renal ischemia-reperfusion (IR) injury (ASN2009). In this study we investigated the effects of RLX on IR induced injury and expression of hemeoxygenase-1 (HO-1) in kidneys. Male rats underwent unilateral nephrectomy and contralateral renal IR (45 min of renal pedicle clamping). A sham-operated group served as the control. Four groups were studied: (1) Sham, (2) IR, (3) RLX before IR, (4) RLX after IR. RLX was infused at 0.8 µg/h via subcutaneous osmotic minipump for 24 h beginning 2 h before renal IR (RLX before IR) or just after IR (RLX after IR). At 24 h of reperfusion, renal function was assessed and kidneys were removed. There was no significant difference in blood pressure among 4 groups. Increased levels of serum creatinine and urea nitrogen (BUN) were identified in the IR group compared to the Sham group. The RLX treated groups showed significantly lower serum creatinine (mean +/- SEM: IR, 3.12 +/- 0.48; RLX before IR, 1.70 +/- 0.33; RLX after IR, 1.37 +/- 0.48 ; Sham, 0.17 +/- 0.01 mg/dl) and BUN. Semi-quantitative assessment of the histological lesions showed that the IR rats developed marked structural damage, whereas significantly less tubular damage was observed in the IR-RLX rats. The expression of HO-1 was significantly increased in the IR and IR-RLX kidneys compared

to the Sham kidneys. Despite significantly decreased serum creatinine and histological injury in the RLX treated group, the increase of HO-1 of the RLX-treated group was comparable to the IR group.

These results clearly demonstrate that administration of RLX before or after ischemia attenuated tubular injury, suggesting that RLX can be a promising protective and therapeutic tool for renal IR injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1000**

**Pituitary Adenylate Cyclase-Activating Polypeptide Prevents Cyclosporine A-Nephrotoxicity** Altaf-M. Khan,[1] Min Li,[1] Elizabeth Jane Brant,[1] Kristine E. Gullo,[1] Anna Wei Cai,[1] Dewan S. Majid,[2] Eric E. Simon,[1,3] Vecihi Batuman.[1,3] *[1]Section of Nephrology and Hypertension, Tulane University School of Medicine; [2]Physiology, Tulane University School of Medicine; [3]Veterans Affairs, Southeast Louisiana Veterans Health Care System, New Orleans, LA.*

Cyclosporine A (CsA) is a widely used drug for organ transplantation. Acute and long-term toxic effects on the kidney are the major dose-limiting factors. We recently showed that pituitary adenylate cyclase-activating polypeptide (PACAP) protects the kidney from ischemic and nephrotoxin-induced injury both *in vitro* and *in vivo*. This study evaluates the effects of PACAP38 on CsA nephrotoxicity in human renal proximal tubule epithelial (HK-2) cells and in intact mice. CsA caused marked morphological alterations, apoptosis in cell cultures, increased expression of transforming growth factor (TGF)-β1, and evidence of oxidative injury (NADPH oxidase and $H_2O_2$ assays). The addition of $10^{-8}$ M PACAP38 restored cell confluency, reduced TGF-β1 secretion and preserved cell integrity as indicated by lactate dehydrogenase and TUNEL assays. For studies *in vivo*, male BALB/c mice (8-10 wk old) were given a single intraperitoneal injection of CsA (5 mg/kg bw). Treatment groups received 20 μg of PACAP38 2 h before exposure to CsA, and additional doses daily for 10 days. CsA caused severe tubular injury characterized by extensive loss of tubular epithelial cell brush border membranes, tubular collapse, cellular necrosis, interstitial fibrosis, elevated serum creatinine (3.39 ± 0.21 vs 0.13 ± 0.02 mg/dl in controls, $p < 0.01$, N= 5 in each group), and significantly stimulated the production of TGF-β1 in mouse kidneys. Treatment with PACAP38 resulted in a decrease TGF-β1 and tumor necrosis factor-α production in kidney, and reduced serum creatinine levels to 1.01 ± 0.18 mg/dl, $p < 0.01$ vs CsA group. PACAP38 ameliorated renal tubular injury, inhibited the expression of proinflammatory and profibrotic cytokines, and reactive oxygen species production in CsA-exposed murine kidneys, and prevented epithelial-mesenchymal transition of the renal cells. PACAP could be a novel renoprotective agent for CsA-induced nephrotoxicity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1001**

**Mitochondrial Injury of Collecting Duct Aggravates Cisplatin Nephrotoxicity** Dae Eun Choi,[1] Jin Young Jeong,[1] Ji Yoon Jung,[1] Dong-Suk Chang,[1] Sarah Chung,[1] Yoon-Kyung Chang,[2] Ki Ryang Na,[1] Kang Wook Lee,[1] Young Tai Shin.[1] *[1]Internal Medicine, Chungnam National University Hospital, Daejeon, Korea; [2]Internal Medicine, Daejeon St Mary's Hospital, Daejeon, Korea.*

**Introduction:** It is known that cisplatin induces injury of mitochondria in tubular cells in kidney. However, there are confusing reports whether inhibiton of mitochondrial function aggravates cisplatin nephrotoxicity. Some reported that inhibition of mitochondrial function aggravates cisplatin injury of tubular cell. However, others reported opposite results. We made collecting duct specific mitochondrial injury model using Cre-lox system. We investigated whether mitochondrial inhibition aggravates cisplatin nephrotoxicity.

**Methods:** In vitro, we used mIMCD cell, and divided 4 groups ;scRNA-mIMCD cells, crif1-siRNA-mIMCD cells, cisplatin treated scRNA-mIMCD cells, and cisplatin treated crif1-siRNA-mIMCD cells. In vivo, we got crif1 flox mice (crif1 (flox/flox)) and collecting duct cell specific crif1 knockout (KO) mice (crif1 (Δ/Δ)) via mating C57Bl/6 background HoxB7 Cre mice and crif1 flox mice. We divided 4 groups, crif1 (flox/flox), crif1 (Δ/Δ)), cisplatin treated crif1 (flox/flox), and cisplatin treated crif1 (Δ/Δ)). We evaluated functional, histologic, and molecualr studies.

**Results:** There were significant decreased in mitochondrial membrane potential, O2 consumption, and complex I, II, III, and IV in crif1-siRNA-mIMCD cells compared to scRNA-mIMCD cells. Viable cell were significantly decreased in cisplatin treated crif1-siRNA-mIMCD cells compared to cisplatin treated scRNA-mIMCD cells. The level of BUN and serum creatinine and damaged collecting tubular cells were significantly increased in cisplatin treated crif1 (Δ/Δ)) compared with cisplatin treated crif1 (flox/flox). There were no significant difference in TUNEL positive cells in both cisplatin treated crif1 (Δ/Δ)) and cisplatin treated crif1 (flox/flox).

**Conclusions:** Collecting duct cell specific crif1 deletion induced collecting duct specific mitochondrial injury including functional and structural injury. Collecting duct cell specific mitochondrial injury aggravates cisplatin nephrotoxicity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1002**

**Phosphodiesterase 5 Inhibitor Can Ameliorate Radiocontrast-Induced Nephropathy in Rat Model of Chronic Kidney Disease** Byoung Geun Han, Jae Won Yang, Seung Tae Han, Seung-Ok Choi. *Nephrology, Yonsei University Wonju College of Medicine, Wonju, Gangwon, Republic of Korea.*

Backgrounds: Radiocontrast agent causes damage to the kidney by contracting renal arterioles and by direct toxic effect to tubular epitheliums. Phosphodiesterase-5 inhibitors (PDEi-5) are known to have not only vasodilative but also anti-oxidative effect. We investigate whether PDEi-5 ameliorates radiocontrast induced nephropathy (CIN) and N-acetylcysteine (NAC) has benefits and/or synergistic effects with PDEi-5 in rat model of chronic kidney disease.

Methods: 48 male SD rats were performed 5/6 nephrectomy and divided into 4 groups: vehicle, PDEi-5 (1.5mg/kg) treated group, NAC (60 mg/kg) treated group, and combination group. All rats received drugs 4 times every 12 hrs. Radiocontrast agent (Iopromide 10ml/kg) was injected 1 hour after 3rd injection. Blood and 24 hours urine were taken every 48 hrs for 6 days. Rats were sacrificed at 2nd and 6th day of experiment. Serum NGAL, cystatin C, BUN, and creatinine were measured. The expression of iNOS, eNOS, MCP-1, and nitrotyrosine were measured and TUNEL stain were performed in kidney.

Results: There were no significant changes of BUN and creatinine levels among groups. PDEi-5 (0.43±0.13mg/ml/100g) and NAC group (0.39±0.79mg/ml/100g) had significant improvements of creatinine clearance compared to vehicle group (0.25±0.87 mg/ml/100g) at 6th day. Serum NGAL significantly decreased in PDEi-5 (68.3±5.5 vs 82.79±11.25ng/ml, 64.1±10.1 vs 83.6±7.6ng/ml) and NAC group (58.3±19.6 vs 82.79±11.25ng/ml, 53.8±19.3 vs 83.6±7.6ng/ml) compared to vehicle group at 2nd and 4th day respectively. Serum cystatin C also significantly decreased in PDEi-5 (5.28±0.9ng/ml) and NAC group (5.8±0.66ng/ml) compared to vehicle group (7.53±1.3ng/ml) at 2nd day. At 2nd day, eNOS expression was significantly decreased in PDEi-5 group. MCP-1 and nitrotyrosine expression decreased in PDEi-5 and NAC group. At 6th day, the expression of MCP-1 and nitrotyrosine increased in vehicle and combination group. TUNEL stain showed decreased apoptosis in PDEi-5 and NAC group but not in combiantion group.

Conclusion: PDEi-5 may prevent and ameliorate CIN via anti-oxidative and anti-apoptotic potentials.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1003**

**Beneficial Effects of Darbepoietin Alpha Administration in Renal Ischemic Injury: Promotion of Tubular Adhesion** Ignacio Blanco Sanchez,[1] Elisa Conde,[1] Elia Aguado Fraile,[1] Edurne Ramos,[1] Marta Martinez,[1] Rafael Selgas,[2] Jose-Antonio Sanchez-Tomero,[3] Laura Garcia-Bermejo.[1] *[1]Pathology, Hospital Univ. Ramon y Cajal; [2]Hospital Univ. la Paz; [3]Hospital Univ. la Princesa.*

Recent studies suggest that exogenous EPO administration can exert a cytoprotective effect in renal ischemic injury although mechanisms underlying this effect are still poorly understood. We assess the effect of Darbepoetin α (DPO) during renal ischemia/reperfusion (I/R) using two experimental models: 1. an *in vivo* mouse model of renal bilateral clampling, using different DPO concentrations: high dose (HD) 25mg/kg and low dose (LD) 10mg/kg, administrated as a single injection after ischemia; 2. an *in vitro* model of hypoxia/reoxygenation (H/R) in mouse proximal tubular cells (MCT), were DPO is added at different concentrations: 100/250/500 ng/ml starting reoxygenation. Our previous studies demonstrate that HD of DPO protects from I/R injury and LD of DPO accelerates recovery. Here, we asses the mechanisms responsible for both effects. *In vitro* studies demonstrate that cell adhesion is compromised during H/R. X-celligence equipment and fluorescent microscopy after BCECF-AM labelling was used. DPO increase impedance levels in a dose-dependent manner, indicating promotion of adhesion. Moreover, adhesion assays in DPO-treated MCT cells after hypoxia confirming this. By immunofluorescence (INF), we studied localization and expression of adhesion molecules, i.e: ZO-1 for tight junctions, paxillin and actin for focal adhesions and tubulin for cytoskeleton. We found that DPO prevent ZO-1 disruption, reduces loss of actin-paxillin colocalization and prevent tubulin network impairment. In agreement, DPO reduces cell detachment and improves monolayer integrity. Immunohistochemistry and INF of those adhesion molecules in renal tissue of DPO treated mice subjected to I/R, confirms DPO capacity to protect adhesive structures. In summary, we prove the beneficial effect of DPO during I/R by maintaining the localization of proteins responsible for cellular adhesion and structure. These results strongly suggest a potential DPO therapeutic effect in nephropathies associated to tubular epithelium damage.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1004**

**Amphiregulin Promotes Renal Recovery after Acute Kidney Injury** Monika Gupta,[1] Peifeng Deng,[1] Peter Green,[1] Judit Megyesi,[2] Rick G. Schnellmann.[3] *[1]Medicine, Medical University of South Carolina; [2]Medicine, University of Arkansas for Medical Science, Little Rock, AR; [3]Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

While molecular mechanisms that regulate renal tissue repair and regeneration after acute kidney injury (AKI) are not fully understood, epidermal growth factor receptor (EGFR) appears to be critical for renal recovery. **The aim of the current study was to determine the role of amphiregulin (AR), an EGFR ligand, in AKI using a renal ischemia–reperfusion (I/R) mice model.** C57/BL6 mice underwent bilateral renal I/R injury and either received an intravenous dose of AR (10μg) or saline 2 hr after injury. Serum creatinine levels (mean ± SEM) 24, 48 and 72 hr post surgery was 2.3 ± 0.1 mg/dL, 2.5 ± 0.2 mg/dL, and 2.2 ±

0.4 mg/dL, respectively in the control group, and 1.8 ± 0.1 mg/dL, 1.4 ± 0.1 mg/dL, and 1.0 ± 0.1 mg/dL, respectively in the AR group. Histological changes were examined 72 hr after ischemia. While tubular dilation, loss of brush border, tubular necrosis and number of tubular cast were similar between the two groups, distal tubular damage was significantly lower in mice that received AR. Sham-operated mice had no tubular injury. We then examined the effect of AR on apoptosis using TUNEL assay and immunohistochemistry for active caspase-3. Compared to the I/R group, mice that received AR had lower numbers of TUNEL and caspase-3 positive cells. We further analyzed leukocyte infiltration in injured kidneys by staining neutrophils and monocytes with naphthol AS-D chloroacetate esterase. Prominent interstitial infiltration of neutrophils and monocytes was observed at 72 hr in the I/R group. Treatment with AR decreased leukocyte infiltration. We next examined the effect of AR on tubular cell proliferation by evaluating proliferating cell nuclear antigen (PCNA) positive cells. There was no difference in the number of PCNA positive cells between the two groups. **Hence, amphiregulin promotes renal recovery by decreasing inflammation, and apoptosis of tubular epithelial cells.**

Disclosure of Financial Relationships: nothing to disclose

**F-PO1005**

**Platelets Are Relevant Mediators of Renal Injury Due to Primary Endothelial Lesions** Claudia Schwarzenberger, Sandy Walther, Christian Hugo, Bernd Hohenstein. *Department of Internal Medicine III, Division of Nephrology, Dresden, Germany.*

**Introduction:** Platelets are an important part of the coagulation system. While studies have shown angiogenic factors in platelets others suggest a more (pro)inflammatory role. We investigated platelets' role in a mouse model of selective endothelial cell (EC) injury using the P2Y12 blocker clopidogrel (clopi) or platelet depletion and tested whether platelet microparticles (PMP) reflect disease activity.

**Material and Methods:** First, 5 C57Bl/6 mice received 0.2mg anti-GPIb ab to induce rapid platelet depletion, 5 mice served as controls. 24 h later EC injury was induced by selective renal arterial perfusion of ConA/anti-ConA, sacrificial biopsies were taken after 24h. PMP were extracted from 20 mice sacrificed either 0.5h, 2h or 5d after EC injury and platelet rich plasma was analyzed by FACS (CD41 positive PMP). Next, 14 C57Bl/6 mice were treated with clopi (20mg/kg bw) or placebo via oral gavage once daily starting day -1 until sacrifice on d3. We assessed tail bleeding time, urine and blood samples and renal tissues from all mice by immunohistochemistry.

**Results:** Clopi treatment prolonged tail bleeding compared to controls ($P<0.05$). On d3, by PAS and AFOG stains glomerular injury and glomerular fibrin thrombus formation were reduced in clopi mice ($P<0.05$). EC injury (by CD31/MECA32) and EC proliferation (PCNA) demonstrated preservation of glomerular/peritubular capillaries and cell proliferation ($P<0.05$) in clopi treated mice. In parallel, platelet depletion prevented EC loss on day 1 ($P<0.05$) and EC proliferation was reduced, indicating reduced repair subsequent to reduced EC injury ($P<0.01$).

PMP in diseased animals demonstrate a rapid reduction of circulating platelets (30min: 38%, 2h: 19%) and the generation of large amounts of PMP (30min: 50%, 2h: 60%). A significant number of PMP can be found even 5 days after disease induction (51%) compared to controls (16%).

**Conclusion:** Platelets are important mediators of injury after primary EC lesions. Clopi treatment can prevent injury /improve EC repair. PMP persist beyond the time of initial EC injury indicating the presence of a long lasting procoagulant state.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1006**

**Osteopontin Predicts Survival in Critically Ill Patients with Acute Kidney Injury** Johan Lorenzen M. Lorenzen,[1] Robert Faulhaber-Walter,[1] Hermann G. Haller,[1] Jan T. Kielstein,[1] Danilo Fliser.[2] *[1]Nephrology, Hanover Medical School, Hannover, Germany; [2]Nephrology, Saarland University Medical Center, Homburg, Germany.*

Background: The cytokine osteopontin is involved in the pathophysiology of experimental acute kidney injury. We have tested the hypothesis that osteopontin levels might serve as a biomarker predicting outcome in critically ill patients requiring renal replacement therapy after acute kidney injury.

Methods: We measured circulating plasma osteopontin levels in 109 critically ill patients with acute kidney injury at inception of renal replacement therapy and 4 weeks thereafter. Critically ill patients without acute kidney injury served as controls. Osteopontin was measured with ELISA.

Results: Baseline osteopontin levels in patients with acute kidney injury were significantly higher compared to controls ($p<0.0001$). Baseline osteopontin levels in patients recovering from acute kidney injury were significantly elevated compared to patients with permanent loss of kidney function after acute kidney injury ($p=0.01$). In addition, in patients recovering from acute kidney injury without further need for renal replacement therapy osteopontin levels were significantly lower 4 weeks after initiation of renal replacement therapy ($p=0.0005$). Moreover, multivariate Cox analysis revealed osteopontin levels at renal replacement therapy inception as an independent and powerful predictor of mortality ($p<0.0001$). In the ROC-curve analysis an osteopontin cut-off value of 577 ng/mL separated survivors from non-survivors with a sensitivity of 100% and a specificity of 61% (AUC 0.82; 95% confidence interval: 0.74 to 0.89; $p<0.0001$).

Conclusions: Osteopontin may serve as a novel biomarker for both, overall survival and renal outcome in critically ill patients with acute kidney injury that require renal replacement therapy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1007**

**Cyto-Protector Effect of a VDR Activator at the Mitochondrial Level in an Obstructive Nephropathy Model** Leon F. Ferder,[1] Walter Ariel Manucha.[2] *[1]Physiology and Pharmacology, Ponce School of Medicine, Ponce, PR; [2]Área de Fisiopatología, Facultad de Ciencias Médicas, UN de Cuyo, Mendoza, Argentina.*

Activators of vitamin D receptors have suppressant effect on the RAS, as well as anti-inflammatory and anti-fibrotic effects. This study investigates, in an obstructive nephropathy animal model, structural and functional changes in the kidney, and paricalcitol effects on these changes.

Methods: Ten adult female Wistar rats were obstructed surgically at urethero-pelvic level. They were divided in two groups: control and treated. The treatment was done for a 15 days, injecting intraperitoneally 30 ng/Kg/day. We evaluated blood pressure (BP), PTH, calcium and phosphorous levels. Kidney was also evaluated histologically for fibrosis with Masson´s trichomic, for apoptosis with TUNNEL technique, and electronic microscopy (EM) for mitochondrial evaluation.

Results: The biochemical changes in calcium, phosphorous, and PTH were not significant.

*BP*: controls 110±5mmHg, treated controls 112±7 mmHg, obstructed 145±8 mmHg ($p<0.01$) and treated obstructed 130±9 mmHg ($p<0.05$). Blood pressure was taken by plethysmography and checked intra-arterially.

*Masson Trichromic (fibrosis)*: obstructed 60±10% vs. control 5±2% ($p<0.01$) and vs. treated obstructed 20±5% ($p<0.01$).

*TUNNEL*: positive cells in obstruction 20±7 vs. controls 3±2 ($p<0.01$) and treated obstructed 10±2 ($p<0.01$). Use of this improved technique that revealed apoptotic cells, as well as those previous to apoptosis.

*Tubular Dilation*: obstructed 30 ±7 μm vs. control 10±4 μm ($p<0.01$) and treated obstructed 15±3 μm ($p<0.01$).

Changes in the kidney showed interstitial fibrosis with increased apoptosis. Both results decreased in the animals treated with paricalcitol. EM revealed, in obstructed non-treated animals, electronically luminous nuclear material in the nucleus. We also noted that the mitochondria were increased in size with dilated crests and spaces in their interior. These mitochondrial changes were not present in the paricalcitol-treated animals.

Conclusions: These results show a cyto-protected effect of the activator of Vitamin D receptors, paricalcitol, revealing for the first time a possible protective effect at mitochondrial level.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1008**

**Could Cyclosporine Be Renal Protective in Acute Kidney Injury?** Xiao Yan Wen,[1] Zhiyong Peng,[1] Yingjian Li,[2] Hongzhi Wang,[3] Kai Singbartl,[1] John A. Kellum.[1] *[1]CRISMA Center, Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA; [2]Department of Pathology, University of Pittsburgh, Pittsburgh, PA; [3]Intensive Care Unit, Beijing Cancer Hospital and Institute, Beijing, China.*

Introduction: Acute kidney injury (AKI) often represents the combined effects of ischemic, toxic and inflammatory insults. Cyclosporine mediates T-cell receptor signaling, suppresses inflammatory cytokine expression, reduces apoptosis and inhibits leukocyte migration. These properties make it a potentially valuable drug to prevent or treat AKI. However, cyclosporine carries a significant risk of nephrotoxicity especially with chronic use; though a single dose of cyclosporine might be protective while limiting nephrotoxicity.

Methods: This study was designed to evaluate the effect of cyclosporine on apoptosis, inflammation and interstitial fibrosis during folic acid(FA)-induced AKI. We hypothesize that a single, low dose of cyclosporine can ameliorate FA-induced AKI without causing nephrotoxicity. We randomly assigned mice receiving FA to one of three different doses of cyclosporine or vehicle.

Results: Intraperitoneal injection of FA consistently induced AKI, characterized by significant increases in serum creatinine, and tubular epithelial cell apoptosis. Plasma IL-6 and urinary NGAL rose 1 day after FA-injection, indicating a strong inflammatory reaction. Compared to sham treatment, low dose (1 and 5mg/kg) cyclosporine significantly reduced kidney tubular cell apoptosis, serum creatinine, plasma IL-6 and urinary NGAL 2 days after FA-injection. Moreover, low dose cyclosporine blocked the inflammatory mediator TWEAK expression, NF B activation, inflammatory cell infiltration, and interstitial fibrosis 2 weeks after treatment in a dose dependent fashion. By contrast, 10mg/kg dosing of cyclosporine resulted in nephrotoxicity.

Conclusion: Low dose cyclosporine significantly protects mice from FA-induced AKI, presumably through inhibition of inflammation, tubular cell apoptosis, interstitial cell infiltration, and fibrosis. The protective effects of a single dose of cyclosporine have the potential to open a completely new line of investigation to prevent or treat AKI.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1009**

**AMPK Inducer, AICAR, Is Protective in Ischemia-Reperfusion Injury**
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Ischemia reperfusion (IR) causes a cascade of cellular events prompting cellular damage leading to cell death. A response to ischemia is the activation of AMP-activated protein kinase (AMPK). AMPK induces a number of protective, cell survival mechanisms. We propose that inducing AMPK activity prior to IR could prove beneficial.

Sprague-Dawley rats were divided into 4 groups: 1) Control, 2) IR, 3) AMPK inducer, AICAR, 4) IR+AICAR. AICAR (0.1g/kg) was administered 24h prior to ischemia. Three weeks after nephrectomy (all groups) kidney ischemia was performed for 30 min, with reperfusion for 24 hrs. AICAR significantly increased AMPK activity (pAMPK/AMPK ratio: C 1.13±0.32, IR 1.82±0.19, AICAR 2.39±0.35, IR+AICAR 4.15±0.32*, N=4-5 for each group, mean±SEM; * P<0.05 v IR).

Immunoblotting showed an increase in autophagy protein LC3-I in AICAR samples (C 3608±174; IR 3687±309, AICAR 6183±285, IR+AICAR 7137±326*; relative densitometric units). During autophagy, cytosolic LC3-I is converted to an active autophagosome bound LC3-II form. LC3-II increases with IR and significantly further in IR+AICAR (C 318±103, IR 1179±111, AICAR 743±144, IR+AICAR 3629±629*). Autophagy rids the cell of damaged proteins and organelles, suppressing oxidative stress and cell death. In accord with increased autophagy we observed a decrease in the apoptotic execution caspase-3 expression in IR with AICAR (C 1353± 100, IR 4286± 246, AICAR 2039± 62, IR+AICAR 3208± 255*). IR decreased the gap junction protein Cx43, limiting the spread of IRI associated signaling molecules, and increased cyclin kinase inhibitor p27KIP1 and ROS generating NOX4 expressions. AICAR significantly attenuated these responses to IR, again implying less extensive injury (Cx43: C 697±104, IR 345±32, AICAR 1258±71, IR+AICAR 544±30*; p27: C 622±151, IR 4983±515, AICAR 1010±80, IR+AICAR 2709±154*; NOX4: C 624±108, IR 4275±665, AICAR 769±130, IR+AICAR 2858±346*). Parallel changes occurred in response to the AMPK agonist, metformin.

There are currently no clinical procedures in place to safeguard the kidney from IRI. This data implies AMPK induction, in part through induction of autophagy, can provide protective effects in IR.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1010**

**Hepatocyte Growth Factor /c-Met Signaling Contributes to the Acquired Resistance after Acute Subclinical Kidney Damage with Uranyl Acetate**
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Animals recovered from acute kidney injury (AKI) are resistant to subsequent nephrotoxic insults, which is called acquired resistance. We previously reported that rats recovered from subclinical kidney damage without renal dysfunction induced sub-toxic dose of uranyl acetate (UA) showed partial resistance to subsequent nephrotoxic dose of UA in association with reduced renal dysfunction and accelerated cell proliferation compared with vehicle treated as the first insult.(Sun et. al. 2010)

In the present study, we examined possible roles of HGF/c-Met signaling in this acquired resistance model in vivo and in vitro. Rats recovered from subclincal kidney damage by sub-toxic dose of UA (0.2mg/kg), followed by nephrotoxic dose of UA (4mg/kg), were analyzed. After the second nephrotoxic insult, c-Met proteins in the renal tissues were higher than in vehicle pre-treated rats. Using confluent monolayer of primary cultured tubular cells from rats treated with sub-toxic dose of UA or vehicle by collagenase digestion, the cell migration was analyzed by wound-healing assay with or without hepatocyte growth factor (HGF). Wound closure of cells from UA (0.2mg/kg) treated rats was as same as cells from vehicle-treated rats without HGF. However, wound closure was significantly different with HGF after 12hr culture between them(91.8±8.7% vs. 74.0±5.8%). These results suggest that HGF/c-Met signaling may contribute accelerated tubular cell migration in rats that recovered from subclincal kidney damage, followed by nephrotoxic insult, resulting in the partial acquired resistance.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1011**

**SIRT1 Activation by Resveratrol Ameliorates Cisplatin-Induced Renal Injury through Deacetylation of p53**
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Nephrotoxicity is one of the important dose-limiting factors during cisplatin treatment. There is a growing body of evidence that activation of p53 has a critical role in cisplatin-induced renal apoptotic injury. The nicotinamide adenine dinucleotide-dependent protein deacetylase, SIRT1 decreases apoptosis through deacetylating of p53 and resveratrol is known as an activator of SIRT1. To study the role of SIRT1 in cisplatin-induced renal injury through interaction with p53, the mouse proximal tubular cells (MPT) were treated with cisplatin and examined the expression level of SIRT1, acetylation of p53, PUMA-α, Bax, the ratio of cytosolic/mitochondrial cytochrome c, and active caspase-3. The expression of SIRT1 was decreased by cisplatin. Resveratrol, a SIRT1 activator, ameliorated cisplatin-induced acetylation of p53, apoptosis and cytotoxicity in MPT cells. In addition, resveratrol remarkably increased cisplatin-induced decrease of Bcl-xL in MPT cells. Further specific SIRT1 inhibition with EX 527 or siRNA specific to SIRT1 reversed the effect of resveratrol on cisplatin-induced toxicity. SIRT1 protein expression after cisplatin treatment was significantly decreased in kidney. SIRT1 activation by resveratrol decreased cisplatin-induced apoptosis as demonstrated by TUNEL assay. Taken together, our findings suggest that the modulation of p53 by SIRT1 could be a possible target to attenuate cisplatin-induced kidney injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1012**

**Beneficial Effect of Mineralocorticoid Receptor Antagonism in Acute Cyclosporine A Nephrotoxicity**
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Cyclosporine A (CsA) nephrotoxicity is one of its most frequent adverse effect but its physiopathology remains unclear. The implication of aldosterone has been proposed based on experimental studies in rat showing that pharmacological blockade of the Mineralocorticoid Receptor (MR) prevents nephrotoxicity of the CsA by modulating the expression of vaso-active factors (Perez-Rojas et al. AJPRP 2005). We have recently shown that MR is expressed in the endothelium of the renal vasculature and that endothelial-specific MR overexpression alters vascular function (Nguyen Dinh Cat et al. FASEBJ 2010). Our working hypothesis is that the activation of vascular MR plays a key role in CsA nephrotoxicity. The first step of our work was to study the effect of pharmacological blocade of MR on acute CsA toxicity in mice.

We used three groups of 8 weeks FVB/N male mice under low salt diet: control (Ctrl, vehicle), CsA (CsA 100mg/kg/d) and CsA+C (CsA + canrenoate 30mg/kg/d). CsA was administered daily by subcutaneous injection and canrenoate in the drinking water until sacrifice at day 10. Canrenoate had a clear beneficial effect on the survival of CsA mice. At day 7, 40% of the CsA mice were dead versus 0 in the CsA+C mice ($p<0.05$). CsA nephrotoxicity was prevented by canrenoate. Creatinine clearance (in mL/min/100g) was : in Ctrl : 1.15±0.13 ; in CsA : 0.74±0.17 ; in CsA+C : 1.31±0.24, $p<0.05$. CsA-induced proximal tubular vacuolisations were partially prevented by canrenoate. Canrenoate also prevented the increase in urinary NGAL, a biomarker of renal damage, observed in CsA. However, CsA and CsA+C groups presented similar increase in urinary excretion of 8-isoprostane; a global marker of oxidative stress.

In conclusion, we demonstrate that pharmacological MR antagonism has beneficial effects on survival and prevents histological and functional alterations in a mouse model of acute CsA nephrotoxicity. The implication of the endothelial MR is currently under investigation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1013**

**Inhibition of Renin Angiotensin System Decreases TLR2 and TLR4 in Obstructed Kidney**
Jin Young Jeong,[1] Ji Yoon Jung,[1] Dong-Suk Chang,[1] Sarah Chung,[1] Dae Eun Choi,[1] Yoon-Kyung Chang,[2] Ki Ryang Na,[1] Kang Wook Lee,[1] Young Tai Shin.[1] *[1]Internal Medicine, Chungnam National University Hospital, Daejeon, Korea; [2]Internal Medicine, Daejeon St Mary's Hospital, Daejeon, Korea.*

**INTRODUCTION:** Many studies show TLR signaling and renin angiotensin system (RAS) respectively are important role inflammation and fibrosis of obstructed kidney. However, there are little study of relationship between RAS and TLR signaling in obstructed kidney. We evaluated that inhibition of RAS may modulate TLR2 and TRL4 and its ligands and renal inflammation and fibrosis.

**METHODS:** We use unilateral ureteral obstruction (UUO) model and female C57Bl/6 mice weigh 25~28g which were divided into 4 groups; sham group, enalapril treated sham group, UUO group, and enalapril treated UUO group. Enalapril, angiotensin converting enzyme inhibitor was administrated via drinking water (100mg/L) 1 day before sham and UUO operation and continued until harvest of kidneys 5 days after operation. We performed realtime RT PCR and immunohistochemistry for molecular study and H&E stain and Masson trichrome (MT) stain for histological examination.

**RESULTS:** The levels of mRNA expression of TLR2, TLR4, Myd88, renin, renin receptor in UUO kidneys were significantly increased compared to sham operated group (all, $p<0.05$). The mRNA levels of TLR2, TLR4 and Myd88 of enalapril treated UUO group were significantly lower than those of control UUO group (all, $p<0.05$). The renal mRNA expressions of TNF-α, MCP-1, TGF-β, and α-SMA in enalapril treated UUO group ($p<0.05$) were significantly lower than those of control UUO group. Enalapril also significantly reduced number of CD68 positive cell, MT stained area, and immunostained area of TGF-β and α-SMA in UUO kidneys ($p<0.05$).

**CONCLUSIONS:** Enalapril attenuated renal inflammation and fibrosis in obstructed kidney via both inhibition of RAS and reducing TLR 2 and TLR4.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1014**

**Hypoxia and GSK3β/β-Catenin Signaling in Wound Healing of Renal Proximal Tubular Cells** Jianping Peng, Zheng Dong. *Department of Cellular Biology and Anatomy, Medical College of Georgia and Charlie Norwood VA Medical Center, Augusta, GA.*

Wound and subsequent healing in tissues are frequently associated with hypoxia due to vascular damage and decreased blood supply. While hypoxia induces angiogenesis for tissue remodeling during wound healing, it may also affect the healing response of the parenchymal cells. Whether and how wound healing is affected by hypoxia in renal cells and tissues are largely unknown. Here we used a scratch wound healing model to examine the effect of hypoxia in cultured renal proximal tubular cells (RPTC). The results show that wound healing was significantly slower in hypoxic (1% oxygen) RPTC than the cells cultured in normal (21%) oxygen. To explore the signaling pathway, we initially focused on GSK3β and β-catenin. It was shown that β-catenin expression increased in the cells at the wound edge. Hypoxia led to the inactivation of GSK3β and activation of β-catenin. Pharmacologic inhibition of GSK3β with LiCl and SB216763 could also activate β-catenin, resulting in the suppression of wound healing. Consistently, knockdown of β-catenin could improve wound healing in hypoxic cells. These results suggest that GSK3β/ β-catenin signaling may be involved in the defective wound healing in hypoxic renal cells and kidney tissues.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1015**

**cGMP Phosphodiesterase-5 Inhibitor Ameliorates Renal Injury in Rats with Cyclosporine A Induced Nephrotoxicity Via eNOS and VEGF** Byoung Geun Han, Jae Won Yang, Seung Tae Han, Seung-Ok Choi. *Nephrology, Yonsei University Wonju College of Medicine, Wonjuj, Gangwon, Republic of Korea.*

Backgrounds: The mechanism of Cyclosporine-A (CsA) induced nephrotoxicity have been suggested vasoconstriction due to the reduction of nitric oxide (NO), VEGF. The cyclic guanosine monophosphate (cGMP) phosphodiesterase (PDE)-5 inhibitor has been reported to ameliorate renal injury. The PDE-5 inhibitor was studied to determine whether it ameliorated CsA nephrotoxicity and altered the expression of eNOS and VEGF compared to nitroprusside.

Methods: All groups (SD, N=24, 200-250g, male) were divided 4 groups: Control group (N=6), CsA group (N=6) treated with 15 mg/kg cyclosporine-A, Nitroprusside group (N=6) treated with 5mg/kg nitroprusside on CsA group, PDEI group (N=6) treated with 10 mg/kg PDEI (udenafil) on CsA group for 28 days.

Results: In comparison with the CsA group, the Nitroprusside group showed no significant reduction of the BUN and creatinine (Cr), but the PDEI group showed a significant reduction of the BUN and Cr ($p$=0.041, $p$=0.004). In H&E stain, the CsA group showed that the shape of the proximal tubules was not maintained and that vacuolization of the cytoplasm as well as reduction of nuclei were present compared with Control group. In the Nitroprusside and PDEI group, the vacuolization of the cytoplasm of the proximal tubules was observed, nonetheless, the shape was maintained, and the number of nuclei was partially recovered. The protein expression of eNOS and VEGF in the CsA group were decreased compared with the control group, after the administration of nitroprusside or PDEI, were increased compared with the CsA group. The expression of eNOS mRNA was reduced in the CsA group ($p$=0.029) compared with Control group,and increased in the PDEI group ($p$=0.029) compared with CsA group. The expression of VEGF mRNA was reduced in the CsA group ($p$=0.027) compared with Control group, increased in the PDEI group ($p$=0.027) compared with CsA group.

Conclusion: We suggest that cGMP PDE inhibitor ameliorated kidney injury in a rat model of CsA-induced nephrotoxicity via eNOS and VEGF.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1016**

**Mesenchymal Stem Cells and Darbepoetin-alpha Ameliorate Acute Kidney Injury** Rahmi Yilmaz,[1] Bulent Altun,[1] Hadim Akoglu,[1] Serhan V. Piskinpasa,[1] Mustafa Arici,[1] Duygu U. Cetinkaya,[2] Petek Korkusuz,[3] Nuhan Purali,[4] Fazil T. Aki,[5] Cetin Turgan.[1] *[1]Nephrology, Medical Faculty of Hacettepe University, Ankara, Turkey; [2]Pediatric Hematology, Medical Faculty of Hacettepe University, Ankara, Turkey; [3]Histology and Embryology, Medical Faculty of Hacettepe University, Ankara, Turkey; [4]Biophysics, Medical Faculty of Hacettepe University, Ankara, Turkey; [5]Urology, Medical Faculty of Hacettepe University, Ankara, Turkey.*

**Introduction**

Bone marrow originating stem cells and erythropoietin have a possible role in recovery of ischemia induced acute kidney injury (AKI). We aimed to compare the therapeutic potential of mesenchymal stem cells (MSCs), darbepoetin-alfa (DPO) or combination of them in a rat model of ischemia/ reperfusion (I/R) AKI.

**Methods**

In a parallel group study design, rats (Male, Spraque Dawley) were randomly assigned to groups treated with % 0.9 NaCl (n=5) (control), subcutaneous DPO (1mcg/kg) (n=5), intra-arterial MSCs ($1.5x10^6$/animal) (n=5) or combination of DPO and MSCs (n=5) immediately after I/R. We monitored hematocrit, serum creatinine (SCr) (24, 48, 72 hours after operation) and obtained renal tissue on 3rd day after nephrectomy. Tissue injury was quantified by application of a standardized histological scoring system using light and electron microscope.

**Results**

Renal function was significantly improved by treatment with MSCs or DPO (mean SCr levels on 3rd day; in control group:2.00±0.14 mg/dL, in MSCs group:1.16±0.69 mg/dL, in DPO group:1.44±0.31 mg/dL, $p<0.05$) although hematocrit levels were similar in all groups. Improvement of renal function in rats treated with combination of MSCs and DPO was better than the other groups (mean SCr: 0.76±0.19mg/dL, $p<0.05$). Histological analysis demonstrated a significantly decreased tissue injury in rats treated with MSCs or DPO (Histological score in control group:108±10, in MSCs group: 57±10, in DPO group: 74±17 $p<0.05$). Best histological score was observed in rats treated with combination of MSCs and DPO (Histological score:37±11 $p<0.05$).

**Conclusion**

These results suggest the potential clinical application of DPO and MSCs in combination as a novel renoprotective therapy for patients who developed ischemic renal injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1017**

**Endogenous BMP-7 Is a Critical Molecular Determinant of the Reversibility of Obstruction-Induced Renal Injuries** Scott R. Manson,[1] Keith A. Hruska,[2] Paul Austin.[1] *[1]Surgery, Washington University, Saint Louis, MO; [2]Medicine, Washington University, Saint Louis, MO.*

**PURPOSE:** Although obstructive nephropathies are frequently correctable through surgical intervention, the potential for permanent renal injury remains even following surgery. Little is known about the intrinsic mechanisms that determine the reversibility of renal injuries. We and others have found that exogenous BMP-7 inhibits the pathogenesis of renal injury. Here, we examine the role of endogenous BMP-7 in the outcome of renal recovery following the correction of obstructive nephropathies.

**METHODS:** A reversible murine model of ureteral obstruction was used to characterize renal recovery following the correction of short-term obstructions that lead to 'reversible' renal injuries and prolonged obstructions that lead to 'irreversible' renal injuries. The role of BMP-7 was examined by treatment with either BMP-7-neutralizing antibodies or exogenous BMP-7.

**RESULTS:** While BMP-7 levels are upregulated following the correction of obstruction in 'reversible' renal injuries, the upregulation of BMP-7 is diminished in 'irreversible' renal injuries. The activation of the BMP-7 pathway is required for several processes that contribute to renal recovery including the suppression of TGF-β-dependent pro-fibrotic pathways, the resolution of fibrotic lesions, and the regeneration of renal architecture. Importantly, the therapeutic restoration of BMP-7 in 'irreversible' renal injuries enhances renal recovery following the correction of obstruction.

**CONCLUSIONS:** While BMP-7 plays a critical role in the repair of obstruction-induced renal injuries, the potential for renal recovery in prolonged obstructions is diminished, in part, due to the dysregulation of BMP-7. Accordingly, renal recovery from obstructive nephropathies may be optimized through timely intervention and adjuvant approaches to restore BMP-7 activity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1018**

**Cytosolic Phospholipase A2α (IVa) Interacts with Sirt2 and Attenuates Its Deacetylase Activity** Said Movahedi Naini, Joseph V. Bonventre. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Cytosolic phospholipase A2α (cPLA2α) has been shown to play a role in different physiological and pathological conditions such as ischemia/reperfusion (I/R), inflammatory and infectious diseases and progression of cancer. To identify potential mechanisms of action of cPLA2α, we searched for its potential interacting partners. We show by two hybrid screening and by co-immunoprecipitation that cPLA2α interacts with Sirt2. Sir2 (Sirtuin) family of proteins are involved in aging, life span due to calorie restriction and cell cycle regulation. We have identified the region of interaction to within 88 amino acids within a recognized RING domain of SIRT2. We show that Sirt2 is expressed in the kidney after I/R with highest levels at 5 days after injury. We show that endogenous Sirt2 interacts with cPLA2α in primary mouse mesangial cells. We also show that overexpression of cPLA2α decreases tubulin deacetylase activity of Sirt2 in vivo and in vitro and this inhibition is independent of phospholipase activity of cPLA2α. We further show that overexpression of cPLA2α can inhibit the checkpoint function of Sirt2 during mitosis in responce to Microtubule inhibitors (MTIs). These data suggest an important role for cPLA2α in the regulation of Sirt2 function.

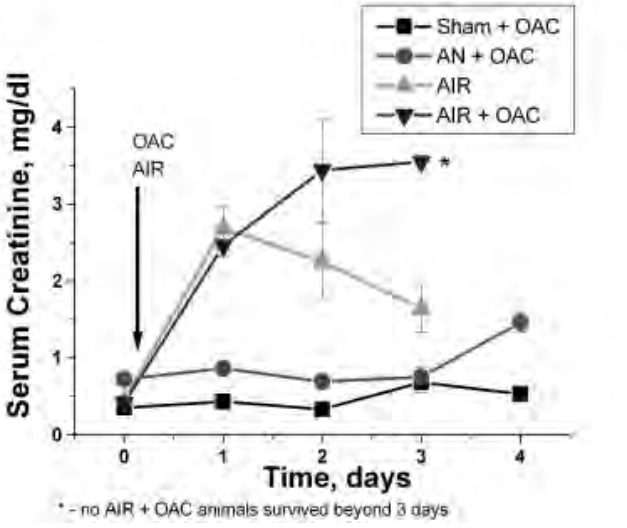
Disclosure of Financial Relationships: nothing to disclose

**F-PO1019**

**Overanticoagulation Causes Acute Kidney Injury (AKI) in Experimental Animals** Sergey V. Brodsky, Kyle M. Ware, Jon R. Von Visger, Lee A. Hebert, Gyongyi Nadasdy, Anjali A. Satoskar, Tibor Nadasdy. *The Ohio State University, Columbus, OH.*

We recently reported that acute increase in international normalized ratio (INR)>3.0 may associate with AKI in patients with chronic kidney disease (CKD). Morphological findings included glomerular hemorrhage with formation of obstructive red blood cell (RBC) casts. We referred this condition as warfarin related nephropathy (WRN). WRN often resulted in CKD acceleration. We tested the possibility that overanticoagulation (OAC) may affect renal function in experimental animals. OAC was induced by feeding Sprague Dawley rats with brodifacoum. We used acute ischemia-reperfusion injury (AIR) and 5/6 nephrectomy

[ablative nephropathy, (AN)] as experimental models. Treatment with brodifacoum resulted in a significant increase in prothrombin time in all experimental groups. Morphologically, sham-OAC did not have RBC casts associated with OAC, although hematuria was present. Both AIR-OAC and AN-OAC groups had features of glomerular hemorrhage and occlusive RBC casts on histological examination. AN animals showed significant proteinuria and increase in serum creatinine (SC) levels before OAC was initiated. SC levels were not changed in sham-OAC group, but AN-OAC animals had an increase in SC associated with OAC. In AIR-OAC group SC levels did not improve after the surgery, as compared to AIR only. The mortality was significantly increased in the AIR-OAC group.




Based on these data, we conclude that OAC results in glomerular hemorrhage with formation of occlusive RBC casts and impairment of renal function in animals with underlying kidney conditions. These findings are similar to our previous observation on patients with increased INR. Therefore, we can model OAC-associated kidney injury in experimental animal, which will allow to study the WRN pathogenesis.

Disclosure of Financial Relationships: nothing to disclose

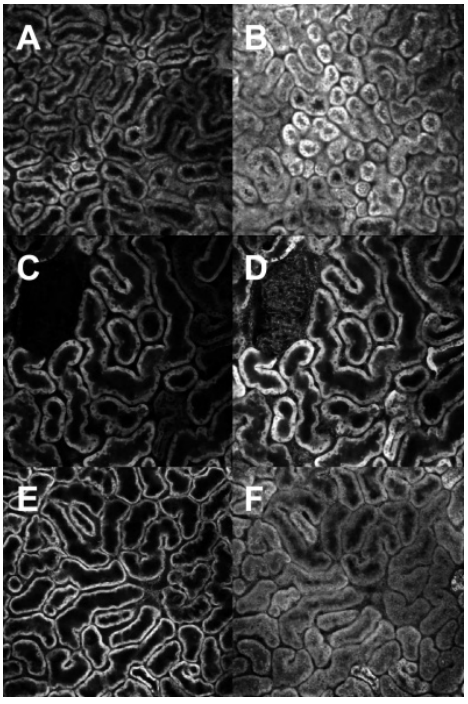
**F-PO1020**

**In Vivo Multiphoton Imaging of Mitochondrial Function in the Rodent Kidney** Andrew Michael Hall,[1] George Rhodes,[2] Ruben M. Sandoval,[2] Bruce A. Molitoris.[2] *[1]Centre for Nephrology, University College London, United Kingdom; [2]Indiana Centre for Biological Microscopy, Indiana University School of Medicine, Indianapolis, IN.*

Mitochondrial dysfunction has been implicated in the pathogenesis of ischemia-reperfusion induced acute kidney injury (AKI). We have demonstrated, using *in vivo* multiphoton microscopy, that mitochondrial function can be imaged in kidneys of anesthetized rats.

Ischemia-reperfusion was induced by clamping then releasing the renal artery. Mitochondrial NADH was excited at 720nm (Fig 1A) and increased in response to ischemia (1B). Mitochondrial membrane potential ($\Delta\Psi_m$) was measured using rhodamine 123 (1C) or tetramethylrhodamine methyl ester (TMRM) (1D) excited at 800-850nm; following bolus intravenous injection rapid uptake was observed in mitochondria in proximal tubules (PTs). Fluorescence signals decayed over time in PTs, probably due to dye extrusion. Neither verapamil (inhibitor of MDR) nor cimetidine (inhibitor of OCTs) prevented the decay; however, a steady state could be achieved using a constant dye infusion over 50 minutes. TMRM signal in distal tubules (DTs) did not decay; 20 minutes post injection mean signal in DTs (1248.7±229.5 A.U.) was greater than in PTs (464.4±83.4, $p<0.05$). Uptake of rhodamine 123 into DTs was low; after 20 minutes mean signal was higher in PTs (1224.7±156.3) than DTs (644.0±132.9, $p<0.05$). Rhodamine 123 signal in glomeruli was also low compared to TMRM. Following the onset of ischemia, control TMRM signal (1E) decreased rapidly in PTs (1F) consistent with dissipation of $\Delta\Psi_m$. Injection of TMRM post reperfusion lead to heterogeneous uptake.

In summary, mitochondrial function was imaged in a rat model of AKI using *in vivo* multiphoton microscopy. Constant intravenous infusion of TMRM leads to a steady state signal in the PT and better uptake in glomeruli and DTs when compared to rhodamine 123.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1021**

**Dendritic Cells Induce an Anti-Inflammatory Phenotype on Tubule Epithelial Cells after Kidney Injury** Suetonia Palmer, Takaharu Ichimura, Benjamin D. Humphreys, Joseph V. Bonventre. *Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

The mechanisms by which dendritic cells (DCs) modulate kidney tissue responses to injury remain incompletely understood. The aim of our study was to determine whether DCs interact with tubular epithelial cells to alter the inflammatory response of the kidney after injury.

DCs and macrophages accumulate in the renal interstitium adjacent to injured tubules that express Kim-1. Cultured mouse proximal tubule cells express Kim-1 and release high levels of TNFα and monocyte chemoattractant protein (MCP-1). Conditioned media from normal tubular epithelial cells markedly increases TNFα and MCP-1 production by RAW 264.7 cells, a macrophage cell line, suggesting kidney epithelial cells are pro-inflammatory *in vitro*. Pre-incubation of kidney epithelial cells with bone marrow-derived DCs significantly reduces the pro-inflammatory effect of proximal tubule cells on macrophages. Direct cell-to-cell contact between DCs and kidney epithelial cells is necessary for this anti-inflammatory effect of DCs to be observed. DCs maintain steady-state expression of antigen-presentation and co-stimulatory molecules when in contact with kidney epithelial cells. DCs strongly stimulate epithelial cell expression of MCP-1, IL-10, and IL-6. In addition, DCs completely protect cultured kidney epithelial cells (LLC-PK1) from necrotic injury after exposure to oxidative stress induced by $H_2O_2$ (1 mM).

In conclusion, tubular epithelial cells are important targets of DC actions in the kidney. DCs may induce an anti-inflammatory epithelial cell phenotype after kidney injury via both alterations in epithelial cell cytokine expression and protection against tubule cell death. Increased anti-inflammatory cytokine and MCP-1 expression by renal epithelial cells in direct contact with resident and infiltrating DCs may promote the homing of a subpopulation of macrophages to the peritubular interstitium that drives tissue healing and repair.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1022**

**The Effect of CCR5 and CCR2 Deficiency on Renal Ischemia-Reperfusion Injury in Mouse Model** Ran-Hui Cha,[1] Seung Hee Yang,[2] Yon Su Kim.[1,2] *[1]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Kidney Research Institute, Seoul National University Medical Research Center, Seoul, Korea.*

***Background*** Various factors including chemokines participate in ischemia-reperfusion injury (IRI). CCR2 is known to be responsible for renal IRI and CCR5 together with its ligand RANTES are also assumed to be associated with renal IRI. We evaluated the role of CCR5 and CCR2 on renal IRI via *in vitro* hypoxia-reoxygenation experiment using human renal tubular epithelial cells (TECs) and *in vivo* IRI experiment using CCR5 and CCR2 knock out mice.

***Methods*** Human renal TECs were incubated at a hypoxic condition (1% $O_2$) for 6 hours and then, cells were placed under a normoxic condition (20% $O_2$) for 18 hours. Proliferation of TECs was determined using colorimetric MTS assay kits. IRI was induced in C57BL/6

wild, CCR5$^{-/-}$, CCR2$^{-/-}$, and CCR5$^{-/-}$CCR2$^{-/-}$ mice. Kidney tissues were assayed for cytokines using a multiplex cytokine bead array system (Bio-Plex).

***Results*** *In vitro*, human renal TECs proliferated well under normoxic condition, but their proliferation was significantly hampered under hypoxic condition. Recombinant CCR5 more inhibited the proliferation of TECs with dose-dependent pattern, on the contrary, neutralizing antibody to CCR5 partially restored. *In vivo*, the absence of CCR2 and CCR5 lessened the severity of renal IRI, histologically and functionally. IRI induced tubular necrosis and the damage was more extensive in wild type mice than in knock out mice. The intra-renal infiltration of T cells and macrophages was markedly inhibited in knock out mice than in wild type mice. Tubular CCR2 expression was reduced in CCR5$^{-/-}$ mice and CCR5 expression was reduced in CCR2$^{-/-}$ mice, either. Level of serum BUN and creatinine was lower in CCR5$^{-/-}$ and CCR2$^{-/-}$ mice than in wild type, furthermore, CCR5$^{-/-}$CCR2$^{-/-}$ mice showed the least level of those markers. Results from Bio-Plex revealed that these were mediated by IL-4, IL-10, and IL-13 pathway activation in knock out mice.

***Conclusion*** These findings demonstrate that absence of CCR5 together with CCR2 exert protective effects on kidneys, suggesting that CCR2 and CCR5 may be a feasible target in protecting kidneys from IRI.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1023

**Kidney Injury Molecule-1 (Kim-1) Down-Regulates the Proximal Tubule Innate Immune Response to Injury** Li Yang,[1, 2] Craig R. Brooks,[1] Joseph V. Bonventre.[1] [1]*Renal Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA;* [2]*Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China.*

Our laboratory has shown that Kidney Injury Molecule-1 (Kim-1) is a phosphatidylserine receptor, which mediates phagocytosis of apoptotic cells and oxidized lipids by renal proximal tubular cells. In unpublished studies of Kim-1 mutant and wild-type mice we have found that Kim-1 is protective against acute ischemic and toxic kidney injury. The purpose of this study was to investigate how human Kim-1 (KIM-1) expression and facilitated phagocytosis of apoptotic cells modulates the kidney innate immune response to acute injury.

In control pCDNA vector-transfected HEK293 cells and LLC-PK1 cells, LPS treatment (5mg/ml) for 24 hours increased mRNA and protein levels of Toll-like receptor 4 (TLR4). Increased expression of KIM-1 resulted in decreased TLR4 mRNA and protein levels in both cell lines. Pre-feeding epithelial cells with apoptotic T-lymphocytes 2 h before LPS treatment further decreased TLR4 expression in KIM-1-expressing cells. The supernatant from LPS-treated control LLC-PK1 cells markedly increased the mRNA levels of MCP-1, TNF-$\alpha$ and IL-1$\beta$ in RAW264.7 cells. The expression of KIM-1 in LLC-PK1 cells mitigated the RAW cell activation. Feeding KIM-1-expressing cells with apoptotic cells resulted in further reductions in supernatant-induced RAW activation.

In primary cultured mouse proximal tubular cells, approximately 30% of cells expressed Kim-1. LPS treatment increased TLR4 expression in non-Kim-1 expressing cells, but not in Kim-1 expressing tubular cells, presumably because Kim-1 expression suppressed TLR4 expression. In human kidneys from patients with AKI, TLR4 was detected in proximal tubular cells, with either apical membrane linear or intracellular granule-like distribution. TLR4 expression was markedly reduced in proximal tubule cells that express KIM-1.

In conclusion the expression of Kim-1 in proximal tubules, especially in the presence of apoptotic cell uptake, down-regulates the innate immune response, which may explain the protective effects of Kim-1 in models of acute kidney injury.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1024

**SIRT1, a Stress-Responsive Deacetylase, Mediates Age-Related Response to Acute Kidney Injury** Hong Fan,[2] Haichun Yang,[1,2] Wenjuan He,[1] Li You,[2,1] Chuan-Ming Hao.[1, 2] [1]*Nephrology, Vanderbilt University, Nashville, TN;* [2]*Nephrology, Huashan Hospital Fudan University, Shanghai, China.*

It has been documented that older individuals are more susceptible to acute kidney injury (AKI). The mechanism underlying this age-related susceptibility to AKI is incompletely defined. Sirt1, a NAD+ dependent deacetylase and stress response molecule, has been demonstrated to mediate caloric restriction associated life-span extension. The aim of the present study was to investigate whether SIRT1 is associated with age-related response to AKI in mice.

Immunoblot shows that the Sirt1 expression in the kidney of 7-week old mice (younger mice) is significantly higher compared with 22-week old mice (older mice) (1.0±0.2 vs 0.45±0.13, p<005). Following renal ischemia-reperfusion (I/R, 40 min bilateral renal artery clamp), the BUN was markedly increased in older mice, but only mildly elevated in younger mice (day 2, 190.9±20 vs 38.6±6.8 mg/dl, n=8, p<005), suggesting that younger mice were more resistant to AKI. Loss of one allele of Sirt1 significantly enhanced the kidney injury following I/R (BUN 141.4±14.4 of SIRT1+/- mice vs 94.0±13.7 mg/dl of SIRT1+/+ mice, n=12, p<0.05). The kidney from SIRT1+/- mice following I/R also exhibited more apoptotic cells assessed by TUNEL assay and activated caspase 3 levels compared with wild type mice. In contrast, a Sirt1 selective activator, SRT1270, protected the kidney from I/R induced injury (BUN 113.4±14.8 vs 169.2±19.0, n=13, p<005). All the renal function results (BUN) were supported by histology scores. SRT1720 treatment was associated with lower levels of activated caspase 3 compared with vehicle treatment. In the older mice following I/R, the expression levels of p53 and p21 in the kidney were significantly higher than younger mice (p53 2.3±0.4 vs 0.9±0.4, p21 2.4±0.4 vs 0.3±0.04, p<005). SRT1720 treatment significantly attenuated, while deletion of one allele of Sirt1 gene increased p53 and p21 protein expression levels in the kidney.

These results suggest that reduced expression of SIRT1 is associated with increased susceptibility to AKI, and therefore Sirt1 could be a potential target for the prevention and treatment of AKI.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1025

**Thick Limb-Associated Tamm-Horsfall Protein Regulates MIP-2 Expression in the S3 Segment: A Case for Tubular Cross-Talk** Tarek M. Elachkar, Ruth A. Mccracken, Michael I. Rauchman. *Medicine, Saint Louis University and the Saint Louis VA, Saint Louis, MO.*

Tamm-Horsfall Protein (THP) is a glycoprotein with unclear functions expressed exclusively in thick ascending limbs (TAL) of the kidney. We recently described a novel protective role for this protein in Acute Kidney Injury (AKI), whereby THP decreased inflammation in the outer medulla. Using an ischemia-reperfusion model, we showed that S3 proximal segments and not TAL are the main tubules injured during AKI. To explain how TAL-associated THP affects S3 segments, we hypothesize that THP released basolaterally from TAL modulates the expression of pro-inflammatory mediators by S3 segments. Using Immunofluorescence microscopy and 3D volumetric imaging, we show that THP expression is increased with ischemia at the basolateral side of TAL and in the interstitial area around S3 segments. The scavenger receptor SRB-1, a potential receptor for THP, is also expressed at the basolateral aspect of S3 segments. Using Real-time PCR performed on whole kidney extracts from THP-/- and THP+/+ mice, we measured mRNA of the cytokine TNF$\alpha$ and the chemokines MCP-1 (macrophage chemo-attractant) and MIP-2 (neutrophil chemo-attractant). Only MIP-2 showed a differential increased expression in THP knockout vs. wild type mice at baseline and after ischemia. MIP-2 protein expression was localized predominantly to S3 segments in THP-/- mice after ischemia. This also correlated with neutrophil infiltration around these tubular segments. To validate this direct effect of THP on proximal cells, MIP-2 mRNA was measured in HK-2 proximal tubular cells subjected to chemical anoxia with or without pre-incubation with THP. MIP-2 expression was significantly increased after anoxia, but this expression was markedly diminished by pretreatment with purified THP. In conclusion, our findings support that THP released from TAL down-regulates MIP-2 in neighboring proximal cells, an effect consistent with its protective propertied during AKI. We propose that THP is a mediator of tubular cross-talk aimed at stabilizing the outer medulla in the face of injury.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1026

**Identification of Stage-Specific Markers of Acute Kidney Injury** Anne Katharina Wübken,[1] Kai M. Schmidt-Ott.[1, 2] [1]*Max-Delbrueck Center for Molecular Medicine, Berlin, Germany;* [2]*Experimental and Clinical Research Center, Charité Berlin, Berlin, Germany.*

Several biomarkers of acute kidney injury have been proposed, including neutrophil gelatinase-associated lipocalin and kidney injury molecule-1. While these markers detect the presence and intensity of renal tubular injury, they are elevated for extended periods following tubular damage. Hence, an elevated level of these markers at a single given time point does not differentiate the hyperacute stage of tubular injury (e. g. 2-24 hours after ischemic injury in mice) from the regeneration phase (e. g. 2-5 days after ischemic injury). Yet a differentiation of these stages will be clinically relevant to allow for an accurate assessment of the timing of disease progression and to target stage-specific pharmacotherapy. To identify stage-specific markers of acute kidney injury, we analyzed gene expression signatures in mouse kidneys at three different time points (6h, 48h and 7d) following ischemia reperfusion injury using microarray analysis and identified time point-specific transcripts. In the early, hyperacute phase (6h post injury), we found an antiproliferative and proapoptotic gene signature, including several components of the ATF4-ATF3-CHOP pathway. Two genes of this hyperacute signature, cation transport regulator-like protein 1 (Chac1) and angiopoietin-like 7 (Angptl7), were highly responsive to injury and rapidly normalized at later time points. Immunofluorescence staining indicated induction of Chac1 in damaged tubular cells. Conversely, 48 hours post injury a gene signature representative of tubular proliferation was observed. Of these genes, baculoviral IAP repeat-containing 5 (Birc5/survivin) displayed co-induction with the proliferation marker Ki-67, a marked degree of responsiveness, and tubular-restricted expression according to immunofluorescence staining. Together, our data identify Chac1/Angptl7 and Birc5 as stage-specific markers of acute kidney injury representing the hyperacute and proliferative phases, respectively. In the future, these markers may facilitate risk assessment and guidance of pharmacotherapy in patients with acute kidney injury.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1027

**Inducible PAX8-Driven Knockout of the Von-Hippel-Lindau Protein Protects from Acute Kidney Injury** Christian Rosenberger,[1] Alexander Paliege,[2] Harm Peters,[1] Hans-Hellmut Neumayer,[1] Robert Koesters,[3] Sebastian C. Bachmann.[2] [1]*Nephrology and Renal Transplantation, Charité Universitaetsmedizin, Berlin, Germany;* [2]*Anatomy, Humboldt University, Berlin, Germany;* [3]*Hopital Tenon, Inserm/Université Pierre et Marie Curie, Paris, France.*

The Von-Hippel-Lindau (VHL) protein is a key suppressor of hypoxia-inducible factors (HIFs). We generated a mouse strain with conditional paired box protein (PAX)8-driven knockout of VHL gene (VHL-KO) that provides a model of normoxic HIF activation. In the mice, conditional PAX8-VHL-KO led to robust activation of both HIF-1$\alpha$ and -2$\alpha$ in

all nephron segments and approximately 20% of hepatocytes. VHL-KO mice developed significant polyglobulia within 2 wk due to liver EPO production and showed increased renal capillary density in parallel with increased VEGF expression. Protein excretion, renal function and gross renal and liver histology were not altered in VHL-KO mice over 9 mo (kept on a normal Hb by repeated bleeding). When VHL-KO mice were subjected to systemic hypoxia by subacute bleeding anemia, there was no change in pattern or expression intensity of the renal and liver HIF-1α signals. When VHL-KO animals were exposed to rhabdomyolysis-induced acute kidney injury (AKI), which serves as a model of hypoxic damage to proximal tubules, kidneys showed persistently enhanced HIF-1α, but de novo activated cell protective HIF target genes in proximal tubules, such as heme oxygenase-1 and glucose transporter-1. At 48 h after injury, VHL-KO mice had better renal function and less proximal tubular damage, including necrosis as compared to wildtype mice with similar rhabdomyolysis-induced AKI. We conclude that 1) *in vivo* VHL-KO activates both HIF-1α and -2α and leads to polyglobulia and renal angiogenesis; and 2) VHL-KO ameliorates AKI associated with tubular hypoxia, potentially through tubular survival genes and/or renal angiogenesis.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1028

**Mitochondria Targeted Heme Oxygenase-1 (HO-1) Reduces Oxidative Stress in Renal Epithelial Cells** Subhashini Bolisetty,[1,2,3] Amie Traylor,[1] Karina Claudia Ricart,[3] Michelle S. Johnson,[3] Aimee L. Landar,[3] Victor M. Darley-Usmar,[3] Anupam Agarwal.[1,2,3] *[1]Medicine, Division of Nephrology, University of Alabama at Birmingham, Birmingham, AL; [2]Cell Biology, University of Alabama at Birmingham, Birmingham, AL; [3]Pathology, University of Alabama at Birmingham, Birmingham, AL.*

Oxidative stress and mitochondrial dysfunction play a major role in the pathogenesis of acute kidney injury. One of the key mediators of oxidative stress is the generation of reactive oxygen species (ROS) by mitochondria, leading to damage of several cellular components. HO-1, an anti-oxidant enzyme, is induced during oxidative stress and is protective against cell death. The purpose of this study was to determine the role of HO-1 in modulating mitochondria-derived oxidative stress, mitochondrial function and cell death. We generated a mitochondria-targeted HO-1 cell line by transfecting HEK293 cells with the MnSOD mitochondria leader sequence fused to HO-1 cDNA (Mito-HO-1). Non-targeted HO-1 overexpressing cells (3.1/HO-1) and empty vector cells (control) served as controls. Both Mito-HO-1 and 3.1/HO-1 cells showed a significant increase in HO-1 expression by western blot analysis. Furthermore, Mito-HO-1 cells showed an increase in mitochondrial HO-1 expression compared to 3.1/HO-1 cells. Mitochondrial localization of HO-1 in Mito-HO-1 cells was confirmed by co-localization with mitotracker and cytochrome C oxidase. Additionally, to evaluate the functional significance of mitochondria-targeted HO-1, cells were exposed to the electrophilic lipid, 15d-$PGJ_2$, a product of the cyclooxygenase pathway. Mito-HO-1 cells generated significantly lower levels of ROS as compared to control cells by the DCF assay and were also protected against heme cytotoxicity. These results suggest that HO-1 can be targeted to specific organelles within the cell and that overexpression of HO-1 in the mitochondrial compartment is protective. Targeted HO-1 expression represents a novel strategy to overcome mitochondria-derived oxidative stress and may be more beneficial than generalized overexpression of HO-1 in acute kidney injury.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1029

**The Outcome of Lipocalin-Positive Acute Kidney Injury: A Multicenter Study** Michael Haase,[1] Anja Haase-Fielitz,[1] Rinaldo Bellomo,[2] Prasad Devarajan,[3] Dinna N. Cruz,[4] Gebhard Wagener,[5] Catherine D. Krawczeski,[3] Jay L. Koyner,[6] Patrick T. Murray,[7] Michael Zappitelli,[8] Stuart Goldstein,[3] Konstantinos Makris,[9] Claudio Ronco,[4] Peter R. Mertens.[10] *[1]Nephrology, Charite Berlin; [2]Melbourne; [3]Cincinnati; [4]Vicenza; [5]NY; [6]Chicago; [7]Dublin; [8]Montreal; [9]Athens; [10]Nephrology, Magdeburg.*

**Background**

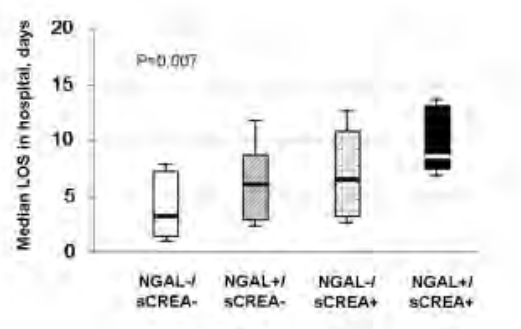
Increases in neutrophil gelatinase-associated lipocalin (NGAL) detect acute kidney injury (AKI) hours to days before serum creatinine and may be clinically significant. We aimed to test the hypothesis that, independent of changes in serum creatinine, increased NGAL levels identify patients with AKI and therefore, worse prognosis.

**Methods**

We analyzed pooled data from 1,217 patients from seven prospective observational studies of NGAL. We used the terms NGAL(-) or NGAL(+) to study-specific NGAL cut-off values for optimal AKI prediction and the terms sCREA(-) or sCREA(+) according to RIFLE-AKI.

**Results**

Of study patients, 661 (54.3%) were NGAL(-)/sCREA(-), 231 (19.0%) NGAL(+)/sCREA(-), 80 (6.6%) NGAL(-)/sCREA(+) and 245 (20.1%) NGAL(+)/sCREA(+). There was a progressive increase in median number of intensive care and in-hospital days with increasing biomarker positivity: NGAL(-)/sCREA(-): 3.2 and 8.3 days; NGAL(+)/sCREA(-): 5.8 and 9.3 days; NGAL(-)/sCREA(+): 6.9 and 18.0 days; NGAL(+)/sCREA(+): 8.3 and 19.8 days; P=0.007 and P=0.04, respectively.




There was a similar and consistent stepwise increase in subsequent renal replacement therapy initiation (0%, 1.3%, 8.8% and 11.4%, respectively), hospital mortality (4.1%, 6.1%, 8.8%, 16.7%, respectively) and their combination (P<0.001).

**Conclusions**

Irrespective of associated diagnostic increases in serum creatinine, NGAL detects patients with AKI who have an increased risk of adverse outcomes including death. The concept and definition of AKI may need re-assessment.

Disclosure of Financial Relationships: Honoraria: Abbott, Biosite.

## F-PO1030

**p66shc and Cisplatin-Mediated Neprotoxicity** Istvan Arany, Jeb S. Clark, Radhakrishna Baliga. *Pediatrics, University of Mississippi Medical Center, Jackson, MS.*

Cancer patients often develop serious renal failure due to treatment with the anticancer drug cisplatin (CP) that induces apoptotic death of renal proximal tubules. The p66shc adaptor protein plays a pivotal role in apoptosis but its role in CP-mediated nephrotoxicity is unknown. Here, we show that CP treatment phosphorylates p66shc at its Serine36 residue both in the kidney and in cultured renal proximal tubule cells. We found that overexpression of p66shc exacerbates while knockdown of p66shc or mutation of its Serine36 phosphorylation site to alanine (S36A) ameliorates CP-induced caspase-3 activation, membrane permeabilization, LDH release, collapse of the actin cytoskeleton and consequent apoptosis *in vitro*. We also determined that the extracellular signal regulated kinase (ERK) serves as the kinase that phosphorylates p66shc at its Serine 36 residue after treatment with CP. Accordingly, pharmacologic or dominant negative inhibition of ERK mitigates while adenoviral overexpression of activated ERK exacerbates CP-induced Ser36 phosphorylation of p66shc and consequent activation of caspase-3 and LDH release.

These results suggest that CP activates the ERK-p66shc pathway that leads to apoptosis in renal proximal tubule cells. Thus, manipulation of p66shc expression and/or phosphorylation might offer new therapeutic means to ameliorate CP-induced nephrotoxicity in cancer patients.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1031

**Up-Regulated HNF-1β during Experimental Acute Kidney Injury Plays a Crucial Role in Renal Tubular Regeneration** Koji Ogata. *Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Nankoku, Japan.*

HNF-1β is a transcription factor expressed in the kidney, liver, and other epithelial organs.HNF-1β was reported binds to the SOCS3 promoter and represses SOCS3 transcription. However, the signaling pathways and tubular regeneration controlled by HNF-1β are poorly understood in ischemia/reperfusion renal injury.

The aim of this study is to understand the functional roles of HNF-1β, SOCS3 and STAT3 in tubular damages in AKI in vivo and in vitro, and examine the effect of HNF-1β on renal tubular formation using 3-D-gels.

To clarify the significance of HNF-1β pathway in AKI, we used a rat AKI model *in vivo* and cultured renal tubular cells (NRK-52E cells) as an *in vitro* model. After clamping renal artery for 1 h, kidney homogenate at 2, 3, 6, 12, 24, 48, and 72 h after reperfusion was extracted. In Western blot analysis, HNF-1β and phosphorylated-STAT3 expressions were increased at 2-12h and 6-24h. In immunohistological examination, expression of HNF-1β was increased in proximal tubules at 12h. To understand the downstream signaling of HNF-1β in renal tubular cells, we transfected HNF-1β construct to NRK-52E cells. Overexpression of HNF-1β increased $^3$H-thymidine uptake in NRK-52E cells. Furthermore, we used 3-D-gels to examine the role of HNF-1β for tubular formation. Overexpression WT HNF-1β promoted tubular formation. Tubular formation is inhibited by overexpression of DN HNF-1β.

In conclusion, HNF-1β is up-regulated in ischemia/reperfusion renal tubular injury at proximal tubular cells in vivo, and HNF-1β regulates cell proliferation and tubular formation by regulating SOCS3 expression and STAT3 activation. The current study therefore unravels both physiological and pathological significance of HNF-1β pathway in ischemia/reperfusion renal injury in vivo and in vitro.

Disclosure of Financial Relationships: nothing to disclose

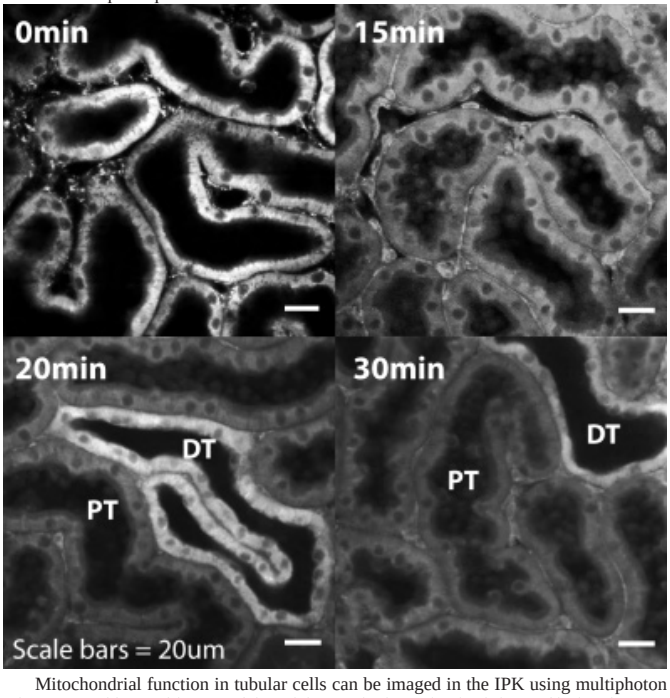
**F-PO1032**

**Multiphoton Imaging of Mitochondrial Function in the Isolated Perfused Rat Kidney** Andrew Michael Hall,[1] Carol Crawford,[2] Robert J. Unwin,[1] Michael Duchen,[3] Claire M. Peppiatt-Wildman.[2] *[1]Centre for Nephrology, University College London, United Kingdom; [2]Urinary Systems Physiology Unit, Royal Veterinary College, London, United Kingdom; [3]Cell and Developmental Biology, University College London, United Kingdom.*

Mitochondrial dysfunction is central to the pathogenesis of ischemic acute kidney injury (AKI). We used multiphoton microscopy to image mitochondrial function in isolated perfused kidneys (IPKs).

Adult male Sprague-Dawley rats were anesthetized with intra-peritoneal pentobarbitone. The right renal artery was cannulated and perfusion was commenced immediately with a HEPES-buffered solution at 37ºC. The preparation was moved to a custom built chamber and imaged with a Zeiss LSM510 microscope coupled to a Coherent Chameleon laser. Dyes were loaded using a recirculating perfusion system. Ischemia was induced by stopping perfusion.

Mitochondrial signals were co-imaged with proximal tubule (PT) uptake of fluorescent-labeled insulin, to provide simultaneous measurements of transport and metabolism. Reactive oxygen species production was measured using dihydroethidium; after 20min of perfusion, the mean signal was higher in PTs (1582.8±150.9 A.U.) than in distal tubules (DTs: 694.9±133.6, $p<0.05$). Mitochondrial NADH was used as a readout of redox state and increased in response to hypoxia. Mitochondrial membrane potential was measured using tetramethyl rhodamine methyl ester (TMRM). Control TMRM signal in the PT (1496.8±132.7) decreased after 30min of ischemia (870.4±92.8, $p<0.05$), but there was no significant change in the DT (1772.7±149.3 vs 1458.5±359.9, $p>0.05$) (Figure 1). Widespread cellular debris was observed in the PT lumen during ischemia, which impaired tubular flow upon reperfusion.




Mitochondrial function in tubular cells can be imaged in the IPK using multiphoton microscopy; this provides a useful model to study the pathophysiology of ischemic AKI.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1033**

**Experimentally Induced Overload Albuminuria Increases Urinary Cystatin C Excretion** Maryam Nejat, Jonathan V. Hill, John W. Pickering, Zoltan H. Endre. *Christchurch Kidney Research Group, Department of Medicine, University of Otago Christchurch, Christchurch, New Zealand.*

Introduction:

Megalin mediated transport is responsible for the reabsorption of most filtered proteins including cystatin C (CysC), neutrophil gelatinase-associated lipocalin (NGAL), and liver fatty acid binding protein (L-FABP). We used a model of transient proteinuria induced by intraperitoneal (IP) administration of Bovine Serum Albumin (BSA), to examine whether competition for megalin mediated transport by albumin could cause increased urinary CysC excretion in the presence of proteinuria.

Methods:

Seven male and female (6-8 week old) Sprague-Dawley rats received 5 $mg.day^{-1}.g$ body $wt^{-1}$ BSA in saline twice daily for 2 consecutive days (Day 0 and 1). Four rats of each sex received saline only. Albumin, total protein and CysC were measured in timed collection of spontaneously voided urine on Day -1, Day 2, and Day 6. Repeated measures two-way ANOVA was used to explore the effects of proteinuria, sex, and time.

Results:

The CysC excretion rate was correlated with albumin ($r^2=0.29$, $p<0.01$) and total protein ($r^2=0.58$, $p<0.001$) excretion rates. BSA treatment increased excretion rates of protein, albumin, and CysC ($p<0.01$, $p<0.001$, and $p<0.001$ respectively) by Day 2. All excretion rates returned to pre-treatment rates by Day 6. Male rats excreted more protein, albumin, and CysC than females ($p<0.001$, $p<0.001$, and $p<0.05$, respectively). The control groups showed no significant changes in urine analytes over time.

Conclusion:

Overload proteinuria resulted in a proportional increase in albumin and CysC excretion. Male rats had higher albuminuria and cystatinuria than females. Increased urinary CysC excretion may reflect increased permeability of the glomerular filtration barrier or impaired proximal tubule reabsorption. These data suggest the clinical interpretation of urinary CysC and other filtered biomarkers of AKI transported by megalin require concomitant assessment of proteinuria.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1034**

**Heat Shock Protein 72 (Hsp72) Is an Early and Sensitive Biomarker To Detect Acute Kidney Injury (AKI)** Jonatan Barrera-Chimal,[1,2] Rosalba Pérez-Villalva,[1,2] Marcos Ojeda,[1,2] Gerardo Gamba,[1,2,3] Luis E. Morales-Buenrostro,[2] Norma Bobadilla.[1,2] *[1]Molecular Physiology Unit, Instituto de Investigaciones Biomédicas, UNAM; [2]Instituto Nacional de Ciencias Médicas y Nutrición SZ; [3]Instituto Nacional de Cardiología ICh.*

Given that Hsp72 is induced in renal tubules during AKI and that proximal tubular detachment is projected to the urinary space, we reasoned that the urinary Hsp72 levels could serve as an early biomarker to detect, monitor and/or stratify AKI. For this purpose, 72 Wistar rats were divided into six groups: sham-operated and rats subjected to 10, 20, 30, 45 and 60 min of bilateral-ischemia (I) and 24-h of reperfusion (R). Different times of reperfusion were also evaluated in 30 other rats subjected to 30 min of ischemia. Hsp72 mRNA and protein levels were determined in both kidney and urine by RT-PCR and WB, respectively. Tubular injury (TI) was scored in kidney sections by morphometry. In addition, Hsp72-specificity as a biomarker to assess the success of a renoprotective intervention was evaluated in spironolactone treated rats before I/R.

Urinary Hsp72 mRNA and protein levels, gradually increased relative to the extent of renal injury induced by different periods of ischemia quantified by histomorphometry as a benchmark of TI ($r=0.82$ and $r=0.94$, respectively). Urinary Hsp72 increased significantly after 3-h, it continued rising until 18-h, and a restoration was seen after 120-h of reperfusion, consistent with TI ($r=0.74$). Spironolactone renoprotection was associated with normalization of urinary Hsp72 levels. Accordingly, urinary Hsp72 levels significantly increased in patients with clinical AKI (58.3 ± 8.5 arbitrary units), compared to healthy volunteers in which, Hsp72 levels were almost undetectable (3.7 ± 0.7).

Our results show that urinary Hsp72 is a reliable biomarker for the early detection of AKI. This novel biomarker was adequately sensitive for stratifying different degrees of tubular injury, tubular regeneration and recovery, as well as for monitoring a renoprotective intervention. These findings were confirmed in patients diagnosed with AKI, revealing that this protein is a promising biomarker for both early detection and stratification of AKI.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1035**

**Nitric Oxide Induces Tubular Cell Hypertrophy Via Cyclic GMP-Dependent Protein Kinase in Rat Compensatory Renal Hypertrophy** Hajime Nagasu, Minoru Satoh, Naruya Tomita, Tamaki Sasaki, Naoki Kashihara. *Division of Nephrology and Hypertension, Department of Internal Medicine, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Renal hypertrophy as an early compensatory mechanism to replace the loss of functioning tissue ultimately turns into a maladaptive process leading to progressive deterioration of renal function. We previously reported that nitric oxide (NO) production in the kidney is increased in the early phase after nephrectomy. However, the precise mechanism of increased NO production and its role in compensatory renal hypertrophy following nephrectomy remain unclear. Endothelial nitric oxide synthase (eNOS) is the key enzyme of vascular homeostasis involved in the pathophysiology of cardiovascular and renal diseases. We therefore hypothesize that the eNOS-soluble guanylate cyclase (sGC)-protein kinase G (PKG) pathway plays a crucial role in compensatory renal hypertrophy following unilateral nephrectomy.

Methods and results: We used the following three groups of mice: wild-type (WT), eNOS knockout (eNOS-KO) and endothelial specific eNOS transgenic (ECeNOS-TG) mice. These mice underwent left unilateral nephrectomy (i.e., WT-Nx, eNOS-KO-Nx and ECeNOS-TG-Nx), and their right kidney and serum were obtained for examination 2 weeks after nephrectomy. A control group of mice underwent sham operation (i.e., WT-sham, eNOS-KO-sham and ECeNOS-TG-sham) and these mice were similarly examined. Compensatory renal hypertrophy was markedly suppressed in the eNOS-KO-Nx group compared with the WT-Nx group. On the other hand, the ECeNOS-TG-Nx group exhibited enhanced compensatory renal hypertrophy than the other two groups. In addition, we examined the effects of BAY 41-2272 in vivo by treating eNOS-KO mice with BAY 41-2272 after unilateral nephrectomy. GSNO and BAY 41-2272 treatments stimulated the PKG activity and phosphorylation of Akt, s6 kinase and 4E-binding protein in hPTECs. Treatment with BAY41-2272 significantly enhanced compensatory renal hypertrophy in the eNOS-KO-Nx mice.

Conclusion: These results indicate that eNOS-sGC-PKG pathway plays a crucial role in the compensatory renal hypertrophy following unilateral nephrectomy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1036**

**Urinary Thioredoxin Is a Quick and Predictive Biomarker of Acute Kidney Injury** Kenji Kasuno,[1] Eri Muso,[2] Daisuke Mikami,[1] Naoki Takahashi,[1] Hideki Kimura,[1] Tomomi Kurose,[1] Yasunari Nobukawa,[1] Haruyoshi Yoshida.[1] *[1]Nephrology and Clinical Laboratories, University of Fukui Faculty of Medical Sciences, Fukui, Japan; [2]Nephrology and Dialysis, Kitano Hospital, The Tazuke Kofukai Medical Research Institute, Osaka, Japan.*

Thioredoxin (TRX) is a redox regulating protein induced by oxidative stress, however, little is known concerning the role of urinary TRX. We measured urinary levels of TRX by an ELISA in patients with acute kidney injury (AKI) (N=10), mesangial proliferative glomerulonephritis (mPN) (N=10), tubulointerstitial nephritis (TIN) (N=13), IgA nephropathy (N=20), membranous nephropathy (N=6), non-IgA mesangial proliferative glomerulonephritis (N=10), focal segmental glomerulonephritis (N=10), lupus nephritis (N=6), and healthy individuals (N=11). In addition, we performed an experimental study on mice with ischemia-reperfusion, and investigated the origin of urinary TRX. As a result, urinary levels of TRX were predominantly increased in patients with AKI. The quickness of the elevation of urinary TRX was equal or superior to neutrophil gelatinase-associated lipocalin in urine samples from patients on cardiopulmonary bypass surgery. Patients with full recovery of the urinary TRX elevation to the bottom levels showed better outcomes of renal function. A positive correlation was observed between urinary TRX and N-acethy-β-D-glucosaminidase levels in these patients. The urinary levels of TRX correlated with the degree of renal tubular atrophy. In an immunohistochemical study on mice with ischemia-reperfusion, stains of 8-Hydroxydeoxyguanosine and TRX were co-localized. Whereas a diffuse staining in the tubular cytosol in sham-operated mice, TRX became to eccentrically-locate in the apical side of the tubular cytosol, and then only in the urinary lumen in ischemia/reperfusion condition. Western blot analysis in the kidney tissue showed that ischemia/reperfusion caused a drastic decrease of TRX compared with the sham-operation. Urinary TRX excretion increases rapidly as patients with AKI. These findings suggest that urinary TRX dominantly originates from tubular cells and the measurement is useful in the diagnosis and the prognostic prediction of AKI.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1037**

**Histone Deacetylase 1/2 Mediates Proliferation of Renal Interstitial Fibroblasts and Expression of Cell Cycle Proteins** Shougang Zhuang. *Department of Medicine, Brown University School of Medicine, Rhode Island Hospital, Providence, RI.*

Proliferation and activation of renal interstitial fibroblasts is associated with the development and progression of renal fibrosis. We recently reported that the histone deacetylase (HDAC) activity *is required for* activation of renal interstitial fibroblasts. In this study, we further determined the role of HDACs, in particular, HDAC1 and HDAC2, in proliferation of renal interstitial fibroblasts and expression of cell cycle proteins. Inhibition of HDAC activity with trichostatin A (TSA), blocked cell proliferation, decreased expression of the, positive cell cycle regulator cyclin D1, and increased expression of p27 and p57, two negative cell cycle regulators. Silencing HDAC1 or HDAC2 with siRNA also significantly inhibited cell proliferation, decreased expression of cyclin D1, and increased expression of p57. Down-regulation of HDAC2, but not HDAC1, resulted in increased expression of p27. Further, we revealed that HDAC1 and HDAC2 are required for tyrosine phosphorylation and deacetylation of STAT3 (signal transducer and activator of transcription 3), and we showed that blockade of STAT3 with S3I-201 or siRNA decreased renal fibroblast proliferation. Finally, mouse embryonic fibroblasts (MEFs) lacking STAT3 demonstrated a reduced inhibitory effect of TSA on cell proliferation; add-back of wild type STAT3 to STAT3$^{-/-}$ MEFs restored the effect of TSA. Collectively, our results reveal an important role for HDAC1 and 2 in regulating proliferation of renal interstitial fibroblasts, expression of cell cycle proteins and activation of STAT3. Further, STAT3 mediates the proliferative action of HDACs.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1038**

**The Kidney Defends the Urinary System from Infection by Secreting NGAL** Neal A. Paragas, Ritwij Kulkarni, Andong Qiu, Adam J. Ratner, Jonathan M. Barasch. *Medicine, Columbia University, New York, NY.*

NGAL is a critical component of innate immunity because it binds catecholate-siderophores which microorganisms require for the capture of iron. Urinary NGAL (uNGAL) is expressed at mg/L levels after either septic or aseptic diseases of the kidney and has two potential functions, epithelial growth and/or bacteriostasis. Here we examined the activity of uNGAL in the infection of the urogenital tract with uropathogenic E. coli (CFT073). We found that NGAL significantly inhibited bacterial growth in vitro, an effect which could be rescued by the addition of iron. In a murine model of E. coli cystitis without pyelonephritis, the intensity and timing of urinary colony forming units was mirrored by the intensity and timing of uNGAL, including a decrease in uNGAL coincident with the resolution of infection. NGAL-deficient mice cleared E. coli urinary tract infections with delayed kinetics compared to matched wild-types. Likewise, conditional knockout of NGAL in the HoxB7 domain of the CD resulted in a similar phenotype despite normal levels of NGAL in other tissues. We determined the relative contribution of bladder and kidney to the uNGAL pool.Surprisingly, the kidney had a 168.10% percent increase in NGAL message while the bladder had a 7.68% increase in NGAL message in response to infection. Primary cells from the mouse kidney upregulated NGAL transcription in response to lipid A or heat-inactivated CFT073, indicating that the kidney was the critical responsive organ to infections of the bladder. Additional studies suggest that toll-like receptors are critical local sensors of infection. We conclude that uNGAL is essential for rapid clearance of uropathogenic E. coli in a mouse model of acute urinary tract infection and that the kidney responds to even a localized infection by secreting NGAL. These findings provide an explanation for the abundant NGAL secretion from the kidney observed in both septic and some aseptic states, demonstrating that the kidney defends the urinary system via exocrine delivery of NGAL.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1039**

**A High Throughput Respirometric Assay for Mitochondrial Biogenesis and Toxicity** Craig Cano Beeson, Gyda C. Beeson, Rick G. Schnellmann. *Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Mitochondria are a common target of toxicity for drugs and other chemicals, and injury results in decreased aerobic metabolism and cell death. In contrast, mitochondrial biogenesis restores cell vitality and there is a need for new agents to induce biogenesis. Current cell-based models of mitochondrial biogenesis or toxicity are inadequate because cultured cell lines are highly glycolytic with minimal aerobic metabolism and altered mitochondrial physiology. In addition, there are no high-throughput, real-time assays that assess mitochondrial function. We adapted primary cultures of renal proximal tubular cells (RPTC) that exhibit in vivo levels of aerobic metabolism, are not glycolytic, and retain higher levels of differentiated functions and used the Seahorse Biosciences analyzer to measure mitochondrial respiration in real time in multi-well plates. Changes in carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP)-uncoupled respiration, a measure of electron transport chain (ETC) integrity, were used to quantify mitochondrial toxicity and biogenesis. The nephrotoxicants cisplatin, mercury(II) chloride and gentamicin induced statistically significant decreases in the FCCP-uncoupled rates of 13 – 35% relative to controls prior to decreases in basal respiration and cell death. Conversely, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), SRT1720, resveratrol, daidzein, and metformin produced mitochondrial biogenesis in RPTC as measured from statistically significant increases in FCCP-uncoupled respiration rates ranging from 20 – 55% of control. The latter results demonstrate that an increased FCCP-uncoupled rate is a new, phenotypic biomarker of biogenesis. The merger of the RPTC model and multi-well respirometry results in a single high-throughput assay to measure mitochondrial biogenesis and toxicity, and nephrotoxic potential.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1040**

**MicroRNA687 Is a Novel Regulator of PTEN during Renal Hypoxia and Ischemia-Reperfusion** Kirti Bhatt, Qingqing Wei, Zheng Dong. *Department of Cellular Biology and Anatomy, Medical College of Georgia and Charlie Norwood VA Medical Center, Augusta, GA.*

MicroRNAs are small endogenous non-coding RNAs that have recently emerged as important regulators of gene expression in various pathophysiological conditions. In kidneys, conditional knockout of Dicer (a key enzyme for microRNA production) from podocytes leads to glomerular defects and kidney failure, suggesting a role for microRNAs in the maintenance of podocyte homeostasis and function during kidney development. On the other hand, targeted deletion of Dicer from proximal tubules protects against renal ischemia-reperfusion injury (IRI), suggesting the involvement of microRNAs in the pathogenesis of renal IRI. Despite these findings, the specific microRNA species that contribute to renal IRI are not known. To identify the pathogenic microRNAs, we analyzed microRNA expression by microRNA microarray. Out of 220 detectable microRNAs, 138 (~60%) were decreased to varying degrees while 70 microRNAs (~30%) showed up-regulation during renal IRI in C57BL/6 mice. Fifteen microRNA species showed significant changes. Among these microRNAs, we confirmed by real-time PCR and Northernblot analysis that microRNA-687 (miR-687) was drastically up-regulated during renal IRI. Consistently, miR-687 was induced during hypoxic incubation of renal proximal tubular cells in vitro. Bioinformatic analysis suggested that the tumor suppressor PTEN is a likely target gene of miR-687. We further showed that, when miR-687 was induced by hypoxia, there was a concomitant decrease of PTEN expression. Notably, blockade of miR-687 with anti-miR-687 LNA could prevent PTEN decrease during hypoxic incubation and enhance apoptosis. The 3'UTR of PTEN gene contains miR-687 binding sequence and miR-687 mimic could decrease luciferase activity in cells transfected with a luciferase reporter vector bearing PTEN 3'UTR. Together, this study has identified miR-687 as a novel regulator of PTEN during renal hypoxia and ischemia-reperfusion injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1041**

**Novel Use of Ultrasound To Assess Renal Reperfusion and P-Selectin Expression Following Unilateral Renal Ischemia in Mice** Erika I. Boesen, Jennifer C. Sullivan. *Vascular Biology Center, Medical College of Georgia, Augusta, GA.*

Incomplete restoration of blood flow following ischemia likely contributes to hypoxia-induced injury. We tested if high resolution ultrasound (Vevo 770) is a viable non-invasive alternative to traditional perivascular flow probe methods to assess reperfusion after 60 min of unilateral renal ischemia in mice. Renal blood flow velocity was measured by pulse-wave Doppler in anesthetized C57Bl/6 mice prior to renal ischemia/reperfusion (I/R) surgery,

and at 1 and 24 h post-ischemia. Male and female mice (n=6-7) displayed similar deficits of renal blood flow at 1 h (56±9% and 69±10% of baseline; P=0.4) and 24 h post-ischemia (40±8% and 46±7% of baseline; P=0.6). In a separate group of male mice, perivascular flow probes revealed a similar deficit of blood flow recovery at 1 h post-ischemia (66±6%, n=8) to that seen with ultrasound. Additional studies used antibody-tagged contrast agent to test if ultrasound imaging could be used to measure I/R-induced increases in endothelial adhesion molecule expression. P-selectin targeted contrast agent was infused i.v. 1 h post ischemia, and contrast agent signal intensity compared in post-ischemic and contralateral kidneys before and after a high frequency "destruction sequence" that disrupts adherent contrast agent. Thus, a fall in signal intensity is proportional to the adherence of contrast agent to the endothelium. Male and female mice (n=5-6) displayed a significant fall in signal intensity in the post-ischemic renal cortex (-35±6% and -33±6%; P<0.001 versus contralateral kidney), whereas the contralateral kidney showed virtually no change in signal intensity (-7±3% in both groups). These data indicate a significant up-regulation of P-selectin in the cortical endothelium of the post-ischemic kidney after 1 h reperfusion. There were no sex differences in any variables measured. This study validates the use of high resolution ultrasound as a non-invasive means to assess both renal reperfusion and vascular endothelial adhesion molecule expression in mice. This methodology has the potential to address key mechanistic questions relating to the pathogenesis of renal damage in multiple disease models.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1042

**The Contribution of EGR-1 to Renal Injury Following Renal Ischaemia and Reperfusion Injury Is Dependent on PAR-1** Jonathan H. Erlich.[1] *[1]Dept of Nephrology / Prince of Wlaes Clinical School, UNSW, Sydney, NSW, Australia; [2]Dept of Paediatrics/ Prince of Wlaes Clinical School, UNSW, Sydeny, NSW, Australia; [3]Departement of Nephrology, Prince of Wales Hospital, Randwick, NSW, Australia.*

Background: EGR-1 is a zinc finger transcription factor that is normally expressed in cells at low level but upon appropriate cellular stress such as hypoxia is found to be rapidly elevated and then translocated to the nucleus where it stimulates pro inflammatory mediators. We have shown that protease activated receptor -1 (PAR-1) is an important mediator in renal IR injury and EGR-1 has been suggested to regulate PAR-1. Further EGR-1 has also been shown to be an important mediator in various models of ischaemia reperfusion injury.

Methods and Results: Using a mouse model of renal IR with 25 min ischaemia and up to 24 h reperfusion we found that EGR-1 assessed by real time RT PCR was similarly and maximally elevated in both C57BL6 mice and PAR-1-/- mice at 2 h reperfusion (C57BL6 19.7±7.48 vs 0.13±0.04, and PAR-1-/- 22.9±4.9 vs 0.8±0.3, WT vs Sham p<0.05) decreased at 5 h (C57BL6 11.8±3.9 vs 0.25±0.18 and PAR-1-/- 3.2±1.43 vs 0.42±0.26 IR vs sham P< .05) with a decrease toward baseline by 24 h reperfusion. However, PAR-1-/- mice developed less severe renal injury compared to WT mice (123 ± 24.8 micromol/L vs 216.2 ± 7.2 micromol/L, p< 0.01). Further, an EGR-1 regulated gene tissue factor was similarly expressed in PAR-1-/- and WT mice. The data suggests that EGR-1 is an upstream mediator of PAR-1 and that in this model EGR-1 effects are PAR-1 dependent.

This has important implications for designing therapeutic strategies to limit the effect of inflammatory injury following renal IRI.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1043

**Diagnosis and Staging of Acute Renal Failure in Cardiac Surgery: Comparison between AKIN and RIFLE Criteria** Carmen Bernis,[1] Ana Perez de José,[1] Rosario Madero,[2] Pablo Alonso,[3] Juan Bustamante,[4] Jose-Antonio Sanchez-Tomero.[1] *[1]Nephrology, H.U. Princesa, Madrid, Spain; [2]biostatistics unit; [3]ICU; [4]Cardiac Surgery, .*

Several diagnostic criteria have been proposed for AKI classification .The aim of this study is to compare AKIN ( Crit Care; 2007) and RIFLE (Critical Care ;2004) classifications for AKI diagnosis and for predicting outcome in a cohort of patients who underwent cardiac surgery

**Methods**

Patients who underwent coronary artery bypass(30%), valve heart surgery(70%) or both, between January 2007 and December 2008 were retrospectively evaluated. Data were prospectively collected. The statistical package SPSS was used.

**Results**

601 patients (mean age 65,9 ± 11,7 years; 40,3 % females; mean Cleveland Score 3.3 ± 1.8 were evaluated.

AKIN criteria compared to RIFLE allowed the identification of more patients as having any stage of AKI (16.6% vs 7.2%). AKIN increases patients classified as Stage I (category R in RIFLE) from 4.2% to 13.6%. 57 patients (9,5%) were not considered AKI according to RIFLE classification and were stage 1 according to AKIN criteria. No significant differences were observed for stage 2 (injury in RIFLE) and for stage 3 (failure in RIFLE). RIFLE and AKIN concordance is moderate, kappa index 0,55 (IC 95% 0,45-0,65, p<0,05). Using Mc Nemar test discrepancies are significant (p<0,001).

The area under the ROC curve for hospital mortality was 0.70 for AKIN criteria ($P < 0.001$) and 0.66 for RIFLE criteria ($P < 0.001$). However, in these 57 patients with AKIN stage 1 , (and no AKI according to RIFLE), mortality was 18.3% higher than in patients with no AKI ( 4,6%, p=0,05) with a risk of death 3,4 times higher (OR 3.4, IC 95% 1,44-7,99).

**Conclusions**

1- AKIN criteria improves the sensitivity compared to RIFLE. 2-As global classifications, considering all stages, there are no significant differences between RIFLE and AKIN . 3- However, patients with AKIN stage 1( considered as no AKI according to RIFLE), have a significant higher mortality than patients with no AKI.4-The modification of the RIFLE criteria proposed by AKIN, has epidemiological relevance , is associated with mortality and can help to identify more patients at risk

Disclosure of Financial Relationships: nothing to disclose

## F-PO1044

**BMP-2 Induces a Pro-Fibrotic Phenotype in Adult Renal Progenitor Cells (ARPC) through Nox4 Activation** S. Simone, C. Cosola, Fabio Sallustio, A. Loverre, G. Grandaliano, Francesco Paolo Schena, G. Pertosa. *Nephrology, Dialysis and Transplantation Unit, Univ. of Bari, Italy.*

CD133+CD24+Pax-2+ ARPCs, isolated in human adult kidney, contribute to repair processes featuring acute kidney injury (AKI). BMPs are involved in differentiation, modeling and regeneration. The role of BMP-2 in AKI is still unclear. Aim of the study is to evaluate the expression of BMP-2 in ARPCs and its biological action on these cells.

BMP-2 expression in ARPCs was studied in vivo by confocal microscopy on adult human renal tissue. ARPCs were isolated by magnetic cell sorting. BMP-2 and BMP receptors (BMPRs) gene and protein expression were evaluated by RT-PCR and ELISA/ immunoblotting. Myofibroblastic markers (alpha-SMA, collagen-I, fibronectin) and Nox4 (NADPH oxidase renal isoform) protein expression were studied by immunoblotting. Intracellular reactive oxygen species (ROS) production was measured by 2',7' dichlorodihydrofluorescein fluorescence. Superoxide production by NADPH oxidase was studied by chemiluminescence.

BMP-2 is basally expressed in normal adult human kidney by ARPCs. In vitro, these cells express BMPRs, ALK-2, 3 and 6. TNF-alpha (200 U/ml) significantly induced both BMP-2 gene [fold change (fc) 1.7±.2 vs basal, p=.03] and protein expression (basal 48h 65±.1; TNF-alpha 48h 152±19 pg/ml, p=.002) in ARPCs. After incubation with BMP-2 (30 ng/ml) ARPCs showed an increased ROS production (basal 36±15; BMP-2 15' 65±17 AU, p=.05), NADPH oxidase activity (basal 86±38; BMP-2 15' 212±75 AU, p=.01) and Nox4 protein expression (basal .3±.2; BMP-2 5' 1±.6 AU, p=.03). BMP-2 incubation for 5 days induced α-SMA (basal .3±.1; BMP-2 .8±.2 AU, p=.02), collagen-I (fc 2.4±.6 vs basal, p=.03) and fibronectin (fc 1.4±.1 vs basal, p=.04) protein expression in ARPCs, but not in HK-2 cells. Incubation with the anti-oxidant N-acetyl-cysteine reverses BMP-2-induced alpha-SMA expression (fc BMP-2 2.2±.6 vs basal p=.03; BMP-2+NAC 1.3±.4 vs BMP-2 p=.04).

We demonstrated for the first time that: 1. ARPCs express BMP-2; 2 this expression is significantly induced by inflammatory stimuli; 3. BMP-2 may commit ARPCs towards a myofibroblastic phenotype. 4. This pro-fibrotic effect is mediated by Nox4 activation.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1045

**Trends in Acute Kidney Injury Associated with Coronary Stenting in the United States from 2000 to 2007** Ankit Sakhuja, Abhishek Deshmukh, Nilay Kumar, Rahul S. Nanchal, Aaron T. Dall, Gagan Kumar. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

**Introduction:**

In hospital mortality of acute myocardial infarction (AMI) has improved over the past few years due to early cardiac catheterization. Acute Kidney Injury (AKI) can occur either due to hypotension resulting from AMI, athero-embolic phenomenon or as a complication from the contrast dye. We studied the trends of AKI in patients admitted with AMI.

**Methods**

Retrospective observational study was utilized to analyze the Nationwide Inpatient Sample from year 2000 to 2007. The adult patients (>18 years age) with a primary discharge diagnosis of AMI were identified using ICD-9 code. Pearson correlation and Chi square were used to compare the variables for unadjusted analysis and logistic regression was used to obtain adjusted odds ratios. The model adjusted for age, sex, race, hospital characteristics and the risk factors for developing AKI like diabetes mellitus, hypertension, chronic kidney disease and hypovolemia including shock. α was set at 0.05.

**Results**

There were an estimated 15,459,503 admissions with the primary diagnosis of AMI over 8 years. The frequency of AKI increased from 2.6% in 2000 to 6.5% in 2007. The odds for developing AKI was 1.52 times higher in 2007 when compared to 2000 (OR 1.52, 95%CI 1.48-1.57). AKI was an independent predictor of mortality in patients after AMI (OR 4.04; 95%CI 3.89-4.19). There was a significant decrease in mortality in patients with AMI with AKI (2.8% in 2000 vs. 1.4% in 2007).

**Conclusion**

The in hospital frequency of AKI has increased 1.5 times in patients admitted with and undergoing catheterization for AMI over this new millennium. The all cause in hospital mortality has nevertheless decreased in patients following treatment of AMI whom developed AKI.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1046**

**Distinct Effects on Long Term Function of Injured and Contralateral Kidneys Following Acute Unilateral Ischemia Reperfusion** David P. Basile, Ellen Leonard, Jessica Friedrich. *Cellular & Integrative Physiology, Indiana University, Indianapolis, IN.*

AKI induced by ischemia reperfusion injury in rats can have persistent effects function, predisposing salt-sensitive hypertension and CKD. Immune suppression can ameliorate salt-sensitive hypertension and renal fibrosis following recovery from AKI. The current study was designed to investigate further the hypothesis that the chronic effects of AKI on salt sensitivity are mediated, in part, via circulating factors secondary to injury. SD rats (maintained 0.4% NaCl diet) were subjected to 40 minutes of left unilateral renal ischemia or sham-surgery. The effect of unilateral I/R on the contralateral kidney was evaluated by removal of the (left) injured kidney at 5 weeks of recovery and subjecting rats to elevated salt intake (4% NaCl) for an additional 4 weeks. Blood pressure values, measured by telemetry, were significantly higher in animals from which a previously injured kidney had been removed vs sham-operated Unx controls (MAP 124 ± 7 vs 108± 3 mm Hg). Rats from which an injured kidney had been removed had similar creatinine clearance and protein excretion rates as sham-operated UNx controls. The remaining untouched kidney from I/R treated rats showed significantly greater hypertrophy relative to the sham-operated UNx controls (3.5 ± 0.2 g vs 2.9 ± 0.3g, P < 0.05), showed moderate evidence of increased interstitial SMA and CD4+ interstitial cells relative to shams. A third group of animals evaluated the effect of a solitary injured kidney (by removing the untouched contralateral kidney) and subsequent exposure to elevated salt intake. Animals with a solitary post I/R kidney showed an elevation in blood pressure, a 5-fold increase in protein excretion and a significant ~75% reduction in creatinine clearance. The solitary post I/R kidney was significantly atrophied relative to shams (2.2 ± 0.3 g) and had widespread interstitial fibrosis. The data suggest that the chronic effects of AKI on hypertension and CKD are mediated primarily via direct effects on tissue injury, but are also partially mediated by indirect effects of I/R injury mediated possibly via circulating humoral factors.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1047**

**Relation between Obstructive Sleep Apnea and Acute Kidney Injury** Michal P. Nowicki,[1] Anna Zawiasa,[1] Malgorzata Kolodziejska,[1] Maciej Banasiak,[1] Piotr Bialasiewicz,[2] Dariusz Nowak.[2] *[1]Department of Nephrology, Hypertension and Kidney Transplantation, Medical University of Lodz, Lodz, Poland; [2]Chair of Clinical and Experimental Physiology, Medical University of Lodz, Lodz, Poland.*

**Introduction**. Obstructive sleep apnea (OSA) is characterized by recurrent episodes of hypoxia and hypercapnia that may cause repeated ischaemia-reperfusion injury and thereby subclinical acute kidney injury (AKI).

The aim of the study was to assess the effect of OSA on urine markers of AKI, arterial stiffness and central aortic pressure.

**Patients and methods**. 20 males with polysomnography-based diagnosis of sleep apnea syndrome and $GFR_{CKD-EPI}$> 60ml/min/1,73 m² were recruited to a prospective, interventional pilot study. The patients were divided into two groups, i.e. with mild OSA (8 patients, age 39,6±9 years, BMI 29±4 kg/m², $GFR_{CKD-EPI}$ 88,2±17,7 ml/min, apnea-hypopnea index (AHI) 5,8±3,8 episodes/hour) and severe OSA (12 patients, age 49,6±9,4 years, BMI 38,0±6,0 kg/m ², $GFR_{CKD-EPI}$ 82,9±14,1 ml/min, AHI 68,5±24,3 episodes/hour). Central blood pressure in the aorta and pulse wave velocity **(**PWV) were measured with ECG-synchronized tonometry (PulsePen, DiaTecne., Milan, Italy). Serum creatinine hsCRP and urine AKI markers: cystatin C, NGAL, L-FABP were assessed both before and after polysomnography (diagnostic night). Patients with severe OSA were treated with the continuous positive airway pressure (CPAP). After 6 to 8 weeks of CPAP therapy all measurements were repeated as at baseline (therapeutic night).

**Results**: Both urine NGAL and L-FABP tended to increase after both diagnostic and therapeutic night but the changes were not significant. Only urine cystatin C significantly increased after the diagnostic night in patient with severe OSA (from 84.1±37.4 to 117.5±33.3 ng/ml; p=0,002). The increase of cystatin C was lower after CPAP therapy (from 93.2±48.4 to 108.9±41.9 ng/ml; p=0,045). Aortic stiffness (PWV and Aortic Index) did not correlate with severity of OSA.

**Conclusion**: This pilot study results seem to support the concept that OSA may cause subclinical acute kidney injury and that the treatment of severe OSA with CPAP may reduce an extend of the injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1048**

**Impact of Acute Kidney Injury after Acute Myocardial Infarction on Short- and Mid-Term Survival: A Contemporary Analysis** Ana Cortesao Costa, Luis Resende, José Lopes, Antonio Gomes da Costa. *Nephrology and Kidney Transplantation, CHLN-HSM, Lisboa, Portugal.*

Acute kidney injury (AKI) often complicates patients with Acute Myocardial Infarction (AMI) and carries an ominous prognosis. We retrospectively evaluated the impact of AKI, defined and stratified based on RIFLE (Risk, Injury, Failure, Loss and End-stage kidney disease) criteria, on in-hospital mortality, and on survival at 3 years of follow-up among patients who had hospital discharge. Chronic kidney disease patients were excluded from analysis. In all, 264 patients with AMI hospitalized in the Department of Cardiology of our Hospital in 2006 were studied. AKI occurred in 65 patients (24.6%) as follows: 8.7% were on Risk, 8.3% were on Injury and 7.6% were on Failure. In-hospital mortality was higher in AKI ($P$<0.0001), as compared with no AKI patients, and it increased in accordance to AKI severity (Risk, Injury, Failure; $P$=0.009). Adjusting for age, gender, race, ST-segment elevation, AMI location, ejection fraction, vasopressors use, and need of mechanical ventilation, AKI (AKI odds ratio 4.57, 95% confidence interval 1.48-14.16, $P$=0.008) was as independent predictor of in-hospital mortality. Injury and Failure classes also predicted independently in-hospital mortality (Injury odds ratio 5.36, 95% confidence interval 1.25-23 $P$=0.024; Failure odds ratio 13.34, 95% confidence interval 2.83-62.93, $P$=0.01), while Risk class did not (Risk odds ratio 2.1, 95% confidence interval 0.45-9.7, $P$=NS). Survival at 3 years of follow-up was lowest among AKI patients ($P$<0.0001), as compared with patients with no AKI. The negative impact of AKI on 3-year survival was particularly relevant for moderate and severe AKI forms (Risk, Injury, Failure; $P$<0.0001). After adjusting for age, gender, race, and comorbidity (diabetes mellitus, hypertension, previous AMI, heart failure, cerebrovascular disease, and peripheral vascular disease), AKI increased 3-year mortality. In addition, Injury and Failure classes also has an independent detrimental effect on 3-year mortality, while Risk class did not. AKI is frequent after AMI and it has a negative impact on short-and mid-term survival, mainly in its moderate and severe forms.

Disclosure of Financial Relationships: nothing to disclose

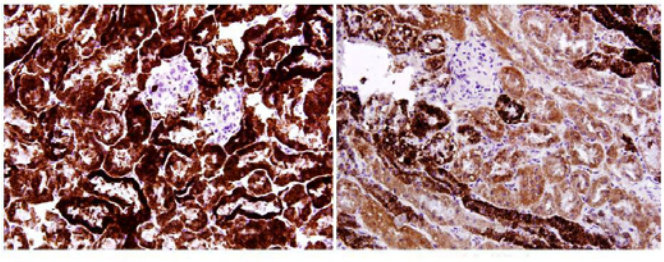
**F-PO1049**

**Mitochondrial Impairment without Cell Death in Sepsis-Induced Acute Kidney Injury** Amit Bardia,[1] Zsuzsanna Zsengeller,[2] S. Ananth Karumanchi,[1] Isaac E. Stillman,[2] Samir M. Parikh.[1] *[1]Nephrology, Beth Israel Deaconess Medical Center, Boston, MA; [2]Pathology, Beth Israel Deaconess Medical Center, Boston, MA.*

BACKGROUND: Recent case series and meta-analyses have shown a paucity of structural changes in kidney specimens obtained during sepsis-induced AKI. We used the murine endotoxemia (lipopolysaccahrides, LPS) model of sepsis-induced AKI to explore the role of functional, rather than structural, impairments in the septic kidney.

RESULTS: 10 mg/kg LPS given intraperitoneally to 8-week old BL6/J male mice increased Blood urea nitrogen (BUN) and creatinine at 18 and 42 hrs. Apoptosis, as measured by cleaved caspase-3 immunohistochemistry, and necrosis were unchanged compared to controls. Despite a marked reduction in renal blood flow, renal tissue oxygenation, as measured by BOLD MRI, was also unchanged, suggesting an impairment of oxygen consumption in the septic kidney. Enzyme activity of cytochrome C oxidase, a key member of the electron transport chain, was greatly diminished in the septic kidney.

Figure 1: Cytochrome C oxidase staining in control (A) and septic (B) mice kidneys.



A (Control kidneys) B (Septic kidneys)

Moreover, treatment of cultured renal proximal tubular cells with TNF-alpha reduced both basal oxygen consumption and uncoupled oxygen consumption without reducing mitochondrial abundance, thus specifically implicating a functional mitochondrial impairment.

CONCLUSIONS: Mitochondrial dysfunction is more strongly associated with septic AKI than cell death. We speculate a contributory role for this phenomenon in the development of depressed renal function during sepsis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1050**

**Genetic Polymorphism of TGB-β and IFN – γ and Its Influence on the Occurrence of Acute Kidney Injury (AKI)** Caren Cristina Grabulosa, Maria Dalboni, Marie Beata Redublo Quinto, Edgar Ferreira Cruz, Julio Cesar Monte, Marcelino Durao, Oscar Pavao Dos Santos, Miguel Cendoroglo, Marcelo Costa Batista. *Medicine/Nephrology, Universidade Federal de São Paulo, Sao Paulo, Brazil.*

Introduction: We conjecture that genetic polymorphisms of TGF-beta and INF-gamma cytokines have crucial role in the outcome to AKI in critically patients.

Objective: To assess the genetic polymorphism of TGF - β and IFN-γ in patients with SIRS in the occurrence of AKI in patients admitted in intensive care unit (ICU).

Materials and Methods: We performed a nested case-control study with 797 critically patients admitted in ICU. The AKIN criteria were used to characterize patients with AKI. We evaluated polymorphism TGF-β and IFN -γ in 150 patients who developed AKI (case group) and a control group of 150 individuals without AKI. We investigated the polymorphism codon 10T/C and codon 25C/G of TGF-β and intron +874T/A IFN-γ by PCR-SSP.

Results: Patients who progressed to AKI showed higher APACHE score (20+/- 7 vs. 17+/- 5, p < 0.01) and CRP (9+/- 9 vs. 7+/- 8, p = 0.01). In univariate analysis we observed that patients with AKI had a higher frequency of TC GG genotype (high producer) for TGF-beta polymorphism compared to non-AKI (19.5% vs. 7.6%, p < 0.01). Similarly, patients

with AKI showed a higher frequency of TC genotype (intermediate producer) for IFN-Y polymorphism than non-AKI group (39.8% vs. 17%, p<0.01). In respect to the phenotype, we observed an increased prevalence of high producers of TGF-beta (58.4% vs. 26.6%, p< 0.01) and IFN-Y (50.7% vs. 21 0%, p<0.01) in patients with AKI compared without AKI. However, in multivariate analysis model taking both polymorphisms as possible independent predictors adjusted by ethnicity, age, gender and APACHE II score, the TC GG and TC genotypes did not remained statistically related with AKI occurrence.

Conclusions: Although we found an increased prevalence of high producer phenotypes and a high expression of 10T/C 25C/G TGF-β and +874T/A INF-γ polymorphism in patients who developed AKI in ICU setting, this study did not show that these polymorphism could independently determine AKI occurrence.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1051

**Microarray Analysis of Isolated Proximal Tubules in Rats with Cisplatin-Induced Nephrotoxicity** Satohiro Masuda,[1] Kumiko Nishihara,[1] Atsushi Yonezawa,[1] Aiko Ozawa,[1] Takaharu Ichimura,[2] Joseph V. Bonventre,[2] Ken-Ichi Inui.[1] [1]*Pharmacy, Kyoto University Hospital, Kyoto, Japan;* [2]*Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

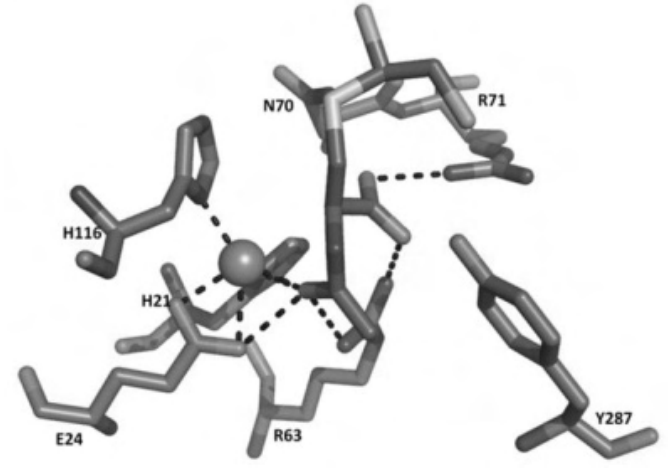
**[Backgrounds]** Cisplatin has been identified as a substrate of the kidney specific organic cation transporter OCT2/SLC22A2, and its cellular uptake into the tubular epithelium was found to be a crucial step for subsequent nephrotoxicity (Yonezawa et al., Biochem Pharmacol, 70, 1823-31, 2005; Tanihara et al., Biochem Pharmacol, 78, 1263-71, 2009). Therefore, cisplatin-induced nephrotoxicity may be characterized by a gene expression profile in proximal tubules, which may serve as an early specific marker for detection of cisplatin-induced tubular damage. **[Aim]** we have used microarray analysis to evaluate gene expression in isolated proximal tubules of the rats with cisplatin-induced nephrotoxicity. **[Methods]** Renal proximal tubules were isolated under microscopy, and transcriptome data were collected with Rat Genome Survey Microarray® (Applied Biosystems) (Nishihara et al., Am J Physiol Renal Physiol, 298, F923-34, 2010). **[Results]** Among 17,000 transcripts examined, the mRNA expression levels of chemokine (C-C motif) ligand (Ccl) 2 (as also called MCP-1) as well as kidney injury molecule 1 (Kim-1) was significantly increased after treatment with cisplatin. Immunofluorescent analysis showed that the positive signals for Ccl2 were initially and specifically detected in the proximal tubular epithelial cells. In addition, urinary Ccl2 as well as Kim-1 protein was significantly increased approximately 3-fold compared to controls on the day after administration of cisplatin (5mg/kg intraperitoneal administration), respectively. However, no changes were observed in the levels of plasma creatinine, blood urea nitrogen and plasma Ccl2. **[Conclusions]** Urinary Ccl2 and Kim-1 are suggested to serve as sensitive and noninvasive markers for early detection of tubular injury secondary to cisplatin treatment. The production of Ccl2 at the proximal tubular cells may play a pathophysiological role contributing to the deterioration the proximal tubule that characterizes cisplatin-induced nephrotoxicity.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1052

**Structural Aspects of Mercapturate Deacetylation by Aminoacylase 3 in Renal Proximal Tubule** Kirill Tsirulnikov,[1] Jennifer Hsieh,[2] Natalia Abuladze,[1] Nathaniel Magilnick,[1] Debra Newman,[1] Jeff Abramson,[2] Ira Kurtz,[2] Alexander Pushkin.[2] [1]*Medicine/Nephrology, UCLA, Los Angeles;* [2]*Physiology, UCLA, Los Angeles.*

Toxic mercapturates (N-acetyl conjugates of cysteine) are known to selectively damage renal proximal straight tubule. We have recently shown that their deacetylation mediated by aminoacylase 3 (AA3) is a key step in the mercapturate induced nephrotoxicity. Although inhibition of AA3 may be used to ameliorate nephrotoxic effects of mercapturates, the absence of a high-resolution structure of AA3 significantly limits generation of highly specific inhibitors of AA3. Here we report the crystal structure of AA3 in complex with a toxic mercapturate N-acetyl-1,2-dichlorovinyl-L-cysteine (shown in purple).




The substrate binds through a hydrogen bond network (dashed lines) to the *N*-acetyl-α-amino carboxylic acid component of the substrate while the side chain constituent is secured solely by van der Waals interactions (not shown). Comparison of the structures of free AA3 and AA3 complexed with the mercapturate in conjunction with biochemical analysis indicate that AA3 has a dynamic substrate recognition mechanism capable of accommodating a broad range of substrates. The results provide the basis for generation of highly specific inhibitors of AA3 that may be used for amelioration of mercapturate nephroxicity. Funding Source: NIH ES012935.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1053

**Involvement of DDAH-ADMA Axis in Accelerated Renal Injury in Acute Ischemia-Reperfusion Injury** Yosuke Nakayama, Seiji Ueda, Yusuke Kaida, Ryotaro Ando, Kei Fukami, Seiya Okuda. *Division of Nephrology, Department of Medicine, Kurume University, Kurume, Fukuoka, Japan.*

***Background.*** Recent evidence suggests that injury to the renal vasculature may play an important role in the pathogenesis of ischemic acute kidney injury (AKI). Since nitric oxide (NO) is a vasodilator and known to play an important role in the maintenance of renal microvasculature and its flow and may exert a protective role against AKI, it is conceivable that asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, could enhance tubulointerstitial injury during AKI by decreasing NO bioavailability. However, the association between ADMA and AKI remain to be elucidated.

***Methods.*** To determine the role of ADMA in AKI, eight-week-old male C57BL/6J (wild) mice and DDAH-1, a key enzyme for ADMA degradation, transgenic (Tg) mice were used in this study. Ischemia- reperfusion (IR) injury were performed in wild mice with (n=10) or without (n=15) ADMA infusion (0.01mg/kg/min) and Tg mice (n=5). Sham operated mice (n=10) were used as control. Renal function, morphology of acute renal injury,and rarefaction rate of renal capillaries were compared after IR. Tissue or plasma levels of ADMA were measured by HPLC. ADMA-related related enzymes such as DDAH and PRMT, an enzyme for ADMA synthesis, were measured by western blot analysis.

***Results.*** Western blot analysis revealed significant decreases in renal DDAH1 expression levels during IR injury associated with increased ADMA levels in wild mice. Compared with IR-treated wild mice, ADMA infusion markedly enhanced capillaries rare faction, tubular injury and increases in BUN levels, whereas these changes were significantly attenuated in IR-treated DDAH Tg mice.

***Conclusion.*** These results strongly suggest that the active involvements of ADMA-DDAH axis in the pathogenesis of IR injury. ADMA-DDAH axis could be a novel target for patients with AKI.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1054

**Development of an *In Vitro* Model To Investigate Changes in Kidney-Derived Exosomes with Disease** Jonathan M. Street, Matthew A. Bailey, David J. Webb, James W. Dear. *Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom.*

Acute kidney injury (AKI) is a significant cause of mortality. Our ability to detect and treat AKI has been limited and mortality has remained high. Although a number of new biomarkers for the detection of AKI are currently under development our understanding of AKI is still limited. A number of groups have studied exosomes, small lipid membrane bound vesicles released by cells, as a method to study cellular state within the kidneys. We have confirmed the presence of exosomes in the urine using the markers Tsg-101, Flotillin-1 and CD24, density determination on a sucrose gradient and by direct visualisation using transmission electron microscopy. Using GeLC-MS/MS we have identified 88 proteins within the urinary exosomes which represent all regions of the nephron. To study the factors effecting exosome release and the effect of exogenous exosomes on the kidney we have developed an *in vitro* murine cortical collecting duct (mCCD) cell model. These cells constitutively release exosomes as determined by western blot for known exosomal markers and density determination. Release of exosomes can be enhanced by ionomycin, a known promoter of exosome release. We have previously shown that the transcription factor cJun is present in the urinary exosomes of septic patients but not healthy controls. In contrast cJun was present in the mCCD cell derived exosomes prior to stimulation and was not increased following stimulation with TNFα. We also investigated the effect of cisplatin treatment on the mCCD cell derived exosomes. It has previously been suggested that exosomal fetuin-A is a marker of cisplatin toxicity. In our model cisplatin treatment, at sub-apoptosis inducing concentrations, did not increase fetuin-A in exosomes. Characterisation of exosome release under physiological, inflammatory and toxic conditions is ongoing. The developed cell model has the potential to connect changes seen in the exosomal proteome to the changes seen in the kidney with AKI.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1055**

**Monitoring of Urinary L-FABP and Renal Microcirculation in Mouse Radiocontrast-Induced Acute Kidney Injury** Kousuke Negishi,[1] Kent Doi,[1] Tokunori Yamamoto,[2] Takeshi Sugaya,[3] Toshiro Fujita,[1] Eisei Noiri.[1] *[1]Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan; [2]Urology, Nagoya University, Nagoya, Aichi, Japan; [3]CMIC Co. Ltd., Tokyo, Japan.*

Radiocontrast-induced acute kidney injury (RC-AKI) remains to be a critical issue because of its association not only with renal outcome but with the impact on survival. Since L-type fatty acid-binding protein (L-FABP), expressed in proximal tubules of the human kidney, is established as a fine sensor of renal hypoxia by basic and clinical studies (*J Am Soc Nephrol 18:2894-902, 2007*), we evaluated whether urinary L-FABP is a useful marker for early diagnosis of RC-AKI using human L-FABP transgenic mice.

Mice were subjected unilateral nephrectomy 7 days before RC injection. After water restriction for 24 h, mice were intraperitoneally injected indomethacin (I), L-NAME (L) and low-osmolar RC iohexol (3 g Iodine/kg, R). Equivalent volume of saline was injected as control for each reagent (S). Using metabolic cages, serial urine and blood sampling was carried out. Moreover, renal peritubular capillary (PTC) blood flow at 12 h was directly monitored by intravital CCD videomicroscope. All mice were sacrificed at 48 h.

Urinary L-FABP in the animals with indomethacin, L-NAME, and RC injection (I-L-R) at 12 h significantly increased up to 3000-fold from the baseline. BUN was also peaked at 12 h [0 h; 44.6 ± 8.7, 12 h; 92.1 ± 5.1 (mg/dl, Mean ± SE)]. Significant increase of urinary NAG was shown at 24 h, but not at 12 h. PTC flow velocity in I-L-R was significantly decreased than that in S-S-S.

Histological analysis revealed vacuolation and increased L-FABP expression in proximal tubules were limited only in ILR. Moreover, ROC analysis for predicting vacuolation at 48 h demonstrated an excellent accuracy of urinary L-FABP at 12 h and at 24 h (area under the ROC curve; 0.917, 0.955, respectively). Furthermore, Log converted urinary L-FABP showed a positive correlation with BUN at 12h ($r^2 = 0.790$, $p = 0.00001$).

In summary, urinary L-FABP demonstrated good performance as a RC-AKI biomarker for detecting both structural and functional change of the kidney.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1056**

**Mitochondrial Damage Is Attenuated during Hypoxia/Reoxygenation by Delipidated Bovine Serum Albumine and Glutamine** Özlem Köse, Oliver Witzke, Tobias Tuerk, Andreas Kribben, Thorsten Feldkamp. *Nephrology, University Duisburg-Essen, Essen, Germany.*

We have shown that hypoxia induced accumulation of nonesterified fatty acids (NEFA) plays a pivotal role in the pathogenesis of mitochondrial damage in proximal tubule (PT) during hypoxia and reoxygenation (H/R). We studied, whether binding of NEFA during H/R with delipidated bovine serum albumine (dBSA) attenuates mitochondrial damage. Mitochondrial function was assessed by measuring ATP production of freshly isolated rat PT and accumulation of NEFA was assessed during H/R. 45 min of H lowered tubular ATP content after 60 min of R significantly compared to 105 min of normoxia (Tab. 1, $p<0.01$ vs. normoxia n=5-8). Addition of glutamine during R resulted in an increased ATP content compared to H/R alone, even though it was still significant lower than during normoxia (Tab. 1, $p<0.05$ vs. H/R alone, n=6). Addition of dBSA during R to bind NEFA resulted in an increased ATP content after R compared to H/R alone (Table 1, $p<0.05$ vs. H/R alone n=4). The combination of glutamine with dBSA during R fully recovered ATP content to levels in normoxia (Tab. 1, $p<0.01$ vs. H/R+glutamine, n=4). Tubular NEFA levels were significantly higher after H/R without or with glutamine compared to normoxia (Tab. 1, $p<0.01$ vs. normoxia, n=4-8). Addition of dBSA during H/R without or with glutamine further increased NEFA levels compared to H/R alone, because NEFAs bound to dBSA were also detected by the assay (Tab. 1, $p=0.075$ and $p<0.01$ resp. vs. H/R alone, n=4-8).

| Condition | ATP (nmol/mg protein) | NEFA (nmol/mg protein) |
|---|---|---|
| Normoxia | 11.01± 0.38 | 3.32±0.21 |
| H/R | 2.88± 0.40 | 7.90±1.20 |
| H/R + Glutamine | 5.88± 0.37 | 6.68± 2.02 |
| H/R +dBSA | 4.08± 0.37 | 12.14± 2.38 |
| H/R+ Glutamine+ dBSA | 11.75±0.98 | 15.79± 2.16 |

Table 1

In summary, dBSA and glutamine improve mitochondrial function during H/R induced mitochondrial damage. NEFA accumulate during H/R which is further enhanced by dBSA, presumably by binding NEFA and preventing mitochondrial NEFA metabolism. A mechanism for the attenuation of mitochondrial damage by dBSA could be the prevention of oxygen radical production induced by mitochondrial NEFA metabolism.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1057**

***In Vivo* Architecture and Regulation of Heme Oxygenase-1 Gene Expression in "Humanized" Transgenic Mouse** Junghyun Kim, Abolfazl Zarjou, Amie Traylor, Reny Joseph, Carl A. Frizell, Anupam Agarwal. *Medicine, Division of Nephrology, University of Alabama at Birmingham, Birmingham, AL.*

Heme oxygenase-1 (HO-1) catalyzes the degradation of free heme and produces carbon monoxide, iron, and biliverdin. In addition to heme degradation, HO-1 is known to protect against various cellular insults and disease states including acute kidney injury, atherosclerosis, vascular restenosis and transplant rejection. HO-1 gene expression is regulated at the level of transcription initiated by changes in chromatin conformation. However, there is no currently available *in vivo* model to study mechanisms of the human HO-1 gene transcription. To enable such an in vivo analysis, we generated transgenic mice to study transcription of the human HO-1 gene by integrating a 87-kb bacterial artificial chromosome (BAC), a portion of human chromosome 22 containing the human HO-1 gene and its regulatory elements with the mouse genome. We obtained two founders and confirmed the BAC DNA integration by PCR-based genotyping. Immunohistochemistry on tissues from brain, heart, lungs, liver, spleen, and kidneys revealed that the HO-1 is overexpressed in all organs tested. HO-1 mRNA and protein were also overexpressed from multiple organs from the BAC transgenic mice. Using these BAC transgenic mice, we generated a "humanized" BAC transgenic mouse (hHO-1 BAC) by crossing HO-1 BAC transgenic mice with HO-1 knockout ($HO\text{-}1^{-/-}$) mice. In addition to the overexpression of human-specific HO-1 mRNA and protein expression, the human HO-1 gene in hHO-1 BAC mice rescued the typical phenotype observed in the $HO\text{-}1^{-/-}$ mice such as increased incidence of abortion and embryonic lethality, heightened sensitivity to acute kidney injury, iron overload, anemia, and splenomegaly. These humanized HO-1 BAC transgenic mice will serve as a valuable model system to study the regulation of the human HO-1 gene and will provide information on the *in vivo* architecture of the HO-1 gene in acute kidney injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1058**

**Exercise Exacerbates Ischemia-Reperfusion (IR) Induced Acute Kidney Injury (AKI) in the Sprague Dawley (SD) but Not Fisher 344 (F344) Rat** Natasha C. Moningka,[1] Mark W. Cunningham,[1] Myrline Sterling,[1] Christine Baylis.[1,2] *[1]Physiol., University of Florida, Gainesville, FL; [2]Med., University of Florida, Gainesville, FL.*

If renal blood flow falls (RBF) during exercise, this may reduce the shear stress induction of endothelial nitric oxide synthase (eNOS) and extracellular superoxide dismutase (EC-SOD), and increase susceptibility to oxidative stress mediated AKI. Here, we investigated the impact of 12 weeks low intensity exercise (EX) by voluntary wheel running, compared to rats that remained sedentary (SED) in two rat strains, the SD and F344, subjected to renal IR. The right kidney was removed as a control and the left kidney received 35 min. of ischemia and 24 hr. reperfusion, followed by renal function measurements. In the control kidney cortex of the SD rat, EX reduced eNOS and EC SOD, did not change renal NOx (metabolites of NO) levels, decreased the NADPH oxidase subunit p22phox, and had no effect on H2O2. In the control kidney cortex of the F344 rat, EX increased eNOS, EC-SOD, NOx, p22phox and H2O2 . In response to IR, SD EX rats experienced a greater fall in inulin clearance (CIN) and further increase in plasma creatinine (PCr) compared to SED SD IR. In contrast, EX F344 displayed less functional impairment with IR than the SED F344 IR, exactly opposite to the SD (Table). Conclusion: Susceptibility to IR was predicted by the directional change in eNOS and EC-SOD in response to EX, with the SD showing increased vulnerability and the F344 showing relative protection. Mechanisms for these marked differences in renal responses to EX remain to be determined. We speculate that the SD reduce RBF with EX (consistent with decreased p22phox), whereas the F344 may increase cardiac output more efficiently with preservation, or even some increase in RBF, consistent with increased renal p22phox and H2O2.

Table. *p<0.05 vs. SED IR.

| | SD | | F344 | |
|---|---|---|---|---|
| | $P_{Cr}$ (mg/dl) | $C_{IN}$ (ml/min/100g BW) | $P_{Cr}$ (mg/dl) | $C_{IN}$ (ml/min/100g BW) |
| SED IR | 1.2±0.2 | 0.17±0.04 | 1.9±0.2 | 0.06±0.02 |
| EX IR | 2.4±0.4* | 0.04±0.01* | 1.5±0.3 | 0.15±0.06 |

Disclosure of Financial Relationships: nothing to disclose

**F-PO1059**

**Acute Renal Failure Induced by Hantavirus Infection Differs from Acute Tubulointerstitial Nephritis by Affecting Podocytes** Ellen Krautkrämer,[1] Stephan Grouls,[1] Jochen Reiser,[2] Martin G. Zeier.[1] *[1]Nephrology, University of Heidelberg, Heidelberg, Germany; [2]Nephrology and Hypertension, University of Miami.*

Purpose:

The typical histopathological findings in hantavirus-induced acute renal failure correspond to acute tubulointerstitial nephritis. However, these lesions do not explain the massive glomerular-type proteinuria observed in the infection. Therefore, we compared structure of glomeruli of patients with acute renal failure induced by hantavirus infection and of patients with tubulointerstitial nephritis.

Methods:

Cryo-sections of renal biopsies of patients with serologically confirmed hantavirus infection and of patients with non-hantaviral acute tubulointerstitial nephritis were analyzed by immunofluorescence. Sections were stained for proteins of tight and adherens junction, for marker proteins of podocytes and their glomerular slit diaphragms (GSD). The localization of proteins was analyzed by microscopy and expression levels were quantified by analyzing mean fluorescence intensity. To examine mechanisms of hantavirus-induced alterations, we infected human epithelial tubular cells and podocytes in vitro.

Results:

Our analysis of renal biopsies of patients suffering from hantavirus infection demonstrate that expression levels of specific proteins of tight and adherens junctions in tubular epithelia and the GSD in podocytes are mislocalized and dysregulated in hantavirus-infected patients. In contrast, biopsies of patients with tubulointerstitial nephritis showed no alteration of GSD structures or expression levels of marker proteins. Infection of tubular epithelial cells and podocytes in vitro results in relocalization and decrease of junctional proteins and the breakdown of the integrity of the epithelial barrier.

Conclusion:

The disassembling of GSD in podocytes that is specific for the hantavirus infection may explain the clinical picture of hantavirus-induced acute renal failure that is characterized by massive proteinuria compared to the tubulointerstitial nephritis. These findings provide useful insights in the pathogenesis of hantavirus-induced renal failure accompanied with massive proteinuria.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1060**

**Global Gene Expression Analyses in Renal Ischemia-Reperfusion Injury (IRI) from Mice Lucking Hif-1a Gene** Toshiaki Tamaki,[1] Shuji Kondo,[2] Shoji Kagami,[2] Shuhei Tomita.[1] *[1]Pharmacology, The University of Tokushima Graduate School, Tokushima-city, Tokushima, Japan; [2]The University of Tokushima Graduate School, Tokushima-city, Tokushima, Japan.*

We have shown that amelioration of acute tubular necrosis (ATN) in ischemic acute renal failure was impaired in mice lucking hypoxia inducible factor-1α gene in the previous ASN meeting. However, there are many unanswered questions as to how HIF-1α causes protective response to the ischemic acute renal injury. To investigate molecular mechanisms how HIF-1α-downstream molecules contribute to the protective events against ischemic renal injury, we subjected Hif-1$\alpha^{+/-}$ mice to renal IRI and performed global gene expression analyses.

Transcripts were obtained from corticomedullary junction in the left kidney of Hif-1$\alpha^{+/+}$ of Hif-1$\alpha^{+/-}$ mice, at 6 hrs, 12 hrs and 24 hrs after initiation of the reperfusion, and were used for gene expression analyses. Global gene expression profile in time-coursed transcripts from the kidneys after I/R injury showed that increase of the expression levels of HIF-1α gene after I/R injury in the Hif-1$\alpha^{+/-}$ mice was relatively lower than that of the control mice. Majority of extracted genes from time-point 6 hrs after I/R injury included functions related to cellular stress response, such as, redox response, immune response, energy metabolism. In the latter phases, 12-24 hrs after I/R injury, the functions included in majority of extracted genes were changed to a restore and regeneration of tissues and cells, such as growth signaling, cell cycle, cytoskeleton associated genes. Consistent with the results described above, immunohistochemical study showed that the number of proliferating cells stained for Ki-67 and tubular epithelial cells stained for Pax-2, a marker of renal cell differentiation, were both significantly decreased in the mutant kidney, compared to that in the controls. Additionally, 63 genes in which expression levels in the mutant mice were different from the controls at the time point 6 hrs after initiation of reperfusion, were extracted, and we identified 7 genes including chemokine, matrix metallopeptidase, cation transport regulator, by qPCR experiments.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1061**

**Connexin43 Hemichannels Exaggerate Cadmium-Induced Oxidative Stress and Cell Injury in Renal Tubular Epithelial Cells** Jian Yao,[1] Wei Sun,[2] Tao Huang,[1] Shotaro Nakajima,[1] Masanori Kitamura.[1] *[1]Department of Molecular Signaling, University of Yamanashi, Chuo, Yamanashi, Japan; [2]Department of Nephrology, Affiliated Hospital of Nanjing University of Traditional Chinese Medicine, Nanjing, Jiangsu, China.*

Gap junctions, formed by special protein called connexin (Cx), play important roles in the determination of cell survival in various pathological situations, especially those involving oxidative stress. Here we investigated whether and how gap junctions were involved in the cadmium ($Cd^{2+}$)-elicited renal tubular cell injury. 1) Transfection of renal tubular epithelial cell line LLC-PK1 with a wild-type connexin43 (Cx43) significantly sensitized them to $Cd^{2+}$-elicited cell injury, which was associated with increased JNK activation. Inhibition of JNK could largely prevent $Cd^{2+}$-elicited tubular cell injury. 2) The LLC-PK1 cell susceptibility to $Cd^{2+}$ was increased by depletion of glutathione (GSH) with DL-Buthionine-[S,R]-Sulfoximine (BSO), and decreased by N-acetyl-cysteine (NAC) or glutathione reduced ethyl ester, indicating an determinant role of oxidative status in JNK activation and cell survival. 3) To analysis the mechanisms, fibroblasts derived from Cx43 wild-type (Cx43+/+), heterozygous (Cx43+/-) and knockout (Cx43-/-) fetal littermates were used. These cells also displayed different susceptibility to $Cd^{2+}$. The severity of cell injury in these cells was closely related to the intracellular levels of reactive oxygen species (ROS) and c-Jun N-terminal kinase (JNK) activation. 4) $Cd^{2+}$ caused a reduction in intracellular GSH, but an elevation in extracellular GSH. This effect of $Cd^{2+}$ was significantly more dramatic in Cx43-positive cells than Cx43-negative cells. 5) Treatment of Cx43-positive cells with $Cd^{2+}$ caused a Cx43-hemichannel-dependent intracellular influx of Lucifer Yellow (LY) and extracellular efflux of ATP. Collectively, our study demonstrates that connexin43 sensitizes renal tubular cells to $Cd^{2+}$-initiated cytotoxicity through hemichannel-mediated regulation of intracellular redox status. Manipulation of connexin-forming channels could be a novel approach for prevention and treatment of $Cd^{2+}$-induced renal tubular cell injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1062**

**H2O2 but Not ATP Depletion Activates Gα12/Src To Stimulate Disruption of TJs** Wanfeng Yu, Sarah Beaudry, Bradley M. Denker. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Acute kidney injury(AKI) is a frequent complication of hospitalization and is associated with substantial morbidity, mortality and health care expenditure. Ischemia/reperfusion(I/R) is among the most common causes of AKI. With I/R injury, generation of ROS is an important mechanism leading to cellular damages including loss of tight junction(TJs).

Our previous studies indicate that Gα12 activation delays TJ recovery,stimulates apoptosis and may play a pivotal role in epithelial injury. To examine Gα12 in injury,we examined TJ loss/recovery in two models of stress:ATP depletion/repletion and H2O2/calatase. MDCK cells with inducible Gα12 over-expression or shGα12 were compared with controls and barrier function monitored by transepithelial resistance (TER),biochemical and immunofluorescent analysis. ATP depletion(10μM antimycin A+2mM 2-deoxy-D-glucose) for one hour,was followed by repletion with normal media. H2O2(5mM) for one hour was followed by 5000U/ml catalase. ATP depletion caused a rapid drop of TER in both celllines plus controls. Recovery of TER from ATP repletion was delayed in Gα12 over-expressing cells and accelerated in shGα12 cells,which is consistent with Gα12 functioning to delay TJ assembly. H2O2 promoted rapid loss of the barrier in Gα12 over-expressing and control cells but shGα12 cells were completely protected from TJ disruption. GST-TPR pulldowns revealed activated Gα12 within 30 min of H2O2 exposure but not with 30 min of ATP depletion. H2O2 significantly increased Src and RhoA activities in control cells and led to disruption of ZO-1 and phalloidin staining. Inhibiting Rho kinase had little effect on loss of barrier while Src inhibition was fully protective. H2O2 treated shGα12-MDCK cells were protected from loss of TER and changes in ZO-1/phalloidin staining. Taken together,these findings indicate that H2O2 and ATP depletion in MDCK cells activate different injury pathways. H2O2 induces Gα12/Src but not Rho for TJ disruption. However, the recovery of TJs is also affected by Gα12, but was independent of the specific stress. Defining these mechanisms in vivo may provide a novel target to uncouple numerous adverse responses to injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1063**

**NGAL Scavenges Iron by Forming a Complex with Catechol, an Endogenous "Siderophore"** Jonathan M. Barasch, Andong Qiu, Neal A. Paragas, Kristen Mcnierney. *Columbia.*

Ngal is a carrier protein that is expressed at low levels, until the kidney is stimulated by ischemia, hypoxia, nephrotoxins, sepsis. R. Strong demonstrated that Ngal binds bacterial siderophores which are essential for iron capture and microbial growth but yet Ngal is generally expressed in aseptic diseases. To determine whether Ngal can bind additional ligands we turned to the urine as a source of biomolecules because upon acute kidney injury Ngal is abundantly expressed in this compartment. We collected 400 liters of urine from our medical students, and using an Ngal:ligand:iron type binding assay we identified the catechol family of amino acid and polyphenol metabolites as Ngal dependent iron chelators. The catechols demonstrated the greatest iron binding activity of many aromatic compounds. These included Catechol, 4-methylcatechol, 3-methylcatechol, benzene triol which are present in micromolar levels in the urine. Remarkably, each of these bound Ngal with poor affinity (200nM), but when iron was added the affinity for ligand rose 100-500 fold (2nM, 0.4nM) and the solution of catechol:Fe changed from blue to bright red in color as tris-catechol:Fe formed within the binding cleft of Ngal. X-ray crystallography showed the catechol:Fe moiety sandwiched between amino acids which bound the catechol moiety of the bacterial siderophores, defining a critical site of molecular recognition. The Ngal:tris-catechol:Fe complex could form in vivo, in serum, and then traffic iron to the kidney which was absorbed in the megalin pathway of the proximal tubule. Iron delivery could be seen by autoradiography. The complex was pH sensitive and released its iron below pH 6.5-6.0, resulting in iron delivery. These data define the Ngal pathway of iron traffic based on a novel extracellular co-factor. The authors are grateful for the collaboration of Matt Clifton and Roland Strong, and Trisha Hoette and Kenneth Raymond.

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**F-PO1064**

**Tropomyosin-1 Acts Through RhoA Pathway To Regulate Cofilin Activity** Morgan S. A. Gilman,[1] Sarah N. Young,[1] Mark A. Hallett,[2] Sarah E. Wean,[2] Simon J. Atkinson,[2] Bruce A. Molitoris,[2] Sharon L. Ashworth.[1] *[1]Molecular and Biomedical Sciences, University of Maine, Orono, ME; [2]Division of Nephrology, Indiana University School of Medicine, Indianapolis, IN.*

The actin cytoskeleton in kidney proximal tubule cells is essential for maintenance of proper cellular structure. The actin cytoskeleton is dynamic, relying on the ability of actin to exist both as a filament (F-actin) and as a monomer (G-actin) to fulfill its many unique roles in the cell. Destruction of F-actin has been shown to occur in response to models of renal ischemia, including clamping of the renal pedicle in the rat and ATP depletion of cultured cells. ADF/cofilin, an actin binding and severing family of proteins, is activated in response to ischemia. Binding of ADF/cofilin to F-actin results in F-actin severing and an increase in free actin monomers. However, tropomyosin-1 (TM1) binding protects F-actin from the activity of ADF/cofilin. The competitive roles of these two proteins assist in maintaining proper balance between F-actin and G-actin in accordance with the existing needs of the cell. Cofilin activity is suppressed by phosphorylation at serine 3, a process

occurring downstream of the GTPase RhoA. A decrease in RhoA activity leads to a decrease in cofilin phosphorylation and an increase in active cofilin. We sought to further understand the role TM1 plays in the regulation of actin dynamics. LLC-$PK_{A4.8}$ cells were transfected with pEYFP-C1 plasmid containing TM1 cDNA. EYFP-TM1 localized to actin stress fibers, cell edges and cellular extensions, which were present 3.6 times more often in these cells. EYFP-TM1 expression also led to a 51.9% decrease in cofilin phosphorylation, a 10.9% decrease in RhoA activity and a 29.5% increase in expression of death associated protein kinase 2 (DAPK2). In EYFP-TM1 expressing cells, cofilin was dephosphorylated at an earlier time point than in non-expressing cells in ATP depletion studies. In a cellular pull down assay, histidine tagged TM1 bound to DAPK1, a protein known to phosphorylate TM1, and the DAPK1 related protein, DAPK2. These findings suggest TM1 plays a role in the regulation of cofilin phosphorylation, a process mediated by RhoA.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1065**

**Renal Tubular Fluid Shear Stress Stimulates Inflammation: Role of TNF-α**
Mathieu Miravete,[1] Julie Klein,[1] Julien Gonzalez,[1] Christianne Pecher,[1] Jean-Loup Bascands,[1] Joost Schanstra,[1] Muriel Mercier-Bonin,[2] Benedicte Buffin-Meyer.[1] *[1]U858, Inserm, Toulouse, France; [2]UM 14/LISBP, INSA, Toulouse, France.*

Renal tubulointerstitial fibrosis (TIF) is strongly correlated with the progression towards end stage renal disease. TIF is in most cases initiated by aggression of renal tubular cells. Injured cells subsequently produce cytokines leading to inflammation followed by interstitial extracellular matrix accumulation. In most chronic renal diseases, urinary fluid flow and composition are changed leading to modified tubular shear stress. This could be an early tubular aggression and contribute to the development of TIF. Therefore the aim of our study was to evaluate the contribution of fluid shear stress (FSS) on inflammation, a key and early event in TIF.

For this purpose, we exposed human renal tubular cells (HK-2) to laminar FSS of 0.01 Pa for 30 min. Then we studied the ability of resulting conditioned medium (FSS-CM) to stimulate monocytes (THP-1) and endothelial cells (HMEC-1). HK-2 cells maintained in static conditions served as controls.

48 h treatment of THP-1 cells with FSS-CM inhibited mRNA expression of monocyte marker C-C Chemokine receptor 2 and stimulated that of macrophage marker CD68. It also increased mRNA levels of inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β, but did not modify mRNA expression of the anti-inflammatory cytokines transforming growth factor-β and interleukin-10.

Incubation of HMEC-1 cells with FSS-CM for 6h increased the expression of vascular cell adhesion molecule-1 (VCAM-1) and stimulated the endothelial adhesion of phorbol ester activated-monocytes. These effects were mediated by TNF-α since incubation of FSS-CM with a TNF- α neutralizing antibody or soluble TNF-α receptor abolished both VCAM-1 induction and monocyte adhesion.

These results suggest that renal tubular cells stimulated by FSS release inflammatory mediators including TNF- α which improve the adhesion of monocytes on endothelial cells and activate their differentiation into inflammatory macrophages. Consequently, we suggest that modification of FSS during nephropathies plays an important role in the development of TIF by promoting inflammation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1066**

**A Microfluidic Model of Albumin Handling by Proximal Tubule Cells**
Nicholas J. Ferrell,[1] Kevin Ricci,[1] Joseph J. Groszek,[1] William Fissell.[1,2] *[1]Biomedical Engineering, Cleveland Clinic; [2]Nephrology and Hypertension, Cleveland Clinic.*

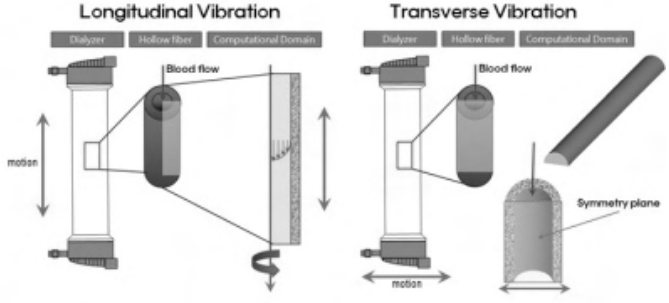
We have developed two *in vitro* microfluidic models to evaluate albumin handling by proximal tubule cells. For the first system, cells were grown on solid glass substrates with apical microchannels. In the second system, a porous membrane was added to provide both an apical and basolateral compartment. Opossum kidney (OK) epithelial cells were grown in the microchannels and exposed to physiologically relevant fluid flow. Cells were grown to confluence, then exposed to 50 μg/ml of fluorescein isothiocyanate (FITC) labeled albumin. Uptake of FITC-albumin was determined by measuring the fluorescence of cell lysates. Confocal fluorescence microscopy was used to compare uptake profiles in cells grown under flow and static conditions. Albumin processed by the cells was examined by size exclusion chromatography (SEC) and SDS-PAGE. Results showed that uptake per unit of cell growth area was significantly higher under flow compared to static conditions. This was confirmed by confocal microscopy. Size exclusion chromatography and SDS-PAGE showed that FITC-albumin was broken down into small molecular weight fragments and excreted by the cells. No trace of intact albumin was detectable by either SEC or SDS-PAGE. These results show that fluid flow has a significant impact on cellular uptake of albumin by OK cells. Analysis of processed albumin further confirms that it is broken down into small molecular weight fragment, likely via lysosomal degradation, and these fragments are excreted from the cells. This microfluidic *in vitro* model provides a novel system to further investigate tubular protein handling.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1067**

**Effects of Mechanical Vibration on Solute Removal in Hollow-Fiber Hemodialyzer: Numerical Analysis**
Jeong Chul Kim,[1,2,3] Francesco Garzotto,[1,2] Dinna N. Cruz,[1,2] Alessandra Brendolan,[1,2] Federico Nalesso,[1,2] Claudio Ronco.[1,2] *[1]Nephrology Dialysis & Transplantation, S. Bortolo Hosp, Vicenza, Italy; [2]International Renal Research Institute Vicenza (IRRIV), Vicenza, Italy; [3]Institute of Medical & Biological Engineering, Medical Research Center, Seoul National Univ., Seoul, Korea.*

Blood-membrane interaction during dialysis forms secondary protein layer on the surface of membrane. Operating condition control to inhibit concentration polarization and membrane fouling is very important to achieve targeted dialysis dose. Mechanical vibration can be one of useful methods to maintain the membrane mass transfer performance during dialysis. In this study we analyzed the effects of mechanical vibration on hemodyanmics in hollow fiber and their effects on mass transfer to optimize the operating condition dialysis.




Using finite volume based commercial code we simulated the vibrating hollow-fiber system. Wall shear stress (WSS) distributions were analyzed to different frequencies, directions and accelerations. The mechanical vibrations enhanced the relative motion of boundary layer, which increased WSS at the surface of membrane as shown in Table.

Time-averaged wall shear stress (TAWSS) for vibration amplitude of 10 mm

| Motion | Direction | TAWSS @ 1Hz | TAWSS @ 2Hz | TAWSS @ 4Hz |
|---|---|---|---|---|
| Sinusoidal | Longitudinal | 1.97 | 2.16 | 4.4 |
| Sinusoidal | Transverse | 4.38 | 4.52 | 4.83 |
| Square wave | Longitudinal | 1.9 | 1.95 | 3.59 |
| Square wave | Transverse | 4.2 | 4.2 | 4.5 |

Sinusoidal vibration was more effective than square wave vibration and transverse motion enhanced WSS compared to longitudinal one. However, acceleration induced by vibration may provide more absorption capacity to low molecular proteins.

The optimal mechanical vibration of hollow-fiber dialyzer during dialysis can be used to improve the dialysis efficiency.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1068**

**A Longitudinal Study of Hemodynamic and Luminal Changes in a Porcine Synthetic Arteriovenous Graft Model**
Yan-Ting E. Shiu,[1,2] Randy Jay Christopherson,[1] Christi M. Terry,[2] Huan Li,[2] Ilya S. Zhuplatov,[2] Alfred K. Cheung.[2,3] *[1]Bioengineering, Univ. of Utah, SLC, UT; [2]Medicine, Univ. of Utah, SLC, UT; [3]Medical Service, VASLCHCS, SLC, UT.*

A common complication of hemodialysis arteriovenous (AV) grafts is neointimal hyperplasia (NH) occurring at the venous anastomosis, leading to stenosis. Abnormal blood flow patterns and their associated wall shear stress (WSS) in the graft likely modulate vessel wall remodeling and the development of NH, but these relationships have not been well characterized. The purpose of this study is to use contrast-free magnetic resonance imaging (MRI) and computational fluid dynamics (CFD) to elucidate how the changing hemodynamic characteristics lead to anatomical changes in the lumen of the graft and adjacent vessels over a 6-week time course in a porcine model of synthetic AV graft stenosis. The graft was placed bilaterally between the common carotid artery and the external jugular vein. Time-of-flight MR images collected weekly after graft placement were used to create 3D reconstructions of the luminal geometry of the graft and adjacent vessels. Cine phase-contrast MR data collected at the same time points were used to obtain flow rates and direction in the graft and adjoining proximal and distal segments of artery and vein. The in vivo flow data and luminal geometries were used as input for detailed hemodynamics analysis using CFD. We found that 5 days after graft placement, the WSS increases in the arterial and venous anastomoses were 1.5- and 8-fold, respectively, when compared to the WSS in the control artery and vein. There was no remodeling of the arterial anastomosis over 6 weeks, though there was a slight and gradual narrowing of the distal artery. In contrast, the vein underwent substantial changes over the 6 weeks with the most notable feature being a substantial narrowing of the lumen over time. This suggests a thickening of the venous wall as an adaptive response to the significantly increased WSS seen in the vein. Work is ongoing to further examine the relationship between abnormal WSS and the location and degree of NH at the venous anastomosis determined from MRI.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1069**

**Intracellular Volume Shift: Standard vs UF-Control Treatment** Francesco Garzotto,[1,2] Flavio Basso,[1,2] Jeong Chul Kim,[1,2] Dinna N. Cruz,[1,2] Massimo De Cal,[1,2] Nicola Marchionna,[1,2] Alessandra Brendolan,[1] Federico Nalesso,[1,2] Claudio Ronco.[1,2] *[1]Nephrology Dept., S Bortolo Hosp, Vicenza, Italy; [2]IRRIV, Vicenza, Italy.*

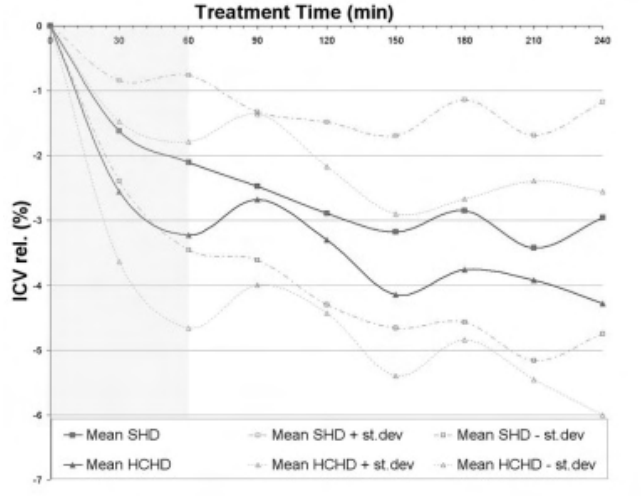
Body Fluid shifts are natural phenomena during hemodialysis (HD). Ultrafiltration (UF) results in intravascular hypovolemia and, correspondingly, in an increase in plasma colloid osmotic pressure, initiating a volume shift from interstitial to vascular space. The relative contribution of Extra (ECV) and Intra (ICV) volume to the UF volume is poorly defined. Our aim was to evaluate the ICV movement during a standard HD treatment (SHD) vs UF controlled (Hemocontrol, Gambro) dialysis (HCHD).

Methods

Whole body bioimpedance spectroscopy (50 frequencies, 5-1,000 kHz)(Fresenius Medical Care) was performed during HD every 30 min in 20 stable ESRD patients, 10 on HCHD. Weight Loss (WL), Dialysate (DNa) and Plasma (PNa) were recorded.Relative ICV (ICVrel) was calculated as % of ICV loss during HD.

Results

During HD PNa may change as a function of the sodium gradient between dialysate and plasma (ΔD-PNa).When effective ECV osmolarity exceeds that of ICV, fluid exits from the ICV to the ECV compartment.In the first section (FS) of HCHD, the dialysate fluid has the maximum permitted value of Na (range within -5, +10 mmol/L of prescribed in the Hemocontrol settings) reaching the higher ΔD-PNa.In the FS, ICVrel decreases more rapidly in the HCHD treatments (where ΔD-PNa is higher), than in SHD and its final value is lower in HCHD than in SHD.




Conclusion

Bioimpedence during HD treatment may improve fluid management giving information regarding the volume shift between body compartments. ICVrel seems strongly correlate with (ΔD-PNa).A biofeedback system in the sodium profile of the HD treatments may be potentially implemented.

| | SHD | HCHD |
|---|---|---|
| WL | 3.1± 1.0 | 3.3± 1.1 |
| ΔD-PNa | 5.4±3.2 | 10.2±2.9 |
| ICV | 20.3±3.19 | 21.2±3.9 |
| UFV | 3.1±1.0 | 3.0±0.7 |

Disclosure of Financial Relationships: nothing to disclose

**F-PO1070**

**Intravital Measurement of Glomerular Capillary Displacement in Rodents** Joel M. Henderson,[1] George Rhodes,[2] Lyne Lucien,[1] Philip A. Bondzie,[1] Bruce A. Molitoris.[2] *[1]Pathology, Boston University, Boston, MA; [2]Medicine, Indiana University, Indianapolis, IN.*

PURPOSE: Glomerular capillary hypertension and structural abnormalities of the glomerular capillary wall are associated with glomerular injury, and highlight the role of biomechanics in glomerular disease. To fully understand this relationship, methods are needed for measuring mechanical phenomena in the intact, living glomerulus. The aim of this project is to develop methods for intravital dynamic measurement of glomerular capillary displacement, in response to changes in capillary blood pressure.

METHODS: Renal capsular surface of 2 Munich-Wistar rats and surgically exposed deep renal cortex of 1 BL6 mouse were examined with a Biorad MRC1024 two-photon inverted microscope under general anesthesia. Fluorescent high molecular weight dextrans and Hoechst 33342 were injected to label vascular space and nuclei. Stable, clearly visible cross sections of glomerular capillaries were identified and imaged with a single scan line at 512 lines/s to create a 2D image of capillary width over time. Time-varying capillary width (displacement) was extracted by image segmentation to compute % change in width (strain). Ultrastructural examination was performed on murine kidney post-procedure to evaluate tissue integrity.

RESULTS: Between 4 and 11 glomerular capillaries were measured in each rodent. Capillary width cyclically varied 6.1% and 5.7% in rats, and 8.1% in the mouse, with regularly cyclic (heart rate) width variation most apparent in murine capillaries. Corresponding cyclic variation in intracapillary pressure was inferred by RBC shadow indications of changing flow rate. Ultrastructural examination of the cut surface of murine kidney post-procedure revealed intact glomerular capillary wall structures in exposed glomeruli.

CONCLUSION: The methods applied here successfully resolved cyclic changes in glomerular capillary width. Measurements obtained in this way suggest that rodent glomerular capillaries are relatively rigid compared to other vascular structures. This approach may be applied to studies of glomerular capillary structure and mechanics, and their role in glomerular disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1071**

**Different Glomerular Permeability between Munich Wistar Rat Strains** Ruben M. Sandoval, Monika D. Gandhi, Mark C. Wagner, George Rhodes, Exing Wang, Silvia B. Campos-Bilderback, Sarah E. Wean, Bruce A. Molitoris. *Nephrology, Indiana University, Indianapolis, IN.*

Recent studies using intravital imaging of rat kidneys has provided new insight and raised new questions into the mechanisms that lead to proteinuria. In particular, these studies provide data supporting an increased glomerular permeability and indicating that the proximal tubule, via its absorptive capacity, has an increased regulatory role in determining levels of proteinuria.

To investigate this further and attempt to reconcile some of the clear differences between earlier studies, we have compared permeability between the Simonsen Munich Wistar (used for intravital imaging studies) and the Fromter Munich Wistar (used for most micropuncture studies). Note that the Simonsen MW rats have fewer surface glomeruli, do not develop spontaneous proteinuria with age and are therefore rarely used to study disease progression. These studies all utilized rats of 180-270g, all imaging was done under identical conditions using maximized parameters on the same scope (Olympus FV1000 microscope adapted for two-photon microscopy using high sensitivity Gallium Arsenide non-descanned 12-bit detectors), and rat temperature, BP, & hydration were monitored and maintained.

We calculated the glomerular sieving coefficient using TxR-albumin and measuring the fluorescence ratio of Bowman's space/capillary lumen. Background fluorescence was subtracted from each compartment and no saturation was present. Our data show that Fromter MW rats have a lower GSC (0.011) than the Simonsen MW rats (0.036) of the same weight and age, $p<0.0001$. In addition, we addressed the important issue of dietary state by comparing fasting versus fed Simonsen rats. Interestingly, the fasted rats had a significantly reduced GSC (0.016) compared to either the fed ($p<0.0001$) or the Fromter rat ($p=0.0014$). These studies support that some of the variability between investigators is the result of strain differences, and that feeding increases the GSC in Simonsen MW rats.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1072**

**Different Gene Expression Profiles between Arterial and Venous Anastomoses in a Porcine Arteriovenous (AV) PTFE Graft Model** Li Li,[1] Randy Jay Christopherson,[2] Mary Carlson,[1] Donald Blumenthal,[3] Christi M. Terry,[1] Yan-Ting E. Shiu,[1,2] Alfred K. Cheung.[1,4] *[1]Internal Medicine, University of Utah; [2]Bioengineering, University of Utah; [3]Pharmocology, University of Utah; [4]Medical service, VASLC Healthcare System, SLC, UT.*

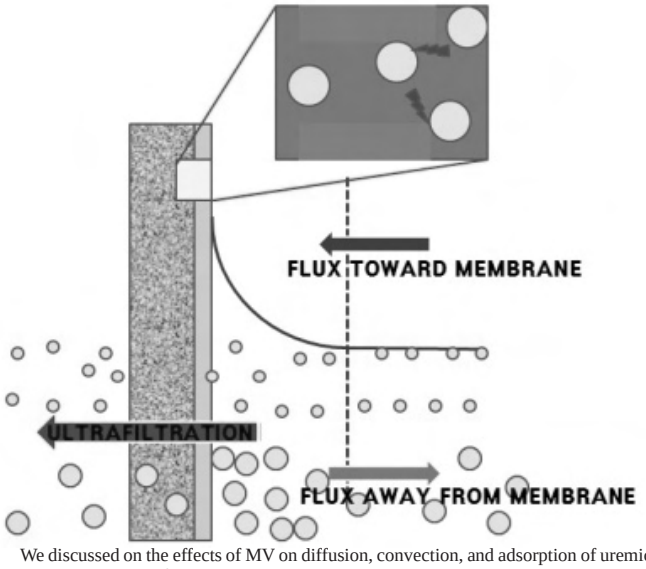
Neointimal hyperplasia (NH) is the predominant cause of AV PTFE graft failure and occurs mostly at the venous anastomosis (VA). The underlying molecular mechanisms of the different propensities for stenosis between the arterial anastomosis (AA) and VA are unclear. This study was conducted to compare the temporal gene expression between AA and VA through microarray analysis. Carotid-jugular PTFE grafts were placed in 4 pigs. Five days (n=2) or 14 days (n=2) post-operatively, the AV graft lumen geometry and blood flow data were obtained from black blood and cine-phase contrast MRI. The animals were then immediately euthanized. The anastomotic tissues and contralateral control carotid artery and jugular vein were explanted. The RNA from these tissues was subjected to cDNA microarray analysis. Statistical analysis revealed significant differences in expression of 70 genes between AA and VA. Specifically at 14 days, complement 3 and somatostatin were up-regulated (8-fold and 16-fold, respectively, above control vein) in VA, but not in AA. Computational fluid dynamics (CFD) simulations were used to assess the average wall shear stress (WSS) in these regions based on MRI results. The WSS in VA and AA at 5 days were the same order of magnitude, $60 \pm 28.3$ dyn/cm$^2$, which was higher than the WSS in control vein ($7.5 \pm 3.5$ dyn/cm$^2$) and control artery ($40 \pm 0$ dyn/cm$^2$). The WSS increase at the AA at 5 days was less profound (1.5-fold). Further investigations are required to determine (1) if the changes in WSS and gene expression in the VA are causally related to each other; and (2) if the differences in the magnitude of WSS changes explain the differential propensity to neointimal hyperplasia between VA and AA. Understanding the molecular mechanisms and their relationships to alterations in hemodynamic stresses will facilitate the identification of novel targets and strategies to inhibit stenosis in hemodialysis vascular accesses.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1073**

**Enhancement of Solute Removal in Hollow-Fiber Hemodialyzer by Mechanical Vibration** Jeong Chul Kim,[1,2,3] Francesco Garzotto,[1,2] Dinna N. Cruz,[1,2] Alessandra Brendolan,[1,2] Federico Nalesso,[1,2] Claudio Ronco.[1,2] *[1]Nephrology Dialysis & Transplantation, S. Bortolo Hosp., Vincenza, Italy; [2]International Renal Research Institute Vicenza (IRRIV), Vicenza, Italy; [3]Institute of Medical & Biological Engineering, Medical Research Center, Seoul National Univ., Seoul, Korea.*

Blood-membrane interaction during dialysis results in development of secondary layer on the surface of membrane. The vibratory shear enhanced process was recently introduced in membrane technology to minimize the secondary layer development at membrane surface. The mechanical vibration (MV) produces high wall shear stress (WSS) at the surface of membrane, which reduces the thickness of concentration boundary layer and inhibits gel layer development.




We discussed on the effects of MV on diffusion, convection, and adsorption of uremic solutes during dialysis for sinusoidal and triangle wave vibratory motions. Longitudinal vibration generates reverse flow inside membrane by moving wall motion, which increases WSS magnitude of hollow fiber. Transverse vibration generates swirling motion of fluid inside membrane, which enhances mixing and inhibits the secondary layer formation. Acceleration induced by triangle wave vibration could provide more absorption capacity to middle molecular solutes. MV could enhance solute removal by minimizing change in membrane morphology by secondary layer formation during hemodialysis.

Effects of mechanical motions on mass transfer in hollow-fiber hemodialyzer

| **Motions** | **Longitudinal** | | **Transverse** | |
|---|---|---|---|---|
| | Sinusoidal | Square wave | Sinusoidal | Square wave |
| Diffusion | ++ | + | ++ | + |
| Convection | ++ | + | ++ | + |
| Adsorption | + | + | + | ++ |

However, safety aspects such as hemolysis, user interface membrane mechanical property should be considered for clinical application of MV. Optimal MV conditions are to be investigated with simulation and experiments in the future.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1074**

**Novel Cell and Gene Therapy for Anemia of CKD** Matthew H. Wilson, Joseph Edward Doherty, Daniel L. Galvan, Sunandan Saha. *Medicine, Baylor College of Medicine, Houston, TX.*

EPO-deficient anemia of CKD is currently treated with recombinant EPO analog injections which are recently associated with undesired side effects such as increased risk of stroke, heart attacks, and deep vein thrombosis that complicate use of this therapy. Although the mechanisms of these side effects are unclear, it is clear that bolus dosing of EPO analogs either weekly or monthly does not recapitulate the physiologic regulation of this important hormone. Thus, there is a need to develop alternative therapies for anemia of CKD. We are developing an innovative approach for therapy of anemia of CKD using genetically modified T lymphocytes whose specificity is directed to persistent (latent) viruses such as Epstein-Barr virus (EBV) and survive long-term (>8 years) in stable numbers *in vivo* due to chronic viral antigen stimulation. Moreover, preclinical and recent clinical studies have shown T cells can be readily induced to apoptose by activation of a co-transferred suicide gene, providing an additional layer of safety and control. We *hypothesize* that virus specific T cells genetically modified to inducibly express EPO and a separately inducible suicide gene represent an ideal candidate cell population for sustained and safe treatment of anemia of CKD. We have successfully used the non-viral *piggyBac* transposon system to efficiently genetically modify mouse and human T cells achieving long-term gene expression. We have achieved inducible (tetracycline regulated) EPO expression in vitro in human cells and in vivo after somatic cell gene transfer with regulation of hematocrit levels in intact mice. We are modifying virus specific murine T cells to inducibly express EPO and a suicide gene that will be infused into wild type and CKD mice to measure their effectiveness in regulating hematocrit levels *in vivo*. We are also modifying human T cells and testing them *in vitro* for their ability to be propagated long-term via chronic viral antigen stimulation, as well as inducibly express EPO and undergo selectively induced cell ablation if needed. This innovative approach has the potential to provide safe cell therapy for anemia of CKD and can easily be adapted for a variety of other human diseases.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1075**

**Endothelial Progenitors Encapsulated in Bioartificial Niches Are Insulated from Systemic Cytotoxicity and Are Angiogenesis-Competent** Brian B. Ratliff,[1] Tammer Ghaly,[1] Philip Brudnicki,[1] Kaoru Yasuda,[1] Antonis K. Hatzopoulos,[2] Michael S. Goligorsky.[1] *[1]New York Medical College, Valhalla, NY; [2]Vanderbilt University, Nashville, TN.*

Intrinsic stem cells (SC) participate in tissue remodeling and regeneration in various diseases and following toxic insults. Failure of tissue regeneration is in part attributed to lack of SC protection from toxic stress of noxious stimuli, thus prompting intense research efforts to develop strategies for SC protection and functional preservation for in vivo delivery. One strategy is creation of artificial SC niches in attempt to mimic the requirements of endogenous SC niches by generating scaffolds with properties of extracellular matrix. We investigated the use of hyaluronic acid (HA) hydrogels as an artificial SC niche and examined regenerative capabilities of encapsulated embryonic endothelial progenitor cells (eEPC) in vitro and in three different in vivo models. Hydrogel encapsulated eEPC preserved their phenotype and survived better in HA-hydrogels coated with pronectin. Hydrogel encapsulated vis-à-vis surface-cultured eEPC demonstrated improved resistance to cytotoxic adriamycin insult in vitro. Implantation of HA-hydrogels containing eEPC to mice with adriamycin nephropathy or renal ischemia resulted in eEPC mobilization to injured kidneys (and to a lesser extent to the spleen) and improvement of renal function, which was equal or superior to adoptively transferred EPC by IV infusion. In mice with hind limb ischemia, EPC encapsulated in HA-hydrogels and timely released by local application of hyaluronidase dramatically accelerated the recovery of collateral circulation with the efficacy superior to IV infusion of EPC. In conclusion, HA-hydrogels protect eEPC against adriamycin cytotoxicity and implantation with timely release of eEPC encapsulated in HA-hydrogels supports renal regeneration in ischemic and cytotoxic (adriamycin) nephropathy and neovascularization of ischemic hind limb, thus establishing their functional competence and superior capabilities to deliver stem cells stored in and released from this bioartificial niche.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1076**

**Novel Growth Factor Containing Hydrogel To Support Tubular Repair after Acute Kidney Injury** Peter V. Hauser,[1] Mikhail V. Tsurkan,[2] Raquel Carvalhosa,[1] Benedetta Bussolati,[1] Carsten Werner,[2] Giovanni Camussi.[1] *[1]Center for Molecular Biotechnology, University of Torino, Torino, TO, Italy; [2]Leibniz-Institut of Polymer Research, Dresden, Germany.*

**Aim:** Stem cell injection contributes to regeneration after acute kidney injury (AKI), most likely by endo- or paracrine action. To test if a novel hydrogel could serve as a local deposit for growth factors, we induced AKI in mice and measured the tubular proliferation in kidneys after injection with growth factor containing hydrogel.

**Methods:** A hydrogel was developed by carbodiimide chemistry crosslinking of end-functionalized polyethylene glycol (sPEG) and heparin (Mw≈14kDa) in a ratio of 1:1,5. The gel was grounded and extensively washed with PBS. Next, EGF or bFGF were disolved in the gel with a concentration of 2µg/ml. AKI was induced by injecting c57b WT mice intramuscular with glycerol (7.5 g/g bw). On day 3, 20 animals were injected with 100µl hydrogel containing EGF (n=10) or bFGF (n=10) subcapsular into the left kidney. Biopsies were taken on days 2 and 5 after hydrogel injection, and the proliferative response of treated kidney ($k_t$) was compared to the untreated contralateral kidney ($k_{ctrl}$) by counting Pcna and BrdU positive tubular cells. Serum creatinine and BUN was measured in treated animals and in controls.

**Results and Conclusion:** The PEG-gel has been shown to release bFGF at a 2% rate over 48 hours in *in vitro* studies and similar activity was expected *in vivo*. Visual examination revealed stability of the injected gel during the *in vivo* experiments. Preliminary results showed that at day 2 after injection, the bFGF-hydrogel injected kidneys (n=5) exhibited a significant higher number of BrdU positve cells ($k_t$:13.9±8.6; $k_{ctrl}$:7.5±7.9) and Pcna ($k_t$:28.8±16.3; $k_{ctrl}$:13.1±16.7) positive cells per high power field (hpf). Kidneys injected with EGF containing hydrogel (n=5)showed a non-significant increase of BrdU ($k_t$:22.9±6.8; $k_{ctrl}$:20.6±4.1) and Pcna ($k_t$:51.7±1; $k_{ctrl}$:44.2±3.6) positive cells/hpf. Serum creatinine and BUN of treated animals did not differ from control animals.

Preliminary results suggest that the hydrogel can serve as deposit and deliver growth factors locally in order to support regeneration.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1077**

**Developing the Optimal siRNA/Nanocarrier Complex for Glomerular-Targeted Molecular Therapy in Mice** Hideki Shimizu,[1] Yuichi Hori,[1] Shinya Kaname,[2] Kanjiro Miyata,[3] Nobuhiro Nishiyama,[3] Akira Yamada,[2] Kazunori Kataoka,[3] Toshiro Fujita.[1] *[1]Department of Nephrology and Endocrinology, University of Tokyo Graduate School of Medicine, Tokyo, Japan; [2]First Department of Internal Medicine, Kyorin University School of Medicine, Tokyo, Japan; [3]Department of Materials Engineering, University of Tokyo School of Engineering, Tokyo, Japan.*

We have recently developed a gene delivery system using new blockcopolymers composed of polyethylene glycol-poly(L-lysine) (PEG-PLL) in experimental glomerulonephritis (JASN 2010;21:622). However, it is not known what is the best design of the nanocarriers with regard to the gene silencing effect. To answer this question, we prepared a complex of MAPK1 siRNAs /PIC (polyion complex) nanocarriers at various N/P ratios, i.e. primary amino groups in PLL (N) / phosphate units in siRNA(P), and examined the delivery and gene-silencing effect in cultured mesangial cells and in MRL/lpr mice. *In vitro* study showed that the complexes formulated at an N/P ratio of 1.0 to 1.4 were most efficient in transfection and also in an inhibitory effect of MAPK1 expression. *In vivo* study showed that intraperitoneal administration of the PIC nanocarriers at an N/P ratio of 1.4 was most effective in inhibiting the glomerular expression of MAPK1 and also in ameliorating glomerulosclerosis and BUN. The complexes were proven to be neutral or slightly positively charged and had a size of several ten nanometers. In summary, we were able to determine the best design of PIC nanocarriers by modifying PEG-PLL-based blockcopolymers, which may contribute to the development of molecular therapy in glomerular diseases.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1078**

**Enhanced β2-Microglobulin Clearance by Silicon Nanopore Membranes** William Fissell,[1,2] Nicholas J. Ferrell,[1] Joseph J. Groszek,[1] Albert Terrence Conlisk,[3] Dharmeshkumar M. Kanani,[4] Andrew L. Zydney.[5] *[1]Biomedical Engineering, Cleveland Clinic, Cleveland, OH; [2]Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH; [3]Mechanical Engineering, The Ohio State University, Columbus, OH; [4]Chemical Engineering, The Pennsylvania State University, University Park, PA.*

Background: Conventional polymer hollow-fiber dialysers tend to retain B2-microglobulin (B2mg) and other middle-molecular weight solutes, as performance is constrained by the absolute need to retain albumin and the limited selectivity of the membrane material. Selectivity is governed by pore shape and polydispersity of pore size. We tested predictions of enhanced selectivity of silicon nanopore membranes with monodiperse slit pores using bovine serum albumin and human B2-mg. Methods: Silicon nanopore membranes with nominal pore size of 7nm x 45 microns were manufactured, coated with polyethylene glycol, and placed in an ultrafiltration cell as previously described. Human serum albumin and B2-mg were dissolved in phosphate buffered saline. After wetting the membrane, the albumin-B2mg solution was filtered under pressure and feed and ultrafiltrate samples collected (n=5). The first 2 samples were discarded as they were diluted by wetting solution. Protein concentrations in feed and permeate solutions were measured by size-exclusion chromatography with online UV absorption at 280 nm. Results: albumin was not detectable in the permeate solution, and the apparent sieving coefficient for B2mg was 0.52 +/- 0.04. The data matched a steric-electrostatic prediction model for hindered transport of albumin and B2-mg in slit shaped pores. Discussion: A membrane of uniform slit shaped pores appears to maximize selectivity. A novel membrane for renal replacement assembled using top-down nanotechnology demonstrated very high clearance of a uremic solute while retaining albumin.

Disclosure of Financial Relationships: Consultancy: Medtronic, Inc.; Gambro Renal Products, Inc.; Davita Clinical Research, Inc.; Patent: US Pat No 09/949,575.

**F-PO1079**

**Development of an Open-Source Platform To Support Analysis, Integration and Representation of Kidney and Urinary Pathways (KUP)-Related –Omics Data** Julie Klein,[1] Simon Jupp,[2] Jean-Loup Bascands,[1] Robert Stevens,[2] Joost Schanstra.[1] *[1]U858, Inserm, Toulouse, France; [2]School of Computer Science, University of Manchester, Manchester, United Kingdom.*

Kidney research continues to produce large data sets obtained from different biological levels. Mining this data for new knowledge is hindered by the size of these data sets and the absence of user-friendly data analysis tools. To facilitate data exploration and analysis, and to help generate new hypothesis in kidney research, a supporting database of biological knowledge related to the KUP, that allows integration of existing knowledge from databases and scientific literature, would be of great help.

We have developed a prototype KUP Knowledge Base (KUPKB) that integrates KUP related data across the different –omic levels. A specialised KUP Ontology (KUPO) provides a common schema for annotating the data and allows for richer queries over the data to obtain information on genes, proteins, and pathways in the context of the kidney. Currently the KUP KB consists of gene expression data in specific kidney compartments linked to several public biological databases including Entrez gene, Uniprot, GO Annotations and Kegg. Simple queries in the KUPKB are as follows: "In which cells is gene "x" expressed?" or "Which genes are upregulated in the glomerulus compared to other renal compartments?". Answering those queries is interesting for two reasons: the kidney is highly cellular with each cell having distinct functions, and most of the large scale data obtained in human disease comes from analysis of urine, that needs to be put into the 'kidney' context. Short terms aims of the KUP KB are to allow queries more specific such as: "In which renal diseases my differentially expressed genes have the same regulation profile?" or "Which urinary proteins are expressed in the proximal tubule cells and are linked to inflammation". In conclusion the KUPKB prototype is a flexible tool allowing data integration, query and representation and will help to generate new knowledge and hypothesis in kidney research.

***Acknowledgements***

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Disclosure of Financial Relationships: nothing to disclose

**F-PO1080**

**NHLBI *AbDesigner*: Artificial Intelligence-Based Software for Design of Peptide-Directed Antibodies** Trairak Pisitkun, Mark A. Knepper. *NHLBI, NIH.*

Over the years, our laboratory has developed a large number of polyclonal antibodies to transporters and regulatory proteins expressed in the kidney. The choice of the sites in target proteins to use for synthesis of immunizing peptides has been based on a desire to optimize immunogenicity, antibody specificity, multi-species conservation, and robustness in the face of post-translational modifications. To create a tool for semi-automatic choice of immunizing peptides that optimize the above factors, we are developing an artificial intelligence-based software program called NHLBI AbDesigner written in *Java*. It predicts optimal sites along the primary sequence of individual proteins by calculating and extracting information as follows: 1) immunogenicity score based on secondary structure prediction and hydropathy; 2) uniqueness score [predicting specificity of an antibody produced by a particular peptide]; 3) conservation score [predicting likelihood of multi-species recognition of an antibody produced by a particular peptide]; and 4) protein features that describe regions or sites of interest in the protein sequence such as post-translational modifications, binding sites, enzyme active sites, sequence conflicts, and other characteristics reported in the appropriate Swiss-Prot records. It displays the prediction in an easy-to-view graphic user interface including heat maps for the scored predictors. To test the software algorithm, we have used NHLBI AbDesigner to select immunizing peptides for producing peptide-directed antibodies against the water channel aquaporin-2 and two podocyte-specific proteins, nephrin and podocin. These 3 antibodies have performed remarkably well in terms of both sensitivity and specificity. In summary, NHLBI AbDesigner is a Java-based "expert system" that codifies the principles developed for optimal antibody design developed in prior studies and it is currently being made available in the form of stand-alone and web applications.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1081**

**The Engineering of Anemia Management Protocols in Chronic Kidney Disease** Michael J. Germain,[5] William C. Vogt,[2] Rajiv P. Shrestha,[1] Brendan Nichols,[1] Christopher V. Hollot,[4] Joseph Horowitz,[3] Yossi Chait.[1] *[1]Mechanical & Industrial Engineering, University of Massachusetts, Amherst, MA; [2]Biomedical Engineering & Sciences, Virginia Polytechnic Institute & State University, Blacksburg, VA; [3]Mathematics & Statistics, University of Massachusetts, Amherst, MA; [4]Electrical & Computer Engineering, University of Massachusetts, Amherst, MA; [5]Nephrology, Baystate Medical Center, Springfield, Ma.*

Background. Anemia management protocols (AMP) for EPO-dosing based on hemoglobin measurements inherently form a feedback system wherein the protocol acts as a feedback controller. As such, the design of an AMP should be informed by the dynamic structure of the erythropoiesis system, together with models of the system's uncertainties, including variations in EPO responsiveness and intercurrent events such as infection and internal bleeding.

Methods. This study combines PK/PD models, retrospective data of 49 HD patients collected over two years, and modern robust control system design to synthesize new protocols.

Results. The system comprises models of PK, early-stage erythropoiesis dynamics, and reticulocyte and RBC compartments. For each subject, we quantified parameter ranges and designed a robust feedback controller (protocol) as shown in Figure 1. This protocol, simulated in feedback with a subject-specific erythropoiesis model, was successfully tested against Hgb measurement noise and system uncertainties. Figure 2 shows a simulation comparing the ability of a standard protocol and the new protocol to maintain a target Hgb level of 11.5.

Conclusions. The design of an AMP for EPO dosing in ESRD should use robust control methods. Such protocols can maintain Hgb target levels and reduce Hgb variability in the face of intercurrent events and variations in EPO responsiveness.

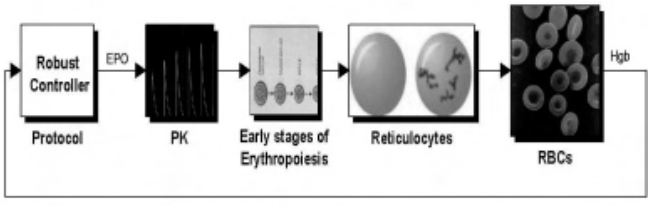



*Figure 1: The feedback control system comprised of the erythropoiesis system and robust controller (protocol).*

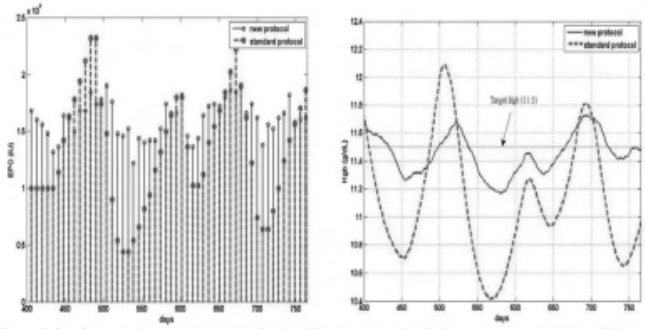


*Figure 2: Simulation results comparing a standard and the new protocol in the face of measurement noise; Values of weekly EPO doses (3x per week) are shown to the left and resulting subject's Hgb values are shown to the right.*

Disclosure of Financial Relationships: nothing to disclose

**F-PO1082**

**Regulatory Networks at Work in IgA Nephropathy Elucidated through Contextual Modeling of CCL5 Transcription** Peter J. Nelson,[1] Matthias Kretzler,[4] Bernhard Banas,[2] Elisabeth Groene,[3] Clemens D. Cohen,[5] Dilip Kumar.[1] *[1]Medical Policlinic, Ludwig-Maximilians-University Munich, Munich, Germany; [2]Internal Medicine II, Nephrology, University of Regensburg, Regensburg, Germany; [3]Division of Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Germany; [4]Internal Medicine, University of Michigan, Ann Arbor, MI; [5]Institute of Physiology, University of Zurich, Zurich, Switzerland.*

The prediction and analysis of the regulatory networks underlying gene expression is an important challenge in systems biology. Molecular characterization of the contextual transcription of the chemokine CCL5 was used to help elucidate regulatory networks linked to the development of IgA nephropathy, a glomerular disease characterized by activation of renal mesangial cells. CCL5 is associated with amplification and propagation of the disease. In mesangial cells CCL5 is optimally induced in response to TNF-alpha and IFN-gamma stimulation where JNK, IRF and NF-kappaB regulatory pathways independently converge on the promoter to control CCL5 transcription through p50/p65, IRF-1 and JunD/Fra1/CREB binding to their cognate sites. Convergent evolution can lead to a core organization of conserved transcription factor binding sites within gene promoters whose products need to be co-expressed within the same biologic context. A model based on the spatial orientation of functional binding elements for CCL5 was used to identify similar hierarchical features in a human promoter database. The results were interfaced with transcriptomic data from microdissected glomerular renal biopsies from patients with IgA nephropathy (n=27). Overlapping genes were identified, and of these, RIG I and IL-32 were demonstrated to be tightly co-regulated with CCL5 in mesangial cells in response to TNF-alpha/IFN-gamma. The approach demonstrates that experimental analysis of a promoter in a specific tissue context can be used to identify networks of co-regulated genes independent of a priori knowledge about their biological connection and provides information about common regulatory pathways.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1083**

**Reduction of Glomerular Injury by Raising Podocyte pH** Mehmet M. Altintas,[1] Kumiko Moriwaki,[1] Clemens C. Möller,[2] Jan Flesche,[2] Changli Wei,[1] Jing Li,[1] Suma Yaddanapudi,[2] Jochen Reiser.[1] *[1]Division of Nephrology and Hypertension, University of Miami Miller School of Medicine, Miami, FL; [2]Division of Nephrology and Program in Glomerular Disease, Massachusetts General Hospital, Boston, MA.*

Podocytes are highly differentiated cells and represent critical elements for the filtration barrier of the kidney. Loss of their foot process architecture results in urinary protein loss, a hallmark of kidney disease. Here we show a novel role for the neutral amino acid glutamine in structural and functional regulation of the kidney filtration barrier. Based on proteomic and genetic information, we created a metabolic map of podocytes and analyzed their metabolism using Metabolic Flux Analysis. We characterized amino acid fluxes in podocytes using genetic, toxic and immunologic models and identified commonly dysregulated glutamine utilization pathways in podocytes that were not uniformly present in fibroblasts or other eukaryotic cells. Glutamine uptake is increased in diseased podocytes to couple the increased demand for nutrient support during the disease state. This feature can be further exploited to pharmacologically increase glutamine uptake into podocytes. Glutamine uptake raised the podocyte cytosolic pH from 7.0 to >7.2. This pH increase was functionally significant as the determined pH optimum for cytosolic cathepsin L lies at 7.0 and showed a 60% reduction at pH 7.2. Glutamine supplementation did not change the normal functioning of healthy podocytes but led to a strong reduction of cytosolic cathepsin L protease activity in diseased podocytes resulting in protection of the known cathepsin L targets, dynamin and synaptopodin. We tested these findings in mice and supplemented high doses of glutamine before challenging the animals with LPS. The glutamine-loaded animals had alkalinized glomeruli shown by pH indicator labeling, reduced foot process effacement and lower levels of proteinuria. In summary, our data unravels specific amino acid pathways that provide a metabolic opportunity to combat urinary protein loss through modulation of podocyte pH.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1084**

**PodoNet – A Podocyte Protein Interaction Network for the Analysis of Expression Data** Gregor Warsow,[1,2] Nicole Endlich,[1] Sandra Schordan,[1] Eric Schordan,[1] Georg Fuellen,[2] Karlhans Endlich.[1] *[1]Anatomy and Cell Biology, Ernst Moritz Arndt University, Greifswald, Germany; [2]Institute for Biostatistics and Informatics in Medicine and Ageing Research, University of Rostock, Rostock, Germany.*

In recent years, microarray and proteomic analyses have become frequently used methods to assess gene and protein expression in podocytes and glomeruli. However, tools for the analysis of large data sets specifically for podocyte or glomerular genes and proteins are not available. In view of the large amount of data that is generated by microarray and proteomic experiments, meaningful data reduction represents a critical step. We built a protein interaction network for the podocyte (PodoNet), and we prepared an electronic representation of the network in Cytoscape software. PodoNet consists of 148 nodes and 239 edges that represent proteins and protein-protein interactions, respectively. The network is based on expert knowledge; podocyte proteins and protein-protein interactions were selected by literature search. Gene or protein expression data can be mapped onto the network and differential expression can be visualized. As protein-protein interactions depend on the amounts of interacting proteins, differential expression changes the strengths of interactions. Using protein expression data or assuming proportionality between transcript and protein expression, differential interaction strengths can be visualized in PodoNet. Selecting edges with highest differential interaction strength, the network size can be interactively reduced to core network modules. Using publicly available microarray data obtained from freshly isolated and cultured mouse podocytes as a test input for PodoNet, differentially regulated core modules are identified, e.g. the nephrin/ podocin module. In summary, we have developed PodoNet, a protein interaction network of podocytes, as a valuable tool for the analysis of podocyte and glomerular expression data.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1085**

**Metabonomic Analysis of Serum Metabolites in Kidney Transplant Recipients with Cyclosporine A- or Tacrolimus-Based Immunosuppression** Chan-Duck Kim,[1] Sun-Hee Park,[1] Yong-Lim Kim,[1] Geum-Sook Hwang,[2] Tae-Hwan Kwon.[3] *[1]Dept of Internal Medicine, School of Medicine, Daegu, Republic of Korea; [2]Joint Bioanalytical Analysis Team, Korea Basic Science Institute, Seoul, Republic of Korea; [3]Dept of Biochemistry and Cell Biology, School of Medicine, Daegu, Republic of Korea.*

**Background:** Cyclosporine (CsA) and tacrolimus (TAC) affect the body metabolism of renal transplant recipients differently. We applied a novel method of $^{1}$H-nuclear magnetic resonance (NMR)-based metabonomics to integrate the serum metabolic profiles of transplant recipients with normal allograft function and identify time-dependent changes in the levels of serum metabolites in response to CsA- or TAC-based immunosuppression after kidney transplantation (KT).

**Methods:** Fifty-seven consecutive renal transplant recipients were treated with CsA-based (CsA, MMF, and steroid, n = 27) or TAC-based (TAC, MMF, and steroid, n = 30) regimens. Serum samples were analyzed at baseline (pre-transplant), and 1, 3, and 6 months after KT.

**Results:** The PLS-DA score plots showed a clear separation between levels at baseline and at 1, 3, and 6 months after KT in both groups. The levels of lipid metabolites were increased after KT in both groups and importantly CsA-group demonstrated higher levels than TAC-group. The metabolites for which the levels differed between the CsA-group and TAC-group and that changed according to treatment duration were glucose, hypoxanthine, lactate, succinate, and taurine. In contrast, trimethylamine-N-oxide levels, known to be associated with graft dysfunction, did not differ between the two groups.

**Conclusions:** These data indicate that CsA- and TAC-based immunosuppression elicits unique changes in serum metabolic profiles after KT. $^{1}$H-NMR-based metabonomics could provide new insights regarding the side effects of immunosuppressive regimens.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1086**

**Application of a Uromodulin Exclusion List Improves Depth of Urine Exosomal Protein Coverage by LC-MS/MS** Thomas F. Hiemstra,[1, 2] Svenja S. Hester,[2] Kathryn S. Lilley,[2] Fiona E. Karet.[1] *[1]Medical Genetics and Renal Medicine, University of Cambridge, United Kingdom; [2]Centre for Proteome Research, University of Cambridge, United Kingdom.*

Introduction: Discovery of urinary biomarkers for detection and monitoring of kidney disease is of considerable health-economic importance. Advances in the sensitivity of mass spectrometry (MS) have encouraged interest in its deployment in urine biomarker studies, but success has been limited. The abundant urinary protein uromodulin (UMOD) represents a practical contaminant which limits MS sensitivity, and may account for this paradox. UMOD depletion has been attempted, but is labour- and time-intensive, and may alter or remove other proteins. We describe the application of an exclusion list (EL) of UMOD-related peptide ions coupled with high sensitivity mass spectrometric analysis to increase the depth of coverage of the urinary exosomal (UEx) proteome.

Methods: UEx were differentially centrifuged from the urine of 5 healthy male volunteers. Separate exosomal samples were subjected to GelC-MS/MS on an LTQ-Orbitrap instrument. Spectra were analyzed using MASCOT-PERCOLATOR. Samples were then re-run, with m/z corresponding to the 20 most intense UMOD spectra rejected by forced exclusion. Spectra were searched against the human IPI protein database (v4.3), setting a false discovery rate of 0.1 for all analyses.

Results: A mean of 158 (range 114–231) UMOD spectra were identified per run by conventional methods (CM) whereas 0 UMOD spectra were identified by applying a UMOD EL, indicating the list's effectiveness. We identified 476 proteins overall by CM, of which 188 were also present on the EL list. With the application of the EL, an additional 80 proteins were identified that CM had not found, representing an increase of 17%.

Conclusion: CM give greater coverage of abundant proteins, whereas EL identifies an additional set of proteins but with fewer identifications overall. The combined use of UMOD EL and CM significantly improves depth of UEx protein coverage, providing an attractive alternative to UMOD depletion.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1087**

**Comparison of High-Throughput Sequencing (HTS) and Quantitative Realtime (qRT)-PCR for MicroRNA Profiling in Murine Kidneys** Jennifer Yi-Chun Lai,[1] David L. Turner,[2] Andrew S. McLellan,[3] Robert C. Thompson,[2] Markus Bitzer.[1] *[1]Internal Medicine, University of Michigan, Ann Arbor, MI; [2]Biological Chemistry, University of Michigan, Ann Arbor, MI; [3]Genetics, Einstein, Bronx, NY.*

Background: miRNA play important roles in disease development and their expression profiles can identify disease subtypes. Because accurate determination of miRNA expression is essential, potential differences between miRNA profiling methods need to be identified. Therefore, we compared HTS and qRT-PCR to determine miRNA expression.

Methods: Total RNA was extracted from whole kidneys of 3, 12 and 20 months old C57BL/6 mice using TRIzol. For HTS, the small RNA fraction (18 to 40 nucleotides) was sequenced using Illumina/Solexa genome analyzer standard protocol. The program *Bowtie* was used for genome alignment and specific criteria were implemented to optimize mapping accuracy. For miRNA profiling by qRT-PCR in the same RNA-pool miRNA Taqman Array (Applied Biosystems) were used.

Result: HTS output provided 2 to 6 million reads per sample up to 36 nucleotides length. The mean mapping efficacy was 69%. An average of 83% of the aligned reads matched to annotated miRNAs (miRbase V14). Out of 827 annotated miRNAs, approx. 18% were highly expressed (>1000 sequence reads). Furthermore, sequence polymorphism and length variations were detected for most miRNAs. I.e. 261850 total reads were identified for mir192, of which 84% (220761) exhibit 3' and 4% (11332) 5' terminal end-variations, and 2% have sequence mismatches. miRNA profiling by qRT-PCR detected 58% out of 517 murine miRNAs on the array. Only 50% of all expressed miRNAs were detected by both assays. No significant correlation between these methods was identified (R2 = 0.0082, P value = 0.07).

Conclusion: Absolute miRNA levels differ substantially between HTS and qRT-PCR with looped reverse transcriptase primers. Most likely, miRNAs with 3'end variations (miRNA isoforms and post-transcriptional modifications) are not detected by this qRT-PCR method. Comparisons of relative expression levels between samples are underway. These insights will improve our understanding of miRNA expression profiling and miRNA biology.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1088**

**Non-Invasive Assessment of Disease Activity in Lupus Nephritis by MRI-Based Molecular Imaging** Siranush Anna Sargsyan,[1] Kendra Hasebroock,[2] Brandon Renner,[1] Natalie J. Serkova,[2] Conrad R. Stoldt,[3] V. Michael Holers,[1] Joshua M. Thurman.[1] *[1]Medicine, University of Colorado School of Medicine, Aurora, CO; [2]Anesthesiology and Radiology, University of Colorado School of Medicine, Aurora, CO; [3]Mechanical Engineering, University of Colorado Boulder, Boulder, CO.*

Lupus nephritis is characterized by immune-complex deposition and complement activation within the glomeruli. We have developed a non-invasive method for detecting tissue-bound iC3b/C3d using superparamagnetic particles of iron oxide (SPIO) linked to the iC3b/C3d binding region of complement receptor type 2 (CR2). SPIO cause a reduction in the spin-spin T2-relaxation time and cause negative enhancement (i.e. darkening) of T2-weighted MRI images of tissues in proportion to the abundance of iC3b/C3d. We hypothesized that this agent could be used to monitor disease activity in a murine model of lupus nephritis. SPIO were synthesized and encapsulated using amine-functionalized phospholipids as ~75 nm aggregates. The particles were conjugated to a recombinant protein that contains the SCR1-2 iC3b/C3d binding region of CR2, generating CR2-targeted SPIO. MRL/lpr mice spontaneously develop a progressive lupus-like glomerulonephritis as they age. MRL/lpr mice and control MRL/MpJ mice were injected with CR2-targeted SPIO at 12, 16, 20 and 24 weeks of age. T2-relaxation times were acquired prior to and at 48 hours post injection. We found that CR2-targeted SPIO reduced the T2-relaxation time in the cortex and outer medullas of MRL/lpr mice, but did not affect the T2-relaxation time in the kidneys of control mice. Histological examination demonstrated that degree of iC3b/C3d deposition in the glomeruli of diseased animals in the diseased kidneys positively correlated with disease severity. Negative enhancement of the renal cortex and outer medulla after injection with CR2-SPIO was strongest in the 20 week-old MRL/lpr mice, corresponding to the peak glomerular iC3b deposition. MRI of the kidneys after injection with CR2-targeted SPIO may provide a quantitative non-invasive alternative for monitoring disease activity in patients with lupus nephritis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1089**

**Diagnostic Accuracy of Dual-Source 64-Slice Multidetector CT Coronary Angiography for the Evaluation of Coronary Artery Disease in Patient with ESRD** Joon Seok Oh, Joong Kyung Kim, Yong Hoon Sin, Seong Min Kim, Ji-Min Jeon, Yong-Ki Park, Gun Ung Jeon. *Internal Medicine, Bong Seng Hospital, Busan, Korea.*

Background: Invasive coronary angiography remains the gold standard in the diagnosis of coronary artery disease. However, multidetector computed tomography (MDCT) coronary angiography is an emerging technique that is available for the non-invasive detection of coronary artery stenoses. Few studies about diagnostic accuracy of MDCT coronary angiography in patients with end stage renal disease (ESRD) were performed. So we investigate the diagnostic accuracy of dual-source 64-slice MDCT coronary angiography in patients with ESRD. Methods : 24 patients (11 males and 13 females; mean age, 59.1 ± 9.5 years) were included in this study. All segments of coronary arteries based on the ACC/AHA classification system were analyzed for the presence of significant stenosis (≥50% diameter stenosis) and compared with the quantitiative coronary angiographic findings. Results : 360 coronary artery segments were assessed quantitatively by both dual-source 64-slice MDCT and conventional coronary angiography. 138 significant stenoses were detected by conventional coronary angiography. On a segment-based analysis, the senisitivity, specificity, and positive and negative predictive values of dual-source 64-slice MDCT were 96, 100, 100, and 50%, respectively.

Diagnostic accuracy of dual-source 64-slice MDCT compared to conventional coronary angiography for detection of significant coronary stenoces in patients with ESRD

| Coronary segment | Number | Sensitivity(%) | Specificity(%) | Positive predictive value(%) | Negative predictive value(%) |
|---|---|---|---|---|---|
| All segments | 360 | 96 | 100 | 100 | 50 |
| LM | 24 | 67 | 95 | 67 | 95 |
| LAD | 120 | 80 | 95 | 89 | 90 |
| LCX | 96 | 92 | 81 | 65 | 97 |
| RCA | 120 | 92 | 76 | 73 | 93 |

LM, Lt. main; LAD, Lt. ant. descending; LCX, Lt. circumflex; RCA, Rt. coronary artery

Conclusions : This study demonstrated that dual-source 64-slice MDCT coronary angiography shows the similar diagnostic accuracy as conventional coronary angiography for the detection of coronary artery disease. In patients with ESRD, dual-source 64-slice MDCT may replace the more invasive coronary angiography.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1090**

**Evaluation of Renal Function by Means of Diffusion Tensor MR Imaging (DTI) and Its Relationship with CKD Stages: Preliminary Results** Emiliana Ferramosca,[1] Marcora Mandreoli,[1] Caterina Gaudiano,[2] Fiorenza Busato,[2] Rita Golfieri,[2] Antonio Santoro.[1] *[1]Nephrology, Dialysis and Hypertension, S. Orsola-Malpighi Hosp, Bologna, Italy; [2]Radiology Institute, S. Orsola-Malpighi Hosp, Bologna, Italy.*

Diffusion Tensor MR Imaging (DTI) is a MR imaging technique used to show molecular diffusion. The apparent diffusion coefficient (ADC) combines the effects of capillary perfusion and water diffusion in the extracellular space. DTI provides information on perfusion and diffusion simultaneously. Fractional anisotropy (FA) is a quantitative value that provides information on direction of water molecules flow within a tissue.

We evaluated feasibility of DTI in assessment of renal function in 2 groups of patients (pts): **A** (CKD 1-2) and **B** (CKD 3-4).

Ten CKD 1-2 (3M, 7F, 50 ±18 ys) and 10 CKD 3-4 (4M, 6F, 58 ±10 ys) pts were enrolled. All pts underwent morphological MR evaluation of kidneys with transverse T1 and T2 weighted acquisition, followed by transverse fat-saturated echo-planar DTI during breath-holding (with a *b*-value 500 sec/mm$^2$). ADC and FA in cortex and medulla were evaluated. Cockcroft and Gault formula was used for GFR assessment. T-test was performed for statistical analysis.

In **A**, GFR was 76±12 ml/min, in B was 33±10 ml/min. In **A**, ADC was higher in cortex than in medulla (2.55±0.09 and 2.10 ±0.08 x $10^{-3}$ mm$^2$/sec), while FA was higher in medulla than in cortex (0.280±0.04 and 0.195±0.03 mm$^2$/sec).

In **B**, ADC and FA were distributed as in **A** (ADC 2.0±0.04 and 1.80±0.05 $mm^2$/sec; FA 0.130±0.03 and 0.160±0.04), but with lower values ($p<0.05$). Our finding was the result of the water molecules flow, that in medulla has a radial direction due to the presence of tubular structure.

DTI of kidneys is feasible with excellent cortex-medulla differentiation. According to literature, we found lower ADC values in patients with moderate-severe renal failure, than in pts with mild degree of renal impairment. In **B**, the reduction in FA is more significant than the reduction in ADC, and particularly evident in medulla. FA may play an important role in detecting early renal damage, even before the onset of renal function impairment. More data are needed to confirm our preliminary results.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1091**

**Direct Visualization of the Oscillations in Proximal Tubule NADPH Oxidase Activity In Vivo** Zalan Peterfi, Arnold Sipos, Janos Peti-Peterdi. *University of Southern California, Los Angeles, CA.*

Multiphoton fluorescence microscopy is a non-invasive, quantitative imaging tool to study important kidney functions, e.g. single nephron GFR and renal blood flow (RBF). RBF and GFR are autoregulated by adjustments in afferent arteriolar diameter. An intrinsic action of vascular smooth muscle cells, known as the myogenic mechanism, and tubuloglomerular feedback (TGF) mediate autoregulation. Both the myogenic mechanism and TGF operate in the vasculature of every nephron, and each mechanism generates its own characteristic autonomous oscillation with a frequency of 0.12 and 0.023 Hz respectively. Proximal tubule (PT) reabsorption is regulated by reactive oxygen species (ROS) and the flow rate of tubular fluid. Also, it is established that tubular flow increases the production of ROS by NADPH oxidase (NOX). PT flow rate oscillates as part of the TGF oscillation, but it is not known whether the production of ROS and reabsorption in the PT oscillate in response. We aimed to directly and quantitatively visualize NADPH oxidase activity in vivo by imaging tissue autofluorescence in C57Bl6 mice with multiphoton microscopy. The PT autofluorescence intensity was the highest in the brush-border membrane area, suggesting that the highest NADPH concentration is present close to apical microvilli (membrane localization). Autofluorescence changed rapidly in response to variations in TGF phase and tubular flow. Also, autofluorescence measured in real-time showed regular oscillations with a frequency that matched TGF characteristics. Furthermore, we detected a transitional increase in autofluorescence after the iv administration of the NOX inhibitor apocynin, confirming the origin of the fluorescence signal (NADPH) and suggesting the involvement of a NOX enzyme in the process. These novel findings demonstrate the feasibility of in vivo autofluorescence measurements in the intact PT, and support our hypothesis that PT reabsorption, controlled by ROS physiologically oscillates with a frequency similar to that of TGF. Moreover, this reproducible method to measure NOX activity in vivo is a high impact finding useful to directly assess the specific role of ROS, NOX in PT function.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1092**

**High Frequency Ultrasound Assessment of Arterio-Venous Fistulae Vessel Wall Thickness To Track Fistula Maturation and To Predict Cannulation Readiness** Sandra A. Donnelly, Rosa M. Marticorena. *Medicine-Nephrology, St. Michael's Hospital, Toronto, ON, Canada.*

**Objectives:**

Post operative wall thickness changes of newly created native arterio-venous fistulae (AVF) measured by high frequency ultrasound might provide a quantitative assessment of vascular remodeling associated with fistula maturation and cannulation readiness.

**Methods:**

High frequency ultrasound evaluation (35-60 MHz) of vessel wall thickness was performed at baseline, 2, 4 and 6 weeks post AVF creation in 17 hemodialysis (HD) patients with newly created AVF (6 radiocephalic, 7 brachiocephalic, 3 brachiobasilic, 1 ulnarcephalic; mean fistula age 0.23 +/-0.16 years). Fistula diameter, size, volume flow, with particular attention to the near field fistula wall within 7 days of cannulation were also made. Images were obtained at 5, 10 and 15 cm from the anastomosis by a single sonographer and measurements were generated by 3 independent and blinded observers. Intra-class correlation analysis was performed. The decision to cannulate the new fistula was made using standard clinical assessment and cannulation was performed by two experienced HD nurses. The definition of "Cannulation readiness" was operationalized as "no extravasation" during the dialysis treatment at 200-250 ml/min blood pump speeds.

**Results:**

Fifteen fistulae met the criteria of cannulation readiness, where 6 treatments were associated with extravasation and further time for maturation was needed. Intimal medial thickness at the arterial puncture site was 0.169 +/-0.02 mm; in the no extravasation group compared to 0.103 +/-0.02 mm in the extravasation group ($p<0.001$). A minimum wall thickness of 0.13 mm ($p<0.001$) is associated with successful cannulation, particularly for the arterial site.

**Conclusions:**

Preliminary data suggests that arterio-venous fistulae wall thickness assessed by high frequency ultrasound predicts cannulation readiness. This may be used clinically to avert fistula damage by premature cannulation and to maximize successful cannulation.

Disclosure of Financial Relationships: Other Relationship: Patent to use doppler has been signed off to St. Michael's Hospital by Dr. Donnelly and Dr. Muradali.

**F-PO1093**

**High Field MRI Methods for the Assessment of Mouse Kidney Structure and Function** Feng Wang,[1] Rosie T. Jiang,[2] Mohammed Noor Tantawy,[1] Keiko Takahashi,[2] Raymond C. Harris,[2] Christopher Chad Quarles,[1] Takamune Takahashi.[2] [1]*Vanderbilt University Institute of Imaging Science, Nashville, TN;* [2]*Vanderbilt O'Brien Mouse Kidney Physiology and Disease Center, Nashville, TN.*

In vivo imaging techniques are widely used for the evaluation of functional, structural, and metabolic integrity of the compromised kidney in human renal pathologies. However, the application of these methods to investigate disease progression in mouse models of renal pathology is highly limited. The overall goal of this study is to develop, optimize, and apply qualitative and quantitative non-invasive MRI methods for the assessment of mouse renal function and injury.

In normal and unilateral ureteral obstruction (UUO) injured mice, $T_1$/$T_2$/$T_2$*-weighted, diffusion-weighted, contrast enhanced perfusion and magnetization transfer imaging protocols were optimized on a high-field 7T MRI system. To minimize the influence of motion artifacts we compared multiple rapid acquisition MRI methods such as coherent gradient echo, multi-slice inversion-recovery fast gradient echo, fast spin echo, and multi slice multi-gradient echo MRI sequences. Relevant imaging parameters and contrast agent doses were varied to optimize structural delineation, signal to noise and image contrast. We also evaluated the use of respiratory gating and fat saturation. The relative blood volume (rBV) measurement was utilized for mapping and characterizing vascular beds in mouse kidney.

Here, we demonstrate that these methods can provide the quantitative evaluation of structural (size, shape, contrast, thickness) and vascular variation (rBV) of mouse kidney during the progression of disease such as UUO. Fine structural changes of kidney were detected at the day (day 0) of UUO surgery and progressive reduction of each renal compartments and the rBV were quantitatively assessed. Thus, high-field MRI measurements allow the non-invasive assessment of kidney structure and function during disease progression in mice. These imaging efforts complement the physiological information obtained from conventional assays of kidney function and facilitate the understanding of pathological mechanisms of kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1094**

**Evaluation of Left Ventricular Hypertrophy in Patients with CKD Stage 3-4 – Which Is the Best Imaging Technique?** Gavin Dreyer,[1] Tom Burchell,[2] Mark Westwood,[2] Martin J. Raftery,[1] Magdi Yaqoob.[1] [1]*Renal Unit, Royal London Hospital, United Kingdom;* [2]*Cardiology, London Chest Hospital, United Kingdom.*

Introduction

Cardiac magnetic resonance imaging (CMR) is considered the gold standard to assess LVH in end stage kidney disease since 2D echocardiography consistently overestimates these parameters probably due to fluid shifts during dialysis. However, CMR is expensive (approximately $750) and not widely available. We studied patients with early CKD (stage 3-4) to determine if 2D echocardiography compared to CMR can determine the presence of LVH with similar accuracy in this patient group.

Methods

Nineteen patients with CKD stage 3-4 (9 male, mean eGFR 37.4 ml/min/$m^2$, mean age 43.3 yrs) underwent CMR and 2D echocardiography. CMR was performed using a Philips Achieva CV 1.5T. LV mass was measured from the steady state free precession contiguous short axis cine stack (8mm slice thickness and 2mm interslice gap, with whole LV coverage) using Philips MR WorkSpace software. LV mass by echocardiography was calculated by the 2D truncated ellipsoid method. Left ventricular mass index (LVMI) is normalized to body surface area (g/$m^2$).

Results

The mean LV mass and LVMI were significantly higher by echocardiography compared to CMR.

LV mass and LVMI values

| | Echocardiography | CMR | p value |
|---|---|---|---|
| LV mass (g) | 142.1 | 97.3 | <0.001 |
| LVMI (g/m2) | 79.3 | 51.9 | <0.001 |

There is a strong positive correlation between LV mass (r=0.64) and a positive but weaker correlation between LVMI (r=0.42) by both techniques. Two patients (10.5%) are classified with LVH by echocardiography but matched values for these patients by CMR are within established normal ranges. No patients with normal values at CMR were misclassified with LVH by 2D echocardiography.

Conclusion

2D echocardiography performs well compared to CMR for evaluation of LVH in CKD 3-4. However, 10.5% of patients in this series are misclassified with LVH by echocardiography. Based on the cost and availability of CMR, we recommend echocardiography as a first line investigation for LVH in patients with CKD 3-4. The presence of LVH on echocardiography should be confirmed by CMR prior to diagnosing, starting or intensifying therapies for LVH.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1095**

**Detecting Microchimerism with Real-Time Quantitative Polymerase Chain Reaction (qPCR) Based on Insertion-Deletion Polymorphisms** Suzanne Wilhelmus, Mathilde M. M. Almekinders, Juan D. Diaz de Pool, Hans J. Baelde, Jan A. Bruijn, Ingeborg M. Bajema. *Pathology, Leiden University Medical Center, Leiden, Netherlands.*

Microchimerism is an intriguing phenomenon playing a role in both renal transplantation and autoimmune diseases such as SLE. Microchimerism is defined as the presence of < 1% of cells present in one individual which are derived from another individual. Previous studies on microchimerism have been limited by the detection of chimeric cells on the basis of a sex-difference: in these studies, Y-chromosome positive cells were used to determine the amount of chimerism in female tissues and/or blood. We here present a technique based on the detection of a mismatch in polymorphisms in a previously described set of bi-allelic insertion deletion polymorphisms (Indels) by qPCR (Alizadeh, Blood 2002).

Twenty couples consisting of related subjects (parent and child) donated oral mucosal cells for the determination of the presence of Indel polymorphisms from our selected set. These Indels are bi-allelic polymorphisms that are common in the population and are located on 9 different chromosomes.

Using the Indel-set we were able to distinguish 85% of related couples. In tested samples, a sensitivity of 1:100.000 genome equivalents and specificity of 100% could be reached. We set up a real-time qPCR using SYBR Green for the detection of all Indels.

Detection of microchimerism is technically challenging. It requires the presence of allogeneic markers and a method with high specificity and sensitivity. Using real-time qPCR with SYBR Green intercalation to detect microchimerism based on different Indels, we were able to distinguish offspring derived chimeric cells in healthy individuals with a sensitivity of 1:100.000 genome equivalents. This method has the ability of quantifying a small amount of DNA in the presence of a large amount of background DNA. This technique bypasses the gender-mismatch limitation, and is applicable for research on chimerism in renal transplantation and autoimmunity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1096**

**Measuring Nephron Endowment in the Intact Kidney Using MRI** Scott Charles Beeman, Teresa Wu, Min Zhang, Kevin Bennett. *Arizona State University, Tempe, AZ.*

The goal of this work was to nondestructively measure nephron endowment using MRI. A reduction in the number of nephrons is associated with a host of renal and systemic diseases, though this information is unavailable in the clinic due to the need for sectioning or maceration of the kidney for counting. Were a nondestructive method available, counting glomeruli could serve an important clinical role. We show that nephrons may be counted in a whole kidney using targeted MRI contrast agents. This, along with previous detection in-vivo (Bennett, Mag. Res. Med., 2008), suggests the possibility of counting nephron in the clinic.

Rats were given bolus IV injections of either cationized ferritin (CF) or native ferritin (NF). Rats were perfused and the kidneys removed. The left kidney was imaged and the right kidney was acid macerated for histological counting. Glomeruli were counted in the MRI volumes using morphological algorithms. Right kidney histological counts were estimated based on observed glomeruli per unit area using a counting chamber.

Imaging showed glomeruli exposed to CF were labeled while glomeruli exposed to NF were not. Counting of labeled glomeruli from MRI yielded 31,046±2,429 glomeruli (n=3). The same counting algorithm yielded 2,788 glomeruli for the control kidney. This number of false positives is approximately equal to the difference between the MRI counting of labeled glomeruli and the histological count of 29,212±1,856 glomeruli (n=3). We conclude that our MRI technique yields comparable results to histological methods with the advantage of maintaining the entire organ. This result, along with previous in-vivo detection of glomeruli, suggests the possibility of counting glomeruli in the clinic.

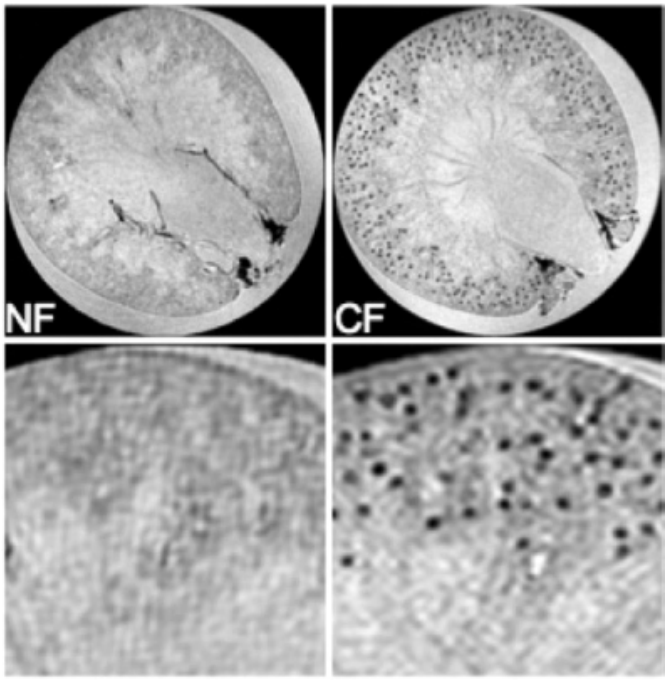



Figure: MRI of excised kidneys with magnifications below. Dark spot to the right represent individual glomeruli labeled with CF. No glomeruli were labeled with NF.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1097**

**Improved Efficacy with Increasing Dose of Biomimetic Membrane Device in Septic Shock** L. Lou,[1] M. Wang,[1] P. Smith,[1] A. Westover,[1] J. Jung,[2,3] D. Buffington,[1] K. Johnston,[1] David Humes.[1,2,4] *[1]Innovative BioTherapies; [2]Univ of Michigan; [3]Chungnam Nat Univ; [4]CytoPherx.*

Sepsis is the leading cause of death in critically ill patients in the United States. Sepsis syndrome is defined as the systemic inflammatory response to infection. We have developed a selective cytopheretic device (SCD) composed of a synthetic biomimetic membrane that binds and sequesters activated leukocytes from the systemic circulation along an extracorporeal blood circuit. This device was evaluated in an established acute porcine model of septic shock. **Methods:** The circulating blood entering the extracapillary space of the SCD was anticoagulated with either citrate (0.7 $m^2$ or 1.8 $m^2$ SCD-C) or heparin (SCD-H). Cardiovascular and pulmonary function, systemic inflammatory indices and activated neutrophil levels were measured. **Results:** The SCD-C maintained cardiovascular, pulmonary and renal function, ameliorated the degree of capillary leak, minimized the systemic level of neutrophil activation compared to SCD-H group. Cardiovascular, pulmonary and renal parameters were significantly better ($p<0.05$) in the SCD-C groups, with 1.8 $m^2$ showing most improvement, versus SCD-H group. These differences in cardiovascular and hemodynamic parameters were associated with significantly longer survival times in the SCD-C groups compared to the SCD-H group, with the 1.8 $m^2$ showing longest survival times. SCD-C treatment has a dampening effect on circulating neutrophil activation levels when compared to the SCD-H treatment group ($p=0.031$). Significant differences were found between the SCD-H and SCD-C treated groups with respect to both total eluted cells and the differential profiles, with the most significant difference seen between the SCD-H and 1.8 $m^2$ SCD-C treated animal groups. **Conclusions:** The results indicate efficacy of SCD-C compared with SCD-H in the amelioration of cardiovascular parameters, inflammatory indicators and increased survival time in a porcine model of septic shock, with increasing SCD membrane surface area as a dose adjustment.

Disclosure of Financial Relationships: Employer: Innovative BioTherapies, Inc.; Ownership: Innovative BioTherapies, Inc.

**F-PO1098**

**The CARPEDIEM Project: Cardio Renal Pediatric Dialysis Emergency Machine. A New CRRT Equipment for the Treatment of AKI in Infants** Claudio Ronco,[1] Dinna N. Cruz.[1] *[1]Department of Nephrology and IRRIV, San Bortolo Hospital, Vicenza, Italy; [2]Pediatric Intensive Care, Ospedale Bambin Gesù, Roma, Italy.*

Acute Kidney Injury may occur in infants due to severe dehydration or complicated cardiac surgery. This syndrome can be considered an orphan disease since only a few cases are reported annually in pediatric intensive care centers and these cases may not receive optimal renal replacement therapy because of the lack of dedicated technology. Infants with body weigth between 2 and 8 Kg must in fact be treated with dialysis machines designed for adults in which pediatric blood lines are utilized. In spite of all adjutments, most machines

are used off label in patients below 15 Kg of body weight. In these circumstances, fluid balance may not be sufficiently accurate and circuit priming volume may result excessive putting the small patients at risk.

Based on these considerations, we designed a miniaturized machine for renal replacement therapy to be used specifically in patients under 10 Kg. The machine isdesigned based on specifications for small infants featuring a low priming extracorporeal circuit (less than 35 ml), a 0.3 sqm hemofilter, a small blood pump delivering a blood flow between 0 and 80 ml/min, small dialysate/filtrate pumps with an operational range between 0 and 15 ml/min, and scales with accuracy of 0.1 g. The equipment can perform ultrafiltration, dialysis and hemofiltration in continuous mode providing continuous feedback on the operational parameters and featuring all classic safety characteristics of a dialysis machine. Sterile fluids for dialysate or replacement solutions are provided in 0.5-1 liter bags. Heparin pump and a drug delivery pump are incorporated in the machine for anticoagulation or combined drug therapies.

This is the first machine in the world specifically designed for infants with body weight below 10 Kg. The project has been supported by a consortium of 3 non profit organizations and the construction has been commissioned to an industry of the field who accepted a non commercial partnership. The machine is CE maked and it will be tested in a multicenter prospective trial.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1099**

**First Implantation of Silicon Nanopore Membrane Hemofilter** William Fissell,[1,2] Joseph J. Groszek,[1] Shuvo Roy,[4] Matthew N. Simmons.[3] *[1]Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH; [2]Biomedical Engineering, Cleveland Clinic, Cleveland, OH; [3]Urology, Cleveland Clinic, Cleveland, OH; [4]Department of Biopharmaceutical Sciences, University of California, San Francisco, San Francisco, CA.*

**Background:** An implanted hemofiltration membrane might offer patients the benefits of quotidian hemodialysis with the ease of use of peritoneal dialysis. A novel membrane technology based on silicon nanotechnology appears enabling for implanted devices. **Methods:** Silicon nanopore membranes (SNM) were coated with PEG as described previosuly. Computational fluid dynamics simulations were used to design a pumpless implant cartridge, which was machined out of medical-grade polycarbonate, and the SNM were installed in the cartridge. Yorkshire breed pigs (n=2) were anesthetised with isofluorane and underwent uninephrectomy. The SNM hemofilter was flushed and connected to remnant renal artery and vein via 7mm PTFE end-to-end grafts and the animals heparinized. Brisk blood flow was observed. Ultrafiltrate production was measured gravimetrically over the 4 hour experiments. **Results:** Stable ultrafiltrate production over 4 hours was observed in both animals. In one animal, accidental kinking of the PTFE graft resulted in cessation of blood flow for 45 minutes, but not cartridge or membrane thrombosis occurred, and repositioning restored blood flow and ultrafiltrate production. Discussion: these pilot large animal experiments demonstrate that SNM are sufficiently robust to withstand packaging and surgical handling, as well as arterial blood pressure, without membrane leakage or thrombosis. more study is needed to estimate in-vivo lifetime and optimal configuration.

Disclosure of Financial Relationships: Consultancy: Medtronic, Inc.; Gambro Renal Products, Inc.; Davita Clinical Research, Inc.; Patent: US Pat No 09/949,575.

**F-PO1100**

**The Effects of a Novel Selective Cytopheretic Device on Mortality and Renal Function in the Intensive Care Unit: A Pilot Study** David Humes,[3] James A. Tumlin,[2] Alexander S. Yevzlin.[1] *[1]Internal Medicine, University of Wisconsin, Madison, WI; [2]Internal Medicine, SERRI, Chattanooga, TN; [3]Internal Medicine, University of Michigan, Ann Arbor, MI.*

Acute kidney injury initiates a systemic inflammatory response state often leading to multiorgan dysfunction. A novel biomimetric membrane called a selective cytopheretic device (SCD), was developed to bind activated leukocytes. The SCD is incorporated in series with a standard hemofilter along a continuous renal replacement therapy (CRRT) extracorporeal blood circuit and pump system coupled with regional citrate anticoagulation. This system has been shown in a porcine model of septic shock to ameliorate cardiovascular instability, minimized the sequestration of activated leukocytes in lung tissue, reduced renal dysfunction and improved survival times compared to control groups. This effect was associated with minimal elevations of systemic neutrophil activation. Two exploratory Phase I/II clinical studies of 10 and 24 patients have been completed (Humes, HD, Sobota, JT, Ding, F, Song, JH. A Selective Cytopheretic Inhibitory Device to Treat the Immunological Dysregulation of Acute and Chronic Renal Failure. Blood Purif 2010;29:183-190; and Ding F, Yevzlin AS, Humes HD. A Selective Cytopheretic Inhibitory Device (SCD) Accelerates Renal Recovery and Improves Mortality in ICU Patients with AKI and MOF in an Exploratory Clinical Study. Abstract: ASAIO annual scientific meeting 2010) and have shown improved survival rates and faster time to renal recovery than concurrent or historical controls utilizing conventional CRRT. No major safety concerns have been observed. Accordingly, an FDA approved IDE pilot study has been initiated in 7 U.S. medical centers. This pilot study is a single arm study with SCD treatment in ICU patients with AKI and multiorgan failure or sepsis requiring CRRT. Up to 35 patients will be recruited. This study is designed to evaluate the safety of this device and early efficacy parameters of mortality at 28 and 60 days and time to renal recovery. Full results of this trial will be presented at the time of these scientific meetings.

Disclosure of Financial Relationships: Employer: Innovative BioTherapies, Inc.
CytoPherx, Inc.; Consultancy: Renal Solutions, Inc.; Ownership: Innovative BioTherapies
CytoPherxResearch Funding: Innovative BioTherapies
CytoPherx; Honoraria: AmGen; Patent: University of Michigan; Scientific Advisor: Innovative BioTherpies
CytoPherx
Renal Solutions
Natural Therapeutics
Michigan Center for Regenerative Medicine.

**F-PO1101**

**Integration of a Selective Cytopheretic Inhibitory Device (SCD) into a Portable Sorbent Dialysis System** K. Johnston,[1] L. Lou,[1] D. Buffington,[1] K. Lee,[2] A. Rojas,[2] David Humes.[1,2] *[1]Innovative BioTherapies; [2]Univ of Michigan.*

Sorbent based dialysis systems regenerate dialysate, permitting the use of only 6L tap water per treatment. When these cartridges are exhausted, ammonium is produced from the breakdown of urea. Certain chelators, i.e. citrate, inadvertently bind the cartridges resulting in premature exhaustion. A novel selective cytopheretic inhibitory device (SCD) requiring citrate anticoagulation has been shown to improve survival in systemic inflammatory response syndrome (SIRS) and associated septic shock, which often lead to multiple organ dysfunction (MOD) and acute kidney injury (AKI). Integration of these two technologies would readily adapt to treating trauma victims from military engagements or natural disasters in remote areas. **Methods:** 60kg pigs (n=7) were made acutely uremic via bilateral nephrectomy and intravenous urea load to achieve systemic BUN >75mg/dl. After heparinization, 6 hours of hemodialysis was administered using the Aliant® sorbent system connected in series with the SCD. Six liters of tap water was used to create the dialysate. Citrate was infused into the blood line pre-SCD with 2% CaCl infused into the venous return post-SCD to compensate for iCa bound by citrate. Blood gas, electrolytes, BUN, CREA, and citrate levels were monitored. Dialysate was tested hourly to detect ammonia breakthrough. **Results:** Serum BUN levels declined throughout the study. Ammonia breakthrough was observed in 3 animals as was predicted based on urea load. Systemic levels of iCa decreased over time but remained within the normal range. **Conclusion:** Successful setup of a Sorbent-SCD circuit capable of the regeneration of dialysate throughout a 6 hour treatment with only 6L of tap water allows for treatment benefits of both dialysis and the use of the SCD in a format that is ideal for field hospital application. This initial study demonstrates the feasibility of using the SCD in a Sorbent circuit for the treatment of SIRS and septic shock.

Disclosure of Financial Relationships: Employer: Innovative BioTherapies, Inc.

**F-PO1102**

**Rapid Determination of Kidney Function in Dogs with Acute Kidney Injury Using a Portable Fiber Optic Fluorescence Ratiometric Analyzer** Vanessa E. Von Hendy-Willson,[1] Barrak M. Pressler,[1] Daniel J. Meier,[3] Ruben M. Sandoval,[2] Exing Wang,[2] Bruce A. Molitoris.[2] *[1]Purdue Univeristy; [2]Indiana University School of Medicine; [3]Fast Diagnostics, Inc.*

Rapid quantification of glomerular filtration rate (GFR) in acute kidney injury (AKI) is important for initial diagnosis and for determination of disease severity. We have previously presented our design of a fiber optic fluorescence ratiometric kidney function analyzer that in theory should allow bed-side calculation of GFR and plasma volume within one hour of marker administration using a two compartment model. This novel system consists of dual LED excitation sources, two PMTs, a sub-millimeter optic fiber, and computer hardware with software handling control, data acquisition, and analysis capability. We now report our further work on optimization of the fluorescence markers/reporter molecules and the suitability of the ratiometric analyzer for determination of GFR in dogs. The optimized conjugates (freely filterable 5 kDa FITC-dextran and non-filterable 150 kDa 2-sulfohexamine-rhodamine-dextran) are both much brighter than alternative commercially available fluorescent markers, thus permitting a large reduction in injected conjugate volume. We also compared iohexol clearance and fluorescence-determined GFR values in healthy laboratory dogs under normal physiological conditions and following gentamicin- or LPS-induced AKI. A wide range of GFR values expected in naturally-occurring AKI was successfully generated using these models. Very good correlation existed between the two methods of GFR determination (r=0.89). As expected, occasional movement by the dogs during the data acquisition period resulted in background noise when measuring fluorescence; however, the analyzer's software was able to remove these data points, resulting in a characteristic smooth elimination curve, and an associated increase in accuracy. Finally, no apparent toxic effects were noted following repeated administration of both fluorescent markers over a 4-month period to study dogs. Based on the success of these pilot studies we anticipate progression to a phase I human study with rapid bedside monitoring of GFR and plasma volume.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1103**

**First Prototype of a Peritoneal Dialysis-Based Automated Wearable Artificial Kidney (AWAK)** Christian G. Bluchel,[1] Martin Roberts,[2-4] David B. Lee,[2-4] Jui Pin Er.[1] *[1]Temasek Engineering School, Temasek Polytechnic, Singapore; [2]VAGLA Healthcare System, Los Angeles, CA; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]AWAK Technologies, Singapore.*

Continuous peritoneal dialysis (CAPD) leads to "patient fatigue" and provides marginal dialytic adequacy, particularly in anuric patients. AWAK is a wearable system that automates dialysate exchange and markedly enhances adequacy. This is accomplished by automated and continuous regeneration of spent dialysate and recycling of the regenerated, fresh dialysate at a high flow rate (4L/h) using tidal PD (TPD). TPD will be conducted with low reserve (RV) and tidal (TV) volume (RV+TV≤ 1.5L) for minimizing both the weight of AWAK and patient discomfort. The prototype weighs about 3 kg and consists of inter-connected disposable and non-disposable assemblies. Operation initiates with the "outflow mode" (OM), during which spent dialysate is drained from the peritoneal cavity (PC) through a sorbent cartridge, where it is regenerated. The regenerated dialysate is then reconstituted with electrolytes and glucose as prescribed. The OM is followed by the "inflow mode" (IM), during which the regenerated and reconstituted dialysate stored in a module in the disposable assembly, is returned into the PC. An ammonia detector monitors for sorbent cartridge exhaustion. OM-IM cycle is continuously repeated in sequence. Prior to replacing the sorbent cartridge (every 12 hours), the PC is completely drained into an UF module in the disposable assembly. The prescribed RV and TV is then pumped back into the PC. The remainder fluid is discarded as UF. Preliminary TPD studies indicate a weekly Kt/V of 3.4 to 6.5 and a daily UF of 1.8 to 3.9 L can be attained.

Disclosure of Financial Relationships: Research Funding: AWAK Technologies, Singapore.

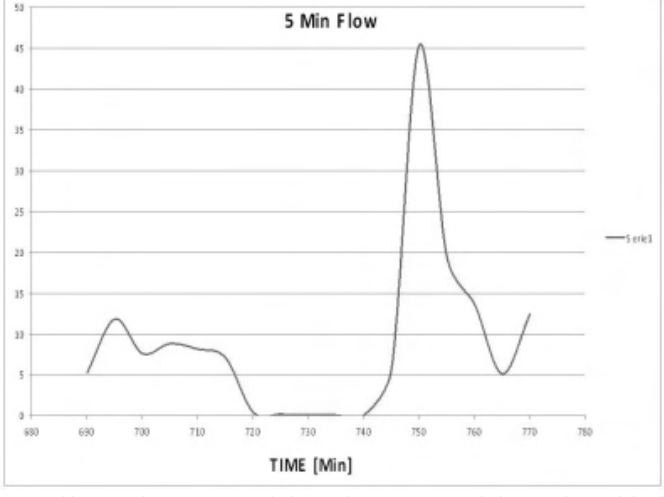
**F-PO1104**

**URINFO® in the Early Detection of Acute Kidney Injury** Anthi Panagiotou,[1,3] Francesco Garzotto,[1,3] Matteo Floris,[1,3] Federico Nalesso,[1,3] Dinna N. Cruz,[1,3] Claudio Ronco.[1,3] *[1]Nephrology, S Bortolo Hosp, Vicenza, Italy; [2]IRRIV, Vicenza, Italy.*

INTRODUCTION: RIFLE consensus classification was the first accepted definition for acute kidney injury (AKI). RIFLE criteria are based on creatinine and urine output changes and define severity classes of AKI. However, many patients with AKI are not identified early enough since changes in creatinine level have a 48 hours time window even though AKI is already present. Standardized manual urine output (UO) measurements in most of critical care units are inaccurate and inconsistent. We describe the first preliminary results from a clinical trial that was conducted in our center using the URINFO® System.

METHODS: In critical ill patients urine volume and flow variation are important parameters. URINFO® system is an innovative digital urine meter that provides continuous minute based monitoring of UO allowing kidney monitoring and reliable information for UO in real time. Since all data are automatically stored in a designated data logger, urine monitoring does not require manual intervention. Continuous urine measurement allows physicians to identify early UO variations and to obtain rapid information about kidney function and potential development of AKI, making an earlier clinical patient's management.

RESULTS: Analyzing our data, urine flow diagram can be used as other vital signs for patient's monitoring.




In this case, that we presented above, the UO curve variation can be explained as mechanical problem due to catheter kicking only and not as real AKY because after the kicking detection UO was restored immediately as the curve demonstrated. CONCLUSIONS: The use of URINFO® system can be a new and easy approach for the UO monitoring in order to consent an earlier AKI identification by RIFLE criteria. This approach can consequently allow rapid therapeutic interventions.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1105**

**LNA Antisense Are Potent, Specific and Long Lasting Inhibitors of mRNA in the Kidney** Bo R. Hansen. *Research Division, Santaris Pharma A/S, Hørsholm, Denmark.*

BACKGROUND: Oligonucleotides comprising Locked Nucleic Acid (LNA) exhibits unprecedented high RNA binding affinity. The high affinity offers the opportunity to reduce the oligonucleotide length and at the same time maintaining the potency. LNA improves the *in vivo* potency/efficacy significantly, and notably without conjugation ligands and special lipid formulations. The biodistribution of LNA oligonucleotides is broad and shows a high uptake into kidney tissue with a long tissue half-life due to the much improved stability of LNA oligonucleotides. Hyperlipidemia and hypercholesterolemia has shown to contribute to the pathogenesis of progressive renal disease and apolipoprotein B-100 (ApoB) has been found to play a significant role. METHODS: The 13-mer LNA SPC3833 was selected from a series of LNA oligonucleotides targeting the human and murine ApoB mRNAs and tested *in vitro* and *in vivo* in two different rodents. C57BL/6 mice (5 animals/group) were dosed i.v. with oligonucleotides diluted in saline at doses of 0.2 mg/kg and 1.0 mg/kg per day or saline for three consecutive days and sacrificed 24 hours after the last dosing. The expression levels of ApoB mRNA were analyzed in kidney tissue. Wistar rats (5 animals/groups) were dosed s.c. with SPC3833 diluted in saline at doses of 5 mg/kg and 10 mg/kg per day or saline once weekly for four consecutive weeks and sacrificed 7 days after the last dosing. ApoB mRNA was analyzed in kidney tissue. RESULTS: In the mouse studies the groups of mice dosed with 13-mer LNA oligonucleotide SPC3833 resulted in a 70% reduction of the target mRNA at 0.2mg/kg and a 85% reduction of the target mRNA at 1.0mg/kg compared to saline treated control animals. In the rat studies SPC3833 resulted in a 70% reduction of the target mRNA in the kidney tissue at the 5 mg/kg dose compared to the saline treated control animals while the target mRNA was hardly detectable in the kidney tissue in the 10 mg/kg treated animals even 7 days after the last dosing. CONCLUSIONS: These studies showing the high potency and long duration of action of a short LNA oligonucleotide *in vivo* demonstrate the broad possibilities of using LNA antisense therapeutics for ApoB and other RNA targets in renal disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1106**

**Assessment of Glomerular Filtration Rate in Chinese Patients with Chronic Kidney Disease Using a Radial Basis Function Neural Network** Xun Liu,[1,2] Xiaoming Wu,[2] Ningshan Li,[2] Tan-Qi Lou.[1] *[1]Division of Nephrology, Department of Internal Medicine, The Third Affiliated Hospital of Sun Yet-Sun University, Guangzhou, Guangdong, China; [2]College of Biology Engineering, South China University of Technology, Guangzhou, Guangdong, China.*

Background Artificial neural networks have been widely used in the field of engineering forecasting. This paper attempts to predict glomerular filtration rate (GFR) in Chinese patients with chronic kidney disease (CKD) by radial basis function (RBF) neural network.

Materials and methods 327 patients with CKD who had undergone 99mTc-DTPA-glomerular filtration rate (GFR) estimation were enrolled. SC was determined enzymatically. The 99mTc-DTPA-GFR was used as the reference standard GFR (sGFR). The input layer of the RBF network consisted of only one unit, the patients' records of SC. The output layer consisted of one unit representing sGFR. Average sGFR was 44.5 (±26.9) ml/min/1.73 m2 (range, 2.8-140.0 ml/min/1.73 m2). The Cockcroft-Gault-equation, Jelliffe-1973-equation, Bjornsson-equation, Ruijin-equation and RBF network were compared. The accuracy of estimated GFR (eGFR) was compared with sGFR in various stages of CKD.

Results Bland-Altman analysis demonstrated that RBF network was better than the other equations. Only the agreement limits of the RBF network exceeded the prior acceptable tolerances defined as 60 ml/min/1.73 m2. The slope of the regression line estimated by the RBF network was smaller than those of the other equations. Median % absolute difference of the RBF network was significantly less than those of the other equations. The accuracy with a deviation less than 30% from the sGFR of the RBF network was significantly higher than those of the Jelliffe-1973-equation and Ruijin-equation. When compared the bias as well as accuracy of eGFR with sGFR in different stages of CKD, GFR estimated by RBF network showed good results.

Conclusion Our data indicated that when SC was measured by the enzymatic method, based on both overall performance as well as performance in different CKD stages, this RBF network model is suitable for the specific Chinese population tested. Further procedures are needed to facilitate the validation of the model.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1107**

**Modeling of Individual Hemoglobin Responses to Erythropoietin (EPO) in DCI Hemodialysis Patients Using Retrospective Data** John Mcmichael,[1] Klemens B. Meyer,[3] Dana C. Miskulin,[3] Kevin Ho.[2] *[1]Dimensional Dosing Systems Inc., Wexford, PA; [2]University of Pittsburgh Medical Center, Pittsburgh, PA; [3]Tufts University, Boston, MA.*

We used the Intelligent Dosing System (IDS) to model a retrospective DCI EPO dosing dataset. The IDS is an FDA Class II medical device that uses a nonlinear equation to correlate changes in dose to changes in level or response. The IDS equation consists of 3 equations: (1) calculates EPO dose based on dose- response Hgb, (2) stochastic loop modifies result of 1st equation to account for non-linear, patient-specific effects, (3) calculates expected Hb following the dose adjustment. The retrospective analysis was conducted on a dataset with 1.9 million dosing observations on 11,083 chronic DCI hemodialysis patients which

included EPO dose, Hgb level, date of EPO administration. Data was organized into a tabular view so that each row contained the appropriate data elements which represented a dosing interval. Variability when no dose change is made and non-logical observations could be attributed to changes in the patient's clinical status; these was not analyzed. We used 5 different strategies to select the best input parameters. To conduct this retrospective analysis, EPO Dose1, EPO Dose2 and Hgb1 were used as inputs to the IDS equation to calculate Hgb2. We calculated the absolute error between the observed next Hgb2 and the IDS calculated Hgb2. The IDS also uses a stochastic loop to individualize therapy based on previous dosing experience to further increase predictive accuracy. Since this would necessitate a prospective study, this component could not be tested.

Results

| | Total # Observations | # Abs Err <0.3 g/dL | % <0.3 g/dL | # Abs Err <0.5 g/dL | % <0.5 g/dL | # Abs Err < 1.0 g/dL | % <1.0 g/dL |
|---|---|---|---|---|---|---|---|
| Model 1 | 730787 | 57432 | 30.7 | 86889 | 41 | 138897 | 65.6 |
| Model 2 | 1182835 | 94751 | 25.7 | 144688 | 39.2 | 237210 | 64.2 |
| Model 3 | 534708 | 43328 | 33.5 | 64482 | 49.8 | 96940 | 74.9 |
| Model 4 | 730787 | 83666 | 30.7 | 125253 | 46 | 192633 | 70.7 |
| Model 5 | 1182835 | 152498 | 28.7 | 230360 | 43.3 | 366847 | 68.9 |

Disclosure of Financial Relationships: Ownership: I am a shareholder and President of Dimensional Dosing Systems Inc.; Patent: I have patents on the Intelligent Dosing System (IDS).

## F-PO1108

**Bayesian Classifier vs Relevant Physiological Parameters** Francesco Garzotto,[1,3] Matteo Recchia,[1,3] Dinna N. Cruz,[1,3] Matteo Floris,[1,3] Alessandra Brendolan,[1,3] Federico Nalesso,[1,3] Pasquale Piccinni,[1,2] Claudio Ronco.[1,3] *[1]Nephrology, St. Bortolo Hosp, Vicenza, Italy; [2]Intensive Care Unit, St. Bortolo Hosp, Italy; [3]IRRIV.*

Acute kidney injury (AKI) is a common problem, especially in critically ill patients. Prediction of AKI in a simple manner without costly biomarkers is a desirable clinical goal. The aim of the study is use the Bayesian classifier (BC) approach to predict an event AKI in order to provide the intensivist with a tool to aid in medical decision-making

Methods

Data from the NefroInt project (Italian prospective multicenter study) were used. Database requires clinician to indicate the patient's renal outcomes as A)Dialysis dependent,B)Dialysis-independent but renal function not at baseline,C)Renal function returned to baseline & D)No renal dysfunction during ICU stay. For the model, we defined only 2 classes: AKI (A+B+C) vs Normal(D).Using 6670 days recorded from 600 patients of 10 different ICU, we derived a probabilistic classifier based on Bayes theorem, using the same parameters found in severity of illness scores (APACHE II,SOFA,SAPSII), as well as several variables commonly considered by clinicians (e.g. baseline renal function, diuretic use, fluid balance).Forward linear method was used to identify parameters that give maximum accuracy. Models design was based on a leave-one-out supervised classification implemented in the software Rapidminer.

contingency table

| | TRUE | | | |
|---|---|---|---|---|
| | AKI | Normal | | |
| AKI | 320 | 78 | 80.40% | ppv |
| Normal | 39 | 165 | 80.88% | npv |
| | 89.14% | 67.90% | | |
| | sensitivity | specificity | | |

Accuracy of BC to classify AKI vs Normal patients was 80.56%.The forward linear technique give 7 parameters necessary to attain this level of accuracy: creatinine, urine output 24h, worst urine output 6h, MAP, chronic disease, urinary pathology, sepsi.Sensitivity was 89.14% and specificity was 67.90%

Conclusion

A tool based on BC without any type of medical information could predict with an high accuracy AKI in critically ill patients. The seven clinical parameters used by BC to obtain the results are physiologically relevant for a nephrologist

A more sophisticated classifier capable of predicting more detailed renal outcome (e.g.4 classes) would be the logical next step.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1109

**Evaluation of Bioartificial Renal Epithelial Cell System (BRECS) Therapy in a Porcine Septic Shock (PSS) Model** A. Westover,[1] D. Buffington,[1] L. Lou,[1] M. Wang,[1] P. Smith,[1] K. Johnston,[1] C. Pino,[1] J. Jung,[2,3] David Humes.[1,2] *[1]Innovative BioTherapies; [2]U Michigan; [3]Chungnam Nat U.*

Renal cell therapy incorporated into conventional CRRT circuit has shown metabolic, immunologic and survival benefits in renal failure in preclinical and clinical studies. To improve upon previously used hollow fiber based delivery platforms, a miniaturized, freezable BRECS has been developed. The device is fabricated with approximately $10^8$ renal epithelial cells seeded onto trabeculated carbon disks maintained by continuous perfusion. BRECS are evaluated using a previously established model of sepsis.

**Methods**: Hemofiltration is established in a PSS model with incorporation of the BRECS into an ultrafiltrate (UF) loop with processed UF returned to the animal allowing for maintenance of cell viability and communication between the device and host. SS is simultaneously induced by the introduction of bacteria into the peritoneum resulting in multiorgan dysfunction. Hemodynamic parameters including cardiac output, mean arterial pressure and systemic vascular resistance were monitored through death or up to 12 hours. Systemic inflammation was evaluated using serum cytokines, neutrophil (NE) expression of CD11b and evaluation of NE extravasation into lung tissue by post mortem immunohistochemistry. Cell Viability within BRECS was verified post study using $O_2$ consumption and using a fluorescein diacetate based viable dye.

**Results:** To date 8 BRECS and 7 acellular BRECS have been evaluated. A significant improvement in cardiovascular parameters (CVP) was observed T=2 hours through study termination. Average survival time was increased from 7.7 to 10.9 hours, with 4 animals in the treated group surviving up to the predetermined stop point of 12 hours, even though CVP indicated that survival times would be much longer. Significant differences in serum cytokines were not observed; the CD11b expression by NEs was significantly altered, peaking at T=4 vs T=10 hours. Aggregation of activated NEs into lungs was 50% lower in BRECS treated animals compared to sham controls.

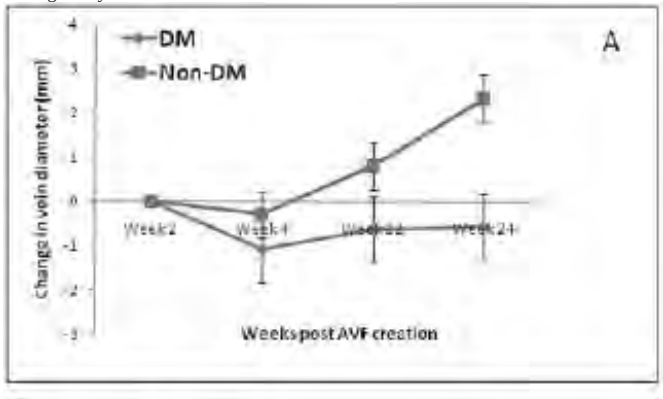
**Conclusion**: BRECS therapy may ameliorate the course of SS by modulating inflammation and providing cardiac support.

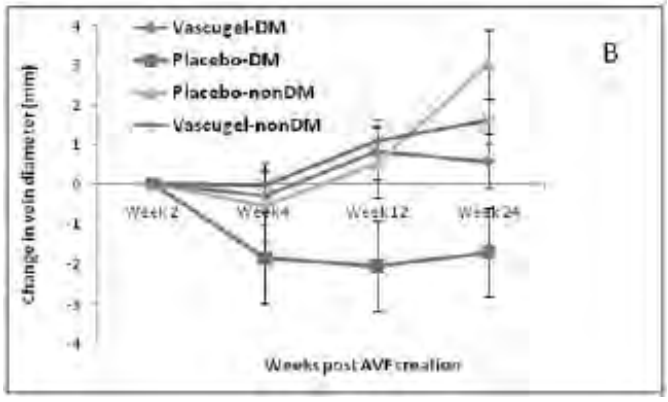
Disclosure of Financial Relationships: Employer: Innovative BioTherapies, Inc.; Ownership: Innovative BioTherapies, Inc.

## F-PO1110

**Arteriovenous Fistula Remodeling from a Multi-Center Phase I/II Trial Investigating the Use of Allogeneic Endothelial Cell Implants: V-HEALTH Study** Michael S. Conte,[1] Helen Nugent,[2] Prabir Roy-Chaudhury.[3] *[1]University of California, San Francisco, CA; [2]Pervasis Therapeutics, Cambridge, MA; [3]University of Cincinnati, Cincinnati, OH.*

Arteriovenous fistula (AVF) is the preferred type of access for hemodialysis to treat end stage renal disease. However, a high proportion of AVF are never used for dialysis because the vein fails to mature. We previously described the safety of Vascugel (allogeneic aortic endothelial cells in a gelatin matrix) when placed around the anastomotic and venous outflow sites of AVFs (V-HEALTH study). In this retrospective analysis, we describe the remodeling of AVF assessed by serial ultrasound measurements of lumen diameter. Thirty-one AVF patients received either Vascugel or control matrices (placebo) at surgery and were followed for 24 weeks. Venous lumen diameter was measured at 1, 3 and 5 cm from the anastomosis. Vein remodeling (mm change in lumen diameter at 4, 12 and 24 weeks compared to baseline diameter at 2 weeks) was analyzed using a mixed model repeated measures analysis of covariance. The results indicate that diabetes (DM) is a significant predictor of poor venous remodeling post AVF creation ($P$<0.03, Figure 1A). The average change in lumen diameter from 2 to 24 weeks was -0.75 mm in DM patients (N=12) and +0.94 mm in non-DM patients (N=15), with the major effect seen at 24 weeks (difference of 2.3 mm, 95% CI 1.2-3.4, p=0.0002). Treatment with Vascugel showed a positive trend in change in lumen diameter (0.62 mm vs. -0.44 for placebo, 95% CI for difference is -0.33, 2.46, p=0.13). Specifically, comparing Vascugel-treated DM patients to placebo-treated DM patients, this difference was 2.24 mm (95% CI 0.1-4.4, p=0.04), indicating that treatment with Vascugel positively mitigated the effect of DM on remodeling (Figure 1B). We conclude that DM negatively impacts AVF remodeling, and Vascugel may ameliorate this effect.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1111**

**Interaction of ELMO1 with COX-2 Increases COX-2-Mediated Fibronectin Upregulation** Andrey Sorokin, Chen Yang. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

Renal glomerulosclerosis, the final major lesion of many primary and secondary glomerular diseases, is characterized by accumulated deposits of extracellular matrix (ECM) proteins in glomeruli. Renal glomerular mesangial cells (GMC) play a pivotal role in the development of glomerular injury and in response to pathological stimuli produce ECM proteins such as collagens and fibronectin which result in irreversible glomerular injury. Increased glomerular cyclooxygenase-2 (COX-2) expression has been reported in many glomerular diseases and even though selective inhibition of COX-2 retarded the progression of glomerular injury the precize mechanisms by which COX-2 causes glomerular injury have not yet been elucidated. Engulfment and cell motility 1 (ELMO1), a bipartite guanine nucleotide exchange factor for the small GTPase Rac1, was identified as a susceptibility gene for glomerular disease. To elucidate the role of ELMO1 in the development and progression of glomerular injury, we examined the expression of ELMO1 protein in anti-Thy-1 glomerulonephritis rats. By immunofluorescence double staining, we demonstrated that the expression of ELMO1 and COX-2 was increased in the glomeruli of these rats. We detected co-localization of ELMO1 with COX-2 in cultured human GMC by immunofluorescence and proved interaction of ELMO1 with COX-2 by co-immunoprecipitation. Furthermore, we identified ELMO1 as a posttranslational regulator of COX-2 activity. We found that COX-2 increased fibronectin upregulation through its cyclooxygenase activity and protein-protein interaction between ELMO1 and COX-2 increased COX-2-mediated fibronectin expression. We also found that ET625, the dominant negative form of ELMO1 lacking Rac1 activity, still interacted with COX-2, increased cyclooxygenase activity of COX-2 and enhanced COX-2-mediated fibronectin upregulation. We also evaluated effect of the constitutive active Rac1 ($Rac1^{Q63E}$), and found that activation of Rac1 signaling had no effect on COX-2-mediated fibronectin promoter activity. Our data suggest that ELMO1 contributes to the development of glomerular injury at least partially acting as a posttranslational regulator of COX-2 activity by Rac1-independent mechanism.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1112**

**The Regulation of EDA+ Fibronectin Production in Human Tubule Cells** Mysore Keshavmurthy Phanish, Ioana Niculescu-Duvaz, Elisha Seah, Mark Edward Dockrell. *SW Thames Institute for Renal Research, London, United Kingdom.*

Fibronectin (Fn) is an important extracellular matrix component which is expressed as several variant proteins produced as a result of pre-mRNA alternative splicing. The EDA+ variant of Fn is particularly associated with fibrotic diseases including renal fibrosis. EDA+ Fn accelerates fibrosis in a number of diverse ways. In this work, we investigate the regulation of EDA+ Fn in human proximal tubule epithelial cells (PTEC).

Primary and transformed (HKC8) cells were used. Fn expression was assessed by immunofluorescence, Western Blotting (with antibodies to total and EDA+ Fn) and RT-PCR (with a single primer pair producing separate products for EDA+ and EDA-).

Involvement of the splicing factor SRp40 was investigated using RNA interference, Western blotting and immunoprecipitation.

The role of CLK/Sty was investigated using the inhibitor TG003 (10 μM). The role of PI 3-kinase/Akt pathway was investigated using the PI 3-kinase inhibitor LY294002 (5-10 μM) and Western blotting for p-AKT following immunoprecipitation (IP) with SRp40 antibody.

TGFb1 (2.5ng/ml) induced EDA+Fn expression, which was selectively inhibited by SRp40 knock-down. TG003 did not alter TGFb1-induced EDA+ Fn expression; however LY294002 did significantly reduce EDA+ expression. Furthermore LY294002 treatment altered the intracellular distribution of SRp40. TGFb1 also increased the association of SRp40 and p-AKT in IP experiments, which was LY294002 sensitive.

We have presented the first evidence for the regulation of Fn RNA splicing by SRp40, a member of the SR protein family of splicing factors in renal tubule cells. We provide evidence that PI3kinase-AKT signaling regulates this process by a direct interaction between pAKT and SRp40. Targeting the splicing of Fn pre-RNA such that EDA+ inclusion is prevented is an attractive and novel option to combat fibrosis while leaving the more physiological isoforms of Fn intact.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1113**

**A Feedback Loop in High Glucose-Induced Inactivation of FoxO1 Regulates Akt Activation, Mesangial Cell Hypertrophy and Fibronectin Expression Via Downregulation of Catalase** Falguni Das,[1] N. Ghosh-Choudhury,[2] C. Mandal,[2] Nirmalya Dey,[1] B. S. Kasinath,[1] Goutam Ghosh-Choudhury.[1] *[1]Medicine, UTHSCSA, San Antonio, TX; [2]Pathology, UTHSCSA, San Antonio, TX.*

We have recently shown that Akt kinase contributes to increased expression of matrix protein fibronectin and hypertrophy of mesangial cells (MC) in response to high glucose (HG; 25 mM). As a mechanism, we considered the Akt substrate transcription factor, FoxO1. HG increased phosphorylation of FoxO1 in MC in a time-dependent and sustained manner, concomitant with enhanced phosphorylation/activation of Akt kinase. To elucidate the role of FoxO1, phosphorylation-deficient constitutively active mutant of FoxO1 (FoxO1/A3) was used. Expression of FoxO1/A3 inhibited and dominant negative FoxO1 (DN FoxO1) increased HG-induced fibronectin expression, respectively. Furthermore, FoxO1/A3 blocked HG-stimulated phosphorylation of the negative regulator of TORC1, PRAS40, resulting in inhibition of TORC1 activation and suppression of protein synthesis, which caused attenuation of MC hypertrophy. Since Akt kinase regulates MC hypertrophy and fibronectin expression, we examined the effect of FoxO1 on Akt activation. FoxO1/A3 decreased HG-stimulated Akt phosphorylation while DN FoxO1 increased it. To elucidate the mechanism, we considered the anti-oxidant protein catalase, which quenches H2O2. HG significantly reduced the expression of catalase in MC in a time-dependent manner, which followed the same kinetics of phosphorylation of Akt and FoxO1. HG induced transport of FoxO1 from the nucleus to cytosol, indicating a role of this transcription factor in reduction of catalase expression. Expression of FoxO1/A3 increased expression of catalase, resulting in reduced abundance of reactive oxygen species. DN FoxO1 decreased the expression of catalase. Furthermore, FoxO1/A3 increased transcription of catalase in a reporter transfection assay. These results represent the first evidence for the presence of a positive feedback loop for phosphorylation of Akt in response to HG involving inactivated FoxO1-dependent catalase downregulation, which may contribute to fibronectin expression and hypertrophy of MC.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1114**

**C-Terminal CTGF Fragment Alone Selectively Modulates Pro-Fibrotic Signaling in PTECs** Simon Winn, Ekram Nabi, Mysore Keshavmurthy Phanish, Mark Edward Dockrell. *SW Thames Institute for Renal Research, London, United Kingdom.*

As a matricellular modulator, full-length Connective Tissue Growth Factor (flCTGF) is associated with fibrosis. Cleavage products of CTGF are found in various biological fluids and organ systems. We have previously demonstrated binding to BMP-7 and attenuation of its canonical signaling pathway by a purified 11kDa C-terminal fragment of CTGF (cCTGF). We have further investigated binding to TGFβ and whether other BMP-7 and TGFβ signaling pathways implicated in fibrosis might be similarly affected.

Transformed HKC-8 proximal tubule epithelial cells, known to possess the full complement of ALK receptors, were used to compare the effects of "in-house" flCTGF and cCTGF (Peprotech) on BMP-7 and TGFβ induced signaling using immunoblotting techniques. Real time molecular binding of cCTGF to TGFβ was investigated using Surface Plasmon Resonance, by immobilizing carrier-free cCTGF (129fM/$mm^2$) to a CM5 sensor chip.

TGFβ demonstrated dose dependent binding to the cCTGF peptide across a range of 10 - 40nM; comparable to that seen with BMP7. Unlike BMP7-induced Smad signaling, BMP-7-induced p38 activation was not reduced by co-incubation with flCTGF (100 and 200ng/ml) nor cCTGF (25 and 50ng/ml). Furthermore, the significant induction of pSmads and pp38 by TGFβ (1.25ng/ml) were differentially modulated by co-incubation with similar doses of CTGF; adjunct flCTGF (200mg/ml) caused a further 10-30% increase in the activation of pSmads 1, 2 and 3 as well as pp38 whilst, in the presence of cCTGF, this synergism was only seen for pSmad 3 and pp38 (for TGFβ 5ng/ml) but absent for pSmads 1 and 2.

These results identify cCTGF as a binding partner for TGFβ and BMP-7 and further indicate the emergence of a differential role for flCTGF and cCTGF in the modulation of pro-fibrotic stimuli. The effects on BMP-7 and TGFβ signaling cannot simply be ascribed to extracellular trapping phenomena and likely involve as yet undefined interactions of CTGF peptides with their target receptors. Interestingly, cCTGF by promoting TGFβ induced activation of Smad 3 and p38, as well as selectively attenuating BMP-7 induced Smad signaling may favor a pro-fibrotic phenotype.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1115**

**Inhibition of PAI-1 Enhances Transforming Growth Factor β (TGF-β)-Induced Endothelial-to-Mesenchymal Transition (EndMT)** Li-Jun Ma,[1] Dan Gao,[1, 2] Bridgette Corsa,[1] Agnes B. Fogo.[1] *[1]Department of Pathology, Vanderbilt University; [2]Department of Nephrology, First Affiliated Hospital of ZhengZhou University, ZhengZhou, China.*

Background: TGF-β induces both epithelial-to-mesenchymal transition (EMT) and endothelial-to-mesenchymal transition (EndMT), processes that may contribute to kidney fibrosis. TGF-β also induces plasminogen activator inhibitor-1 (PAI-1). We investigated whether inhibition of endogenous PAI-1 in endothelial cells affects TGF-β-induced endMT.

Methods: Mouse glomerular endothelial cells (GENs) were transfected with PAI-1 siRNA (PAI-1 KD, n=3) or CONT siRNA (CONT, n=3) or untreated, and were then stimulated by TGF-β (5 ng/ml) for 48 hrs. Expression of PAI-1 protein, pSmad2 and claudin 5 was examined by Western blot. EndMT was assessed morphologically as well as by expression of mesenchymal marker α-smooth cell actin (α-SMA). Data are expressed as mean±SE.

Results: TGF-β treatment in GENs induced marked transition of endothelial cells into smooth muscle-like cells in vitro, which was associated with increased p-Smad2 expression (11.8-fold, $p<0.05$ vs baseline), and prominent expression of α-SMA, a mesenchymal marker (3.1-fold vs baseline, $p<0.05$). Targeting endogenous PAI-1 in GENs using siRNA reduced PAI-1 protein levels about 80-90%. When siRNA treated GEN cells were stimulated by TGF-β, p-Smad2 levels were significantly but similarly increased in PAI-1 knockdown GENs vs CONT. However, α-SMA protein level was 5.3-fold higher (α-SMA/GAPDH 0.16±0.04 vs 0.03±0.01, $p<0.05$) and claudin 5, a marker of endothelial cells, protein expression was 23% lower in PAI-1 knockdown GEN cells vs CONT, indicating enhanced TGF-β-induced EndMT.

Conclusions: Inhibition of PAI-1 enhances TGF-β-induced endothelial-to-mesenchymal transition through a non-pSmad2 pathway. We speculate that altered proteinases and/or metalloproteinase by PAI-1 in GENs may play an important role in TGF-β-induced EndMT.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1116**

**αvβ3-Integrin Activation Promotes TGF-β1 Induction of Collagen Production Via Enhanced Rac1 Activity in Human Kidney Epithelial Cells** Tomoko Hayashida, H. William Schnaper. *Pediatrics, The Feinberg School of Medicine, Northwestern University and Childrens' Memorial Research Center, Chicago, IL.*

We previously showed that integrin engagement-dependent focal adhesion kinase (FAK) phosphorylation modulates TGF-β/Smad fibrogenic signals, via ERK-MAP kinase-mediated phosphorylation of the Smad3 linker region (Hayashida, J Cell Sci, 2007). Utilizing β1-integrin knockdown (k/d) cells, we unmasked the function of αvβ3-integrin, which promotes renal cell collagen production in culture via enhanced Rac1 and ERK activity (ASN 2009). Here, we have applied these findings in regular, β1-integrin-expressing kidney epithelial cell lines (HKC) and sought to further define a mechanism by which αvβ3-integrin interacts with TGF-β/Smad signaling. Phosphorylation of both tyrosine 747 and -759 residues of the β3-integrin cytoplasmic tail in HKCs was induced after 30 min treatment with TGF-β. However, phosphorylation of Tyr 747, but not Tyr 759, was detected in immunoprecipitates with an antibody to αvβ3-integrin, suggesting that phosphorylation of the former is specific to αvβ3-integrin activity. Indeed, in β1-k/d cells where αvβ3-integrin activity is increased, Tyr 747, but not Tyr 759, was constitutively phosphorylated. TGF-β induction of type I collagen promoter activity, determined with a -378 COL1A2-luciferase construct, was enhanced in β3-integrin-overexpressing HKCs compared to control HKCs, whereas Y747F-, but not Y759F mutant of β3-integrin abrogated the response. On the other hand, the collagen response was significantly diminished in CT26 fibroblast-like colon carcinoma cells that lack β3-integrin. Furthermore, β3-integrin-overexpressing HKCs showed enhanced Rac1 activity, similar to those in β1-integrin k/d cells. These findings support the notion that the level of β3-integrin expression is a critical factor for αvβ3-integrin activity that promotes TGF-β-mediated cellular collagen production via Rac1-mediated ERK activity, and that αvβ3-specific tyrosine 747 phosphorylation of β3-integrin is essential for the interaction between the Smad and integrin signals.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1117**

**ITA-1 – A Novel Inhibitor of TGF-β1 Signalling** James B. Corcoran, Sarah Mccarthy, Una Bhreathnach, Brenda Griffin, Fionnuala B. Hickey, Finian Martin, Catherine Godson, Madeline Murphy. *Diabetes Research Centre, University College Dublin, Dublin, Ireland.*

We have recently described *Induced in high glucose-1 (IHG-1),* an evolutionarily conserved gene transcript increased in diabetic nephropathy. IHG-1 enhances the actions of TGF-β1 and may contribute to the development of tubulointerstitial fibrosis (TIF) (Murphy et al., J. Am Soc Nephrol 2008) TGF-β1 plays a key role in TIF. Although highly conserved IHG-1 has no functional homology to other proteins apart from a putative mitochondrial targeting sequence (mts). We have confirmed that IHG-1 localizes primarily to mitochondria in mammalian cells. Here we investigate whether mitochondrial localization of IHG-1 is necessary to amplify TGF-β1 signal transduction. Amino acids 9-22 of the putative mts were deleted (Δmts-IHG-1) and the mutant IHG-1 protein expressed in human renal epithelial (HK-2) cells. Overexpressed Δmts-IHG-1 localised to the cytoplasm indicating the putative mts is essential for mitochondrial localization of IHG-1. Δmts-IHG-1 inhibited TGF-β1- fibrotic responses including increased expression of fibronectin, CTGF and the notch ligand Jagged1. Δmts-IHG-1 expression also decreased TGF-β1-driven activation of the PAI-1 promoter, pARE-lux, a Smad 2 responsive promoter and SBE-luc a Smad3 responsive promoter. Δmts-IHG-1 inhibited gene expression of fibronectin, CTGF and Jagged1 induced by overexpression of the constitutively active TGF-β type 1 receptor and the TGF-β1 signal mediators Smad 2 and Smad3, indicating modulation of Smad function. Increased migratory ability of epithelial cells is a feature of epithelial-to-mesenchymal transition, a process believed to contribute to TIF. Δmts-IHG-1 inhibited TGF-β- stimulated HK-2 cell migration. These data indicate that (1) the putative mts is necessary for localization of IHG-1 to mitochondria (2) IHG-1 must retain the potential to associate with mitochondria in order to amplify TGF-β1 induced transcriptional responses and (3) Δmts-IHG-1 is an inhibitor of TGF-β1 induced cellular responses. We have renamed Δmts-IHG-1 as ITA-1 i.e. inhibitor of TGF-β1 activity 1 and propose ITA-1 as a novel biotherapeutic for the treatment of fibrotic kidney disease

Disclosure of Financial Relationships: nothing to disclose

**F-PO1118**

**Active PI3-Kinase Maintains Expression of the EMT-Antagonist Protein SARA** Constance Runyan, Xiaoying Liu, H. William Schnaper. *Division of Kidney Diseases, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

TGF-β1 promotes renal fibrosis in part, by promoting epithelial to mesenchymal transition (EMT). We have previously shown that the Smad anchor for receptor activation (SARA) helps maintain an epithelial cell phenotype, and that reduction of SARA by TGF-β1 or other means stimulates events associated with EMT. Here we investigated signaling pathways that might regulate SARA expression. Treatment of human kidney proximal tubular epithelial (HKC) cells with a panel of kinase inhibitors demonstrated variable effects on SARA expression levels. The PI3-kinase (PI3K) inhibitor LY294002 caused a reduction in SARA protein expression independently of TGF-β1. Conversely, expression of the constitutively active catalytic subunit of PI3K increased SARA expression. Additionally, we found a correlation between basal SARA expression and PI3K activity in a variety of cell types. Together these data suggested that PI3K activity levels might be related to SARA expression with high levels of active PI3K being protective for expression of SARA. In agreement with our previous findings that a loss of SARA expression was sufficient to induce de novo expression of the EMT marker αSMA, the expression of αSMA was elevated in LY294002-treated HKC. Treatment of HKC with an inhibitor specific for the PI3K downstream signaling-mediator Akt (Akt inhibitor IV) caused a similar reduction in SARA to that of LY294002, suggesting that downstream signaling is required. Neither LY294002 nor Akt inhibitor IV affected SARA mRNA expression, so the stabilizing effect of PI3K activity is likely at the level of SARA protein. Although endosomal localization is considered crucial for SARA function, we found no appreciable difference in co-localization between SARA and the early endosomal marker EEA1 in the presence of LY294002. Taken together these results suggest that high levels of active PI3K and downstream Akt may stabilize SARA protein expression by some means other than modification of SARA transcription or localization.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1119**

**Renal Lipocalin-Type Prostaglandin D2 Synthase (L-PGDS) Exerts Reno-Protective Effects on Unilateral Ureteral Obstruction (UUO)** Hideyuki Ito,[1] Motoaki Sano,[2] Yasunori Utsunomiya,[1] Keiichi Fukuda.[2] *[1]Division of Kidney and Hypertension, The Jikei University School of Medicine, Tokyo, Japan; [2]Division of Cardiology, Keio University School of Medicine, Tokyo, Japan.*

L-PGDS is responsible for the synthesis of PGD2 from common precursor PGH2. We recently reported that L-PGDS-mediated PGD2 *de novo* synthesis plays a cardioprotective role in ischemia/reperfusion injury (JCI 2009). In addition, several clinical studies have linked L-PGDS to a variety of pathological conditions in human. The serum and urinary L-PGDS level, for instance, is increased in patients with hypertension and with diabetic nephropathy. Recent studies revealed that L-PGDS is endogenously expressed in kidneys, especially in the proximal tubules. However, the precise roles for L-PGDS mediated $PGD_2$ constructional system in the tubulointerstitium remain to be elucidated. Then, to investigate whether renal L-PGDS have reno-protective effects, we performed UUO in L-PGDS knockout (LKO) mice and wild-type (WT) mice.

As the results, L-PGDS protein and gene expression drastically increased in the cortexes from UUO kidneys of WT mice and that L-PGDS immunoreactivity was significantly increased in the proximal tubules. Furthermore, UUO-induced tubular dilatation and tubulointerstitial fibrosis deteriorated in UUO kidneys of LKO mice compared to those of WT mice. At that point, the gene expression of IL-1β was significantly up-regulated, and those of IL-6, TGF-β and TNF-α also pointed to increase in WT/UUO kidneys comparing with LKO/UUO kidneys. In contrast, the expressions of local renin and angiotensinogen in LKO/UUO kidneys resembled those in WT/UUO kidneys. Of note, quantitative RT-PCR showed that the gene expression of angiotensin II type 2 receptor (AT2R) was down-regulated in LKO/UUO kidneys, whereas AT1R expressed in LKO/UUO kidneys as the same to WT/UUO kidneys. In addition, AT2R gene expression was down-regulated not only in kidney, but in brain and in heart obtained from LKO mice.These data suggest that L-PGDS is up-regulated in the proximal tubules of UUO kidneys and that L-PGDS may attenuate tubulointerstitial injury through modulating the gene expression of AT2R.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1120**

**Sphingosine-1-Phosphate Is One of the Key Molecules Directly/Indirectly Mediating Fibrosis in the Kidney** Shunji Shiohira, Takumi Yoshida, Junko Kohei, Hidekazu Sugiura, Michihiro Mitobe, Ken Tsuchiya, Kosaku Nitta. *Department of Medicine IV, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan.*

The major sphingolipid metabolite, sphingosine-1-phosphate (S1P), is now attractive and bioactive molecule for a potential mediator of renal fibrosis. In our past report, S1P induced fibrosis *in vitro* in normal rat kidney interstitial fibroblast cells (NRK-49F), and *in vivo* in the kidneys of a mouse model of unilateral ureteral obstruction (UUO). S1P is likely to have potentiality to mediate directly renal fibrotic processs in addition to via inflammaroy pathway. To clarify the mechanism of S1P in renal fibrosis, we adopted UUO model using nude mice which were characterized by deficit of immune response. The expression of S1P receptor subtype was analyzed in UUO and NRK-49F. The expression (mRNA and Western blot) of fibrosis marker, such as α-SMA, E-cadherin, etc., were examined and also histological changes were analyzed. (fibronectin (FN) and collagen type1 (COL1)). S1P receptor subtype (1-5) mRNA expression was examined by RT-PCR in NRK-49F and UUO. Fibrotic change was successfully induced in nude mice in which the staining intensity of FN and COL1 was prominent. The change was attenuated directly by FTY720 (S1P receptor 1,3,4,5 as antagonist), or DMS (sphingosine kinase inhibitor), suggesting another possible mechanism of S1P-induced fibrosis in addition to the inflammatory pathway. On the other hand, S1P is known to have unique tissue distribution of the receptor subtypes and the differing signaling pathways resulting from S1P receptor (S1PR) subtype activation. NRK-49F showed mRNA expressions of S1PR 1, 2, 3 and 5, but not S1PR 4. FTY720 is S1PR 1,3,4,5 antagonist. S1PR 1 is mostly related with inflammatory pathway and S1PR 5 is involved in the regulation of nervous system. Thus, these results suggest that S1PR 3 is likely to play main role in renal fibrosis. In conclusion, S1P is a hitherto unrecognized

profibrotic mediator in the renal tubulo-interstitium that functions through S1PR signaling pathways, in part, via direct fibrotic effect.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1121

**Nephrogenic Systemic Fibrosis: The Gadolinium-Based Magnetic Resonance Imaging Contrast Agent, Omniscan, Triggers Fibroblast Proliferation and Fibronectin Synthesis in a Redox-Dependent Manner** Brent Wagner.[1,2] *[1]Audie L. Murphy Memorial Hospital, South Texas Veterans Health Care System, San Antonio, TX; [2]Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX.*

**Purpose of the study:** Nephrogenic systemic fibrosis (NSF) is a recently recognized disorder in acute kidney injury, chronic kidney and end stage renal diseases. NSF is strongly correlated with exposure to gadolinium-based magnetic resonance imaging (MRI) contrast. Little is known about the mechanisms that trigger NSF and current treatments do not yield promising results. This study was conducted to examine potential mechanisms by which a common contrast reagent, Omniscan, elicits fibrosis.

**Methods:** Human foreskin fibroblasts (HFFs) were treated with clinically-relevant doses of Omniscan. Fibronectin and levels of phosphorylated protein were assessed by immunoblot. DNA synthesis was measured by incorporation of [$^3$H]-thymidine into TCA-precipitable material. Reactive oxygen species generation was assessed by confocal microscopy of 2',7'-dichlorodihydrofluorescein- (DCF-) loaded cells.

**Results:** Omniscan induced fibronectin synthesis in HFFs by 24 and 48 h in dose-dependent manners. MRI contrast-treated cells expressed $\alpha$-smooth muscle actin$^+$ stress fibers to a similar degree as TGF-$\beta$ treatment. Time-dependent reactive oxygen species generation was also induced by Omniscan. Both Omniscan-induced fibronectin synthesis and DNA synthesis were suppressed by pretreatment with *N*-acetyl cysteine (NAC). After Omniscan treatment, levels of phospho-extracellular signal-regulated kinase (ERK) and phospho-Akt increased in time-dependent manners. Both NAC and the ERK inhibitor U0126 suppressed the Omniscan-induced fibronectin increase. Omniscan treatment decreased the cell cycle inhibitor $p27^{Kip1}$.

**Conclusions:** Pharmacologically-relevant doses of the MRI contrast agent Omniscan led to an increase in fibronectin and DNA synthesis in HFFs *in vitro*. These data demonstrate that the mechanisms are redox- and ERK pathway-dependent. Elucidation of the cellular effects of gadolinium-based contrast on fibroblasts may aid in tailoring therapies to this devastating disease.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1122

**Activated EPAC-Rap1 GTPase Signaling Pathway Attenuates Membrane Trafficking of $Na^+/H^+$ Exchanger 3 (NHE3) Induced by Angiotensin II in Proximal Tubular Cells** Ping Xie,[1] Lin Sun,[1] Fu-You Liu,[2] Yashpal S. Kanwar.[1] *[1]Department of Pathology, Northwestern University Medical School, Chicago, IL; [2]Department of Nephrology, Second Xiangya Hospital of Central South University, Changsha, Hunan, China.*

Angiotensin II (ANG II) acts directly on the proximal tubules in promoting $Na^+$ reabsorption by modulating Na/H exchanger 3 (NHE3). NHE3 is considered to be regulated by cAMP stimulation. The purpose of the present study was to determine the effect of EPAC, a guanine nucleotide exchange factor for Rap1, on the ANG II modulation of NHE3 in Sprague-Dawley rat kidney slices and LLC-PK1 cells. Incubation of kidney slices up to 15 min with $10^{-11}$M ANG II induced the NHE3 redistribution from base to the tip of microvilli in proximal tubules. This process was attenuated by the addition of EPAC activator (8-pCPT-2'-O-Me-cAMP-AM) without any discernible change in total and phosphorylated NHE3. In LLC-PK1 cells, the membrane trafficking of NHE3 induced by ANG II was also attenuated by activation of EPAC pathway, and this effect of EPAC was blocked by transfection of LLC-PK1 cells with dominant negative mutant of EPAC1. In addition, the activity of Rap1, downstream effector of EPAC, was noted to be decreased by stimulation of ANG II in LLC-PK1 cells, and this effect was significantly diminished by blockade of ANG II type I receptor with losartan. To confirm the role of Rap1 in the regulation of NHE3, constitutively active mutants of Rap1a (G12V and T35A) as well as the dominant negative mutant of Rap1a (S17A) were introduced into the LLC-PK1 cells. The activated Rap1 reversed the NHE3 membrane trafficking induced by ANG II. Inhibitory effect of the EPAC activator on NHE3 membrane trafficking was blocked by the dominant negative mutant of Rap1a. No significant differences in the expression of total NHE3 or phosphorylated NHE3 in various groups were observed. These results suggest that EPAC1-Rap1 axis may be a novel potential pathway in the NHE3 regulation by ANG II in proximal tubules.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1123

**Attenuation of Angiotensin (Ang) II-Induced Cells Signalling by Ang1-7 Is cAMP-PKA Dependent** George Chu Liu,[1] Xiaohua Zhou,[1] James W. Scholey.[1] *[1]Medicine, University of Toronto, Toronto, ON, Canada; [2]Medicine, University of Alberta, Edmonton, AB, Canada.*

The renin-angiotensin system (RAS) plays an important role in renal physiology and pathophysiology. Although the cellular effects of the RAS activation are generally attributed to AngII, the recent identification of angiotensin converting enzyme-2 (ACE2) has shifted focus to the biologic effects of other angiotensin peptides, including Ang1-7. The G protein coupled receptor for Ang1-7, mas, is expressed by mesangial cells (MC), and Ang1-7 has been reported to attenuate AngII cell signalling in MC. Accordingly, we sought to better understand both the signal transduction pathways activated by Ang1-7, and the interaction between Ang1-7 and AngII in MC. We first observed that there was a time and concentration-dependent effect of Ang1-7 on ERK phosphorylation. Pre-treatment of MC with the mas receptor antagonist D-Ala$^7$ but not the $AT_1$ antagonist, loasrtan, or the $AT_2$ antagonist, PD11042, abrogated Ang1-7 induced ERK activation. Neither pre-treatment with the NADPH oxidase inhibitors, DPI (10$\mu$M) and apocynin (100$\mu$M), nor pre-treatment with the EGF receptor antagonists, AG1478 (0.2$\mu$M) and PD158780 (10$\mu$M), attenuated Ang1-7 activation of ERK, although each of these compounds abolished activation of ERK by AngII. Ang1-7 increased intracellular cAMP levels and activated protein kinase A (PKA) in MC, and inhibition of adenylyl cyclase (SQ22536, 0.1mM) or inhibition of PKA activity (H89, 1$\mu$M) attenuated Ang1-7 induced ERK activation. Pre-treatment with Ang1-7 reduced Ang II-induced NADPH oxidase activation and ERK activation in MC in a cAMP-PKA-dependent manner. In conclusion, Ang1-7-induced activation of ERK is cAMP/PKA dependent in MC, and independent of NADPH oxdase and the EGF receptor. Ang1-7 also inhibits AngII-induced NADPH oxidase thus preventing ERK activation by AngII. These effects may account, at least in part, for the attenuation of experimental models of glomerular injury by Ang1-7.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1124

**Ryanodine Receptors Mediate Calcium Sensing Receptor-Induced Inhibition of Renin** Mahendranath Reddy, Cecilia Ortiz-Capisano, Jeffrey L. Garvin, William H. Beierwaltes. *Dept. Int Med, Hypertension Research Div., Henry Ford Hospital, Detroit, MI.*

Renal juxtaglomerular (JG) cells contain G-protein coupled calcium-sensing receptors (CaSR) which sense changes in extracellular calcium, stimulating calcium-mediated intracellular signaling. The ryanodine receptor regulates release of calcium from intracellular stores. We hypothesized calcium activation of JG cell CaSR initiates a pathway involving the ryanodine receptor, release of intracellular calcium, inhibiting cAMP production, reduced protein kinase A, retarding cAMP-mediated renin secretion. We measured renin released from primary cultures of JG cells isolated from C57 mice, plated at 80-90% confluence for 48 hrs. Activation of JG cell CaSR using the calcimimetic Cinacalcet ($1_\mu$M) in media containing low, normal or high calcium decreased renin release by 70% in all three (0.88±0.15 to 0.24±0.05, 0.84±0.18 to 0.20±0.04, and 0.50±0.12 to 0.15±0.04 ug ANG-I/ml/hr/mg prot, ($p<0.005$). In contrast, adding the calcilytic Ronacaleret ($1_\mu$M) completely inhibited extracellular calcium-mediated activation of CaSR. This shows that changes in extracellular calcium are sensed by JG cell CaSR to regulate renin release. Inhibiting the ryanodine receptors with high concentrations ($0.1_\mu$M) of ryanodine reversed Cinacalcet inhibition of renin (0.13±0.02 to 0.07± 0.01 ugAng1/ml/mg prot with Cinacalcet, $p<0.01$, returning to 0.13±0.01 uAng-I/ml/hr/mg prot with Cinacalcet plus Ryanodine). Similarly, cinacalcet reduced JG cell cAMP formation (from 1.09±0.to 0.75±0.26 pM/mg prot, $p<0.025$, but returned to 1.50±0.35 pM/mg prot with cinacalcet plus ryanodine. This suggests CaSR activation of the ryanodine receptor results in calcium-mediated inhibition of cAMP formation. To mimic the effect of cAMP, we added NZ-B6, a Protein Kinase-A (PKA) agonist (0.2 mM), which increased renin release from 0.51±0.11 to 0.85±0.18 uAng-I/ml/hr/mg prot ($p<0.025$). Our data suggest extracellular calcium activation of the JG cell CaSR results in activation of a pathway involving ryanodine receptor induced release of intracellular calcium stores, inhibiting adenylyl cyclase cAMP production, diminishing PKA, and retarding cAMP-mediated renin secretion.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1125

**Convergence of Serine/Threonine and Tyrosine Signaling on Synaptopodin Balances Cathepsin-Regulated Actin Dynamics** Lisa Buvall,[1] Hoon Young Choi,[3] Christian Faul,[2] Peter H. Mundel.[1] *[1]Dep of Medicine/Division of Molecular Medicine, University of Miami, Miami, FL; [2]Dep of Medicine/Division of Nephrology, University of Miami, Miami, FL; [3]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea.*

Serine/threonine and tyrosine signal-transduction pathways influence many aspects of cell behavior, including spatial and temporal regulation of cytoskeletal dynamics. We previously reported a physiological role for cathepsin L (CatL) in the cytoplasm, where it mediates the calcineurin-dependent, CatL-mediated proteolysis of synaptopodin, a regulator of Rho GTPases in kidney podocytes. Phosphorylation of synaptopodin by PKA or CaMKII promotes 14-3-3 binding and protects synaptopodin from cleavage by CatL. Dephosphorylation of synaptopodin by calcineurin abrogates 14-3-3 binding, thereby allowing the degradation of synaptopodin by CatL. Here we identify a signaling pathway that directly links serine/threonine to tyrosine phosphorylation-controlled actin remodeling. We found that the tyrosine kinase Src can bind to synaptopodin and induce the tyrosine phosphorylation of synaptopodin. This, in turn increases the binding of calcineurin to synaptopodin, thereby promoting serine/threonine dephosphorylation and CatL-mediated synaptopodin degradation and loss of stress fibers. Src-resistant Synpo-TA, calcineurin-resistant Synpo216E619D, and CatL-resistant Synpo-CM1+2 are protected from Src-induced, CatL-mediated degradation of synaptopodin. Our results unveil how dual serine/threonine and tyrosine phosphorylation of an actin organizing protein can regulate cytoskeletal dynamics in an antagonistic fashion.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1126**

**Podocytes Lacking CD2AP Have Less Non-Muscle Myoin II Activity** Hani Suleiman, Andrey S. Shaw. *Pathology & Immunology, Washington University, Saint Louis, MO.*

CD2-associated protein (CD2AP) is a scaffold protein that plays a critical role in the maintenance of the kidney filtration barrier. Previous works suggested a role for CD2AP in regulating the actin cytoskeleton. We show that conditionally immortalized mouse podocytes lacking Cd2ap (-/-) have less prominent actin stress fibers. Moreover, Cd2ap (-/-) podocytes have a defect in focal adhesion turnover. This phenotype can be partially rescued by introducing Cd2ap back to the cells. Non-muscle myosin II is an actin-binding protein important for actin cross-linking as well as providing the cell with contractile properties. Here, we show that Cd2ap (-/-) podocytes lacks myosin light chain phosphorylation (pMLC) on Ser19, a key regulated for non-muscle myosin II activity. Data from our lab suggests a defect in RhoA-Rock pathway, a major regulator of pMLC, to be the cause of this phenotype.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1127**

**Pdlim2 Is a Novel Podocyte Protein Involved in Actin Dynamics** Laleh Sistani, Karl Tryggvason, Jaakko Patrakka. *Division of Matrix Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden.*

Pdlim2 is a member of a family of ALP proteins containing both PDZ and LIM domains. These proteins have been implicated in the actin filament organization. We have identified Pdlim2 as a novel podocyte foot process protein by microarray analyses of the mouse glomerulus transcriptome, and subsequent generation of an antibody against the corresponding human protein. Using this antibody, Pdlim2 was detected in immunofluoresence staining exclusively in podocyte foot processes. This specific expression pattern was confirmed by Western blotting. To define the role of Pdlim2 in podocytes, we used yeast-2-hybrid screening, and found interaction with the disease-associated protein, α-actinin-4, which was confirmed with co-immunoprecipitation. This interaction was mapped to the C-terminus of α-actinin-4, whereas pdlim2 needed both pdz- and lim-domains for the interaction with α-actinin-4. In cultured podocytes, Pdlim2 localizes to stress fibers and cortical actin. Both pdz- and lim-domains were required for the targeting of pdlim2 to these structures. In podocytes, pdlim2 stabilized stress fibers as these structures were more resistant to latrunculin A treatment in the presence of pdlim2. In conclusion, we have identified Pdlim2 as a new molecular component of podocyte foot processes in where it seems to play a role in regulating actin cytoskeleton dynamics.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1128**

**Nephrin Recruits the Cellular Inositol Phoshpatase SHIP2 To Regulate Actin Filament Morphology** Puneet Garg, Leslie Cook, Kamal Abuarquob, Madhusudan M. Venkatareddy. *Department of Internal Medicine, University of Michigan, Ann Arbor, MI.*

Regulated actin dynamics determines podocyte morphology during development and in response to podocyte injury. Nephrin-Neph1 receptor complex has been shown to regulate actin dynamics by assembling a protein complex including Nck, Grb2, Arp2/3 and nWASp which are involved in nucleation of the actin filament. To identify proteins which interact with Nephrin we generated a library of 140 SH2 domain containing recombinant proteins. We report here that Nephrin recruits SHIP2 a SH2-containing 5'-inositol phosphatase2 in a phosphorylation dependent manner and in doing so regulates the morphology of actin filament. SHIP2 is a phosphatase that dephosphorylates phosphoinositol 3,4,5 phosphate (PIP3) to generate phosphoinositol 3,4 phosphate [PI(3,4)P2]. We have previously shown that Nephrin interacts with p85 subunit of PI3 Kinase. Activation of PI3 kinase at the membrane by Nephrin results in generation of PIP3. Nephrin further recruits Ship2 which dephosphorylates PIP3 to PI(3,4)P2 providing a lipid signaling platform essential for the specific recruitment of the cytoskeletal regulator Lamellipodin to control actin pedestal morphology. We hypothesized that Nephrin-p85 interaction results in generation of PI(3,4) P2 in a SHIP2 dependent fashion. We were able to co-immunoprecipitate Nephrin and SHIP2 from glomerular lysates, and also in lysates from transfected podocytes. Using a cultured podocyte model of Nephrin activation described previously by us (induced clustering of CD16/7-Nephrin chimeric protein and various mutants), we found that nephrin activation and clustering results in recruitment of GFP-SHIP2 to Nephrin. Furthermore RNAi mediated Ship2 knock down in cultured cells resulted in distinctly different and aberrant actin tail morphology in response to clustering of CD16/CD7/ Nephrin chimeric protein. These observations provide evidence that Nephrin is not only able to initiate actin polymerization but also assembles/regulates a complex of proteins which not only maintains the actin filament but also determines the architecture of the generated actin network.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1129**

**Fluid Shear Stress Induces Renal Epithelial Gene Expression through Polycystin-2 Dependent Trafficking of Extracellular Regulated Kinase** Rajeev Rohatgi,[1, 2, 3] G. Luca Gusella,[1] Daniel Armando Flores.[1] *[1]Medicine, The Mount Sinai School of Medicine, New York, NY; [2]Medicine, The James J. Peters VA Medical Center, Bronx, NY; [3]Pediatrics, The Mount Sinai School of Medicine, New York, NY.*

The cilium and cilial proteins have emerged as the principal mechanosensors of renal epithelial cells responsible for translating mechanical forces into intracellular signals. Polycystin-2 (PC-2), a cilial protein, regulates shear induced changes in intracellular $Ca^{2+}$ and, recently has been implicated in the regulation of mitogen activated protein (MAP) kinases. We hypothesize that fluid shear stress (FSS) activates PC-2 which regulates MAP kinase and, in turn, induces MAP kinase dependent gene expression, specifically, monocyte chemoattractant protein-1 (MCP-1) mRNA. To test this, PC-2 expression was constitutively reduced in a murine inner medullary collecting duct (IMCD3) cell line, and the expression of FSS-induced MCP-1 mRNA expression (by quantitative RT-PCR) and MAP kinase signaling (by Western blotting) compared between the parental (PC-2 expressing) and PC-2 deficient IMCD3 cells. FSS of 0.4 dynes/cm$^2$ (a physiologic level of FSS) for 2 hrs induced MCP-1 mRNA expression by ~10 fold in IMCD3 cells compared to static controls ($p<0.05$). IMCD3 cells treated to the same level and duration of FSS induced extracellular regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) phosphorylation, as early as 10 and 60 min, respectively, after exposure to FSS. Inhibition of the ERK (U0126, 10μM) or JNK (SP600126, 30μM) pathways either abrogated or reduced the FSS-induced MCP-1 mRNA response, respectively, in IMCD3 cells. In contradistinction, FSS **did not** induce MCP-1 mRNA expression in PC-2 deficient cells, but **did** increase phosphorylation of the upstream ERK and JNK proteins. Immunocytochemistry of PC-2 expressing and deficient cells did not reveal gross abnormalities in cilia structure. However, wildtype cells exposed to FSS augmented the nuclear abundance of phospho-ERK in 3 of 4 experiments while PC-2 deficient cells did not (n=2). We conclude that PC-2 is a regulator of FSS-induced ERK trafficking and gene expression in collecting duct cells.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1130**

**Epidermal Growth Factor (EGF) Suppresses Chaperone-Mediated Autophagy (CMA) Via FoxO1 Transcription Factors in Kidney Cells** Harold A. Franch,[1,2] Changlin Ding,[2] Sara Zoromsky.[2] *[1]Atlanta VAMC; [2]Emory University.*

Reduced CMA during diabetic renal hypertrophy causes accumulation of oxidized proteins. Kidney growth suppresses CMA via Akt signaling. However, oxidative stress (OS) increases CMA but also increases Akt signaling. How can Akt suppress CMA when OS activates Akt? Perhaps isoform differences in EGF and OS Akt-mediated phosphorylation (P) of forkhead transcription factors (FoxOs 1,3a, and 4) explain different CMA activities. At 48 hrs in NRK-52E tubular cells, 10 nM EGF reduced proteolysis, increased CMA marker protein pax2(2.7 fold), and decreased the CMA receptor, LAMP2a (54%, $p<0.05$, n=4), consistent with reduced CMA. OS ($H_2O_2$100μM) did not reduce proteolysis, increase pax2, or decrease LAMP2a. EGF and OS both increased Akt phosphorylation equally and,using a FoxO response element luciferase reporter, reduced FoxO transcription. Akt regulates FoxO abundance and thus activity by P on T and S sites. Using phospho-specific antibodies, EGF for one hour induced FOXO1 T34 P(255%, $p<0.05$) and FoxO3a T24 P(>10-fold), while OS had little effect (40% & -4%, NS, n=3). EGF increased FoxO4 S193 P (220% $p<0.05$), while OS induced phosphorylation of FoxO4 S193 (>10-fold). EGF decreased FoxO1 and FoxO4 abundance by 40% and 31%($p<0.05$)at one hour, but did not change FoxO3a. OS decreased FoxO4 abundance by 69%($p<0.05$), but not FoxO3a or FoxO1. Adenoviral expression of constitutively active (CA) FoxO1 activated FoxO luciferase 3-fold, reversed EGF-induced Pax2 and GAPDH accumulation, and increased the expression of the LAMP2a consistent with increased CMA. CA FoxO1 accelerated lysosomal protein-degradation by 21+4% (n=12, $p<0.01$) and FoxO1 DN reduced proteolysis. siRNA against FoxO1 or 3a reduced these proteins by >60% or >80% and FoxO luciferase activity 50% or 20% respectively. EGF or FoxO1 siRNA, but not OS, scrambled or FoxO3a siRNA, reduced CMA as measured by peri-nuclear LAMP2a aggregates. Thus EGF and OS both activate Akt, but EGF reduces FoxO1 abundance which regulates CMA, while OS reduces FoxO3a and FoxO4 abundance. FoxO isoform specificity may allow for growth factor suppression of CMA in the presence of oxidative stress during diabetes.

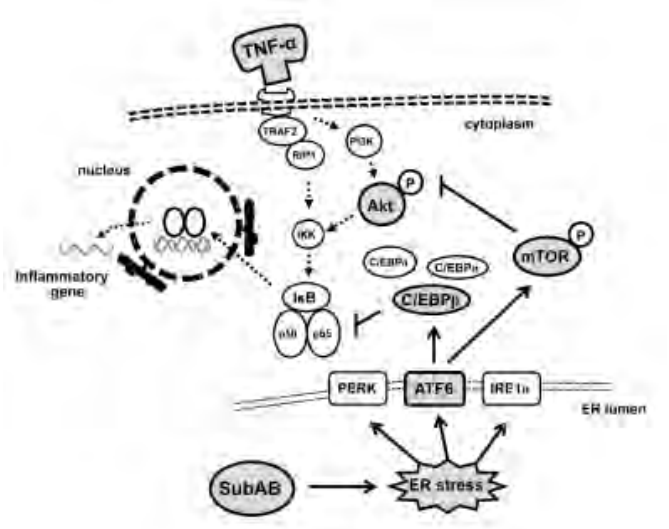
Disclosure of Financial Relationships: nothing to disclose

**F-PO1131**

**Anti-Inflammatory Potential of Subtilase Cytotoxin: Dual Suppression of NF-κB Via Induction of C/EBPβ and mTOR-Mediated Dephosphorylation of Akt** Shotaro Nakajima, Tao Huang, Jian Yao, Masanori Kitamura. *Department of Molecular Signaling, University of Yamanashi, Chuo, Yamanashi, Japan.*

We recently reported that administration of subtilase cytotoxin (SubAB) triggers the unfolded protein response (UPR) and protects mice from endotoxic lethality and collagen arthritis (J Immunol. 183: 1368-1374, 2009). We found that pretreatment with SubAB suppressed TNF-α-induced activation of NF-κB and NF-κB-dependent gene expression. To elucidate underlying mechanisms, we investigated involvement of C/EBPβ and Akt, the putative regulators of NF-κB. Among members of the C/EBP family, SubAB preferentially induced expression of C/EBPβ. Overexpression of C/EBPβ suppressed TNF-α-induced NF-κB activation, and knockdown of C/EBPβ blunted the suppressive effect of SubAB on NF-κB. We identified that the ATF6 branch of the UPR played a crucial role in the

induction of C/EBPβ. In addition to this effect, SubAB depressed basal and TNF-α-inducible phosphorylation of Akt via mTOR, which was also mediated by the ATF6 pathway. Several inducers of the UPR shared this suppressive effect, and chemical chaperones reversed the inhibition of Akt by SubAB. Blockade of Akt phosphorylation attenuated activation of NF-κB by TNF-α, suggesting that Akt is another target of SubAB-triggered, UPR-mediated NF-κB suppression. These results elucidated that SubAB depresses activation of NF-κB through dual mechanisms; *i.e.,* ATF6-mediated induction of C/EBPβ and suppression of Akt via mTOR.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1132**

**Activation of ERK and mTOR Signalling Pathways Via the Purinergic Receptors in Macrophages** Simona Deplano,[1] Reiko Hewitt,[1] Robert J. Unwin,[2] Frederick W. K. Tam.[1] *[1]Renal Medicine, Imperial College Kidney and Transplant Institute, London, United Kingdom; [2]Nephrology, University College London Medical School, London, United Kingdom.*

**Purpose:**

P2X7 receptor (P2X7R) plays a critical role in the pathogenesis of glomerulonephritis and in the release of active pro-inflammatory cytokines from LPS-primed macrophages. There is an incomplete understanding of the signalling cascade following ATP stimulation.

**Methods:**

Primary bone marrow derived macrophages (BMDM) from wild type (WT) and P2X7R knock out (P2X7-/-) mice were used.

(1). We examined the effects of P2X7R agonist BzATP on IL-1β secretion in both WT and P2X7-/- BMDM. ELISA was used to detect mature IL-1β in cell culture supernatant.

(2). We examined the effect of BzATP stimulation on the activation of two key regulators of cell growth, ERK and mTOR, in WT and P2X7-/- BMDM. Western blotting was used to detect both phosphorylated and total ERK, and S6 proteins.

**Results:**

(1). As already shown in peritoneal macrophages, our data confirm that LPS-primed BMDM from WT mice produce high levels of IL-1β in response to BzATP stimulation, while the P2X7-/- BMDM failed to produce this active cytokine. Furthermore, the WT BMDM produced high levels of active IL-1β when stimulated with the human cathelicidin-derived peptide LL37, a novel P2X7R activator, in a dose-dependent manner.

(2). BzATP stimulation led to a greater increase of ERK phosphorylation in WT BMDM compared with P2X7-/- BMDM, suggesting direct involvement of P2X7 receptor in this event. BzATP stimulation also induced an increase in phosphorylation of S6, a downstream effector of mTOR, but no significant differences were observed between the WT and P2X7-/- BMDM, suggesting that other purinergic receptors may be involved in this process.

**Conclusions:**

These preliminary data show the contribution of the P2X7R in the activation of the ERK signalling pathway, which is known to regulate many important processes such as cell growth and apoptosis. Further, investigations are required to identify the purinergic receptors activating the mTOR signalling pathway.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1133**

**Differential Regulation of Adenylyl Cyclases in Lipid Rafts in Human Kidney Cells** Peiying Yu,[1] Yanrong Zhang,[2] Pedro A. Jose.[3] *[1]Center for Molecular Physiology Research, Children's National Medical Center, Washington, DC; [2]Center for Molecular Physiology Research, Children's National Medical Center, Washington, DC; [3]Center for Molecular Physiology Research, Children's National Medical Center, Washington, DC.*

Dopamine $D_1$-like receptors ($D_1$ and $D_5$) stimulate adenylyl cyclase (AC), whereas the $D_2$-like receptors ($D_2$, $D_3$ and $D_4$) inhibit AC. We tested the hypothesis that $D_1$ and $D_5$ receptors differentially regulate AC isoform expression and activity in lipid rafts (LRs) in human embryonic kidney (HEK-293) cells heterologously expressing either human $D_1$ or human $D_5$ receptor (HEK-h$D_1$ and HEK-h$D_5$ cells). In the basal state, AC6 was expressed in LRs to a greater extent in HEK-h$D_1$ cells (84.5±2.3 %) than in HEK-h$D_5$ cells (68.9±3.1 %) (ANOVA, $P<0.05$, n=4) while AC7 expression in LRs was similar in the two cell lines (12.1± 0.8% and 13.3±4.1%, respectively). The $D_1$-like receptor agonist fenoldopam (1μM/15 min) *increased* AC6 expression in LRs up to 92.5±1.4% ($P<0.01$, vs. control, n=4) in HEK-h$D_1$ cells. In contrast in HEK-h$D_5$ cells, fenoldopam (1μM/l5 min) decreased AC6 expression in LRs down to 48.9±5.3% ($P<0.05$, vs. control, n=4). Disruption of LRs with methyl-β-cyclodextrin (βCD, 2%/1hr) markedly decreased AC activity in both cell lines ($D_1$R: 0.20± 0.08 vs. control=3.54± 0.22 pmol/mg/50μl; $D_5$R: 0.61± 0.11 vs. control=6.01± 0.59 pmol/mg/50μl, $P<0.001$, n=4/groups, ANOVA). Fenoldopam differentially augmented AC activity in cells pre-treated with βCD; a greater stimulatory effect was noted in HEK-h$D_1$ than in HEK-h$D_5$ cells (HEK-h$D_1$: βCD+Fen=+653 ±73% vs. βCD alone; HEK-h$D_5$: βCD+Fen=315.7±14.7% vs. βCD alone, n=4/group, $P<0.05$ ANOVA). These studies show for the first time that: 1) AC6 is differentially distributed in LRs and nonLRs; 2) disruption of LRs also differentially affects agonist-dependent cAMP accumulation in HEK-h$D_1$ and HEK-h$D_5$ cells. We conclude that LRs are essential not only for the proper membrane distribution and maintenance of AC6 activity but also the regulation of $D_1$ and $D_5$ receptor-mediated AC signaling.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1134**

**Endoplasmic Reticulum Stress Disturbs Erythropoietin Gene Expression in a HIF-Dependent Manner** Chih-Kang Chiang, Masaomi Nangaku, Tetsuhiro Tanaka, Toshiro Fujita, Reiko Inagi. *Division of Endocrinology & Nephrology, University of Tokyo School of Medicine, Tokyo, Japan.*

Purpose of study: Some patients with renal anemia suffer from resistance against treatment with erythropoietin (EPO), and this is often associated with chronic inflammatory states. Evidences suggest that impaired endoplasmic reticulum (ER) homeostasis encountered during chronic inflammatory status. Here we investigated a role of disturbance in the homeostasis of ER in hypoxia-induced EPO production.

Methods: HepG2 cells, a hepatoma cell line obtained from RIKEN Japan, were incubated in the hypoxia chamber (1%), or by added cobalt chloride (100uM) in culture medium to activate hypoxia-inducible factor (HIF), the master gene switch of hypoxic responses. Thapsigargin (THG, 50ng/ml) or tunicamycin (TUN, 4ug/ml) were used to induce ER stress in vitro. Male Wistar rats (150~200g) were treated by TUN (i.p. 0.3mg/kg), and then received cobalt chloride (s.c. 60mg/kg) to activate HIF. EPO gene expression was analyzed by the quantitative real-time PCR. Nuclear levels of HIF 1α and 2α were evaluated by Western blotting analysis.

Results: We first tested two representative ER stressors in the HepG2 hepatoma cell line: THG and TUN. As we expected, both compounds induced ER stress (7.7 and 6.8 times increased as compared with control), estimated by the induction of GRP78, an indicator of ER stress. Both of the ER stressors markedly suppressed the basal expression level of EPO mRNA under normoxic conditions (0.55-in TUN and 0.32-fold in THG, $p<0.05$). Further, both THG and TUN suppressed cobalt chloride-induced and hypoxia-induced EPO mRNA expression (0.54-in TUN and 0.34-fold in THG, $p<0.05$), which accompanied by inhibition of HIF-1α and HIF-2α nuclear translocation. We performed further experiments in rats treated with TUN and found that ER stress readily suppressed cobalt chloride-induced hepatic and renal EPO gene expression by 91.5 and 87.6% ($P<0.01$), respectively.

Conclusions: Inhibition of HIF activation seems to be involved in the suppression of EPO gene expression by the induction of ER stress in vitro, and may be responsible for impaired EPO synthesis in chronic inflammatory states in vivo.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1135**

**Impact of Anthracyclines on Hypoxia Response in Tubular Epithelial Cells** Tetsuhiro Tanaka,[1,2] Toshiro Fujita,[2] Masaomi Nangaku.[2] *[1]Division for Health Service Promotion, University of Tokyo, Tokyo, Japan; [2]Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Tokyo, Japan.*

Anthracyclines are widely used to treat tumors of lymphoid and other origins. Upon administration, they distribute in multiple organs including the kidney, at various concentrations and with diverse consequences, but their impact on tubular epithelium remains unknown. In this study, we investigated the effect of anthracyclines on hypoxia response of renal tubular cells, in light of the emerging concept that tubulointerstitial hypoxia plays a critical role in the progression of kidney disease.

In cultured human proximal tubular cells (HK-2), doxorubicin dose-dependently inhibited transcriptional activity of hypoxia-inducible factor-1 (HIF-1), as demonstrated by luciferase reporter assay, which was reflected by transcriptional downregulation of HIF-target genes such as vascular-endothelial growth factor (VEGF) and glucose transporter 1

(GLUT1). On the other hand, doxorubicin neither influenced the expression level of HIF-1alpha and its binding partner, HIF-1beta, nor did it have any effect on the heterodimer formation. However, chromatin immunoprecipitation assays revealed that the recruitment of the HIF-heterodimer to its consensus hypoxia-responsive element was markedly weakened in the presence of doxorubicin, with the following impairment of its target gene transcription. This study uncovers a novel mechanism by which anthracyclines dampen hypoxia response in proximal tubular cells of the kidney. Impaired recruitment of the HIF-heterodimer to its consensus cis-element was mainly responsible for the transcriptional downregulation of HIF-target genes.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1136**

**Fructose Activates the Polyol Pathway in the Development of Metabolic Syndrome** Miguel A. Lanaspa, L. Gabriela Sanchez-Lozada, Carlos Alberto Roncal-Jimenez, Ana Andres-Hernando, Richard J. Johnson. *University of Colorado Denver.*

Excessive fructose intake can induce features of metabolic syndrome in experimental animals and man. Fructose intake, primarily in the form of added sugar correlates closely with the epidemics of obesity and diabetes. These observations have led to much interest in the role of exogenous dietary sugars (containing fructose) in the current epidemic of metabolic syndrome. However, fructose can also be generated from endogenous sources via the polyol pathway. Aldose reductase, the rate limiting enzyme of this pathway converts glucose into sorbitol which is the metabolized to fructose by sorbitol dehydrogenase (SDH). Here, we demonstrate that postpandrial levels of fructose (1 mM) are able to significantly up-regulate aldose reductase expression within 4 hours after exposure in hepatocytes HepG2 cells (2.6-fold increase between control, $p<0.01$). SDH expression is further up-regulated at 8 hours after exposure (0.7-fold increase in the first 4 hours, $p=0.07$ and 2.05-fold increase after 8 hours, $p<0.01$) indicating that AR is creating the pool of sorbitol needed for SDH over-expression. This up-regulation occurs at both the transcriptional and translational level since an increase in both mRNA and protein expression was observed. The mechanism by which fructose up-regulates aldose reductase is mediated by the ability of this sugar to induce intracellular uric acid and reactive oxygen species (ROS). Scavenging ROS with a NADPH oxidase inhibitor (apocynin) or inhibition of uric acid generation (oxypurinol) dramatically diminished the up-regulation of the enzymes of the polyol pathway. This data strongly suggests that the polyol pathway is activated by fructose. Hence it is possible that subjects with metabolic syndrome, who have postpandrial hyperglycemia and hyperuricemia, may have substantial endogenous fructose production that may continue to maintain or accelerate features of the metabolic syndrome even if the original underlying mechanism driving it (exogenous fructose ingestion) is curtailed.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1137**

**Clopidogrel Effectively Suppresses Endothelial Microparticle Generation Induced by Uremic Toxin Via Inhibition of the p38 MAPK Pathway** Jung-Hwa Ryu,[1] Seung-Jung Kim,[1] Dong-Ryeol Ryu,[1] Duk-Hee Kang,[1] Kyu Bok Choi.[1] *[1]Division of Nephrology, Department of Internal Medicine, Ewha Womans University School of Medicine, Seoul, Republic of Korea; [2]Medical Research Institute, Ewha Womans University School of Medicine, Seoul, Republic of Korea.*

Vascular access failure is one of the most common problems in hemodialysis patients and is closely related with endothelial injury. Endothelial microparticles (EMPs) are fragments shed from the activated or apoptotic cell membrane and are closely associated with thrombotic propensity and vascular dysfunction. To identify drugs with beneficial effects on injured endothelium, we investigated the effects of anti-proliferative drugs on EMP generation induced by uremic toxin in human umbilical vein endothelial cells (HUVECs). We then examined the involvement of the MAPK in EMP generation and effects of MAPK inhibitors on EMP production. CD31+CD42-EMP counts were measured by flow cytometry in supernatants of HUVECs incubated with indoxyl sulfate and the EMP responses to anti-proliferative drugs (losartan, lovastatin, clopidogrel, and mesoglycan) were examined. We then measured the effects of MAPK inhibitors on EMPs. The results were as follows: 1) indoxyl sulfate induced EMP release in HUVECs in a dose-dependent fashion; 2) losartan, lovastatin, clopidogrel, and mesoglycan inhibited EMP generation induced by indoxyl sulfate at concentrations of 10-50 μM, with clopidogrel the most effective; 3) the p38 MAPK inhibitor (SB-203580, 20 μM) effectively suppressed EMP generation induced by indoxyl sulfate; And 4) clopidogrel significantly suppressed MAPK signaling pathways; p38, ERK1/2, and JNK activated by indoxyl sulfate, with the most potency on p38. These results collectively suggest that p38 signaling involves EMP generation induced by indoxyl sulfate and is effectively suppressed by clopidogrel. Further studies will be necessary to define the therapeutic benefit of these drugs for prevention of vascular access stenosis *in vivo*.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1138**

**Agonist-Activated, Endocytosed Dopamine D3 Receptor Traffics through the Endosomal Network and Interacts with GADD34** Maria João Pinho,[1] Van Anthony M. Villar,[2] Yanrong Zhang,[2] John Edward Jones,[2] Patricio Soares-da-Silva,[1] Pedro A. Jose.[2] *[1]Instituto de Farmacologia e Terapêutica, Faculdade de Medicina da Universidade do Porto, Porto, Portugal; [2]Center for Molecular Physiology Research, Children's National Medical Center, Washington, DC.*

During conditions of moderate sodium excess, the peripheral dopaminergic system regulates blood pressure and water and electrolyte balance via the dopamine receptors, including the dopamine $D_3$ receptor (DRD3). While the association of DRD3 dysfunction with hypertension is fairly established, not much is known about how the activated receptor is subsequently processed and modified for eventual recycling or degradation. This study aimed to demonstrate the trafficking of DRD3 in human proximal tubule cells (hPTCs) and to characterize its interaction with GADD34, a regulatory subunit of phosphatase 1 (PP1) that recruits the catalytic subunit that dephosphorylates target proteins. Confocal microscopy revealed that DRD3 is localized in the cytoplasm and partially at the plasma membrane. DRD3 agonist PD128907 treatment resulted in receptor endocytosis and accumulation at the perinuclear area, and increased receptor colocalization with EEA1 (sorting endosome marker), Rab4 (fast recycling endosome) and Rab11 (slow recycling endosome) in hPTCs. Agonist stimulation markedly enhanced the colocalization with Rab7 (late endosome), LAMP-1 (lysosome) and $p44S^{10}$ (proteasome); endocytosed receptors are degraded via the lysosome or proteasome. Agonist treatment increased DRD3 phosphorylation. The DRD3/GADD34interaction was confirmed through a His pulldown between heterologously expressed DRD3 and endogenous GADD34. Overexpression of GADD34 increased the phosphatase activity in hPTCs, while RNAi silencing of GADD34 did not change the activity. DRD3 and GADD34 colocalized in the proximal tubules of human kidney, and agonist stimulation resulted in increased colocalization of DRD3 with GADD34 and PP1γ in hPTCs. Our data indicate that agonist activation promotes DRD3 phosphorylation, endocytosis, endosomal trafficking, and possible degradation via the lysosomes and proteasome, and suggest an important role for GADD34 in DRD3 function in hPTCs.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1139**

**Protein Kinase C-ε Activation Induces Mitochondrial Dysfunction in Renal Proximal Tubules** Grazyna Nowak, Diana Bakajsova. *Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR.*

Activation of protein kinase C-ε (PKC-ε) mediates protection from ischemia/reperfusion injury in the heart. Mitochondria are a subcellular target of these protective mechanisms of PKC-ε. We have previously shown that PKC-ε activation and translocation to mitochondria is involved in mitochondrial dysfunction in oxidant-injured renal proximal tubular cells (RPTC). However, mitochondrial targets of PKC-ε were unknown. The goal of this study was to determine the role of PKC-ε activation in mitochondrial dysfunction and to identify mitochondrial targets of PKC-ε in RPTC. The constitutively active (caPKC-ε) and inactive (dnPKC-ε) mutants of PKC-ε were overexpressed in primary cultures of RPTC using adenoviral technique. The cells were collected at different time points after transfection to measure mitochondrial functions, ATP production and content, oxidant generation, and cell viability. Protein levels of caPKC-ε increased at 12 hr after transfection and remained elevated for another 36 hours. Increases in caPKC-ε levels in RPTC were accompanied by caPKC-ε translocation to mitochondria. Sustained PKC-ε activation resulted in decreases in state 3 respiration, electron transport rate, activities of complexes I and IV, ATP production, ATP content, and $F_0F_1$-ATPase activity. Furthermore, PKC-ε activation increased $\Delta\Psi_m$ and oxidant production and induced mitochondrial fragmentation and RPTC death. Antioxidants blocked PKC-ε-induced increases in oxidant generation but did not prevent mitochondrial fragmentation and cell death. Inactive PKC-ε mutant had no effect on mitochondrial functions and morphology, reactive oxygen species production, and RPTC viability. We conclude that activation of PKC-ε in RPTC targets complex I, complex IV, and $F_0F_1$-ATPase, mediates mitochondrial dysfunction, hyperpolarization and fragmentation, and induces RPTC death but oxidative stress is not the mechanism of PKC-ε induced loss of RPTC viability. These results show that, in contrast to protective effects of PKC-ε activation in cardiomyocytes, sustained PKC-ε activation is associated with oxidant generation and is detrimental to mitochondrial function and viability in RPTC.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1140**

**Functional Discrimination of Intrarenal and Renal Pelvic Capsaicin-Sensitive Peptidergic Afferent Nerve Fibers** Tilmann Ditting, Wolfgang Freisinger, Kristina Rodionova, Sonja Heinlein, Karl F. Hilgers, Roland Veelken. *Nephrology, University Erlangen, Erlangen, Germany.*

Kidneys have a complex sympathetic efferent and peptidergic afferent innervation expressing TRPV1. The renal pelvis is very densely innervated with afferent nerves which are functionally far better understood than the intrarenal afferents, that are however likely to be important in health, renal disease and hypertension. However, a clear-cut functional distinction of renal pelvic and intrarenal afferents has not yet been described. We tested the hypothesis that intrarenal and renal pelvic afferent nerve fibers can be discriminated functionally using a multifiber nerve recording approach.

8 anesthetized SD rats were equipped with art. and ven. catheters for blood press. recording and application of fluids and drugs, a stainless steel electrode for recording of afferent renal nerve activity (ARNA), a triple lumen renal pelvic (rp) catheter for urine drainage and increasing rp pressure (+15 cmH20) and for rp drug perfusion to stimulate

(CAP=Capsaicin 3.3*10-7M, 10µl/1min) or block (CPZ=Capsacepine 3.3*10-4M, 90µl/9min) renal pelvic ARNA via TRPV1; a renal artery catheter for intrarenal ARNA stimulation (CAP=Capsaicin 3.3, 6.6, 10*10-7M 10µl bolus). Mechanical stimulation of rp ARNA (pressure increase) was followed by chemical stimulation of rp and intrarenal ARNA (CAP). This was repeated after rp perfusion with CPZ to block rp ARNA and once more after rp perfusion with saline to unblock rp ARNA

Mechanical and chemical rp stimulation significantly increased ARNA (289±39%, 299±47%, respectively). To achieve a similar peak ARNA increase (302±51%) by intrarenal chemical stimulation, via renal artery, higher CAP concentrations were necessary (2.5-fold). Renal pelvic perfusion with CPZ reversibly blocked renal pelvic mechanic and chemical stimulation effects efficiently. Interestingly, the intrarenal CAP effect was only just sligthly diminished (-9%; n.s.) during blockade of pelvic fibers.

For the first time, our study provides direct neurophysiological evidence for the existence of a putatively functionally relevant intrarenal peptidergic innervation that is independent from renal pelvic innervation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1141**

**Rosuvastatin Reduces Pressure-Induced Fibrotic Signals in Rat Renal Tubular Cell** Tso Hsiao Chen,[1] Yen Cheng Chen,[1] Cheng-Hsien Chen,[1] Yung-Ho Hsu.[2] *[1]Division of Nephrology, Taipei Medical University-Wan Fang Medical Center, Taiwan; [2]Division of Nephrology, Taipei Medical University-Shuang Ho Hospital, Taiwan.*

HMG-CoA reductase inhibitors, a class of drugs known as statins and initially described as lipid-lowering drugs, have anti-inflammatory and immunomodulatory actions. Recently, many studies revealed that the pressure force is an important mechanisms contributing to the induction and progression of tubulo-interstitial fibrogenesis in diabetic nephropathy and ureteric obstruction. In this study, we established an *in vitro* pressure culture system to study the influence of statins on renal fibrosis in rat renal tubular cells (NRK-52E). NRK-52E cells were cultured under 60 mmHg pressure, and we found that the fibrotic signals were significantly induced depend on pressured time. Connective tissue growth factor (CTGF) was induced at 2 and 8 h, and Transforming growth factor beta (TGF-b) as well as fibronectin increased at 8 and 24 h. These pressure-induced CTGF and fibronectin were reduced by rosuvastatin in a dose-dependent manner, but pressure-induced TGF-b was not influenced by rosuvastatin. We further found that rosuvastatin also reduced the TGF-b-induced expression of fibronectin, CTGF and COX-2 in a dose-dependent manner. Both pressure and TGF-b induced PGE2 secretion and this induction was inhibited by rosuvastatin in NRK-52E cells. The specific inhibitor for COX-2, NS398, inhibited pressure-induced PGE2 secretion and enhanced the anti-fibrotic effect of rosuvastatin. When we added PGE2 back to the pressure-treated cells, rosuvastatin-reduced fibrotic signals were recovered. Therefore, rosuvastatin may prevent rat renal tubular cells from pressure-induced fibrosis by inhibiting PGE2 generation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1142**

**Rapid, Non-Genomic Regulation of Multidrug Resistance Protein 2 by Glucocorticoids in Renal Proximal Tubules** Rosalinde Masereeuw,[1] Brigitte Prevoo,[1,3] Kimberley Wever,[1] David S. Miller,[2] Gert Flik.[3] *[1]Pharmacology and Toxicology (149), Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Laboratory of Pharmacology, NIH/NIEHS, Research Triangle Park, NC; [3]Dept. Organismal Animal Physiology, Radboud University, Nijmegen, Netherlands.*

Multidrug resistance protein 2 (Mrp2) in renal proximal tubules is concerned with active transport of many compounds, including drugs and metabolic wastes, into the urine. Upon exposure to nephrotoxic agents or during endotoxemia, Mrp2 activity and expression are tightly controlled. This may result from an induced de-novo synthesis of Mrp2, or from post-transcriptional regulation involving receptors and signaling pathways. Here we investigated glucocorticoid signaling to Mrp2. For transport experiments, freshly isolated killifish renal tubules were exposed to dexamethasone without and with inhibitors of signaling. Fluorescein-methotrexate (FL-MTX) was used to measure Mrp2-mediated transport by confocal microscopy and quantitative image analysis. Exposure of renal tubules to dexamethasone (0.25 – 10 µM) for 3 h increased FL-MTX transport at all concentrations tested ($p<0.01$). Other glucocorticoid receptor (GR)-ligands, cortisol and triamcinolone acetonide (both at 1.0 µM), also stimulated Mrp2-mediated transport ($p<0.01$). Cortisone, an inactive metabolite, was without effect. The GR-antagonist, RU-486 (0.5 µM), abolished the effects ($P<0.01$ for all three ligands). Consistent with action through a non-genomic mechanism, dexamethasone up-regulation of Mrp2-mediated transport was insensitive to cycloheximide. Immunohistochemistry revealed that dexamethasone did not alter Mrp2 expression at the luminal membrane. K252a, an inhibitor of the tyrosine kinase subfamily, reduced the dexamethasone effect, as did the specific c-Met kinase inhibitor, PHA-665752 ($p<0.001$). Protein kinase C signaling was not involved. In conclusion, we demonstrate a novel signaling pathway for the rapid induction of Mrp2-mediated transport in renal proximal tubules. Signaling involved glucocorticoids acting through GR and tyrosine kinases, but did not involve increases in protein synthesis or Mrp2 expression.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1143**

**Coordination of ARLs in Cilia** Yujie Li, Jinghua Hu. *Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Joubert syndrome (JS) is the most common inherited cerebellar malformation disorder that belonging to a rapidly expanding group of human diseases called ciliopathies. All identified JS loci encode cilia-related proteins, however, with the precise roles enigmatic. Here, we address the function of the small GTPase ARL-13, the sole *C. elegans* orthologue of human ARL13B, in regulating cilia biogenesis. In *C. elegans*, ARL-13 localizes to ciliary doublet segment in its proline-rich C-terminus dependent manner. *arl-13* animals exhibit shortened cilia with various cilia ultrastructural defects as well as disrupted association between intraflagellar transport (IFT) subcomplex A and B. Intriguingly, depletion of ARL-3, another evolutionarily conserved ciliary small GTPase, can suppress ciliogenesis defects in *arl-13* via a microtubule associated deacetylase HDAC-6 dependent pathway by restoring the binding efficiency between IFT complex A and B. We further identified several ARL-13 interacting proteins in yeast-two-hybrid screens and will elucidate their roles in ARL-13-mediated ciliogenesis and IFT regulation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1144**

**AOPPs-Induced Endothelial Ezrin Activation Potentiates Monocyte Transendothelial Migration through a RAGE-Rho/ROCK-NADPH Oxidase-Mediated Pathway** Zhijian Guo, Ying Zhang, Fan Fan Hou, Xun Zhang. *Nephrology, Nanfang Hospital, Guangzhou, Guangzhou, Guangdong, China.*

The accumulation of advanced oxidation protein products (AOPPs), a class of potential proinflammatory mediators, has been linked to the increased monocyte infiltration into vessel intima in the previous study. However, the underlying mechanism is still unknown. The hypothesis that AOPPs modified human serum albumin (AOPPs-HSA) may contribute to the monocyte transendothelial migration (TEM) process by modulation of endothelial permeability was investigated. Incubation of AOPPs-HSA with human umbilical vein endothelial cells (HUVECs) resulted in increased enzyme activities of Rho and Rho-associated kinase (ROCK). This Rho/ROCK activation enhanced the activity of endothelial NAD(P)H oxidase as demonstrated by inducting phosphorylation of p47phox and p47phox membrane translocation. Exposure of HUVECs to AOPPs-HSA also increased the phosphorylation of ezrin, a key protein regulating endothelial cytoskeleton rearrangement, causing a shift in F-actin distribution from web-like structure to polymerized stress fiber. The cytoskeleton rearrangement was accompanied by increased endothelial permeability to monocytes as detected by a double-chamber culture system. This cascade of events could be largely inhibited by blocking the receptor for advanced glycation end products (RAGE), by the special inhibitor of Rho/ROCK and NADPH oxidase, and by the small interfering RNA to ezrin. These data suggested that AOPPs-HSA induced endothelial ezrin activation precipitating cytoskeleton rearrangement and endothelial permeability via a RAGE-mediated signals involving Rho/ROCK and NADPH oxidase.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1145**

**Caveolin/Caveolae Expression in Proximal Tubule (PT) Cells in Culture but Not In Situ: Isolated PTs Are Contaminated with Endothelial Cells** Zhenjie Jane Zhuang, Vladimir Marshansky, Sylvie Breton, Dennis Brown. *Center for Systems Biology, Program in Membrane Biology and Division of Nephrology, Massachusetts General Hospital, Boston, MA.*

Kidney proximal tubules (PT) are composed of epithelial cells that are specialized for the uptake and transport of ions, solutes, peptides and proteins. These functions are often regulated by hormones that signal at the cell surface and are subsequently internalized by clathrin-mediated endocytosis. However, the caveolin/caveolae pathway has also been implicated in normal proximal tubule function, often based on data from isolated PTs or PT cells in culture. Although we reported previously that caveolae and caveolin 1 are not detectable in PTs in situ, reports of caveolin expression and its functional consequences in PT cells appear periodically in the literature. Therefore, we re-examined caveolin expression in PTs in situ, in isolated, purified PTs, and in cultured PT cells. Caveolin 1 and 2 immunofluorescence was undetectable in PTs in situ using different specific antibodies, but all PT cell cultures examined (MTC, IRPT and LLC-PK1) and IMCD cells expressed caveolin 1 and 2. Furthermore, both caveolin 1 and 2 mRNAs were detected in collagenase-isolated PTs along with the endothelial markers CD31 and ICAM1. In contrast, no caveolin or endothelial marker mRNAs were detectable in PT samples isolated from snap frozen kidneys by laser cut microdissection, which eliminates contamination by endothelial cells. We conclude: a) caveolin 1 and 2 are not normally expressed by PT cells in situ, and caveolae are not present; b) caveolin expression is activated in cultured PT cells; c) endothelial cell contamination is a potential source of caveolins that must be taken into account when collagenase-isolated PT are used in studies to correlate expression of these proteins with proximal tubule function.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1146**

**Exosomes Derived from Primary Renal Cells Contain microRNAs That Can Potentially Drive Therapeutically-Relevant Outcomes in Models of Chronic Kidney Disease** Roger Ilagan, Sumana Choudhury, Andrew T. Bruce, Joydeep Basu, Kelly I. Guthrie, Bryan R. Cox, Chris Genheimer, Rusty Kelley, John W. Ludlow, Sharon C. Presnell. *Tengion, Inc., Winston-Salem, NC.*

Paracrine effects have become a focus for understanding mechanisms of regeneration. MicroRNAs (miRNAs) transferred through the extracellular space via exosomes can affect mRNAs of other cells. Here, we correlated specific exosome-derived miRNAs with functionally-relevant outcomes in target cells *in vitro* to inform the design of *in vivo* renal regeneration mechanistic studies.

Conditioned media effects on signaling pathways associated with regeneration were investigated with human cell lines: HK-2 (proximal tubule), HRMC (mesangial), and HUVEC (endothelial). RNA content in exosomes from human and rat primary renal cell cultures (UNFX) was screened by PCR-based array designed to detect known miRNAs. Low oxygen can affect exosome shedding; therefore, some cultures were exposed to low oxygen (2% $O_2$) for 24 hours prior to media collection. Exosomes were separated from cellular debris in UNFX conditioned media by FACS.

UNFX-conditioned media affected specific signaling pathways associated with regeneration; non-conditioned media did not. NFkB (immune response) and epithelial-to-mesenchymal transition (fibrotic response) was attenuated in HK-2 cells, PAI-1 (fibrotic response) was attenuated in HRMC cells, and angiogenesis was promoted in HUVEC. PCR array screening of exosome content from UNFX-conditioned media indicated that UNFX produces exosomes containing miRNA sequences consistent with the observed responses.

UNFX cells shed exosomes containing known miRNAs. UNFX-conditioned media affects responses associated with regeneration in human cell lines. The cause and effect relationship between detected miRNAs and observed regenerative responses is under investigation; however, results achieved to date suggest that UNFX cells have the potential to produce therapeutically-relevant paracrine effects via exosome-mediated transfer of miRNAs to target cells and tissues.

Disclosure of Financial Relationships: Employer: Tengion, Inc.

**F-PO1147**

**Regulation of HSP27 in Unilateral Obstructed Kidney and in RMIC Subjected to Mechanical and Inflammatory Stress** Inge Gram Carlsen,[1,2] Rikke Norregaard,[1,2] Jorgen Frokiaer.[1,2] *[1]The Water and Salt Research Center, University of Aarhus; [2]Institute of Clinical Medicine, University of Aarhus, Aarhus University Hospital-Skejby, Aarhus, Denmark.*

In vivo, renal medullary interstitial cells (RMIC) are subjected to mechanical and inflammatory stress as a result of ureteral obstruction. It has previously been demonstrated that heat shock protein (HSP) 27 is expressed in RMIC in vivo and in vitro. HSP27 exists in a phosphorylated active form (pHSP27) which plays a role in stabilization of actin filaments and has anti-apoptotic function.

In vivo studies using rats subjected to unilateral ureteral obstruction for 6h and 12h; showed unchanged HSP27 mRNA and protein level in kidney inner medulla (IM). However, pHSP27 protein level was increased in obstructed kidney IM compared to both sham and contralateral kidney. To further examine the activation of HSP27 in response to mechanical and inflammatory stress in vitro we subjected RMIC to pressure and stimulation with interleukin 1β (IL-1β). Using a novel pressure apparatus, pressure of 60 mm Hg over time (2-8 h) was explored directly in RMIC which increased expression of HSP27 protein level after 6h and remained increased at 8h. Using the p38 MAPK inhibitor (SB202190), it was demonstrated that the induction of pHSP27 was down-regulated in a dose-dependent manner.

During inflammatory stress, stimulation with IL-1β over time (2-24h) showed unchanged HSP27 mRNA and protein level whereas pHSP27 protein was increased in response to 8 h stimulation.

These data indicate that different forms of cellular stress activate HSP27 and pressure induced HSP27 phosphorylation in RMICs are p38 MAPK dependent. This may affect the ability of RMIC to mount an effective cytoprotective response.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1148**

**Phosphatidylinositol 3-Kinase (PI3K) p85α Subunit Expression Is Downregulated in the Kidney of Spontaneously Hypertensive Rats** Maribel Ch. Chavez,[1] Jose L. Arcaya,[1] Jose D. Herrera,[1] Richard J. Johnson,[3] Bernardo Rodriguez-Iturbe,[2] Freddy J. Romero.[2] *[1]Universidad del Zulia, Maracaibo, Venezuela; [2]Instituto Venezolano de Investigaciones Cientificas (IVIC-Zulia), Maracaibo, Venezuela; [3]University of Colorado, Denver.*

**Background:** Mice with reduced expression of the insulin receptor in renal tubules, resulting from renal epithelial cell-specific knockout, have significant increments of systolic blood pressure and reduced sodium and nitric oxide excretion, which suggest a potential role of the insulin receptor-activated pathways in blood pressure control. Previous studies have demonstrated that the density and mRNA levels of insulin receptors in the kidney of spontaneously hypertensive rats (SHR) do not differ from those in Wistar Kyoto (WKY) rats. However, is not known if the phosphatidylinositol 3-kinase (PI3K) pathway, one of the major downstream mediators of insulin receptor, is altered in the kidney of SHR. The present study was carried out to investigate the PI3K pathway components in the kidney of SHR.

**Methods**: Studies were done in male SHR and WKY rats aged 10 weeks (n=6 each) in which harvested kidneys were used for mRNA and protein expression of the constituents of the insulin PI3K pathway.

**Results:** No differences were detected in the renal transcript abundance of regulatory subunit p85β, and catalytic subunits p110α, p110β, and IRS-1 in the SHR and WKY rats. However, SHR had reduced renal mRNA levels of PI3K p85α subunit (SHR=0.28 ± 0.15 versus WKY=1.00 ± 0.12, respectively, $p< 0.01$). Western blot analysis demonstrated a reduction in the expression of PI3K p85α subunit in the renal cortex of SHR versus WKY (0.36 ± 0.05 vs. 1.04 ± 0.08, $p<0.01$)

**Conclusions**: PI3K p85α subunit expression is downregulated in the kidney of SHR. Suppressed activity of insulin receptor-activated pathways, resulting from reduced renal PI3K signaling may play a role in the pathogenesis of hypertension in the SHR.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1149**

**Diabetes and Mitochondrial Dysfunction; IHG-1 Binds to Mitofusion 1 & 2 and Regulates Mitochondrial Dynamics** Brenda Griffin, Fionnuala B. Hickey, James B. Corcoran, Una Bhreathnach, Catherine Godson, Madeline Murphy. *Diabetic Research Centre, University College Dublin, Dublin, Ireland.*

Mitochondrial dysfunction is a major contributor to hyperglycaemic-induced renal damage. In mammalian cells mitochondria undergo continuous changes in morphology as they constantly divide and fuse. Morphology and function are closely related. Fusion is regulated by the GTPAse proteins, mitofusin 1 and 2 (Mfn1 and Mfn2) and OPA1 while fission is regulated by DRP1 and Fis1.

The glucose regulated gene transcript Induced in High Glucose I (IHG-1) is associated with the development of tubulointerstitial fibrosis in diabetic nephropathy. We have previously shown that IHG-1 contributes to the TGF-ß1 induced profibrotic changes in tubular cells that prime for TIF. On discovery IHG-1 had no known function or functional classification apart from a predicted mitochondrial localisation signal sequence.

Here we show that IHG-1 is localised to mitochondria in mammalian cells by immunogold cryosection electron microscopy. Using fluorescent confocal microscopy, IHG-1 overexpression demonstrated an altered mitochondrial morphology consistent with increased fusion. Conversely, loss of IHG-1 expression using shRNAmir resulted in a fragmented mitochondrial morphology consistent with increased fission or loss of fusion. Additionally we overexpressed known mediators of fusion (i.e. Mfn1, Mfn2, OPA1) and fission (Drp-1, Fis1) by transient transfection in cell culture. Characteristic mitochondrial morphologies were demonstrated by fluorescent confocal microscopy.

In order to investigate how IHG-1 mediates mitochondria fusion we investigated whether IHG-1 could interact with these mediators of fusion and fission. Co-immunoprecipitation experiments indicate that IHG-1 binds to Mfn1 and Mfn2 but not OPA1, DRP1 or Fis1.

In summary these data indicate that IHG-1 regulates mitochondrial dynamics by binding to the GTPase proteins Mfn1 and Mfn2. Future work will elucidate whether binding is necessary or sufficient for IHG-1's profibrotic role.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1150**

**Zebrafish (*Danio Rerio*) Require Cofilin 1 for Renal Function** Sharon L. Ashworth,[1,2,4] Hannah B. Marquis,[1] Christopher Preziosi,[2] Jessica K. Kaufeld,[3,4] Mario Schiffer.[3,4] *[1]Biology and Ecology, University of Maine, Orono, ME; [2]Molecular and Biomedical Sciences, University of Maine, Orono, ME; [3]Division of Nephrology, Hanover Medical School, Hanover, Germany; [4]Mount Desert Island Biological Laboratory, Salisbury Cove, ME.*

Regulated actin dynamics is critical for normal kidney cellular functions. Cofilin 1, an 18 kDa non-muscle actin binding protein, is an essential regulator of actin dynamics in renal function. The zebrafish model system is an ideal vertebrate model organism to study kidney function due to large brood sizes, rapid generation time, embryo transparency, availability of morpholinos and mutants, and accessibility to a simplified kidney structure or pronephros. To investigate the role of cofilin in zebrafish development and renal function, we studied cofilin 1 mutants and morphants. The cofilin 1 mutant embryos contained the *hi3736aTg* proviral insertion downstream of the first exon of the *cofilin 1* gene. This recessive mutation required PCR identification and maintenance of a heterozygous cofilin 1 mutant population to breed homozygous cofilin 1 mutant embryos. Cofilin 1 zebrafish mutants developed pericardial edema at 48 hours post fertilization (hpf). At 72 hpf pericardial edema increased and an abnormal heart structure was observed. At 148 hpf the heart rate was 20% slower than the wildtype larva heart rate. Atypical head and jaw development, smaller eyes with deformed pupils and lack of a swim bladder were also observed. Integrity of the glomerular filtration barrier function was tested by measuring clearance of 70 kDa FITC-dextrans from the retinal blood vessels over a 48 hour time course. Both the cofilin 1 mutant and morphant embryos exhibited a significant decrease in FITC fluorescence compared to wildtype controls which suggested the glomerular filtration barrier was compromised. TEM studies demonstrated podocyte effacement in both cofilin mutant and morphant zebrafish larva. Using fluorescent dyes, leakage from the pronephric tubules was detected by confocal microscopy at 120 hpf. These studies demonstrated cofilin 1 is essential to normal zebrafish development and pronephros function.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1151**

**Influence of Tumor-Necrosis-Factor (TNF) on Tunneling Nanotube (TNT) Mediated Cell-to-Cell Communication** Julia Ranzinger,[1] Amin Rustom,[2] Lars Kihm,[1] Margarete Witkowski,[3] Peter E. Scheurich,[3] Martin G. Zeier,[1] Vedat Schwenger.[1] *[1]Nephrology, University of Heidelberg, Heidelberg, Germany; [2]Dept. of Biophysical Chemistry, University of Heidelberg, Heidelberg, Germany; [3]Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, Germany.*

**Background**

TNTs allow intercellular communication between various cells and have important physiological functions in health and disease. Here, we show for the first time the existence of TNTs between human primary peritoneal mesothelial cells and the effect of TNF on TNT-mediated intercellular communication.

**Methods**

Fluorescence microscopy was applied to detect TNTs in i) mesothelial cells from omentum of healthy donors and ii) mesothelial cells isolated from effluents in dialysis fluid from patients undergoing continuous ambulatory peritoneal dialysis (CAPD). To investigate both the effect of a changed cholesterol content in the plasma membrane and the presence of TNF on the TNT-formation, cells were treated with Methyl-ß-Cyclodextrine. Moreover, microinjection experiments were performed to elucidate a shuttling of molecules between TNT-bridged cells.

**Results**

The experiments show that the number of TNTs strongly depends on the concentration of cholesterol available in the plasma membrane. Incubation of cells with TNF reveals a stimulating effect on the TNT-formation. Analysing the ability of cells from patients undergoing CAPD to form TNTs, our study show that the number of TNTs built is significantly higher as compared to cells from healthy donors. In the course of microinjection experiments, we further provide evidence for a directed transport of molecules via TNTs.

**Conclusions**

The existence of TNTs connecting mesothelial cells points to a distinctive communication among the cells under *in vitro* conditions. We show that the cholesterol content of the cell membrane, as well as the presence of TNF have a strong influence on the TNT-formation. The significantly higher TNT number among cells from patients undergoing CAPD reflects the stimulating effect of TNF, given that the TNF concentration is elevated in patients undergoing CAPD due to the durable exposition of dialysis solution in the peritoneal cavity.

Disclosure of Financial Relationships: nothing to disclose

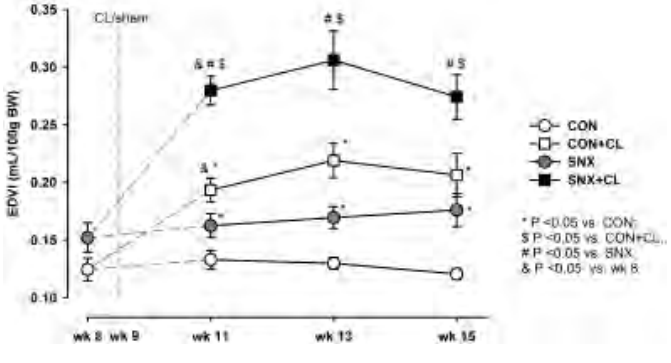
**F-PO1152**

**Reciprocal Organ Damage in Combined Experimental Chronic Kidney Disease and Myocardial Infarction** Lennart G. Bongartz,[1] Jaap A. Joles,[1] Marianne Christina Verhaar,[1] Maarten Jan M. Cramer,[2] Roel Goldschmeding,[3] Carlo A. Gaillard,[4] Pieter Doevendans,[2] Branko Braam.[5] *[1]Nephrology, UMC; [2]Cardiology, UMC; [3]Pathology, UMC, Utrecht; [4]Nephrology, Meander Medical Center, Amersfoort, Netherlands; [5]Nephrology & Immunology, University of Alberta, Edmonton, AB, Canada.*

In humans co-existence of chronic kidney disease (CKD) and heart failure (HF) is associated with accelerated failure of each organ and worse outcome. However, the reciprocal nature of cardiorenal interactions has scarcely been studied in animal models. We hypothesized that pre-existent chronic CKD worsens cardiac outcome after MI, and conversely that MI worsens CKD.

We subjected male Lewis rats to subtotal nephrectomy (SNX) or sham (CON). In wk 9, we performed coronary ligation (CL) or sham-surgery to realize 4 groups: CON, SNX, CON+CL, and SNX+CL. *In vivo* cardiorenal measurements were performed in wk 8, 11, 13 and 15. In wk 16, hemodynamic studies were performed and end-organ damage was assessed.

Proteinuria per level of SBP was significantly higher in SNX+CL vs. SNX, whereas there was no difference between CON+CL and CON. Correspondingly glomerulosclerosis was worsened in SNX+CL vs. SNX. Two wks after MI, rats with SNX+CL had more cardiac dilatation compared to CON+CL (end-diastolic volume index (EDVI): 0.28±0.01 vs. 0.19±0.01 mL/100g BW; *P*<0.001), despite similar infarct size.




During follow-up, SNX+CL rats developed a lower ejection fraction and increased mortality rate (only SNX+CL rats died, 2 out of 9). In SNX+CL, end-diastolic pressure (18±2 mmHg) and *tau* (29±4 msec), the time-constant of active relaxation, were significantly higher compared to SNX (13±1 mmHg, 20±1 msec; *P*<0.01) and CON+CL(11±2 mmHg, 17±1 msec; *P*<0.01).

This study demonstrates the existence of reciprocal organ damage in a model of combined CKD and MI, which cannot be explained by hemodynamic changes.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1153**

**A New Mechanism for Angiotensin II-Induced Cardiac Fibrosis and Dysfunction: A New Role for SGK-1 Signaling** Jizhong Cheng, Anlin Liang, William E. Mitch, Jie Du. *Internal Medicine, Baylor College of Medicine, Houston, TX.*

It is widely agreed that the inflammation induced by an infusion of angiotensin II (Ang II) contributes to cardiac interstitial fibrosis. To understand how angiotensin II causes cardiac fibrosis, we studied Ang II-infused mice and evaluated a new cell signaling pathway based on serum- and glucocorticoid-regulated kinase 1(SGK-1) plus activation of NF-κB. First, we used an echocardiography analysis comparing mice infused with either Ang II or saline for one month. Ang II caused a decrease in left ventricular fractional shortening and an increase in left ventricular diameters (P<0.05) during diastole. In the heart, we also found that Ang II induced phosphorylation and nuclear translocation of the p65 subunit of NF-κB. Second, we evaluated downstream signaling responses to NF-κB (i.e., MCP-1 and IL-8) in cardiac endothelial cells. Both mediators were upregulated by Ang II infusion. The hearts also exhibited increased infiltration with macrophages, accompanied by cardiac remodeling and an increase in fibrosis. Third, we found that Ang II stimulated SGK-1 expression in both cultured SMCs and cardiac fibroblasts. However, SGK-1 mRNA and protein levels were inhibited > 95% by the MEK1/2 inhibitor, U0126, indicating that Ang II induces SGK-1 expression via a MEK-ERK signaling pathway.

To explore whether SGK-1 influences cardiac fibrosis, SGK-1 knockout (KO) mice were studied. In hearts of SGK-1 KO mice infused with Ang II: 1) there was a sharp decrease in p65 NF-κB nuclear translocation and the expression of inflammatory markers; 2) cardiac fibrosis and inflammation were absent; and 3) the absence of SGK-1 dramatically decreased expression of α-smooth muscle actin, collagen I, fibronectin and decreased fibrosis. Importantly, echocardiography analysis of Ang II infused, SGK-1 KO mice showed no signs of heart dysfunction. Thus, we have uncovered a new pathway causing fibrosis and functional impairment in the heart. Inhibition of SGK-1 function could be a therapeutic target for preventing cardiac dysfunction in conditions associated with excess Ang II.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1154**

**Rapamycin Reduces Cardiac Fibrosis in Experimental Uremic Cardiomyopathy** Steven T. Haller,[1] George Budny,[1] Joe Xie,[1] Jiang Tian,[1] Mohammad Taleb,[1] Sankaridrug Periyasamy,[1] Deepak K. Malhotra,[1] Olga Fedorova,[2] Alexei Bagrov,[2] Joseph I. Shapiro.[1] *[1]Medicine, University of Toledo Medical Center, Toledo, OH; [2]National Institute on Aging, Baltimore, MD.*

Experimental uremic cardiomyopathy causes cardiac fibrosis and is associated with increased levels of the cardiotonic steroid marinobufagenin (MBG), a ligand of the Na/K-ATPase. The mammalian target of rapamycin (mTOR) is a serine/threonine kinase implicated in tissue fibrosis. Treatment with rapamycin (an mTOR inhibitor) has been shown to attenuate renal fibrosis in experimental renal failure. Rapamycin has also been noted to decrease the activity of CYP27A1, an enzyme believed to be important in MBG synthesis. To address how rapamycin would attenuate uremic cardiomyopathy, male Sprague Dawley rats weighing between 250-300 gms were divided into six groups consisting of sham surgery, partial nephrectomy (PNx), MBG infusion (10 ug/kg/day) and the combination of rapamycing (0.2 mg/kg/day) with the other manipulations.The PNx-rapamycin group demonstrated a significant decrease in systolic BP by the third week of treatment compared to PNx (149 ± 2 vs 166 ± 1 mmHg, P<0.01). Plasma MBG levels were significantly decreased in the PNx-rapamycin animals compared to PNx (248 ± 30 vs 684 ± 40 pmol/L, P<0.01). The PNx-rapamycin animals showed a substantial decrease in cardiac fibrosis compared to PNx animals as assessed by both tissue collagen expression and quantitative histology. MBG treated animals also had significant increases in systolic BP, and cardiac fibrosis compared to controls, and rapamycin treatment did not significantly lower MBG levels in these animals. However, rapamycin did appear to lower fibrosis as assessed by collagen expression and quantitative histology. In vitro studies in cardiac fibroblasts confirmed that rapamycin at doses as low as 10 ng/ml prevented MBG induced increases in collagen expression. Our results suggest that rapamycin exerts its protective effects both through inhibition of MBG synthesis and also through the mTOR pathway. Treatment with rapamycin may provide a novel therapy for reducing cardiac fibrosis in the setting of uremic cardiomyopathy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1155**

**Treatment with Nrf-2 Activator Ameliorates Uremia-Induced Endothelial Dysfunction and Hypertension** Stanislav Shelkovnikov,[1] Zhenmin Ni,[1] Hyun Ju Kim,[1] Colin Meyer,[2] Ardeshir Rahimi,[1] Nosratola D. Vaziri.[1] *[1]Medicine, University of California, Irvine, Irvine, CA; [2]Reata Pharmaceuticals, Inc, Irving, TX.*

Chronic kidney disease (CKD) results in endothelial dysfunction, hypertension, accelerated atherosclerosis and cardiovascular disease, events which are driven by oxidative stress (OS) and inflammation. The nuclear factor, Nrf2 regulates expression of genes encoding antioxidant and cytoprotective molecules which protect against OS and inflammation. We recently found that OS and inflammation in 5/6 nephrectomized rats are associated with impaired Nrf2 activation and downregulation of the corresponding antioxidant molecules [Am J Physiol, 298:F662, 2010]. Bardoxolone methyl and its analogs are potent Nrf2 activators that reduce production of reactive oxygen species and activation of NF-κB in endothelial, renal and cardiac cells. Therefore, we hypothesized that Nrf2 activator therapy may attenuate CKD-induced OS and endothelial dysfunction. To this end 5/6 nephrectomized rats were treated with a bardoxolone methyl analog (RTA 405, 20 mg/kg/day) or vehicle for 6 weeks. Acetylcholine, phenylephrine and sodium ntroprusside (SNP) concentration–response curves were obtained, and EC50 values were determined in isolated carotid artery rings by cumulative addition of the given agonists. Compared to the Sham-operated controls, CKD rats showed significant elevation of blood pressure and plasma lipid peroxidation product, malondialdehyde (MDA, 3.05±0.26 vs. 2.18±0.22 nM, P<0.01) and reduced vasodilatory response to acetylcholine (EC50; 181±20 nM vs. 38±3 nM, P<.01), denoting the presence of OS and endothelial dysfunction. RTA 405 therapy reversed CKD-associated hypertension, lowered plasma MDA (2.56±0.28 nM) and restored vasodilatory response to acetylcholine (EC50, 29±4 nM, P<.01). The response to phenylephrine and SNP was not altered by either CKD or RTA 405 therapy. Thus, Nrf2 activator ameliorates oxidative stress, hypertension and endothelial dysfunction, representing a potentially novel tool for management of CKD-associated endothelial dysfunction and cardiovascular disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1156**

**Luminal Alkalization Attenuates Superoxide Production Induced by Fatty Acid-Bound Albumin in Renal Proximal Tubular Cells Through Pyk2-Dependent Pathways** Tomokazu Souma,[1,2] Michiaki Abe,[1] Takashi Moriguchi,[2] Takaaki Abe,[1,3] Sadayoshi Ito.[1] *[1]Department of Nephrology, Endocrinology and Vascular medicine, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan; [2]Department of Medical Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan; [3]Division of Medical Science, Tohoku University Graduate School of Biomedical Engineering, Sendai, Miyagi, Japan.*

Proximal tubular cells are physiologically exposed to a highly acidic environment as a consequence of their reabsorption of HCO3-. However, it remains unclear whether this luminal acidity affects the progression of tubular cell damage induced by proteinuria. In the present study, we investigated the contribution of luminal acidity to O2•- production induced by oleic acid-bound albumin (OA-Alb) in proximal tubular cells. Acidic media significantly enhanced the OA-Alb induced O2•- production in the HK-2 proximal tubular cell line up to 7-fold (p<0.001). Of note, Proline-rich tyrosine kinase 2 (Pyk2), an intracellular pH sensor, was strongly phosphorylated by simultaneous treatment with both OA-Alb and acidic media. Highly phosphorylated Pyk2 was associated with activation of Rac1, a subcomponent of NAD(P)H oxidase. Furthermore, Pyk2-specific siRNA-knockdown attenuated the OA-Alb induced O2•- production by 70% (p<0.05). Assuming that luminal alkalization possibly exerts therapeutic effects on proteinuria-induced tubular damage, we employed a mouse model of protein overload nephropathy. Proximal tubules of protein-overloaded mice showed marked accumulation of oxidative DNA damage; oxidative stress was assessed by measuring accumulation of 8-OHdG immunoreactivity and augmentation of DCFDA, oxidation-sensitve dye, -intensity by flowcytometry. NaHCO3-feeding selectively alkalized the urine and dramatically attenuated the accumulation of proteinuria-induced oxidative DNA damage and subsequent tubulointerstitial injury (p<0.05). Overall, these observations suggest that luminal acidity is an intrinsic precipitating factor that aggravates proteinuria-induced tubular damage, and that modulation of this acidic environment is a potential therapeutic target for chronic proteinuric kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1157**

**Knockout of Nuclear Receptor 4a1 on a Hypertensive Genetic Background Results in Severe Renal Injury** Kevin R. Regner,[1] Jessica A. Eisenhauer,[1] Cary T. Stelloh,[1] David L. Mattson,[2] Michael R. Garrett.[1] *[1]Nephrology, Medical College of Wisconsin, Milwaukee, WI; [2]Physiology, .*

The NR4A subgroup of nuclear hormone receptors (NR) have been implicated in apoptosis, cancer, atherosclerosis, and most prominently in metabolic disease. However, the role of NR4A in the kidney has not been well characterized. Nr4a1 knockout rats generated using an ENU mutagenesis strategy on the genetic background of the fawn-hooded hypertensive (FHH) rat were originally characterized using a high throughput phenotypic protocol for heart, vascular, and renal parameters through the PhysGen program at the Medical College of Wisconsin. Increased proteinuria was the most prominent phenotype observed in Nr4a1-/- compared to FHH controls. To more closely evaluate the onset and progression of renal injury, the temporal relationships in blood pressure, proteinuria, renal function and metabolic parameters in the Nr4a1-/- and FHH were evaluated from 4-24 weeks of age. Nr4a1-/- rats exhibited decreased creatinine clearance and ~4-fold higher proteinuria (81±11.5 mg/24hrs/100g BW) compared to FHH (21±1.0) at week 24. No differences in telemetry measured BP were observed between groups. At week 24, Nr4a1-/- animals also showed 3-4 fold higher cholesterol and triglyceride levels compared to the FHH. FHH and Nr4a1-/- kidneys exhibited similar glomerular pathology at each time point as evaluated by light microscopy and electron microscopy (EM) and was characterized by mesangial expansion and podocyte foot process effacement. Compared to the FHH, Nr4a1-/- kidneys demonstrated a significant increase in tubulointerstitial injury characterized by severe tubular atrophy, protein casts, and interstitial fibrosis. At the EM level, proximal tubule cells demonstrated increased lipid-like inclusions along with an increased number of autophagosomes and/or lysosomes. Immunohistological localization demonstrated that NR4a1 is expressed in all tubular components, but not observed to any great extent in the glomeruli or vessels. These data demonstrate that loss of NR4a1, on a genetic background predisposed to hypertension, results in progressive renal injury and loss of renal function likely through a tubular mediated mechanism.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1158**

**Obesity Induced-Proteinuria Modulates Tight Junction Protein Expression and Function** Li-Jun Ma,[1] Fnu Bhawana,[2] Amar B. Singh.[2] *[1]Departments of Pathology, Vanderbilt University, Nashville, TN; [2]Surgery, Vanderbilt University, Nashville, TN.*

Background: Obesity induces proteinuria and chronic kidney injury. Tight junctions help maintain paracellular permeability and integrity of renal tubular epithelial cells. However, effect of obesity-related proteinuria upon tight junction structure/function is poorly understood. We investigated the effects of obesity-induced proteinura upon tight junction composition and function.

Methods: 8-10 weeks old male DBA mice were fed standard normal chow (NC, n=8) or a high-fat diet (HFD, n=9) for 24 wks. Metabolic parameters and kidney morphology were assessed. Expression of tight or adherent junction proteins in kidney was assessed. In addition, effects of conditioned medium from differentiated adipocytes upon permeability and resistance were determined using MDCK-II cells. Data are expressed as mean±SE.

Results: HFD feeding significantly increased body weight (39.3±2.9 vs 29.8±0.8 g, p<0.01), albumin-creatinine ratio (ACR, 242±56 vs 95±33 ug/mg, p<0.01), and induced extensive tubular vacuolization and dilation (0-4 score: 2.5±0.4 vs 0.6±0.1 p<0.01), although mesangial expansion was not different in HFD vs NC (0-4 score: 1.2±0.3 vs 1.1±0.1). Interestingly, expression of all tight junction proteins investigated was highly increased in HFD-fed vs NC-fed mice: Occludin (2.58 fold), claudin-1 (1.54 fold) and claudin-4 (6.02 fold). Notably, the HFD-induced increase for tight junction proteins was highest for claudin-2 (19.69 fold, p<0.05 HFD vs NC), a claudin family member expressed exclusively in the proximal tubular epithelial cells. No change in the expression of E-cadherin or β-catenin in the same samples confirmed specificity. In vitro, exposure of polarized MDCK II cells to conditioned medium from adipocytes increased paracellular permeability for FITC-dextran (50.50±4.0 vs. 33.75±2.1, p<0.05).

Conclusions: Our data demonstrate, for the first time, that obesity induced-proteinuria is associated with significant and specific changes in tight junction composition and function and thus suggest important role of paracellular transport in the pathology of obesity-induced proteinuria.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1159**

**Mechanism of Glomerular Disease Initiation in Alport Syndrome** Dominic E. Cosgrove. *Boys Twon National Research Hospital, Omaha, NE.*

Alport syndrome is a delayed onset progressive glomerular disease caused by mutations in type IV collagen genes. Studies using animal models for Alport syndrome have revealed many aspects of the pathobiology of the glomerular disease, and have suggested several therapeutic avenues for treatment. The mechanism of Alport glomerular disease initiation, however, has remained elusive. It has been known for some time that abnormal laminins (laminin 111 and 211) accumulate in the GBM of Alport mice, dogs and humans. The functional significance of this observation was unclear. Here we show that activation of abnormal GBM laminin expression results in activation of focal adhesion kinase (FAK) in podocytes. FAK activation is associated with cytoskeletal rearrangements with the formation of large stress fibers and the induction of maladaptive genes including MMP-10 and MMP-12, both of which are implicated in GBM proteolysis. Both FAK siRNA knockdown and small molecule inhibitors of FAK reverse the actin cytoskeletal phenotype and block MMP induction. CD151 knockout mice, which have weakened α3β1 integrin adhesion to laminin 521 also show laminin 111 and 211 accumulation in the GBM. Both SiRNA knockdown of integrin linked kinase (ILK) and biomechanical strain of cultured podocytes induces abnormal laminin expression, and abnormal laminin accumulation is exacerbated in Alport mice by hypertension. Collectively, these data suggest that reduced ILK activity due to strained α3β1 integrin interaction with laminin 521 results in activation of abnormal laminin expression and accumulation in the GBM, which in turn activates FAK, resulting in maladaptive gene regulation. We suggest these events constitute the molecular mechanism of Alport glomerular disease initiation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1160**

**Defining an Angiotensin II-Stimulated Proteome in Human Kidney Cells** Ana Konvalinka,[1] Eleftherios P. Diamandis,[2] James W. Scholey.[1] *[1]Medicine, University of Toronto, Toronto, Canada; [2]Pathobiology, Mount Sinai Hospital/ University Health Network, Toronto, Canada.*

**PURPOSE:** Activation of the renin angiotensin system and the generation of Angiotensin II (ANGII) is an important determinant of kidney disease progression, but there are currently no measures of renal ANGII activity. Accordingly, we sought to define an ANGII-stimulated proteome in primary human proximal tubule epithelial cells (PTEC) in order to identify potential biomarkers of ANGII activity in the kidney.

**METHODS:** We utilized stable isotope labeling with amino acids (SILAC) in PTECs under two sets of paired conditions: 1) ANGII and Control, and 2) ANGII and ANGII plus AT-1 receptor blockade (Losartan). Lysates from each SILAC pair were dialyzed, lyophilized, reduced, alkylated, trypsin-digested and fractionated using high performance liquid chromatography (LC). Fractions were zip-tipped and injected on the LC coupled to LTQ-Orbitrap MS. The resulting raw mass spectra were analyzed using Mascot on the IPI Human database. Protein identification and calculation of peptide ratios was performed by MaxQuant software.

**RESULTS:** The labeling efficiency of SILAC was confirmed by comparing lysates from untreated labeled cells. In each experiment more than 2500 proteins were identified. Of these proteins 349 were regulated by ANGII – 198 proteins were up-regulated and 151 were down-regulated. Ontology analysis revealed that metabolic processes were over-represented in the differentially-expressed proteins. Ingenuity Pathway Analysis determined that NFkB associated protein network was upregulated by ANGII. Several interesting candidate biomarkers emerged: for example, myosin heavy chain9 (MYH9), macrophage migration inhibitory factor (MIF) and cysteine-rich protein (CYR61) which is related to connective tissue growth factor. Interestingly, Losartan blocked ANGII-stimulated expression of CYR61, but not expression of MYH9 and MIF.

**CONCLUSIONS:** We have successfully utilized SILAC with human PTECs to define proteins differentially expressed by ANGII, and this strategy has identified novel proteins that may serve as biomarkers of ANGII activity in the kidney.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1161**

**TGF-β Was Upregulated in Renal Fibrosis Model of Klotho Defect Mouse and Affected Renal Klotho Expression Level** Hidekazu Sugiura, Takumi Yoshida, Junko Kohei, Shunji Shiohira, Michihiro Mitobe, Ken Tsuchiya, Kosaku Nitta. *Fourth Department of Internal Medicine, Tokyo Women's Medical University, Tokyo, Japan.*

Klotho is well known as an anti-aging gene that is mainly expressed in the kidney, which protein is essential for the functioning of endogenous fibroblast growth factor 23 (FGF23). We previously reported more severe tubulointerstitial fibrosis in klotho defect mice (Klotho (-/-) mice) comparing with wild type mice (Klotho(+/+) mice). It is well known that TGF-β is one of major fibrotic progression gene, so we investigated to clarify whether TGF-β is Klotho mediating signaling pathway in the process of renal fibrotic change in *in vivo* and *in vitro*. In practice, the relationship of the expression between Klotho and TGF-β in mice deficient for the klotho gene and in HK2 cells (in which Klotho expression is confirmed) adding TGF-β. We investigated renal fibrosis in the classical model of unilateral ureteral obstruction (UUO) in klotho hetero defect mice (Klotho (+/-) mice), then HK2 cells incubated with TGF-β (10 ng/ml) for 24, 46, 72, 96 and 120 hours with/without TGF-β1 receptor inhibitor (ALK5 inhibitor). Expression of renal TGF-β was analyzed by ELISA and that of renal fibronectin and Klotho was by Western blotting and/or immunohistochemical staining. UUO treatment induced the expression of TGF-β (1.4-fold, $p<0.05$) and fibronectin (1.6-fold, $p<0.05$) in the kidney of Klotho (+/-) mice comparing with Klotho (+/+) mice. Klotho expression was significantly reduced (0.4-fold, $p<0.05$) in the Klotho (+/-)-UUO-mice comparing with the Klotho (+/-)-sham-mice and the Klotho (+/+)-UUO-mice. Klotho (+/-)-UUO-mice demonstrated prominent fibrosis of kidney tissues. Klotho expression was significant suppression was observed in late phase, 72 to 120 hours in cultured HK2 cells in a time dependent manner, suggesting not acute but chronic effects of by TGF-β on Klotho expression. The effect was attenuate by co-incubation with ALK5 inhibitor. Together with our previous observations, there is a cross-talk between Klotho and TGF-β in the kidney, and Klotho is likely to play its potential roles, in part, mediating TGF-β signaling pathway.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1162**

**Dynamic Analysis of Renal Autoregulation in Experimental Chronic Kidney Disease** Maarten P. Koeners,[1] William A. Cupples,[2] Jaap A. Joles.[1] *[1]Nephrology, UMC, Utrecht, Netherlands; [2]Biology, Univ. Victoria, BC, Canada.*

Renal autoregulation, the kidneys' intrinsic property to maintain constant renal blood flow (RBF) and glomerular filtration (GFR) with fluctuating renal perfusion pressures, is necessary for normal renal function and volume homeostasis, and to protect the kidney against increased pressures. Impaired renal autoregulation is believed to be responsible for enhanced susceptibility to renal damage in chronic kidney disease (CKD).

In contrast to steady-state, dynamic analysis of autoregulation can be used to study multiple controllers operating in parallel under physiologically relevant conditions. As the level and pattern of BP fluctuation can vary significantly, BP was forced by a computer-driven suprarenal aortic clamp. Such broadbandforcing ensures that all relevant input frequencies are equally represented and that relevant control loops are opened. Dynamic analysis of renal autoregulation was assessed under isoflurane anesthesia in male CKD rats (5/6 subtotal nephrectomy) vs. Sprague Dawley control. Male Fawn-Hooded Hypertensive (FHH) rats, a genetic model for defective autoregulation and progressive glomerulosclerosis, were used as positive controls.

Body weight, mean arterial pressure and left kidney RBF were similar between groups. GFR was lower in CKD than in control rats (0.55±0.03 vs 1.24±0.19 ml/min, $p<0.001$).

Renal blood flow dynamics

| | | RPP, forced | Slope of gain reduction | Phase Peak |
|---|---|---|---|---|
| | *n* | *mmHg* | *dB/decade* | *rad* |
| Control | 8 | 95±2 | 24±3 | 1.11±0.12 |
| CKD | 5 | 95±4 | 12±3# | 0.56±0.15# |
| FHH | 4 | 85±3 | 6±1# | 0.33±0.11# |

# $P<0.05$ vs. Control

Dynamic analysis of renal autoregulation revealed gain reduction <~0.2 Hz, and associated phase peak in controls, indicating effective autoregulation mediated by the myogenic mechanism (0.06 to 0.2 Hz). CKD rats displayed marked impairment of the myogenic mechanism shown by inhibition of slope of gain reduction ($p=0.03$ vs control) and lower phase peak ($p=0.012$ vs control), both of which approached values seen in FHH rats.

In conclusion, these data are consistent with pressure-passive behavior of the renal circulation in experimental CKD resembling an accepted model of impaired renal autoregulation and progressive injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1163**

**Pro-Fibrotic Role of Matrix Metalloproteinase-9 in a Murine Fibrotic Model of Unilateral Ureteral Obstruction** Thian Kui Tan,[1] Guoping Zheng,[1] Tim Tzu-Ting Hsu,[1] Xinrui Tian,[1,2] Qi Cao,[1] Ya Wang,[1] Yiping Wang,[1] Vincent W. S. Lee,[1] Dong Zheng,[1] David C. Harris.[1] *[1]Centre for Transplantation and Renal Research, Westmead Millennium Institute, Sydney, New South Wales, Australia; [2]Department of Respiratory Medicine, The Second Hospital of Shanxi Medical University, Shanxi, China.*

Matrix metalloproteinase-9 (MMP-9) appears to promote renal fibrosis. Previously, we showed that macrophage MMP-9 causes tubular cell (TEC) epithelial-mesenchymal transition (EMT); TGF-β-induced TEC EMT is dependent on MMP-9 activity; and MMP-9 is upregulated in murine unilateral ureteral obstruction (UUO). The aim of this study was to determine the profibrotic role of MMP-9 in murine UUO via stage-specific inhibition of MMP-9 activity. Renal MMP-9 expression in BALB/c mice with UUO was examined on day 1, 3, 5, 7, 9, 11 and 14. To inhibit MMP-9 activity, MMP-2/9 inhibitor (Inh) or MMP-9 neutralising antibody (Ab) (2mg/kg) was administered daily for 4 consecutive days from day 0-3, 6-9, or 10-13 and tissues harvested at day 14. In UUO, there was a biphasic (early and late-stage but not mid-stage) upregulation of MMP-9 activity. Double immunofluorescence staining revealed co-localisation of MMP-9 with TEC, macrophages and myofibroblasts in UUO kidney. Early- and late-stage but not mid-stage inhibition of MMP-9 by MMP-2/9 Inh or MMP-9 Ab resulted in a significant (at $P<0.05$) reduction of fibrosis [51% & 29% Inh; 31% & 73% Ab]; macrophage infiltration [20% & 34% Inh; 27% & 33% Ab], α-SMA expression [46% & 76% Inh; 51% & 62% Ab] and MMP-cleaved osteopontin expression [61% & 57% Inh; 78% & 91% Ab] in obstructed kidney. Cytoplasmic & nuclear translocation of membrane β-catenin, indicating EMT, was also reduced by the MMP-2/9 Inh and MMP-9 Ab. In conclusion, our study has demonstrated an important role of MMP-9 in causing renal fibrosis in UUO, the time dependency of its expression and its potential as a therapeutic target.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1164**

**Renal Phenotypes in Mice with Homozygous Mutation of the Gene Encoding Canonical Transient Receptor Potential 1 Channel (TRPC1): Evidence for Renal Failure in a Novel Mouse Model of Chronic Kidney Disease** Kai Lau,[1,5] Bonnie Eby,[1] Chris Skaggs,[4] Erin Johnston,[1] Richard Matthew Atkins,[1] Jan L. Guz,[2] Joel Abramowitz,[3] Lutz Birnbaumer,[3] Leonidas Tsiokas.[2] *[1]Medicine, University of Oklahoma, Oklahoma City, OK; [2]Cell Biology, University of Oklahoma, Oklahoma city, OK; [3]Intramural Research, NIEHS, Research Triangle Park, NC; [4]Radiology, University of Oklahoma, Oklahoma City, OK; [5]Medicine, VA Hospital, Oklahoma City, OK.*

TRP is a superfamily of various cation-permeable channels implicated in ion homeostasis and/or signal transduction. We found a critical role of TRPC1 in cardiac hypertrophic signaling. Others showed its role in vascular smooth muscle proliferation & hyperplasia and in mesangial cell contractility. TRPC1 heteromultimerizes with polycystin 2 to form a ion channel distinct from that created by the complexes of polycystins 1 & 2, suggesting a possible role in cystogenesis. Its expression is down-regulated in diabetes. Since its in-vivo role is unknown, we studied serial renal functions & structures in 12 age-matched pairs of male TRPC1 -/- & +/+ mice of the 129 Sv background. At 7 mon, kidney volume by echo (0.38 vs. 0.46 cc., $p<0.01$) & kidney weight (KW) to body (B) W (1.2 vs. 1.45 %, $p<0.04$) were 17% lower in -/- mice. At 11 mon, echogenicity was similar, but volume remained 16% lower (0.42 vs. 0.5 cc., $p<0.01$) & KW:BW fell further in -/- mice (1.1 vs. 1.53 %, $p<0.01$). At 12 mon, serum creatinine by HPLC was similar but by 17 mon increased by 87% in -/- mice (0.103 vs. 0.055 mg/dl, $p<0.01$). Creatinine clearance by HPLC fell by 42 % (0.66 vs. 1.13 ml/min/mouse, $p<0.01$) & by 50% when factored for BW (1.7 vs. 3.4 ml/100g, $p<0.01$). Hypercalciuria or proteinuria were absent. Concentration ability was intact by urine osmolality after 17 h dehydration. Acidification was impaired

by urine pH (6.0 vs. 5.2, p<0.01). Since -/- mice developed diabetes at 12 mon, our data support the hypothesis that TRPC1 protein plays a key role in normal renal development & in the adaptive hypertrophic & hyperfiltration responses to diabetes. Its absence may accelerate progression to renal failure induced by diabetes.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1165**

**Lysosome Membrane Protein-2, a Urinary Exosomal Marker for Idiopathic Membranous Nephropathy** Michael Merchant,[1] Jeroen Deegens,[2] Ilse M. Rood,[2] Daniel W. Wilkey,[1] Terry Zhang,[3] Brad H. Rovin,[4] Vlad Zabrouskov,[3] Jack F. Wetzels,[2] Jon B. Klein.[1,5] *[1]University of Louisville; [2]Radboud University Nijmegen Medical Center; [3]Thermo Fisher Scientific; [4]Ohio State University; [5]Veterans Administration Medical Center.*

Urinary exosomes are the intraluminal vesicles of multi-vesicular bodies and contain proteins specific for podocytes and tubular cells. We hypothesized that the proteome of urinary exosomes isolated from patients with idiopathic membranous nephropathy (iMN) and focal segmental glomerular sclerosis (FSGS) would be different from normal control (NC) urine samples. Exosomes were isolated from urine collected from iMN (n=5) and FSGS (n=5) patients with biopsy proven disease and compared with those from NC urine (n=3). Exosomes were analyzed using a multiplexing approach (8-plex iTRAQ) and LCMS methods (1D-RP-HPLC-ESI-LTQ-VELOS-Orbitrap and Proteome Discover Software). Stringent analysis of iTRAQ labels (one way ANOVA and t-test p-value less than 0.01) indicated changes in abundance of nine proteins. One protein, lysosome membrane protein 2 (LIMP2) also known as scavenger receptor class B, member 2 (SCARB2), was increased greater than two-fold in iMN exosomes above FSGS and control samples. To explore our proteomic findings, IHC analysis of human renal biopsies from FSGS, iMN, disease controls (diabetic nephropathy with proteinuria) and normal controls suggests a striking increase in tubular and glomerular staining for LIMP2 in iMN and FSGS sections over controls. LIMP2 gene mutations have been associated with action myoclonus-renal failure syndrome (AMRF) that presents with focal collapsing glomerulosclerosis. These mutations lead to LIMP2 retention in the endoplasmatic reticulum. In the puromycin aminonucleoside rat model of FSGS, the loss of podocyte foot processes correlated with LIMP2 over-expression. We conclude that renal LIMP2 is upregulated in iMN and FSGS. However, as shown in AMRF, LIMP2 may be unable to reach lysosomes and exosomes in patients with FSGS, resulting in glomerulosclerosis. Urinary LIMP2 is a candidate biomarker for iMN and demonstrates the potential value of urinary exosomes in the diagnosis of renal diseases.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1166**

**Role of Mineralocorticoid Receptor/Rho/Rho-Kinase Pathway in Obesity-Related Renal Injury** Hirobumi Tokuyama, Shu Wakino, Koichi Hayashi. *Medicine, Keio University, Tokyo, Japan.*

Both aldosterone and Rho-kinase are demonstrated independently to play an important role in renal injury and impaired insulin signalling. We therefore examined whether aldosterone/Rho/Rho-kinase pathway contributed to obesity-associated nephropathy. C57BL/6J mice were fed a high fat (HFD) or low fat diet (LFD) and mice on HFD were treated with a mineralocorticoid receptor (MR) antagonist, eplerenone. The mice on HFD developed obesity, and hyperglycemia compared with those on LFD, but manifested similar levels of systolic blood pressure. In HFD-fed mice, glomerular hypercellularity and increased mesangial matrix were noted, which paralleled the increase in albuminuria (HFD, 0.13±0.04mg/gCr, P<0.01 vs LFD, 0.02±0.01mg/gCr). The treatment with eplerenone alleviated the histological changes and albuminuria (0.04±0.01mg/gCr, P<0.01 vs HFD) without alterations in systemic blood pressure. Adipose tissue weight and adipocyte size of HFD-fed mice were increased, which were ameliorated by eplerenone. Furthermore, enhanced Rho-kinase activity was noted in kidneys (2.0-fold, p<0.05) and adipose tissues (1.8-fold, p<0.05) from HFD-fed mice, as well as increased expressions of MCP-1, TNF-α and PDGF-B. All of these changes were attenuated by eplerenone. In HFD-fed mice, MR protein levels in the nuclear fraction were increased in kidneys (2.3-fold, p<0.05) and adipose tissues (2.0-fold, p<0.05) without elevation in serum aldosterone levels (LFD, 278±14mg/dl, HFD, 297±12mg/dl). Finally, in mesangial cells, stimulation with aldosterone increased Rho-kinase activity, and pre-incubation with eplerenone prevented the aldosterone-induced activation of Rho-kinase. In conclusion, excess fat intake causes obesity and renal injury in C57BL/6J mice, and these changes are mediated by an enhanced mineralocorticoid receptor /Rho/Rho-kinase pathway and inflammatory process. Mineralocorticoid receptor activation in the kidney and the adipose tissue and the subsequent Rho-kinase stimulation are likely to participate in the development of obesity-associated nephropathy without elevation in serum aldosterone levels.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1167**

**Targeted Degradation of Cytosolic β-Catenin Dissociates Profibrotic from Anti-Inflammatory Effects of TGF- β1** Xinrui Tian,[1,2] Guoping Zheng,[1] Thian Kui Tan,[1] Tim Tzu-Ting Hsu,[1] Ya Wang,[1] Qi Cao,[1] Dong Zheng,[1] Yiping Wang,[1] Vincent W. S. Lee,[1] David C. Harris.[1] *[1]Centre for Transplantation and Renal Research, Westmead Millennium Institute, Sydney, New South Wales, Australia; [2]Department of Respiratory Medicine, The Second Hospital of Shanxi Medical University, Shanxi, China.*

**Aim**: To differentiate the profibrotic role of TGF- β1 from its anti-inflammatory effect by targeting cytosolic β-catenin.

**Background:** TGF- β1 is known to be both anti-inflammatory and profibrotic. β-catenin, as a central mediator of some TGF- β1 pathways is activated during fibrosis and tissue remodeling and was found dispensable for hematopoiesis, lymphopoiesis and B cell development and function under physiological conditions. Targeting β-catenin holds a potential to dissect the profibrotic property of TGF- β1 from its immunosuppressive effect.

**Methods:** A protein knockdown chimera (F-Trcp-Ecad) in murine C1.1 tubular epithelial cell and J774 murine macrophages to decrease the levels of cytosolic and presumably nuclear β-catenin was used to test its roles in TGF- β1-induced epithelial-mesenchymal transition (EMT) in C1.1 cells and in inhibition of macrophage activation.

**Results:** F-trcp-Ecad preferentially targeted soluble nuclear/cytosolic β-catenin for degradation. TGF-β1-induced EMT and E-cadherin, snail transcription and MMP-9 activity in C1.1 cells was reduced by cytosolic β-catenin knockdown. However, the steady state level of β-catenin in J774 cells was low and there was no change when J774 cells were exposed to IFN-γ or LPS with or without TGF-β1. TGF-β1 inhibition of LPS-induced TNF-α mRNA and IFN-γ-stimulated iNOS mRNA expression was not affected in J774 cells expressing wild type β-catenin or F-trcp-Ecad.

**Conclusio**n: In this study, we present novel evidence that degrading unphosphorylated β-catenin in the cytosol resulted in the inhibition of EMT induced by TGF-b1 in tubular epithelial cells but had no effect on TGF- β1 inhibition of macrophage activation. This suggests that the profibrotic and anti-inflammatory pathways of TGF- β1 can be dissociated, with implications for therapy targeting TGF- β1.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1168**

**Correction of Lysosomal Dysfunction and Autophagy in Diabetes by Tunneling Nanotube (TNT) Exchange between Endothelial and Endothelial Progenitor Cells (EPC)** Kaoru Yasuda, Leonid Buryanovskyy, Anupama Khandare, Peter Jose Hayek, Frank Fan Zhang, Alberto Nasjletti, Michael S. Goligorsky. *New York Medical College, Valhalla, NY.*

We have demonstrated that diabetic milieu triggers collapse of lysosomal pH gradient, lysosomal permeabilization, and frustrates autophagy - leading to developing stress-induced premature senescence (SIPS) of vascular endothelium and vasculopathy. Adoptive transfer of bone marrow-derived cells markedly improved macro- and microvasculopathy. In the present investigation we analyzed the ability of EPC to transfer lysosomes to HUVEC (and vice-versa) via TNT. We examined the transfer ratio, HUVEC viability and SIPS under diabetic-like milieu. Application of non-enzymatically glycated collagen (GC) for 3 days resulted in a dramatic increase in the population of non-viable, senescent, and apoptotic cells, which were decreased approximately 2-fold after 24 h co-incubation of HUVEC with intact EPC. Disruption of TNT formation with cytochalasin B annulled this effect of EPC. Differential labeling of HUVEC and EPC with the Celltracker CFDA and Lysotracker provided direct evidence of TNT formation between these cells. The rate of exchange was 29.97±0.80% after co-culture for 24 hours. Egress of highly acidic lysosomes from EPC along the TNT toward GC-treated HUVEC, which express lysosomes with the collapsed pH gradient was examined using acridine orange labeling of acidic compartments. Transferred lysosomes maintained pH gradient associated with the improved fusion of lysosomes and autophagosomes. In vivo, STZ-treated diabetic mice received an adoptive transfer of intact EPC or EPC pretreated with cytochalasin B. EPC treatment resulted in improved acetylcholine-induced relaxation of aortic rings and reduced number of senescent cells in the adventitial layer. However, prevention of TNT formation resulted in the reduced efficacy of adoptively transferred EPC. In conclusion, in vitro TNT formation between EPC and injured endothelial cells is partially responsible for restoration of functional lysosomal pool resulting in correction of autophagy with improved cell viability and in vivo improvement in macro- and micro- vasculopathy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1169**

**A Novel Role for miR-382: Targeting of SOD2 during TGFβ1-Induced Loss of Epithelial Characteristics in Human Renal Epithelial Cells** Alison J. Kriegel,[1] Yong Liu,[1] Yi Fang,[2] Domagoj Mladinov,[1] Xiaoqiang Ding,[2] Mingyu Liang.[1] *[1]Department of Physiology, Medical College of Wisconsin, Milwaukee, WI; [2]Division of Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

We recently identified microRNA-target pairs showing differential expression after TGFβ1 treatment of HK-2 human renal epithelial cells by combining microRNA and proteomic analysis. That analysis revealed that TGFβ1 treatment (3 ng/ml, 24 hours) increased miR-382 expression and suppressed protein expression of predicted miR-382 target Mn-superoxide dismutase (SOD2), an enzyme important for the protection against

mitochondrial oxidative stress. Here we validated these results and investigated the functional role of miR-382 interaction with SOD2 in the development of TGFβ1-induced loss of epithelial characteristics in HK-2 cells. Targeting of SOD2 transcript by miR-382 was confirmed by 3'-untranslated region reporter assay. Knockdown of miR-382 by anti-miR-382 transfection attenuated the loss of epithelial marker E-cadherin with TGFβ1 treatment. In addition, the reduction in SOD2 protein levels with TGFβ1 treatment was blunted by knockdown of miR-382. Overexpression of SOD2 significantly attenuated TGFβ1-induced reduction in E-cadherin. These results indicate that TGFβ1 up-regulates miR-382, which contributes to down-regulation of SOD2 and loss of renal epithelial characteristics. This novel mechanism might be relevant to the development of renal interstitial fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1170**

**MicroRNA miR-382 Contributes to the Development of Renal Interstitial Fibrosis** Alison J. Kriegel, Kristie Usa, Mingyu Liang. *Department of Physiology, Medical College of Wisconsin, Milwaukee, WI.*

We previously found an increase in miR-382 expression in cultured human renal epithelial cells treated with TGFβ1. In the current study we examined the in vivo role of miR-382 in the development of renal interstitial fibrosis. Exposure of CD-1 mice to 3 days of unilateral ureteral obstruction (UUO) increased miR-382 expression in the kidney by 363 ± 121% (vs. sham operated controls, $p<0.05$; n=6/group). Locked nucleic acid (LNA)-modified anti-miR-382 or scrambled anti-miR (10 mg/kg) was delivered by tail vein injection prior to UUO or sham surgery and repeated 24 hours following surgery (n=8-10/group). Fibrosis measurements were made in the cortex, outer medulla, and inner medulla of trichrome stained kidney sections after 3 days of UUO. UUO, again, produced a nearly 3 fold increase in miR-382 expression in mice receiving scrambled anti-miR. The anti-miR-382 treatment completely prevented this increase in miR-382. In scrambled anti-miR treated mice, UUO significantly increased the percent areas of fibrotic tissue in the outer medulla and inner medulla. Treatment with anti-miR-382 significantly reduced percent fibrotic areas in the inner medulla from 16.0 ± 1.3% in UUO mice treated with scrambled anti-miR to 10.2 ± 1.5% in UUO mice treated with anti-miR-382 ($P<0.05$), the latter no longer significantly different from sham animals receiving scrambled anti-miR (9.3 ± 2.0%). Moreover, anti-miR-382 reduced baseline miR-382 expression in sham animals by 46.5 ± 8.2% ($P<0.05$). Concomitantly, sham animals treated with anti-miR-382 had significantly less fibrosis in the cortex and outer medulla than animals receiving scrambled anti-miR (1.4 ± 0.1% vs. 2.1 ± 0.2% and 1.6 ± 0.1% vs. 2.9 ± 0.4 %, respectively; $p<0.05$ for each). These data indicate that miR-382 contributes importantly to the development of renal interstitial fibrosis in vivo.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1171**

**Administration of Adiponectin Retards the Progression of Diabetic Nephropathy in db/db Mice through Targeting the RAS** Xiaohua Guo,[1] Yufeng Huang,[1] Guangyu Zhou,[1] Alfred K. Cheung,[1,2] Srinivasan Beddhu.[1,2] *[1]Univ Utah; [2]VA, SLC, UT.*

Adiponectin is an anti-inflammatory adipokine and its deficiency in obesity is considered to play a major role in insulin resistance. However, some large epidemiological studies suggest that higher levels of serum adiponectin are associated with kidney disease progression. Because of these counter-intuitive data, we sought to examine whether administration of recombinant ADPN retards or accelerates the progression of glomerulosclerosis in the db/db mice uninephrectomized at week 8 (a model of type II diabetes where overt nephropathy is established at 20 weeks of age). Recombinant human ADPN was administered at a dose of 30μg or 150ug per day (ip, twice-daily) from week 19 for two consecutive week. Rosiglitazone (20mg/kg BW by gavage daily) served as therapeutic control. Untreated uninephrectomized db/db mice developed progressive albuminuria and glomerular mesangial matrix expansion associated with increased expression of TGFß1, PAI-1, type 1 collagen and fibronectin, compared with nondiabetic db/m mice. Although body weight and blood glucose were comparable in all diabetic mice, treatment with ADPN at either 30μg or 150μg prevented the increases in albuminuria and markers of renal fibrosis seen in db/db mice. The effect of ADPN on Ang II-related fibrogenic action in cultured glomerular mesangial cells (GMCs) was further investigated. Ang II (10-6M) increased the mRNA expression of TGFß1 by 3 fold ($P<0.01$) and PAI-1 by 2 fold ($P<0.01$) at 24h and increased the mRNA expression of collagen I by 2.3 fold ($p<0.01$) and fibronectin by 2.2 fold ($P<0.01$) in mouse GMCs. Interestingly, ADPN obliterated the stimulatory effect of Ang II on GMCs mRNA expression in a dose-dependent manner. These observations suggest that ADPN treatment halts glomerulosclerosis resulting from type 2 diabetes through targeting the renin-angiotensin-system. Thus, the therapeutic administration of ADPN has promise in preventing the progression of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1172**

**ARF6, a Novel Target for Renal Fibrosis, Mediates TGFβ-Induced Epithelial Mesenchymal Transition in Renal Tubular Epithelial Cells** Xiaohua Guo, Dean Y. Li. *Molecular Medicine Program, University of Utah, Salt Lake City, UT.*

Renal fibrosis is as a result of aberrant accumulation of extracellular matrix in kidney produced by myofibroblasts which mainly originate from renal tubular epithelial that undergo epithelial mesenchymal transition (EMT). ADP-ribosylation factor (ARF6), of the ARF family of small GTP-binding proteins, has been shown to participate in a wide variety of cellular events including regulation of membrane trafficking and actin cytoskeleton remodeling. In our experiment, we found markedly increase of ARF6 activity either in a mouse model of unilateral ureteral obstruction (UUO) induced renal fibrosis or in TGFβ induced EMT in renal tubular epithelial cells when compared with normal control group. Administration of inhibitor of Arf6 activity significantly attenuates extracellular matrix production in UUO mouse either Day 5 or Day 10, as well as myofibroblast markers αSMA and vimentin. Inhibitor of Arf6 activity also reverses EMT induced by TGFβ in renal tubular epithelial cell. Further evidence showed Arf6 plays a critical role in the breakdown of adherens junction, a key event during EMT process. The new finding indicating that inhibition of Arf6 activity is a promising strategy to treat renal fibrosis disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1173**

**IL-25 Promotes Th2 Response and Induces Alternatively Activated Macrophages in Protection of Renal Injury** Qi Cao,[1] Changqi Wang,[1] Dong Zheng,[1] Ya Wang,[1] Yuan Min Wang,[2] Vincent W. S. Lee,[1] Guoping Zheng,[1] Thian Kui Tan,[1] Stephen I. Alexander,[2] David C. Harris,[1] Yiping Wang.[1] *[1]Centre for Transplantation and Renal Research, Westmead Millennium Institute, Sydney, NSW, Australia; [2]Centre for Kidney Research, Children's Hospital at Westmead, Sydney, NSW, Australia.*

IL-25 is a recently described cytokine which has a role in regulation of inflammation in the gastrointestinal tract and autoimmune diseases. In the present study, the effects of IL-25 in chronic kidney disease were examined in a murine model of adriamycin nephropathy (AN). **Methods:** Murine AN was induced by 10 mg/kg adriamycin in BALB/c mice and 5.4 mg/kg in SCID mice. IL-25 was given by 7 consecutive daily injections starting at day 5 after adriamycin administration. **Results:** Kidneys from AN BALB/c mice administered with IL-25 had less glomerulosclerosis, tubular atrophy and interstitial expansion than did control AN BALB/c mice. AN mice infused with IL-25 also had significantly reduced proteinuria compared to AN alone mice. The possible mechanisms underlying the protective effect of IL-25 were examined. *In vivo*, IL-25 increased the levels of IL-4, IL-5 and IL-13 in serum and induced alternatively activated macrophages (M2) in the kidney. To determine whether the protective effect of IL-25 in AN BALB/c mice was Th2 response dependent or due to a direct effect on M2, IL-25 was further examined in AN SCID mice in which T and B cells were deficient. IL-25 failed to induce M2 or protect against renal structural and functional injury in AN SCID mice. **Conclusion:** IL-25 protected against renal injury in AN model. Its protective effects are dependent on induction of Th2 immune responses.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1174**

**Ex Vivo Modified Plasmacytoid Dendritic Cells but Not Bone Marrow Dendritic Cells Ameliorate Experimental Chronic Inflammatory Renal Disease** Dong Zheng, Qi Cao, Changqi Wang, Ya Wang, Vincent W. S. Lee, Guoping Zheng, Thian Kui Tan, Yiping Wang, David C. Harris. *Centre for Transplantation and Renal Research, The University of Sydney, Sydney, NSW, Australia.*

Plasmacytoid dendritic cells (pDC), which are regarded usually as being of lymphoid origin, display a regulatory role in autoimmune diseases and graft-versus-host disease. Dendritic cells (DC) from bone marrow (BM) have also been shown to induce immune tolerance and prevent rejection in transplantation. However, the effect of pDC and DC from BM in chronic kidney disease (CKD) is unknown. Our study aimed to explore the role of pDC and DC from BM in treating murine adriamycin (ADR) nephropathy (AN), and possible mechanisms involved in their protective effects.

pDC were separated from spleen of BALB/c mice using anti-mPDCA-1 magnetic microbeads (MACS) (naïve pDC) and stimulated with LPS (0.5μg/ml) for 24 hrs to become regulatory pDC. BM DC were prepared from BM, and cultured with GM-CSF and IL-10 for 7 days, followed by LPS for 24h. One million cells were transferred into each BALB/c mouse on day 5 after ADR administration. Renal function and histology were analysed quantitatively at 4 weeks. pDC localised to renal cortex, but BM DC did not appear in kidney. pDC, but not BM DC, reduced glomerulosclerosis (normal vs ADR vs naïve pDC vs regulatory pDC: 2.3±0.3 vs 28.0±4.6 vs 28.4±3.7 vs 12.6±1.8%, $p<0.01$), tubular atrophy (tubular cell height 7.34±0.38 vs 1.45±0.23 vs 1.11±0.29 vs 5.72±0.91μm, $p<0.05$), and interstitial expansion (interstitial volume 1.8±0.6 vs 26.8±1.1 vs 28.3±2.5 vs 12.6±1.0%, $p<0.01$). Transfusion with regulatory pDC also significantly reduced proteinuria and impairment of creatinine clearance. The mechanisms underlying the protective effects of modified pDC in AN involved their ability to transform naïve T cells into Foxp3+ Tregs (naïve pDC vs regulatory pDC 6.67±0.76% vs 18.69±2.52%, $p<0.01$) and to deactivate effector macrophages.

Modified pDC, but not BM DC protect against structural and functional injury in AN, providing a potential therapeutic strategy for CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1175**

**The Transcription Factor ETS-1 Mediates Angiotensin II Induced Fibronectin Transcription in Mesangial Cells Via Cooperative Interactions with CREB** Ping Hua,[1] Wenguang Feng,[1] Shaonin Ji,[1] Gabriel Rezonzew,[1] Edgar A. Jaimes.[1 2] *[1]Medicine, University of Alabama at Birmingham, Birmingham, AL; [2]VA Medical Center, Birmingham, AL.*

Angiotensin II (Ang II) plays a critical role in the pathogenesis of CKD in large part by increasing the glomerular deposition of extracellular matrix. We have previously shown that Ang II increases the expression of the transcription factor ETS-1 in mesangial cells (MC) and that ETS-1 mediates MC fibronectin (FN) expression (AJP'08) by directly binding and activating the fibronectin promoter (ASN' 09). In the current studies we further investigated the transcriptional regulation of FN by ETS-1 in rat MC. Mutation of two putative ETS-1 binding sites (M1 and M2) on the FN promoter fragment nt-943/+106 were generated by site-directed mutagenesis and ligated to reporter plasmid pGL3/basic. When pGL3/M1 or pGL3/M2 were co-transfected with a mouse ETS-1 expression vector into MC, the promoter activities decreased by 32.5% and 37.5% respectively in comparison with the WT construct pGL3/-943/+106. Double mutation of both M1 and M2 did not further decrease promoter activities. Mutation of a cAMP-responsive element binding site (CM) decreased promoter activity by 63.5% in comparison with the WT construct pGL3/-943/+106, thus indicating that cAMP-responsive element binding protein (CREB) is needed for FN promoter activity transducted by ETS-1. To better study the interaction between ETS-1 and CREB, we performed Co-IP and Electromobility Shift Assays (EMSA) in MC stimulated with Ang II ($10^{-7}$ M for 2 hours)**.** By Co-IP we determined direct binding of ETS-1 and CREB in nuclear protein extracts from MC treated with Ang II. By EMSA we determined that oligonucleotides covering either M2 or CM, but not M1, bind strongly to nuclear protein extracts from MC stimulated with Ang II. The specificity of the DNA/protein complexes was confirmed by supershift assay with an ETS-1 antibody (M2) and a CREB antibody (CM). In summary, we have identified two active ETS-1 binding sites on the rat FN promoter and have also established the role of CREB as a critical co-activator in the transcriptional regulation of FN by ETS-1 in MC.

Disclosure of Financial Relationships: nothing to disclose

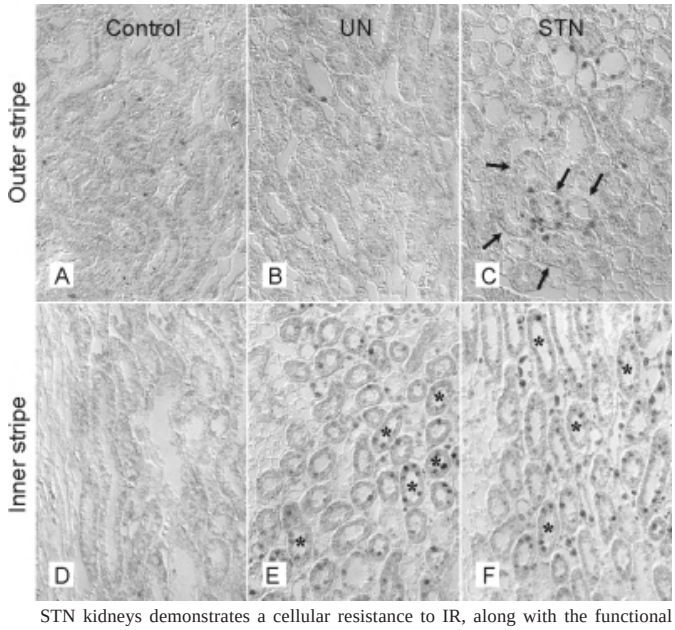
**F-PO1176**

**Pre-Conditioning by Hypoxia Inducible Factor in Subtotal Nephrectomy** Prabhleen Singh,[1] Christian Rosenberger,[2] Francis B. Gabbai,[1] Scott C. Thomson,[1] Roland C. Blantz.[1] *[1]UCSD&VASDHS; [2]Charité - Universitätsmedizin Berlin.*

We have reported an unexpected, functional resistance to ischemia reperfusion (IR) in rats after subtotal nephrectomy (STN) despite major reduction in renal mass and function (ASN 2009). After IR in STN, the GFR was lowered by only 25%, but in unilateral nephrectomy (UN) controls, a precipitous drop in GFR of 75% (p=0.0001) was observed. We have also reported that early hemodynamic adaptations in STN, such as lack of normal tubuloglomerular feedback (AJP 2009), which typically curtails the GFR after IR, may pre-condition the kidney against IR.

Presently, we examined the morphological and molecular components of this pre-conditioning response. We assessed tubular necrosis after IR by semiquantitative histological scoring, and found significantly lower scores for severe tubular necrosis in STN compared to UN (78 vs. 189, p=0.03).

We, then, examined the expression of hypoxia inducible factor (HIF) by immunohistochemistry, as prior activation of HIF is known to afford cellular protection against IR. In STN only, we observed clusters of HIF positive cells in the S3 segment of the proximal tubule (arrows in Figure 1C), the usual site of injury during IR. Medullary thick ascending limb cells (asterixes in Figure 1) were also positive for HIF with a modestly stronger signal in STN vs. UN. This pattern of HIF expression has never been reported. In further support, GFR was nearly preserved after IR in UN treated with cobalt chloride, a known HIF inducer (1.4 vs. 0.5 ml/min in untreated, p=0.0001).




STN kidneys demonstrates a cellular resistance to IR, along with the functional preservation. This suggests that molecular events in STN confer cellular protection beyond what can be afforded simply by the hemodynamic adaptations. An important role of HIF may be implicated in this pre-conditoning phenomenon naturally occuring in early STN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1177**

**Localization and Functional Role of Thioredoxin-Related Protein 14 (TRP14) in the Mouse Kidney** Sunjoo Park,[1] Su-Youn Lee,[2] Woojin Jeong,[1] Ki-Hwan Han,[2] Sue Goo Rhee.[1] *[1]Division of Life and Pharmaceutical Sciences, Ewha Womans University, Seoul, Korea; [2]Department of Anatomy, Ewha Womans University, Seoul, Korea.*

A novel 14 kDa thioredoxin-related protein (TRP14), which shares 20% sequence identity and 37% similarity with thioredoxin 1 (Trx1), is highly expressed in the kidney, but its exact localization and role have not been determined. To study the function of TRP14, we generated transgenic knockdown (KD) mice by siRNA. Kidney tissues were processed for immunohistochemistry and immunoblot analysis. TRP14 was expressed primarily in the proximal tubule and medullary collecting duct in the mouse kidney, showing similar expression patterns of Trx1. There were no detectable changes in renal function in young TRP14 KD mice. However, in old aged mice, genetic ablation of TRP14 resulted in tubular damages and significant elevation in serum BUN and creatinine. Electron microscopy demonstrated severe mitochondrial damage and accumulation of numerous autophagic vacuoles in the proximal tubule. Ratios of LC3 II to LC3 I and Atg5 expression increased in old TRP14 KD mice. These findings suggest that TRP14 may play an important role in protecting kidney during normal aging process.

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**F-PO1178**

**In Contrast to Healthy Bone Marrow, Uremic Bone Marrow Cannot Restore GFR in CKD** Arianne van Koppen, Jaap A. Joles, Marianne Christina Verhaar. *Nephrology & Hypertension, UMC, Utrecht, Netherlands.*

Healthy bone marrow derived cells (BMDC) confer functional protection in a rat model of CKD (ASN 2009). Hence we compared functional and structural effects of healthy and uremic BMDC in this model. In Lewis rats, CKD was induced by 5/6 nephrectomy (SNX), plus L-NNA, a NO-synthase inhibitor (20 mg/L for 9 wk), and 8% salt diet until sacrifice. Six wk after SNX, CKD rats received 50*106 BMDC (N=7) obtained from healthy eGFP transgenic Lewis rats, or 50*106 BMDC (N=7) obtained from uremic eGFP transgenic Lewis rats, or 0.5 ml vehicle (N=9) by single renal artery injection. CKD in donor $GFP^+$SNX and recipient SNX rats was confirmed by uremia (resp. 14±1 and 13±2 vs. 6±1 mmol/L in healthy controls) and hypertension (resp. 143±19 and 140±14 vs. 101±6 mm Hg). Plasma urea, systolic blood pressure (SBP) and 24h-proteinuria were assessed fortnightly. 14 wk after BMDC or vehicle, GFR (inulin) and effective renal plasma flow (RPF; PAH) were determined under barbiturate anesthesia. Renal injury was scored.

Both healthy and uremic BMDC reduced SBP, proteinuria, glomerulosclerosis and tubular atrophy compared to vehicle in CKD rats. However, uremic BMDC was not effective after 14 wk: GFR: 1.1±0.4 vs. 1.0±0.6 ml/min, RPF: 4.6±1.4 vs. 3.1±1.8 ml/min and renal blood flow: 7.7±2.5 vs. 5.3±3.1 ml/min were not significantly different. In contrast, healthy BMDC administration vs. vehicle was functionally effective in CKD

rats after 14 wk: GFR: 1.8±0.4 vs. 1.0±0.6 ml/min ($p<0.05$), RPF: 5.5±1.8 vs. 3.1±1.8 ml/min ($p<0.05$) and renal blood flow: 9.4±2.9 vs. 5.3±3.1 ml/min ($p<0.05$). Healthy but not uremic BMDC decreased tubular inflammation and fibrosis.

In sum, in CKD rats, intrarenal injection of healthy $eGFP^+$ BMDC, but not of uremic $eGFP^+$ BMDC, ameliorates loss of kidney function.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1179**

**Dysregulation of Hepatic β-Chain ATP Synthase, SRB-1 and PDZK1 in Nephrotic Syndrome: Contribution to Defective HDL-Mediated Reverse Lipid Transport** Hamid Moradi, Nosratola D. Vaziri. *Medicine/Nephrology, University of California, Irvine, Irvine, CA.*

Heavy proteinuria (nephrotic syndrome) is associated with profound dysregulation of lipid/lipoprotein metabolism, impaired HDL-mediated reverse cholesterol transport and a high risk of atherosclerosis. HDL serves as a vehicle for uptake and transport of surplus lipids from the peripheral tissues for disposal in the liver. The latter involves two important receptors: a – SRB-1 which serves as the HDL docking receptor enabling HDL to unload its lipid cargo in the liver and return to circulation to repeat the cycle; and b – β chain ATP synthase which serves as an HDL endocytic receptor mediating the removal and catabolism of lipid-poor HDL particles. Expression of SRB-1 is regulated by PDZK1, a multifunctional membrane-associated protein which promotes SRB-1 production at the post-translational level. Given the critical role in hepatic SRB-1, β chain ATP synthase and PDZK1 in HDL-mediated reverse lipid transport, we studied expression of these proteins in the liver of rats with chronic puromycin-induced nephrotic syndrome and normal control rats. The nephrotic animals exhibited severe proteinuria, hypoalbuminemia, hypercholesterolemia, hypertriglyceridemia, reduced HDL/total cholesterol ratio and normal glomerular filtration rate. This was accompanied by a significant upregulation of the endocytic HDL receptor, β chain of ATP synthase (+62%, $P = <0.01$). In contrast, SRB-1 abundance was significantly reduced (-50%, $P = <0.01$) in the liver of nephrotic animals confirming our earlier studies (Kidney Int 56:621-626, 1999), which showed marked reduction of SRB-1 protein abundance despite normal SRB-1 mRNA abundance. The reduction in SRB-1 protein abundance was accompanied by a parallel decline in PDZK1 abundance (-24%, $P=0.01$). Thus, nephrotic syndrome results in a rise in hepatic β chain ATP synthase and a decline in SRB-1 protein abundance. The latter is associated with and, in part, mediated by the reduction of PDZK1 abundance. Together, these abnormalities can increase catabolism and diminish recycling of HDL and contribute to the defective reverse cholesterol/lipid transport in nephrotic syndrome.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1180**

**Urinary HIFs and miRNA 29b as Biomarkers in a New Rat Fibrosis Model** Hua Zhou, Asada Leelahavanichkul, Xuzhen Hu, Takayuki Tsuji, Zachary Dezman, Lara Zafrani, Peter S. Yuen, Robert A. Star. *NIDDK, NIH, Bethesda, MD.*

Chronic tubulointestitial fibrosis, a histological finding of many forms of chronic kidney disease, initially progresses insidiously without significant renal dysfunction, as measured by creatinine/eGFR. Typically, rat folic acid models produce transient renal tubular damage and renal dysfunction without long-term sequelae. We developed a new rat model of chronic progressive renal fibrosis and searched for early urinary biomarkers for renal fibrosis. Sprague Dawley rats were injected with folic acid (FA, 400mg/kg, i.v.) to induce acute kidney injury (AKI), and 4 injections were given weekly starting at day 14 (post-AKI). Blood and urine were obtained at pre-, day 2, week 1, 2, 3, 4, 5, 7, 20, or 32; organs were harvested at pre-, day2, week 2, 7, 20, or 32. Serum creatinine (Scr) peaked (3.43±0.62 mg/dL at day 2, $p<0.01$) then returned to baseline (0.45±0.03 mg/dL at 2 weeks vs preinjection 0.43±0.03 mg/dL). Albumin to creatinine ratio (ACR) did not increase until 20 weeks (293±102 μg/mg vs. baseline 3.4±3.2 μg/mg); Scr increased 30% at 32 weeks in both FA-treated and age-matched controls (0.6 vs 0.45 mg/dL). Despite apparently normal values for conventional renal biomarkers, we could detect significant fibrosis in kidney, abdominal aorta, and heart at 7 wks by Masson's trichrome staining; kidney scarring was detected by microCT. Urinary exosomal HIF-1α and HIF-2α were undetectable in normal rats and increased in 5/6 FA rats; urinary exosomal miR29b decreased ~8-fold at 7 wks when Scr and ACR remained normal. Urinary microRNA-29b at 7 weeks correlated with the tubulointerstitial fibrosis score at at 7 weeks ($R^2=0.68$, $p=0.012$). We conclude that 1) multiple injections of folic acid can induce chronic progressive tubulointerstitial fibrosis in rats along with aortic and cardiac fibrosis; 2) urinary exosomal HIF-1α and HIF-2α increased and miR29b decreased in the early, silent phase of chronic tubulointerstitial fibrosis; 3) urinary HIFs and miR 29b are potentially useful biomarkers that can non-invasively diagnose chronic renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1181**

**Differential Activations of Unfolded Protein Response in Unilateral Ureteral Obstruction Renal Fibrosis Model** Chih-Kang Chiang,[1,2,3] Kuan-Yu Hung,[2] Shing-Hwa Liu.[3] *[1]Department of Integrated Diagnostics & Therapeutics, National Taiwan University Hospital; [2]Department of Internal Medicine, National Taiwan University Hospital; [3]Institute of Toxicology, School of Medicine, National Taiwan University, Taipei, Taiwan.*

The aims of this study are to investigate whether endoplasmic reticulum (ER) stress involved in renal tubular cell loss and renal interstitial fibrosis by unilateral ureteral obstruction (UUO) rat model. Either candesartan (25 mg/kg/d) or vehicle alone was orally administered to male wistar rats from day 1 after unilateral ureteropelvical ligation was performed. As our hypothesis, ureteral ligation induced 3 times of GRP78 protein expression (an indicator of GRP78) within 24 hours, and then was maintained until day 14. The three ER stress branches (Inositol-requiring 1,IRE1; PKR-like ER kinase, PERK; and transcription factor 6, ATF6) were activated in response to UUO stress. We found that IRE1-splicing XBP-1 activities were attenuated by persistent ER stress induced by UUO. By contrast, PERK signaling, including translational inhibition and proapoptotic transcription regulator C/EBP homologous protein (CHOP) induction, was maintained. These results suggest a causal link between the differential expression of UPR branch signaling leading to tubular cell apoptosis and development of interstitial fibrosis. Candesartan-treated UUO rats showed less tubulointerstitial apoptosis and renal fibrosis. In contrast to vehicle-treated UUO kidney, candesartan successfully maintained the expression of splicing XBP1 and attenuated ATF4, splicing ATF6 and CHOP1 protein expression. Procaspase 12, an ER stress-related apoptosis signal, was also abolished by candesartan treatment. Base on these findings, we suggested that candesartan ameliorates renal apoptosis and fibrosis induced by UUO model is associated with the differential activation of the ER stress signaling.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1182**

**PI3K and Smad3 Mediate Murine Renal Fibrosis through Distinct Mechanisms** Gal Finer,[1] Tomoko Hayashida,[2] Yashpal S. Kanwar,[2] Leileata M. Russo,[3] Herbert Y. Lin,[3] H. William Schnaper.[2] *[1]Pediatrics, Children's Memorial Hospital, Northwestern University, Chicago, IL; [2]Pediatrics, Northwestern University, Feinberg School of Medicine, Chicago, IL; [3]Massachusetts General Hospital, Harvard Medical School.*

TGF-β plays a central role in renal fibrogenesis. We previously showed in vitro that PI3K and TGF-β-stimulated Smad3 activity lead to collagen I expression; this work is aimed to extend these findings to an in-vivo model. Balb/c mice treated with ADR (0.15 mg, iv) showed massive albuminuria and advanced global glomerulosclerosis, as described by others. Last year we showed that PI3K is activated in this model and its inhibition by LY294002 (25mg/kg, i.p, 2x/week) prevents ADR-induced fibrotic changes. Further work implicated PI3K p110 γ catalytic unit in this model, with increased mRNA expression of the isoform in ADR-treated kidneys and in cultured mouse podocytes treated with TGF-β. Here, we report studies in a mouse strain that more closely models human focal sclerosis/hyalinosis (FSGS) and implicates TGF-β/Smad signaling. ADR-treated 129/sj mice manifested nephrotic syndrome including albuminuria, hypoalbuminemia and hypercholesterolemia, and elevation of serum creatinine and BUN starting at fifth day after ADR administration. Histological changes, including segmental sclerosis and crescent formation, were more consistent with FSGS than those in Balb/c. PI3K activation was similar in the two strains. In ADR-treated 129/sj mice, Smad3 phosphorylation and nuclear localization shown by immunostaining, implicated TGF-β. A TGF-β inhibitor, soluble TGF-β type II receptor (sTβRII.Fc), ameliorated Smad3 activation and protected 129/sj mice from ADR-induced glomerular changes. Inhibition of TGF-β signaling diminished collagen I, αSMA and fibronectin mRNA expression, without effecting albuminuria. Thus, ADR-induced nephropathy in 129/sj mice shows features similar to human FSGS, and can be used to dissect disease mediated signaling mechnisms. PI3K plays a role in fibrogenesis and proteinuria whereas TGF-β is implicated only in extracelluar matrix accumulation, suggesting distinct mechanisms for proteinuria and fibrogenesis in kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1183**

**Association of Proteinuria with Urinary Concentration Defect in Puromycin Aminonucleoside Nephrosis** Gheun-Ho Kim, Sua Kim, Chor Ho Jo, Joon-Sung Park, Chang Hwa Lee. *Department of Internal Medicine, Hanyang University College of Medicine, Seoul, Republic of Korea.*

**Purpose**: Puromycin aminonucleoside (PA) can induce nephrotic syndrome in rats. In glomerular disease, proteinuria is known to be an important mediator of tubulointerstitial injury. Thus, it is conceivable that glomerular proteinuria may affect tubular function such as urinary concentration. We investigated whether urinary concentration defect is associated with proteinuria in PA-induced nephrosis and whether it is relieved by angiotensin converting enzyme (ACE) inhibition.

**Methods**: Glomerular proteinuria was induced by a single intraperitoneal injection of PA (150 mg/kg BW) in male Sprague-Dawley rats. In a half of them, enalapril (35 mg/kg BW) was daily given in food mixture for two weeks. After the animal experiment, kidneys were harvested for immunoblot analysis and histopathologic examination.

**Results**: Compared with controls (n=4), PA-treated rats (n=8) had overt proteinuria, polyuria and a lower urine osmolality. PA treatment induced remarkable tubulointerstitial injury and significant reductions in protein abundances of aquaporin-1 and Na-K-2Cl

cotransporter type 2 (NKCC2). Proteinuria was significantly correlated with osteopontin expression in the kidney (r=0.79, P<0.01), and inversely correlated with renal expression of aquaporin-1 (r=-0.85, P<0.01), aquaporin-2 (r=-0.79, P<0.01) and NKCC2 (r=-0.67, P<0.05) as well. The degree of tubulointerstitial injury was significantly correlated with proteinuria (r=0.90, P<0.01), urine output (r=0.67, P<0.05), and osteopontin expression (r=0.73, P<0.01), and inversely correlated with urine osmolality (r=- 0.77, P<0.01) and renal expression of aquaporin-1 (r=- 0.81, P<0.01), aquaporin-2 (r=- 0.86, P< 0.01) and NKCC2 (r= - 0.78, P<0.01). No differences in parameters between PA-treated rats with and without ACE inhibition were found probably because of large individual variations.

**Conclusion**: In acute PA-induced nephrosis, glomerular proteinuria was associated with tubulointerstitial injury and urinary concentration defect. Altered expression of osteopontin, aquaporin-1, aquaporin-2 and NKCC2 may be involved in this glomerulotubular connection.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1184**

**Aggravated Renal Tubular Damage and Interstitial Fibrosis in Mice Lacking Guanylyl Cyclase-A (GC-A), a Receptor for Atrial and B-Type Natriuretic Peptides** Fumiki Yoshihara,[1] Takeshi Tokudome,[1] Ichiro Kishimoto,[2] Takeshi Horio,[1] Yuhei Kawano.[1] *[1]Division of Hypertension and Nephrology, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan; [2]Division of Endocrinology and Metabolism, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan.*

Background: Chronic angiotensin II (Ang II) infusion causes to an increase of renal interstitial fibrosis. Aim: We infused Ang II (1.0 mg/kg/day) in GC-A-deficient mice (GC-A-KO), to evaluate the pathophysiological significance of natriuretic peptides-GC-A system. Methods and results: We created 5 groups (Wild-Saline: n=12, Wild-Ang II: n=14, GC-A-KO-Saline: n=11, GC-A-KO-Ang II: n=13, GC-A-KO-Ang II-Hydralazine: n=10). Saline or Ang II were infused subcutaneously using osmotic minipump for 3 weeks. Hydralazine was administered orally (0.05 g/L in drinking water). Systolic blood pressure in GC-A-KO-Saline (130±12 mmHg) was significantly higher than that in Wild-Saline (105±30 mmHg), and was comparable to Wild-Ang II (141±17 mmHg) and GC-A-KO-Ang II-Hydralazine (140±20 mmHg). Systolic blood pressure in GC-A-KO-Ang II (159±21 mmHg) was significantly higher than the other 4 groups. The severity of renal tubular atrophy and interstitial fibrosis were significantly aggravated in GC-A-KO-Ang II (atrophy: 13.4%, fibrosis: 12.0%) compared to Wild-Saline (0%, 2.0%), Wild-Ang II (2.9%, 4.4%) and GC-A-KO-Saline (0%, 2.6%). Hydralazine could not inhibit the aggravation (GC-A-KO-Ang II-Hydralazine: 13.5%, 11.3%). Ang II infusion promoted the immunohistochemical staining intensities of monocyte chemoattractant protein-1 in medullary collecting duct cells and of F4/80 and alpha-smooth muscle actin in the interstitium, whereas Ang II infusion reduced the staining of E-cadherin in atrophic renal tubular cells. Conclusions: Natriuretic peptides-GC-A system might play inhibitory roles for Ang II-induced renal tubular atrophy, interstitial fibrosis, and phenotypic transformation in renal tubular cells and fibroblasts.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1185**

**Role of Glomerular Proteoglycans in IgA Nephropathy** Kerstin Ebefors,[1] Anna Granqvist,[1] Madeleine Ingelsten,[1] Johan C. Molne,[2] Jenny C. Nystrom,[1] Borje Haraldsson.[1] *[1]Clinical and Molecular Medicine, University of Gothenburg, Gothenburg, Sweden; [2]Pathology, University of Gothenburg, Gothenburg, Sweden.*

**Purpose:** IgA nephropathy (IgAN) is the most common glomerulonephritis, still the mechanisms behind the disease are relatively unknown. To find molecular markers and improve the understanding of the disease, the expression of proteoglycans was investigated in biopsies from patients with IgAN and correlated to clinical data. Proteoglycans are negatively charged matrix molecules also acting as binding sites and signaling molecules.

**Methods:** We collected and microdissected biopsies from patients with IgAN (n=19) and from healthy kidney donors (n=14). Gene expression of proteoglycans and related genes were separately investigated in glomerular and tubular parts. Paraffin embedded sections of the biopsies were used for protein staining. Gene expression levels were correlated to morphological and clinical data.

**Results:** Patients were followed for an average time of 4 years and blood pressure was according to target guidelines. Three of the proteoglycans investigated were found to be of special interest and upregulated in glomeruli; perlecan (x1.9, p<0.01), decorin (x1.4, p<0.05) and biglycan (x2.4,p<0.001). Perlecan gene expression negatively correlated to albumin excretion (r=-0.58, p<0.05) and progress (r=-0.52, p<0.05) of the disease. Abundant decorin protein expression was found in sclerotic glomeruli, but not in normal glomeruli from IgAN patients or controls. Also, the decorin and biglycan-binding protein, transforming growth factor beta (TGF-β) was upregulated in IgAN both on gene (x2.5, p<0.001) and protein (p<0.05) level.

The tubular gene expression differed from the glomerular compartment, with a down regulation of perlecan (x0.6, p<0.01), and unchanged expression of decorin, biglycan and TGF-β.

**Conclusion**: We found that a high perlecan expression correlates to a slow progress of the disease, and may thus be used as a predictor for patient outcome. Also, the upregulation of the TGF-β associated proteoglycans biglycan and decorin, as well as TGF-β itself, in IgAN patients indicate that regulation of TGF-β, and other profibrotic markers may play a role in IgAN pathology.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1186**

**Clearance of Renal IgA1 Deposits Using a Bacterial Protease: A Treatment Option under Investigation for IgA-Nephropathy** Elaine Phan, Shinong Long, Rajeev Mahimkar, Amanda N. Kovach, Andreas Taylor, Sherron Bullens, Paul A. Fitzpatrick. *BioMarin Pharmaceutical Inc., Novato, CA.*

This poster describes the development of a mouse model for IgA Nephropathy (IgAN) using human IgA1 as well as ability of the *H. Influenzae* IgA1 protease to clear IgA1 deposits in the model.

Previous studies by Hiki *et. al.* (1999), showed that IgAN patients have aberrantly undergalactosylated IgA1. High levels of this aberrant IgA1, both circulating freely and in immune complexes, lead to deposition and accumulation within the kidney glomerulus. Accumulation is causally linked to pathological development including hematuria, proteinuria and progression to end stage renal failure.

The mouse model was developed by injecting different doses of human IgA1 and assessing the renal deposition. IgA1 from plasma of normal human subjects was purified to near homogeneity by a series of enrichment and chromatographic steps including ammonium sulfate precipitation and by column chromatography including ion exchange, Jacalin lectin and size exclusion. Purified normal IgA1 was modified with specific deglycosidases to confer properties similar to undergalactosylated IgA1 (degly-IgA1). The mouse model developed renal IgA1 deposits.

The pathogenic bacterium, *H. Influenzae*, expresses an endogenous IgA1 protease that is specific to human IgA1 but is not suited for large scale IgA1 protease production. A recombinant form of IgA1 protease was produced in *E. coli* as inactive and insoluble inclusion bodies (IBs). The IBs were solubilized by 8M urea and refolded by dilution into an arginine-containing buffer. The refolded recombinant protein was purified by metal chelating chromatography, ion exchange chromatography, and size exclusion chromatography.

The IgA1 protease produced in *E. coli* was tested for its ability to clear renal IgA1 deposits in the mouse model. A two week study was conducted to treat the degly-IgA1 deposits in mice by injecting them with IgA1 protease intravenously, resulting in clearance of the degly-IgA1 deposits. Thus, the mouse IgAN model and clearance of deposits via the IgA1-Protease provide a platform for development of protease therapeutics for treatment of IgA Nephropathy.

Disclosure of Financial Relationships: Employer: BioMarin Pharmaceutical Inc.; Ownership: BioMarin Pharmaceutical Inc.

**F-PO1187**

**Renal Klotho Expression May Be Modulated by Recombinant Human Erythropoietin in Experimental Chronic Kidney Disease** Hidekazu Sugiura, Takumi Yoshida, Junko Kohei, Shunji Shiohira, Michihiro Mitobe, Ken Tsuchiya, Kosaku Nitta. *Fourth Department of Internal Medicine, Tokyo Women's Medical University, Tokyo, Japan.*

Erythropoietin (EPO) and Klotho expression have both been detected in the kidney. Klotho is well known as an anti-aging gene, which protein is essential for the functioning of endogenous fibroblast growth factor 23. Since a recent study suggested that both erythropoietin and Klotho mitigate kidney damage, we explored the relation between erythropoietin and Klotho in a doxorubicin hydrochloride (DXR)-induced rat nephropathy model treated with recombinant human erythropoietin (rhEPO). Male Sprague-Dawley (SD) rats were subjected to DXR-induced nephropathy. The rhEPO group was intracutaneously injected with rhEPO twice weekly at 4 to 16 weeks after the DXR injection. On the other hand, to examine the direct effects of rhEPO on Klotho expression, SD rats were pretreated with rhEPO (200 U/kg s.c. three times every other day). Expression of renal Klotho and heat shock protein 70 (HSP70) were assessed using real-time PCR or Western blotting. The hematocrit, serum creatinine (SCr) and phosphate (iP) levels were also determined. Histological assessment was performed by immunohistochemical and Masson-trichrome staining. Renal Klotho expressions was significantly reduced (0.5-fold, p<0.05) in the DXR nephropathy group. Treatment with rhEPO improved the SCr, iP level and histological changes observed in the DXR nephropathy group. The reduction in Klotho expression induced by DXR nephropathy was mitigated by rhEPO administration. Normal rats with rhEPO treatment showed slight but significant increase in Klotho and HSP70 (1.2-fold, p<0.05), which was heat shock protein family and could be expected anti-oxidant activity, nevertheless the levels of hematocrit, hemoglobin, SCr, iP were not significantly alternated in this group. The precise mechanism of cross-talk between Klotho and EPO could not be clarified in this study, however, both of them, being expressed in the renal interstitium, may have interaction between them. In conclusion, favorable effects of rhEPO against renal damage are likely, in part, mediated via the expession of Klotho.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1188**

**Detection of Calcineurin Expression in Podocytes of Patients with Membranous Nephropathy** Zhao-Hong Chen, Yongchun Ge, Xi Tang, Ming-Chao Zhang, Xiao-Dong Zhu, Ke Zuo, Chun-Xia Zheng, Cai-Hong Zeng, Zhi-Hong Liu. *Research Insitute of Nephrology, Jinling Hospital, Nanjing University of Medcine, Nanjing, Jiangsu, China.*

Calcineurin activity relates with the regulation of podocyte cytoskeleton stability and the development of proteinuria. It is interesting to know the expression of calcineurin in podocyte of patients with membranous nephropathy (MN) and its change after treatment with calcineurin inhibitor (CNI). 49 patients with idiopathic MN were recruited. Repeated renal biopsy tests were performed in 2 idiopathic MN patients with complete remission after tacrolimus treatment. Immunohistochemistry was performed to detect the expression

of calcineurin A alpha subunit (CnAα) and synaptopodin in renal tissue. Furthermore, the expression of CnAα was compared between patients with idiopathic MN, type V lupus nephritis and hepatitis B virus associated MN. The results showed that increased expression of CnAα in podocytes were observed in 20 idiopathic MN patients (40.8%), accompanied by decreased expression and abnormal distribution of synaptopodin. Tacrolimus treatment results in the significant decrease of CnAα expression, with improved expression of synaptopodin in the podocytes. No podocyte CnAα expression was observed in patients with hepatitis B virus associated MN, but the positive rate for CnAα expression was similar between patients with type V lupus nephritis and those with idiopathic MN. Therefore, detection of podocyte calcineurin expression may be used as a diagnostic test to evaluate the efficacy of CNI in the treatment of patients with MN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1189**

**Albumin Overload Impairs Autophagy in Proximal Tubular Cell** Raymond Yan, Jonathan M. Gall, Zhiyong Wang, Steven C. Borkan, John H. Schwartz, Andrea Havasi. *Renal Section, Boston University Medical Center, Boston, MA.*

Proteinuria predicts progressive chronic kidney disease (CKD). Exposure of proximal tubular epithelial cells (PTEC) to albumin promotes tubular atrophy and fibrosis but the mechanism(s) of albumin toxicity have yet to be elucidated. We hypothesize that exposing PTEC to albumin inhibits autophagy, a critical cellular function responsible for turnover of macromolecules and organelles, including dysfunctional mitochondria. Autophagy inhibition is a potential cause of cell stress, tubular dysfunction and atrophy. To test this hypothesis, autophagy was measured in cultured mouse tubular cells exposed to albumin. LC3, an established autophagy protein marker, undergoes LC3-I to LC3-II conversion during autophagy induction and becomes tightly membrane-bound to autophagosomes. To assess autophagy LC3-II content was detected by immunoblot and autophagosomes (AP) were visualized in cells expressing an LC3-GFP fusion protein. Autophagic flux was measured in the presence of bafilomycin, an inhibitor that prevents lysosomal LC3-II degradation. In mouse tubular cells, albumin exposure at concentrations of 0.5-25 mg/ml decreased LC3-II content in a concentration-dependent manner. Similar results were obtained using recombinant human albumin or bovine albumin, and either normal media (basal autophagy) or starvation media (induced autophagy). In the presence of bafilomycin, increasing albumin concentrations caused a stepwise decrease in autophagic flux. In addition, by immunofluorescence, albumin treatment decreased the number of GFP positive punctate structures that represent AP. A few larger GFP positive structures were observed in albumin-treated cells, especially over the first 24 hr, raising the possibility that albumin inhibits fusion between AP and lysosomes.

Taken together, these data show that albumin exposure inhibits PTEC autophagy. As a result, we propose that inhibition of autophagy contributes to CKD progression in proteinuric states.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1190**

**A Functional Local Vitamin D System in Human Podocytes: Target for Anti-Proteinuric Therapy?** Katarina Mirkovic, Carolina R. C. Doorenbos, Jacob Van den Born, Wendy Dam, Gerjan Navis, Martin H. De Borst. *Department of Nephrology, University Medical Center Groningen, Groningen, Netherlands.*

**Background:**

Podocytes play a main role in glomerular permselectivity. In patients with chronic kidney disease the synthetic vitamin D receptor agonist paricalcitol has an additional anti-proteinuric effect when given on top of RAAS blockade, but its mechanism is not fully understood. To investigate whether podocytes could be a target for the antiproteinuric effect of vitamin D agonists, we characterized the local vitamin D system in cultured human podocytes.

**Methods:**

In human podocytes from a conditionally immortalized podocyte cell line, the presence of a local vitamin D system was investigated by measuring vitamin D receptor (*VDR*), 1α-hydroxylase and 24-hydroxylase (*CYP24A1*) mRNA expression. Cells were treated with increasing doses of calcidiol, calcitriol or paricalcitol (5 nM to 500 nM) or vehicle, and *VDR* activation (defined as *CYP24A1* gene expression) was determined by qPCR.

**Results:**

Human podocytes expressed *VDR*, 1α-hydroxylase and *CYP24A1* mRNA under basal conditions. Calcitriol dose-dependently induced *VDR* activation, with 100 nM inducing *VDR* activation already at 1 h (5-fold vs vehicle), reaching a plateau at 12 h (33-fold, $p<0.05$ vs vehicle) after stimulation. Similarly, paricalcitol dose-dependently induced *VDR* activation up to 10-fold ($p<0.05$) at 100 nM. Of interest, calcidiol (100 nM) also induced *VDR* activation strongly during the first 6 h after stimulation (up to 30-fold), but a rapid decline was observed thereafter.

**Conclusion:**

Our results support the presence of a functional local vitamin D system in human podocytes. Similar to many extrarenal cell types, also podocytes express 1α-hydroxylase. *VDR* activation by calcidiol suggests local (autocrine/paracrine) conversion to calcitriol or direct *VDR* activation by calcidiol in cultured human podocytes. The podocyte may be a target for direct anti-proteinuric effects by vitamin D analogues.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1191**

**A Dynamic Retinoic Acid, Wnt and Hedgehog Signalling Network in Experimental Renal Chronic Damage** Christine Von Toerne,[1] Peter J. Nelson,[1] Elisabeth Groene,[2] Hermann-Josef Groene.[2] *[1]Department for Biological Chemistry, University Hospital LMU Munich, München, Germany; [2]Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Germany.*

Chronic renal allograft damage (CAD) is a common feature in transplanted kidneys. CAD is manifested by a smouldering inflammation leading to transplant glomerulopathy, diffuse interstitial fibrosis and tubular atrophy. In this regard, CAD can also be seen as a general model for chronic renal failure. Retinoic acid (RA)is an important regulator of differentiation during vertebrate embryogenesis; it can moderate the damage observed in experimental models of CAD (Adams J. et al. Am J Pathol. 167:285-98, 2005). The Wnt and Hedgehog (Hh) signalling pathways are also relevant for tissue homeostasis and have been shown to interact during embryogenesis. Using a Fischer 344 (RT1lvl) to Lewis (RT1l) rat renal allograft model with analogies to human CAD (Kiss E. Am J Pathol. 176:2150-62, 2010) and enhanced microarray analysis linked to a systems biology approach, we show that Wnt and Hedgehog pathway signalling is dysregulated during CAD where subsets of Hh/Wnt modules can be linked to the pathophysiology of progressive fibrosis, loss of cilia in epithelia and chronic dysfunction. Nuclear accumulation of β catenin protein also strenghthened the finding of the array analysis of canonical Wnt pathway activation in CAD. Amongst other critical components of the Hh pathway, the receptor Ptch1, an indicator for Hh pathway induction was increased with CAD. Nuclear receptors such as the RAR can bind β catenin, compete with TCF/LEF1 for DNA binding and help regulate activation of Wnt target genes.

Treatment with 13cis-retinoic acid selectively ameliorated the dysregulation of the Hh and canonical Wnt pathways associated with CAD and preserved cilia structures. We could verify direct effects of RAR activation on tubular epithelial cells by showing effects of 13CRA on mRNA expression of Ihh, Shh, Gli1, Ptch1, Lrp2, Bmp7, Wnt6 and Wnt7a genes. Thus, the interplay between these pathways helps explain the therapeutic effects of retinoic acid treatment in CAD and may suggest future directions for moderating chronic fibrosing renal damage.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1192**

**Improvement in Diabetes Care and Immunization Rates with a Disease Management Program in End-Stage Renal Disease Patients** Tami Deeb, Deborah Murray, Jess Parks, Allen R. Nissenson. *DaVita Inc., Denver, CO.*

Introduction: Both infections and complications from diabetes lead to poor outcomes in ESRD patients. Disease management (DM) programs may be effective ways to administer vaccinations to prevent pulmonary infections and improve diabetes care. We evaluated the impact of a DM program on pneumococcal and influenza vaccine rates and diabetes processes of care.

Methods: The ESRD DM Demonstration, implemented by CMS, studied the impact of DM by DM organizations (DMO) in Medicare Advantage ESRD patients on clinical, patient-centered outcomes. The impact of one participating DMO, VillageHealth (VH), is described. Data from Medicare ESRD pts who voluntarily enrolled in VH were compared to fee-for-service (FFS) data from the 2008 and 2009 United States Renal Data System (USRDS) Annual Data Report.

Results: From 2006-2009, 894 patients enrolled in VH. Immunization rates increased after program initiation, rising to a 95% annual influenza vaccination rate in 2009. For pneumococcal vaccination, the 2007 VH rate exceeded the USRDS FFS comparison of 22% for 2006-2007. In 2009, the pneumococcal vaccination rate was 97%.

Care for diabetic patients was also examined. In 2009, 61% of VH diabetic patients received retinal exams compared to 47% of FFS USRDS patients. Low density lipoprotein levels were tested in 78% of diabetic VH patients compared to 73% of FFS USRDS patients in 2009. Foot exams were received by 98% of diabetic VH patients. Compared to available USRDS FFS comparison rates, 71% of VH pts received ≥4 HbA1c tests in 2009, well above the 60% reported in the FFS population in 2009.

Conclusions: Within the VH program, DM leads to increased rates of influenza and pneumococcal immunizations and improved diabetes care processes. In both categories, the VH DM program exceeded rates in the FFS.

Disclosure of Financial Relationships: Employer: DaVita Inc.

**F-PO1193**

**Characterizing Pre-ESRD Care in the Era of eGFR Reporting** Khaled Abdel-Kader,[1] Gary S. Fischer,[2] James R. Johnston,[1] Chen Gu,[2] Charity G. Moore,[2] Mark L. Unruh.[1] *[1]Renal-Electrolyte Division, University of Pittsburgh, Pittsburgh, PA; [2]Department of General Internal Medicine, University of Pittsburgh, Pittsburgh, PA.*

**Background:** PCPs care for the majority of pre-dialysis CKD patients; however, they often fail to recognize the presence of CKD based on serum creatinine levels. One strategy to improve disease awareness and perhaps treatment is eGFR reporting. We examined practices in PCP-managed and co-managed patients before and after routine eGFR reporting.

**Methods:** We conducted a retrospective cohort study of patients with CKD 3b-4 seen at a university-based, outpatient general internal medicine clinic. We compared co-management rates, CKD documentation, renal protective strategies, and laboratory monitoring in 274 and 266 patients seen in a 6-month period prior to and following eGFR implementation, respectively.

**Results:** Pre-eGFR patients were less likely to be men (29% vs. 39%, P=0.02), black (24% vs. 38%, P<0.001), or have HTN documented at baseline (63% vs. 80%, P<0.001) than post-eGFR patients. Following eGFR use, the prevalence of co-management increased from 23% to 49% (P<0.001). In addition, CKD documentation, NSAID avoidance, and annual monitoring of phosphorus and PTH improved following eGFR use.

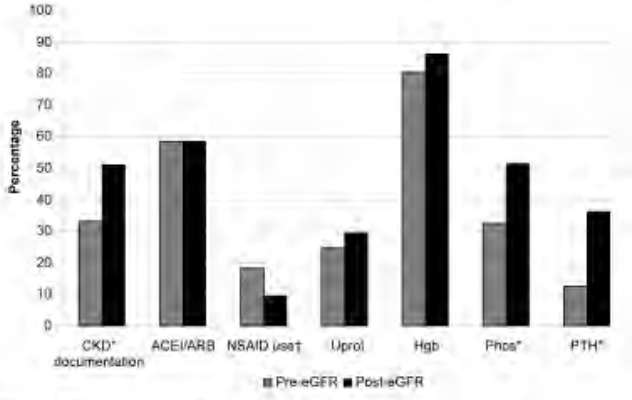



**Figure 1.** Processes of care in CKD 3b-4 patients.
Uprot: annual quantitative urinary albumin/protein testing.
Hgb/Phos/PTH: annual monitoring of respective lab parameter
*P<0.001, † P=0.003.
Pre-eGFR N=274, Post-eGFR N=266.

However, ACEI/ARB use, quantitative urinary protein testing, and Hgb monitoring were unchanged. Despite improvements following eGFR reporting, PCP-managed patients still had significantly lower rates of CKD documentation (31% vs. 81%, P<0.001), NSAID avoidance (86% vs. 97%, P=0.003), and Hgb (81% vs. 94%, P=0.001), Phos (33% vs. 79%, P<0.001), and PTH (17% vs. 65%, P<0.001) monitoring than co-managed patients.

**Conclusions:** Although CKD co-management increased and some CKD processes of care improved following eGFR implementation, overall care remained suboptimal. Further strategies are needed to improve PCP and nephrologist CKD care.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1194

**Ten-Site Implementation of the Advanced Chronic Kidney Disease Patient Management Toolkit (ACPMT)** Uptal D. Patel,[1] Jameta N. Barlow,[1] William E. Haley.[2] *[1]Duke Univ, Durham, NC; [2]Mayo Clinic, Jacksonville, FL.*

**Background:** Appropriate management of advanced CKD improves patient outcomes, however, care is often suboptimal. We implemented the Renal Physicians Association's ACPMT in 10 community nephrology practices and evaluated whether adherence to clinical practice guidelines for patients with advanced CKD improved.

**Methods:** Ten willing US sites were selected after stratifying by practice characteristics. Patients with advanced CKD (eGFR 15-30 ml/min) were identified and random samples of 30 charts were abstracted at each site before and after implementation by an independent abstractor. ACPMT implementation was supported by site visits and monthly conference calls with site champions over the 6 month intervention period. Differences in adherence to guidelines between pre- and post-implementation periods were estimated using chi-squared tests.

**Results:** Study sites varied according to practice characteristics, patient compositions, and baseline conformance to practice guidelines. Quantitative analyses demonstrated relatively high (>75%) baseline conformance to routine monitoring guidelines within categories of anemia, metabolic bone disease, hypertension, and nutrition. However, baseline assessment of any abnormalities present (60-80%) and management with treatments (40-60%) were somewhat lower. Documentation for counseling (eg, nutrition, social work, others) and renal replacement preparatory activities (eg, referral for transplant evaluation, vein preservation, vascular access referral, others) was low (<20-40%). Although conformance to various guidelines changed for some measures (increased [n= 2], decreased [n= 4]), most remain unchanged (n= 19) perhaps due to ceiling effects, especially for monitoring guidelines. Median use of tools within the ACPMT was high (13 of 16 available tools), but varied across sites (range, 9-14).

**Conclusion:** There was limited impact on adherence to clinical practice guidelines for patients with advanced CKD during a 6 month implementation of the ACPMT. Consistent tool usage for a longer duration may be required to improve the management of advanced CKD.

Disclosure of Financial Relationships: Research Funding: Merck & Co, Co-Investigator

Renal Physicians Association, Co-Investigator; Scientific Advisor: National Kidney Foundation, Kidney Learning System, Editorial Board; National Kidney Foundation of North Carolina, Medical Advisory Board.

## F-PO1195

**Use of NSAIDS and Awareness of CKD in Patients of Internal Medicine Residency Clinic** Devathi Sreedhar, John E. Prior. *Internal Medicine, Scranton Temple Residency, Scranton, PA.*

Background : Many patients are unaware that they have CKD. In these patients the use of over the counter drugs like NSAIDS is associated with drug related toxicity. It is shown previously the use of NSAIDS is highly prevalent in CKD patients. The aim of this study is to survey the awareness of CKD and the use of NSAIDS in our patients cared for in our internal medicine clinic.

Methods: Patients with CKD (GFR < 60 ) who visited the clinics in a period of of one year ( n=100 ) were identified by EMR and then were interviewed over the telephone to survey their awareness of CKD, education of CKD and to document their use of NSAIDS.

Results: Overall, 60% of the patients with a GFR of <60 were aware of they had CKD, 60% of the patients stated that they were educated by their physicians and 1% of the patients of CKD reported using NSAIDS. Conclusion : In contrast to the results reported by the prior studies, our patients showed a greater awarenss of their diagnosis of CKD ( 60% ) than reported in the literature ( 10% ). This awareness was associated with less use of potentially nephrotoxic drugs such as NSAIDS. The use of EMR, lab reporting of eGFR and education of patients all likely had a positive impact on patients' CKD awareness

Disclosure of Financial Relationships: nothing to disclose

## F-PO1196

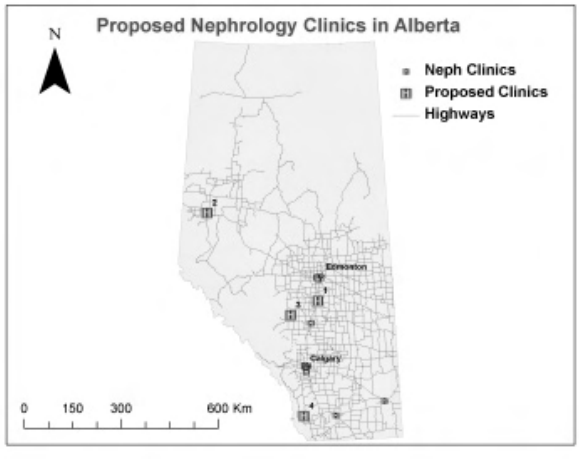
**GIS Techniques To Optimize Locations of New Nephrology Clinics for Remote Areas** Bharati Ayyalasomayajula,[1] Brenda Hemmelgarn,[2] Natasha Wiebe,[1] Marcello Tonelli.[1] *[1]Medicine, U. Alberta; [2]U. Calgary.*

**Background**: The province of Alberta, Canada has a geographic area of 660,000 km$^2$ but only 15 nephrology clinics, which are chiefly located in major cities. Although most CKD patients with eGFR<45 mL/min/1.73m$^2$ live within 30 min (by road) of the closest clinic, a substantial proportion live >2 hours away, which may compromise their care. Given limited resources, a systematic approach to determining the optimal location for 4 new nephrology clinics is required.

**Methods**: GIS techniques such as buffer analysis, network analysis and spatial analysis were used to determine the ideal locations for new clinics. Travel times were calculated using posted speed limits and road network data. Service area polygons for different travel time intervals (0-30 min, 30-60 min, 60-90 min, 90-120 min) were generated and used to determine the best location for the 4 new facilities that would maximize the number of patients travelling < 30 min. Clinical characteristics by travel time category were determined and cartographic techniques used to visualize results.

**Results**: We studied a population-based sample of 31,452 patients with eGFR<45 mL/min/1.73m$^2$, most of who lived <30 min from a nephrology clinic (Table). Adding the 4 new facilities would increase the number of patients living <30 min from a clinic by 1,154 (5.0%) and reduce the number living >120 min away by 960 (35%).

**Conclusion**: GIS techniques facilitate optimum resource allocation and perhaps better patient care. These techniques could be applied in other settings for management of CKD and other non-communicable illnesses.




| Scenario | Total travel time (h) | 0-30min, n(%) | 30-60min | 60-90min | 90-120min | >120min |
|---|---|---|---|---|---|---|
| Status Quo | 19,352 | 23,230(74) | 2,524(8) | 2,041(6) | 903(3) | 2,754(9) |
| 4 New Facilities | 13,783 | 24,384(78) | 2,611(8) | 1,680(5) | 983(3) | 1,794(6) |

Disclosure of Financial Relationships: nothing to disclose

**F-PO1197**

**HALT-PKD Clinical Trials, Characteristics of Baseline Liver Cystic Volume** Arlene B. Chapman,[1] Robert W. Schrier,[2] Vicente E. Torres,[3] Ronald D. Perrone,[4] Kyong Tae Bae,[5] Marva M. Moxey-Mims,[6] Theodore I. Steinman,[7] William E. Braun,[8] Franz Winklhofer,[9] Kaleab Z. Abebe,[5] James E. Bost.[5] *[1]Emory U; [2]U Colorado; [3]Mayo Clinic; [4]Tufts; [5]U Pittsburgh; [6]NIH/NIDDK; [7]Beth Israel; [8]Cleveland Clinic; [9]Kansas U; [10]HALT PKD Study Group.*

The HALT-PKD clinical trial network currently has two randomized trials, Study A (548 participants, eGFR >60 ml/min/1.73 m$^2$, 2-by-2 design randomization to ACEi/ARB *vs* ACEi alone and to low *vs* standard BP targets) and Study B (470 participants, eGFR 25-60 ml/min/1.73 m$^2$, randomized to ACEi/ARB *vs* ACEi alone). All Study A patients underwent magnetic resonance imaging to determine total kidney volume (TKV) and liver cyst volume (LCV) to estimate the severity of polycystic liver disease. We ascertained associations of baseline parameters and LCV in Study A participants.

462/558 (83%) of HALT A participants demonstrated liver cysts, similar to the overall CRISP cohort (85%). Mean LCV was 248 ± 762 mLs. LCV were greater in women vs. men (343±801vs.148±706 mL, p=0.006). Those without liver cysts were significantly younger (31.2±10.0 vs. 37.2±7.5 years), more often male (51.5 vs. 48.5%), with greater eGFR (93.8 ±21.4 vs. 84.9±17.1 mLs/min) all P<0.0001. No differences in TKV were found in those with or without liver cysts (1101±660 vs. 1235±732 mLs). LCV significantly correlated with age (r = 0.22**)** and eGFR (-0.11), but not urine albumin excretion or TKV.

Conclusion: Polycystic liver disease is present in the majority of HALT A participants consistent with other cohorts. Female gender, increased age and reduced eGFR associated with larger LCV. Further investigation to determine the factors associated with polycystic liver disease is underway.

Disclosure of Financial Relationships: Consultancy: Novartis Pharmaceuticals Corp. Otsuka.

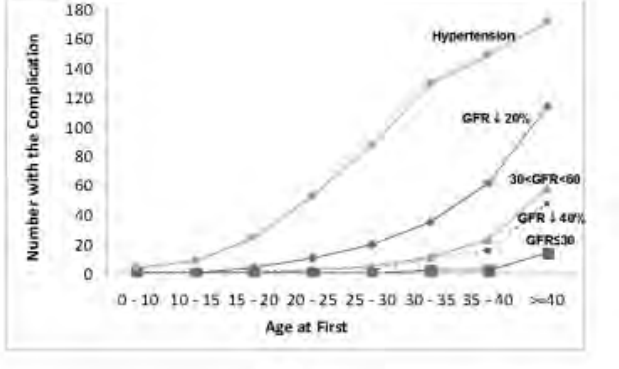
**F-PO1198**

**Cyst-Dependent Renal Complications in Autosomal Dominant Polycystic Kidney Disease** Arlene B. Chapman,[1] James E. Bost,[3] Vicente E. Torres,[2] Michal Mrug,[5] Kyong Tae Bae,[3] Jared J. Grantham.[4] *[1]Emory U; [2]Mayo Clinic; [3]U of Pittsburgh; [4]Kansas U Medical Ctr; [5]U of Alabama, Birmingham.*

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive kidney enlargement due to expanding cysts, which account for increased kidney volume (TKV). We test the hypothesis that TKV predicts the development of renal complications in ADPKD.

**Methods:** We determined the age of onset of hypertension (HBP), gross hematuria (GH), pain, nephrolithiasis (NL), urinary tract infection (UTI) and renal insufficiency (RI) in 200 CRISP study participants followed from 2001 to 2009. Serial measurements of TKV adjusted for height (htTKV, cc/m) using magnetic resonance images and kidney function assessed by iothalamate clearances (IC) were obtained. HtTKV associations with the occurrence of renal complications in ADPKD were determined.

**Results:** Renal complications began in the first decade of life with eight participants free of complication at end of follow up. The average number of complications excluding RI was 2.7 with > 50% having 3 or more. HtTKV was associated with the number of renal complications experienced independent of age. 50% of CRISP participants had developed HBP by age 30, UTI by age 40, GH by age 45, ≥ 20% reduction in IC by age 45, and K/DOQI stage 3 CKD by age 49. One episode of symptomatic NL had occurred by age 45 in 25%. Plots of cumulative onset curves for HBP and decreased IC were exponential (Figure 1). HtTKV predicted the onset of HBP, GH and RI (p<0.001) in multivariable analysis. Receiver operator curves for baseline htTKV and K/DOQI stage 3 demonstrated an AUC 0.83, with high sensitivity (81.0%) and specificity (72.1%) at htTKV value of 600 c/m.




**Summary:** Major life-altering complications occur early and throughout the course of ADPKD. The development of HBP, GH and RI are predictably related to TKV.

Disclosure of Financial Relationships: Consultancy: Novartis Pharmaceuticals Corp. Otsuka.

**F-PO1199**

**Effect of Pioglitazone, a PPAR-γAgonist, on Non-Diabetic Chronic Kidney Disease with Proteinuria. A Prospective, Randomized Study** Takashi Oda,[1] Kojiro Yamamoto,[1] Keishi Higashi,[1] Taketoshi Kushiyama,[1] Toshitake Hyodo,[1] Naoki Oshima,[1] Hiroki Sato,[2] Yutaka Sakurai,[3] Soichiro Miura,[1] Hiroo Kumagai.[1] *[1]Nephrology, National Defense Medical College, Tokorozawa, Saitama, Japan; [2]Medical Informatics, National Defense Medical College; [3]Preventive Medicine and Public Health, National Defense Medical College.*

We have reported that pioglitazone (Pio), a PPAR-γ agonist, significantly reduced proteinuria and exerts renoprotective effect in spontaneously hypercholesterolemic (SHC) rats, a model of chronic kidney disease (CKD) with massive proteinuria (Am J Physiol 2007; 293: F1292-8). We therefore conducted a prospective and randomized trial comparing the antiproteinuric effect of Pio with that of dipyridamole (Dip) in nondiabetic CKD patients. Twenty four nondiabetic CKD patients, whose systolic blood pressure have been controlled below 135 mmHg, and who have been receiving a renin-angiotensin system (RAS) inhibitor (4 of them couldn't use it because of low blood pressure or high potassium concentration), were randomly assigned into Pio or Dip group. Pio (at a dose of 15~30 mg/day) or Dip (at a dose of 150~300 mg/day) was added on the regular treatment and continued for 6 month. The primary endpoint was a decrease in the urinary protein to creatinine ratio. One patient in Pio group was eliminated because of the usage of corticosteroid during the study due to the asthmatic attack, and one patient in Dip group stopped continuation of Dip because of severe headache. Thus, 11 patients with Pio and 11 patients with Dip were compared. There was no significant difference in baseline parameters between two groups. In the Pio-treated group, the urinary protein was significantly decreased from 1.41 ± 0.33 to 0.76 ± 0.19 g/gCr (p< 0.01) after the therapy, but it did not change in the Dip-treated group (p=0.873). In both groups, no significant difference was found in other parameters, such as body weight, systolic blood pressure, serum creatinine, estimated GFR, HbA1c, LDL-cholesterol, Total-cholesterol, or uric acid. This study suggests that Pio significantly decrease the proteinuria of non-diabetic CKD and may be useful as a therapeutic option secondary to RAS inhibitors.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1200**

**Study of Reduction of Albuminuria with Ramipril Therapy in Obese vs Non-Obese Chronic Albuminuric Kidney Disease Patients** Sanjib Kumar Sharma,[1] Mahesh Bhattarai,[1] Prahlad Karki,[1] Nirmal Baral.[2] *[1]Department of Internal Medicine, B P Koirala Institute of Health Sciences, Dharan, Sunsari, Nepal; [2]Deparment of Biochemistry, B P Koirala Institute of Health Sciences, Dharan, Sunsari, Nepal.*

Background: Obesity increases the risk for chronic kidney disease (CKD). Albuminuria predicts renal disease progression, and its reduction by angiotensin converting enzyme inhibitor is renoprotective.

Objectives: To evaluate the extent of reduction of albuminuria in patients treated with ramipril in chronic albuminuric kidney disease and to compare the reduction of albuminuria in obese vs non-obese.

Methodology: In this prospective, open label, interventional study, the effect of 8 weeks of treatment with ramipril was compared in obese chronic albuminuric kidney disease patients with age and sex matched non-obese chronic albuminuric kidney disease patients.

Results: Despite comparable changes in blood pressure and glomerular filtration rate, 8 weeks of ramipril therapy decreased albuminuria significantly in non obese as compared to obese (50.14% Vs 10.72% p=0.028). Furthermore, in macroalbuminuric patient reduction was significant in non obese (58.16% Vs 7.34% p = 0.038) whereas in microalbuminuric group reduction was (25.48% Vs 18.01%, p = 0.380) not significant.

Conclusion: Eight weeks of ramipril therapy reduced albuminuria more effectively in non obese patients as compared to those of obese patients. Superior anti albuminuric effect in non obese was independent to blood pressure reduction. Reduction of albuminuria is more in macroalbuminuric non obese chronic kidney disease patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1201**

**Renal and Cardiovascular Outcomes with Telmisartan, Ramipril or Both in People at High Renal Risk: Results from the ONTARGET and TRANSCEND Studies** Sheldon W. Tobe,[1] Catherine M. Clase,[4] Peggy Gao,[2] Salim Yusuf,[2] Johannes F. Mann.[3] *[1]Medicine, Sunnybrook Health Sciences Centre, Toronto, ON, Canada; [2]Population Health Research Institute, McMaster University, Hamilton, ON, Canada; [3]Medicine, Schwabing General Hospital, and KfH Kidney Center, Erlangen, Germany; [4]Medicine, McMaster University, Hamilton, ON, Canada.*

**Background and Objective:** The relationship between proteinuria reduction, with ACE inhibitors and angiotensin receptor blockers (ARBs) and improved renal and cardiovascular outcomes comes from observational data. Dual therapy with both agents has demonstrated greater proteinuria reduction than either alone but has yet to be shown to also improve renal or cardiovascular outcomes. This analysis was designed to evaluate and compare renal and cardiovascular outcomes in patients with normal and low glomerular filtration rate (GFR) and normal, micro- or macroalbuminuria in the ONTARGET and TRANSCEND studies.

**Methods:** Post-hoc analysis of renal subgroups of ARB versus placebo for the TRANSCEND study and dual therapy versus monotherapy for the ONTARGET study. Hazard ratios of events by subgroups and Cox regression models with factors for treatment, subgroup and interactions. Main renal outcome was the composite of chronic dialysis or doubling of creatinine and the main cardiovascular outcome, the composite of cardiovascular death, MI, stroke or hospitalization for heart failure.

**Results:** No renal or cardiovascular benefit from dual over mono-therapy in any subgroup. In TRANSCEND comparing ARB to placebo, there was a significant interaction (p= 0.01) in the direction of harm for patients with normoalbuminuria (35% greater renal risk from telmisartan) but a benefit for patients with micro- or macroalbuminuria (40% and 29% lower renal risk from telmisartan).

**Conclusions:** This analysis does not support dual over monotherapy even in patients with low GFR and macroalbuminuria. This observation is a post hoc comparison and should be interpreted appropriately.

Disclosure of Financial Relationships: Honoraria: Boehringer Ingelheim, Bristol Myers Squibb, Sanofi Aventis, Servier, Novartis.

## F-PO1202

**Tacrolimus Versus Intravenous Pulse Cyclophosphamide Therapy in Adults with Steroid-Resistant Idiopathic Minimal Change Nephropathy: A Nonrandomized Controlled Trial in China** Heng Li,[1] Xiangdong Shi,[2] Hong Shen,[3] Xiayu Li,[1] Jianghua Chen.[1] *[1]Kidney Disease Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China; [2]Department of Nephrology, Huzhou Central Hospital, Huzhou, China; [3]Department of Nephrology, Taizhou Hospital, Taizhou, China.*

**Background:** Treatment of steroid-resistant minimal change nephropathy (SR-MCN) remains a challenge to nephrologists. The aim of this study was to compare the efficacy and safety of tacrolimus (TAC) with that of pulse intravenous cyclophosphamide (CTX) therapy in the management of SR-MCN.

**Methods:** We performed a nonerandomized controlled trial in three Chinese medical centers. Totally 33 Chinese adults with SR-MCN, and serum creatinine level of 133 mmol/L or less, were enrolled. Patients were self assigned to: (1) combination therapy with prednisone and oral TAC or (2) combination therapy with prednisone and intravenous CTX. TAC began at 0.05 mg/kg/day and adjusted to maintain a trough blood level of 5 to 10 ng/mL for 1 year ($n$ = 19). CTX began at 1.0 g/1.73 $m^2$ for a total dosage of 10.0 g/1.73 $m^2$ over 1 year ($n$ = 14). In both groups, oral prednisone began at 0.5 mg/kg/day for 3 months, which was tapered off to complete cessation by 6 months.

**Results.** The remission rate was 57.9%, 73.7%, and 78.9% in TAC group and 14.3%, 42.9%, and 50.0% in CTX group after 2, 4, and 6 months, respectively. The remission rate at 2 months was significantly higher in TAC group ($P < 0.05$). The remission rate during the 1-year therapy and the 1-year follow-up was higher in the TAC group than that in the CTX group (Kaplan-Meier, log-rank test, $P < 0.001$). For patients who achieved remission, the mean time needed for remission was 48.7 ± 36.0 days in the TAC group and was 85.3 ± 40.6 days in the CTX group ($P < 0.05$). During the 1-year therapy and 1-year follow-up periods, 6 of 15 TAC patients and 1 of 7 CTX patients relapsed ($P > 0.05$).

**Conclusions.** TAC therapy is safe and effective for treating adults with SR-MCN and induces remission more rapidly than does pulse intravenous CTX therapy, but it has a high relapse rate after withdrawal.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1203

**Long-Term Outcome of Paediatric Patients with Primary Systemic Vasculitis** Nishkantha Arulkumaran,[1] Helen Doolittle,[1] Susan Jawad,[1] Stuart W. Smith,[3] Lorraine Harper,[3] Paul Brogan,[2] Charles D. Pusey,[1] Alan D. Salama.[1] *[1]Renal Section, Division of Medicine, Imperial College Kidney and Transplant Institute, London, United Kingdom; [2]Institute of Child Health, Great Ormond Street Hospital, London, United Kingdom; [3]Division of Renal Immunobiology, University of Birmingham, Birmingham, United Kingdom.*

Purpose of study

We have described the long-term outcome of patients with primary systemic vasculitides presenting in childhood.

Methods

Retrospective analysis was performed to identify patient demographics and clinical course.

Results

Nine patients were identified- 6 had Wegeners granulomatosis (WG), 2 had Polyarteritis nodosa (PAN), and 1 had Microscopic Polyangiitis (MPA). The mean age at presentation was 11 years. The median follow up is 14 years. Mean presentation creatinine was 0.84mg/dL for 8 patients and one patient presented with end- stage renal failure (ESRF). 2 patients had a renal biopsy, - both were consistent with acute vasculitis. At induction all patients had steroids and 8 had cyclophosphamide. Eight patients achieved complete remission. Maintenance is with azathioprine or mycophenolate mofetil. Biological agents were used in 4 patients in adulthood for relapsing disease. All patients had at least 1 relapse, 5 were infertile and 7 had recurrent infections. Patient 2 died from disease- related complications after 25 years. Mean follow up creatinine is 0.87mg/dL.

Demographics and clinical outcomes of patients

| Patient | Diagnosis | Age at presentation (Years) | Serum Cr at presentation (mg/dL) | Current Cr (mg/dL) | Infertility | Recurrent infections | Relapses |
|---|---|---|---|---|---|---|---|
| 1 | PAN | 14 | 0.85 | 0.70 | + | - | 8 |
| 2 | PAN | 11 | 0.96 | 0.87 | + | + | 4 |
| 3 | MPA | 14 | 0.86 | 1.36 | + | + | 4 |
| 4 | WG | 9 | 0.72 | 0.76 | - | + | 3 |
| 5 | WG | 0.75 | 0.89 | 1.17 | + | + | 6 |
| 6 | WG | 9 | 0.76 | 0.77 | + | + | 8 |
| 7 | WG | 12 | 0.68 | 0.49 | - | + | 2 |
| 8 | WG | 14 | ESRF | ESRF | - | + | 6 |
| 9 | WG | 15 | 0.97 | 0.86 | - | - | 1 |

Abbreviations: Cr= Creatinine

Conclusions

Patients with primary systemic vasculitides presenting in childhood have similar long-term survival to adult cohorts. Renal function is usually preserved, but relapse rates and treatment-related morbidity is high. Close follow-up and identification of new treatment regimes are imperative.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1204

**Quality of Anemia Management and Healthcare Costs in CKD and Other At-Risk Patients** Anthony Staresinic. *Pharmacy Services, Physicians Plus Insurance Corp., Madison, WI.*

**Background:** Adherence to KDOQI, CKD testing, and other guidelines for anemia management in our HMO remains problematic despite ongoing improvement efforts. This study was undertaken at an integrated health system in Madison, WI to evaluate changes in anemia management quality and costs. **Methods:** Members were screened for anemia using administrative claims data. The anemia index date (AID) is the date of the member's first anemia claim. Members had to meet eligibility requirements before and after the AID. Anemia cohorts underwent further analysis: chronic kidney disease (CKD), female 18 to 49 years of age (FEM), diabetes, hypertension or congestive heart failure (DHC) and Other (OTH). To qualify for KDOQI adherence hemoglobin, iron, iron binding capacity and ferritin tests had to be done within 90 days before and 30 days after the AID. This study was reviewed and approved by our local institutional review board. **Results:** Study included 4177 members with anemia. Compared to DHC, the CKD group was more likely to have diabetes but less likely to have hypertension.

| Variable | CKD | DHC | FEM | OTH |
|---|---|---|---|---|
| KDOQI adherent | 25 (19) | 101 (8)* | 125 (11)* | 2 (<1)* |
| SCr, No. (%) | 82 (61) | 398 (33)* | 165 (14)* | 382 (23)* |
| CBC, No. (%) | 111 (83) | 825 (69)* | 668 (58)* | 974 (58)* |
| Ferritin, No. (%) | 22 (16) | 71 (6)* | 77 (7)* | 101 (6)* |
| Fecal occult, No. (%) | 7 (5) | 70 (6) | 22 (2) | 73 (4) |
| ESA Use, No. (%) | 4 (3) | 0 (0) | 0 (0) | 3 (<1) |
| IV Iron, No (%) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| ACE Use, No. (%) | 54 (40) | 387 (32) | 3 (<1)* | 63 (4)* |
| *p<0.05 | | | | |

Post AID, there were no differences in rates of myocardial infarction or unstable angina. The CKD cohort had significantly higher rates of emergency room visits post AID. Post AID, CKD and DHC experienced 30% and 75% increases in medical costs. The FEM and OTH group experienced increases of 114% (P< 0.0001) and 138% (P<0.0001) respectively. **Conclusion:** Adherence to anemia testing and management guidelines remain suboptimal. There was no change in the drug management of anemia. Our HMO experienced increased costs post-AID. Interventions are needed to improve anemia-based care in CKD and at-risk member groups.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1205

**SureClick® (Darbepoetin Alfa, DA) Improves Perceived Satisfaction and Competence for Anemia Treatment and Increases Self-Administration in Non-Dialyzed Patients with Chronic Kidney Disease (CKD)** Xavier Bonafont,[1] Ramon Romero,[1] Isabel Martínez,[2] Mª Dolores Del Pino Pino,[3] José Manuel Gil,[4] Pedro Aranda,[5] Ramon Roca,[6] Joana Claverol,[7] Ignacio Arístegui.[7] *[1]H.G.Trias i Pujol, Badalona, Spain; [2]H.Galdakao, Galdakao, Spain; [3]H.Torrecárdenas, Almería, Spain; [4]CH.Jaén, Jaén, Spain; [5]H.Carlos Haya, Málaga, Spain; [6]H.Mollet, Mollet, Spain; [7]Amgen, S.A., Barcelona, Spain.*

Purpose: The SureClick® device is a pre-filled pen for the administration of DA that requires no preparation and is ready to use. The main objective was to evaluate whether the use of SureClick® increases patient satisfaction compared with pre-filled syringes.

Methods: Multicenter, prospective, 6-months, observational study in non-dialyzed patients with CKD treated with DA in pre-filled syringes (≥6 previous months), with stable hemoglobin, who switched to SureClick® as indicated in clinical practice. The primary endpoint was the Anemia Treatment Satisfaction Questionnaire (ATSQ-S). Secondary endpoints included: Perceived Competence for Anemia Scale (PCAS), degree of self-administration and adverse reactions to DA or device.

Results: 132 patients treated with DA every 2 weeks were included. Mean age (SD) was 71.3(14.6) years and 57.6% were women. Median(Q1;Q3) time since diagnosis of CKD was 5.1(2.6; 8.4) years (73.5% stage 4). Approximately half (47.7%) of patients self-administered DA with pre-filled syringes, vs 74.2% at final visit (p<0.001). Mean(SD) ATSQ-S scores at baseline and final visits were 25.5(7.9) and 31.6(4.9) (on a scale from 0 to 36 –maximum satisfaction), respectively (+6.2 points, 95%CI:4.6-7.8, p<0.05). The

patients' perceived competence (PCAS) also increased significantly (4.3(2.0) vs 5.6(1.6), on a scale from 1 to 7 – maximum competence, +1.3 points, 95%CI:1.0-1.6, p<0.05). Two patients (1.5%) had adverse reactions related to the device (pain on application) which led to discontinuation.

Conclusions: The change from pre-filled syringes to SureClick® for the administration of DA is associated to an increase in satisfaction and competence for anemia treatment in non-dialyzed patients with CKD. The use of SureClick® also achieves self-administration in 3 of 4 patients.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1206

**Predictors of Baseline and *Changes* in Quality of Life among Patients with Diabetes, Chronic Kidney Disease, and Moderate Anemia in TREAT** Eldrin F. Lewis,[1] Marc Pfeffer,[1] Amy Feng,[2] Hajime Uno,[1] John Mcmurray,[2] Robert D. Toto,[2] Shravanthi R. Gandra,[2] Scott D. Solomon,[1] Moustafa Moustafa,[2] Iain C. Macdougall,[2] Francesco Locatelli,[2] Patrick S. Parfrey.[2] *[1]Brigham and Women's Hospital; [2]On Behalf of TREAT Investigators.*

**Background:** Patients (pt) with anemia, diabetes, and kidney disease have variable quality of life (QOL). We aimed to identify *independent* predictors of 1) baseline and 2) changes in QOL.

**Methods:** *Baseline* QOL predictors in pt enrolled in Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) were assessed using 3 pre-specified domains[FACT-Fatigue, SF36 Energy, & SF36 Physical Function (SF36-PF)]. At 25 weeks, predictors of ≥3 point improvement in FACT-Fatigue and ≥5 point improvement in SF36 ("clinically meaningful") were assessed with logistic regression.

**Results:** Baseline QOL was impaired(FACT-Fatigue 30.3±12, SF36 Energy 45.9±22, SF36 PF 47.7±29). Predictors of all 3 baseline QOL domains were identified(*Table*). At 25 weeks, higher physical activity and worse baseline QOL were predictors of *improvements* in all 3 domains. Hospitalizations decreased odds of improved FACT-Fatigue and SF36-PF. Randomization to darbepoetin alfa increased odds of improved FACT-Fatigue and SF36-Energy.

**Conclusion:** Directly assessing QOL may be a useful strategy to determine pt who may benefit from therapy.

**Multivariate Predictors of *Baseline* Quality of Life**

| | PARAMETER ESTIMATES | | |
|---|---|---|---|
| | **FACT-Fatigue** | **SF36 Energy** | **SF36 PF** |
| **Better QOL** | *n=3551* | *n=2295* | |
| Heavy/Medium Recreation Activity | 3.55* | 7.12* | 14.54* |
| Black Race | 3.46* | 7.85* | 2.83 |
| Male | 2.78* | 2.96# | 5.78* |
| Albumin(1 g/dL increase) | 2.05* | 2.70 | 6.45* |
| Alcohol use | 1.68# | 3.25# | 4.21# |
| Potassium(1 mmol/L increase) | 0.88# | 1.70 | 1.93 |
| Hemoglobin(1 g/dL increase) | 0.75* | 1.41# | 2.00* |
| Systolic BP(1 mmHg increase) | 0.04* | 0.05 | 0.06 |
| **Worse QOL** | | | |
| Diabetic Neuropathy | -2.60* | -4.15* | -7.31* |
| CV Disease | -2.22* | -3.40* | -4.33* |
| Lung Disease | -1.71* | -2.43 | -2.90# |
| Former Smoker | -1.24# | -2.04 | -2.17 |
| BMI(1 kg/m2 increase) | -0.20* | -0.28* | -0.63* |
| Ferritin(1 ug/L increase) | -0.002 | -0.004# | -0.004 |

**p<0.001, #p<0.01 (other p<0.05)*

Disclosure of Financial Relationships: Research Funding: Amgen, Inc. for research studies.

### F-PO1207

**Medication Non-Adherence Predicts Hospitalization Rate and Healthcare Costs in Hemodialysis (HD) Patients** Harold J. Manley, Steven Wang, Allen R. Nissenson. *DaVita Inc., Denver, CO.*

Medication non-adherence is common among HD patients. Adherence can be estimated using the medication possession ratio (MPR). According to the USRDS, an MPR > 0.8 for individual ACEI/ARB, Beta-blocker, Statin, or oral hypoglycemic medications are associated with lower healthcare costs. Information on the relationship between entire-medication-regimen MPR and hospitalization risk and healthcare cost is lacking. We evaluated, in a cohort of HD patients, the hypothesis that overall non-adherence is directly related to both hospitalization risk and healthcare costs as part of a CMS ESRD Demonstration.

METHODS: We estimated medication adherence through a retrospective review of all pharmacy and medical claims for 663 HD patients from 1/06 to 6/09. We calculated MPR: MPR = Σ Medication Day's Supply/(# Days between the first fill & the last refill + Day's supply last refill). We used logistic regression models to determine the odds ratio of hospitalization (OR-hosp) for MPR ranges above and below 0.8-0.99, the reference range. We determined patient medication and hospital per member per month (PMPM) costs and hospitalization rate (per member per thousand days, PMPT) for each MPR range.

RESULTS: Risk of hospitalization, PMPM costs and hospitalization rate each increased significantly as overall adherence decreased. MPR values < 0.8 were associated with a greater hospitalization risk.

| MPR | Member Months | Medical Cost | Rx Cost | Total PMPM Cost | Admits PMPT | OR-hosp (CI) |
|---|---|---|---|---|---|---|
| <0.4 | 1444 | $6,266 | $386 | $6.612 | 1820 | 2.57 (1.1,5.3) |
| 0.4-0.59 | 3184 | $6,392 | $553 | $6,944 | 2080 | 2.2 (1.3,3.8) |
| 0.6-0.79 | 4352 | $5,385 | $670 | $6,056 | 1701 | 1.7 (1.1,2.7) |
| 0.8-0.99 | 3117 | $4,493 | $866 | $5,359 | 1178 | 1.0 (ref) |
| 1.0-1.19 | 1053 | $5,313 | $883 | $6,196 | 1641 | 0.6 (0.4,1.1) |
| >1.2 | 460 | $5,962 | $943 | $6,906 | 1826 | 1.3 (0.6,2.6) |

CONCLUSIONS: In HD patients, both hospitalization risk and total PMPM cost are directly related to medication non-adherence. Methods to improve MPR may help reduce morbidity and cost in this fragile patient population.

Note: This is a DaVita analysis of CMS demonstration experience; CMS will conduct an independent evaluation.

Disclosure of Financial Relationships: Employer: DaVita Inc; Ownership: DaVita Inc.

### F-PO1208

**Longitudinal Assessment of Health Related Quality of Life (HRQOL) in Pediatric Dialysis Patients** Shari K. Neul,[1] Stuart Goldstein.[2] *[1]Renal Service / Pediatrics, Texas Children's Hospital, Houston, TX; [2]Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Long-term ESRD pt survival has improved. Optimal pt care now must incorporate HRQOL assessment and management. We developed a pediatric ESRD-specific HRQOL instrument now validated (PedsQL™ 3.0 ESRD Module) in multiple cross sectional analyses. As part of standard clinical care we semiannually assess global (PedsQL™ Generic Scale) and ESRD-specific HRQOL in our dialysis patients. Both measures include patient self-report (5 to 18 yrs) and caregiver-proxy report (caregivers of children 2 to 18 yrs). We now report the first longitudinal assessment of pediatric ESRD HRQOL using these scales.

Eighteen dialysis pts (mean age 14.7 yrs; 14 HD; 4 PD; 61% male) and 15 caregivers had longitudinal data available. Results from the first year of assessment indicate that multiple global and disease-specific indices of HRQOL worsened over time on patient and caregiver-proxy ratings. Caregivers tended to view patients as functioning worse than patients viewed themselves on the global domains whereas patients tended to view themselves as functioning worse than caregivers perceived them to be on the ESRD-specific domains with the exception of communication, worry, and disease knowledge.

Magnitude of change in patient & caregiver-proxy HRQOL ratings over time

| PedsQL™ Generic | Patient | Caregiver |
|---|---|---|
| Physical | -3.65 | -5.74 |
| Emotional | -5.51 | -4.67 |
| Social | -1.39 | -4.67 |
| School | -6.25 | -8.67 |
| Total | -4.39* | -5.94* |
| PedsQL™ 3.0 ESRD Module | | |
| General Fatigue | -17.64 | -12.05 |
| About My Disease | -3.67 | -5.00 |
| Treatment Problems | -5.42 | -1.34 |
| Family & Peers | -8.89 | -3.57 |
| Worry | 3.67 | 0.89 |
| Physical Appearance | -3.89 | 0.00 |
| Communication | -4.33 | -8.57 |
| ESRD Total | -5.74 | -4.23 |
| *Represents a clinically significant change | | |

Our data suggest assessing both pt and caregiver HRQOL perceptions, and using both global and ESRD-specific measures, are key to more aptly understanding HRQOL in dialysis-dependent pts. We now are developing intervention initiatives aimed at patient/family HRQOL treatment planning as well as unit-wide programming based on these longitudinal data to optimally manage HRQOL functioning.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1209

**Trends in Hospital Charges, Length of Stay (LOS), and Mortality for Pediatric Nephrotic Syndrome** Rose M. Ayoob, David S. Hains, William E. Smoyer. *Center for Clinical and Translational Research, Nationwide Children's Hospital.*

**Background**: Nephrotic syndrome (NS) is one of the most common types of kidney disease diagnosed in children, and is characterized by edema, hypoalbuminemia, and proteinuria. Children with NS are also at increased risk of developing serious complications such as infections, acute kidney injury, or thrombosis necessitating hospitalization.

**Objective:** To analyze trends in hospital charges, LOS, and mortality for children hospitalized with NS in the US.

**Methods:** Data were collected for patients ages 0-18 years hospitalized with the principal diagnosis of NS (ICD-9 codes 581,582.1, 583.1) during 2000, 2003, and 2006 using the Healthcare Cost and Utilization Project (HCUP) Kids' Inpatient Database (KID). Hospital charges were not corrected for inflation.

**Results:** From 2000-2006, the mean number of children hospitalized annually with a principal diagnosis of NS was 2,682 and the mean LOS was 4.4 days. These admissions resulted in aggregate hospital charges that increased from $35 to $52 million (48%). The mean total hospital charges per admission increased 46% from $13,120 to $19,549. About 75% of all admissions were to children's as opposed to non-children's hospitals. Of note, the mean hospital charges per day averaged 19% more in children's vs. non-children's hospitals, while the mean LOS averaged 67% longer. Importantly, the mortality rate remained <0.4% during the entire study period.

**Conclusions:** No significant trends were found in the total admissions for NS or the mean LOS during the 6 year time period. However, children's hospitals had higher mean hospital charges per day and LOS than non-children's hospitals. Furthermore, 25% of all children with NS were admitted to non-children's hospitals. We speculate that the larger hospital charges and LOS in children's hospitals may be due to referrals of higher acuity patients requiring specialized care.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1210

**Healthcare Utilization and Costs for Children with Lupus Nephritis** Marie Tanzer,[1] Amber Kroeker,[1] Larysa Wickman,[1] Cheryl Tran,[1] Brett Ehrmann,[1] Peter Song,[2] Debbie S. Gipson.[1] *[1]Division of Pediatric Nephrology, University of Michigan, Ann Arbor, MI; [2]School of Public Health, Biostatistics, University of Michigan, Ann Arbor, MI.*

Introduction:

Systemic Lupus Erythematosus (SLE) is a common cause of vasculitis in children and adults. The presence of lupus nephritis can be a life threatening complication which when defined and treated specifically may be controlled.

Study Aim:

We undertook the present study to define the healthcare utilization and cost of SLE Nephritis in the USA.

Methods:

We used the 2006 data from the Healthcare Cost and Utilization Kids Inpatient Database, sponsored by the US Agency for Healthcare Research and Quality, for this study. Hospitalization data were reported from 38 states. SLE was defined by ICD-9 code 710.0, nephritis was defined as Nephritis NOS 583.81, Chronic Nephritis 582.81, Nephrotic Syndrome 581.81.

Results:

Among children age 1 to 17 years, 2,109 hospitalizations for SLE were reported out of 6,578,069 total hospitalizations during this period, accounting for 3 out of every 10,000 hospitalizations. Nephritis or Nephrotic syndrome was present in 1,258 (59%) and ARF in 138 (6.5%) of these pediataric hospitalizations. Hospitalization for lupus nephritis was most common among children age 15 to 17. The median length of stay was 3 days for all SLE, 2 days for SLE with nephritis NOS, 3 days for chronic nephritis, 4 days for nephrotic syndrome and 10 days for SLE with ARF. The median charge per hospitalization ranged from $12, 034 for lupus nephritis NOS to $63,266 for lupus with ARF. The weighted aggregate charge was $38,982,842 for all pediatric hospitalizations with lupus nephritis. When ARF charges were added, the total bill increased to $53,956,117. The aggregate inpatient charges was strongly correlated with admission from the emergency department (r=0.99).

Conclusions:

Although only a portion of the total cost of care for children with SLE, the cost of inpatient healthcare for children with Lupus Nephritis is large. When SLE is complicated by nephrotic syndrome or ARF, the cost of a typical SLE hospitalization increases approximately 2 to 5 fold, respectively.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1211

**Health Utility at 60 Days Predicts 12-Month Mortality among AKI Survivors** Mark W. Smith,[1] Kirsten L. Johansen,[2] Mark L. Unruh,[3] Andrew M. Siroka,[1] Theresa Z. O'Connor,[4] Paul M. Palevsky.[3,5] *[1]VA Palo Alto; [2]VA San Francisco; [3]University of Pittsburgh; [4]VA Connecticut; [5]Pittsburgh VA.*

We hypothesized that utility 60 days after AKI onset would predict 12-month all-cause mortality. The VA/NIH Acute Renal Failure Trial Network Study was a multi-center, prospective, randomized trial of intensive vs. less-intensive renal replacement therapy in critically ill patients with AKI. Participants completed the Health Utilities Index (HUI3) at days 60 and 365. The total score and subscores for eight attributes range 0-1 where 1 represents highest functioning. Of 1124 participants there were 415 survivors with analyzable utility data at 60 days and 258 at 365 days. Persons still dialysis-dependent at day 60 were less likely to have usable data (48% vs 84%).

Four HUI3 subscales were hypothesized to be affected by AKI: ambulation, cognition, emotion, and pain. We estimated proportional hazard models of 12-month mortality controlling for demographics, baseline health (Charlson Comorbidity Index, admission from home vs. SNF, SOFA score), hospital stay characteristics (study arm, treating specialty, LOS), and dialysis independence at day 60.

Higher scores on the HUI3 and three subscales (ambulation, cognition, and emotion but not pain) were strongly correlated with lower mortality (hazard ratio < 1). Age, LOS, and comorbidity were also significantly associated with mortality.

Health utility can assist clinicians in predicting medium-term mortality risk among AKI survivors.

Cox Proportional Hazard Models of 12-Month Mortality: Partial Results

| | | | | | |
|---|---|---|---|---|---|
| HUI3 total score | 0.27 (<.01) | | | | |
| -- ambulation | | 0.11 (<.01) | | | |
| -- cognition | | | 0.13 (<.01) | | |
| -- emotion | | | | 0.15 (<.01) | |
| -- pain | | | | | 0.44 (0.14) |
| | | | | | |
| Age 60-74 | 1.46 (.10) | 1.41 (.14) | 1.55 (.06) | 1.50 (.08) | 1.54 (.06) |
| Age 75+ | 2.20 (<.01) | 2.15 (.02) | 2.21 (<.01) | 2.43 (<.01) | 2.66 (<.01) |
| Hospital LOS | 1.02 (<.01) | 1.02 (<.01) | 1.03 (<.01) | 1.03 (<.01) | 1.03 (<.01) |
| Charlson Score 4+ | 1.98 (<.05) | 2.05 (.04) | 1.69 (.13) | 1.98 (.05) | 2.12 (.03) |

Figures are hazard ratio and p-value.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1212

**Unintended Consequences of the Proposed Incident Case Mix Adjuster** Zack Burstein, Mahesh Krishnan, Tracy Jack Mayne, Leanne Zumwalt. *DaVita Inc., Denver, CO.*

**Background**: The CMS Notice of Proposed Rule Making (NPRM) for the dialysis prospective payment system (i.e., 'the bundle') included 18 case mix adjusters (CMAs). The single highest CMA (1.47) is for patients during the first 120 days of dialysis. We modeled the impact of a case mix adjuster of this magnitude on reimbursement.

**Methods**: We calculated mean Medicare dialysis reimbursement for incident patients in calendar years 2007, 2008 and 2009 (N=3,831) at a large US dialysis organization. We compared this to average reimbursement for prevalent patients (N=154,851) and calculated average incremental cost per treatment for the first 120 days. We modeled the population effect of the difference between the CMS incident patient CMA, and the CMA calculated based on actual Medicare reimbursement shown here. The analysis excludes costs of oral drugs and lab tests, which are likely to have a minor impact.

**Results**: The actual increment in Medicare reimbursement for the first 120 days of treatment is 11.7%. We applied the resultant 1.117 CMA to the first 120 days of treatment in the 5.6% of incident patients (estimated in the NPRM). Adding the 2% total payment reduction mandated by MIPPA, but not taking into account other case mix adjusters, a 5% reduction in base payment per treatment is necessary to make the incident patient adjuster 'budget neutral.'

| Year | Mean Cost Per Treatment | | Incident increment |
|---|---|---|---|
| | Incident (1st 120 days) | Prevalent (>120 days) | |
| 2007 | $282.33 | $245.02 | 13.2% |
| 2008 | $270.21 | $239.01 | 11.5% |
| 2009 | $269.97 | $241.26 | 10.6% |
| All | $273.82 | $241.72 | 11.7% |

**Conclusions** The first 120 days of dialysis reimbursement are 11.7% higher than prevalent reimbursement, substantially lower than the 47% proposed in the NPRM. The monies taken out of the base payment for the incident adjuster are excessive. These calculations do not take into consideration the multiplier effect that would result when the 1.117 is multiplied by the other co-morbid CMAs, all of which are also positive. CMS should reduce the incident case mix adjuster weighting in the final rule to correct for this.

Disclosure of Financial Relationships: Employer: DaVita Inc.

## F-PO1213

**Using Genetic Polymorphism as a Strategy To Estimate the Potential Cost-Effectiveness of Pharmacological CCR5 Blockade in Dialysis Patients** Stefan Vegter,[1,2] Friso Muntinghe,[1] Marion Verduijn,[4] Elisabeth W. Boeschoten,[3] Friedo W. Dekker,[4] Gerjan Navis,[2] Maarten J. Postma.[1] *[1]Division of Nephrology, University Medical Centre Groningen (UMCG), Groningen, Netherlands; [2]PharmacoEpidemiology and PharmacoEconomics, University of Groningen, Groningen, Netherlands; [3]Hans Mak Institute, Naarden, Netherlands; [4]Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands.*

Background

Pharmacological interventions that are of benefit in non-dialysis populations have thus far been disappointing in dialysis patients. Since clinical trials are expensive and time-consuming, adjunct strategies are needed to support decision making in prioritization of tracks for drug development. Genetic association studies may provide such a strategy when a genotype is associated with a well-defined molecular and functional phenotype. Previously an association with better survival was found in incident dialysis patients with systemic inflammation and a deletion variant of the CC-chemokine receptor 5 (CCR5Δ32). Thus, we hypothesized that pharmacological CCR5 blockade could protect against inflammation associated mortality and estimated if such a treatment would be cost-effective.

Methods

A screen-and-treat strategy was modelled in which patients were screened for the CCR5Δ32 polymorphism and patients with the wild-type genotype and high inflammation status were treated with pharmacological CCR5 blockade. Univariate and probabilistic sensitivity analyses were performed.

Findings

The cost-effectiveness of the screen-and-treat strategy was €18,557 per life-year gained and €21,896 per quality-adjusted life year (QALY) gained. The main drivers of the cost-effectiveness were the costs of pharmacological CCR5 blockade and the reduction in relative risk of mortality.

Interpretation

Pharmacological blockade of the CCR5 receptor in inflamed dialysis patients can be incorporated in a potential cost-effective genetic screen-and-treat program. This study illustrates the potential of genetic association studies in drug-development programs, as a new source of Mendelian randomized evidence from an observational setting.

Disclosure of Financial Relationships: Consultancy: Shire Pharmaceuticals plc. Research Funding: Shire Pharmaceuticals plc.

**F-PO1214**

**The Cost-Effectiveness of Lanthanum Carbonate in the Treatment of Hyperphosphatemia in Chronic Kidney Disease Patients: Pre-Dialysis and Dialysis** Stefan Vegter,[1,2] Keith H. Tolley,[2] Michael S. Keith,[3] Maarten J. Postma.[1] *[1]Department of Pharmacy, University of Groningen, Groningen, Netherlands; [2]Tolley Health Economics Ltd, Buxton, United Kingdom; [3]Shire Pharmaceuticals, Wayne, PA.*

**Purpose:** Hyperphosphataemia is associated with increased mortality in Chronic Kidney Disease (CKD); in dialysis and before dialysis (CKD-ND). First-line treatment consists primarily of calcium based phosphate binders (CB). We determined the cost-effectiveness of the non-calcium based phosphate binder lanthanum carbonate (LC) as second-line treatment from a UK healthcare perspective. **Methods:** A Markov model was developed to determine the cost-effectiveness of LC for patients not achieving target serum phosphate (SP) levels on CB, compared with continued CB treatment. Patient-level data were obtained from two LC trials in CKD-ND (n=56) and dialysis (n=380) for drug efficacy parameters. Data of CKD-ND and dialysis patients were pooled to calculate the drug efficacy of CKD-ND patients. Two 1,000 patient cohorts were modelled: CKD-ND patients and dialysis patients. **Results:** For the CKD-ND cohort, 544 did not achieve target SP levels (<4.6 mg/dl) on CB; of these, 230 were eligible for second-line LC treatment. Of these 230 CB non-responders, 43 (18.8%) now responded to an 8 week trial of LC. Compared to continued CB treatment, 150 life-years (LY) were gained with LC, corresponding to 101 quality-adjusted life-years (QALYs) and 76 dialysis free years were gained. In the cohort of 1,000 dialysis patients, 378 did not achieve target SP levels (<5.5 mg/dl) on CB and were eligible for LC, with 169 (44.6%) additional responders, resulting in 106 LYs and 65 QALYs gained. In CKD-ND, second-line LC resulted in net healthcare cost-savings compared to continued CB treatment. In dialysis patients, second-line LC treatment incurred incremental costs of £6,816 per QALY gained. **Conclusions:** Second line treatment with lanthanum carbonate is cost-effective compared to continued treatment with calcium based binders in CKD, regardless of dialysis status. These results are in line with current K/DOQI guideline recommendations to treat both predialysis and dialysis CKD patients with hyperphosphataemia.

Disclosure of Financial Relationships: Consultancy: Shire Pharmaceuticals plc. Research Funding: Shire Pharmaceuticals plc.

**F-PO1215**

**Comparative Effectiveness of Intravenous Vitamin D Agents** Carey Colson, Mahesh Krishnan, Tracy Jack Mayne, Allen R. Nissenson. *DaVita Inc., Denver, CO.*

**Background:** The topic of comparative effectiveness research (CER) of therapeutic agents has risen to top of the national healthcare debate with the recent passage of healthcare reform. Dialysis electronic health records provide a unique environment for evaluating CER across multiple categories. We undertook an evaluation of two intravenous vitamin D agents used for in-center end stage renal disease (ESRD). **Methods:** December 2009 data was retrieved from the LDO patient database to find hemodialysis patients meeting the following criteria: over age 18 as of the end of the month, at least one treatment in the month, on dialysis for 90 days or more, at least one of the lab tests below within the last 90 days and receiving a single vitamin D preparation via IV at least once during the month. Patients receiving doxercalciferol (n-9,048) were propensity-score matched to patients receiving paricalcitol; the resultant sample included 18,000 individuals. Percent of patients meeting standard mineral and bone disease (MBD) outcomes were determined (using the latest test within 90 days) and compared by Chi-square test. **Results:** The results of these analyses are listed in the table.

| Vitamin D preparation (IV) | Patient Count | Albumin ≥3.5 g/dl | PTH 150-600 pg/ml | Ca 8.5-10.2 mg/dL | Ca >10.2 mg/dL | Phos ≤5.5 mg/dL |
|---|---|---|---|---|---|---|
| Doxercalciferol | 9,000 | 88.1% | 75.4% | 81.8% | 2.6% | 65.8% |
| Paricalcitol | 9,000 | 88.5% | 76.3% | 82.6% | 2.4% | 65.6% |
| p-value | | NS | NS | NS | NS | NS |

**Discussion:** The predominant medication used was paricalcitol but a sizable number of patients also used doxercalciferol, and in a matched sample of 9,000 patients per group no statistically significant differences were noted between doxercalciferol and paricalcitol with regards to albumin, parathyroid hormone (PTH), calcium, or phosphorus. **Conclusions:** In a changing reimbursement environment, comparative effectiveness between the two predominant vitamin D preparations showed no significant differences with regards to important MBD outcomes.

Disclosure of Financial Relationships: Employer: DaVita Inc.

**F-PO1216**

**Cost-Effectiveness of Lanthanum Carbonate vs Sevelamer Hydrochloride for the Treatment of Hyperphosphatemia in Dialysis Patients** Haesuk Park,[1] Karen L. Rascati,[1] Michael S. Keith,[2] Paul S. Hodgkins,[2] Michael D. L. Smyth,[3] David Goldsmith,[4] Ronald L. Akehurst.[5] *[1]University of Texas, Austin; [2]Shire Pharmaceuticals, Wayne; [3]Shire Pharmaceuticals, Basingstoke, United Kingdom; [4]Guy's Hospital London, London, United Kingdom; [5]The University of Sheffield, Sheffield, United Kingdom.*

**Purpose:** To assess the cost-effectiveness of lanthanum carbonate (LC) vs sevelamer hydrochloride (SH) for treatment of hyperphosphatemia in dialysis patients previously treated with calcium-based phosphate binders. **Methods:** A Markov model was developed to estimate health outcomes; quality-adjusted life years (QALYs), life-years saved (LYS), and associated costs. The base-case model included dialysis patients who received a calcium-based binder prior to being included (ITT population) in a randomized head-to-head crossover clinical trial that compared the reduction of serum phosphorus using fixed doses of LC (2250 to 3000 mg/day, N = 148) and SH (4800 to 6400 mg/day, N = 140). A model was also developed for those who completed this trial (i.e. 'completers'). The baseline risks of cardiovascular disease (CVD), overall mortality, and CVD mortality were derived from a large US renal database. Utilities, costs and relative risks of CVD were taken from published sources. Patient outcomes were modeled for 10 years, and incremental cost-effectiveness ratios (ICERs) were calculated for LC vs SH using a 5% discount rate for both costs and outcomes. Deterministic and probabilistic sensitivity analyses (PSA) were performed to test the robustness of the models. **Results:** In the base case model, the ICERs of LC vs SH were $24,724/QALY and $15,053/LYS. The PSA indicated a 61.9% probability of LC being cost-effective at the $50,000/QALY threshold. For completers, the ICERs of LC vs SH were $15,285/QALY and $9,337/LYS, and the PSA indicated an 85.8% probability of LC being cost-effective at the $50,000/QALY threshold. Sensitivity analyses demonstrated the robustness of the pharmacoeconomic model. **Conclusions:** Compared with SH, LC therapy is more cost-effective in the treatment of hyperphosphatemic dialysis patients previously treated with calcium-based binders.

Disclosure of Financial Relationships: Employer: University of TexasResearch Funding: Shire Pharmaceuticals.

**F-PO1217**

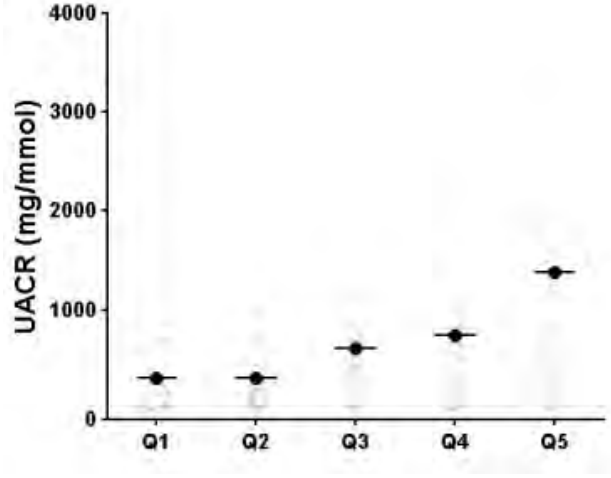
**Baseline Characteristics in the Paricalcitol Capsules Benefits in Renal Failure Induced Cardiac MOrbidity (PRIMO) Trial in CKD** Ravi I. Thadhani. *On Behalf of the PRIMO Steering Committee.*

Background: In subjects with chronic kidney disease (CKD), left ventricular hypertrophy (LVH) is prevalent and associated with morbidity and mortality. Emerging pre-clinical evidence suggests that vitamin D receptor activators (VDRA) may halt LVH progression in animal models of CKD. We sought to evaluate the efficacy of oral paricalcitol (a selective VDRA) to prevent progression or induce regression of LVH in patients with CKD and mild to moderate LVH.

Methods: The PRIMO study (NCT00497146) is an investigator-initiated industry sponsored (Abbott) multinational randomized double-blinded trial using a adaptive sample size re-estimation design to test the hypothesis that paricalcitol 2 mcg daily improves LVH in ~220 patients with stages 3 and 4 CKD (eGFR 15-60 ml/min/1.73m2) and echocardiographic evidence of LVH (septal thickness in men 12-18 mm, 11-17 mm in women) and ejection fraction ≥ 50%. The intervention will be compared with placebo after 48 weeks of treatment. The primary endpoint is change in LV mass index (LVMI) measured by cardiac magnetic resonance. The main secondary endpoint will be a change in diastolic mitral annular relaxation velocity (E') and changes in additional measures of diastolic function (IVRT, E/E', DT) measured by tissue Doppler echocardiography.

Results: 227 subjects (30% Female, 74% Caucasian, age 65±12) from 11 countries were randomized 1:1 to paricalcitol or placebo. At baseline (BL), eGFR was 34±11 ml/min/1.73m2, LVMI was 32 ± 7 g/m2.7, and early diastolic mitral annular relaxation velocity (E') was 8.1±2.4 cm/s. At BL, LVMI correlated with systolic blood pressure (SBP, r= 0.23), urine albumin creatinine ratio (UACR, r=0.32; figure, by quintiles of LVMI), and plasma brain natriuretic peptide (BNP, r=0.23), all with p<0.01.

Conclusion: In multinational subjects with stages 3 and 4 CKD, LVMI correlates with SBP, UACR, and BNP levels.




Disclosure of Financial Relationships: Consultancy: Roche DiagnosticsResearch Funding: Abbott Laboratories; Patent: Preeclampsia Biomarkers; Scientific Advisor: Shire, Chugai, Mitsubishi Tanabe.

**F-PO1218**

**Prospective Evaluation of Pharmacokinetically Guided Dosing of Carboplatin in Japanese Patients with Cancer** Yoshinari Yasuda,[1] Tomoya Shimokata,[2] Yuichi Ando,[2] Shoichi Maruyama,[1] Enyu Imai,[1] Yoshinori Hasegawa,[2] Seiichi Matsuo.[1] *[1]Department of Nephrology/CKD Initiatives, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; [2]Department of Respiratory Medicine, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.*

*Background:* Carboplatin, a second-generation platinum-containing compound, is widely used to treat solid tumors, especially lung and gynecologic cancers. Because carboplatin is eliminated primarily through renal excretory mechanisms, the dosage adjustment is generally conducted by using the Calvert formula, i.e., carboplatin dose (mg) = target area under the concentration versus time curve (AUC) x [glomerular filtration rate (GFR) + 25], however it has not yet been validated among Japanese subjects in whom GFR was accurately measured. *Objective*: We prospectively evaluated the validity of this formula for Japanese patients with cancer and modified it for this population. *Methods:* GFR was measured by inulin clearance. The dose of carboplatin was determined by the Calvert formula, using 24-h creatinine clearance (Ccr). The pharmacokinetics of carboplatin was analyzed during the first cycle of chemotherapy. The relationship between GFR and carboplatin clearance was prospectively evaluated. *Results:* Inulin clearance was examined in 28 patients with cancer. The 24-h Ccr was unbiased (mean prediction error [MPE] +- SE = -2.3 +- 4.5%) and acceptably precise (root mean squared error [RMSE] = 23.7%) for GFR assessment. The pharmacokinetics of carboplatin was analyzed in 21 patients with GFRs of 17.2 to 91.4 ml/min. The Calvert formula using GFR overestimated carboplatin clearance, resulting in an MPE of 14.3%. When we revised the Calvert formula for Japanese patients by substituting a nonrenal clearance of 15 for 25, i.e., dose = target AUC x (GFR + 15), the MPE decreased to -0.1% (P<0.001). *Conclusions:* We conclude that 24-h Ccr is acceptably precise for GFR assessment, and the nonrenal clearance of carboplatin is suggested to be lower in Japanese patients with cancer than in their Western counterparts.

Disclosure of Financial Relationships: Honoraria: Astellas, Banyu, Takeda, Novartis, Daiichi-Sankyo, Dainippon-Sumitomo, Kowa, Kirin, Mochida, Ohtsuka, Chugai, Pfizer, Eisai, Tokyo-Tanabe-Mitsubishi,Fuji; Scientific Advisor: Astellas; Other Relationship: Astellas, Banyu, Chugai, Dainippon-Sumitomo, Phizer, Novartis.

**F-PO1219**

**Quantitative Modeling and Simulation To Optimize Dosing in Renally Impaired Patients: Application to Entecavir** Marc Bifano,[1] Frank Lacreta,[1] Alaa Ahmad,[1] Amit Roy,[1] Dennis M. Grasela,[1] Suzanne K. Swan,[2] Marc Pfister.[1] *[1]Discovery Medicine and Clinical Pharmacology, Bristol-Myers Squibb, Princeton, NJ; [2]DaVita Clinical Research, Minneapolis, MN.*

**Background.** Quantitative modeling and simulation can be applied to (i) characterize the relationship between renal function and exposure; in order to maximize the amount of information extracted from single-dose renal impairment studies, and (ii) evaluate different dosing options (dose, schedule) in 'virtual' patients to make dosing recommendations. Such a quantitative approach is presented with an application to entecavir.

**Methods.** Using nonlinear mixed effects modeling, a compartmental model with a function to characterize the effect of 'renal function' on drug clearance was utilized to simulate 'virtual' patients with selected degrees of renal impairment. Simulated steady state exposures were evaluated for alternative dose and schedule regimens with the goal of identifying dosing regimens that achieve target exposures in at least 80% of 'virtual' patients.

**Data and Results.** Consistent with prior knowledge that entecavir is eliminated primarily via renal excretion, trial simulation indicated that (i) patients with mild renal (CrCL ≥ 50 ml/min) impairment do not need a dose adjustment (0.5 mg Q 24h usual dose, 1 mg Q 24h for refractory patients), (ii) patients with moderate renal impairment (CrCL 30 to <50 ml/min) need a 50% dose reduction (0.25/ 0.5 mg Q 24h) or a change in schedule to 0.5/1 mg Q 48h, (iii) patients with severe renal impairment (10 to <30 ml/min) need a 70% dose reduction (0.15/ 0.3 mg Q 24h or a change in schedule to 0.5/1 mg Q 72h, and (iv) patients on hemodialysis or CAPD (< 10 ml/min) need a dose reduction of 90% (0.05/ 0.1 mg Q 24h) or a change in schedule to 0.5/1 mg Q 168h.

**Conclusions.** Quantitative modeling and simulation was successfully applied to entecavir and the dosing information is reflected in the product label. Such a quantitative approach maximizes the amount of information extracted from single-dose renal impairment studies and allows for virtual clinical trials that optimize dosing in renally impaired patients while saving cost and time.

Disclosure of Financial Relationships: Employer: Bristol-Myers Squibb.

**F-PO1220**

**Obesity, Disease Progression and Ramipril Renoprotective Effects in Proteinuric Chronic Nephropathy: A Post-Hoc Analyses of the REIN Study** Francesca Mallamaci,[1] Piero Ruggenenti,[2] Annalisa Perna,[2] Daniela Leonardis,[1] Rocco Tripepi,[1] Giovanni Tripepi,[1] Giuseppe Remuzzi,[2] Carmine Zoccali,[1] Rein Study Group.[3] *[1]CNR-IBIM, Clin. Epid. and Physiopath. of Renal Diseases and Hypertension, Reggio Calabria, Italy; [2]Mario Negri Institute, Bergamo, Italy; [3]Rein Study Group, Italy.*

In observational studies, obesity has been associated with accelerated renal disease progression in CKD patients. The effect of established pharmacological treatment (such as ACE inhibition) has been never specifically tested in overweight and obese CKD patients. We undertook a secondary analysis in the REIN study cohort (n=337) to investigate whether overweight and obesity influence renal events rate and the response to Ramipril.

In the study cohort, 105 patients were overweight (BMI: 25-30 kg/m$^2$) and 49 obese (BMI >30 kg/m$^2$). GFR was 43±18 ml/min/1.73 m$^2$. During the follow-up (average: 30 months; range: 0.2-76 months), 89 patients had renal events [ESRD in 77 cases and doubling of serum creatinine in 12 cases]. In the placebo group, the renal events rate was similar in overweight and in those with normal BMI but higher in obese [Table 1a: combined renal end point; Table 1b: ESRD].

| Table 1a | Crude incidence rate (and 95% CI) of the combined renal end point *(events/100 person-years)* | | Crude hazard ratio and 95% CI and P value *(Ramipril versus placebo)* |
|---|---|---|---|
| | **Placebo group** | **Ramipril group** | |
| <25 kg/m$^2$ | 14 (9-20) | 8 (5-11) | 0.55(0.31-0.99), P<0.05 |
| 25-30 kg/m$^2$ | 14 (8-21) | 7 (3-13) | 0.52(0.24-1.13), P=0.09 |
| >30 kg/m$^2$ | 25 (11-41) | 5 (1-14) | 0.21(0.06-0.81), P=0.02 |

| Table 1b | Crude incidence rate (and 95% CI) of ESRD occurrence *(events/100 person-years)* | | Crude hazard ratio and 95% CI and P value *(Ramipril versus placebo)* |
|---|---|---|---|
| | **Placebo group** | **Ramipril group** | |
| <25 kg/m$^2$ | 10 (7-16) | 6 (4-10) | 0.58(0.31-1.09),P=0.09 |
| 25-30 kg/m$^2$ | 11 (7-8) | 6 (3-12) | 0.55(0.24-1.23),P=0.14 |
| >30 kg/m$^2$ | 24 (11-45) | 3 (0.4-11) | 0.14(0.03-0.65),P=0.01 |

Ramipril reduced renal events as compared to placebo in all BMI strata (Table 1) but the effect of this drug was much higher in obese (incidence rate reduction: combined end point -79%; ESRD -86%) than in those with overweight (-48% and -45%, respectively) or normal BMI (- 45% and -42%, respectively). The effect modification of BMI on the Ramipril response was confirmed in Cox models adjusting for confounders.

In this secondary analysis of the Rein study, obesity predicted a higher incidence of renal events in the placebo arm but such a risk excess was virtually abolished in Ramipril treated patients and the risk reduction by this drug was actually larger in obese than in non obese patients.

Disclosure of Financial Relationships: nothing to disclose

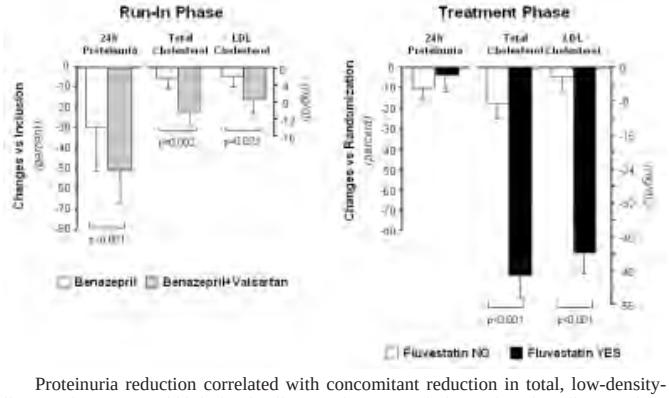
**F-PO1221**

**Effects of Add-On Fluvastatin Therapy in Patients with Chronic Proteinuric Nephropathy on Dual RAS Blockade: Esplanade Trial** Giuseppe Remuzzi. *Mario Negri Institute and Azienda Ospedaliera Ospedali Riuniti di Bergamo, Italy.*

Background: Effects of statins in chronic kidney disease (CKD) patients on optimized antiproteinuric therapy by angiotensin-converting-enzyme (ACE) inhibition and angiotensin receptor blockade (ARB) are not known.

Methods: After 1-month benazepril (10-20 mg/day) therapy followed by 1-month benazepril-valsartan (80-160 mg/day) combined therapy (Run-In), 186 consenting CKD patients with residual proteinuria >0.5 g/24-h were randomized to 6-month benazepril-valsartan therapy alone or combined to fluvastatin (40-80 mg/day). Between-groups changes in proteinuria (centrally measured primary outcome), serum lipids and glomerular filtration rate (GFR, by iohexol plasma clearance) were compared by blinded and intention to treat ANCOVA.

Results: On Run-in, proteinuria decreased more with benazepril-valsartan than benazepril alone.




Proteinuria reduction correlated with concomitant reduction in total, low-density-lipoprotein (LDL) and high-density-lipoprotein (HDL) cholesterol, and apolipoprotein B (p<0.001 for all) and apolipoprotein A (p<0.05) levels. After randomization, proteinuria similarly decreased in the fluvastatin and benazapril-valsartan therapy alone arms. Fluvastatin further reduced total and LDL cholesterol and apolipoprotein B vs benazepril-valsartan alone (p<0.001 for all), but did not affect serum triglycerides and GFR. Treatment was well tolerated.

Conclusions: In CKD patients with residual proteinuria despite ACE inhibitor and ARB therapy, add-on fluvastatin does not affect urinary proteins, but further reduces serum lipids and is safe. Whether combined ACE inhibitor, ARB and statin therapy may help improving cardiovascular outcomes in this high-risk population is worth investigating.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1222**

**Stroke in Patients with Diabetes and CKD Treated with Darbepoetin Alfa. The TREAT Experience** Hicham Skali,[1] Hans-Henrik Parving,[2] Patrick S. Parfrey,[2] Emmanuel A. Burdmann,[2] Eldrin F. Lewis,[1] Peter Ivanovich,[2] Janet B. McGill,[2] Jerome A. Rossert,[2] Ajay K. Singh,[1] Scott D. Solomon,[1] Hajime Uno,[1] Marc Pfeffer.[1] *[1]Brigham & Women's Hospital; [2]On Behalf of TREAT Investigators.*

We sought to investigate baseline and post-randomization (rz) factors that could be associated with the increased incidence of stroke (CVA) observed in the Trial to Reduce cardiovascular Events with Aranesp Therapy (TREAT).

**Methods:** Baseline characteristics were compared between patients with a CVA [n=154/4038 (4%): darbepoetin alfa (darb):101/2012 (5%) and placebo:53/2026 (2.6%)] and those without. A multivariate logistic regression model was used to identify baseline predictors and generate a propensity score for a nested case-control (1:10 matching) analysis that evaluated post-rz blood pressure (BP), hemoglobin level (Hb) and darb dose.

**Results:** Baseline predictors of stroke were: assignment to darb [OR 2.1(95%CI:1.5,2.9)], history (hx) of stroke [2.0(1.4,2.9)], higher proteinuria, hx of CV disease, insulin use and lower Hb or BMI.

Case-control analyses of post-rz factors (1:10 matching), did not show any difference in the most recent value (90 days prior to the stroke) of systolic or diastolic BP, Hb, and darb dose.

| | Darb | | Placebo | |
|---|---|---|---|---|
| **Most recent value-3 months** | **Stroke** | **No Stroke** | **Stroke** | **No Stroke** |
| | **Controls** | **Cases** | **Cases** | **Controls** |
| n | 86 | 923 | 44 | 468 |
| SBP mmHg | 135(120,150) | 134(121,145) | 136(122,148) | 137(124,150) |
| DBP mmHg | 72(64,80) | 71(65,80) | 70(63,80) | 71(64,80) |
| n* | 80 | 866 | 42 | 436 |
| HGB mg/dL | 12.3(11.1,13.1) | 12.5(11.7,13.2) | 10.4(9.4,10.8) | 10.4(9.7,11.3) |
| Darb dose µg/week | 150(100,300) | 150(80,300) | 0(0,0) | 0(0,0) |
| Rescue darb | | | 6(14%) | 82(19%) |

Median(IQR).Stroke vs. no stroke comparisons all NS Wilcoxon test. * lower sample size due to available data

Additional sensitivity analyses using maximal or latest values (or averages) over varying periods of exposure yielded similar results.

**Conclusion:** Rz to darb and prior hx of CVA were strong predictors of a heightened risk of stroke. In the treated group, the increased risk does not appear to be mediated by either higher BP, Hb or darb dose.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1223**

**Prolylhydroxylase Inhibitor Modulation of Erythropoietin in a Randomized Placebo Controlled Trial** Richard A. Brigandi,[1] Steven F. Russ,[2] Mahir Al-Banna,[1] Jianping Zhang,[2] Connie L. Erickson-Miller,[1] Bin Peng.[1, 3] *[1]GlaxoSmithKline, King of Prussia, PA; [2]GlaxoSmithKline, Research Triange Park, NC; [3]Current Address, Novartis, East Hanover, NJ.*

GSK1278863A is a novel small molecule that has demonstrated *in vitro* and *in vivo* inhibition of the hypoxia-inducible factor (HIF) prolyl hydroxylases EGLN1/3. GSK1278863A increases erythropoietin (EPO) protein levels and hemoglobin levels after oral administration in rats, mice and dogs. Through prolylhydroxylase inhibition, GSK1278863A has the potential to modulate HIF targets, including those impacting erythropoiesis, angiogenesis, and iron metabolism and utilization. Study PHX111427 was a single-blind, randomized, placebo controlled, dose-rising, single dose, sequential parallel group study to investigate the safety, tolerability, PK and PD of GSK1278863A in healthy adult subjects. Subjects were dosed sequentially in six ascending cohorts ranging from 2 to 300 mg with randomization to receive GSK1278863A or placebo in a ratio of 2:1. Single dose, oral administration of GSK1278863A was generally well tolerated. Pharmacokinetics were linear with dose proportional increases in plasma exposure as the dose increased from 2 to 300 mg, $T_{max}$ ranged between 1.25-2.00 hours post dose, and $t_{1/2}$ ranged from 0.8-4.0 hours. No consistent statistically significant change, across doses, in pro-hepcidin levels were observed, although there were significant increases in vascular endothelial growth factor (VEGF) observed only at GSK1278863A doses of 150 and 300 mg. As expected, there were no observed effects on hemoglobin following single dose, however, significant increases in circulating plasma EPO were observed at 15, 50, 150, and 300 mg dose levels of GSK1278863A compared to placebo at one or more post-dose time points.

Conclusion: These data indicate that GSK1278863A has the potential to induce erythropoiesis in patients with anemia.

Disclosure of Financial Relationships: Employer: GlaxoSmithKline; Ownership: GlaxoSmithKline.

**F-PO1224**

**Estimated Net Endogenous Acid Production and Serum Bicarbonate in the AASK Cohort Study** Julia J. Scialla,[1] Lawrence J. Appel,[1] Edgar R. Miller,[1] Brad C. Astor,[1] Rulan S. Parekh,[2] Cheryl A. Anderson.[1] *[1]Johns Hopkins University; [2]University of Toronto.*

Metabolic acidosis may contribute to CKD progression. Dietary protein, the major source of fixed acid, and dietary potassium, which is naturally complexed to alkali precursors, can be used to estimate net endogenous acid production (NEAP). We evaluated the cross-sectional association between NEAP and serum bicarbonate (HCO3) in participants from the AASK Cohort Study. Exclusion criteria were >10% decline in weight over 6 months and use of potassium or alkali supplements. Included were 360 African American adults (ages 31 to 76y) with hypertensive CKD (median GFR 44.3 mL/min/1.73 $m^2$). NEAP was estimated using published equations: NEAP (mEq/d) = -10.2 + 54.5 [protein (g/d) /potassium (mEq/d)]. Dietary protein and potassium intake were estimated from the average 24 hour urinary excretion of urea nitrogen and potassium, respectively, during the first year of the study (98% had 2 measurements). Using linear regression adjusted for confounders (age, sex, randomized group during trial phase, BMI, GFR, albuminuria, years of hypertension, diabetes, ACE inhibitor/ARB use, and diuretic use) higher estimated NEAP was associated with lower serum HCO3 in a graded fashion (p trend <0.001). Estimated protein intake was not associated with serum HCO3 (p=0.28), but higher estimated potassium intake was associated with higher serum HCO3 (p trend=0.001).

| | Difference in serum HCO3 (mmol/L) compared to reference | **p-value** |
|---|---|---|
| **Estimated NEAP (mEq/d)** | | |
| Quartile 1 (27.5-57.2) | Ref | |
| Quartile 2 (57.8-70.9) | -0.42 (-1.11, 0.26) | 0.21 |
| Quartile 3 (70.9-86.1) | -0.72 (-1.80, 0.38) | 0.18 |
| Quartile 4 (86.2-199.7) | -1.66 (-2.37, -0.94) | <0.001 |
| **Estimated Potassium Intake (mEq/d)** | | |
| Quartile 1 (10.6-30.2) | Ref | |
| Quartile 2 (30.2-40.2) | 0.57 (-0.47, 1.60) | 0.27 |
| Quartile 3 (40.2-53.4) | 1.33 (0.24, 2.43) | 0.02 |
| Quartile 4 (53.6-106.5) | 1.65 (0.64, 2.65) | 0.003 |

Reducing NEAP through intake of potassium rich foods may prevent metabolic acidosis in patients with CKD, challenging the current practice of potassium restriction.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1225**

**Difference in Age-Related Patterns of Arterial Stiffness among Patients with Kidney Disease** Laurie A. Tomlinson,[1] Helen Eddington,[2] Philip A. Kalra,[2] Chris W. McIntyre,[4] David J. Webb,[5] David C. Wheeler.[3] *[1]University of Cambridge; [2]Salford Hospitals NHS Trust; [3]UCL Medical School; [4]Derby Hospital; [5]University of Edinburgh; [6]University of Birmingham.*

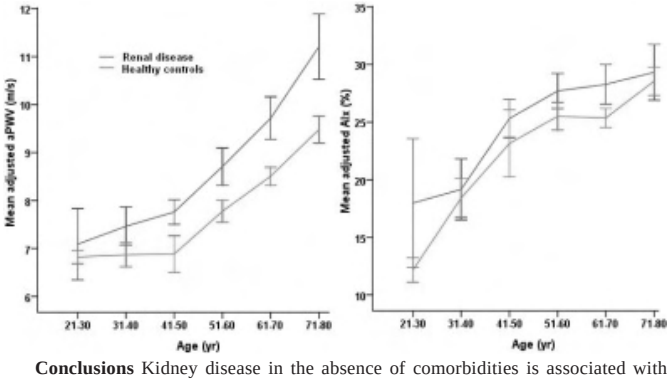
**Background** Patients with CKD may have higher aortic pulse wave velocity (aPWV) and augmentation index (AIx) but studies are limited by size and lack of control population and differences may be due to joint risk factors. The UK Research Alliance Into Kidney Disease And Arterial Stiffness (UREKA) Collaboration examined whether aPWV and AIx are increased in CKD patients with no vascular co-morbidities compared to controls and how change with age varies between populations.

**Methods** Cardiovascular risk factor data, aPWV and AIx were obtained from CKD patients at 8 UK renal centres and participants in ACCT, a community-based study of the general population. Those with diabetes and/or vascular disease were excluded.The relationship of age to aPWV and AIx was compared between patients with CKD (stages 1-5, not on dialysis, non-vascular diagnosis, n=524) and healthy controls (eGFR>60mL/min, n=1535). The control group was stratified by age and gender to ensure comparability.

**Results** Adjusted aPWV and AIx were higher in CKD patients (P<0.001).

| | Kidney disease | Healthy controls |
|---|---|---|
| **Age (y)** | 55±14 | 56±18 |
| | | |
| **eGFR (ml/min)*** | 43±21 | 96±30 |
| **SBP (mmHg)** | 133±20 | 134±19 |
| **DBP (mmHg)** | 80±11 | 80±11 |
| **Adj PWV(m/s)*** | 9.0±2.5 | 7.8±1.8 |
| **Adj AIx (%)*** | 26.0±9.2 | 19.3±8.5 |

There was a significant interaction (P<0.001) between age and the presence of CKD on aPWV, but this was not seen for AIx (P=0.19).



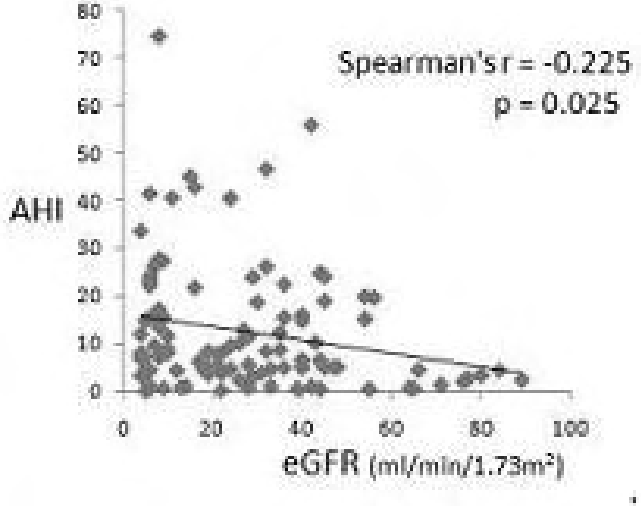

**Conclusions** Kidney disease in the absence of comorbidities is associated with increased arterial stiffness compared to controls. In CKD patients aPWV increases more rapidly with age than controls but there was no difference in the pattern of change of AIx with age between groups.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1226**

**Obstructive Sleep Apnea in Non-Dialysis Chronic Kidney Disease Patients**
Yusuke Sakaguchi, Akira Suzuki, Tatsuya Shoji, Yoshiharu Tsubakihara. *Kidney Disease and Hypertension, Osaka General Medical Center, Osaka, Japan.*

**Background**
Recently, obstructive sleep apnea (OSA) has been widely recognized as an important risk factor for cardiovascular disease. Although a high prevalence of OSA was reported in ESRD patients, there is little information regarding OSA in non-dialysis CKD patients. The aim of this study was to examine OSA in non-dialysis CKD patients and elucidate the relationship between renal function and the prevalence and severity of OSA.
**Study design**
Cross-sectional study.
**Setting** & Participants
Consecutive patients hospitalized mainly for CKD educational program or renal biopsy from June 2009 to November 2009.
**Predictors**
Age, sex, body mass index (BMI), diabetes mellitus, eGFR, history of cardiovascular disease and history of stroke.
**Outcomes**
The prevalence and severity of OSA.
**Measurements**
Apnea-hypopnea index (AHI) measured by type 3 (cardiorespiratory) monitor devices (Morpheus; Teijin Pharma Ltd.).
**Results**
One hundred patients (men 68%), median (interquartile) age 66.5 (58.0, 74.8) years, BMI 23.1 (20.8, 24.8) kg/m$^2$, eGFR 28.5 (8.0, 40.0) ml/min/1.73m$^2$ and 31% with diabetes mellitus were enrolled. Overall, 65% of the subjects were suffering from OSA (mild (5≤AHI<15): 32%, moderate (15≤AHI<30): 25%, and severe (30≤AHI): 8%). In multiple logistic regression analysis, lower eGFR was significantly associated with increased prevalence of OSA adjusted for age, sex, BMI, diabetes mellitus, history of cardiovascular disease and history of stroke (adjusted odds ratio 0.97, 95% confidence interval 0.97 to 0.99). Moreover, in a generalized linear model, eGFR was inversely correlated with AHI after adjusting for covariates (p=0.007).




**Conclusions**
Our data demonstrated that the prevalence of OSA was also high in non-dialysis CKD patients and suggested the vicious cycle between the decline of GFR and OSA.
Disclosure of Financial Relationships: nothing to disclose

**F-PO1227**

**Gender Differences in the Prevalence of Depression and Increased Stress among Older Americans with Chronic Kidney Disease**
Nancy G. Kutner,[1,2,3] John J. Isitt,[2,3] Suzanne E. Judd,[2] Brian D. Bradbury,[2,3] Manjula Kurella Tamura,[2] Virginia G. Wadley,[2] Neil A. Zakai,[2] David G. Warnock,[2] William M. McClellan.[2] *[1]Emory University School of Medicine, Atlanta, GA; [2]For the Renal REGARDS Working Group, UAB, Birmingham, AL; [3]Amgen Inc., Thousand Oaks, CA.*

Introduction. Reduced kidney function may be associated with increased psychological distress, but there has been little investigation of gender differences in the prevalence of depressive symptoms and stress among individuals with impaired kidney function. Methods. Participants in a population-based cohort study aged 45 years with a urine albumin-to-creatinine ratio of >= 30 mg/g or eGFR < 60 ml/min/1.73 m$^2$ were included in the study. Recruitment was conducted between 2003 and 2007. Our main exposure was hemoglobin level. Outcomes included a four-item Center for Epidemiologic Studies Depression Scale (CES-D-4) score4 and a short four-item Cohen's perceived stress scale (PSS-4) score7. Covariates included age, sex, race, education, income, marital status, history of heart disease, hypertension, diabetes, and stroke, cigarette and alcohol use and access to medical care. The association between gender and individual outcomes was assessed by multivariable logistic regression. Results. The 3,817 individuals with CKD, 20.3% of all subjects, had a mean age 68.3 years, 39.8% males and 45.9% African American. Elevated depressive symptoms were reported for 6.9% of men and 10.9% of women, M:F OR (95% CI) adjusted for age and race was 0.63 (0.51, 0.79); 12.4% of men and of 20.0% women reported high stress, age- and race-adjusted M:F OR (95% CI) =0.62 (0.53, 0.73). After adjustment for demographic characteristics, socioeconomic status, behavioral factors and comorbidity, the association between gender and depressive symptoms was attenuated and no longer significant, M:F OR (96%CI), 0.77 (0.56, 1.04). In contrast, age- and race-adjusted associations between gender and stress persisted after adjustment for other covariates, M:F OR (95%CI) = 0.63 (0.50, 0.80). Conclusion. Women with CKD have increased prevalence of high stress compared to men which cannot be fully explained by other participant attributes.
Disclosure of Financial Relationships: nothing to disclose

**F-PO1228**

**Does Lower Hemoglobin Explain Gender Differences in the Prevalence of Depressive Symptoms and Increased Stress among Older Americans with Chronic Kidney Disease?**
Nancy G. Kutner, Suzanne E. Judd, Manjula Kurella Tamura, Virginia G. Wadley, Neil A. Zakai, David G. Warnock, William M. McClellan. *Renal REGARDS Working Group, UAB, Birmingham, AL.*

**Introduction.** We assessed gender differences in the prevalence of depressive symptoms (DS) and stress among individuals aged 45 years and older with a urine albumin-to-creatinine ratio of >= 30 mg/g or an eGFR less than 60 ml/min/1.73 m$^2$ in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study. **Methods.** We defined our exposure as hemoglobin <12 g/dL (HGB); elevated DS as a four-item Center for Epidemiologic Studies Depression Scale (CES-D-4) score ≥ 4; and high stress as a four-item Cohen's perceived stress scale (PSS-4) score ≥ 7. We adjusted for age, race, education, income, marital status, history of heart disease, hypertension, diabetes, and stroke, cigarette and alcohol use and access to medical care. The association between HGB and individual outcomes was assessed by multivariable logistic regression. **Results.** There were 3,817 individuals with CKD, 20.3% of all subjects; mean age 68.3 years, 39.8% men and 45.9% African American. Overall 10.9% of women and 6.9% of men had elevated DS and high stress was reported by 20.0% of women and 12.4% of men (Table). HGB was more prevalent in women (26.3%) then men (9.8%), OR (95% CI) = 2.27 (1.96, 2.64). Men without HGB were substantially less likely to have elevated DS or high stress than women while the prevalence of both increased in men and women with HGB (Table). The fully adjusted M:F OR (95% CI) for elevated DS was 0.67 (0.48, 0.95) for individuals without and 1.74 (0.84, 3.63) for those with HGB. Comparable M:F OR (95%CI) for high stress were 0.53 (0.41, 0.68) and 1.71 (0.96, 3.05). **Conclusion.** Rates of elevated DS and high stress increased substantially more in men than in women with HGB.

| | Elevated DS | | M:F OR (95% CI) | High Stress | | M:F OR (95% CI) |
|---|---|---|---|---|---|---|
| Hgb g/dL | Male | Female | Fully adjusted | Male | Female | Fully adjusted |
| ≥12 | 6.3% | 10.7% | 0.67 (0.48, 0.95) | 11.7% | 20.3% | 0.53 (0.41, 0.68) |
| <12.0 | 12.3% | 11.5% | 1.74 (0.84, 3.63) | 19.6% | 19.2% | 1.71 (0.96, 3.05) |

Disclosure of Financial Relationships: nothing to disclose

**F-PO1229**

**Anemia and Risk of Reduced Health-Related Quality of Life among Older Americans with Chronic Kidney Disease in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study**
John J. Isitt,[1,2] Nancy G. Kutner,[1] Suzanne E. Judd,[1] Manjula Kurella Tamura,[1] Virginia G. Wadley,[1] Neil A. Zakai,[1] David G. Warnock,[1] Brian D. Bradbury,[1,2] William M. McClellan.[1] *[1]Renal REGARDS Working Group, UAB, Birmingham, AL; [2]Amgen Inc, Thousand Oaks, CA.*

**Introduction.** This study examines reduced health-related quality of life (HRQoL) among older adults with chronic kidney disease (CKD). **Methods.** Participants 45 years in a population-based cohort study with a urine albumin-to-creatinine ratio of >= 30 mg/g or an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m$^2$ were included. Primary exposure was hemoglobin (HGB). HRQoL was concurrently assessed by reported health status, frequency and intensity of physical activity and the Medical Outcomes Study Short Form-12 mental (MCS) and physical (PCS) composite scores. Associations between HGB and HRQoL were assessed with multivariable regression models controlled for age, sex, race, socioeconomic status, health behaviors and co-morbidity. **Results:** Among 3,817 individuals with CKD the prevalence of poor health, decreased activity and absence of intense activity all increased as HGB level declined from 12 to <10.0g/dL. In fully adjusted models stratified by gender the mean MCS was not associated with HGB while PCS decreased as HGB decreased. The OR for poor health, being less active, not engaging in intense activity increased, compared to the referent level 12 g/dL, among men as HGB levels declined, while among women only poor health increased (Table). **Conclusions.** In these individuals with CKD an inverse association between HGB levels and measures of physical function and poor health was observed in men and women and lower activity was more evident among men.

| | Men | Women |
|---|---|---|
| Hgb g/dL | Poor Health: OR (95%CI) | |
| 10-11.9 | 1.58 (0.72, 3.45) | 1.31 (0.81, 1.36) |
| <10 | 9.2 (2.08, 41.0) | 3.23 (1.27, 8.22) |
| | Less active: OR (95%CI) | |
| 10-11.9 | 1.14 (0.89, 1.15) | 1.37 (1.07, 1.74) |
| <10 | 4.52 (1.28, 16.4) | 1.30 (0.67, 2.53) |
| | No intense activity: OR (95%CI) | |
| 10-11.9 | 1.48 (0.98, 2.23) | 1.17 (0.93, 1.47) |
| <10 | 2.85 (0.83, 9.78) | 0.70(0.37, 1.32) |

Disclosure of Financial Relationships: Employer: Employer: I work in the Global Health Economics Department at Amgen, Inc.

**F-PO1230**

**Leg Muscle Cross-Sectional Area Is Associated with Serum 1,25-Dihydroxyvitamin D Level in Chronic Kidney Disease Stage 3 and 4** Patricia L. Gordon,[1,2] Julie W. Doyle,[2] Kirsten L. Johansen.[1,2] *[1]Medicine, SFVAMC, San Francisco, CA; [2]Nephrology Division, UCSF, San Francisco, CA.*

Declines in 1,25-dihydroxyvitamin D ($1,25(OH)_2$ D) follow the course of chronic kidney disease (CKD). The molecular mechanisms of vitamin D in skeletal muscle are well known, but there is little information regarding vitamin D status and muscle loss in patients with CKD. Thus we examined baseline data of an ongoing blinded randomized controlled trial of paricalcitol on muscle size in CKD patients stage 3 and 4. We previously reported that $1,25(OH)_2$ D but not 25-hydroxyvitamin D (25(OH)D) was associated with walking speed and distance, leg strength and balance. We now report the results of magnetic resonance imaging (MRI) of the thigh muscle in the same group of patients (22 M, 2 F, 60 ±13 years), stage 3 (n = 14) and 4 (n = 10) CKD. Clinical measures include eGFR (31±9 ml/min by MDRD), iPTH (132, 74-249 pg/ml), albumin (3.6±0.56 gm/dL), plasma calcium ($Ca^{2+}$) (9.01±0.44 mg/dL), 25(OH)D (20.3, 7.0-39.8 ng/ml) and $1,25(OH)_2$D (19.7, 5-74 pg/ml). Muscle cross-sectional area (MCSA) of the quadriceps was determined by MRI. Overall 67% of the subjects were either 25(OH)D deficient (n = 8), or insufficient (n = 8) but there was no association between 25(OH)D levels and MCSA. MCSA was associated with eGFR (r=0.54, p=0.006), but there was no association of age, sex, albumin or iPTH with MCSA. Using least-squares regression, variance in MCSA was best explained (adj $R^2$ = 0.45, p =0.005) by a model containing $1,25(OH)_2$D (β =0.635, p=0.02), plasma $Ca^{2+}$ (β = -13.98, p=0.02), and daily physical activity (PA) measured by accelerometry (β =0.008, p=0.02). The negative coefficient of plasma $Ca^{2+}$ with MCSA is supported by reports that sedentary behavior is associated with higher plasma $Ca^{2+}$ due to cellular damage associated with changes in $Ca^{2+}$ metabolism and transport leading to loss of $Ca^{2+}$ into plasma. There was no association of $1,25(OH)_2$D with plasma $Ca^{2+}$ or PA, although $Ca^{2+}$ was inversely but not significantly related to PA. These results suggest that $1,25(OH)_2$D, known to be essential to muscle function may be an important determinant of muscle size in patients with stage 3 and 4 CKD.

Disclosure of Financial Relationships: Research Funding: Abbott Laboratories.

**F-PO1231**

**Health Related Quality of Life as a Predictor of Adverse Outcomes in African Americans with Chronic Kidney Disease** Anna C. Porter,[1] Deborah Heffley Brooks,[2] Marino A. Bruce,[2] Jennifer J. Gassman,[2] Cynthia A. Kendrick,[2] Keith C. Norris,[2] Michael J. Fischer,[1] Denyse Thornley-Brown,[2] Mark L. Unruh,[2] James P. Lash.[1] *[1]Medicine, U. Illinois, Chicago, IL; [2]For the AASK Study Group.*

Health-related quality of life (HRQOL) has been associated with increased risk for hospitalization and mortality in patients with ESRD, but has not been evaluated as a predictor in CKD. We examined the relationship of baseline measures of HRQOL with CKD progression, cardiovascular (CV) events, and all-cause mortality among 691 participants with hypertensive CKD in the African American Study of Kidney Disease and Hypertension (AASK) Cohort Study. HRQOL was assessed using the Medical Outcomes Study Short Form (SF-36). At baseline, mean participant SF-36 Mental Health Composite (MHC) and Physical Health Composite (PHC) scores were 40.4±10 and 46.3±10.5, respectively. As summarized in the table, Cox proportional hazard models were used to assess associations between HRQOL and risk for CV events, CKD progression, and mortality, adjusting for age, sex, eGFR, proteinuria, and CVD history.

| | No. events | Mean time to event (mos.) | PHC Unadjusted RR per 5-pt. lower score | PHC Adjusted RR per 5-pt. lower score | MHC Unadjusted RR per 5-pt. lower score | MHC Adjusted RR per 5-pt. lower score |
|---|---|---|---|---|---|---|
| CV events | 96 | 47.7 | 1.19 (1.08,1.3) p=0.001 | 1.11 (1,1.23) p=0.04 | 1.09 (0.99,1.2) p=0.07 | 1.03 (0.93,1.13) p=0.6 |
| Doubling of SCr/ESRD | 143 | 48.7 | 1.08 (0.99,1.17) p=0.07 | 0.99 (0.9,1.07) p=0.7 | 1.05 (0.97,1.13) p=0.2 | 0.98 (0.91,1.07) p=0.7 |
| Death | 99 | 54.7 | 1.16 (1.06,1.28) p=0.002 | 1.13 (1.02, 1.26) p=0.02 | 1.00 (0.91,1.1) p=0.9 | 1.00 (0.9,1.12) p=0.9 |

Lower baseline SF-36 PHC but not MHC scores were associated with increased relative risk of CV events and death. Neither PHC nor MHC scores were associated with CKD progression. Future work should focus on mechanisms underlying the association between PHC scores and adverse outcomes.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1232**

**Dyslipidemia and Mortality in CKD: NHANES III** Magdalena B. Sikora,[1] Guo Wei,[1] Alfred K. Cheung,[1,2] Bradley C. Baird,[1] Tom H. Greene,[1,2] Srinivasan Beddhu.[1,2] *[1]Univ Utah; [2]VA, SLC, UT.*

Uremic dyslipidemia is characterized by ↑serum triglyceride (TG) levels and ↓ serum HDL-cholesterol (C) levels. As statins are not effective against these, this might be a potential explanation for the lack of efficacy of statins in dialysis patients. However, it is unclear whether ↑serum TG and ↓ serum HDL-C levels are associated with ↑ mortality in earlier stages of CKD. We examined these associations in National Health And Nutrition Examination Survey (NHANES) III, a complex, multistage survey conducted by the National Center for Health Statistics (NCHS) in 1988 to 1994. Mortality data were obtained by NCHS by linkage with National Death Index records through December 31, 2006. The sub-population with CKD (eGFR < 60 ml/min/1.73 $m^2$) and fasting (8 hrs or more) lipid profile were studied (n=1269).

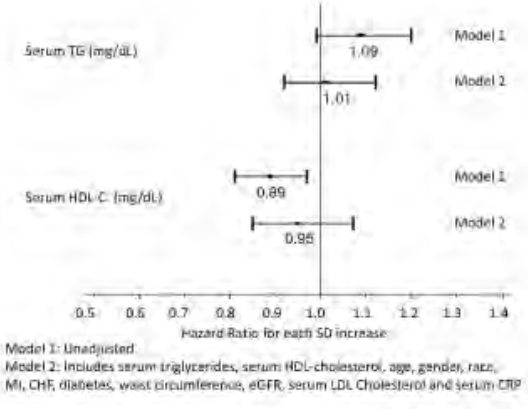
Baseline characteristics by the levels of serum TG are summarized in Table 1.

Baseline Characteristics in CKD Sub-population by Triglyceride Levels

| | TG < 150 mg/dl (42%) | TG ≥ 150 mg/dl (58%) |
|---|---|---|
| TG (mg/dL) | 105±2 | 262±6 |
| Age (years) | 69.7±0.9 | 69.4±0.8 |
| Male (%) | 36 | 37 |
| AA Race (%)** | 10 | 5 |
| MI (%)* | 11 | 20 |
| CHF(%)* | 9 | 15 |
| DM (%)** | 13 | 27 |
| Waist (inch)** | 37±0.4 | 39±0.4 |
| GFR (ml/min/1.73m²) | 49±0.7 | 49±0.4 |
| HDL-C (mg/dL)** | 56±1 | 44±1 |
| LDL-C (mg/dL) | 143±2 | 146 ±3 |

Percentages shown as percent; Continuous measures shown as mean ± standard error; * 0.001< P ≤0.05, ** P ≤0.001.

In unadjusted as well as adjusted analyses, higher serum TG were associated with non-significant ↑ in mortality. Higher serum HDL-C levels were associated with ↓ mortality risk which was attenuated when adjusted for factors that might potentially fall in the causal pathway between HDL-C and mortality (Figure 1).




Lower HDL-C levels might be associated with ↑ mortality in CKD. Interventional trials are warranted to determine the causal effects of increasing HDL-C on mortality in CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1233**

**Barriers to Exercise among Dialysis Patients** Cynthia Delgado,[1] Lindsey Storer,[1] Julie W. Doyle,[1] Kirsten L. Johansen.[1,2] *[1]Nephrology, UCSF, San Francisco, CA; [2]Nephrology, SFVAMC, San Francisco, CA.*

Patients with chronic kidney disease (CKD) are inactive compared to sedentary individuals. Physical inactivity is a strong predictor of mortality in CKD patients and associated with poor physical functioning in this population. Recent evaluation of practice patterns demonstrated low exercise counseling behavior among nephrologists. However, patient barriers must also be identified in order to increase physical activity (PA).

Methods: Patients greater than age of 21 were recruited from dialysis centers affiliated with UCSF and Satellite Dialysis, which provide care for more than 250 dialysis patients. The study survey was composed of questions designed to ascertain information in the following areas: self reported level of physical functioning, PA recall, patient PA preference and barriers to PA. Components of the Physical Activity Scale for the Elderly (PASE) and the 7-day Physical Activity Recall, which are validated in original form, were abstracted to create the study instrument. Univariate and multivariable linear regression analyses were performed to study the association between reported barriers to PA and reported levels of PA.

Results: A total of 100 dialysis patients agreed to participate, the majority were male (73%) mean age of 60 ±15 years. 27% identified themselves as white, 30% Black and 21% Hispanic. The majority of participants strongly agreed that sedentary lifestyle was a health risk (98%) and that increasing exercise was a benefit (98%). Only 8% of participants reported no barriers to PA. Commonly reported barriers to PA were fatigue on dialysis days and non-dialysis days (67% and 40%, respectively) and shortness of breath (48%). Older patients reported fewer barriers to exercise (p<0.002). There were no differences in reported barriers by sex or race/ethnicity. In multivariate analysis, a greater number of reported barriers was associated with lower levels of PA (p <0.02) when adjusted for age and sex.

Conclusion: Low levels of PA were associated with higher numbers of reported barriers. Dialysis fatigue was the most commonly reported barrier. Future studies should focus on addressing such barriers in an effort to increase levels of PA.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1234**

**Extracellular Volume and Disease Progression in Children with Chronic Kidney Disease** Alison G. Abraham,[1] Alvaro Munoz,[2] Susan L. Furth,[2] Bradley A. Warady,[2] George J. Schwartz.[2] *[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health; [2]CKiD Investigator.*

Extracellular volume (ECV) is the fluid contained in all non-cellular compartments of the body and is a tightly controlled quantity. A primary function of the kidneys is maintenance of the fluid-solute balance through volume control and filtration. Thus, there is a strong link between ECV and kidney function. As a physiologic metric, ECV may serve an important role as an indicator of disease progression and cardiovascular disease risk. The direct calculation of ECV is viable only through the direct assessment of plasma disappearance of an injected marker. The Chronic Kidney Disease in Children (CKiD) study uses injected iohexol to obtain direct measures of glomerular filtration rate (GFR) and the descriptors of the fast and slow components of the disappearance curves were used to calculate ECV from 790 iohexol studies with medians for GFR= 43.4 ml/min per 1.73 m², weight= 35 kg and height= 1.4 m. The median ECV was 8.6 L (IQR: 5.9L to 12.2 L) and the median ECV normalized to body weight (ECV/wt) was 0.23 L/kg (IQR: 0.20 to 0.25 L/kg). ECV/wt declined with maturation, which was consistent with previous reports. We found ECV to be a function of height [m] and weight [kg] according to the relationship ECV=√wt×ht ($R^2$=0.92). Using linear regression methods on the log-transformed variables, biomarkers of kidney disease (serum creatinine, BUN and cystatin C) were significantly related to ECV/wt ($P<0.05$) but the strength of association was small compared to relationships with GFR. We found no significant association between ECV/wt and hypertension, defined as systolic blood pressure > 95th percentile for age, sex and height, or edema. However edema was only reported at 3% of study visits. In this pediatric cohort ECV was related to CKD progression but to a lesser degree than to GFR and was not a clear predictor of hypertension or edema, though the small numbers of edematous participants may have hindered the detection of a relationship. Nevertheless, ECV has relevance to fluid and electrolyte management and can be easily estimated from the square root of weight and height.

Disclosure of Financial Relationships: nothing to disclose

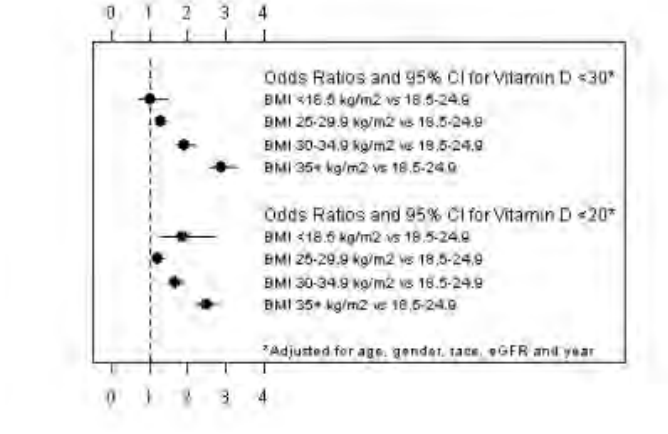
**F-PO1235**

**Obesity Is Associated with Higher Rates of Vitamin D Deficiency among CKD Patients** Sankar D. Navaneethan,[1] Jesse D. Schold,[2] Welf Saupe,[3] Susana Arrigain,[2] James F. Simon,[1] Joseph V. Nally.[1] *[1]Nephrology and Hypertension, Cleveland Clinic; [2]Quantitative Health Sciences, Cleveland Clinic; [3]eResearch, Cleveland Clinic.*

**Purpose:** Prevalence of obesity and chronic kidney disease (CKD) are increasing and vitamin D insufficiency and deficiency is widely prevalent in CKD. Obesity is associated with low vitamin D levels in people without CKD. We examined the association of body mass index (BMI) with vitamin D levels in patients with CKD.

**Methods:** Patients with stage 3 CKD (GFR 30-59 ml/min/1.73m2) who had vitamin D levels measured after their inclusion in our Cleveland Clinic CKD registry (n=11,857) were included in this analysis. We conducted separate analysis for patients with vitamin D levels <30 ng/ml and < 20 ng/ml.

**Results:** Sixty-five % of patients (n=7770) had vitamin D levels <30 ng/ml and 32% had levels <20 ng/ml during follow up. The proportion of patients with vitamin D levels <30 ng/ml increased with BMI (BMI 18.5-24.9: 55% vs. BMI 25-29.9: 63% vs. BMI 30-34.9: 72% vs. BMI >35: 79%). In the multivariable analysis, male gender (OR 1.30, 95% C.I. 1.19-1.41), African American race (OR 1.78, 95% C.I.1.56-2.04), overweight and obesity were associated with vitamin D levels <30 ng/ml.




Increasing age and higher GFR levels were inversely associated with vitamin D levels <30 ng/ml. Analysis restricted to vitamin D levels <20 ng/ml yielded similar results. 7% of patients with vitamin D levels <30 ng/ml died while 5% with vitamin D ≥30 ng/ml died during the follow-up ($p<0.001$).

**Conclusions:** Vitamin D insufficiency and deficiency are widely prevalent in overweight and obese stage 3 CKD patients. We observed a dose-response relationship between BMI and low vitamin D levels and increased mortality among those patients with low vitamin D levels. Future studies should address the impact of these associations and interventions on morbidity and mortality in CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1236**

**Concurrent Complications of Chronic Kidney Disease (CKD) by Level of Albuminuria and Estimated GFR (eGFR)** Lesley A. Stevens,[1] Josef Coresh,[2] Andrew S. Levey,[1] Marcello Tonelli,[3] Paul Muntner.[4] *[1]Tufts Medical Center; [2]Johns Hopkins University; [3]University of Alberta; [4]University of Alabama at Birmingham.*

**Background** Higher levels of albuminuria are associated with increased risk for mortality, cardiovascular disease and kidney outcomes independent of eGFR. It has not been established whether albuminuria is associated with concurrent complications of CKD independent of eGFR.

**Methods** The association of spot albumin-to-creatinine ratio (ACR) level with anemia (hemoglobin < 12 g/dL for women and <13.5 g/dL for men), acidosis (bicarbonate < 22 mmol/L) and hyperphosphatemia (phosphate ≥ 4.5 mg/dL) was assessed using 30,528 participants, ≥ 20 years of age, in the National Health and Nutrition Examination Survey 1988-1994 and 1999-2006. GFR was estimated using the CKD-EPI equation.

**Results** The table shows age adjusted associations of ACR and eGFR with anemia, acidosis and hyperphosphatermia. Results were similar in models adjusted for age, race, sex and eGFR/ACR and stratified by eGFR/ACR.

**Conclusions** After adjustment for eGFR, albuminuria was associated with anemia, but not acidosis or hyperphosphatemia. Algorithms for management of concurrent complications, as well as for risk stratification of concurrent complications and future events, may need to be tailored to specific outcomes.

Table: Age adjusted odds ratios for CKD complications associated with level of albuminuria and eGFR.

| ACR, mg/g | Anemia | Acidosis | Hyperphosphatemia |
|---|---|---|---|
| <10 | 1 (ref) | 1 (ref) | 1 (ref) |
| 10–29 | 1.35(1.31–1.59) | 1.25(1.05–1.49) | 1.13(0.93–1.36) |
| 30–299 | 1.59(1.31–1.92) | 1.37(1.13–1.67) | 1.04(0.82–1.33) |
| ≥300 | 2.85(2.14–3.79) | 1.28(0.89 –1.84) | 0.88(0.60–1.29) |
| p-trend | <0.001 | 0.175 | 0.453 |
| eGFR, ml/min/1.73m² | | | |
| ≥90 | 1 (ref) | 1 (ref) | 1 (ref) |
| 60–89 | 0.70(0.59–0.82) | 1.34(1.07–1.67) | 1.46(1.24–1.71) |
| 45–59 | 1.45(1.19–1.77) | 1.97(1.38–2.80) | 2.30(1.75–3.02) |
| 30–44 | 3.07(2.34–4.01) | 4.81(3.25–7.11) | 2.83(1.81–4.45) |
| <30 | 9.65 (6.29–14.8) | 13.5(9.24–19.7) | 8.61(5.12–14.5) |
| p-trend | 0.006 | <0.001 | <0.001 |

Disclosure of Financial Relationships: Consultancy: Orexigen Therapeutic Inc. Research Funding: Gilead Inc.

**F-PO1237**

**The Relationship between Cognitive and Physical Function in Patients with CKD** Patricia Lynn Painter,[1] Katy Van Schyndle,[1] Deirdre A. Palmer,[1] Marc L. Weber.[1] *[1]School of Nursing, University of Minnesota, Minneapolis, MN; [2]School of Nursing, University of Minnesota, Minneapolis, MN; [3]Division of Renal Diseases, University of Minnesota, Minneapolis, MN; [4]Division of Renal Diseases, University of Minnesota, Minneapolis, MN.*

It is well documented that patients with CKD have low physical functioning (PF) and significant cognitive impairment. It is unclear whether there is an relationship between low PF and cognitive impairment. In this study, we aimed to characterize the relationship between PF and cognitive function (CF) in older patients with CKD and to compare levels in patients treated with hemodialysis (HD) and those with reduced renal function (CKD). Fifty one patients (21 HD and 30 CKD) were tested once using the Montreal Cognitive Assessment tool (MoCA)and the Short Physical Performance Battery (SPPB, which assesses lower extremity function). A shuttle walk test (SWT) (a progressive walking test) was also used to assess PF. Physical activity was assessed using step counters for 7 days. Comparisons were done using independent T-tests and X2 for categorical variables. The HD group (10 males/11females age 63.8± 8.5 y) was on dialysis an average of 45.0±35.8mo. The CKD group (17 males/13 females; 68.7± 9.6 y) had eGFR of 37.4±15.9 ml/min/1.73m2 (range 12 to 71). The CKD group had higher average steps/day (5909±4735 vs. 2713 ± 1996; p=0.009), longer distance on the SWT (297.7±121.1 vs. 121.4 ± 83.8 m' p=0.006), and higher MoCA scores (23.7 ± 4.6 vs 19.9 ± 5.1; p=0.01). More HD had MoCA scores <26 (mild cognitive impairment cutpoint) (95.3%) compared to 56.6% of CKD (p=.002). Only 23.8% of the HD group had SPPB scores in the highest category (10-12) compared to 50% of the CKD group. The MoCA score was significantly correlated with the SPPB total score (r=.39; p=.006), SWT distance (r=.39; p=.007) and peak walk speed on the SWT (r=.438; p=.002). CKD patients, whether treated with HD or not have significant cognitive impairment and low levels of PF. The significant relationship between CF and PF warrants further study to develop interventions to prevent or attenuate deterioration.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1238**

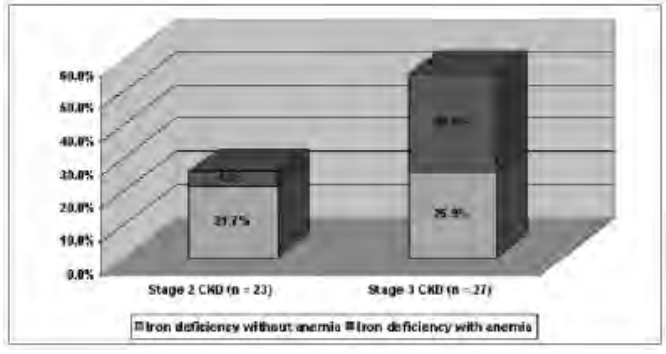
**Anemia and Iron Deficiency in Children with Early Chronic Kidney Disease** Rossana G. Baracco Maggi, Sermin Saadeh, Rudolph P. Valentini, Tej K. Mattoo, Amrish Jain. *Pediatric Nephrology, Children's Hospital of Michigan/Wayne State University, Detroit, MI.*

**Background**: Normocytic, normochromic anemia is a well known complication of advanced chronic kidney disease (CKD). Very little is known about anemia and iron deficiency (ID) in children with early CKD.

**Objectives**: To determine the frequency of ID and iron deficiency anemia (IDA) in early CKD.

**Methods:** Retrospective chart review of 50 patients 0 to 18 years of age with CKD stages 2 and 3. Anemia was defined per KDOQI guidelines, according to the age and sex of the patient. ID was defined as transferin saturation (Tsat) <20% and low ferritin according to patient's age, and/or patient already on iron supplementation. IDA was defined as ID plus low hemoglobin (Hb). GFR was calculated using the Schwartz formula. The lowest hemoglobin while the patient was in CKD stage 2 and 3 was identified and the average of three consecutive levels was used for analysis. The GFR, ferritin level and Tsat done at the time of low Hb were noted.

**Results:** The study included 36 (72%) male and 14 (28%) female patients; 23 (46%) had obstructive uropathy or dysplastic kidneys, 12 (24%) had vesicoureteral reflux, 11 (22%) had focal segmental glomerulosclerosis, the rest had other causes of CKD. The mean GFR was 55.4. 21 (42%) patients had ID and of these, 9 (42.9%) patients had anemia. The frequency of ID without anemia increased from 21.7% to 25.9% from stage 2 to stage 3 and the frequency of ID with anemia increased from 4.3% to 29.6% from stage 2 to stage 3 respectively.




Females had a higher frequency of IDA than males (28.6% vs 13.9% respectively), as well as higher frequency of ID (35.7% vs 19.4% respectively).

**Conclusions:** ID is common in children with CKD 2 and 3, and 42.9% of such patients present with anemia. IDA increases with advancing CKD. Early identification and management of IDA in early CKD is recommended.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1239**

**Supplementary School-Based Education Services for Children with Mild to Moderate Chronic Kidney Disease** Arlene C. Gerson, Susan R. Mendley, Stephen R. Hooper, Matthew Matheson, Shlomo Shinnar, Debbie S. Gipson, Judith Jerry-Fluker, Amira Al-Uzri, Robert W. Butler, Bradley A. Warady, Susan L. Furth. *CKiD Neurocognitive WorkGroup.*

Purpose: Final educational attainment and successful employment lag in adults with childhood onset chronic kidney disease (CKD) compared to their unaffected peers. We sought to determine the proportion of youth with mild to moderate CKD receiving supplementary school services and variables associated with receipt of extra help.

Methods: In a cross-sectional analysis of youth enrolled in the Chronic Kidney Disease in Children (CKiD) study with complete data for all variables of interest, 219 children were available for analysis. Multiple logistic regression was used to identify risk factors for receipt of supplementary school services. We controlled for variables suspected of influencing school performance including age, race, gender, SES, GFR, %life with CKD, low birth weight, proteinuria, height percentile and anemia.

Results: Study population was 15% Black, 40% female, with median age 12.5 (IQR 9.6-15.2), height percentile 23.2 (IQR: 8.0-54.3), GFR 42.4 ml/min/1.73m$^2$ (IQR: 33.3-52.1), percent of life with CKD =90 (IQR: 35-100). The presence of poor academic achievement (WIAT-IIA Total Achievement Score < 85), parent observation of social functioning problems (PedsQL Social Subscale < 62) and/or parent observation of school functioning problems (PedsQL School Subscale <57), were associated with receipt of supplementary school services (OR 7.7, p<.01; OR 2.6, P<.05, OR 3.9, p<.01 respectively). Short stature (p=.04) and marked proteinuria (urine protein/creatinine ratio >2, p=.01)) were also associated with receipt of supplementary school services. 59% of the group could be considered at risk for poor academic performance, poor school functioning and/or poor social functioning problems, yet only 36% received supplementary school services.

Conclusion: A subtantial fraction of youth with CKD and risk factors for poor school performance do not receive supplementary educational services to which they may be entitled. Further prospective studies on the effectiveness of supplementary school interventions are appropriate.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1240**

**Bioimpedance Spectroscopy (BIS) Derived Normalized Extracellular Water (nECW) as an Index Hydration Status: A Tool To Attain Fluid Balance** Mauricio Paredes-Fernandez, Klearly M. Tinoco, Ricardo Correa-Rotter. *Nephrology and MM, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico, DF, Mexico.*

Introduction: BIS is a multifrequency bioimpedance technique that assesses hydration. Volume expansion of dialysis pts can be estimated by BIS, yet comparison with reference values from ECW measurements of a demographically and ethnically similar yet healthy population would enhance accuracy to estimate dry weight.

Purpose: To describe reference values for ECW in a Mexican population and to analyze normalized ECW (nECW) correlations with demographic and somatometric variables to generate a mathemathical model to predict ECW.

Methods: We performed BIS (3 measurements) with a Body Composition Monitor (BCM®, Fresenius Medical Care) in healthy adults, 3 hours fasted. ECW was normalized with: a)intracellular water (ECW/ICW), b)Height (ECW/height), c)total body water (ECW/TBW). We also analyzed correlation of these values with age and body surface area (BSA) to obtain linear regression specific formulas for nECW normal values in euvolemic controls.

Results: 200 subjects (male=90), age 40.6±11.6 y (19-63), weight 71.9±13 kg, height 161.8±9 cm, BMI 27.4±4.5 kg/m2 and BSA 1.76±0.18 m2. The coefficient of variation (CV%) of ECW was 0.33%. ECW normalized by ICW, height, and TBW showed a poor correlation with age (r=0.11, r=0.26 and r=0.12, respectively) in contrast to what others have shown. In our population ECW, normalized by height (ECW/H) showed good correlation with BSA (r=0.86, p<0.001). By multiple regression (stepwise) we obtained a ECW/H formula with R2=0.85 (adjusted R2=0.84, p<0.001) employing BSA, BMI and gender [ECW/H =-0.007594+0.03557BSA+0.001229BMI +0.008608gender (0= females, 1=males)].

Discussion: We find a very low CV% for ECW measurements in euvolemic controls. In this population nECW does not correlate with age. In contrast, we found good correlation with BSA. This phenomenom has not been previously reported. Our nECW formula derived from demographic and somatometric parameters could be useful to objectively estimate overhydration (measured nECW in a dialysis patient minus nECW values from euvolemic controls) and is easily applicable to PD or HD.

Disclosure of Financial Relationships: Employer: Fresenius Medical CareResearch Funding: Fresenius Medical Care.

**F-PO1241**

**CT and MR Imaging Criteria for Diagnosis of Cyst Infection and Intracystic Hemorrhage in Autosomal Dominant Polycystic Kidney Disease** Tatsuya Suwabe, Yoshifumi Ubara, Keiichi Sumida, Rikako Hiramatsu, Junichi Hoshino. *Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

Background and objectives: Cyst infection and intracystic hemorrhage are serious complications of autosomal dominant polycystic kidney disease (ADPKD). However, they are often difficult to differentiate from each other. PET was reported to be useful for detecting infected cysts, however it has limited availability and is an expensive imaging method. PET might also be unable to differentiate infection from intracystic hemorrhage. Magnetic resonance imaging (MRI) and computed tomography (CT) are presumed to be easy and useful imaging methods for detecting infected or bleeding cysts in patients with ADPKD, but the diagnostic criteria have not been clarified. Accordingly, we investigated the MRI and CT findings for diagnosis of cyst infection or intracystic hemorrhage in patients with ADPKD.

Design, setting, participants, & measurements: Patients with definite cyst infection or definite intracystic hemorrhage were enrolled, among all patients with ADPKD who were referred to our institution from January 2004 to December 2009. We compared the MRI and CT features of patients with infected cysts and intracystic hemorrhage. First, we identified intracystic fluid-fluid levels and thickening of the cyst walls. Second, we compared the signal intensity (SI) ratio and CT density among infected cysts, normal cysts, and cysts with hemorrhage.

Results: Thirteen patients with definite cyst infection and 6 patients with definite intracystic hemorrhage were enrolled. With MRI, infected cysts showed a lower intensity on T2WI and a significantly higher intensity on T1WI and DWI than normal cysts. Many infected cysts also had a fluid-fluid level and wall thickening. Cysts with hemorrhage showed a significantly higher intensity on T1WI, T2WI, and DWI than infected cysts, and also had a higher density on CT. Moreover, intracystic hemorrhage displayed an irregular high density on CT.

Conclusions: CT and MRI are both useful for diagnosis of cyst infection and intracystic hemorrhage.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1242**

**Expression of Intrarenal Renin-Angiotensin System and Its Relationship with Clinical-Pathological Injury in Primary IgA Nephropathy Patients** Xiaoyan Zhang, Xiaoqiang Ding, Wenlv Lv, Jie Teng, Yihong Zhong. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

**Introduction**

Little information is available about the RAS expression and regulation in the human kidney and particularly in kidney diseases. These data in humans and in diseased kidneys

would be worthwhile being evaluated because changes in general RAS do not closely reflect local expression and regulation of intrarenal RAS.

**Methods**

Thirty-six biopsy-proved IgA nephropathy patients who were hospitalized between January 2009 and June 2009, had not received ACEI, ARB, glucocorticoids or immunosuppressive agents. We recorded gender, age, height, body weight, blood pressure, urine routine, renal function, serum electrolytes, urinary protein of 24 hours and urinary sodium. We assessed pathological injury by semiquantitative Katafuchi score and assessed intrarenal experssion of all the components of the intrerenal RAS by immunohistochemistry staining. We assessed expression and regulation of all the components of intrarenal RAS and the relationship between intrarenal Ang II activity and clinical-pathological injury index in primary IgA nephropathy patients.

**Results**

Positive IHCS area of intrarenal renin, angiotensinogen and AngII were 26.86±13.66%(7-55%), 38.34±9.71%(12-57%) and 32.73±14.74%(6-70%), respectively. There were positive correlation between positive IHCS area of intrarenal renin and positive IHCS area of intrarenal Ang II ($P<0.01$), positive IHCS area of intrarenal angiotensiongen and positive IHCS area of intrarenal Ang II ($P<0.05$). Average eGFR in 36 IgA nephropathy patients was 55.92±22.87 ml/min/1.73m$^2$(6.62-92.49 ml/min/1.73m$^2$) and there was negative correlation between positive IHCS area of intrarenal Ang II and eGFR($P<0.01$). Average pathological chronicity index in 36 IgA nephropathy patients was 3.06±2.60 (0-10) and there was positive correlation between positive IHCS area of intrarenal AngII and pathological chronicity index ($P<0.05$).

**Conclusion**

Expression of intrarenal Ang II correlates positively with expression of intrarenal renin and angiotensinogen in IgA nephropathy. Intrarenal Ang II activity plays an important role in kidney fibrosis in IgA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1243

**Impaired Maximal Aerobic Capacity Is Parallel to GFR Decline in Elderly Population** Ammar Almehmi,[1] Mike Broce.[2] [1]*Division of Nephrology, The Kidney Institute, Kansas City, KS;* [2]*CAMC Research Institute, Charleston, WV.*

**Background:**

It is well known that adult patients with end stage renal disease have impaired maximal aerobic capacity *($VO_2^{max}$)*. However, studies on $VO_2^{max}$ in elderly patients with early stages of chronic kidney disease (CKD) are limited.

**Objective:**

To examine the relationship between $VO_2^{max}$ and glomerular filtration rate (GFR) in elderly subjects and to determine factors associated with decreased $VO_2^{max}$ in elderly patients with different stages of CKD.

**Methods:**

Subjects aged 60 and over were included in this study. GFR was estimated using Cockcroft-Gault formulae. $VO_2^{max}$ was assessed using the standard graded treadmill test. Dual energy x-ray absorptiometry was utilized to assess lean mass. ANOVA, nonparametric tests, and linear regression were used to explore the relationship between $VO_2^{max}$ and GFR.

**Results:**

This study included 112 subjects, 57.1% were women with mean age of 73 ± 6.9 yr (mean ± SD), blood pressure 130 ±14.9 / 71 ± 9.2 mmHg, LDL-cholesterol 110 ± 27.6 mg/dL, blood urea nitrogen 16 ± 4.7 mg/dL, and serum creatinine 0.85 ± 0.20 mg/dL. Maximal aerobic capacity ($VO_2^{max}$) was reduced in subjects with different GFR: 40.87 mL/Lean mass (kg)/min for GFR ≥ 90 ml/min, 35.9 mL/Lean mass (kg)/min for GFR 60-89 ml/min, and 32.95 mL/Lean mass (kg)/min for GFR < 60 ml/min ($p = 0.0001$). In univariate analysis, $VO_2^{max}$ was associated with age ($p = 0.0001$), total cholesterol (($p = 0.011$), LDL- cholesterol ($p = 0.003$), body mass index ($p = 0.0001$), maximum heart rate ($p = 0.013$), and GFR ($p = 0.001$). In multivariate regression analysis, after controlling for age, sex, BMI, and duration of exercise, GFR was an independent predictor of lower $VO_2^{max}$.

**Conclusion:**

$VO_2^{max}$ is reduced in the early stages of CKD in elderly patients. These results suggest that the ability of cardiovascular system to respond to the metabolic challenge is impaired early in the development of CKD. Longitudinal studies are required to determine if improving physical activity would affect the cardiovascular outcomes in this population.

Disclosure of Financial Relationships: nothing to disclose

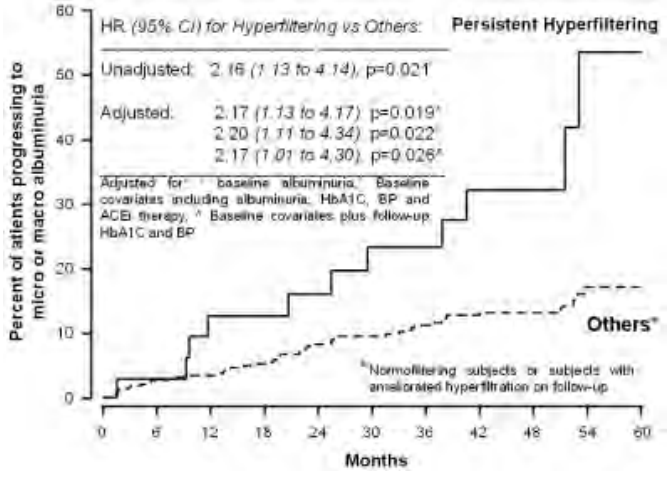
## F-PO1244

**Glomerular Hyperfiltration Accelerates Renal Disease Progression in Type 2 Diabetes** Esteban Porrini, Piero Ruggenenti, Giuseppe Remuzzi. *Mario Negri Institute for Pharmacological Research, Bergamo, Italy.*

Background: The role of glomerular hyperfiltration in onset and progression of human diabetic nephropathy is uncertain.

Methods: To assess the relationships between glomerular hyperfiltration [glomerular filtration rate (GFR) ≥120 ml/min/1.73m$^2$], GFR decline and nephropathy, we centrally measured GFR (iohexol plasma clearance) and albuminuria every 6 months over 4.0 years of intensified metabolic and blood pressure (BP) control in 600 type 2 diabetes subjects with normo- or micro-albuminuria. Glucose disposal rate (GDR) was measured by hyperinsulinemic euglycemic clamps at baseline and 1 year.

Results: GFR declined by a median (interquartile range) of 3.37 (5.26-1.54) ml/min/1.73m$^2$ per year. Independent of metabolic and BP control, concomitant therapy with ACE inhibitors, and level of initial albuminuria, more GFR reduction at 6 months significantly predicted slower GFR decline on subsequent follow-up ($p<0.0001$). The proportion of progressors to micro- or macro- albuminuria (24.4 vs 10.3%) was significantly higher in persistently hyperfiltering subjects than in non-hyperfiltering subjects and subjects who had their hyperfiltration ameliorated on follow-up.




Excess risk in hyperfiltering subjects was independent of initial albuminuria and metabolic and BP control, and was associated with more GDR reduction ($p=0.038$ by ANCOVA) at 1 year. Serum creatinine based prediction formulas failed to detect hyperfiltration at inclusion and to describe short- and long-term GFR changes in all patients.

Conclusions: GFR decline in type 2 diabetes patients with normo- or micro-albuminuria is 3 to 5 folds faster than normal. Hyperfiltration is a determinant of GFR decline and nephropathy, and its amelioration is renoprotective. Prediction formulas should not be used to identify subjects at risk and reliably monitor the effect of treatment in this population.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1245

**Malnutrition-Inflammation Score Is Reliable Tool for Assessment of Malnutrition Status and Mortality in Chinese Peritoneal Dialysis Patients** Huiling Wang, Jinyuan Zhang. *Nephrology Division, Jimin Hospital of Shanghai, Shanghai, China.*

The malnutrition inflammation score (MIS) was reported to be a reliable tool for screening malnutrition and inflammatory status, but has not been validated in Chinese continuous ambulatory peritoneal dialysis(CAPD)patients. We studied 98 adult patients (47 men, 51 women; age 59.1±18.0 years) on CAPD at least 3 months (4~13 months). The MIS and Quantitative Subjective Global Assessment (MQSGA), and the anthropometry, inflammation makers and biochemical assays were measured. The correlation of MIS with the prospective hospitalization, mortality and morbidity of peritonitis were followed by 12months. **Results** The morbidity of malnutrition was 77.9% according to MQSGA, in which the mild 65.3%, moderate 27.5% and severe 7.1% respectively. But according to MIS, the mild 53.1%, moderate 39.8% and severe 7.14% respectively. MIS significantly correlated with the MQSGA score ($r$=0.894, $P$<0.001). The higher MIS score reflects the worse nutritional status and the more severe inflammation status including increased high sensitive-C Reactive Protein and Interleukin-6. A higher MIS score was positively correlation with a bad nutrition (lower Alb, pre-Alb and Serum creatinine) and severe anemia, which also means a higher morbidity of peritonitis(r=0.292; $P$<0.01). Case-mix–adjusted correlation coefficients for the MIS were significant for hospitalization days (r =0.469; $P$<0.001) and frequency of hospitalization (r=0.513; $P$<0.001). MIS was the independent risk factor of peritonitis ($p$=0.013, odds ratio 1.185, 95% confidence interval 1.036~1.335). During the 12-month followed-up, 12 patients died and 8 patients left the cohort. The Cox proportional hazard–calculated relative risk for death for each increased MIS score was 1.242 (95% confidence interval1.102~1.401, $P$<0.001). ***Conclusion:*** The MIS showed a simple and reliable tool for the assessment of malnutrition status in CAPD patients, as well as a predictor of PD outcome and an indicator of MICS. A higher score means a worse nutrition-inflammation status, and a higher hospitalization and mortality.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1246

**The CRISIS Study: Clinical Outcomes of the Elderly in a Referred CKD Cohort** Richard Hoefield,[1] Philip A. Kalra,[1] Robert N. Foley,[2] Donal J. O'Donoghue,[1] Rachel Middleton.[1] [1]*Vascular Research Group, University of Manchester, Salford Royal Hospital Foundation Trust, UK;* [2]*Chronic Disease Research Group and University of Minnesota, Minneapolis, MN.*

The clinical outcomes and natural history of evolution of eGFR in elderly patients with CKD is not well described. CRISIS is a prospective epidemiological investigation of a prevalent population receiving standard nephrological care with CKD stages 3-5, not on dialysis.

The aim of this analysis was to describe the outcomes of death, requirement of renal replacement therapy (RRT) and outline the evolution of eGFR in an elderly cohort of patients in our referred CKD population.

The CRISIS cohort was categorised into 3 groups; ≤ 69, 70-79 and ≥ 80 years, based on age at inception into the study. Cox proportional hazards model was used to identify variables associated with the endpoints of RRT and death.

1317 patients were available for analysis. Median follow-up was 33 months. Mean age 65 years, with 54% ≤ 69 years of age, 32.5% between 70-79 and 13.5% ≥ 80 years of age. Those patients ≥ 70 years demonstrated slower median rates of decline in renal function than patients < 69 years of age in our cohort. Annual median rate of change of eGFR for patients ≤69 years was -1.78ml/min/y, 70-79, -1.35ml/min/y and ≥80 -1.33 ml/min/y ($p<0.01$). Significantly higher rates of death were seen in those patients ≥ 70 **(Table1).**

Multivariate associations of RRT included; greater proteinuria, lower eGFR and serum calcium. Older age, smoking, presence of baseline cardiovascular disease and diabetes, lower eGFR, higher serum phosphate and CRP were associated with death.Non- smoking status and use of statin therapy was associated with a reduced hazard ratio for mortality. This study demonstrates higher risk of death and slower rates of decline in eGFR in elderly patients with CKD in a referred cohort; highlighting the importance of strategic targeting of cardiovascular risk reduction in these older patients.

| **Age(years)** | **≤69** | **70-79** | **≥80** |
|---|---|---|---|
| **RRT %** | 26.2 | 18.6 | 10.1 |
| **RRT Rate (per100 patient years)** | **7.5** | **6.0** | **3.5** |
| **Death %** | 13 | 31.7 | 49.4 |
| **Death Rate (per100 patient years)** | **3.7** | **10.2** | **17.5** |

Disclosure of Financial Relationships: nothing to disclose

## F-PO1247

**Epidemiology and Microbiology of Joint Infections in Chronic Kidney Disease** Vanbric R. Casilla,[1,2] Brenda Hemmelgarn,[1,2] Matthew T. James.[1,2] *[1]Medicine, University of Calgary, Canada; [2]Community Health Sciences, University of Calgary, Canada.*

Patients with end-stage renal disease experience increased morbidity and mortality due to acute infections. Hematogenous spread of bacteria secondary to dialysis vascular access likely contributes to this risk, including that of bone and joint infections. Adults with chronic kidney disease (CKD) not receiving renal replacement therapy also experience an increased risk of hospitalization with joint infections. The purpose of this study was to determine the causative pathogens of these infections among patients with CKD. A cohort of adults from the Calgary Health Region with at least one outpatient serum creatinine measurement between July 1, 2003 and June 30, 2004 were identified. Patients with a prior transplant or who were receiving dialysis prior to study entry were excluded. GFR was estimated using the Modification of Diet in Renal Disease (MDRD) Study equation. The results of the first positive culture from joint and periarticular samples were obtained for patients with and without CKD (eGFR <60 and ≥60 mL/min/1.73m$^2$, respectively) during follow-up from the date of the first available serum creatinine measurement through December 31, 2008. During a median follow-up of 5 years, a total of 332 culture-positive joint or periarticular samples were obtained from 252,508 participants. The unadjusted incidence rates (per 1000 person-years) of culture-positive infections were 0.63 (95%CI 0.52-0.76) for those with CKD and 0.22 (95% CI 0.19-0.25) for those without CKD. Among those with positive cultures, there were no differences in the distribution of micro-organisms isolated from patients with and without CKD (p=0.116); 28.4 vs. 23.2% coagulase-negative *Staphylococcus* species, 35.3 vs. 46.4% *S. aureus*, 20.6 vs. 20.3% other Gram-positive organisms, and 15.7 vs. 10.1% other pathogens. The risk of culture-positive joint and periarticular infections is increased almost 3-fold among patients with CKD. Similar to patients without CKD, Gram-positive organisms, in particular *Staphylococcal* species, cause the majority of these infections. The consequences of these infections on patient outcomes remains to be determined.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1248

**Diabetes, Podocytes and Lectins: A Glycobiology Study with Diagnostic Potential** Alessandra Ravida,[1] Marjut Kreivi,[1] Luca Musante,[1] Ilkka Miinalainen,[1] Mayank Saraswat,[1] Barry Byrne,[1] Moin Saleem,[2] Harry B. Holthofer.[1] *[1]Centre for Bioanalytical Sciences, Dublin City University, Dublin, Ireland; [2]Academic and Children's Renal Unit, University of Bristol, Bristol, United Kingdom.*

**BACKGROUND:** Glycosylation is one of the most common post-translational modifications of proteins, mirroring variations in metabolism, developmental and functional status of cells and organs.

**AIM:** In this study we aim to study weather the development of the diabetic nephropathy onset associates with changes in the glycosylation of key glycoproteins of podocytes.

**METHODS:** Detection and analysis of differential glycosylation patterns as associated with progression of the disease is carried out by the means of lectin assays on immortalized human podocytes cells treated in diabetes-mimicking conditions (high-glucose, insulin starvation, high-insulin). The results obtained in the *in vitro* studies are validated on the well established *in vivo* rat model of Streptozotocin-induced diabetes.

**RESULTS:** In this study we have probed podocyte glycoproteins with 18 commercially available plant lectins by lectin blots and immunocytochemistry. By lectin blots, we have identified 4 lectins which are able to detect specific alterations in the glycoprotein profile of podocytes treated in diabetes mimicking conditions. Furthermore, by immunocytochemistry studies, we have identified one *O*-glycan-binding lectin which selectively recognises glycoproteins secreted by podocytes in an insulin dependent fashion.

**CONCLUSIONS:** This study represents the first systematic approach towards a novel understanding of the glycobiology of diabetic nephropathy. We demonstrated the feasibility of detecting diabetes-induced changes in podocytes glycosylation patterns by lectin assays. Furthermore, it is expected that these promising lectin candidates will pave the way for a novel approach to the early-stage diagnosis of diabetic nephropathy.

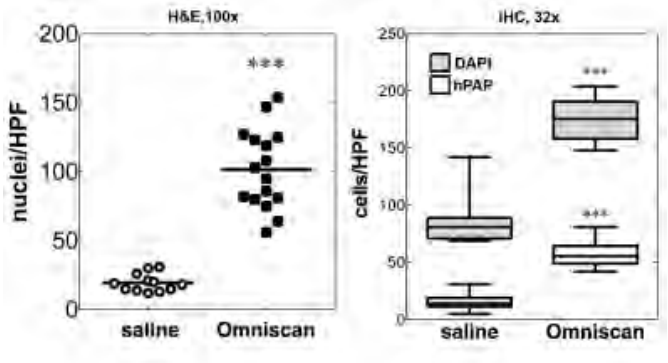
Disclosure of Financial Relationships: nothing to disclose

Disclosure of Financial Relationships: nothing to disclose

## F-PO1249

**Nephrogenic Systemic Fibrosis: Both Resident Tissue and Bone Marrow-Derived Cells Are Increased in the Dermis of Gadolinium-Based MRI Contrast-Treated Rodents** Brent Wagner,[1,2] Seema S. Ahuja,[1,2] Jeffrey L. Barnes.[1,2] *[1]South Texas Veterans Health Care System, San Antonio, TX; [2]Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX.*

**Background:** Nephrogenic systemic fibrosis (NSF) is a disorder associated with gadolinium-based MRI contrast exposure given acute or chronic renal compromise. It has been proposed that circulating fibrocytes mediate the disease, however experimental evidence is lacking. A study was conducted to determine if bone marrow-derived cells are involved in mediating dermal fibrosis in rodents. **Methods:** Fisher 344 rats status post 5/6 nephrectomy were used. A sub-group of rodents underwent lethal irradiation followed by salvage bone marrow transplant (BMT) from human placental alkaline phosphatase-(hPAP-) expressing donors. Animals were treated with the gadolinium-based MRI contrast, Omniscan (2.5 mmol/kg IP), during weekdays or an equivalent volume of saline for 4 weeks. Tissues were fixed in 4% neutral buffered formalin for H&E stain or flash-frozen for immunohistochemistry (IHC) and immunoblot. H&E and trichrome-stained skins were read by a blinded dermatopathologist. Nuclei from H&E-stained tissues and IHC were quantitated. **Results:** Regardless of BMT status, gross dermal signs were mild. Skin from Omniscan-treated animals demonstrated fibrosis and increases in fibronectin and type IV collagen. Dermal cellularity in the Omniscan-treated group was greater than control (*** $P < 0.0001$ by 2-tailed t test). There were significant and proportional increases in dermal hPAP$^+$ and hPAP$^-$ cells.




**Conclusions:** Both resident tissue and bone marrow-derived cells are increased in the skins of MRI contrast-treated rodents. Elucidation of these mechanisms may aid in discovering therapies to this devastating disease.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1250

**Isolation of Urinary Nanovesicles and a Method for Protein-Vesicle Interactions** Luca Musante, Alessandra Ravida, Mayank Saraswat, Barry Byrne, Harry B. Holthofer. *Centre for BioAnalytical Sciences, Dublin City University, Dublin, Ireland.*

BACKGROUND: Urine contains a heterogeneous population of nanovesicles which carry proteins, microRNA, RNA and transcriptional factors. However, little is known about urinary nanovesicles and their interactions with soluble proteins.

AIM: We compared two different methods to isolate exosomes from a pool of healthy donors to evaluate nanovesicle-protein interactions.

METHODS: The urine from 4 healthy volunteers was subjected to serial centrifugations at 1,000g, 18,000g and 200,000g, respectively. The final 200,000g supernatant was further processed by ammonium sulphate precipitation and was subjected to the serial centrifugation steps one more time. In the second method, the urine pool was first dialysed and the volume was reduced by lyophilisation. Nanovesicles were subsequently prepared following serial centrifugations. In order to evaluate co-precipitation of high abundant proteins with exosomes, 200,000g pellets were both treated with either dithiotreitol (DTT) or zwitterionic detergent and re-centrifuged respectively at 200,000g. Zymography was performed to detect enzymatic activities.

RESULTS: Our results show that all pellets expressing exosomal markers are a heterogeneous population of nanovesicles of different size. Surprisingly, we could detect WT-1 antigen in urine of healthy people. Furthermore, our results show that the apparent interference soluble protein is a direct and specific interaction with the 200,000g nanovesicle pellet. Moreover, zymography results showed an association of nanovesicles with protease activity which may be both intra- and extra-vesicular.

CONCLUSION: This study establishes preferential methods to yield a more comprehensive understanding of the physiological role of the abundant nanovesicles in urine and demonstrates the feasibility of detecting the nanovesicle-protein interactions.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1251**

**Assessment of Hydration State in CKD Patients Not on RRT Using Whole Body and Calf Bioimpedance Techniques** Usama T. Hussein,[1] Fansan Zhu,[2] Li Liu,[2] Peter Kotanko,[2] Nathan W. Levin,[2] Fredric O. Finkelstein.[1] *[1]Nephrology Department, St. Raphael Hospital/Yale, New Haven; [2]Renal Research Institute, NY.*

Chronic kidney disease (CKD) is characterized by loss of volume homeostasis, often leading to overhydration that in turn is associated with hypertension(HTN) and increased risk for cardiovascular morbidity and mortality. The aim of this study was to evaluate the assessment of fluid overload in CKD pts not on renal replacement therapy (RRT) utilizing three bioimpedance approaches: ratio of extracellular fluid volume to body weight (wECV/Wt), wECV/TBW and calf normalized resistivity (nRho).

Methods. 50 clinically stable CKD pts without a history of DVT or lower studied. 45 patients (90%) had HTN. Clinical assessment of volume status was determined by the attending nephrologist. Bioimpedance was studied with whole body (wBIS) and calf bioimpedance (cBIS) measurement using Hydra 4200. 20 of the 50 pts were measured for reproducibility in two different ways. In 10 pts wBIS and cBIS measurements were made twice in one hour intervals. In another 10 pts, measurements were made twice on two consecutive days.. Healthy subjects (n=15) were measured once with wBIS and cBIS. Overhydration was defined by cBIS when nRho was less than 0.18 $Wm^3$/kg in males and 0.2 $Wm^3$/kg in females respectively

Results. Although only 24% of CKD pts were assessed clinically to be overhydrated, 86% CKD pts (n=50) were identified as being overhydrated by nRho. nRho was significantly lower in CKD pts than in HS . 28% and 26% were overhydrated by wECV/Wt and wECV/TBW, respectively; wECV/Wt and wECV/TBW were not significantly different between CKD patients and HS . Relative change in -1.14%, 1.7% and 0.42% in the same day measurements and -1.2%, 0.22% 4.6% in two consecutive days study were observed for nRho, wECV/Wt and wECV/TBW respectively.

Discussion and Conclusion. The main finding in this study was that nRho was significantly lower in CKD pts than in HS, however, no difference in wECV/Wt or in wECV/TBW was observed. Given that 86% of patients were hypertensive, calf resistivity could be a useful marker to identify degree of hydration state in CKD pts not on RRT

Disclosure of Financial Relationships: nothing to disclose

**F-PO1252**

**Changes in the Mitochondrial Network and Bioenergetic Capacity of Human Podocytes during Differentiation** Ilkka Miinalainen, Marjut Kreivi, Alessandra Ravida, Barry Byrne, Luca Musante, Louise Sewell, Harry B. Holthofer. *Centre for Bioanalytical Sciences, Dublin City University, Dublin, Ireland.*

Background: Changes in the amount of mitochondria/their functional capacity are associated with several metabolic diseases, including diabetes.

Methods: Mitochondrial functional parameters were determined from transformed human podocytes (AB 8/13) by high-resolution respirometry, fluorescent microscopy and Real-time quantitative PCR.

Results: During differentiation, cellular routine respiration increased from 50 pmols s-1 10-6 cells up to 131 pmols s-1 10-6 cells, and oligomycin-inhibited respiration increased from 7.2 pmols s-1 10-6 to 63.3 pmols s-1 10-6 cells. Uncoupled respiration showed maximal capacity of the respiratory chain was 2.5 times higher in differentiated podocytes. After result normalisation, it was shown that both routine respiration and uncoupled respiration were lower in differentiated podocytes, while leak respiration rates remained significantly higher. In undifferentiated podocytes, mitochondria showed fragmented perinuclear morphologies which changed to filamentous network with long tubules extending to the periphery of the cell during differentiation. Differentiation greatly induced the expression of PGC-1a, while expression of the mitochondrial transcription factor A (TFAM) remained unchanged.

Conclusion: The coactivator PGC-1a plays a central role in podocyte differentiation by promoting the mitochondrial biogenesis, although no large changes are needed for control of transcription and replication of the mitochondrial genome as judged by unchanged levels of TFAM. Respiratory control ratios indicate that approximately 40 % of the electron transport capacity is utilised in the routine respiration in both undifferentiated and differentiated cells, indicating large respiratory excess capacity over routine respiration. In undifferentiated cells, 37 % of the total uncoupled electron transport capacity was activated to ATP production, while in differentiated podocytes this figure was only 19 %. This demonstrates that non-mitochondrial respiration and other oxygen-consuming processes are markedly induced in differentiated podocytes.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1253**

**Role of Glycosylation in the Diabetic Kidney Disease – The Effect of Insulin on Glyco-Gene Expression of Podocytes** Marjut Kreivi,[1] Alessandra Ravida,[1] Ilkka Miinalainen,[1] Suzanne Papp,[2] Lana Schaffer,[2] Luca Musante,[1] Louise Sewell,[1] Barry Byrne,[1] Steve Head,[2] Moin Saleem,[3] Harry B. Holthofer.[1] *[1]Centre for Bioanalytical Sciences, Dublin City University, Dublin, Ireland; [2]DNA Array Core Facility, Scripps Research Institute, CA; [3]Academic and Childrens Renal Unit, University of Bristol, Bristol, United Kingdom.*

**BACKGROUND:** The developmental/functional status of podocytes is reflected by glycodecoration. Podocytes increase glucose uptake when stimulated by insulin via an effect on glucose transporters GLUT1 and GLUT4.

**AIM:** This study aims to map glyco-genes regulated in podocytes under insulin-induced diabetes mimicking stress and those involved in the alteration of the glomerular filtration barrier and podocytes during development of diabetic kidney disease.

**METHODS:** Changes in glyco-gene expression were screened in a human conditionally immortalized podocyte cell line after insulin treatments (normal culture conditions, insulin starvation, and a series of different insulin concentrations). The glyco-gene expression changes were screened using the GlycoV4 oligonucleotide array. A set of genes was further analyzed using real-time RT-PCR, Western blotting and immunocytochemistry.

**RESULTS:** Differential expression compared to the normal control sample was detected both in the insulin-starved and in the insulin-treated samples. Several glyco-genes that are not previously linked to human podocytes under diabetic/insulin stress were also identified. These included glyco-genes from several categories including glycan-transferases, glycan degradation enzymes, growth factors and receptors. In total, 25 glyco-genes were found to be differentially expressed. From these, a set of 6 genes was selected for further analysis.

**CONCLUSIONS:** Diabetes mimicking stress represented by different concentrations of insulin in the culture media resulted in differential glyco-gene expression in cultured human podocytes. Changes in insulin concentration induced differential expression in 25 glyco-genes. The changes in 5 glycan-transferases and a glycan degradation associated protein were confirmed using real-time RT-PCR, Western blotting and immunocytochemistry.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1254**

**High Intake of Insoluble Dietary Fiber (IDF) Is Associated with ↓ Inflammation and All-Cause Mortality (ACM) in CKD: NHANES III** Vidya M. Raj Krishnamurthy,[1,2] Neelakanta A. Dadi,[1,2] Bradley C. Baird,[1] Guo Wei,[1] Tom H. Greene,[1,2] Michel B. Chonchol,[3] Srinivasan Beddhu.[1,2] *[1]Univ Utah; [2]VA, SLC, UT; [3]Univ Colorado, Denver, CO.*

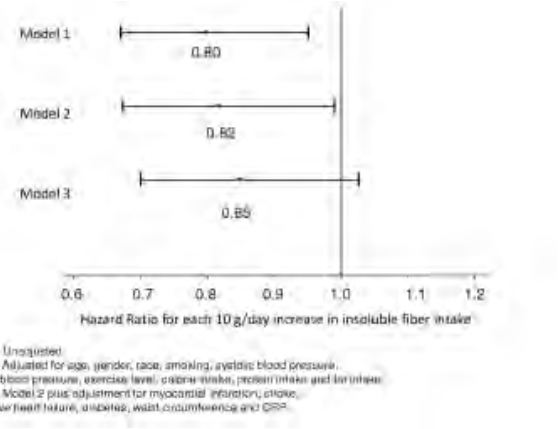
Dietary fiber intake is often low in CKD due to ↓ fruit /vegetable intake. We examined the associations of IDF with elevated serum CRP (>3mg/L) and ACM in CKD sub-population (N=1105) in National Health and Nutrition Examination Survey (NHANES) III, a multistage survey conducted by the National Center of Health Statistics (NCHS) in 1988 -1994. IDF intake was estimated from 24h dietary recalls. Mortality data were obtained by NCHS by linkage with National Death Index records through 12/31/2006.

Characteristics of CKD Population by IDF intake

| | ≤6.4 g/d | 6.5-11 g/d | ≥11.1 g/d |
|---|---|---|---|
| IDF(g/d) | 4.2±0.1 | 8.6±0.1 | 16.7±0.4 |
| Age(yr) | 69±0.9 | 69±1.3 | 70±0.9 |
| Male(%)** | 29 | 31 | 49 |
| AA Race(%)* | 12 | 5 | 4 |
| MI(%) | 16 | 15 | 15 |
| DM(%) | 23 | 20 | 19 |
| Smoking(%)* | 19 | 12 | 9 |
| Inactivity(%)** | 37 | 28 | 20 |
| Waist(inch) | 38.4±0.4 | 38.0±0.4 | 38.3±0.4 |
| Calorie Intake(kcals/d) ** | 1232±42 | 1668±80 | 1953±63 |
| Fat Intake(g/d)** | 48±3 | 63±3 | 72±4 |
| Protein Intake(g/d)** | 50±2 | 63±2 | 78±4 |
| CRP(mg/L) | 8.1±0.8 | 6.1±0.4 | 5.6±0.8 |

* 0.001<P value ≤0.05, ** P value ≤ 0.001

In a multivariable logistic regression model adjusted for age, sex, race, BP, smoking, inactivity, dietary protein, calorie and fat intake, each 10g↑ in IDF was associated with ↓ odds (OR 0.60, 95% CI 0.43 - 0.85) of elevated serum CRP. In a multivariable cox regression model (Model 2 in Figure 1), ↑ IDF intake was associated with ↓ACM which was attenuated when adjusted for factors that are potentially in the causal pathway between IDF intake and ACM (Model 3 in Figure 1).




We conclude high IDF might be associated with ↓ inflammation and ACM in CKD population. Interventional trials are needed to establish the causal relationships of IDF with inflammation and ACM in CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1255**

**Positron Emission Tomography in Diagnosis and Follow-Up of the Infected Cysts in ADPKD and in "Cystic" Kidneys. Report of 9 Cases** Giorgina B. Piccoli, Valentina Consiglio, Maria Chiara Deagostini, Stefania Scognamiglio. *SS Nephrology, ASOU san Luigi, University of Torino, Torino, Italy.*

**Background.** Intracystic infection, both in Autosomal Dominant Polycystic Kidney Disease (ADPKD) and in kidneys with multiple cysts, poses important diagnostic and therapeutic challenges, as conventional imaging techniques do not discriminate among "complicated" cysts. Positron Emission Tomography with fluorodeoxyglucose (FDG-PET) was recently suggested as a tool to detect infection in ADPKD (7 cases published so far).

**Aim.** To report on FDG-PET in the diagnosis and management of 9 cases of suspected cystic infections in ADPKD and in multiple kidney cysts.

**Results**. In 2008-2010, 6 patients with ADPKD and 3 with multiple kidney cysts were referred for suspected intracystic infections (liver and kidney). There were 3 males, 6 females, aged 55-83 years; one patient was on dialysis, one had a kidney graft; all CKD stages were represented. The clinical picture and/or conventional imaging techniques (ultrasounds in all, CT in 7/9) did not allow diagnosis in any patient. All patients displayed moderate-severe inflammatory signs, 8/9 constitutional symptoms; fever was present in 7/9. In 5 cases, FDG-PET was positive (4 kidney and 1 liver cyst); it was repeated during follow-up and the results were used to tailor the duration of antibiotic therapy (1-3 months). No relapse was recorded after discontinuation of therapy. The 4 negative cases did not develop clinically active cystic infection; in one case, FDG-PET identified a positive lymph node, to be further assessed by agobiopsy.

**Conclusion**. FDG-PET is a promising tool to diagnosis intracystic infection and to tailor the duration of antibiotic therapy in ADPKD and in multiple kidney cysts.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1256**

**Determinants of the Levels of Undercarboxylated Osteocalcin in Patients with Chronic Kidney Disease** Mieko Miyazaki, Masahito Tamura, Narutoshi Kabashima, Ryota Serino, Tatsuya Shibata, Tetsu Miyamoto, Yumi Furuno, Junichi Nakamata, Yoko Fujimoto, Emi Hasegawa, Yutaka Otsuji. *Kidney Center, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan.*

Background: Undercarboxylated osteocalcin (ucOC) is a marker of vitamin K deficiency and is associated with an increased risk of bone fractures. We have reported that the levels of ucOC increase along with chronic kidney disease (CKD) stages and are independent risk markers for arteriosclerosis in patients with CKD. We investigated the dietary and nondietary factors that affect ucOC levels in patients with CKD.

Methods: We measured the levels of ucOC and osteocalcin (OC) in 207 outpatients (mean age, 61.2 ± 16.1 years) with CKD stages 1-5, excluding those undergoing renal replacement therapy. Plasma ucOC is expressed as the ratio of ucOC to OC (ucOC/OC). Patient characteristics, including daily diet, medicines, complications, history of fracture, and lifestyle, were examined by performing computer-aided personal interviews and by using self-answered questionnaires. Determinants of ucOC/OC were investigated by analysis of variance, Scheffe test, and logistic regression models.

Results: The median ucOC/OC was 0.71. The levels of ucOC/OC were significantly affected by estimated glomerular filtration rate (eGFR), use of warfarin ($p < 0.0005$) or glucocorticoids ($p < 0.0005$), history of cardiac events ($p = 0.0001$), smoking, and sports activity. However, intake of food with high levels of dietary vitamin K, such as vegetables and beans; history of bone fracture; use of vitamin D or Ca carbonate; and alcohol intake did not influence the levels of UcOC/iOC after adjusting for age, sex, body mass index, and eGFR.

Conclusion: These data suggest that an increase in ucOC is involved in the progression of CKD-mineral and bone disorder and that the levels of ucOC are influenced by medications, complications, and lifestyle in patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1257**

**Metabolic Acidosis Is Associated with Rapid Decline of Renal Function in Pre-Dialysis CKD Patients** Wael F. Hussein,[1] Abdulrahman Esam Alfraih,[2] Ahmad Abdulreda Lari,[2] James Lineen,[1] Patrick O'Kelly,[1] Mark D. Denton.[1] *[1]Department of Nephrology, Beaumont Hospital, Dublin, Ireland; [2]Royal College of Surgeons in Ireland, Dublin, Ireland.*

Metabolic acidosis has been suggested as a risk factor for CKD progression.

**Methods**:

Patients with eGFR 25-30 ml/min/1.73m$^2$ under nephrologists follow up in our centre with at least 12 months follow up were reviewed retrospectively until start of dialysis. In-patient blood results were excluded. Patients were censored for pre-emptive transplantation, death and loss to follow up. Rate of MDRD-eGFR decline per year and 'Average Bicarbonate' were estimated using linear regression for eGFR and bicarbonate values respectively versus time. Associations were studied between quartiles of bicarbonate and (1) Rate of eGFR change/yr, and (2) Incidence of dialysis.

**Results:** 179 patients were followed up for 42±24 months (mean±SD). Mean age was 59±17 yrs. Patients were 56% males, 21% had diabetes, 76% hypertension and 64% were on ACEI/ARB therapy. Nephrotic proteinuria was present in 12% of patients and 21% had 1.0 to 2.9 g/24hr proteinuria at time of inclusion.

For each 1 mmol/L reduction in serum bicarbonate there was a 0.37 (95% CI 0.19 to 0.54) ml/min/1.73m$^2$/yr faster decline in eGFR. This remained statistically significant after adjustment [β 0.22 (0.09 to 0.46)].

Using Cox regression analysis, the second highest bicarbonate quartile (25 to 26 mmol/L) had the least event rates and, compared to the lowest bicarbonate quartile (18 to 22), the OR of starting dialysis was 0.32 (95% CI 0.13 to 0.78) (figure 1).

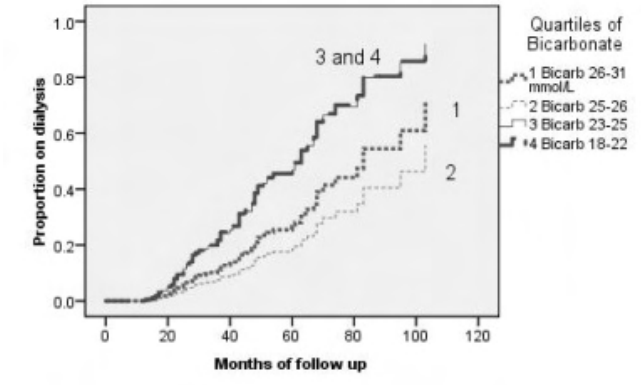



Figure 1. Proportion of patients starting dialysis in each bicarbonate quartile. Model was adjusted for age, gender, hypertension, diabetes, underlying kidney disease, proteinuria and use of ACEI/ARB at time of inclusion.

**Conclusion**: Lower serum bicarbonate level is associated with a more rapid decline in renal function.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1258**

**The Impact of Short Stature on HRQOL in Children with CKD** Amira Al-Uzri,[1] Arlene C. Gerson,[2] Matthew Matheson,[2] Debbie S. Gipson,[3] Ora Yadin,[4] Susan L. Furth,[5] Bradley A. Warady.[6] *[1]Oregon Health & Science Univ; [2]Johns Hopkins; [3]Univ Mich; [4]UCLA; [5]Children's Hosp of Phili; [6]Children's Mercy.*

Short stature (SS) and Health-related quality of life (HRQOL) impairments are common in children with chronic kidney disease (CKD). Our hypothesis is that children with CKD and SS, defined as height z score < 3rd percentile for age and gender, have lower QOL scores compared to those with CKD and normal height (NH).

Methods: This was a cross-sectional review of HRQOL in 483 children with mild-moderate CKD who were enrolled in the NIH multicenter CKiD study. HRQOL was assessed using the Child and Parent Report Pediatric Inventory of Quality of Life Core Scales (PedsQL, V4.0). Uni and multivariate regression analyses were used.

Results: Mean age was 10.4 ± 4.5 in SS and 11.3 ± 4.3 in NH groups. 51% (n =36) in SS and 63% (n =261) in NH group were male ($p>0.05$), with no differences in ethnicity/race. In univariate analysis, only parent-report PedsQL physical domain was different in the SS vs. NH group ($p=.02$) (Table), and GH therapy did not appear to impact QOL scores. Multivariate analysis adjusted for height Z-score, GH use, age, and gender, showed that higher height z-scores were associated with an increase in parent-reported PedsQL physical domain ($p=0.03$) and PedsQL social domain ($p=0.01$). No significant associations were seen with any of the child self-reported PedsQL domains.

Conclusion: Adjusted child and parent reported HRQOL scores in children with SS are not different compared to NH children with mild-moderate CKD, except in parent-reported physical and social domains. Longitudinal analysis on the effect of SS on QOL in CKD is ongoing.

| | SS/NH | SS group | NH group |
|---|---|---|---|
| P-Physical | 69/399 | 72.7 ±24.1 | 79.0±20.9* |
| P-Emotional | 69/398 | 73.5±16.6 | 74.5±18.3 |
| P-Social | 69/398 | 74.0±23.0 | 79.0±21.0 |
| P-School | 66/379 | 63.7±22.1 | 65.4±21.5 |
| P-Overall | 69/399 | 71.4±17.9 | 75.3±16.9 |
| C-Physical | 45/304 | 78.3±18.6 | 80.2±15.5 |
| C-Emotional | 45/304 | 73.8±19.5 | 73.5±18.0 |
| C-Social | 45/304 | 78.7±22.6 | 80.8±18.7 |
| C-School | 45/304 | 62.2±20.5 | 64.4±18.1 |
| C-Overall | 45/304 | 73.9±15.3 | 75.4±14.1 |

(* P< 0.05), P=Parent, C=Child

Disclosure of Financial Relationships: nothing to disclose

### F-PO1259

**Upregulation of HDAC2 Expression by Oxidative Stress Is Associated with Interstitial Fibrosis in Experimental Model of Chronic Cyclosporine Nephropathy** Ji-Hyun Song,[1] Yeon Tae Jeon,[2] Jungyeon Ghee,[1] ShangGuo Piao,[1] Sol Kim,[3] Chul Woo Yang.[1] *[1]Transplant Research Center, The Catholic University of Korea, Seoul, Republic of Korea; [2]Integrative Research Support Center, The Catholic University of Korea, Seoul, Republic of Korea; [3]Department of Bioengineering, University of Pennsylvania, Philadelphia.*

**Background**: Cyclosporine A (CsA)-induced chronic nephrotoxicity is associated with oxidative stress. It is well known that the histone deacetylases (HDACs) is activated by oxidative stress, and that activation of HDAC leads to interstitial fibrosis in diabetic nephropathy. We performed this study was performed to evaluate the association between oxidative stress and expression of HDAC2 in an experimental model of chronic CsA nephropathy.

**Methods**: Sprague-Dawley rats were maintained on a low-salt diet (0.05% sodium) and treated daily for 4 weeks with vehicle (olive oil; 1 ml/kg sc) and CsA (15 mg/kg sc). Induction of chronic CsA nephropathy was confirmed with typical histopathological findings. HDAC2 expression was evaluated with immunohistochemistry and immunoblot of HDAC2. Oxidative stress was detected with immunoblotting, 24-h urinary excretion, and tissue expression of 8-hydroxy-2'-deoxyguanosine (8-OHdG).

**Results**: Four-week treatment of CsA developed interstitial inflammatory cells infiltration and typical tubulointerstitial fibrosis(TIF). Immunoblot of HDAC2 revealed that the expression of HDAC2 increased in CsA treatment group of rat kidney, and immunohistochemical analysis showed that HDAC2 expression was localized to renal tubular cells. Oxidatve stress measured with 24-h urinary excretion of 8-OHdG was significantly increased in CsA group (61.2±7.9 ng/day) compared with VH group (39.1±4.7 ng/day), and it was confined to renal tubular cells. There was a close correlation between HDAC2 expression and 8-OHdG ($r$=–0.64, $P$<0.01).

**Conclusions**: Increased HDAC2 expression by oxidative stress may be associated with interstitial fibrosis in chronic CsA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1260

**Metabolic Syndrome, Its Components, and Chronic Kidney Disease: A Systematic Review** George Thomas, Titte Srinivas, Sankar D. Navaneethan. *Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH.*

**Purpose**: Metabolic syndrome (MetS) and its components are widely prevalent in patients with chronic kidney disease (CKD). However, MetS as a risk factor for development of CKD in the general population has not been sufficiently evaluated. We conducted a systematic review to assess the associations between MetS, its components, and the risk for development of CKD and microalbuminuria.

**Methods**: We searched MEDLINE (August 2009) for relevant studies that examined the associations between MetS (using NCEP-ATP III and modified NCEP-ATP III criteria) and kidney disease in adults. Two reviewers independently assessed eligibility and extracted data using a standardized form. Odds ratios (OR) reported in individual studies were pooled using a random effects model.

**Results**: Eighteen studies (132,678 patients) were included in this review. Thirteen studies defined MetS according to the NCEP-ATP III diagnostic criteria, 3 studies using the modified NCEP-ATP III criteria, and 2 studies according to the definition proposed by the Japanese Society for Internal Medicine. MetS was associated with development of CKD (11 studies, OR 1.70, 95% CI 1.52-1.90) and microalbuminuria (7 studies, OR 2.04, 95% CI 1.72-2.42). The associations became stronger as the number of components of MetS increased from 1 to 5.

| Variable | Pooled Odds Ratio | 95% CI | $p$ – value |
|---|---|---|---|
| 1 component of MetS | 1.35 | 1.03 – 1.79 | 0.03 |
| 2 components of MetS | 1.68 | 1.37 – 2.06 | <0.001 |
| 3 components of MetS | 2.36 | 1.83 – 3.05 | <0.001 |
| 4 components of MetS | 2.64 | 1.74 – 4.00 | <0.001 |
| 5 components of MetS | 3.88 | 2.24 – 6.70 | <0.001 |
| Metabolic syndrome | 1.70 | 1.52 – 1.90 | <0.001 |
| Impaired fasting glucose | 1.61 | 1.28 – 2.03 | <0.001 |
| Hypertension | 1.48 | 1.14 – 1.91 | 0.002 |
| Triglycerides | 1.34 | 1.26 – 1.42 | <0.001 |
| HDL | 1.25 | 1.03 – 1.55 | 0.04 |
| Obesity | 1.20 | 1.06 – 1.35 | 0.003 |

Among components of MetS, patients with impaired fasting glucose had a 61% higher risk of developing CKD, followed by hypertension (48%), elevated triglyceride levels (34%), low HDL levels (25%), and obesity (20%).

**Conclusion**: MetS and its components are independently associated with a dose-dependent increased risk for CKD and microalbuminuria. This emphasizes the need for identifying and studying interventions for individual components of MetS, and its effects on CKD.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1261

**Elevated Endogenous Erythropoietin Levels Are Associated with Mortality in Diabetic Chronic Kidney Disease** Martin Wagner,[1] Ahsan Alam,[2] Angelika Koljaja-Batzner,[1] Josef Zimmermann,[1] Christoph Wanner,[1] Lothar Schramm.[1] *[1]Div. of Nephrology, University Hospital Wuerzburg, Germany; [2]Div. of Nephrology, McGill University, Montreal, Canada.*

Anemia and chronic inflammation are prevalent in diabetic chronic kidney disease (CKD) and are both associated with increased mortality. Previously we found elevated endogenous erythropoietin (EPO)-levels that were associated with inflammation in advanced CKD. Resistance of the bone marrow to EPO might explain this phenomenon. In this prospective cohort study we evaluated the impact of EPO on mortality.

Between 2004 and 2005, 214 patients with type 2 diabetes were enrolled. Exclusion criteria included end-stage renal disease and EPO-stimulating agent-therapy. The association of baseline EPO with mortality was investigated using Cox proportional hazards models, while controlling for age, estimated glomerular filtration rate (eGFR) and C-reactive protein (CRP) at baseline.

Patients (median age 67 yrs, 51% male, median duration of diabetes 10 yrs, median eGFR 50 ml/min/1.73 m$^2$, mean hemoglobin 13.1 g/dl) were followed for up to 6.5 yrs (median 3.8 yrs). Forty-one patients died (19.2%). Elevated EPO levels were associated with increased mortality, even after adjustment for age and GFR (table). The effect of CRP as a predictor of mortality became non-significant when including EPO in the model.

| | univariate | multivariate |
|---|---|---|
| | HR (p-value) | HR (p-value) |
| EPO | 2.56 (<0.001) | 2.88 (0.001) |
| age | 1.07 (<0.001) | 1.05 (0.03) |
| eGFR | 0.97 (<0.001) | 0.98 (0.04) |
| CRP | 1.52 (0.002) | 1.18 (0.28) |

In our cohort of diabetic patients with CKD, elevated EPO-levels were independent risk factors for mortality. The impact of EPO was even stronger than that of CRP, indicating that not only inflammatory processes play a role. We propose a role for EPO-resistance of the bone marrow as a symptom of aggravated inflammatory processes as well as of potentially inflammation-independent mechanisms, which confer a detrimental effect on mortality.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1262

**Coincidence of HCV and HGV Infections in Hemodialysis Patients** Wladyslaw Sulowicz, Jerzy Kopec, Przemyslaw Miarka, Katarzyna Janda, Barbara Tabor-Ciepiela. *Department of Nephrology, Jagiellonian University, Krakow, Poland.*

HCV and HGV were recognized as highly prevalent in maintenance dialysis population and variable from country to country and unit to unit. The introduction of serologic tests, for detecting antibodies to HCV (anti-HCV) and HGV (anti-HGV) antigens, has facilitated the study of the epidemiology as well as clinical significance of HCV and HGV infections in patients on maintenance dialysis. The implementation of molecular biology techniques (polymerase chain reaction) for detecting HCV and HGV viremia (HCV-RNA, HGV-RNA) facilitate diagnosis and transmission routes of the infection.

The aim of the study was to evaluate the coincidence of HCV and HGV infections in dialysis population. The study was performed in the group of 215 patients aged 26-81 years (mean 53.2) on 3 x week maintenance hemodialysis from 33 to 301 months (mean 101.8 months). Anti-HCV and anti-HGV antibodies were determined based on immunoenzymatic, III$^{rd}$ generation, methods. HCV-RNA and HGV-RNA were estimated using polymerase chain reaction (PCR).

The anti-HCV antibodies were present (+) in 40 (18.6 %) of patients and negative (-) in 175 (81.4%) patients. From the group of patients with anti-HCV (+) antibodies (42.5%) were also HCV-RNA (+) whereas from the patients with anti-HCV (-) antibodies 10.4% were HCV-RNA (+).

Anti-HGV antibodies tested in the group of 32 anti-HCV (+) patients and 61 anti-HCV (-) patients were positive in 20 (62.5 %) and 22 (36.1 %) patients, respectively. In the group of patients with anti-HGV (+) antibodies HGV-RNA (+) were detected in 63.6%.

Anti-HCV (+) and anti-HGV (+) patients were dialysed longer as compared with negative ones: 144.5 vs. 83.5 months, p<0.001 and 119 vs. 96.4 months, p NS, respectively.

**Conclusions**: 1. The prevalence of HCV and HGV infections in the dialysis population is very high. 2. The coincidence of HCV and HGV infections is frequent. 3. Our result showed that even anti-HCV (-) and anti-HGV (-) patients carry the risk of viral infection due to detected HCV-RNA and HGV-RNA in some of them.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1263**

**Large Scale Genotype-Phenotype Integration of GFR Susceptibility Loci Identifies CKD Associated Molecular Pathways** Sebastian Martini,[1] Katherine Gurdziel,[1] Carsten A. Böger,[4] Viji Nair,[1] Caroline S. Fox,[3] Clemens D. Cohen,[2] Matthias Kretzler.[1] *[1]University of Michigan; [2]University of Zürich; [3]National Institutes of Health; [4]Regensburg University Medical Center.*

Despite recent progress determining risk alleles for chronic kidney disease (CKD), translating novel insights into underlying pathophysiologic mechanisms remains a challenge. Integrating genome-wide association studies (GWAS) with gene expression data sets might help to define associated molecular pathways. The study's aim was to merge CKDGen GWAS with European Renal cDNA Bank (ERCB) CKD transcript profiles to define CKD associated regulatory networks.

CKDGen recently reported 18 susceptibility loci for glomerular filtration rate (GFR) and CKD in European-ancestry participants. At these loci, 42 genes were located within 60 kb of the lead SNP. Thirty of 42 genes were detected by expression profiles from glomerular compartments patients with 10 different diseases in the ERCB (n=196; eGFR range 6–193 mL/min/1.73m2) and a living kidney donor control group (n=41). Ten of 30 CKDGen-associated transcripts showed significant correlation with GFR in the ERCB CKD cohort (|R|>0.25, FDR<0.01). Monte Carlo simulations confirmed significant enrichment of GFR correlation compared to randomly selected gene sets (p<0.001).

Next, CKDGen associated transcripts that correlated with GFR were interrogated for shared biological functionality. Starting from the hypothesis that genes expressed in the same tissue with concordant disease specific regulation share common transcriptional regulators, co-regulated mRNAs for each CKDGen associated transcript were identified in the ERCB CKD gene expression cohort (|R|>0.6, FDR<0.01,). In the correlated gene sets (106 transcripts/CKDGen candidate gene), 88 KEGG pathways were significantly enriched, e.g. transcripts co-regulated in CKD with VEGFA were enriched in ischemia and IL-8 pathways, PIP5K1B co-regulated transcripts in Rac signaling pathways.

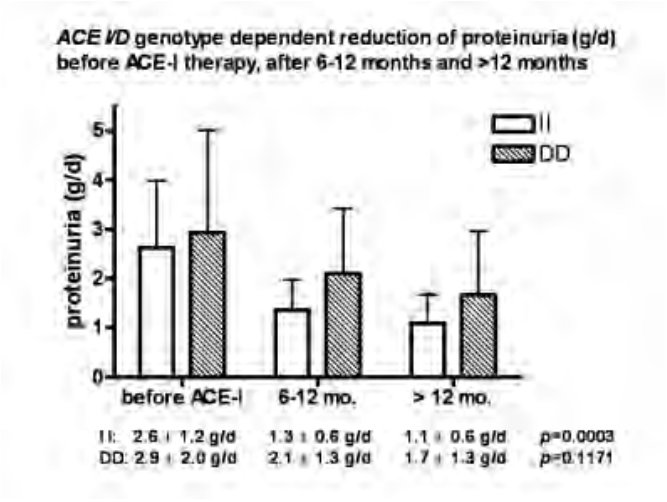
CKDGen associated transcript levels were correlated with GFR in the ERCB CKD cohort. CKDGen transcripts and co-regulated mRNAs identified known and novel pathways of progressive kidney disease for further analysis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1264**

**The Antiproteinuric Efficacy of an ACE-Inhibitor Treatment Depends on the *ACE I/D* Genotype in Children with Chronic Nephropathies** Rainer Buescher, Ilja Finkelberg, Anja K. Büscher, Peter F. Hoyer. *University of Duisburg-Essen, Pediatrics 2, Pediatric Nephrology, Essen, Germany.*

**BACKGROUND:** A successful inhibition of the renin-angiotensin-aldosterone system (RAS) using ACE-inhibitors (ACE-I) has been shown to be renoprotective in adult and pediatric patients. However, it remains unclear whether the ACE-genotype has an impact on the effect of the ACE-I therapy. The aim of our retrospective study was to analyze whether the antiproteinuric efficacy of an ACE-I treatment is influenced by the *ACE I/D* genotype in children with chronic nephropathies. **METHODS:** We included 60 children and adolescents (28 male/32 female, age 5-18 years, mean age 14.4±3.8 years) with different proteinuric nephropathies in our study. A final ACE-I dosage of 5.3±1.6 mg/m$^2$ was administered. In 26 children arterial hypertension could also be confirmed. **RESULTS:** Allelic frequencies of the *ACE I/D* polymorphism were not different from healthy controls. Long-term treatment with ACE-I exceeding 6 months led to a slight decline of the glomerular filtration rate (GFR). However, this GFR-reduction was not different among carriers of the different genotypes. Proteinuria significantly dropped in the overall population within 6-12 months after initiation of ACE-I treatment. Homozygous carriers of the D-allele showed a diminished therapeutic response when compared to homozygous I-allele carriers (Figure 1) and the long-term therapeutic effect was much shorter in carriers of the D-allele (Δ proteinuria II: 61% of baseline after 6 months and 48% of baseline after 12 months vs. DD: 94% of baseline after 6 months and 99% of baseline after 12 months). **CONCLUSIONS:** Children carrying the D-allele of the ACE I/D polymorphism show a reduced antiproteinuric response when treated with an ACE-I. Our retrospective data suggest that adapted therapeutic concepts are necessary in children receiving ACE inhibitors.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1265**

**Nonmuscle Myosin Heavy Chain IIA Encoded by *MYH9* Is Localized in the Primary Process of Podocytes and Its Expression Is Significantly Decreased in Human FSGS** Ken-Ichiro Miura,[1] Hidetake Kurihara,[2] Hiroko Chikamoto,[3] Motoshi Hattori,[3] Satoshi Sasaki,[4] Takashi Igarashi,[1] Takashi Sekine.[1,5] *[1]Pediatrics, The University of Tokyo, Tokyo, Japan; [2]Anatomy, Juntendo University School of Medicine, Tokyo, Japan; [3]Pediatric Nephrology, Tokyo Women's Medical University, Tokyo, Japan; [4]Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan; [5]Pediatrics, Toho University Ohashi Medical Center, Tokyo, Japan.*

Objective: To investigate the precise localization of nonmuscle myosin heavy chain (NMMHC)-IIA encoded by *MYH9* in podocytes and analyze its expression in experimental and human proteinuric diseases.

Methods: Localization of NMMHC-IIA in podocytes was determined by immunofluorescence and immunogold labeling analyses in normal rat kidneys. Dual immunostaining of NMMHC-IIA and podocyte-associated molecules was performed in normal and puromycin aminonucleoside (PAN) nephrosis rats. Immunohistochemical analysis of NMMHC-IIA was performed in patients with childhood-onset idiopathic nephrotic syndrome (6 patients with FSGS and 2 with minimal change disease (MCD)) and a variety of chronic glomerulonephritis (CGN) with heavy proteinuria.

Results: Immunofluorescence analysis demonstrated the colocalization of NMMHC-IIA and nestin, a protein located in the cell body and the primary processes of the podocyte. This finding was confirmed by immunogold labeling analysis. The glomeruli of the PAN-treated rats (day 11) showed significantly decreased staining of NMMHC-IIA, while the staining of podocalyxin and ZO-1 was preserved. Immunohistochemical analysis in the human proteinuric diseases revealed that the staining of NMMHC-IIA was markedly decreased only in steroid resistant FSGS, while that in MCD and CGN was preserved.

Conclusion: Our results demonstrated that decreased expression of NMMHC-IIA is specific to FSGS in the human, suggesting that NMMHC-IIA is a key molecule participating in the pathophysiology of steroid resistant FSGS.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1266**

**Dependency on Immunosuppressants in Children with Steroid-Resistant Nephrotic Syndrome after Cyclosporine and Steroid Therapy** Yuko Hamasaki, Norishige Yoshikawa, Satoshi Sasaki, Kazumoto Iijima, Koichi Nakanishi, Shuichi Ito, Takeshi Matsuyama, Kenji Ishikura, Tetsuji Kaneko, Masataka Honda. *Pediatric Nephrology, Japanese Study Group of Renal Disease in Children, Fuchu, Tokyo, Japan.*

Background

We previously showed in a prospective, multicenter trial in children with steroid-resistant nephrotic syndrome (SRNS) that high remission rates (>80%) were obtained after 12 months of treatment with cyclosporine and steroid therapy (Hamasaki et al., PN 2009). Recently, remission rates of SRNS have improved with cyclosporine; however, there are few data about the long-term prognosis.

Methods

We analyzed the outcome at 5 years after being enrolled in the previous trial. The statuses were classified as complete remission (CR), partial remission (PR), persistent nephrotic syndrome (NS) and over stage 3 chronic kidney disease (CKD). Steroid-sensitive nephrotic syndrome (SSNS) and frequently relapsing nephrotic syndrome (FRNS) were included in CR.

Results

We obtained follow-up data for 5 years in 32 of the 35 patients. The first renal biopsy showed 20 cases with minimal changes, 5 with diffuse mesangial proliferation and 7 with

focal segmental glomerulosclerosis. The patients' statuses at five years were as follows (the median age, 7.6 years): 28 patients had CR, 1 had PR, 1 had persistent NS and 2 had CKD (including 1 with end stage renal failure). Among the 28 patients with CR, 5 had no relapse and 3 had SSNS without immunosuppressants; however, 20 required immunosuppressants (71.4%). The most commonly used immunosuppressant was cyclosporine. Nine of the 20 patients continued to show FRNS with immunosuppressants. The frequent adverse events were hypertension (n=7), osteoporosis (n=2), cataracts (n=2) and hypertrichosis (n=2). The mean standard deviation score for height at the last follow-up was -0.45.

Conclusion

The present results show that high renal survival and remission rates can be obtained. However, many children require immunosuppressants, which can cause serious problems. The advancement of treatment for SRNS has resulted in a shift of management of SRNS from prevention of end stage renal failure to long-term maintenance of relapse after induction therapy.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1267

**Immunosuppressive-Dependent Glomerular Minimal Change Disease – A Case for Rituximab?** Elion Hoxha, Rolf A. Stahl, Sigrid Harendza. *Department of Internal Medicine III, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.*

Introduction

Glomerular minimal change disease (MCD) is a major cause of nephrotic syndrome. Steroid therapy is effective in achieving remission but relapses, steroid-dependence and steroid-resistance are therapeutic challenges. The use of second-line agents such as cyclophosphamide is associated with toxicity and adverse effects. Therefore we studied the effect of rituximab (RTX) on proteinuria in adult patients with immunosuppressive (IS) -dependent MCD.

Methods

Seven patients with IS-dependent MCD and frequent relapses on IS regimes were included. Patients needed steroids or some other IS agents in order to maintain remission and tapering of IS medication lead to a relapse of proteinuria. Clinical data were gathered and blood tests and urinalysis were performed before RTX therapy, one week, four weeks and then every three months afterwards. Patients were treated with a single dose of RTX (375 mg/m²). An additional dose of RTX was administered-if necessary-depending on B-cell count and proteinuria.

Results

After RTX treatment all patients remained relapse-free, all additional IS agents were tapered and eventually stopped. Three patients needed more than one RTX treatment. All patients had a reduction in peripheral CD20+ B cells, which returned to almost normal level six months after RTX therapy. Return of B cells was not associated with relapses of the disease. Proteinuria ceased completely after RTX treatment in five patients, another patient achieved complete remission at the end of follow-up. Serum albumin increased in all seven patients, reaching normal range at the end of the follow-up period. Blood levels of cholesterol and triglycerides decreased to normal range at the end of the follow-up period. Further medication-besides IS-was reduced from 3.6 drugs/patient to 1.2 drugs/patient during the follow-up period.

Conclusions

Our data show that RTX could be an alternative in the therapy of patients with IS-dependent MCD. These promising results have to be further investigated in larger, randomized patient cohorts. RTX may also allow to better understand the factors and cells that may play a role in the pathogenesis of this disease.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1268

**The Impact of Antiproteinuric Therapy on the Prothrombotic State in Patients with Overt Proteinuria** Bakhtawar Khan Mahmoodi,[1] André Mulder,[2] Femke Waanders,[3] Henri Spronk,[4] René Mulder,[2] Maartje C. J. Slagman,[3] Gerjan Navis,[3] Hugo Ten Cate,[4] Hanneke Kluin-Nelemans,[1] Gozewijn Dirk Laverman.[3] *[1]Dep. Hematology; [2]Dep. Laboratory Medicine; [3]Dep. Nephrology, University Medical Center Groningen, Groningen, Netherlands; [4]Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, Netherlands.*

**Background:** Overt proteinuria is a strong risk factor for thromboembolism due to changes in levels of various coagulation proteins and urinary antithrombin loss. The described coagulation disturbances in these patients are based on outdated studies conducted primarily in the 1970's and 80's. Whether these coagulation disturbances resolve with anti-proteinuric therapy has yet to be studied.

**Methods:** A total of 32 adult patients with overt proteinuria (median 3.7 g/d; interquartile range 1.5-5.6) were matched for sex and age with 32 healthy volunteers. Patients were participants of an intervention trial designed to assess optimal antiproteinuric therapy with losartan and diuretics. Levels of various coagulation factors and inhibitors, and two thrombin generation assays (i.e., Calibrated Automated Thrombogram [CAT] and prothrombin fragment 1+2) were performed at baseline and 36 weeks after antiproteinuric treatment initiation.

**Results:** Whereas levels of various procoagulant proteins were higher in patients, levels of antithrombin were comparable in patients versus controls. Based on both thrombin generation assays, patients were substantially more prothrombotic than controls (P<0.001). After 36 weeks of antiproteinuric therapy, median proteinuria was reduced to 0.9 g/d (interquartile range 0.6-1.4). Similarly, levels of various liver-synthesized coagulation factors and inhibitors as well as prothrombin fragment 1+2 levels were significantly reduced; however, in CAT parameters only moderate amelioration in activated protein C resistance was observed. Increased levels of endothelial derived factor VIII and von Willebrand factor persisted.

**Conclusions:** Patients with overt proteinuria are in a more prothrombotic state with increased thrombin generation, as compared to non-proteinuric controls. Antiproteinuric therapy reverses the protrombotic state.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1269

**Anti-Protease Drugs To Treat Idiopathic Nephrotic Syndrome: A New Working Hypothesis** Roberta Camilla, Maria Gabriella Porcellini, A. Amore, Bruno Gianoglio, Valentina Daprà, Elisa Loiacono, Pier Angelo Tovo, Rosanna Coppo. *Nephrology, Dialysis and Transplant, Regina Margherita Hospital, Turin, Italy.*

**INTRODUCTION**

Podocyte alterations characteristic of nephrotic syndrome (NS), i.e.changes in slit diaphragm and cytoskeleton reorganization, are regulated by enzyme pathways. Interest has been focused on the lysosomal cystine-protease cathepsin L. We have been treating some cases of steroid resistant or steroid-dependent NS (SRNS or SDNS) with protease inhibitors adopted in HIV infection, aiming at depressing the proteasome and the consequent activation of NF-kB. The interest of our pilot study cohort has been increased not only for a higher number of cases with longer follow-up, but also on the light that an unsuspected target of these antiprotease drugs could be the podocytary proteases regulating cathepsin L.

**MATERIALS AND METHODS** We used the protease inhibitor saquinavir (750 mg/m2 bid) in 7 patients, 5 SDNS and 2 SRNS (aged 11 years, range 8-24), with history of NS lasting 7 (1-14) years already treated with prednisone and iv metylprednisolone (7/7), cyclosporine A (CyA) (6/7), tacrolimus (TAC) (6/7), mycophenolate (4/7), plasma-exchange (2/7) and rituximab (4/7).

**RESULTS** In 5 cases the follow-up was longer than 6 months. In 4/5 patients we obtained a complete remission or a partial remission with infrequent relapses easily controlled with minimum increase in steroid therapy; in 1/5 a decrease of proteinuria below nephrotic range was obtained.

Saquinavir was given in association very low doses of calcineurin-inhibitors (CyA 2 mg/Kg/day, TAC 0.007-0.07 mg/Kg/day) and prednisone (0.1-0.5 mg/Kg/day). In these 5 cases the cumulative dose of steroids during the follow-up (6 months-4 years) was reduced of an average of 53% (range 32%-85%) in comparison with the 12 months before saquinavir.

In 2 cases the therapy was started < 6 months ago: one patient achieved a good result with remission of proteinuria, while the other one has failed to show response so far.

**CONCLUSIONS** This pilot study using anti protease drugs for multidrug resistant/dependent NS is providing some interesting results, opening new perspectives for NS unresponsive to traditional treatments.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1270

**Circulating Angiopoietin-2 but Not Angiopoietin-1 Can Be Removed through Plasma Exchange – A Pilot Study in Critically Ill Patients with Thrombotic Microangiopathy and Anti-Glomerular Basement Membrane Disease** Svjetlana Lovric,[1] Alexander Lukasz,[1] Carsten Hafer,[1] Jan T. Kielstein,[1] Marion Haubitz,[1] Hermann G. Haller,[1] Philipp Kümpers.[2] *[1]Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [2]Medicine D, Divison of General Internal Medicine, Nephrology and Rheumatology, University Hospital Münster, Münster, Germany.*

Introduction: In critically ill patients, the massive release of angiopoietin-2 (Ang-2) from Weibel-Palade bodies interferes with protective angiopoietin-1 (Ang-1)/Tie2 signaling in endothelial cells, thus leading to vascular inflammation and subsequent organ-dysfunction. We hypothesized that plasma exchange (PE) is efficient for lowering excess Ang-2 levels in critically ill patients with thrombocytic microangiopathy (TMA) or anti-glomerular basement membrane (anti-GBM) disease.

Methods: Plasma Ang-1 and Ang-2 were measured by immuno-luminometric assays in patients with TMA (n=9) or anti-GBM disease (n=4) before and after up to four PE sessions. Twenty apparently healthy volunteers served as controls.

Results: Median (IQR) plasma levels of Ang-2 were markedly increased in patients with TMA (7.3 (2.4-21.1) ng/mL) and anti-GBM disease (5.8 (3.4-7.0) ng/mL) compared to healthy controls (1.0 (0.9-1.4) ng/mL, P <0.001). Moreover, Ang-1 plasma levels were decreased in both, TMA (1.02 (0.62-1.62) ng/mL) and anti-GBM disease patients (0.74 (0.59-3.62) ng/mL) compared to healthy controls (2.5 (1.93-3.47) ng/mL, P <0.005). During a total of 32 treatments, PE effectively lowered elevated mean (SD) Ang-2 plasma levels by 36.7 ± 19.6 % per treatment (P <0.0001), whereas low Ang-1 plasma levels remained unchanged ( 0.3 ± 58.5 %; P =0.147). Ang-2 levels declined to almost normal values during ≤ 4 PE treatments (Friedman 's test P <0.0001).

Conclusions: PE effectively restored the balance between Ang-1 and Ang-2 by lowering excess systemic Ang-2 levels. It remains to be elucidated if the removal of Ang-2 is crucial to ameliorate endothelial damage in critically ill patients with severely altered endothelial integrity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1271**

**Efficacy and Safety of Low Dose of Oral Sirolimus on Refractory Nephrotic Syndrome** Jian Hui Yang. *Renal Division, The 456th Hospital Kidney Center of P.L.A., Jinan, Shandong, China.*

**Purposes**: To evaluate the efficacy and safety of sirolimus on refractory nephrotic syndrome.

**Methods**: Twenty-five patients with primary nephrotic syndrome were refractory to regular dose of prednisone and other immunosuppressors such as intravenous cyclophosphamide, oral cyclosporine or mycophenolate mofetil. After cessation of immunosuppressors for more than 3 months, they were administrated with 0.3~0.5mg/d.kg$^{-1}$ oral prednison and 2 mg/d sirolimus (NCPC GENETECH BIOTECHNOLOGY CO. LTD) for 6 to 12 months. Sirolimus doses were adjusted so that the serum sirolimus concentration could be maintained between 4 to 10 ng/ml. No angiotensin-converting enzyme inhibitors or angiotensin receptor blockers were taken in these patients. The clinical manifestations, laboratory parameters and adverse reactions were observed.

**Results**: After 3 months of therapy, complete remission was induced in fourteen patients while partial remission was found seven patients. Mesangial proliferative glomerulonephritis (MsPGN) had the higher remission rate and shorter time to relieve compared with IgA nephropathy, membranoproliferative glomerulonephritis and focal segmental glomerulosclerosis. Their serum creatinine level decreased markedly. The most common side effects were dyslipidemia. Thirteen patients' hyperlipidemias were obviously exacerbated during the early stage of therapy. However, their serum triglyceride, cholesterol, low density lipoprotein and ApoB levels declined drastically after the patients got complete or partial remission. On the contrary, those parameters remained elevated in patients who failed to response to sirolimus. Acnes were seen in six patients. Influenza-like symptom happened in four patients. Slightly elevated serum aspartate aminotransferase or creatinine level was only seen in one patient respectively. Bone marrow depression was not found in all these patients. During follow-up, the relapse rate in MsPGN was much lower than that in other types of glomerulonephritis.

**Conclusions**: Low dose of oral sirolimus was effective and safe for refractory nephrotic syndrome. Dyslipidemia in nephrotic syndrome was not the contradictions of oral sirolimus.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1272**

**The Combination of Very Low-Dose Prednisolone with Cyclosporine as an Initial Treatment for Minimal Change Nephrotic Syndrome (MCNS) in Adults** Yoshihiko Inoue, Takahiro Nakayama, Kiyoko Inui, Ashio Yoshimura. *Division of Nephrology, Showa University Fujigaoka Hospital, Yokohama, Kanagawa, Japan.*

MCNS is characterized by good response to glucocorticoid (prednisolone, PSL), however frequent relapses may occur and increasing amount of PSL dosage is a critical problem. We tried combination of very low-dose of PSL to 50% of conventional therapy with cyclosporine (CsA) as an initial treatment for adult MCNS patients. Prospectively 14 patients who had all biopsy-proven MCNS were studied for one year. They were randomly assigned to two groups, Group A (PSL 0.8mg/kg/day, n=7) and Group B (PSL 0.4mg/kg/day + CsA 1.5~2 mg/kg/day, n=7). PSL tapering was begun three days after complete remission. The daily PSL dosage was gradually reduced by 5mg/day every two weeks, by 5mg/day every four weeks after 20mg/day, and 5mg/day every six months after 10 mg/day in each group. The daily CsA doseage was sustained by using concentration monitoring with 2 h post-dosing level (C2) (600~1000ng/ml). There were no differences in serum albumin levels, renal function, or protein excretion between both groups before treatment. Initial PSL dosage was 48.6±6.3 mg/day (mean±SE) in Group A and 22.9±3.9 mg/day in Group B. Total PSL dosage in Group A (6455.0±764.6 mg/year) was more than in Group B (3917.5±450.3 mg/year ($p<0.01$). However, there were no differences in duration for complete remission between Group A (14.3±5.5 days) and Group B (15.1±3.3 days). Average hospitalization was significantly longer in Group A (51.6±19.5 days) than in Group B (19.4±4.2 days) ($p<0.01$). Furthermore, There were no relapse and no renal impairment of both groups in one year. Side effects by PSL treatment was observed in two patients in Group A (steroid induced DM and hypercholesterolemia) but none in Group B. C2 level was 628.4±178.1ng/ml after one year. In conclusion, combination of 50% of PSL dosage with CsA as an initial treatment was effective as well as the conventional therapy with PSL alone. The combination treatment may provide a new way to decrease PSL dosage and reduce length of hospital stay for adult MCNS patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1273**

**Urinary Biomarkers for the Clinical Efficacy in FSGS Patients Treated with GC1008** Nikolay Bukanov, Sarah E. Moreno, Oxana Beskrovnaya, Steven R. Ledbetter. *Department of Cell Biology, Genzyme Corporation, Framingham, MA.*

We used SELDI-TOF technology to identify biomarkers of efficacy as well as response-predicting biomarkers from the phase I clinical trial on steroid-resistant FSGS patients treated with a single dose of GC1008 (anti-TGFb antibody). In this clinical trial, four cohorts of patients (four patients in each cohort) were treated with four different single doses of GC1008 ranging from 0.3 to 4 mg/kg. From each patient, multiple urine samples were collected over a 16 week period (at 0, 0.5, 1, 2, 3, 4, 8, 12, and 16 weeks) after treatment with GC1008 and subjected to proteome profiling with SELDI-TOF technology. A three-step approach was used for analysis. First, we used proteinuria measurements to identify responders to treatment. Analysis of protein/creatinine ratio in patients from the GC1008 study suggested that there are two responders in cohort A and two responders in cohort C (treated with 1 and 4 mg/kg, respectively) that showed a significant reduction in proteinuria by weeks 12-16 after treatment. Cohorts B and D (treated with 2 and 0.3 mg/kg, respectively) showed no obvious responders. All non-responder patients had sustained urine protein levels through out the 16 week study within the range observed prior to treatment. Second, we performed comparative responder/non-responder proteome analysis in the strongest responders from cohort A and identified a set of 44 anonymous urinary biomarkers: 7 biomarkers of efficacy and 37 predicative biomarkers of a response to treatment. Finally, we conducted comparative analysis of these markers with less prominent responders from cohort C and identified a subset of 7 biomarkers that showed similar trends in all four responders from both cohorts A and C.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1274**

**Safety & Efficacy of Eculizumab in aHUS Patients on Chronic Plasma Therapy: Interim Analysis of a Phase II Trial** Petra Muus,[1] Christophe M. Legendre,[2] Kenneth Douglas,[3] Maryvonne Hourmant,[4] Yahsou Delmas,[5] Birgitta Maria Herthelius,[6] Antonella Trivelli,[7] Chantal Loirat,[8] Timothy H. Goodship,[9] Christoph Licht.[10] *[1]Radboud Univ Med Ctr, Netherlands; [2]Hôpital Necker, France; [3]Beatson, West Scotland Cancer Ctr, United Kingdom; [4]CHU Nantes-Hôtel Dieu, France; [5]CHU Bordeaux - Pellegrin, France; [6]Huddinge Hospital, Sweden; [7]Istituto G. Gaslini, Italy; [8]Hopital Robert Debré, France; [9]Newcastle Univ, United Kingdom; [10]Hospital for Sick Children, CA.*

Atypical hemolytic uremic syndrome (aHUS) is a rare, chronic, life-threatening disease due to constitutive complement dysregulation resulting in ongoing, diffuse thrombotic microangiopathy (TMA).TMA causes organ ischemia, renal failure, and hemolytic anemia, leading to 1 year 60% ESRD/death. TMA continues despite chronic plasma therapy (PT), a debilitating procedure that subjects patients (pts) to blood-induced injuries, toxicity and poor quality of life. The safety/efficacy of terminal complement inhibitor eculizumab (Ecu) to prevent TMA, maintain renal function, and eliminate PT in 20 adult/adolescent aHUS pts treated with chronic PT is evaluated in a 26-week (wk) open-label single-arm clinical trial. Ecu dosing:900mg/wk x4; 1200mg Q2 wks starting week 5. Neisseria vaccine ≥14 days before Ecu. Primary endpoint(endpt): TMA-Event Free Status (stable platelets, no PT, no new dialysis). Interim analysis (12 wk; n=15) was positive: 87% (13/15; 95% CI 60-98) TMA-Event Free. Median time to first TMA-Event not reached. Secondary endpt: With Ecu, TMA Intervention (TMAI; PT or dialysis) not observed, median time to first TMAI not reached. Exploratory endpt: eGFR stabilized/increased with Ecu vs chronic PT(30.5 vs 27 mL/min/1.73*m∧2 respectively). Similar results in pts with/without mutations. Ecu was well tolerated. Most frequent AEs: diarrhea, nausea, headache and hypertension(all mild-moderate). All pts remain alive. These interim results show that Ecu led to discontinuation of chronic PT, sustained suppression of TMA, maintained renal function, and was well tolerated.

| Cohort | Value |
|---|---|
| Age: median yrs (range) | 28 (13 -63) |
| Complement Mutation/Auto-antibody | 14 (70%) |
| Prior Kidney Transplant | 8 (40%) |
| Dialysis | 5 (25%) |

Disclosure of Financial Relationships: Research Funding: Alexion Pharmaceuticals; Scientific Advisor: Alexion Pharmaceuticals.

**F-PO1275**

**Phase 1, Single-Dose Study of Fresolimumab in Patients with Treatment-Resistant (TR) Primary Focal Segmental Glomerulosclerosis (pFSGS)** Howard Trachtman,[1] Fernando C. Fervenza,[2] Debbie S. Gipson,[3] Peter J. Heering,[4] David R. W. Jayne,[5] Harm Peters,[7] Giuseppe Remuzzi,[6] Lars C. Rump,[8] Lorenz Sellin,[8] Flavio G. Vincenti,[9] Stefano Rota.[10] *[1]Cohen Children's Medical Center, New Hyde Park, NY; [2]Mayo Clinic, Rochester, MN; [3]University of Michigan, Ann Arbor, MI; [4]Städtisches Klinikum, Solingen, Germany; [5]Addenbrooke's Hospital, Cambridge, United Kingdom; [6]Mario Negri Institute for Pharmacological Research, Bergamo, Italy; [7]Charité Universitätsmedizin, Berlin, Germany; [8]Universitätsklinikum, Düsseldorf, Germany; [9]University of California, San Fransisco, CA; [10]Ospedali Riuniti, Bergamo, Italy.*

This open-label, dose-escalating study was performed to test safety, tolerability and PK of fresolimumab, an IgG4 antibody that neutralizes all 3 isoforms of TGF-β, in patients with TR pFSGS.

Patients with biopsy-confirmed pFSGS, resistance to steroid/immunosuppressive therapy, GFR ≥25 mL/min/1.73m$^2$ and persistent proteinuria (urine protein:creatine ratio [UPC] >200 mg/mmol creatinine) were eligible. Patients were infused fresolimumab (0.3, 1, 2, or 4 mg/kg) over 30 min, then followed for 112 days or until complete antibody clearance.

All 16 patients enrolled completed the study, 4 per dose level. Mean age was 37 yr, disease duration 3 yr, half were male, 13 White and 3 Black. Antibody infusion was well-tolerated and there were no SAEs; only 1 AE occurred in >1 patient (Grade 1 pustular rash, n=2). 1 patient was reported to have a new primitive neuroectodermal tumor 2 yr after dosing. Overall, GFR declined; median change from Baseline to Day 112 was -5.85 mL/min/1.73m$^2$. Proteinuria fluctuated during the study; median UPC change from Baseline to Day 112 was -133.31 mg/mmol creatinine. Proteinuria declined substantially in the 3 Black patients. Half-life of fresolimumab was 14 days, mean dose-normalized $C_{max}$ and

AUC values were 0.27-0.37 µg/mL/mg and 48.3-88.1 ng*hr/mL/mg, respectively, and appeared independent of dose.

Fresolimumab, up to 4 mg/kg, was safe and well-tolerated in patients with TR pFSGS. Results suggest inhibition of TGF-β with fresolimumab should be evaluated in a larger dose-ranging study.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1276**

**Long-Term Morbidity in Children with Frequently Relapsing Nephrotic Syndrome after a Randomized Controlled Trial with Cyclosporine** Kenji Ishikura, Norishige Yoshikawa, Satoshi Sasaki, Kazumoto Iijima, Koichi Nakanishi, Shuichi Ito, Takeshi Matsuyama, Nahoko Yata, Takashi Ando, Masataka Honda. *The Japanese Study Group of Renal Disease in Children, Japan.*

We previously conducted a randomized controlled trial (RCT) with cyclosporine (Sandimmune®) in children with frequently relapsing nephrotic syndrome (FRNS)(Ishikura et al., KI 2008). Although safety and effectiveness of 2-year treatment with cyclosporine have been established, concern for cyclosporine dependency has emerged and the long-term prognosis is unknown.

After 2 years of treatment, management was at the discretion of the physicians in charge. In October 2009, a follow-up study was completed. The primary end point was the nephrotic syndrome relapse at the last observation. Multiple logistic regression analysis estimated the relationships between age, previous protocol treatment (high dose or low dose of cyclosporine), and previous administration of cyclophosphamide before RCT to relapse.

Valid information from 46 out of 56 patients was gathered. The median follow-up period from the administration of cyclosporine was 10.3 years (2.9-13.5). At the last follow-up (median age, 17.6 years), 10 patients (21.7%) showed disease-free remission (no relapse for at least 2 years), whereas 23 (50.0%) continued to relapse frequently or were on immunosuppressants including cyclosporine, cyclophosphamide, and high dose mizoribine, 5 (10.9%) were on low dose mizoribine, and 8 (17.4%) had infrequent relapses. Younger age (less than 8 years) was a significant risk factor contributing to relapse (odds ratio; 4.95, P=0.02). The adverse effects of steroids and immunosuppressants were: short stature in 6 patients, osteoporosis in 6, obesity in 4, cataracts in 3, and hypertension in 2. None developed decreased renal function.

In summary, after a 2-year treatment of cyclosporine, the majority of children with FRNS continued to experience relapses for approximately 10 years; particularly, younger patients were at risk. A well-planned strategy for treatment consisting of steroids and immunosuppressants is necessary for long-term management to avoid complications of disease as well as adverse effects of treatment.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1277**

**Effect of Rituximab on T-Cell Subsets in Nephrotic Children with Focal Segmental Glomerulosclerosis (FSGS)** Wee Song Yeo,[1] Chang Yien Chan,[1] Tarun K. Maheshwari,[1] Henry Yang,[2] Kong Peng Lam,[3] Hui Kim Yap.[1] *[1]Pediatrics, National University of Singapore, Singapore; [2]SIgN, A-Star, Singapore; [3]Bioprocessing Technology Institute, A*STAR, Singapore.*

FSGS is an important cause of steroid-resistant nephrotic syndrome (SRsNS) in children. Recently, rituximab (anti-CD20 monclonal antibody) has been used successfully to treat therapy resistant cases, suggesting a possible role for B-cells in the pathogenesis of FSGS, either directly or indirectly through B-T interactions. This study aimed to delineate the effect of B-cell depletion through rituximab therapy on T-cell subsets, in an attempt to gain a further understanding of the B-T interactions that may be important in the pathogenesis of FSGS. Six patients (median age 14 years) with SRsNS or cyclosporine-dependent FSGS were given 2-weekly doses of rituximab (375 mg/m$^2$) for 4 doses. Blood was obtained on D-1, D+14 and 6 months post-rituximab infusion or when the patient goes into remission (end of study) for quantification of B- and T-cell subsets using multi-color flow cytometry. Fifty percent of the patients achieved complete remission defined as urine protein:creatinine (PCR) ratio of <0.02 g/mmol, whereas the rest only had partial remission with urine PCR 0.02-0.2 g/mmol. The percent CD19+ cells decreased significantly from 15.29±7.63% (D-1) to 0.61±0.50% (D+14) and 0.43±0.28% (end of study) (p=0.004). In all patients, analysis of T-cell subsets showed a mean percentage decrease of 42.2% in CD3+CD4+CD25+CD45RA+ and 8.3% in CD3+CD4+CD25+CD45RO+, whereas the T-regulatory cell population (CD3+CD4+CD25+FoxP3+) showed a mean percentage increase of 38.5% during the study period. The CD3+CD8+CD25+CD45RA+ subset showed a similar decrease post-rituximab of 51.4%, whereas there was minimal change in CD3+CD8+CD25+CD45RO+ subset. None of these reached statistical significance. In conclusion, rituximab therapy in patients with FSGS did not appear to affect the activation profile of either naive or memory CD4+ and CD8+ cell subsets, but its effect on T-regulatory cells remains to be determined and awaits studies involving a larger pool of patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1278**

**Tacrolimus as a Rescue Therapy in Steroid-Resistant Minimal Change Nephrotic Syndrome with Reversible Acute Renal Failure** Xiayu Li, Heng Li, Jianghua Chen. *The Kidney Disease Center The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.*

A proportion of patients with steroid-resistant minimal change nephrotic syndrome (SR-MCNS) developed reversible acute renal failure. Tacrolimus may be a rescue agent for such patients. Ten patients with steroid-resistant nephrotic syndrome biopsy proven minimal changes who developed reversible acute renal failure were studied from January 2005 to October 2008. Eight patients were also refractory to treatment with cyclophosphamide (n=5) and MMF(n=3)before this observational study. Oral tacrolimus was administered (target trough levels of 3-6 ng/ml) before serum creatinine (SCr) decreasing to ≤ 133µmol/L, and then increased doses were given (target trough level of 5-10 ng/ml) when SCr decreased to ≤133µmol/L. Outcome variables included recovery of acute renal function, complete remission (CR), partial remission (PR), change in renal function, relapse rate, and tacrolimus dosing and serum levels. One patient discontinued treatment for deterioration of renal failure. All patients who completed at least 12 weeks of tacrolimus therapy had recovery of renal function. Recovery of renal function paralleled with the increasing of urine output or reduction of proteinuria. The mean time required for achieving recovery of renal function is 14.7 ± 4.5 (7-21) days. Complete remission was achieved in 7 patients (70%) and partial remission was achieved in 1 (10%). The mean time for achieving CR or PR is 6.1 ± 2.0 (4-10) weeks and 2.9 ± 0.8 (2-4) weeks. Among patients who achieved complete remission or partial remission, 3 (37.5%) patients experienced relapses during follow-up. One patient had a doubling of SCr level because of primary resistance to tacrolimus therapy during follow-up. Tacrolimus may be an effective rescue therapy for SR-MCNS with reversible acute renal failure.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1279**

**Efficiency of Plasmatherapy in Factor H-Associated Hemolytic Uremic Syndrome** Chantal Loirat,[1] Marie-Alice Macher,[1] Patrick Niaudet,[1] Albert Bensman,[1] Michel Tsimaratos,[2] Georges Deschenes,[1] Veronique Fremeaux-Bacchi.[1] *[1]AP-HP, Paris, France; [2]AP-HM, Marseille, France.*

During the last decade, a clear link has been demonstrated between mutations in complement genes and atypical hemolytic uremic syndrome (aHUS). Factor H (FH)-associated HUS has been shown to have a poor prognosis and recent guidelines recommend early intensive plasmatherapy (PT). We report the first evaluation of the efficacy of PT in a group of 22 children with aHUS and mutations in FH gene.

We retrospectively analyzed the outcome of 22 children, of whom 11 (Group 1, 1984-2002) received conservative treatment and 11 (Group 2, 2003-2010) received intensive and prolonged plasmatherapy (daily plasma exchange (PE) or infusion (PI) for ≥ 10days, tapered to intervals of 1/week to 1/month on an individual basis and maintained as long as efficient). Patients carried homozygous FH mutation (n=6), compound heterozygous FH mutation (n=1) or heterozygous FH mutation located in SCR19-20 (n=9) or SCR1-18 (n=6).

Mean age at onset was 11.5m (3d-3.4y) and 10.9m (12d- 4.2y) in group 1 and 2 respectively. All patients presented hemolytic anemia, thrombocytopenia and acute renal failure, of similar severity in both groups. At 1 year follow up, 10 of the 11 untreated patients (91%) had died (n=2) or reached ESRD (7 at first episode), whereas 10 of the 11 patients receiving plasmatherapy (91%) were in remission with normal GFR. One severe recurrence leading to ESRD occurred at 4.5y during the total follow-up of 30.6 patient-years on plasmatherapy. The overall renal survival was significantly better in group 2 than in group 1 (p = 0.001).This study highlights for the first time the efficiency of intensive plasmatherapy to rescue the most severe forms of aHUS. In addition, it gives evidence that long term plasmatherapy prevents recurrence. This treatment has modified early and mid-term prognosis. However it requires trained centers and central vascular access for PE, and affects quality of life.

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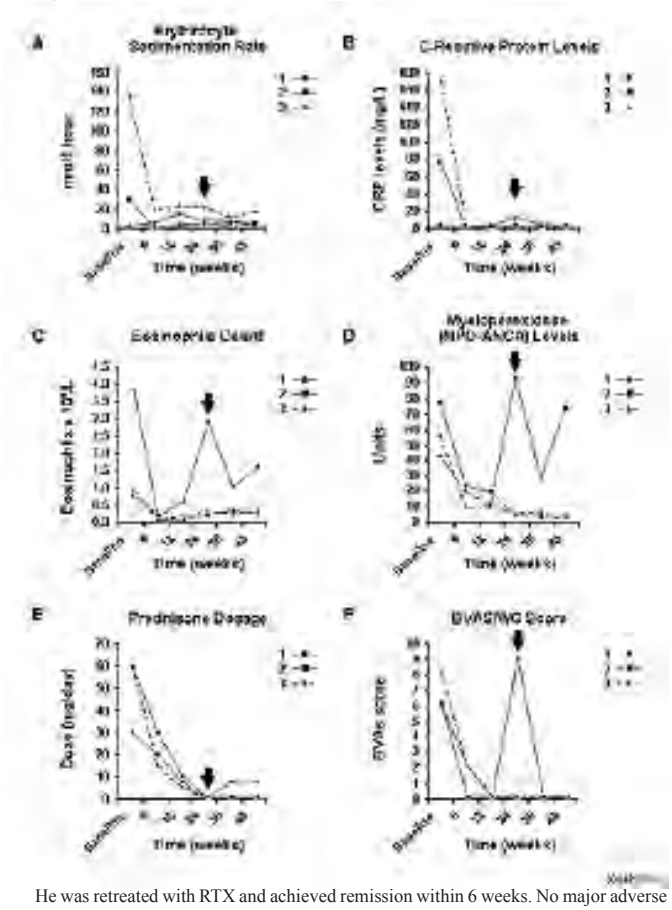
**F-PO1280**

**Rituximab for Churg-Strauss Syndrome with Renal Involvement** Rodrigo Cartin-Ceba,[1] Ulrich Specks,[1] Karina A. Keogh,[1] Fernando C. Fervenza.[2] *[1]Division Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN; [2]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

**Introduction and aim:** Churg-Strauss Syndrome (CSS) is a small vessel vasculitis that causes glomerulonephritis in about 25% of patients. Rituximab (RTX) is an anti-CD20 monoclonal antibody which depletes B-cells and has been effective in ANCA-associated vasculitis. We aim to evaluate the safety and efficacy of RTX in inducing remission of renal disease activity (RDA) in patients with CSS.

**Methods:** Single center, open label pilot study using RTX (375 mg/m2 per week x 4) for induction of remission in CSS patients with RDA (>25% dysmorphic red cells, RBC casts, or pauci-immune GN on biopsy). Patients were eligible if untreated, had failed glucocorticoid therapy, or had failed glucocorticoid dose reductions because of disease relapses. Primary outcome was remission of RDA defined as stability or improvement of creatinine clearance, absence of active urinary sediment and reduction of the glucocorticoid dose to <50% of the average dose received over 3 months before enrollment or <10 mg/day at 6 months. Patients were followed for 1 year.

**Results:** Three patients (ages 54, 55, 65) were enrolled. All patients had MPO-ANCA and active urinary sediments. Two patients had biopsy-proven pauci-immune GN. All achieved the primary end-point within the first three months and remained in renal remission during the year following RTX treatment. Patient 1 experienced a non-renal relapse (eye and joint involvement) at 6 months coinciding with the reconstitution of CD 19 cells and eosinophilia as seen in the figure(black arrow).




He was retreated with RTX and achieved remission within 6 weeks. No major adverse effects were recorded.

**Conclusions:** In this pilot study, RTX was safe and successful in controlling RDA in patients with CSS. This agent deserves further study in CSS.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1281

**Cardiac Troponin T (Tnnt2) Expression Is Induced by TGFβ Signaling in Podocytes** Christina M. Bracken, John N. Vassiliadis, Susan Schiavi, Stefan Wawersik. *Endocrine and Renal Sciences, Genzyme Corp., Framingham, MA.*

TGFβ signaling has long been recognized as a regulator of podocyte apoptosis and survival. More recently, however, evidence has emerged suggesting that TGFβ may also regulate podocyte phenotype by inducing changes in cytoskeleton and cell adhesion. To further explore podocyte phenotypic changes induced by TGFβ, we performed microarray analysis on RNA from cultured immortalized podocytes treated with 5 ng/ml TGFβ for 24 hours. Consistent with other reports, we find that TGFβ upregulates mesenchymal markers (MMP9, α-smooth muscle actin, Fibronectin 1, Col1a1), markers associated with Notch signaling (Jagged 1, Hes1), and elements of the integrin signaling pathway (ILK, Integrin-β3). In addition, we note substantial upregulation of a number of cytoskeletal constituents, including keratin 8, myosin light chain 9, myosin 7a, palladin, and the most highly upregulated gene in this study (>77 fold), Tnnt2. Tnnt2 encodes cardiac troponin T, a thin filament protein known to be involved in muscle contraction, but whose expression has not been reported in the podocyte. Tnnt2 is also affected by glomerular injury in vivo, as we find altered Tnnt2 expression in kidney samples from several in vivo models of proteinuria. We are currently examining functional roles of Tnnt2 in the podocyte to determine whether Tnnt2 expression alters podocyte behavior during injury.

Disclosure of Financial Relationships: Employer: Genzyme; Ownership: Genzyme Stock.

## F-PO1282

**Complement Dysregulation in Hemolytic Uremic Syndrome (HUS)** Arvind Bagga, Aditi Sinha, Ashima Gulati, Pankaj Hari. *Pedaitric Nephrology, All India Institute of Medical Sciences, New Delhi, India.*

**Background:** Mutations in genes encoding complement regulatory proteins are an important cause of HUS

**Objectives:** To prospectively screen for abnormalities in complement regulatory proteins & antibodies in consecutive patients with HUS

**Methods:** Levels of C3, C4, CD46, factors H (CFH), I & B, & anti-CFH antibodies were estimated in 25 patients (19 boys) with HUS during Jan 2007-Dec 2009. Those with antibodies were tested for deletion of CFH-related genes *CFHR1/CFHR3* by multiplex ligation probe amplification. These patients received plasmapheresis, IVIG (2 g/kg), IV cyclophosphamide (500 mg/m$^2$ monthly x 6) & prednisone (1 mg/kg/d for 4 wk, taper). Levels of creatinine, C3 & CFH antibodies were monitored; outcome was assessed by eGFR & dialysis dependence.

**Results:** The mean age was 102±52 (7-190) months; a diarrheal (D+) prodrome was seen in 7. Low levels of C3 & C4 were seen in 7 and 1 patient respectively. Low levels of CFH were seen in 4, factor B in 7 & factor I in 2. *CFH* gene had a homozygous deletion (c.3693-3696delATAG) in an infant with D+ HUS; the parents were heterozygous for this deletion. Sixteen patients (64%) had anti-CFH antibodies (180-60000 U/ml), with high titer in those with dialysis dependence. All these patients showed a homozygous *CFHR1/CFHR3* gene deletion. Immunosuppressive therapy resulted in decline of antibody titers. Six patients had CKD 1-3 at follow up of 1-yr; 4 had ≥1 relapses. The patient with homozygous deletion of CFH progressed to ESRD; one with factor I deficiency died in the acute stage. Of 8 patients without demonstrable complement abnormalities, 4 survived with CKD 1-3 at 1-yr follow up.

**Conclusions:** Our observations highlight that majority of patients with D+/D- HUS in India have anti-CFH antibodies or mutations in the genes encoding complement regulatory proteins. In the former, therapy with plasmapheresis, IVIG & immunosuppressive agents translate into better clinical outcomes. The reasons for the high prevalence of anti-CFH antibodies and their isotype & epitope specificity need to be studied.

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Disclosure of Financial Relationships: nothing to disclose

## F-PO1283

**Uridine Adenosine Tetraphosphate Acts as an Autocrine Hormone Affecting Glomerular Filtration Rate** Vera Jankowski,[1] Raymond C. Vanholder,[2] Walter Zidek,[1] Joachim Jankowski.[1] *[1]Charite, Germany; [2]University of Ghent, Belgium.*

**Objectives:** Recently, uridine adenosine tetraphosphate (Up4A) was described as a strong vasoconstrictor released from endothelial cells after stimulation with mechanical stress. In this study, we isolated and identified Up4A from kidney tissue, and we characterized the essential varying effects of Up4A on the afferent and efferent arterioles.

Methods: [/bold]Porcine and human kidney tissue was fractionated by size-exclusion-chromatography affinity-chromatography, anion-exchange-chromatography, and reverse phase-chromatography In fractions purified to homogeneity, Up4A was identified by matrix assisted laser desorption/ionisation mass-spectrometry (MALDI-TOF-MS), MALDI-LIFT-fragment-massspectrometry (MALDI-TOF-TOF-MS), retention-time comparison, and enzymatic cleavage analysis. We analysed the release of Up4A from cultivated renal proximal tubule cells after stimulation of protein kinase C with OAG. Up4A was identified in renal tissue, and the effect of Up4A on the vascular tone of isolated perfused afferent and efferent arterioles was tested. **Results:** Stimulation of tubule cells with OAG increased the release-rate of Up4A from tubule cells about ten fold. Up4A acts as a strong vasoconstrictive mediator on afferent arterioles, but has no significant effect on the tone of efferent arterioles, suggesting a functional role of Up4A a an autocrine hormone for glomerular perfusion. Because of the predominant effect of the Up4A on afferent arterioles, we assume that Up4A may decrease glomerular perfusion, intraglomerula pressure, and hence glomerular filtration rate. The release of Up4A from renal tubular cells may be an additional mechanism whereby tubular cells could affect renal perfusion. Up4A release may further contribute to renal vascular autoregulation mechanisms. **Conclusion:** As Up4A occurs in renal tissue and has marked effects on afferent but not efferen arterioles, Up4A may play a role in renal hemodynamics and blood pressure regulation.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1284

**Podocytes Have a Primary Cilium and Calcium Mediated Response to Fluid Flow Shear Stress** Robert J. Kolb,[1] Martin R. Pollak,[3] P. Darwin Bell,[4] Jing Zhou.[3] *[1]Pediatrics, Medical University of South Carolina, Charleston, SC; [2]Pharmacology, The University of Toledo, Toledo, OH; [3]Renal Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [4]Division of Nephrology, Department of Medicine, Medical University of South Carolina, Charleston, SC.*

Primary cilia are sensory organelles found at the cell surface of most cell types in the human body. However, it is not known if cilia develop in podocytes, the unique cell type that wraps around the glomerular tuft to help form the filtration barrier. Here we report that podocytes develop a cilium in vitro and in vivo. Cultured podocytes can be stimulated by a fluid shear stress triggering a transient increase in cytosolic calcium. This result is consistent with our hypothesis that the podocyte cilium monitors fluid movement within

Bowman's space. Previously, we have shown that this podocyte flow response is dependent upon an intact cytoskeleton and that the actin binding protein, α-actinin-4, is a prerequisite to the flow response. Recently, we have identified podocytes as having multiple cilia in both cultured Actn4-/- cells over-expressing a human disease mutant variant and in tissue sections from patients with FSGS. The functional significance of multiciliated podocytes and a potential diagnostic role is the focus of our future studies. We propose that the podocyte cilium is a sensory organelle that functions to monitor fluid flow in Bowman's space where it acts to modulate the quality of glomerular filtration through foot process contraction or relaxation. A loss in podocyte cilia function may be contributing factor to human glomerular diseases such as FSGS.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1285**

**Platelet Uptake and Release of Complement Factor H** Viola F. van Eimeren,[2] Fred G. Pluthero,[2] Walter H. Kahr,[2] Christoph Licht.[1] *[1]Nephrology, The Hospital for Sick Children, Toronto, ON, Canada; [2]Hematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada.*

Complement Factor H (CFH) inhibits alternative complement pathway activation on the surface of vascular endothelial and blood cells, including platelets. In a recent study of the role of platelet-complement interactions, we observed uptake of CFH by platelets *in vivo* and *in vitro*. Platelets take up proteins that are stored in and released from secretory granules. Surface glycoprotein IIb/IIIa (GPIIb/IIIa) is involved in the endocytotic uptake of fibrinogen from plasma and has been implicated in platelet uptake of CFH, which we have shown is not stored in secretory granules.

The possible role of GPIIb/IIIa in CFH uptake of resting platelets was examined by comparing the results of incubating cells with fluorescently-labeled CFH under 3 conditions: 1) platelets from patients with Glanzmann thrombasthenia lacking functional GPIIb/IIIa; 2) normal platelets; 3) normal platelets treated with the GPIIb/IIIa blocker abciximab. In each case similar uptake of CFH was observed, indicating that loss or blockage of GPIIb/IIIa receptor does not prevent platelet CFH uptake. When normal cells were activated in the presence or absence of abciximab similar surface uptake of CFH was observed, indicating that GPIIb/IIIa does not play a prominent role in binding of CFH to the surface of activated platelets.

The surface mobilization and release of CFH from normal platelets was investigated by stimulating quiescent cells *in vitro* and monitoring the protein content of releasates and lysates via immunoblotting, and assessing surface CFH by flow cytometry. We observed that platelets can release CFH in conditions that do not trigger α-granule secretion or the release of other cytoplasmic proteins. Full activation of platelets results in release of CFH and granule contents, but not in significant changes in cell surface CFH, indicating that CFH is released from within platelets rather than from the surface.

We conclude that the uptake and release of CFH in platelets is not dependent on interactions with GPIIb/IIIa, and likely involves mechanisms distinct from those involved in the uptake and release of granule-borne proteins.

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**F-PO1286**

**A New Disease Mechanism for MPGN II/DDD: Increased CFHR1 Expression Results in Competitive Loss of CFH Cofactor Activity** Christoph Licht,[1] Paul S. Thorner,[2] Christine Skerka,[3] Peter F. Zipfel.[3] *[1]Nephrology, The Hospital for Sick Children, Toronto, ON, Canada; [2]Laboratory Medicine and Pathobiology, The Hospital for Sick Children, Toronto, ON, Canada; [3]Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany.*

Membranoproliferative glomerulonephritis II (MPGN II) / dense deposit disease (DDD) is characterized by electron dense deposits within the glomerular basement membrane (GBM). The pathogenesis has been recently linked to impaired alternative complement pathway (AP) control caused by Factor H (CFH) deficiency, and by antibodies to the alternative C3 convertase – C3 nephritic factor – or complement Factor B (CFB). In addition, we recently identified CFB mutations and deletions in CFH related proteins (CFHR) 1 and 3 in MPGN patients. Despite these advances, the pathogenesis is still only poorly understood.

We present an 11 y/o boy with biopsy-proven MPGN II. Despite treatment with prednisone and mycophenolate mofitil, he progressed to ESKD within months and was started on PD. After 4 years he received a deceased donor transplant but developed disease recurrence within 1 week (graft biopsy on day 6). He was treated with plasmapheresis followed by plasma infusions. Following transient success for 9 months, renal function deteriorated and graft nephrectomy became necessary due to hypertension. Complement work-up revealed AP activation and a positive C3NeF. Genetic analysis identified 3 copies of CFHR1 along with increased plasma levels (150%). By immunofluorescence, a graft biopsy at 6 months and the graft removed at 17 months showed glomerular CFHR1 staining while control tissue was negative. *In vitro*, CFHR1 competed with CFH for binding to endothelial cells and C3b. In addition, CFHR1 affected CFH cofactor activity with increasing CFHR1 levels causing decreasing CFH cofactor activity.

In conclusion, we identified a new pathomechanism for MPGN II. A patient with 3 allelic copies of CFHR1 showed increased CFHR1 levels causing local loss of CFH cofactor activity. Plasma therapy after early posttransplant recurrence was transiently successful, implying more efficient treatment strategies like targeted complement blockade (e.g. Eculizumab) are required.

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Ophtherion
CSL Behring.

**F-PO1287**

**Impaired Ability of GCs To Induce IL-10 and/or Inhibit TNF-alfa Secretion by LPS-Stimulated PMBC Might Underlie Modifications in Steroid Sensitivity of Patients with INS** Martina Cizmarikova,[1] Katarina Szilagyi,[1] Ludmila Podracka,[2] Jan Mojzis,[1] Ladislav Mirossay.[1] *[1]Department of Pharmacology, Faculty of Medicine, University of Pavol Jozef Safarik, Kosice, Slovakia (Slovak Republic); [2]Department of Paediatrics, Faculty of Medicine, University of Pavol Jozef Safarik, Kosice, Slovakia (Slovak Republic).*

**Aim:** The influence of IL-10 and TNF-alfa on sensitivity to glucocorticoids (GCs) was investigated in children with idiopathic nephrotic syndrome (INS).

**Methods:** 43 patients with INS (13 girls, 30 boys, mean age 9.8 years) and 13 healthy children (mean age 14.5 years) were enrolled into the study. Patients were classified according to GCs sensitivity as responders (RE, n=16), partial responders (PR, n=19) and non-responders (NR, n=8). Patients were also subdivided based on the onset/relapse and remission of NS. No immunosuppressants were administered at the time of blood collection. Secretion of IL-10 and TNF-alfa was measured and IL-10/TNF-alfa ratio was determined after LPS stimulation and/or concomitant dexamethasone (DM) therapy to asses dynamicity of cytokine production by the PMBC. Percentage of IL-10/TNF-alfa increase by DM was calculated and correlated to LPS stimulation.

**Results:** Significantly higher increase in IL-10/TNF-alfa after GCs was observed in relapsing RE than in PR and/or NR. In particular, DM concentration of $10^{-8}$M significantly increased IL-10/TNF-alfa ratio in relapsing RE in comparison to relapsing NR ($p=0.049$). Moreover, higher DM concentration of $10^{-7}$ M significantly increased this ratio in relapsing RE in comparison to both PR and NR ($p<0.05$). Surprisingly, these changes were not observed in the remission of the disease. Stimulated PMBC of PR or NR in relapse failed to increase IL-10/TNF-alfa after DM in contrast to the RE group and that might contribute in some patients to lower response to GCs.

**Conclusion:** We hypothesized that impaired ability of GCs to induce IL-10 and/or inhibit TNF-alfa secretion by LPS-stimulated PMBC might underlie modifications in steroid sensitivity of patients with INS. Further data are needed to confirm our hypothesis. Supported by Norway grant through the EEA and the Norwegian Financial Mechanisms.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1288**

**Screening Characteristic Protein Profiles of Primary Glomerular Diseases Using Proteomics** Nan Chen, Fang Zhong, Weiming Wang, Xie Yinyin. *Nephrology, Ruijin Hospital, Shanghai Jiao Tong Univeristy, Shanghai, China.*

Objective: Primary glomerulonephritis was the main cause of end-stage renal disease in China. Diagnosis of these diseases needs to renal biopsy, which is invasive inspection. Early diagnosis of the pathological types by the biological markers of blood or urine has a high practical value.

Methods: Forty-five patients with primary glomerulonephritis: IgA nephropathy (IgAN, n=15) , focal segmental glomerulosclerosis (FSGS, n=15) and membranous nephropathy (MN, n=15) were enrolled. All these patients had proteinuria (> 0.5g / day), GFR> 15ml/min/1.73m$^2$. Those patients who took immunosuppressant before renal biopsy were not enrolled. These patients were excluded with hepatitis, cancer and immune and other secondary diseases. And 15 healthy subjects were used as a control group. 20ul sera were collected for every subjects and patients. The samples were removal of high abundance proteins using the Multiple Affinity Removal LC Column, desalinated, quantified and marked. Then we applied mass spectrometry HPLC-MS/MS and statistics.

Results: 15 IgA patients(M/F: 7/8, average age 29.6 ± 10 yrs)had 24-hr urine protein output (1491 ± 752 mg/d ), serum creatinine (Scr) (112 ± 56 μmol/L ). 15 FSGS patients(M/F: 7/8, average age 39.8 ± 13.4 yrs)had 24-hr urine protein output (877 ± 640 mg/d), Scr(121 ± 50 μmol/L). 15 MN patients(M/F: 7/8, average age 53.2 ± 12.6 yrs)had 24-hr urine protein output (3988 ± 2332 mg/d),Scr(91 ± 55 μmol/L ).1956 proteins were identified with mass spectra (MS) in these patients . Compared with normal healthy controls, serum of patients with primary glomerulonephritis exist 447 differentially expressed protein peaks. Compared with the control , 48 proteins in IgA nephropathy patients, 68 proteins in FSGS patients and 120 proteins in MN patients were significantly different. In the expression profile, there are some differences in the expression of specific proteins.

Conclusions: There are differences in serum protein expression profiles in primary glomerulonephritis compared with normal healthy people and difference protein expression profiles in different pathological types of primary glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1289**

**Electrical Forces Determine Glomerular Permeability** Marcus J. Moeller,[1] Christoph Kuppe,[1] Wilhelm Kriz,[2] Sylvia Menzel,[1] Jurgen Floege.[1] *[1]Department of Nephrology and Clinical Immunology, University Hospital of RWTH Aachen University, Aachen, Germany; [2]CBTM, Anatomy and Developmental Biology, Medical Faculty Mannheim, University Heidelberg, Mannheim, Germany; [3]Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN.*

The functioning of the glomerular filter is an intriguing and still only partially resolved question in the field of Nephrology. Per day, about 180 l of plasma are filtered across the glomerular filtration barrier. During this process, more than 99% of the plasma proteins are retained, yet the filter never clogs.

We tested the hypothesis that extracellular electrical potential differences are generated by the flow of the filtrate across the charged glomerular filter. For this purpose, glomerular capillaries were for the first time successfully micropunctured in Necturus maculosus (common mudpuppy), a model organism with an unusually large capillary diameter. With this setup, a potential difference was directly measured across the glomerular filtration barrier, which depended directly on glomerular filtration pressures. The potential difference was generated without temporal delay. It was negative in Bowman's space and it was completely reversible when decreasing the perfusion pressures to baseline levels.

To compare the relative contributions of diffusion, convection and electrophoretic effects on the total flux of albumin across the filter, an independent mathematical model was created using published experimental data. The model predicted that potential differences within a similar range as in our experiments induce an electrophoretic flux that significantly influences the glomerular sieving coefficient of albumin.

In summary, the new model provides a mechanistic theory on the filtration characteristics of the glomerular filtration barrier and is based on experimental data. It provides a novel (patho-) physiological explanation for the microanatomy of the glomerulus, renal autoregulation, why the filter does not clog and the pathogenesis of proteinuria.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1290**

**Blood Pressure Abnormalities in Children with Sickle Cell Anemia** Amy M. Becker,[1] Jordan H. Goldberg,[1] Michael Henson,[2] Liyue Tong,[3] Michel G. Baum,[1] George R. Buchanan.[1] *[1]Pediatrics, UT Southwestern Medical Center, Dallas, TX; [2]Pediatrics, Children's Medical Center Dallas, Dallas, TX; [3]Clinical Sciences, UT Southwestern Medical Center, Dallas, TX.*

Kidney disease is a major cause of morbidity and mortality in adults with sickle cell anemia (SCA). Many patients will develop proteinuria and progress to end stage renal disease requiring dialysis and/or transplantation. The factors that modify progression of renal disease are unknown. Alterations in blood pressure (BP), including hypertension and lack of the normal nocturnal dip in blood pressure, are important factors in the progression of kidney disease. Mild increases in BP, termed relative hypertension, are associated with increase risk of stroke, end stage renal disease, and death in adults with SCA. This prospective study of children with SCA characterizes ambulatory BP profiles and presence of microalbuminuria (MA). 46 adolescents (25 females) with SCA mean age of 14.7 years (range 12-18 yrs) underwent 24 hour ambulatory BP monitoring and collected a first morning urine sample to measure MA. 17% of subjects have relative hypertension, and 59% lack the normal nocturnal dip in BP. 33% have previously unrecognized hypertension. 15% have MA with 86% lacking the normal nocturnal dip and 43% with hypertension. Univariate analysis shows that MA is not significantly associated with age, gender, and number of previous blood transfusion, hospitalized pain episodes, or splenic sequestration episodes. Fewer acute chest syndrome events was seen in the MA group (p=0.04). The percent of systolic dip in BP is significantly lower (5.2±3.8%) in the subjects with MA compared to those without MA (9.1±3.6%), p=0.01. Stepwise logistic regression analysis identifies a 26% less chance of having MA with every 1% increment increase in systolic dip. Comparison of clinic BP to mean day ambulatory BP reveals no significant difference. In conclusion, MA is associated with a lack of normal nocturnal variation in blood pressure. Blood pressure abnormalities are common in adolescents with SCA and may be an important modifiable risk factor in the progression of sickle cell nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1291**

**Urinary Hepcidin: A Marker of Renal Tubular Dysfunction** Hilde P. Peters,[1] Coby M. M. Laarakkers,[2] Rosalinde Masereeuw,[3] Dorine W. Swinkels,[2] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Nijmegen Medical Center; [2]Laboratory of Genetic, Endocrine and Metabolic Diseases, Radboud University Nijmegen Medical Center; [3]Toxicology and Pharmacology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.*

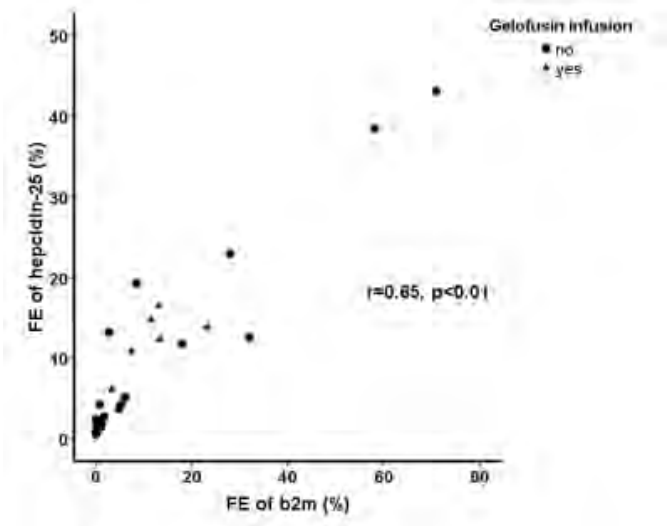
Urinary (U) hepcidin is elevated in various renal diseases and may serve as a biomarker. It is unknown which processes – filtration, reabsorption, local production, and/or degradation - contribute to elevated Uhepcidin levels. We aimed to assess whether tubular dysfunction affects Uhepcidin levels, through comparison with $\beta_2$-microglobulin ($\beta_2$m) excretion. $\beta_2$m is filtered by the glomerulus and reabsorbed by the proximal tubules.

**Method**

Serum and urine levels of hepcidin-25 were determined by a MALDI-TOF Mass Spectrometry based assay in controls and patients with varying degrees of tubular dysfunction due to glomerular disease. U$\beta_2$m was measured by ELISA. Gelofusine, an inhibitor of tubular protein reabsorption, was administered to 2 healthy volunteers. Uhepcidin was measured in megalin deficient mice and wt controls.

**Results**

We studied 21 patients with glomerulopathies, median serum creatinine was 116 (range 70-301) µmol/L, proteinuria 7.1 (1.7-19.1) g/d, and fractional excretion (FE) of $\beta_2$m 1.3 (0.1-71)%. FE of hepcidin was 2.8 (0.6-43) vs 1.9 (range 0.2-5.8)% in 24 controls (p=0.04). FE of hepcidin correlated strongly with FE of $\beta_2$m (r=0.85, p<0.01).




During Gelofusine infusion a rapid increase in FE of $\beta_2$m and hepcidin was observed; ~150 (0.1⇒16.5%), and ~7 fold (3.0⇒15.4%), respectively. Uhepcidin in megalin deficient mice was increased 7-fold compared to controls (n=5, p<0.01).

**Conclusion**

Hepcidin-25 is reabsorbed by the tubules, a process mediated by megalin. Increased levels of Uhepcidin may largely reflect tubular dysfunction. This should be considered when evaluating Uhepcidin as a biomarker in patients with kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1292**

**Altered Activity of Drug Metabolism and Transport Pathways in Glomerulonephritis** Melanie S. Joy,[1] Kim L. R. Brouwer,[2] Joyce A. Goldstein,[3] Mary Anne Dooley,[4] Ronald J. Falk,[4] Reginald F. Frye.[5] *[1]Schools of Medicine and Pharmacy, University of North Carolina, Chapel Hill, NC; [2]School of Pharmacy, University of North Carolina, Chapel Hill, NC; [3]NIH, NIEHS, Research Triangle Park, NC; [4]School of Medicine, Kidney Center, University of North Carolina, Chapel Hill, NC; [5]College of Pharmacy, University of Florida, Gainesville, NC.*

Cytochrome P450 (CYP) pathways metabolize numerous drugs and the P-glycoprotein drug transporter is linked to chemotherapy resistance. The objectives of the research were to characterize the activities of drug metabolizing enzymes and P-glycoprotein in glomerulonephritis.

Glomerulonephritis patients (n=12) with lupus(n=5) and small vessel vasculitis(n=7) received a drug cocktail containing selective probe drugs; fexofenadine 60mg (P-glycoprotein), flurbiprofen 50mg (CYP2C9), bupropion 150mg (CYP2B6), and intravenous $^{14}$C N-methylerythromycin 3µcuries (CYP3A4). Serial blood and urine was collected and analyzed by LC/MS and liquid scintillation counting. Pharmacokinetics were assessed with WinNonlin®.

Patient were 9F/3M, 42% Caucasian, serum creatinine 1.3±0.7mg/dL, UP:Cr 1.6±1.5, creatinine clearance 102±43mL/min, and serum albumin 2.0±0.2g/dL. The pharmacokinetics for glomerulonephritis versus ESRD and healthy controls are provided in Table (a-clearance, b-metabolic ratio).

| | Glomerulonephritis | ESRD | Healthy Controls |
|---|---|---|---|
| CYP3A4 (erythromycin) | 2.0±0.8% dose/h | 1.9±0.7% dose/h | 2.7±1.0% dose/h |
| CYP2C9 (flurbiprofen)[a] | 1.9±0.5 L/h | 1.3 L/h | 1.1±0.4 L/h |
| CYP2B6 (bupropion) [b] | 8.9±5.0 | NR | 35.8 |
| P-glycoprotein (fexofenadine)[a] | 58.8±34.5 L/h | 37.9±19.5 L/h | 103±38 L/h |

The activity of each pathway was reduced in glomerulonephritis, with the exception of increased CYP2C9. CYP3A4 activity in glomerulonephritis was consistent with reported activity in ESRD, despite minimal kidney function declines.

Glomerulonephritis patients with minimal kidney function declines can have significant alterations in activity of drug metabolizing enzymes and transporters. These findings could have repercussions in terms of treatment-related toxicities and responses in patients.

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**F-PO1293**

**Increased Cardiac Troponin T Levels Are Correlated with Inflammatory Markers and Various Indices of Renal Function in Chronic Renal Disease Patients** Siren Sezer, Sebnem Karakan, Nurhan Ozdemir. *Nephrology, Baskent University Hospital, Ankara, Turkey.*

**Background/Objectives:** Serum cardiac troponin T (cTnT) concentrations are commonly increased in end-stage renal disease in the absence of an acute coronary syndrome. Cardiovascular disease begins early in the course of chronic kidney disease

(CKD), and the glomerular filtration rate (GFR) is an independent risk factor for it. Evidence suggests that raised troponin concentrations in uraemic patients do indeed reflect myocardial injury. There is little information on cardiac troponin concentrations in patients with chronic kidney disease (CKD) who have not commenced dialysis. However, factors associated with this deleterous process are not completely understood. We aimed to determine associated laboratory abnormalities of increased cTnT in patients with CKD.

**Methods:** Between 2008-2009 104 patients were recruited to the study. Sixty-three (60%) and 79 (75%) of patients had diabetes mellitus and hypertension, respectively. Exclusion criteria included patients <18 years of age or other vulnerable groups; patients with acute renal failure, a functioning renal transplant, or receiving dialysis; and patients having had a recent (<1 month) cardiac event. Glomerular filtrasyon rate (GFR) was estimated (44,62±14,38 mL/min/1,73 m²) based using the Modification of Diet in Renal Disease (MDRD) study formula.

**Results:** cTnT is correlated with blood urea ($r=0.262$, $p<0.05$), uric acid ($r=0.399$, $p<0.001$), blood phosphourus ($r=0.550$, $p<0.001$), trigliceride ($r=0.329$, $p=0.011$), ferritin ($r=0.325$, $p=0.021$), C-reactive protein ($r=0.768$, $p<0.001$), vitamin $B_{12}$ ($r=0.672$, $p<0.001$), GFR ($r=-0.755$, $p=0.011$), renal resistive index ($r=-0.412$, $p=0.017$). Linear regression analysis revealed that CRP ($\beta=+0,72$, $p<0.001$), serum phosphorus ($\beta=+0,22$, $p<0.05$) were statistically significant risk factors for increased cTnT.

**Conclusion**: Increased cTnT shows not only ongoing inflammation but is a sensitive marker of functioning renal mass. It is strongly correlated with factors influencing the decline in renal function, thus can be used as a renal risk parameter.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1294

**CT Scan Guided Method More Effective Than Non-Real Time Ultrasound for Kidney Biopsy** Amit J. Joshi, George Dunea, Peter D. Hart. *Division of Nephrology, Stroger Hospital of Cook County, Chicago, IL.*

Objective: To examine the diagnostic yield and complications of percutaneous kidney biopsy using either ultrasound or CT scan guidance.

Methods: We retrospectively reviewed 201 native percutaneous kidney biopsies done over a period of 3 years at an urban teaching hospital, using ultrasound in 100 patients and CT scan guidance in 101 patients. In the ultrasound group we used a 14 -gauge needle manually without real time imaging; in the CT scan group we used an 18- gauge needle with an automated gun and confirmed the position of the needle by CT scanning. Biopsy was considered optimal when adequate tissue was obtained without complications.

Results: The mean number of glomeruli and of complications from the procedure was similar in both groups. There were more cases with adequate glomeruli for histological diagnosis in the CT scan group. More patients in the CT scan group required blood transfusions while non-renal tissue was obtained in more cases in the ultrasound group.

Outcomes

| | CT scan n=101 | Ultrasound n=100 | p-value* |
|---|---|---|---|
| **Effectiveness** | | | |
| ≥6 glomeruli n (%) | 98 (98%) | 85 (85%) | 0.003 |
| ≥10 glomeruli n (%) | 90 (90%) | 82 (82%) | 0.15 |
| Glomeruli mean±SD | 21.5±1.04 | 21.7±1.39 | 0.94 |
| **Safety** | | | |
| Complications n(%) | 9 (9%) | 9 (9%) | 0.98 |
| Non-renal organ biopsy n(%) | 1 (1%) | 4 (4%) | 0.17 |
| Blood transfusion n(%) | 8 (8%) | 5 (5%) | 0.4 |
| Sepsis, surgery, death | 0 | 0 | |
| **Optimal biopsy** | 90 (89%) | 81 (81%) | 0.117 |

* Adjusted for all baseline characteristics: age, sex, ethnicity, hemoglobin, platelet count, creatinine, BUN, INR, PTT, bleeding time, blood pressure

Conclusion: Compared to ultrasound without real time imaging, the CT-scan method, even though using a smaller needle, resulted in better localization of the kidney, consistently yielding adequate tissue samples for diagnosis, decreasing the risk of injuring non-renal tissue, and indicating that accurate localization is more important than needle size in renal biopsy.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1295

**Elevated Uremic Toxins in Anephric Sheep** Garry J. Handelman,[1] Isso Bayala,[1] Mary Carter,[2] D. Buffington,[4] L. Charles,[5] K. Johnston,[4] L. Lou,[4] J. Jung,[3] David Humes,[3] Nathan W. Levin,[2] Peter Kotanko.[2] *[1]University of Massachusetts, Lowell, MA; [2]Renal Research Institute, New York, NY; [3]University of Michigan, Ann Arbor, MI; [4]Innovative Biotherapies, Ann Arbor, MI.*

Uremic toxins are of great interest because of the challenge of removing these molecules with standard dialysis procedures, and because of their potential for long-term harmful effects. Sheep were made anephic and established on peritoneal dialysis, and maintained in this conditions for 9 days. BUN increased from 19(±3) to 73(±13) mg/dL ($p<0.001$), and creatinine from 0.72(±.04) to 9.6(±1.4) md/dL ($p<0.001$). Using HPLC, we characterized the profile of low-MW molecules in the sheep; para-cresol sulfate (PCS), indoxyl sulfate (IS), hippuric acid (HA), and tryptophan (Try). PCS and IS reached high levels, compared to baseline.

Changes in molecules, mg/dL (mean ± SD)

| Time point | Para-cresol sulfate | Indoxyl sulfate | Hippuric acid | Tryptophan |
|---|---|---|---|---|
| BL | 1.8±0.4 | 0.03±0.02 | 0.02±0.01 | 0.81±0.11 |
| 2 days | 34±11# | 1.21±0.22# | 0.93±0.15# | 0.23±0.03# |
| 7 days | 57±6# | 1.71±0.17# | 0.61±0.48(NS) | 0.28±0.05# |
| 9 days | 51±9# | 1.99±0.11# | 0.38±0.34(NS) | 0.26±0.09# |

#, $p<0.001$ vs BL, paired t-test, NS, not significant

The decrease in tryptophan is may result from the loss of tryptophan in the dialysate, with no renal mechanism to conserve this amino acid. A similar change needs to be examined in human subjects on dialysis. The transient increase in hippuric acid, with a trend back to lower levels, corresponds to the effectiveness of dialysis treatment to clear this highly-soluble non-protein bound compound.

A similar HPLC pattern was observed in measurement of these compounds in a sample from a human ESRD patient. Several other new molecules were also observed in the analysis of the uremic sheep. The sheep model of uremia, with provision of dialysis therapy, provides an ideal strategy for understanding the accumulation and depletion of several low-MW substances during development and management of renal failure.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1296

**Discovery and Validation of Biomarkers for Diagnosis of Early and Late Diabetic Nephropathy** Mary Y. Yeh,[1] Wei-Ya Lin,[1] Hung-Yi Li,[1] Tsai-Wei Shu,[1] Ping-Fu Cheng,[1] Chih-Jen Wu,[2] Chi-Hung Cheng,[3] Kuo-Hsiung Shu,[3] Chwei-Shiun Yang,[4] Han-Hsiang Chen,[2] Yen-Peng Li,[1] Yuh-Feng Lin,[5] Jin-Shuen Chen,[5] Tzu-Ling Tseng.[1] *[1]Biomedical Engineering Research laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan; [2]Nephrology, Mackay Memorial Hospital, Taipei, Taiwan; [3]Nephrology, Taichung Veterans General Hospital, Taichung, Taiwan; [4]Nephrology, Cathay General Hospital, Taipei, Taiwan; [5]Nephrology, Tri-Service General Hospital, Taipei, Taiwan.*

**Introduction:** Non-invasive diagnosis biomarkers for diabetic nephropathy (DN) can be used regularly to determine the opportune time to initiate therapy and to evaluate therapeutic response. A panel of biomarkers was discovered in serum and urine. Further testing in expanded patient samples and immunohistochemistry staining (IHC) of renal biopsies were performed to confirm diagnostic value and organ/disease-specificity of these biomarkers. **Methods:** Serum and urine samples were collected from healthy individuals and diabetic patients at various DN stages in participating medical centers of the Taiwan Renal Biomarker Consortium. A panel of five biomarkers was discovered based upon novel proteomic platforms. Verification of panel performance was made in a group of 300 patients. Renal biopsies from DN and minimal change disease (MCD) patients were IHC stained against these markers. **Results:** While none of the individual biomarker performed sufficiently in the group of 300 patients, the biomarkers in combination was highly specific and sensitive for DN diagnosis at various stages, with auROC up to 0.98 and inversely correlated with patient's glomerular filtration rate. These markers stained strongly in DN tissue and were minimally present in MCD demonstrating disease specificity that was independent from proteinuria. Biomarkers were detected primarily in the tubules rather than in glomeruli supporting their complex and relevant roles in the pathogenesis of DN. **Conclusion:** A novel biomarker panel for diagnosing DN was validated and performed well for early and late DN stages and thus holds great promise in facilitating non-invasive DN diagnosis and long-term DN monitoring for effective disease management.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1297

**Low and High Transferrin Saturation Levels Are Associated with Mortality among Persons with Normal and Reduced Kidney Function in the General Population** Austin G. Stack,[1] Hoang Thanh Nguyen,[3] Catherine A. Wall.[2] *[1]Regional Kidney Centre, Letterkenny General Hospital, Letterkenny, Donegal, Ireland; [2]Department of Renal Medicine, Adelaide & Meath, National Childrens Hospital, Tallaght, Dublin, Ireland; [3]MD Anderson Cancer Center.*

**Background:** Serum transferrin saturation (Tsat%) is a commonly used indicator of iron deficiency and iron overload in clinical practice. We determined the relationship between Tsat% and death in the U.S. population and among those with reduced kidney function.

**Methods:** A cohort of 15,823 subjects age ≥ 20, and representative of the U.S. population, was identified from the Third National Health and Nutrition Examination Survey (1988-1994). Vital status was obtained through linkage with the National Death Index. Serum Tsat% (serum iron/total iron binding capacity %) measured at baseline was modeled with all-cause mortality according to estimated glomerular filtration rate (eGFR) (< 60, 60-90 and > 90 ml/min) using Cox regression.

**Results:** Adjusting for demographic characteristics, the Tsat%-mortality relationship followed a u-shaped correlation. Compared to the referent group [Tsat% between 23.7-26.4%: Relative Mortality Risks (RR)=1.00], individuals with Tsat% values within the five lowest deciles of 0-12.5 %, 12.5-16.1 %, 16.1-18.8 %, 18.1-21.3 %, and 21.3-23.7 % experienced significantly higher risks of 2.32 (1.78-3.03), 1.57 (1.17-2.10), 1.44 (1.11-1.88) 1.44 (1.11-1.88), 1.41 (1.03-1.91) respectively while those with values in the highest decile, greater than 39.7 %, also experienced significantly higher mortality risks [RR=1.39 (1.04-1.86)]. Adjusting for comorbid conditions, inflammation markers, haemoglobin & ferritin, the Tsat%-mortality relationship remained unaltered. However, among persons with reduced kidney function, (eGFR< 60 ml/min), the mortality risks associated with low Tsat% values were greatly magnified compared to those with GFR > 60 ml/min. (P-value interaction <0.01).

**Conclusions:** Both low and high Tsat% levels contribute independently to increased mortality in the U.S. especially among those with reduced GFR. Optimal targets for Tsat% appear to be in the range of 24-40 %. These relationships should be considered when interpreting results from clinical trials of anaemia correction.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1298**

**Glomerular and Tubular Damage Markers Are Elevated in Patients with Diabetes Mellitus** Ferdinand L. Nauta,[1] Wendy E. Boertien,[1] Stephan J. L. Bakker,[1] Henk Bilo,[3] Harry Van Goor,[2] Paul E. de Jong,[1] Ron T. Gansevoort.[1] *[1]Nephrology, Groningen, Netherlands; [2]Pathology and Medical Biology, Groningen, Netherlands; [3]Diabetes Center, Isala Clinics, Zwolle, Netherlands.*

Albuminuria is an established risk marker for renal function decline in diabetes mellitus (DM). Although albuminuria is regarded as the consequence of glomerular damage, the renal tubulo-interstitium is plays an important role in the genesis of DM nephropathy.

We aimed to investigate in a cross-sectional study the levels of serum and urinary biomarkers in patients with DM (n=94) and non-diabetic controls (n=45) to study the association between glomerular (IgG and IgG-4), proximal tubular (KIM-1, NAG, NGAL, β-2-microglobulin, cystatin C) and distal tubular damage (H-FABP) markers and kidney disease severity (albuminuria and eGFR (MDRD)). Biomarkers were measured in triplo in fresh morning urine samples and in plasma.

Of the DM patients 40 were normo-, 40 micro- and 14 macro-albuminuric. Compared to non-diabetic controls urinary IgG-4 (13 fold), NAG (8.5 fold), NGAL (4.6 fold) and H-FABP (16 fold) were already significantly elevated in normo-albuminuric diabetic patients. Urinary concentrations of glomerular and tubular biomarkers increased per albuminuria stratum, except KIM-1 and β-2-microglobulin. All these urinary biomarkers concentrations were significantly associated with albuminuria, also in multivariate analyses. (Std β 0.34-0.78; all $p<0.003$). All urinary biomarkers except KIM-1 and β-2-microglobulin were significantly associated with eGFR in univariate models (Std β-0.38- -0.21; all $p<0.04$). However, after correction for age, sex, albuminuria and plasma concentration of the corresponding biomarker, only H-FABP was significantly associated with eGFR (Std β-0.26, $p=0.037$).

In conclusion, glomerular and tubular markers are associated with albuminuria, independently of eGFR, suggesting that albuminuria reflects both glomerular and tubulo-interstitial damage. Only urinary H-FABP is associated with eGFR independently of albuminuria and may thus be a promising urinary biomarker for diabetic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1299**

**A New Index To Characterize Iron Status in CKD Patients. Nephrotest Cohort** Lucile Mercadal,[1] Marie Metzger,[1] Martin Flamant,[2] Jean-Philippe Haymann,[3] Benedicte Stengel,[1] Marc C. Froissart.[1,4] *[1]Inserm U1018; [2]Bichat Hospital; [3]Tenon Hospital; [4]G Pompidou European Hospital, Paris, France.*

Taken separately, the markers of iron status do not adequately characterize the mechanisms of anemia in CKD. We investigated the value of an index (table) combining ferritin, transferrin saturation (TSAT) and total iron binding capacity (TBIC) to characterize iron status in 1011 nondialysis CKD patients free from IV iron or ESA. Glomerular filtration rate was measured (mGFR) in all patients by $^{51}$Cr-EDTA renal clearance. Functional ID without inflammation and transferrin deficiency were the 2 most common pathologic profiles. They both steadily increased from 10% and 13% to 28% and 31% with decreasing mGFR from > 60 to < 15ml/min/1.73m², respectively. The relation of Hb with iron index classes differed by mGFR in 2 categories (≥40 and <40 mL/min/1.73m², interaction $p=0.02$). Hb was lower in all ID classes as compared with the reference one before and after adjusting for gender, age, ethnicity, diabetes, BMI, albumin, alpha 1 acid glycoprotein, oral iron therapy, RAS inhibitor use, and mGFR. But except for real ID, relations between Hb and ID classes were stronger in the lower mGFR category. This iron status index made it possible to define 2 main abnormal iron profiles associated with CKD anemia. While noninflammatory functional deficiency may be linked to hepcidin increase with renal function decline, transferrin deficiency may reflect the impact of malnutrition.

| | TSAT (%) | TBIC µmol/L | Ferritin ng/ml | N (%) | Crude Hb mean (g/dL) (sd) | Change in Hb (g/dL), adjusted estimates (sd) | |
|---|---|---|---|---|---|---|---|
| | | | | | | mGFR≥40 | mGFR<40 |
| Normal | ≥20 | ≥50 | ≥ 40 | 546 (53) | 13.0 (1.5)* | reference | |
| Real iron deficiency (ID) | <20 | ≥50 | <40 | 62 (6) | 11.6 (1.7)* | -1.1 (0.3)** | -0.7 (0.2)** |
| Functional ID without inflammation | <20 | ≥50 | ≥ 40 | 188 (18) | 12.3 (1.6)* | -0.3 (0.2) | -0.5 (0.1)** |
| Functional ID with inflammation | <20 | <50 | ≥ 40 | 21 (2) | 12.0 (1.4)* | -0.3 (0.5) | -0.8 (0.3)* |
| Transferrin deficiency | ≥20 | <50 | ≥ 40 | 204 (20) | 12.0 (1.6)* | -0.4 (0.2)* | -0.7 (0.1)*** |

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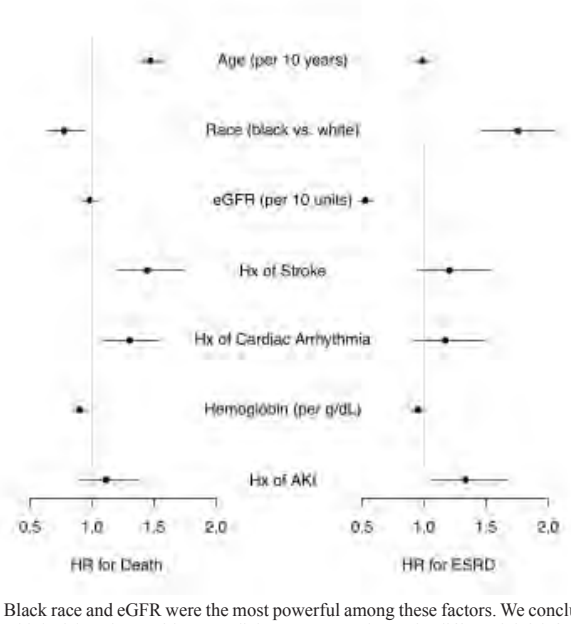
**F-PO1300**

**Differential Risk Factor Profiles for End-Stage Renal Disease (ESRD) and Death in Patients with Type 2 Diabetes, Chronic Kidney Disease (CKD) and Anemia** Robert D. Toto,[1] Andrew S. Levey,[2] Marc Pfeffer,[3] Hajime Uno,[3] Emmanuel A. Burdmann,[4] Chao-Yin Chen,[5] Peter Ivanovich,[6] Eldrin F. Lewis,[3] Julie Lin,[3] Patrick S. Parfrey,[7] Jerome A. Rossert,[5] Kai-Uwe Eckardt.[8] *[1]UT Southwestern, Dallas; [2]NEMC, Boston; [3]Brigham and Women's, Boston; [4]Faculdade de Medicina de Sao Jose do Rio, Sao Paulo, Brazil; [5]Amgen, Thousand Oaks; [6]Northwestern Univ., Chicago; [7]Memorial Univ., Newfoundland, Canada; [8]Univ. of Erlangen-Nuremberg, Erlangen, Germany.*

Preventing ESRD and death in patients with type 2 diabetes and CKD is a major public health priority. We hypothesized that differential risk factor profiles exist for the competing outcomes of: 1) ESRD and 2) Death among 4010 subjects with type 2 diabetes, CKD and anemia in the Trial to Reduce cardiovascular Events with Aranesp Therapy (TREAT). Cox proportional hazards models were used with a stepwise variable selection procedure including the following baseline factors thought to be involved in causal pathways for ESRD and Death: age, sex, race, need for insulin use, body mass index, BUN, eGFR, log urine protein/creatinine ratio, history of acute kidney injury (AKI), stroke, peripheral arterial disease, heart failure, arrhythmia, hemoglobin, serum albumin, CRP, and log serum ferritin. There were 666 (7.4/100 pt-yr) ESRD and 798 (8.1/100 pt-yr) Death events. Hazard ratios with 95% confidence intervals for factors demonstrated to have differential effects on ESRD and Death, based on these preliminary results, are shown.




Age, Black race and eGFR were the most powerful among these factors. We conclude that among high-risk patients with type 2 diabetes, CKD and anemia, differential risk factor profiles exist for ESRD and Death. These can be used to improve bedside risk stratification and design of future clinical trials.

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**F-PO1301**

**Plasma Follistatin Level Is Associated with Protein-Energy Wasting, Bone Mineral Density and Inflammation in Patients with Stage 5 Chronic Kidney Disease** Tetsu Miyamoto, Juan J. Carrero, Abdul Rashid Tony Qureshi, Björn Anderstam, Olof Heimburger, Peter F. Barany, Bengt Lindholm, Peter Stenvinkel. *Division of Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden.*

**Background**: Follistatin, a neutralizer of biological activities of TGF-β superfamily members including myostatin and activin, is known to be involved in the regulation of muscle growth and bone mineralization. As recent animal studies shows that follistatin inhibit myostatin action, this suggests a potential therapeutic use for this molecule. However, possible alterations in this pathway have not been characterized in CKD.

**Methods**: Circulating follistatin levels were analyzed in relation to protein-energy wasting (PEW), bone-mineral density (DEXA) and inflammation in 280 incident dialysis patients, 40 CKD 3-4 patients and 40 healthy controls. Mortality in the dialysis patients was evaluated after a median follow up of 58 months.

**Results**: No difference in follistatin levels was observed between the CKD 5 (1.57 [range 0.9-2.8] ng/mL), CKD 3-4 patients (1.62 [0.91-2.85] ng/mL), and controls (1.45 [1.02-2.14] ng/mL). In CKD 5 patients, plasma follistatin positively correlated with age ($r=0.18$; $p<0.01$), hsCRP ($r=0.35$; $p<0.0001$), IL-6 ($r=0.27$; $p<0.0001$) and negatively

with handgrip strength (HGS, r=-0.28; p<0.0001), s-creatinine (r=-0.13; p<0.05) and bone-mineral density (BMD, r=-0.30; p<0.0001). Patients with PEW (SGA≥2) had higher follistatin levels (1.97 vs 1.65 ng/mL, p<0.01). In multivariate regression analysis, follistatin independently contributed to HGS (β=-4.1, SE=1.5, P<0.01) and BMD (β=-0.03, SE=0.008, P<0.01). Patients with increased follistatin (the middle and highest tertile together) had a worse survival compared to patients within the lowest tertile in crude Cox analysis (HR 1.59, 95%CI 1.1-2.4, p=0.02). However, after adjustment for age, sex and co-morbidities the predictive power disappeared.

**Conclusions**: Elevated follistatin concentration is associated with inflammation, low HGS, low BMD and weakly predicted mortality in incident dialysis patients. Our findings may imply an involvement of the myostatin- and activin- interplay in the renal wasting process.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1302**

**The Appearance of Anemia in Stage 3 CKD Patients Represents a Poor Prognosis Factor. NADIR-3 Prospective 3-Year Study** J. M. Portolés,[1] Jose L. Gorriz,[2] Alberto M. Martinez-Castelao,[3] Fernando Dealvaro-Moreno,[4] Florencio García,[5] Vicente Álvarez,[6] Isabel Martínez,[7] Jesus Arteaga.[8] *[1]H.U.F.Alcorcón, Spain; [2]H.U.Dr.Peset, Spain; [3]H.U.Bellvitge, Spain; [4]GEENDIAB-SEN, Spain; [5]H.12 Octubre, Spain; [6]H.La Princesa, Spain; [7]H.Galdakao, Spain; [8]H.Navarra, Spain.*

Purpose: There are no longitudinal studies about anemia development in CKD-3. Our objective was to estimate time and rate of appearance of anemia and its associated factors.

Methods: Epidemiological, prospective, multicentre, 3-yr study. Inclusion: age 18-78 yrs, CKD-3 (MDRD-4), without anemia or ESA treatment. Data every 6 months (m) until start of renal replacement therapy or death. A specific diagnostic study is conducted if anemia appears (EBPG criteria).

Results: 431 patients: 63.4 yrs, 69.9% male. Nephropathy: vascular 28.4%;DM: 17%;GN 12.3%;CTIN 10.4%;APKD 7.2. Comorbidity: 32.8% with DM; 68.6% dyslipidemia, 92.8% HT and Charlson score: 3.2(1.6). Baseline parameters: Cr 1.8mg/dl, MDRD 39.1ml/min, proteinuria 0.69g/day (20.4%>1g/day), Hb 14.3g/dl, ferritin 131.8ng/ml, TSI 30.2%, PCR 2.1mg/dl and Alb 4.3g/dl. At 3 yrs, 25% had required hospitalization, 9.9% had presented at least one major CV Event, 26 patients had died, 13 had started kidney replacement and 173 reached CKD 4-5. 29.6% of patients were diagnosed with anemia, 85% of them from a renal cause. The likelihood of developing anemia was: 0.12 after 1 year, 0.20 after 2 and 0.25 after 3. Mean time to anemia was 34.9 m 95%CI[33.7-36.1] (Kaplan-Meyer). Those who developed anemia vs those who did not had: greater median age (68.0 vs 65.5yrs), greater % of men (71.6 vs 68.8), lower baseline MDRD (35.9 vs 40.0ml/min), greater baseline proteinuria (0.94 vs 0.62g/day); lower albumin (4.1 vs 4.3g/dl); greater MDRD reduction (6.8 vs 1.6ml/min at 3 yrs; p<0.001), earlier progression to CKD-4 (18.0 vs 28.1 m; p<0.001); greater rate of major CV events (16.1 vs 6.9%, p<0.05), hospitalization (33.7 vs 19.4%, p<0.001) and mortality (10.3 vs 6.6%, p<0.005).

Conclusions: We have the first estimation of the rate of appearance of renal anemia in stage 3 CKD. Those who develop anemia present worse clinical evolution.

Supported by Amgen

Disclosure of Financial Relationships: nothing to disclose

**F-PO1303**

**Successful Weight Loss in Obese Patients with Chronic Kidney Disease Is Associated with Compliance and Maintenance of Kidney Function** Helen L. MacLaughlin,[1] Sharlene A. Greenwood,[1] Katrina L. Campbell,[2] Iain C. Macdougall.[1] *[1]Department of Renal Medicine, King's College Hospital, London, United Kingdom; [2]Department of Nutrition and Dietetics, Princess Alexandra Hospital, Brisbane, Australia.*

Obesity is a risk factor for developing CKD & progression to kidney failure. This study examined factors associated with successful weight loss in obese patients with CKD.

Consenting patients with CKD & BMI >30 kg/m$^2$ were referred to a structured weight loss program of 9 sessions over 12 mths plus a low-fat, 1500 kcal renal diet, physical activity & orlistat. Weight loss, attendance & eGFR (4 variable MDRD Study eq) were measured at 0, 6 &12 mths. Mean % weight loss was determined with an intention to treat analysis. Predictors of weight loss & change in eGFR were assessed using linear regression analysis and ANOVA.

102 patients (57% male), aged 52.2 ±13.2 (±SD) years, weight 101.8 ±21.2 kg, & BMI 35.8 ±5.4 kg/m$^2$ commenced the weight loss program in 2005-2007. 82/102 were in CKD stages 1-4 (17 post transplant) with mean eGFR 36.9 ±32.2 ml/min & 20/102 were on dialysis & otherwise fit for transplantation. 49% of patients were compliant, 21% part-compliant and 30% non-compliant, attending 7-9, 4-6, or 1-3 sessions, respectively. Complete data was obtained for 90 patients, & the last observation carried forward for 12 patients for the primary analysis.

Mean weight loss for all patients commencing the program was 4.1 ±9.0% at 6 mths (p=0.01) & 4.1 ±9.1% at 12 mths (p=0.01). Weight loss was predicted by compliance (adj R2=0.36; p<0.001), adjusted for baseline BMI, weight, CKD stage & age. After 12 mths, compliant patients lost more weight (8.2 ±4.9%) than part-compliant (4.2 ±4.9%) or non-compliant patients (1.7 ±3.8%) (p=0.006), & in stages 1-4 CKD patients, eGFR increased by 1.5 ±2.0 ml/min with >10% weight loss & decreased by 3.3 ±1.2 ml/min, with <10% weight loss (p=0.02).

Significant weight loss was achieved overall, demonstrating the effectiveness of the weight loss program. Compliance predicted weight loss & >10% weight loss was associated with stabilisation of kidney function. Weight loss treatments that can reduce body weight by >10% may slow the progression of CKD in obese patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1304**

**Plasma Tenascin-C, Inflammation, Extracellular Matrix Remodeling and Outcomes in Chronic Kidney Disease Patients – A Pilot Study** Sophie Liabeuf,[1] Daniela Veit Barreto,[1] Axel Kretschmer,[2] Fellype Barreto,[1] Michel Andrejak,[1] Gabriel Choukroun,[3] Ziad Massy.[1,3] *[1]Department of Pharmacology, Amiens University Hospital and INSERM ERI12, Amiens, France; [2]GDD-TD-Biomarker Research, Bayer Schering Pharma AG, Wuppertal, Germany; [3]Department of Nephrology, Amiens University Hospital, Amiens.*

**Background and objectives:** Tenascin-C (TN-C) is an adhesion-modulating extracellular matrix glycoprotein which is overexpressed in various organs under disease conditions (infection, inflammation). Chronic kidney disease (CKD) is associated with a state of chronic inflammation and high cardiovascular morbidity and mortality.

**Design, setting, participants & measurement:** To examine the impact of plasma TN-C levels on cardiovascular outcomes, we studied a cohort of 94 prevalent CKD patients (mean ± SD age: 68 ± 13; 31% at CKD stages 2-3, 31% at stages 4-5, 38% at stage 5D).

**Results:** Plasma TN-C levels were elevated in this population and tended to rise as CKD progressed, with the increase becoming statistically significant at CKD stage 5D. Multivariate linear regression analysis indicated that CKD stage (p=0.04), IL-6 (p=0.02) and albumin (p=0.02) were independently associated with plasma TN-C levels. During follow-up (mean: 969 ± 405 days), 32 patients died (19 from cardiovascular events, 7 from infectious diseases and 6 from other causes). In crude analysis, higher plasma TN-C levels predicted overall and CV mortality (p=0.007 and p=0.003 respectively) and were associated with higher occurrence of CV events. Cox analyses confirmed that elevated plasma TN-C levels were independently associated with cardiovascular events, cardiovascular and overall mortality.

**Conclusions:** Our findings suggest that plasma TN-C levels are independently associated with cardiovascular outcomes in CKD patients. Further studies are needed to confirm our observations and better understand TN-C's role in inflammation and extra cellular matrix remodeling in CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1305**

**Quantification of Inflammatory and Immune Mediators in Serum, Whole Blood and Urine: Correlation with CKD Stage** Alan Perlman,[1] James M. Chevalier,[1] Thomas Parker,[1] Daniel Levine,[1] Patrick F. Wilkinson,[2] Hao Liu,[2] Francis X. Farrell.[2] *[1]The Rogosin Institute and Weill Medical College, Cornell University, NY, NY; [2]Centocor Research and Development, Inc, Radnor, PA.*

Diabetic nephropathy (DN) is caused by uncontrolled hyperglycemia and is the leading cause of ESRD. Traditional risk factors for progression of DN include hyperglycemia, hypertension and genetic predisposition. Recent evidence suggests that inflammatory and immune processes may be pathogenic in the progression of DN. Identification of specific inflammatory and immune mediators and their prevalence during CKD progression is the central objective of this study. We examined the relationship between CKD stage in patients with DN to serum, whole blood and urine concentrations of a variety of inflammatory and immune cytokines. Protein and/or RNA levels of inflammatory and immune mediators were quantified by ELISA, Luminex® and QuantiGene® System. We identified a positive correlation between CKD stage and EGF, EOTAXIN, FGF2, GMCSF, GRO, IL1RA, IL1a, MCP1 and VEGF. Serum IL5, MCP3 and IL8 were inversely correlated with CKD stage. Notably, several cytokine levels increased through stage 4 CKD, then declined in stage 5 suggesting that cytokine elevation was not simply due to a reduction of GFR. Other cytokines increased as early as stage 1 and 2 when GFR was preserved suggesting a possible pathogenic role for these mediators. Inclusion criteria were patients with evidence of diabetic nephropathy. Patients with an active infectious or inflammatory state, exposure to immunosuppressants, renal transplant or dialysis were excluded. 20 patients were targeted from CKD stages 1 or 2 and 20 patients per stages 3,4 and 5. As a comparative group, 5 polycystic kidney disease (PKD) patients without diabetes were also targeted for inclusion from stages 1 or 2 and stages 3, 4 and 5. 90 diabetic and 22 PKD subjects met the inclusion and exclusion criteria. 25 healthy matched subjects were utilized as controls. Patients GFR were estimated from serum creatinine using the MDRD formula. Our results are consistent with other reports that inflammatory and/or immune processes may contribute to the initiation and progression of DN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1306**

**Digital Deconstruction of Heterogeneous Renal Cell Preparations with High-Content Imaging and Cell Specific Biomarkers** Peter A. Antinozzi, Brandi C. Barnes, Daniel Deegan, Lijun Ma, Barry I. Freedman, Mariana Murea. *Wake Forest Health Sciences, Winston Salem, NC.*

Traditionally, detailed analyses of isolated cellular functions are carried out in enriched populations of a single cell type. For many biochemical measurements, isolation of cell sub-types is a necessity to eliminate what may be counter observations from contaminating cell types. In general, efforts to increase purity of specific cell types are often accompanied

by a decrease in quantity, which is further exacerbated by low abundance of the desired cell types. Thus, a major shortcoming of many physical enrichment procedures is they provide biological material of limited quantities, varying quality, and at high labor and reagent costs.

As an alternative to such enrichment procedures, we have developed a strategy to analyze heterogeneous primary cell populations. Typically, mixed cell populations would be regarded as a technical disadvantage; however our imaging-based strategy exploits heterogeneity with the ability to identify cell subtypes with specific fluorescent biomarkers and to measure multiplexed functional readouts. Thus, in a single specimen, multiple cell types can be evaluated simultaneously. The method uses a minimal quantity of biospecimen, where the analyzed area is 3mm x 3mm in a 384 multi-well plate.

The kidney has over 27 cell types, which include multiple types of tubular epithelial cells, glomerular cells, interstitial cells, and those of the vasculature. This heterogeneity and often scarce supply of tissue is well suited for our high-content imaging strategy. Here, we demonstrate two methods to identify individual cell types from primary renal cell preparations: 1) using multiplexed morphology fluoroprobes and 2) using a cell surface marker screen with over 200 probes. Bioinformatic design of cell subtype identifiers enables live cell functional assays to be performed on heterogeneous cultures, where the effects on individual cell types can be evaluated by a cell-by-cell analysis.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1307

**Relationship between the Accuracy of Glycemic Markers and the Stage of CKD in the Patients with Type 2 Diabetes Mellitus** Koji Harada,[1] Yasuhiro Akai,[2] Mikiko Yoshikawa,[1] Hiroki Takahashi,[1] Yukinari Yamaguchi,[2] Masayuki Iwano,[2] Yoshihiko Saito.[2] *[1]Department of Nephrology, Rakuwakai-Otowa Hospital, Kyoto, Japan; [2]First Department of Internal Medicine, Nara Medical University, Kashihara, Nara, Japan.*

**Background:** Adequate glycemic control improves the survival of diabetics by reducing the macrovascular and microvascular complications of diabetes. Although glycated hemoglobin (A1C) is widely used as a reliable marker of glycemic control, A1C could underestimate the hyperglycemia in anemic and dialysis patients. Recently, it is reported that glycated albumin (GA) provide better index to estimate glycemic status in dialysis patients than A1C. This might be true in advanced CKD patients without dialysis because they have similar pathophysiological features to dialysis patients, however, adequate glycemic markers in advanced non-dialysis CKD patients have not been established. We conducted this study to evaluate the accuracy of glycemic markers in advanced CKD patients without dialysis. **Materials and Method:** 139 non-dialysis CKD patients with diabetes were enrolled. The patients were divided into 3 groups; diabetic patients with estimated GFR (eGFR) ≥60 ml/mintute/1.73 m$^2$ were referred to as group 1 (G1), 30≤eGFR< 60 were group 2 (G2), and eGFR <30 were group 3 (G3). **Result:** 28 diabetic patients were in G1, 69 in G2, and 42 in G3. GA was positively correlated with random plasma glucose (PG) in all group, and Spearman rank-correlation coefficient (Rs) decreased with deterioration of eGFR [G1: Rs=0.670, p=0.0005, G2: Rs=0.556, p<0.0001, G3: Rs=0.361, p<0.02]. A1C was positively correlated with random PG in G1 and G2, however, no significant correlation was noted in G3. To clarify the significance of A1C and GA, we examined the relations between A1C, GA and various clinical parameters. Although GA was only correlated with serum albumin, A1C was significantly correlated with hemoglobin, dose of recombinant human erythropoietin, and eGFR. **Conclusion:** Because A1C was affected by various clinical factors, it is necessary to choose the adequate glycemic markers according to the stage of CKD. GA was shown to be superior glycemic index in the patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1308

**ADMA and Endotoxaemia a Newly Recognised Potential Link in the Inflammatory Pathways of Chronic Kidney Disease and Dialysis** Mohamed Tarek Eldehni,[1] Mhairi K. Sigrist,[1] Jan T. Kielstein,[2] Cheuk-Chun Szeto,[3] Philip K. T. Li,[3] Chris W. McIntyre.[4] *[1]Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]Department of Nephrology and Hypertension, Medical School Hannover, Germany; [3]Medicine & Therapeutics, Chinese University of Hong Kong, China; [4]School of Graduate Entry Medicine and Health, University of Nottingham, United Kingdom.*

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) that correlates with markers of cardiovascular disease (CVD) burden. ADMA levels are elevated in chronic kidney disease (CKD) and are implicated in reduced vasodilatory reserve and endothelial dysfunction. Recently endotoxaemia, resulting from probable gut translocation, has been reported across the spectrum of CKD, as a possible factor in systemic inflammation and CVD. We aimed to explore the link between ADMA and endotoxin (ET).

Serum ET and ADMA levels were measured in 134 patients (46 CKD 4, 28 PD and 60 HD). Arg, ADMA, and SDMA were measured by liquid chromatography-mass spectrophotometry and endotoxin quantified by commercially available Limulus Amoebocyte assay.

HD patients had the highest ADMA levels (0.7, IQR= 0.64-0.80) and in this group ADMA significantly correlated with ET levels (r = 0.27, P = 0.05). Overall ADMA maintained its correlation with ET (r = 0.29, P = 0.002) and its levels were highest at the highest ET tertile (Figure). It is recognised that lower serum albumin can result in reduced ADMA protein binding, leading to higher ADMA levels. This was not demonstrated in the HD group (r = - 0.16, P = 0.25).

Although high ADMA is associated with increased systemic vascular resistance; in conditions when NO generation increases (e.g. septic shock), elevated ADMA is a compensatory response to down regulate NO and maintain SVR. Extrapolating from this scenario allows us to postulate that HD patients may exhibit the same compensatory mechanism to the repeated inflammatory stress produced by repetitive and sustained systemic exposure resulting from haemodialysis induced translocation from the gut. This unrecognised link between ADMA and ET may contribute to enhance understanding the pathophysiology of Inflammation and CVD in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1309

**Urinary Proteomics for Early Diagnosis in Diabetic Nephropathy** Petra Zürbig,[1] Sianna Panagaiotopoulos,[2] Stine Nielsen,[3] Richard J. Macisaac,[2] Harald Mischak,[1,4] Hans-Henrik Parving,[5,6] George Jerums,[2] Peter Rossing.[3] *[1]Mosaiques Diagnostics GmbH, Hannover, Germany; [2]Austin Health and University of Melbourne, Heidelberg, Australia; [3]Steno Diabetes Centre, Gentofte, Denmark; [4]University of Glasgow, Glasgow, United Kingdom; [5]University Hospital of Copenhagen, Rigshospitalet, Denmark; [6]Aarhus University, Aarhus, Denmark.*

Diabetic nephropathy may be detectable even at early stages in the urinary proteome. In this study we present recent data indicating that urinary proteome analysis is a valuable tool for early and sensitive detection of diabetes-associated patho-physiological changes, assessment of disease progression and monitoring of therapy success.

High-resolution capillary-electrophoresis coupled to time-of-flight mass-spectrometry (CE-MS) was used to profile the low-molecular-weight proteome in urine of diabetic patients collected in longitudinal trials for up to 15 years at two different clinical centers in Denmark and Australia.

When applying previously defined biomarker patterns for chronic kidney disease onto the data obtained from urine samples of normoalbuminuric subjects, we could demonstrate that these biomarkers enabled prediction of development of macroalbuminuria with an AUC>0.92 (p<0.001) for a period of 3-4 years. One of the hallmarks of diabetes and associated complications appears to be the increase in extracellular matrix (ECM) and the release of its components, most notably collagen. This process appears to be in part due to reduced proteolysis and is reflected at a very early stage by the decrease in urinary collagen fragments. Assessment of these seems to result in a much higher accuracy of predicting DN than clinical parameters like urinary albumin.

Urinary proteome analysis enables the non-invasive prediction of diabetic kidney disease at an early stage in normoalbuminuric patients, via determination of specific collagen fragments. This opens an avenue towards targeted therapeutic intervention towards DN potentially before irreversible damage has occurred.

Disclosure of Financial Relationships: Employer: mosaiques diagnostics GmbH.

### F-PO1310

**Human Urinary Peptides for Use in Diagnosis of CKD** Petra Zürbig,[1] Angel Argiles,[2] Christian Delles,[3] Jochen H. H. Ehrich,[4] Frank Eitner,[5] Danilo Fliser,[6] Mark Girolami,[3] Wilfried Gwinner,[4] Marion Haubitz,[4] Holger Jahn,[7] Bruce A. Julian,[8] Walter Kolch,[9] Andrzej S. Krolewski,[10] Mario Luppi,[11] Ziad Massy,[12] Michael Melter,[13] Jan Novak,[8] Karlheinz Peter,[14] Peter Rossing,[15] Joost Schanstra,[12] Jesns-Uwe Stolzenburg,[16] Dan Theodorescu,[17] Visith Thongboonkerd,[18] Raymond C. Vanholder,[19] Harald Mischak.[1,3] *[1]Mosaiques Diagnostics GmbH; [2]RD Néphrologie; [3]University of Glasgow; [4]Hannover Medical School; [5]RWTH University Hospital; [6]Saarland University Hospital; [7]University Hospital Hamburg-Eppendorf; [8]University of Alabama at Birmingham; [9]University College Dublin; [10]Joslin Diabetes Center; [11]University of Modena and Reggio Emilia; [12]Inserm; [13]University of Regensburg; [14]Baker Heart Research Institute; [15]Steno Diabetes Centre; [16]University of Leipzig; [17]University of Virginia; [18]Mahidol University; [19]University Hospital Ghent.*

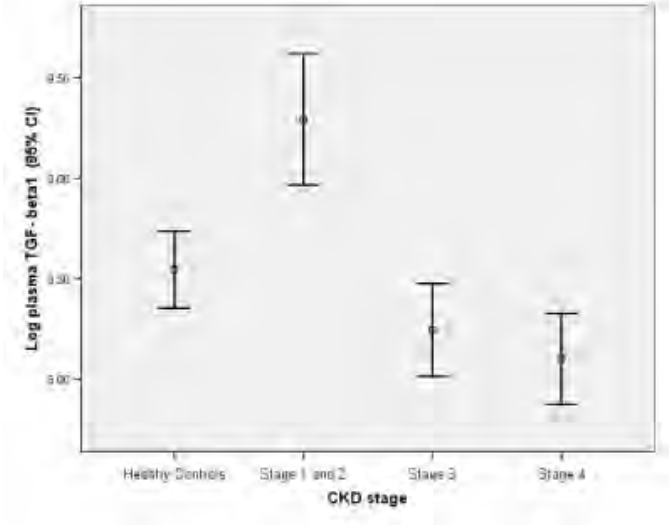
Owing to its availability, ease of collection and correlation with physiology and pathology, urine is an attractive source for clinical proteomics. However, the lack of comparable data sets from large cohorts has greatly hindered the development of clinical proteomics. Here, we report the establishment of a reproducible, high-resolution method for peptidome analysis of naturally occurring human urinary peptides and proteins using samples from 3,600 individuals analyzed by capillary electrophoresis coupled to mass spectrometry (CE-MS). All processed data were deposited in a SQL database. This database currently contains <5,000 relevant urinary peptides that serve as a pool of potential classifiers for diagnosis and monitoring of various diseases. As an example, by using this source of information, we were able to define urinary peptide biomarkers for CKD allowing diagnosis of these diseases with high accuracy. Application of the CKD-specific biomarker set to an independent test cohort in the subsequent replication phase resulted in 85.5% sensitivity and 100% specificity. These results indicate the potential usefulness of CE-MS for clinical applications in the analysis of naturally occurring urinary peptides.

Disclosure of Financial Relationships: Employer: mosaiques diagnostics GmbH.

**F-PO1311**

**Circulating TGF-β1 Levels Predict Progression of Chronic Kidney Disease** Priyanka Jain, Vaidyanathapura S. Balakrishnan, Madhumathi Rao. *Nephrology, Tufts Medical Center, Boston, MA.*

TGF-β1 is a multifunctional cytokine that mediates glomerulosclerosis, interstitial fibrosis and epithelial cell atrophy, processes associated with progression of chronic kidney disease (CKD). The relationship between plasma levels of TGF-β1 and CKD progression has not been explored. In a prospective cohort study we enrolled 161 adult patients with CKD (eGFR >15 ml/min); exclusions were active inflammation, re-transplant, immunosuppression or severe illness. Healthy individuals (N=54) provided baseline control comparisons. Plasma TGF-β1 was measured in patients and controls using the Quantikine Human TGF-β1 Immunoassay (R&D Systems, Inc., Minneapolis, MN). As its distribution was non-normal, TGF-β1 data was log transformed for analyses. The mean±SD age of the CKD cohort was 61±15 years, 61% were male, 81% Caucasian and 30% diabetic; 19% of subjects were CKD KDOQI stages 1 and 2, 51% stage 3 and 30% stage 4. Plasma TGF-β1 levels correlated positively with eGFR (r=0.36, p <0.001), with the highest levels seen in CKD stages 1 and 2 (median levels 9945pg/ml vs. 2836pg/ml for stage 3 and 2627pg/ml for stage 4) (figure). Patients with CKD stages 1 and 2 also showed TGF-β1 levels that were significantly higher than in healthy controls, at comparable levels of GFR (median levels 9945pg/ml vs 4535pg/ml, p<0.001). TGF-β1 levels did not differ by age, race, sex or diabetic kidney disease. TGF-β1 levels predicted CKD progression when adjusted for black race and stage of CKD (doubling of TGF-β1 levels was associated with an HR of 1.8, 95% CI: 1.1-3.0 for the composite outcome of doubling of serum creatinine or need for renal replacement therapy). Our results suggest that TGF-β1 levels predict CKD progression at all stages. However, significant elevations of TGF-β1 levels in the earlier stages of CKD highlights the important of targeted intervention in early kidney disease.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1312**

**Markers of Nutrition and Inflammation Predict Mortality Independent of Each Other in CKD: NHANES III** Nirupama Ramkumar, Bradley C. Baird, Srinivasan Beddhu. *Univ Utah.*

Inflammation and malnutrition are thought to be closely interlinked but there is a paucity of data on whether markers of inflammation and nutrition predict mortality independent of each other in CKD. Therefore, we examined the associations of serum C-reactive protein (CRP), serum albumin and mid-arm muscle circumference (MAMC) with mortality in CKD in National Health And Nutrition Examination Survey (NHANES) III, a complex, multistage survey conducted by the National Center for Health Statistics (NCHS) in 1988 to 1994. The sub-population (N = 1066)with CKD (eGFR < 60 ml/min/1.73 m²) and non-missing data were included in this analysis. Mortality data were obtained by NCHS by linkage with National Death Index records through December 31, 2006.

Baseline characteristics by serum CRP levels are summarized in Table 1.

Baseline characteristics by inflammation in NHANES III CKD subpopulation

| | CRP ≤ 3 mg/L | CRP > 3 mg/L |
|---|---|---|
| Age(years) | 69±1 | 69±1 |
| Male(%) | 38 | 38 |
| Caucasian(%) | 92 | 90 |
| Diabetes(%) | 17 | 22 |
| Htn(%)* | 70 | 77 |
| MI(%)* | 12 | 20 |
| CHF(%)* | 8 | 16 |
| Stroke(%) | 9 | 9 |
| Cancer(%) | 11 | 9 |
| Smoker(%)* | 9 | 18 |
| GFR* | 51±1 | 48±1 |
| BMI(kg/m²)* | 27±2 | 29±1 |
| MAMC(cm) | 26±1 | 26±1 |
| Serum albumin(mg/dl)* | 4.1±0.1 | 3.9±0.1 |

* p ≤ 0.05

There were 715 deaths over average of 76.3 months of follow-up. Because proportionality assumptions were violated by CRP, Cox proportional hazards models were constructed for early (< 36 months of follow-up) and late (≥ 36 months of follow-up) periods. After adjusting for age, gender, race, diabetes, hypertension, stroke, CHF, MI, cancer, smoking, and eGFR, in the early period, serum CRP (for each doubling HR 1.29, 95% CI 1.13-1.47), serum albumin (for each g/dl increase HR 0.48, 95% CI 0.30-0.75) and MAMC (for each cm increase HR 0.87, 95% CI 0.82-0.93) were associated with mortality. In the late period, the corresponding HR (95% CI) for serum CRP, serum albumin and MAMC were 1.02 (0.93-1.12), 0.58 (0.39-0.85) and 0.93 (0.89-0.98), respectively.

We conclude that muscle mass and inflammation are independent predictors of death in CKD. Therefore, interventions that target muscle mass might improve survival in CKD even in those with inflammation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1313**

**The Association of Parathyroid Hormone Levels and the Metabolic Syndrome in Chronic Kidney Disease** Georges Saab,[1] Adam Whaley-Connell,[2] Suying Li,[3, 4] Keith C. Norris,[5] George L. Bakris,[6] Peter A. McCullough.[7] *[1]Washington University School of Medicine, St. Louis, MO; [2]University of Missouri-Columbia School of Medicine, Columbia, MO; [3]KEEP Data Coordinating Center, Minneapolis, MN; [4]Minneapolis Medical Research Group, Minneapolis, MN; [5]David Geffen School of Medicine, Univ of California, Los Angeles, CA; [6]University of Chicago School of Medicine, Chicago, IL; [7]William Beaumont Hospital, Royal Oak, MI.*

OBJECTIVE: To examine the relationship of parathyroid hormone (PTH) and the Metabolic Syndrome (MetS) among non-diabetic persons with chronic kidney disease (CKD).

METHODS: This was a cross-sectional analysis of 3215 non-diabetic participants in the National Kidney Foundation-Kidney Early Evaluation Program (KEEP) found to have CKD (eGFR <60 ml/min/1.73m²) examining the relationship of PTH levels and the MetS.

RESULTS: In unadjusted analysis, the prevalence of the MetS increased along increasing PTH quartiles (31.7%, 33.8%, 37.3%, and 48.7% respectively, p for trend <.0001). After multivariate adjustment, as compared to the first PTH quartile, odds of the MetS were 16% (p=.18), 35% (p=.006), and 89% (p <.0001) higher for the second, third, and fourth quartiles respectively. When taken as a continuous predictor, each standard deviation increase of natural log transformed PTH was associated with a 26% (p<.0001) higher odds of the MetS. The association of PTH with the MetS was not modified by age or gender (p for interaction = NS for both modifiers).

CONCLUSIONS: Among an outpatient non-diabetic population with CKD, higher PTH levels were associated with an increasing prevalence of the MetS.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1314**

**Biomarkers of Endothelial Dysfunction in the Continuum of CKD: Relationship to Medication Use** Darius Mason, Magdalene M. Assimon, Katie E. Cardone, Darren W. Grabe, Amy B. Pai. *ANephRx, Albany College of Pharmacy & Health Sciences, Albany, NY.*

Endothelial dysfunction contributes to the pathogenesis of cardiovascular disease (CVD) in chronic kidney disease (CKD). Soluble adhesion molecules (e.g. sICAM) are easily measured biomarkers that correlate with diagnostic measures of endothelial dysfunction. The aims of this study were to characterize concentrations of adhesion molecules in the continuum of worsening kidney function and to explore possible relationships with commonly used medications.

CKD patients ≥18 years old with an etiology of diabetes and/or hypertension were enrolled in the Albany Pharmacy Bio-surveillance Cohort Study, designed to evaluate the relationship of medications with relevant CVD biomarkers. This study represents a cross-sectional baseline analysis. Medical histories, laboratory values and medication lists were obtained. Plasma markers of endothelial dysfunction including sICAM-1, P-selectin and E-selectin were measured by multiplex bead array assay. Student's t-test was used to compare between CKD groups and within group comparisons were made using Wilcoxon Rank Sum test.

Thirty-two CKD (age 64.8±14.5 years, 40.6% male, GFR 33.8±15.6 ml/min/1.73m2) and forty hemodialysis (HD) (age 64.3±15.6 years, 52.5% male, HD vintage 3.4±2.8 years) were included in this analysis. sICAM concentrations were higher in pre-dialysis patients compared to HD patients (223.41 ng/ml [175.2-403.5] vs. 194.4 ng/ml [105.4-383.7], p=0.009). Conversely, P-selectin and E-selectin concentrations were lower in CKD vs. HD patients: P-selectin (72.3±29.1 ng/ml vs. 102.9±58.2 ng/ml, p=0.0053) and E-selectin (56.2±25.6 ng/ml vs. 88.2±50.5 ng/ml, p=0.0009). Among HD patients, statin use (n=18) was associated with lower P-selectin concentrations (p=0.05) and ergocalciferol use (n=20) was associated with lower sICAM and P-selectin concentrations (p=0.004 and p=0.017, respectively).

These data suggest biomarker profiles of endothelial dysfunction may change as kidney disease progresses. The association of statin and ergocalciferol use with lower adhesion molecules suggests possible non-classical effects of these agents and warrants further investigation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1315**

**The Association of Parathyroid Hormone, Calcium, and Phosphorus Levels and Short Term Mortality in Persons with Chronic Kidney Disease Not on Dialysis** Georges Saab,[1] Adam Whaley-Connell,[2] Suying Li,[3,4] Shu-Cheng Chen,[3] Keith C. Norris,[5] George L. Bakris,[6] Peter A. McCullough.[7] *[1]Washington University School of Medicine, St. Louis, MO; [2]University of Missouri-Columbia School of Medicine, Columbia, MO; [3]KEEP Data Coordinating Center, Minneapolis, MN; [4]Minneapolis Medical Research Group, Minneapolis, MN; [5]David Geffen School of Medicine, Univ of California, Los Angeles, CA; [6]University of Chicago School of Medicine, Chicago, IL; [7]William Beaumont Hospital, Royal Oak, MI.*

To examine the association of Ca, Phos, and PTH levels among persons with CKD (eGFR < 60 ml/min/1.73m²) and short term mortality

This was a cross-sectional analysis of 5780 participants in the National Kidney Foundation-Kidney Early Evaluation Program (KEEP) found to have CKD (eGFR <60 ml/min/1.73m²) and not on dialysis examining the relationship of Ca, Phos, and PTH levels with short term mortality.

Of 5780 KEEP participants with CKD, a total of 152 deaths occurred over a median follow-up of 2.0 years. After adjustment for age, race, gender, smoking, alcohol use, eGFR, education level, recent doctor visits, insurance coverage, prior CVD, each standard deviation increase in natural log transformed PTH was associated with a 20% higher risk of death (HR: 1.20 (95% CI: 1.01-1.44,p=.04). Conversely, each 1 mg/dL increase in serum calcium and phosphorus was not associated with increased mortality (HR: 0.81 (95% CI: 0.58-1.12,p=.20) and 0.86 (95% CI: 0.66-1.13,p=.27 respectively). Since PTH levels are associated with an increased prevalence of the MetS, we additionally adjusted for individual components of the MetS (hypertension, dysglycemia, dyslipidemia, obesity, and microalbuminuria). After additional adjustment, the association PTH and mortality was mildly attenuated (HR 1.18 (95% CI: 0.98-1.41,p=.08) and while there was a strong trend, it was no longer statistically significant.

Higher PTH levels are associated with increased risk of death, possibly due to a higher prevalence of the MetS and possibly through other pathways. Higher calcium and phosphorus levels are not associated with short term mortality.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1316**

**A Low 25D3 Level Is Associated with Increased Prevalence of Comorbidities in the General Population across a Wide Range of eGFR** Francis Dumler. *Division of Nephrology, William Beaumont Hospital, Royal Oak, MI.*

Several epidemiological and clinical observations suggest that a deficient vitamin D status may increase the risk of cardiovascular events, decrease insulin secretion and sensitivity, and enhance inflammation. We evaluated the relationship between vitamin D, and the prevalence of comorbid conditions in 10,617 NHANES participants (age: 47 ± 20 years; 24% black, 76% white, and 47% female. Comorbidities evaluated: congestive heart failure (CHF), stroke (CVA), hypertension (HTN), diabetes mellitus (DM), hypercholesterolemia (CHOL), myocardial infarction (MI), and peripheral vascular disease (PVD). Participants were allocated to one of three vitamin D levels (Group I: < 20; Group II: 29 – 39.9; Group III: 40 – 100) mg/dL.

Biochemical, nutritional and inflammatory parameters are shown below:

Group I: BMI 26 ± 4 kg/m2, serum calcium 9.3 ± 0.4 mg/dL, serum phosphorus 3.5 ± 0.5 mg/dL, CRP 0.44 ± 0.70 mg/dL; Phase Angle 7.47 ± 1.22 º; eGFR 74 ± 17 mL/min/1.73m2.

Group II: BMI 26 ± 4 kg/m2, serum calcium 9.3 ± 0.4 mg/dL, serum phosphorus 3.4 ± 0.5 mg/dL, CRP 0.41 ± 0.80 mg/dL; Phase Angle 7.55 ± 1.13 º; eGFR 68 ± 14 mL/min/1.73m2.

Group III: BMI 24 ± 4 kg/m2, serum calcium 9.3 ± 0.4 mg/dL, serum phosphorus 3.4 ± 0.5 mg/dL, CRP 0.40 ± 0.69 mg/dL; Phase Angle 7.57 ± 1.13º; eGFR 67 ± 14 mL/min/1.73m2.

Differences between groups were statistically significant (P<0.001).

The prevalence for positive comorbid conditions is shown below:

Group I: CHF 3%, CVA 2%, HTN 25%, DM 8%, CHOL 33%, MI 4%, PVD 23%

Group II: CHF 3%, CVA 2%, HTN 24%, DM 6%, CHOL 33%, MI 4%, PVD 21%

Group III: CHF 2%, CVA 2%, HTN 19%*, DM 3%*, CHOL 34%, MI 4%, PVD 18%*

*P<0.0005

Differences between vitamin D groups and biochemical and anthropometric parameters were statistically significance but with no clinical value. However, the decreasing prevalence of the comorbid conditions with incremental vitamin D level indeed suggests a protective effect on HTN, Dm and PVD. These salutary effects remained significant across a wide range of eGR.

These observations add credence to the efforts in raising prevailing serum of vitamin D levels in the general population.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1317**

**Use of Phase Angle Parameter for Identification of Malnutrition and Micro Inflammation in the General Non Dialysis Dependent Chronic Kidney Disease Population** Francis Dumler. *Division of Nephrology, Willaim Beaumont Hospital, Royal Oak, MI.*

Malnutrition, whether alone or in combination with a micro inflammatory is clearly associated with increased morbidity and mortality in dialysis patients. A low bioimpedance phase angle (an index of tissue hydration, cell membrane integrity, and cell mass) may be a consequence of both processes. Our objective was to determine if a low phase angle is associated with the presence of malnutrition and inflammatory markers in 10,242 NHANES III participants (age: 47 ± 20 years; 24% black, 47% female; eGFR 71 ± 16 (range 20-120) mL/min/1.73m2. Study parameters were evaluated by phase angle tertile groupings (< 7.0o, 7.0-8.9 o, and 9.0-12.0 o). Results were as follow:

Low tertile: haemoglobin 13 ± 1 g/dL, lymphocyte/white blood cell ratio 0.316 ± 0.09, serum iron 83 ± 34 µg/dL, CRP 0.52 ± 0.89 mg/dL, serum albumin 40 ± 3 g/L, µalbuminuria 59 ± 440 mg/dL, body cell mass 23 ± 6 kg, fat free mass 49 ± 12 kg.

Middle tertile: haemoglobin 14 ± 1 g/dL, lymphocyte/white blood cell ratio 0.331 ± 0.08, serum iron 90 ± 38 µg/dL, CRP 0.39 ± 0.67 mg/dL, serum albumin 42 ± 3 g/L, µalbuminuria 32 ± 256 mg/dL, body cell mass 26 ± 6 kg, fat free mass 51 ± 12 kg.

Upper tertile: haemoglobin 15 ± 1 g/dL, lymphocyte/white blood cell ratio 0.342 ± 0.09, serum iron 98 ± 39 µg/dL, CRP 0.35 ± 0.54 mg/dL, serum albumin 43 ± 3 g/L, µalbuminuria 23 ± 123 mg/dL, body cell mass 30 ± 6 kg, fat free mass 57 ± 12 kg.

The differences between groups across the phase angle tertiles were statistically significant (P<0.001), and were maintained at a wide range of both eGFR and age during post hoc testing. All potential inflammatory markers outlined above were significantly correlated with body cell mass and fat free mass (P<0.001) by simple regression analysis. In addition, they were found to be independent determinants of a decreased nutritional status by multivariate and logistic regression analysis at a wide range of eGFR and age ranges.

In conclusion, a low phase angle is associated with the presence of a micro inflammatory processes and a lower nutritional status in the general population as sampled by the NHANES III study.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1318**

**Central Fat Distribution Is More Closely Related with Key Risk Factors Than Elevated BMI in Patients with CKD Stage 3** Philip D. Evans,[1] Natasha J. Mcintyre,[1] Richard J. Fluck,[1] Chris W. McIntyre,[1,2] Maarten W. Taal.[1] *[1]Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]School of Graduate Entry Medicine, University of Nottingham, Derby, United Kingdom.*

**Purpose:** Body Mass Index (BMI) as a marker of obesity is an established risk factor for chronic kidney disease (CKD) and cardiovascular disease (CVD). However, BMI can overestimate obesity. Waist-to-hip ratio (WHR), a marker of central obesity, is emerging as a more important risk factor. We studied BMI and WHR in a cohort of patients with CKD stage 3 and compared the results with other known risk factors for CKD progression and CVD.

**Methods:** We recruited 1741 patients with CKD stage 3 from primary care for the Renal Risk in Derby study. Each participant underwent clinical assessment, including measurement of BMI, WHR, pulse wave velocity (PWV) and urine and serum biochemistry tests.

**Results:** The mean age (±SD) of the cohort was 72.9±9yrs with 60% female. Mean BMI was 29.1±5 kg/m² and mean WHR was 0.91±0.09. 37% of the cohort was classified as obese (BMI ≥30 kg/m²) and 88% classified as centrally obese (waist to hip ratio >0.8 for females and >0.9 for males).

Central obesity was associated with a higher mean uric acid, PWV, albumin:creatinine ratio (ACR) and a lower mean eGFR(p=<0.01). BMI ≥30kg/m² was associated with a higher mean uric acid and a lower mean PWV(p=<0.01).

Univariate analysis revealed significant correlations between BMI and age(r=-0.13; p=<0.001), uric acid(r=0.19; p=<0.001) and PWV(r=-0.13; p=<0.001). Significant correlations were also revealed between WHR and age (r=0.11; p=<0.001), uric acid(r=0.31; p=<0.001), PWV(r=0.12; p=<0.001), ACR(r=0.1; p=<0.001) and eGFR (r=-0.17; p=<0.001).

Multivariable linear regression analysis identified WHR as an independent determinant of ACR, uric acid and PWV, when modelled with other known risk factors(p=<0.001). BMI was identified as an independent determinant of PWV (inversely), uric acid and eGFR(p=<0.05).

**Conclusions:** WHR is closely related to more key risk factors in CKD stage 3 patients than BMI. Central obesity as represented by WHR may be of greater importance as a risk factor in CKD than BMI and reliance on BMI alone may therefore underestimate the associated risk.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1319**

**Plasma and Urinary Levels of Vasohibin-1 in Patients with Chronic Kidney Disease** Daisuke Saito,[1] Yohei Maeshima,[1] Tatsuyo Nasu,[1] Hiroko Yamasaki,[1] Norikazu Hinamoto,[1] Masaru Kinomura,[1] Shinji Kitamura,[1] Hitoshi Sugiyama,[2] Hirofumi Makino.[1] *[1]Medicine and Clinical science, Okayama university, Okayama, Japan; [2]Center for Chronic Kidney Disease and Peritoneal Dialysis, Okayama university, Okayama, Japan.*

CKD is associated with cardiovascular complications and tubulointerstitial ischemia is involved in the progression of CKD. Vasohibin-1 (MW: 43 kD) is an unique endogeneous angiogenesis inhibitor, initially identified in endothelial cells induced by proangiogenic factors such as VEGF-A, serving as a negative feedback regulator of angiogenesis. The aim of the present study is to examine the plasma and urinary levels of Vasohibin-1 in patients with chronic kidney disease (CKD) and to evaluate its clinical usefulness. We measured plasma and urinary levels of Vasohibin-1 by ELISA in 69 patients with CKD and assessed the relationship between the levels of Vasohibin-1 and clinical parameters or histological findings of renal biopsy specimens. The levels of plasma Vasohibin-1 were significantly lower in hypertensive patients as compared to non-hypertensive patients and inversely correlated with systolic blood pressure (r=0.551, P<0.0001), but were not correlated with renal function or proteinuria. Urinary Vasohibin-1 levels were significantly lower in patients with CKD stages 3-5 (eGFR <60), and there was a significant positive correlation between urinary Vasohibin-1 levels and eGFR (r=0.369, P=0.0065). Urinary Vasohibin-1 levels tended to be lower in patients with moderate to severe interstitial fibrosis and tubular atrophy compared with those with no or mild involvement. Taken together, these results suggest that plasma levels of Vasohibin-1 may reflect systemic vascular endothelial dysfunction in association with hypertension and that reduction of urinary Vasohibin-1 may reflect tubulointerstitial injuries and ischemia in patients with CKD, potentially counterbalancing excessive anti-angiogenic milieu.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1320**

**Intact PTH as a Predictor of Renal Recovery in Acute Kidney Injury** Hilana H. Hatoum,[1] Ali K. Owda,[1] Fadi S. Rzouq.[2] *[1]IM/Renal Division, MRMC, Flint, MI; [2]IM, University of Washington, Seattle, WA.*

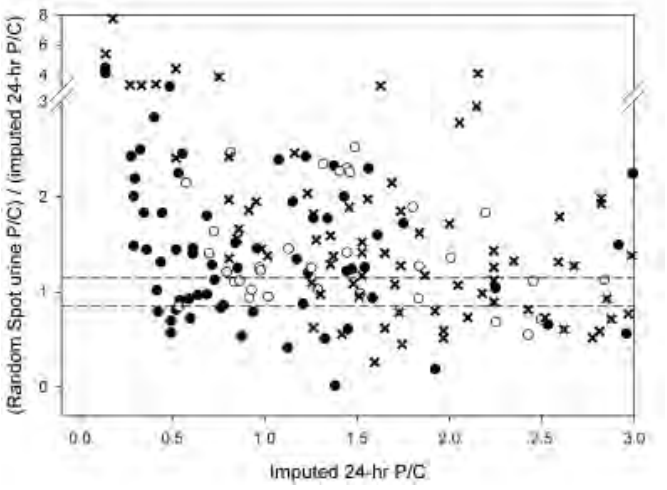
Background:AKI is the abrupt loss of kidney function that results electrolyte dysregulation as well as the accumulation of nitrogenous waste products. Renal recovery (RR) post AKI is an important concept that is understudied. We sought to evaluate the prognostic power of elevated intact-PTH during AKI on RR in the next 6-12 months (T1) after hospital discharge (RR defined as having glomerular filtration rate (GFR) at T1 >75% of baseline (T0) GFR which is measured within the last year before admission for AKI). Method:Patients who were discharged from a tertiary medical center with the diagnosis of AKI were involved in the study. Patients' charts were reviewed and those who had a T0 GFR, PTH level and T1 GFR were involved. Patients were then divided into 2 groups: Group A with incomplete RR (T1 GFR <75% of T0 GFR) and Group B with complete RR (T1 GFR >75% of T0 GFR). Characteristics of patients in both groups were recorded and multiple regression analysis was applied to evaluate if any variable could predict achieving complete Vs incomplete RR.Results:1200 charts of patients with AKI were reviewed, of whom 42 met the criteria and were the final study group. 18 patients had incomplete RR (group A) Vs 24 patients had complete RR (Group B). Patients in both groups were similar in baseline characteristics including age (64.3 Vs 63, expressed as group A Vs B, P of 0.8), gender, race, T0 GFR ( 92.9 Vs 90.8 ml/min, P 0.7), presence of HTN (P 0.7) and DM (P 0.8). None of these variables was able to predict the status of RR. Nevertheless, PTH level was significantly higher among group A patients (109+_68) compared to group B (74.1 +_30), (P 0.03). Logistic regression analysis was applied with a PTH value >100 as the independent variable and achieving complete RR as the dependent variable. Patients with elevated PTH >100 were more likely to have incomplete RR but that didn't reach statistical significance (OR 4.0, 95% CI 0.96-16.5). Conclusion: Intact PTH level during AKI seems like a promising predictor of RR in the future. This may help risk stratify patients discharged after an episode of AKI and may dictate a closer follow up for them in the future.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1321**

**Random Spot Urine Protein/Creatinine (P/C) Ratio Is an Unreliable Estimate of 24-hr Proteinuria in Individual Chronic Kidney Disease (CKD) Patients** Ganesh B. Shidham,[1] Daniel J. Birmingham,[1] Haikady Nagaraja,[2] Brad H. Rovin,[1] Lee A. Hebert.[1] *[1]Internal Medicine/Nephrology, Ohio State University Medical Center, Columbus, OH; [2]Statistics, Ohio State University, Columbus, OH.*

K-DOQI recommends monitoring random spot P/C ratio to monitor proteinuria because of the numerous studies showing high correlation coefficients (CC) between random spot P/C ratio and 24-hr proteinuria. However, the high CC are mainly the result of comparing these variables over a large range (AJKD 47(1):8-14, 2006). In SLE GN patients, 2 independent studies show that, although spot P/C and 24-hr proteinuria are correlated, concordance is poor. Thus, use of P/C ratio would commonly result in diagnostic errors regarding proteinuric flare (Kidney Int 72:865-870, 2007; Neph Clin Pract 113:c177-c182, 2009). The present work uses the approach of the SLE GN study (ibid. Neph Clin Pract) but applies it to CKD patients. A systematic literature review identified 3 CKD studies with suitable measures of spot P/C ratio and data to estimate imputed 24-hr urine P/C ratio (ibid. Neph Clin Pract). Our analysis shows the calibration plot in relationship to the expected limits of agreement (dashed lines) if random spot P/C ratio was as accurate as the 24-hr urine P/C ratio in estimating 24-hr proteinuria (ibid. Kidney Int).




Each study's results are represented by a different symbol. The calibration plot is for those with sub-nephrotic proteinuria. Similar inaccuracy is seen in those with nephrotic proteinuria. Conclusion: Random spot urine P/C ratio is highly inaccurate for estimating 24-hr proteinuria in individuals with CKD. To monitor proteinuria, we recommend the P/C ratio of intended 24-hr collections that are at least 50% complete (ibid. Kidney Int, Neph Clin Pract). These collections can also be used to estimate salt and protein intake, which is important in CKD management.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1322**

**Correlates of Resistin in Children with CKD** Edward Nehus,[1] Susan L. Furth,[2] Bradley A. Warady,[2] Mark Mitsnefes.[1,2] *[1]Cincinnati Children's Hospital Medical Center; [2]CKiD Study.*

Resistin, a 12.5 kDa protein secreted by mouse adipocytes, has been implicated in the development of insulin resistance in murine models. However, its contribution to insulin resistance in humans is uncertain. The aims of this study were: 1) to determine resistin concentrations in children with CKD and their association with demographic and clinical parameters and 2) to evaluate its potential association with insulin resistance.

We cross-sectionally analyzed data from 222 children and adolescents from the CKiD cohort who had serum resistin levels measured. Cohort characteristics were as follows: median age 13 years, median GFR 43ml/min/1.73m2, 58% male, 48% pre-pubertal. GFR was estimated using iohexol disappearance. Univariate analyses were performed to assess associations between resistin and the following data: iGFR, insulin resistance markers, lipid profiles, urine protein/creatinine ratios, and demographic characteristics of interest. Significant associations were then included in a multivariate analysis.

Statistically significant correlations with resistin levels in univariate analyses included average weight (r = 0.15; p<0.05), iGFR (r = -0.029; p<.0001), triglyceride level (r = 0.13; p<.05), HDL (r = -0.17, p<.05), and log urine protein/creatinine ratio (r = 0.24, p<.001). HOMA-IR was not significantly associated with resistin in univariate analysis. In multivariate analysis, higher iGFR and pubertal stage were independently associated with resistin levels.

Multivariate Analysis of predictors of resistin

| Variable | Coefficient | 95% C.I. | p value |
|---|---|---|---|
| Pre-pubertal | -0.25 | -0.39, -0.11 | <.001 |
| iGFR | -0.01 | -0.02, -0.01 | <.0001 |

Our study showed that levels of resistin become increasingly elevated with GFR decline in children with CKD. Furthermore, it demonstrated that elevated resistin levels were not associated with insulin resistance in the CKiD participants.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1323**

**Predictors of Longitudinal Change in Neurocognitive Functioning in Pediatric CKD** Stephen R. Hooper,[1] Arlene C. Gerson,[8] Robert W. Butler,[9] Debbie S. Gipson,[2] Marc Lande,[3] Susan R. Mendley,[4] Shlomo Shinnar,[10] Matthew Matheson,[8] Christopher Cox,[8] Bruce Z. Morgenstern,[5] Susan L. Furth,[6] Bradley A. Warady.[7] *[1]U. North Carolina; [2]U. Michigan; [3]U. Rochester; [4]U. Maryland; [5]Phoenix Children's Hospital; [6]Children's Hospital of Philadelphia; [7]U. Missouri; [8]Johns Hopkins; [9]Oregon Health Sciences Center; [10]Albert Einstein College of Medicine.*

**Purpose:** To determine how changes in kidney function over time relate to changes in neurocognitive abilities.

**Methods:** The sample comprised 224 children and adolescents, average age of 10.9 yrs, with a median baseline iGFR = 44.4 ml/min/1.73m$^2$. About 24% had CKD from a glomerular diagnosis. Measures of IQ and executive functions were obtained at baseline and at 2 year intervals, with 224 participants having at least two assessments. Baseline

cognitive scores were within the average range. Longitudinal mixed models were used to examine the predictive value of CKD-related variables (i.e., glomerular diagnosis, age at CKD diagnosis, CKD duration, iohexol GFR [iGFR] at baseline, iGFR change, degree of proteinuria, hypertension, anemia) on the neurocognitive outcomes.

**Results:** Increasing proteinuria was associated with a decline in Full Scale IQ ($p<.03$) such that having elevated proteinuria (protein/creatinine ratio >2) was associated with Full Scale IQ being 3 points lower. Lower baseline iGFR was associated with a decline in Performance IQ ($p<.03$), although having a baseline iGFR 10 units lower was associated with only a 0.9 point decrease. A greater decline in iGFR predicted lower ratings of Behavior Regulation over time ($p<.05$), such that a 1-unit decline in iGFR was associated with a 0.2 unit worsening of behavioral regulation.

**Conclusions:** The CKD variables of lower baseline iGFR, declining iGFR, and increased proteinuria were significant predictors of declines in neurocognitive functions over time. Glomerular diagnosis, age of diagnosis, CKD duration, anemia, and change in hypertension did not predict neurocognitive changes. Findings suggest that baseline levels of iGFR or changes in selected CKD variables place children at-risk for at least mild neurocognitive deterioration.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1324

**Higher Level of Proteinuria during RAS Inhibitors Treatment Is a Strong Predictor for Renal Outcome in Non-Diabetic Kidney Disease** Di Xie, Fan Fan Hou, Xun Zhang, Min Liang, Biling Fu. *Nephrology, Nanfang Hospital, Guangzhou, Guangzhou, Guangdong, China.*

This study is to investigate whether proteinuria could not only serve as a marker of renal outcome, but also function as a monitor of the renoprotection of renin-angiotensin-system (RAS) inhibitors treatment, in patients with non-diabetic chronic kidney disease (CKD). The data from the Renoprotection of Optimal Antiproteinuric Doses (ROAD) trial were used to examine the contribution to the antiproteinuric effect of benazepril and losartan on the renal outcome (the primary composite end point of doubling of serum creatinine and end stage renal disease or death) in 339 Chinese non-diabetic CKD patients with overt proteinuria and renal insufficiency. The degree of proteinuria at month 6 of treatment (residual proteinuria) and during follow-up (time average proteinuria, TA-proteinuria) showed a close relationship with renal endpoint. Lowering of proteinuria reduced risk of renal progression in patients with high, as well as low proteinuria at baseline. After adjusting for baseline risk markers, therapy-induced change in these variables at month 6 and during follow-up, high residual proteinuria and TA-proteinuria (≥1.0 g/d) remained the independent predictors for renal endpoint. Therefore, minimization of proteinuria at least to the level <1.0 g/d should be a therapeutic goal in the management of non-diabetic patients with heavy proteinuria and renal insufficiency.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1325

**Elevated Serum Intact Parathyroid Hormone May Predict Increased Cardiovascular Events in Moderate Chronic Kidney Disease** Smrita Dorairajan, Anton Lishmanov, Kunal Chaudhary, Anand Chockalingam, Youngju Pak. *Divisions of Cardiology & Nephrology, Harry S Truman Memorial Veterans Hospital, Columbia, MO.*

**Introduction:** Over 8% of adults in the United States are estimated to have moderate (stage III and IV) chronic kidney disease (CKD) which is increasingly recognized as one of the strongest independent predictors for cardiovascular (CV) disease and related mortality. Secondary hyperparathyroidism with elevated serum intact parathyroid hormone (iPTH) is associated with increased all-cause and CV mortality in end stage renal disease, but limited data in CKD.

**Hypothesis -** iPTH elevation is associated with increased CV events in patients with moderate CKD.

**Methods -** Medical records of 196 CKD stage III and IV (glomerular filtration rate, GFR 16-59ml/min/1.73 $m^2$) patients at Harry S. Truman Memorial Veterans Hospital who had iPTH levels determined from 4/2006 to 9/2007 were reviewed. CV event was defined as occurrence of any of the following during follow-up: Myocardial infarction, stroke, coronary/carotid/peripheral artery revascularization and death due to CV reasons. The Wilcoxon rank sum test was used to compare the distribution of iPTH level between the two CV event groups. Multivariate analysis was performed using the multivariate logistic model.

**Results -** During median follow up of 27.2 months, 48 patients had CV events while 148 did not. Both groups were well matched with no significant clinical and CV risk profile differences at baseline. iPTH was elevated (156.43±107.49) for patients who had CV events compared to those without (109.12 ±86.54, p=0.003). Among the covariates studied in the multivariate analysis including history of vascular disease, 25-OH Vit D, corrected calcium, phosphorus, calcium phosphorus product and GFR, iPTH level was found to have a positive association with CV events (odds ratio=1.58 as 10 unit changes in iPTH, p=0.03). Vascular disease history was the only other significant variable with estimated odds ratio of 5.9 (p=0.002).

**Conclusion-** iPTH level in stage III and IV CKD patients is associated with increase in cardiovascular events. This effect was independent of phosphorus level and calcium phosphorus product.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1326

**Proteins Induced by Vitamin K Absence or Antagonism (PIVKA-II); a Comparison of Biomarkers of Subclinical Vitamin K Deficiency in Dialysis Patients** Meghan J. Elliott,[1] Wilma M. Hopman,[2] Jocelyn S. Garland,[3] Alexander R. Morton,[3] Rachel M. Holden.[3] *[1]Medicine, University of Calgary, Calgary, AB, Canada; [2]Clinical Research Center, Kingston General Hospital, Kingston, ON, Canada; [3]Medicine, Queen's University, Kingston, ON, Canada.*

**Introduction**

Vitamin K dependent proteins (VKDPs) play an important role in the inhibition of vascular calcification. We determined the prevalence of subclinical vitamin K deficiency in our hemodialysis population. In this cross-sectional study, vitamin K status was determined as circulating concentrations of phylloquinone, PIVKA-II, percentage undercarboxylated osteocalcin (%ucOC), apolipoprotein E (apoE) genotype and vitamin K Epoxide Reductase (VKOR) polymorphism. 44 patients with a mean age of 64.2 years (SD 14.5) and median dialysis duration of 34.5 months (range 3-183) were enrolled. Criteria for subclinical vitamin K deficiency were met in 13.6% (phylloquinone <0.4 nM/L), 50% (%ucOC > 20%) and 90.9% (PIVKA-II > 2.0 nM/L) of subjects. In bivariate analysis, phylloquinone was positively associated with total cholesterol (r=0.43, p=0.004), triglyceride levels (r=0.45, p=0.002) and non-smoking status (p=0.043). Higher %ucOC was associated with increased serum calcium-phosphate product (r=0.40, p=0.009). Increased PIVKA-II levels were observed with advancing age (r=0.37, p=0.015), reduced dialysis adequacy (r=-0.33, p=0.027), lower HDL (r=-0.31, p=0.046), a history of coronary artery disease (CAD) (p=0.009). In our regression analysis model, serum triglycerides (TG) were the only significant predictor of phylloquinone (p=0.004). Increasing phosphate (p=0.006) and decreasing PTH (p=0.041) were independent predictors of %ucOC. PIVKA-II levels increase by 0.54 nM/L for every 10-year increase in age (p=0.033). In summary, 50% (%ucOC) and 90% (PIVKA-II) of subjects met criteria for sub-clinical vitamin K deficiency. Phosphate concentrations, CAD, VKOR homozygosity (%ucOC); and age (PIVKA-II) were significant predictors of sub-clinical vitamin K deficiency in our models. Future research should examine the impact of sub-clinical vitamin K deficiency on clinical outcomes such as vascular calcification and bone health.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1327

**Complications of Chronic Kidney Disease (CKD) by Level of Albuminuria and Measured Glomerular Filtration Rate (GFR) in the MDRD Study** Gautham Viswanathan,[1] Hocine Tighiouart,[1] Paul Muntner,[2] Mark J. Sarnak,[1] Lesley A. Stevens.[1] *[1]Tufts Medical Center; [2]University of Alabama at Birmingham.*

Based on the improved prediction of cardiovascular and renal outcomes, it has been proposed to revise the classification of CKD using levels of both albuminuria and GFR. However, it is not known whether albuminuria is associated with concurrent complications of CKD.

**Methods** Cross-sectional analysis of 1713 participants screened for the Modification of Diet in Renal Disease (MDRD) Study evaluated the association of albumin-creatinine ratio (ACR) and GFR with anemia (hemoglobin < 12 g/dL for women, <13.5 g/dL for men), acidosis (bicarbonate < 22 mmol/L) and hyperphosphatemia (phosphate > 4.6 mg/dL) using logistic regression.

**Results** The mean GFR was 39 ml/min/1.73$m^2$ (SD 21) and the median ACR was 152 mg/g (IQR 631). The table shows the associations of ACR categories with anemia, acidosis and hyperphosphatemia. In multivariable models adjusted for age, sex, race and ACR, GFR levels lower than 30 ml/min/1.73$m^2$ were associated with higher odds (95% CI) of anemia 13.76 (9.23-20.50), acidosis 7.31 (4.71-11.33) and hyperphosphatemia 31.98 (11.63-87.95) when compared to GFR levels greater than 60ml/min/1.73$m^2$.

**Conclusions** Albuminuria had a significant relationship with hyperphosphatemia and showed a trend with acidosis in non-diabetic kidney disease. The implications of these findings in the management of CKD should be taken into account when considering including albuminuria level in the classification of CKD.

Table: Adjusted OR for CKD complications associated with level of ACR

| | | ACR, mg/g | | | |
|---|---|---|---|---|---|
| | Adjustment | <29 (n=323) | 30-299 (n=721) | > 300 (n=669) | p-trend |
| Anemia | Age | 1 (ref) | 1.71 (1.28-2.28) | 2.81 (2.10-3.76) | <0.001 |
| | MV | 1 (ref) | 1.16 (0.83-1.62) | 1.09 (0.77-1.54) | 0.81 |
| Acidosis | Age | 1 (ref) | 1.75 (1.26-2.43) | 2.54 (1.83-3.52) | <0.001 |
| | MV | 1 (ref) | 1.26 (0.89-1.80) | 1.41 (0.99-2.02) | 0.06 |
| Hyperphosphatemia | Age | 1 (ref) | 4.79 (2.38-9.66) | 10.38 (5.21-20.69) | <0.001 |
| | MV | 1 (ref) | 2.59 (1.21-5.51) | 3.79 (1.80-7.96) | <0.001 |

MV Multivariable adjusted for age, sex, race and GFR

Disclosure of Financial Relationships: nothing to disclose

**F-PO1328**

**25-Hydroxyvitamin D Deficiency Is Associated with an Increased Risk of Metabolic Syndrome in Patients with Severe Chronic Kidney Disease**
Jessica B. Kendrick,[1] Alfred K. Cheung,[2,4] James S. Kaufman,[3] Tom H. Greene,[4] William L. Roberts,[4] Gerard John Smits,[1] Michel B. Chonchol.[1] *[1]University of Colorado Denver Health Sciences Center, Denver, CO; [2]VASLCHCS, Salt Lake City, UT; [3]VA Boston Healthcare System, Boston, MA; [4]University of Utah, Salt Lake City, UT.*

Purpose: 25-hydroxyvitamin D (25(OH)D) deficiency has been associated with insulin resistance and the metabolic syndrome (MetS) in the general population. Patients with CKD have a high prevalence of 25(OH)D deficiency but the relationship between 25(OH) D levels and MetS is unknown.

Methods: This study was conducted among 1,099 subjects with severe kidney disease, not yet on dialysis, who participated in the Homocysteine in Kidney and End Stage Renal Disease study. 25(OH)D levels were measured in stored plasma samples. Participants met criteria for MetS if all three of the following were present: (1) Triglycerides ≥150 mg/dl or drug treatment; (2) high density lipoprotein-cholesterol <50 mg/dl for women or <40 mg/dl for men or drug treatment; and (3) blood pressure ≥130/85 mmHg or drug treatment. Multivariate regression models were used to evaluate the cross-sectional association between 25(OH)D levels and MetS.

Results: The mean (SD) age, eGFR and 25(OH)D was 69±11 years, 18.1± 6.5 mL/min/1.73m2 and 20 ±11 ng/mL. After adjustment for baseline demographics, body mass index, albumin, calcium, phosphorus, eGFR, intact parathyroid hormone (iPTH), calcitriol, and fibroblast growth factor-23 (FGF-23) there was a strong inverse association of 25(OH) D level with MetS. For each log10 increase in 25(OH)D levels, there was a 60% reduction in the risk of MetS (adjusted OR 0.40, 95% CI 0.20 to 0.81, p=0.01). Similarly, when evaluated as a categorical predictor, 25(OH)D < 15 ng/mL vs. higher (adjusted OR 1.63 95% CI 1.16 – 2.28, p= 0.005) and < 20 ng/mL vs. higher (adjusted OR 1.73 95% CI 1.27-2.36, p=0.0005) was also strongly associated with the MetS. Calcitriol, iPTH and FGF-23 levels were not related to the MetS.

Conclusion: 25(OH)D deficiency is strongly associated with an increased risk of MetS in patients with severe CKD. Further studies are needed to confirm our results.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1329**

**Complications of Chronic Kidney Disease (CKD) by Level of Albuminuria and Estimated Glomerular Filtration Rate (eGFR) in the IDNT Trial**
Gautham Viswanathan,[1] Hocine Tighiouart,[1] Paul Muntner,[2] Julia Lewis,[3] Mark J. Sarnak,[1] Lesley A. Stevens.[1] *[1]Tufts Medical Center; [2]University of Alabama at Birmingham; [3]Vanderbilt University Medical Center.*

Based on the improved prediction of cardiovascular and renal outcomes, it has been proposed to revise the classification of CKD using levels of both albuminuria and GFR. However, it is not known whether albuminuria is associated with concurrent complications of CKD.

**Methods** Cross-sectional analysis of 1683 participants screened for the Irbesartan in diabetic Nephropathy (IDNT) trial evaluated the association of albumin-creatinine ratio (ACR) and eGFR with anemia (hemoglobin < 12 g/dL for women, <13.5 g/dL for men) and hyperphosphatemia (phosphate > 4.6 mg/dL) using logistic regression. GFR was estimated using the CKD-EPI equation.

**Results** The mean eGFR was 47 ml/min/1.73m$^2$ (SD 18) and the median ACR was 1467 mg/g (IQR 1932). The table shows the associations of ACR categories with anemia and hyperphosphatemia. In multivariable models adjusted for age, sex, race and ACR, eGFR levels lower than 30 ml/min/1.73m$^2$ were associated with higher odds (95% CI) of anemia 5.59 (3.99-7.83) and hyperphosphatemia 2.33 (1.43-3.8) when compared to eGFR levels greater than 60 ml/min/1.73m$^2$.

**Conclusions** Albuminuria had a significant relationship with anemia and hyperphosphatemia in diabetic nephropathy. The implications of these findings in the management of CKD should be taken into account when considering including albuminuria level in the classification of CKD.

Table: Adjusted OR for CKD complications associated with level of ACR

| | | ACR, mg/g | | | | |
|---|---|---|---|---|---|---|
| | Adjustment | <299 (n=58) | 300-999 (n=515) | 1000-2999 (n=753) | > 3000 (n=357) | p-trend |
| Anemia | Age | 0.77 (0.43-1.38) | 1 (ref) | 1.75 (1.39-2.20) | 2.74 (2.07-3.62) | <0.001 |
| | MV | 0.81 (0.44-1.48) | 1 (ref) | 1.58 (1.24-2.01) | 2.14 (1.58-2.89) | <0.001 |
| Hyperphos-phatemia | Age | 0.64 (0.15-2.74) | 1 (ref) | 2.17 (1.39-3.39) | 4.66 (2.95-7.36) | <0.001 |
| | MV | 0.88 (0.20-3.83) | 1 (ref) | 1.78 (1.13-2.81) | 3.27 (2.03-5.28) | <0.001 |

MV Multivariable adjusted for age, sex, race and eGFR

Disclosure of Financial Relationships: nothing to disclose

**F-PO1330**

**Haptoglobin Phenotype Is Associated with Elevated Levels of High Sensitivity CRP and Interleukin-6 in Patients with Stage 3-5 Chronic Kidney Disease**
Charlotte Strandhave,[1] My Svensson,[2] Jeppe Hagstrup Christensen.[1] *[1]Nephrology, Aalborg Hospital, Aalborg, Denmark; [2]Nephrology, Skejby Hospital, Aarhus, Denmark.*

*Introduction:* Three major phenotypes for the haptoglobin (Hp) gene have been identified: Hp 1-1, Hp 2-2 and the heterozygous Hp 2-1. Due to lower phenotype-dependent antioxidant capacity, Hp 2-2 acts as a weaker innate antioxidant and is associated with a poor outcome in several clinical conditions such as ischemic heart disease and diabetic nephropathy. High sensitivity CRP (hsCRP) and Interleukin-6 (IL-6) are markers of low-grade inflammation and have been implicated as independent predictors of cardiovascular events and mortality among chronic kidney disease (CKD) patients.

*Aim:* To examine whether Hp phenotyping in patients with CKD could identify patients with a pro-inflammatory profile with high levels of hsCRP and IL-6.

*Methods:* We included patients (n = 64) with stage 3-5 CKD and without ongoing infection from our outpatient clinic. The Hp phenotype was determined using a high-performance liquid chromatography. HsCRP was measured using an ADVIA 1650 analyzer and an immunoturbidimetric assay while Il-6 was measured using Luminex multiplex liquid array.

*Results:* The CKD patients were divided in two groups according to haptoglobin phenotypes: Hp 1-1 and 2-1 (n=45) and Hp 2-2 (n=19). They were comparable regarding clinical relevant parameters although a trend was observed towards lower age, lower plasma PTH, and fewer women in the Hp 2-2 group. The median hsCRP was 5.9 mg/L CI 95% (3.4;10.2) among Hp 2-2 patients compared to 2.6 mg/L CI 95% (1.9;3.5) in Hp1-1 and Hp 1-2 patients (p<0.01). The median IL-6 was 5.8 pg/mL CI 95% (4.2;8.1) in the Hp 2-2 patients compared to 4.2 pg/mL CI 95% (3.5;5.2) in the group with Hp1-1 and 2-1, showing a non-significant trend (p=0.08).

*Conclusion:* Haptoglobin phenotyping in patients with CKD revealed that Hp 2-2 patients had significantly higher levels of hsCRP compared to Hp 1-1 and Hp 2-1 patients. Also, a trend towards higher levels of IL-6 was seen. Thus, this phenotype may identify a group of CKD patients with a pro-inflammatory profile potentially predicting a higher risk of cardiovascular disease and mortality.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1331**

**Serum 25-Hydroxyvitamin D and Change in Estimated Glomerular Filtration Rate**
Ronit Katz,[1] Ian H. de Boer,[1] Michel B. Chonchol,[5] Joachim H. Ix,[2] Mark J. Sarnak,[4] Michael Shlipak,[3] David Siscovick,[1] Bryan R. Kestenbaum.[1] *[1]University of Washington; [2]University of California, San Diego; [3]University of California, San Francisco; [4]Tufts Medical Center; [5]University of Colorado Denver.*

**Background:** The prevalence of chronic kidney disease (CKD) is growing most rapidly among older adults, but the determinants of impaired kidney function in this population are not well understood. Vitamin D deficiency may be a novel, modifiable risk factor for the progression of CKD.

**Methods:** We examined associations of 25-hydroxyvitamin D concentration (25OHD) with change in kidney function over four years among 1,705 participants in the community based Cardiovascular Health Study (aged≥65) who were free of cardiovascular disease at baseline. 25OHD was measured by high performance liquid chromatography-tandem mass spectrometry. Change in estimated glomerular filtration rate (eGFR) was calculated using longitudinal measurements of serum cystatin C, measured at baseline and four years later. Rapid loss of GFR was defined as 12mL/min/1.73m$^2$ or more over 4 years. In addition we also looked at a combined endpoint which further included incident ESRD or death prior to follow-up cystatin C measures.

**Results:** The mean age of the cohort was 74 years, 70% were female, 14% had a baseline eGFR<60mL/min/1.73m$^2$ and the mean 25OHD concentration was 26ng/ml. Each 10ng/mL lower 25OHD was associated with a 19% greater risk of rapid GFR loss (p=0.01) in a model adjusted for age, gender, race, site, season, education, income, diabetes, smoking, physical activity, antihypertensive medications, body mass index, blood pressure, and C-reactive protein. Compared with 25OHD≥30 ng/mL, 25OHD<15 ng/mL was associated with a 52% greater risk of rapid GFR loss. Each 10ng/mL lower 25OHD was associated with a 19% greater risk of a combined endpoint of rapid GFR loss, incident ESRD, or death (p=0.002), and 25OHD<15ng/mL was associated with a 76% greater risk of the combined endpoint compared with 25OHD≥30 ng/mL.

**Conclusions:** Lower 25OHD concentrations were associated with increased risk of eGFR loss among community-dwelling older adults.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1332**

**Kidney in Chronic Lymphocytic Leukemia and Related B Cell Lymphoma**
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Introduction: Glomerulonephritis (GN) in chronic lymphocytic leukemia (CLL) and related B cell lymphomas may include membranous GN, membranoproliferative GN, immnunotactoid GN, fibrillary GN, type 1 cryoglobunemia GN, AL amyloidosis.

Method: A retrospective analysis of patients with CLL presenting with proteinuria, haematuria and renal impairment was undertaken. 4 electronic case notes were reviewed. The clinical, demographic, immunological and laboratory data at the time of renal biopsy

were recorded. Creatinine and proteinuria was recorded at the last follow up. All analyses were performed using excel. Results: Presentation of these patients included acute kidney injury (AKI), nephrotic syndrome and heamaturia. Mean proteinuria was [mean+ SD] 2gm+ 2.4 at presentation with mean follow up proteinuria 2.6gms + 2.4. The median age was 70 (range 68 to 72).

| Age | Proteinuria (presentation) | Creatinine (presentation) (mg/dl) | Creatinine (follow up) | Immunology | Light Microscopy | Electron Microscopy |
|---|---|---|---|---|---|---|
| 68 | 5.74gm/24hr | 5.2 | 2.9 | Monoclonal IgG kappa | MCGN type I | Subendothelial deposits |
| 73 | 0.4gm/24hr | 1.5 | 1.4 | Monoclonal of IgG kappa | MCGN type I, infiltrates of monotonous lymphoid cells consistent with infiltrance by CLL | Immunotactoid |
| 70 | 2.2gm/24hr | 1.5 | 1.1 | Low C4, no cryo | Atypical MCGN | Fibillary |
| 72 | 0.54gm/24hr | 3.8 | 1.9 | Raised serum and urine free lambda light chain | lymphomatous infiltration | n/a |

Clinical, Laboratory, Immunological and Renal biopsy features

All patients received some form of chemotherapy. The patient who presented with fibillary GN was diagnosed with CLL 2 years later.

Conclusion: Renal manifestations of CLL appear to be a paraneoplastic phenomenon and may be an under diagnosed entity. It should be considered in any patient with CLL who presents with AKI and proteinuria or those with no obvious cause for slowly deteriorating renal function. Renal histology is an important tool to confirm diagnosis. Treatment is indicated and may lead to remission of renal impairment and is associated with a good outcome. Our data suggest presentations are heterogeneous and kidney involvement may precede the diagnosis of CLL.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1333

**Recurrent Proliferative Glomerulonephritis with Monoclonal IgG Deposits** Samih H. Nasr,[1] Sanjeev Sethi,[1] Lynn D. Cornell,[1] Mary E. Fidler,[1] Mark R. Boelkins,[2] Fernando C. Fervenza,[1] Fernando G. Cosio,[1] Vivette D. D'Agati.[3] *[1]Pathology and Nephrology, Mayo Clinic, Rochester, MN; [2]Pathology, Columbia University, New York, NY; [3]St. Mary's Health Care, Grand Rapids, MI.*

**Introduction:** We have recently described a proliferative glomerulonephritis associated with monoclonal IgG deposition (PGNMID) (JASN 2009, 20, 2055-64).

**Methods:** We reviewed the clinical and kidney biopsy (Bx) data in a cohort of 4 white pts (3F; 1M), mean age at Tx of 58.5 yrs, with PGNMID who underwent kidney transplant (KTX) and developed recurrent disease.

**Results:** Recurrent disease was documented histologically in 14 KTX Bxs in these 4 pts. At the time of first Bx showing recurrence (3-5 mo. post TX), all pts had worsening S. creatinine (SCr; mean 3.1 mg/dl); 3 had proteinuria (mean 4.7 g/d), 2 had nephrotic syndrome, and 3 had hematuria. Serum and urine immunofixation were negative for a monoclonal protein. In each pt, the glomerular deposits in the KTX Bxs were monoclonal (IgG3 κ in 3 pts, IgG3 λ in 1 pt) and had the same heavy and light chain isotypes as the deposits in the native kidney. Ultrastructurally, all native and KTX Bxs had granular glomerular electron dense deposits. In 2 pts, light microscopy (LM) showed endocapillary proliferative glomerulonephritis (EPGN) in both the native and KTX Bxs. In the other 2 pts, native Bxs showed an MPGN pattern while the KTX Bxs showed EPGN in 1 and mesangial proliferative GN in 1. Recurrent disease was treated with high dose prednisone and rituximab in 3 pts and with high dose prednisone and cyclophosphamide in 1 pt. After a mean follow-up of 43 mo. (range 11-83 mo.), proteinuria decreased to a mean of 0.3 g/day in all pts and SCr decreased to a mean of 1.6 mg/dl in 3 pts. Repeat Bxs after therapy showed less disease activity.

**Conclusions:** PGNMID recurs following KTX, and is usually manifested by proteinuria, hematuria, and graft dysfunction. The monoclonal protein deposited in glomeruli of the allograft is identical to that in the native kidney, although the glomerular pattern on LM is not always the same. The recurrent disease appears to respond to immunosuppressive therapy with clinical and histologic improvement.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1334

**Laser Microdissection and Mass Spectrometry in the Diagnosis of Renal Heavy Chain and Light and Heavy Chain Amyloidosis** Sanjeev Sethi,[1] Ahmet Dogan,[1] Lynn D. Cornell,[1] Mary E. Fidler,[1] Nelson Leung,[2] Samih H. Nasr.[1] *[1]Pathology, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic, Rochester, MN.*

The vast majority of cases of primary amyloidosis involving the kidney are composed of immunoglobulin (Ig) light chains only (AL-amyloid). Very rarely, the amyloid deposits are composed of Ig heavy chains alone (AH-amyloid) or Ig light and heavy chains (AL+AH amyloid). The diagnosis of AH amyloid or AL+AH amyloid can be very challenging, particularly in cases in which no tissue is available for immunofluorescence (IF) or when the IF findings and Congo red stain are inconclusive. Herein, we report 4 cases of renal AH amyloid or AL+AH amyloid that were diagnosed by laser microdissection (LMD) and mass spectrometry (MS) based proteomic analysis. All patients were adult males with a mean age of 52 years (range 36-64 years). 3 patients had plasma cell dyscrasia and 1 had MGUS. All patients presented with proteinuria (range 2.5-12.7 g/day) and 2 had renal insufficiency (creatinine range 1.0-1.9 mg/dl). Hematuria was present in all patients and hypoalbuminemia was present in 3 patients. On biopsy, the Congo-red positive amyloid deposits involved glomeruli and vessels in all cases, and the interstitium in 2 cases. Electron microscopy, performed on 3 cases, showed randomly-oriented fibrillar deposits. On IF, performed on 3 cases, the deposits stained only for IgG and lambda in 1, only for IgA and kappa in 1, and equally for IgA, IgG, IgM, kappa, and lambda in 1. By LMD/MS proteomic analysis of the glomerular amyloid deposits, 1 case showed Ig gamma 1 chain constant region and lambda light chains variable and constant regions; 1 case showed Ig alpha chain constant region and kappa light chains variable and constant regions; and 2 cases showed Ig gamma 3 chain constant region and heavy chains variable region I and/or III without light chains. We conclude that LMD/MS is a valuable diagnostic technique for the diagnosis of AH and AL+AH amyloid and is superior to immunofluorescence in some cases.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1335

**The Anion Gap in IgG, IgA, and Light Chain Monoclonal Gammopathies with Focus on the Impact of Serum Free Light Chains** Karen van Hoeven,[1] Laura McBride,[2] Danielle Schillen,[2] Ann McNeill,[2] Linda Schmidt,[2] Elizabeth Bilotti,[2] David Siegel.[2] *[1]The Binding Site Inc, San Diego, CA; [2]Hackensack University Medical Center, Hackensack, NJ.*

**Purpose:** IgG monoclonal gammopathies (MG) are associated with a decreased anion gap (AG), and IgA MG are associated with an increased AG. We explored the AG in IgG, IgA, and light chain MG with a focus on the impact of serum free light chains (sFLC).

**Methods:** $Na^+$- ($Cl^-$ + $HCO3^-$) was calculated in patients (pts) with MG (mostly myeloma) at presentation or at relapse, and in pts with no evidence of MG by serum protein electrophoresis (sPEP), serum immunofixation and sFLC. After raw data analysis, pts were excluded if serum albumin was <2.5 g/dL, serum $Ca^{++}$ was >11.0 mg/dL, or serum creatinine was >2 mg/dL.

**Results:** The AG in 40 pts without MG ranged from 9 to 15 (in 37 of 40 pts, the AG range was 9 to 14). The AG was decreased (<9) in 35% of 102 IgG and increased (>14) in 36% of 50 IgA MG. In 60 LC MG, the AG was increased in 25% and decreased in 3% of pts. Applying exclusion criteria, the AG was decreased in 34% of 89 IgG and increased in 31% of 42 IgA MG. In 40 LC MG, 17% had increased and 3% had decreased AG. Correlation coefficients were poor (strongest *r* was 0.58) between the AG and the paraprotein spike on sPEP, and also poor between the AG and the mean concentration of the involved Ig (IgG 4.64 g/dL±0.18 (SEM), IgA 3.27 g/dL±0.26, free kappa 0.5 g/dL±0.08 [or 4956 mg/L±769; n=20], free lambda 0.36 g/dL±0.08 [or 3607 mg/L±809; n=20]). Only 23 of 40 (58%) LC MG had measurable paraprotein spikes on sPEP, but of 20 kappa LC MG, free kappa ranged from 478 to 14,400 mg/L (normal 3.3 to 19.4 mg/L), and of 20 lambda LC MG, free lambda ranged from 440 to 17,300 mg/L (normal 5.7 to 26.3 mg/L). Mean clonal sFLC in IgG MG with low AG was 620 mg/L±198 vs 967 mg/L±508 in other IgG MG (*P*=NS). Mean clonal sFLC in IgA MG with high AG was 821 mg/L±420 vs 431 mg/L±177 in other IgA MG (*P*=NS).

**Conclusions:** The AG has been used as a tool for detecting IgG and IgA MG, but it has lower sensitivity in LC MG. sFLC had no significant effect on the AG in IgG and IgA MG, likely due in part to relatively low serum concentrations of clonal FLC versus IgG or IgA.

Disclosure of Financial Relationships: Employer: The Binding Site, Inc.

### F-PO1336

**Evaluation of Microscopic Hematuria Using the Urinary Albumin to Total Protein Ratio** Yu Min Lee, Jeong-Min Cha, Seon-Ho Ahn, Ju-Hung Song. *Division of Nephrology, Department of Internal Medicine, Wonkwang University School of Medicine, Iksan City, Jeonbuk, Republic of Korea.*

Background. Hematuria is a clinically significant finding associated with a number of conditions involving the genitourinary tract, including the kidney, ureter, prostate gland, bladder, and urethra. Depending on the source of bleeding, hematuria can be classified as either glomerular or nonglomerular bleeding. The presence of significant number of dysmorphic RBCs, RBC casts and protein excretion > 500mg/d suggest a renal source of the hematuria. In this study, we report on the use of the urinary albumin-to-total protein ratio to accurately differentiate glomerular and nonglomerular bleeding. Methods. This study included 39 microscopic hematuria patients who confirmed by histopathology (30 IgA nephropathy; group 1, 9 non-specific finding; group 2). The following indices were compared with respect to group 1 and group 2: (1) urinary total protein-to-creatinine ratios(UPCR); (2) urinary albumin-to-creatinine ratios(UACR); (3) urinary albumin-to-total protein ratios(UAPR). Received operating characteristic (ROC) curve of UPCR and UAPR obtained from sensitivity and specificity. Results. The mean UPCR, UACR and UAPR for group 1 and group 2 were 1.34±1.76 vs 0.14±0.14 (p=0.05), 1.1±1.58 vs 0.07±0.1 (p=0.05) and 0.71±0.19 vs 0.29±0.23 (p<0.01), respectively. At a cut off 0.12, the UPCR demonstrated a sensitivity 96.7% in detecting IgA nephropathy. And at a cut off 0.38, the UAPR demonstrated a sensitivity 100% in detecting IgA nephropathy. Conclusions. First, our results suggest that the UAPR is potentially a useful index for the differentiation of

glomerular and nonglomerular disease in the presence of microscopic hematuria. Second, the UPCR detected IgA nephropathy at a cut off 0.12. It means that microscopic hematuria with minimal proteinuria (not exceed 500 mg/d) suggest a glomerular origin of the hematuria and should be confirmed by renal biopsy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1337**

**The Optimal Cutoff Value of Urine Albumin Concentration for Albuminuria in Japanese Population: The Takahata Study** Tsuneo Konta, Kazuko Suzuki, Ami Ikeda, Yusuke Mashima, Kazunobu Ichikawa, Satoshi Takasaki, Isao Kubota. *Department of Cardiology, Pulmonology, and Nephrology, Yamagata University School of Medicine, Yamagata, Japan.*

**Background:** To screen albuminuria in spot urine, urine albumin concentration corrected by urine creatinine concentration (urine albumin-creatinine ratio, UACR) is measured. However, the value of UACR is greatly affected by various factors that modulate urine creatinine excretion. Therefore, the optimal cutoff value in urine albumin concentration (UAC) for albuminuria (UACR ≥30 mg/gCr) might be different depending on the characters of examined subjects. To clarify this point we performed the cross-sectional analysis in the general Japanese population.

**Methods:** The participants of this community-based study were 3,114 subjects (average 63 years old, male 44.3%, hypertension 55.1% and diabetes 7.7%). Single spot urine was collected in the morning and the sensitivity/specificity and receiver operation curve (ROC) analyses were performed.

**Results:** In total subjects the sensitivity for albuminuria in the cutoff value in UAC of 5, 10, 15, 20, 25 and 30 mg/L were 100%, 98.2%, 93.2%, 84.7%, 75.7% and 68.3%, respectively. The specificity in these UAC cutoff values were 19.5%, 65.6%, 84.5%, 91.8%, 95.8% and 97.9%, respectively. The median values in urine creatinine concentration (g/L) were significantly different between subgroups (total subjects 1.05, age <65 1.13, age ≥65 0.98, male 1.23, female 0.94, hypertensive 1.02, non-hypertensive 1.09, diabetic 1.11 and non-diabetic 1.04, respectively). The ROC analysis showed the optimal UAC (mg/L) for albuminuria were 16.0 (total subjects), 15.3 (age <65), 20.8 (age ≥65), 16.6 (male), 16.0 (female), 16.0 (hypertensive), 14.4 (non-hypertensive), 22.3 (diabetic) and 15.9 (non-diabetic), respectively.

**Conclusions:** This study revealed that the optimal UAC cutoff level for albuminuria in Japanese population might be lower than the current definition (<20 mg/L). To screen albuminuria using UAC, the characters of subjects (age, ethnicity and comorbidities) should be considered.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1338**

**Diagnosis of Acute Allograft Rejection in Kidney Transplanted Patients by Urinary Proteome Analysis** Christos D. Chatzikyrkou,[1] Jochen Metzger,[2] Verena Broecker,[3] Irina Scheffner,[1] Eric Schiffer,[2] Michael Mengel,[4] Hermann G. Haller,[1] Harald Mischak,[2] Wilfried Gwinner.[1] *[1]Nephrology, Medical School Hannover, Hannover, Germany; [2]Mosaiques Diagnostics, Hannover, Germany; [3]Pathology, Medical School Hannover, Hannover, Germany; [4]Division of Nephrology/Immunology, University of Alberta, Edmonton, Canada.*

Diagnosis of acute renal allograft rejection by non-invasive methods may be advantageous compared to biopsy. In this study, we examine capillary electrophoresis mass spectrometry (CE-MS) of urines for the rejection diagnosis.

In a training set including 16 samples with acute T-cell mediated tubulointerstitial rejection and 23 samples without rejection the low-molecular weight proteome was analyzed. A multi-marker classification model for rejection was defined by multivariate statistical comparison of the CE-MS spectra. This model was extensively evaluated on an independent validation set (n=68) and a blinded set (n=17) of samples.

Application of the rejection model to the validation set resulted in correct classification of 13/14 subclinical and 11/11 clinical rejections (BANFF grades Ia/Ib), and of 31/43 samples without rejection. Comparison of the sub-scores of the BANFF classification with the numerical classifier of the marker set revealed a tight correlation to the i-score and t-score (r=0.62 and 0.63; p<0.0001). Acute tubular injury in the biopsies with and without rejection did not interfere with the CE-MS-based diagnosis. Classification of the blinded test set resulted in an AUC-value of 0.83 (95% CI: 0.57-0.96, p=0.0028) in the corresponding ROC analysis. Sequence information of identified altered collagen alpha(I) and alpha(III) chain fragments in the samples with rejection suggested an involvement of MMP-8. Staining of the biopsies revealed MMP-8 exclusively in polymorphnuclear cells located within peritubular capillaries and sparsely, in the tubulointerstitium of the biopsies with rejection.

CE-MS offers a promising non-invasive method for the diagnosis of acute rejection in the post-transplant surveillance. The established marker set apparently contains peptides which are specifically related to the tubulointerstitial infiltration seen in acute rejection.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1339**

**Impact of Nephrotic Leg Edema on OSA: Gathering a Unifying Concept for the Pathogenetic Role of Nocturnal Rostral Fluid Shift** Sydney C. W. Tang, Bing Lam, Jamie Chung Lam, Desmond Y. H. Yap, Mary S. M. Ip, Kar Neng Lai. *Department of Medicine, The University of Hong Kong, Hong Kong, Hong Kong.*

***Rationale:*** Nocturnal rostral fluid shift has been suggested to be a risk factor for obstructive sleep apnea (OSA) in healthy subjects after lower body positive pressurization and in heart failure subjects. Here, we tested whether this may apply to nephrotic lower limb edema and whether remission of the nephrotic syndrome may reverse OSA.

***Methods:*** In subjects with primary steroid-responsive nephrotic syndrome presenting with bilateral lower limb edema, we examined the prevalence and severity of sleep apnea and related parameters before and after the induction of disease remission.

***Measurements and Main Results:*** Among 16 nephrotic subjects, 7 (43.7%) had sleep apnea (apnea-hypopnea index [AHI]≥5) upon presentation. Overall AHI was 14.8±5.0 and was predominantly obstructive in nature. After steroid-based treatment, there was remission of proteinuria associated with complete disappearance of lower limb edema, significant reduction of body mass index, waist, hip and calf circumferences, and total body water mainly in the extracellular compartment. Polysomnographic analyses, performed 8.3±2.8 months after initial presentation, showed improvement in sleep efficiency (P=0.036), mean nocturnal oxygen saturation (P=0.005), shorter duration with oxygen saturation <95% (P=0.044) and <90% (P=0.02), and reduced desaturation index (P=0.011). AHI reduction was close to statistical significance, particularly when confined to subjects with baseline AHI≥ 5 (P=0.075). There was subjective improvement in self-reported daytime sleepiness (P=0.03).

***Conclusions:*** Nephrotic lower limb edema is a risk factor for nocturnal hypoxemia and possibly also for OSA. This gathers a unifying concept for the pathogenetic role of nocturnal rostral fluid shift in OSA.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1340**

**Hydration Status Influences Kidney Volume in Healthy Adults** Anja M. Jensen,[2] Kristian Karstoft,[2] Bettina Jorgensen,[2] Bente Jespersen,[1] Michael Pedersen.[2] *[1]Nephrology, Aarhus University Hospital, Skejby, Denmark; [2]Institute of Clinical Medicine, Aarhus University Hospital, Skejby, Denmark.*

Kidney volume is positively influenced by BMI, height and male gender whereas older age is associated with reduced kidney volume. Previous studies suggested that the volume of a healthy kidney correlates with the GFR, since a low amount of nephrons (corresponding to a low GFR) occupies a lower volume than a relative large nephron mass. Second, several imaging-based methods allowing estimates of renal function are based on the assumption that GFR is calculated as the average filtration per $cm^3$ in the renal parenchyma multiplied with the cortical volume (CV). Therefore, accurate and reproducible kidney volume measurement is important.

This study aimed to investigate the influence of water intake prior to kidney volume measurements. A total of 8 women and 9 men (all healthy) underwent 2 separate MRI sessions. Before the first MRI, they had no access to water or food for 3 h, whereas the second MRI was commenced after a water intake of 15 ml/kg body weight over a period of 3 h. MRI was performed using a standard clinical system, and high-resolution multislice images were acquired to delineate fine structures of the renal anatomy. Using home-made software, the cortical area was semi-automatically calculated in each slice, and left and right CVs were derived as the sum-of-slice principle.

Male gender was associated with a significantly larger kidney CV adjusted to BMI than female gender. Interestingly, kidney CV was significantly increased in both females (RK: 4.3±0.3 vs. 4.0±0.3 $cm^3$/BMI, $p<0.01$; LK: 4.2±0.3 vs. 3.7±0.3 $cm^3$/BMI, $p<0.01$) and males (RK: 5.2±0.2 vs. 5.0±0.2 $cm^3$/BMI, $p<0.01$; LK: 5.3±0.2 vs. 5.1±0.2 $cm^3$/BMI, $p<0.01$) in response to standardized water intake compared to kidney CV following 3 h dehydration period. Moreover, the hydration-induced increase in kidney CV in percentage was significantly larger in females than males (RK: 8.3±2.1% vs. 3.3±0.7%, $p<0.05$; LK: 12.3±2.7% vs. 3.7±0.9%, $p<0.01$).

In conclusion, standardized protocols for kidney volume measurements including water intake are essential. Interestingly, the response to hydration status was most evident in females.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1341**

**Canakinumab (Fully Human Anti-IL-1β Monoclonal Antibody) vs Triamcinolone Acetonide (TA) for Treatment of Acute Flares in Difficult-to-Treat Gouty Arthritis Patients with Renal Impairment** Naomi Schlesinger,[1] M. De Meulemeester,[2] A. Pikhlak,[3] A. E. Yücel,[4] U. Arulmani,[5] D. Richard,[5] V. Murphy,[5] P. Sallstig,[5] A. So.[6] *[1]UMDNJ-RWJMS, NJ; [2]Gozée, Belgium; [3]MSUMD, Moscow, Russian Federation; [4]Baskent Univ., Ankara, Turkey; [5]Novartis, Basel, Switzerland; [6]CHU Vaudois, Lausanne, Switzerland.*

**Purpose**: Many gouty arthritis patients have renal impairment limiting use of standard treatment like NSAIDs or colchicine. Herein, we report results of an 8-week, multicenter, blinded study of canakinumab vs TA for treatment of acute flares in subgroup of renal impaired gouty arthritis patients.

**Methods**: Patients (≥18–≤80 y) with acute gouty arthritis flare, refractory or contraindicated to NSAIDs and/or colchicine were randomized to 1 subcutaneous dose of

canakinumab (10, 25, 50, 90 or 150 mg) or 1 intramuscular dose of TA 40 mg. Efficacy variables were pain intensity at 72 h post-dose on VAS (0–100 mm), time to 50% pain reduction, recurrence of flares and physician's global assessment.

**Results**: 132/200 patients had renal impairment (canakinumab n=95; TA n=37) (eGFR <90 ml/min/1.73 m$^2$) (mild [eGFR 60–89 mL/min/1.73 m$^2$] n=107; moderate [eGFR 30–59 mL/min/1.73 m$^2$] n=25) at baseline. Canakinumab 150 mg showed superior pain relief compared to TA at 72 h: estimated mean difference in pain intensity was –17.3 ($p<0.05$). Median time to 50% pain reduction was reached at 0.5 day with canakinumab 150 mg vs 2 days for TA ($p=0.003$). At Week 8, recurrent flares occurred in none of the patients on canakinumab 150 mg vs 17 (47.1%) patients on TA. At Week 8, patients on canakinumab 150 mg were 3.2 (95% CI 1.03–9.77) times more likely to have a better physician's global assessment than TA ($p=0.045$). Most frequent AEs were infections; incidence was lower in canakinumab (10.5%) vs TA (16.2%). SAEs (canakinumab n=3, TA n=1) were not considered treatment related by investigators. No discontinuations occurred due to AEs.

**Conclusions**: Canakinumab 150 mg provided rapid and superior pain relief vs TA 40 mg in renal impaired acute gouty arthritis patients. Canakinumab prevented recurrent flares and was well-tolerated. Pain reduction and safety profile was comparable to total population.

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### F-PO1342

**Platelet Activation during Renal Artery Stenting** Steven T. Haller,[1] Satjit Adlakha,[1] Pamela Brewster,[1] David Kennedy,[2] Haifeng Yu,[1] Dong Zhang,[1] Joseph I. Shapiro,[1] Christopher J. Cooper.[1] *[1]Medicine, University of Toledo, Toledo, OH; [2]Cell Biology, Cleveland Clinic, Cleveland, OH.*

Soluble CD40 ligand (sCD40L) is a prominent marker of platelet activation. Platelet activation leading to thrombus formation is common in coronary revascularization. Whether it occurs in the setting of renal artery stenting is unknown. Additionally, the effect of embolic protection devices (EPD) and glycoprotein inhibitors (GP2b3a) on platelet activation during renal artery stenting is unknown. One hundred patients enrolled at 7 centers undergoing renal artery stenting were randomized to an EPD, Angioguard®, or use of abciximab, in a 2x2 design. Platelet activation was assessed by measuring plasma levels of sCD40L. Blood samples were collected at baseline, immediately post, and 24hr post-procedure. Statistical analysis was performed on patients with data available for all time points (n=84). Additional samples were collected from 30 healthy controls, and 30 patients with atherosclerosis, but without renal artery stenosis (RAS). A natural log transformation was performed to normalize the data. Soluble CD40L levels were higher in RAS patients than normal controls ($5.6 \pm 0.7$ vs $4.2 \pm 0.1$ pg/ml, $P<0.01$), but were similar to patients with atherosclerosis but without RAS. In patients that received an EPD, sCD40L levels rose slightly (p=NS) immediately after stenting, and returned to baseline levels at 24hrs. Platelet rich emboli were captured in 26% (9/35) of EPD patients and in these patients sCD40L was elevated prior to the procedure and rose further afterwards. Soluble CD40L declined with abciximab immediately post-procedure ($5.6 \pm 0.6$ vs $4.9 \pm 0.8$ pg/ml, $p<0.001$), which persisted at 24hrs. Patients randomized to both an EPD and abciximab showed a significant decrease in sCD40L immediately after the procedure. Patients with RAS have elevated levels of sCD40L at presentation but this appears to be related to atherosclerosis, not RAS specifically. In patients undergoing stenting embolization of platelet thrombi is common, is associated with systemic evidence of platelet activation that precedes the procedure, and is inhibited with abciximab administration.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1343

**Mycophenolate as Induction Treatment of Lupus Nephritis. Is Its Efficacy Dependent of Initial Renal Function?** Francisco Rivera.[1] *[1]Nephrology, Hospital General de Ciudad Real, Ciudad Real, Spain; [2]Nephrology, Hospital Vall d'Hebron, Barcelona, Spain; [3]Spanish Group for the Study of Glomerulonephritis (GLOSEN).*

Introduction. There is not enough information about efficacy of mycophenolate (M) as induction treatment of lupus nephritis (LN) in patients with initial impairment of renal function.

Aims. To analyse the efficacy of M in LN according its initial renal function.

Patients and Methods. We studied data from 14 Renal Units in Spain. Inclusion criteria: i) biopsy-proven LN, ii) follow-up >3 months, iii) M as induction treatment. Patients were classified according initial Scr in Group 1 (≤1.2 mg/dl) and Group 2 (>1.2 mg/dl). Primary objective: % of patients who reach response (R) defined as proteinuria <3.5 g/24h or stabilization or improvement in whom initial proteinuria ≥3.5 g/24h or when proteinuria decreased ≥ 50% when initial proteinuria was <3.5 g/24h. Secondary objective: % of patients with complete remission (CRem): Scr ≤1.2 mg/dl and proteinuria ≤0.5 g/24h.

Results. We studied 75 patients (Group 1: 57 and Group 2: 18), aged 31±12 year-old, the majority with LN class 4 (80%). The medians of proteinuria and follow-up were 3 g/24h and 36 months, respectively. Scr was 0.8±0.1 mg/dl and 1.5±0.3 mg/dl in Groups 1 y 2, respectively. R: The percentages of patients who reached R was 77% and 78% at 6 and 12 months, respectively; these percentages were maintained for, at least, 60 months since the beginning of treatment. There were any differences in the percentages of cases that reached R in both Groups during the whole follow-up (Kaplan-Meier). Indeed, there was not any relation between initial serum creatinine and R at the end of follow-up (Cox analysis). CRem: The percentages of patients who were in CRem was 28% and 47% at 6 and 12 months, respectively; the percentage of cases who reached CRem was higher in Group 1 compared with Group 2.

Conclusions. M as induction treatment in LN is associated with high percentages of responses that are not related with initial renal function. Complete remissions are more common if initial renal function is well preserved.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1344

**Pantoprazole Reduces the Immunosuppressive Potency of Mycophenolate Mofetil** Matthias Schaier, Vedat Schwenger, Martin G. Zeier, Claudia Sommerer. *Department of Nephrology, University of Heidelberg, Heidelberg, Germany.*

Mycophenolate mofetil (MMF) is cleaved in the acidic milieu of the gastric compartment. However, its absorption might be impeded by proton pump inhibitors (PPIs), which suppress acid production and thus increase stomach pH. Since PPIs are widely used, it is useful to clarify whether the total drug amount of MMF is available in patients undergoing PPI treatment.

We analysed 36 patients with autoimmune diseases (20 with ANCA-associated vasculitis, 16 with systemic lupus erythematosus) under stable MMF maintenance therapy. Twenty-three patients received co-medication with PPIs; 13 patients received no treatment with PPIs or antacids. To assess the immunosuppressive potency, we measured MPA levels and inosin monophosphate dehydrogenase (IMPDH) activity with a validated high-performance liquid chromatography method in plasma samples collected at predose and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12h after oral administration.

The mean MMF dosage of the non-PPI patients was 770±249 mg/12h and 771±291 mg/12h in PPI-treated patients (ns). The total AUC of MMF showed a 37% reduction in PPI patients versus those treated with no PPIs ($p<0.01$), and the maximum peak concentration of MMF was 60% lower in the PPI patients ($p<0.001$). The MMF exposure correlated to the inhibition of IMPDH activity. The area of enzyme activity curve (AEC) was 42% higher in the PPI patients ($p<0.01$). Minimal IMPDH enzyme activity was lower in the non-PPI patients than in the PPI patients.

The co-medication of PPIs with MMF significantly influences the drug exposure and immunosuppressive potency of MMF in patients with autoimmune diseases. This finding might at least partly explain the different outcomes in studies using MMF for maintenance therapy.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1345

**Renal Lesions in Rheumatoid Arthritis (RA) Patients on Anti-Tumor Necrosis Factor-alpha (Anti-TNF-α) Therapy** Steven P. Salvatore, Surya V. Seshan. *Weill Cornell Medical College, New York City.*

Purpose: Anti-TNF-α agents are anti-inflammatory medications used to mitigate severe RA symptoms. These drugs are associated with formation of auto-antibodies against anti-nuclear antigen (ANA), anti-neutrophilic cytoplasmic antibody (ANCA), and others. This study describes the varied renal disease associated with anti-TNF-α therapy in patients with RA.

Methods: A retrospective clinicopathologic review of renal biopsies was performed on eight patients in the past nine years with RA who have received anti-TNF-α therapy.

Results: All patients were female, between 37 and 65 years old, and had severe RA from 3 to 35 years. Four patients were given Etanercept (a soluble TNFα receptor inhibitor), three Infliximab (a chimeric monoclonal IgG1 anti-TNF-α antibody), and one Adalimumab (a fully humanized anti-TNF-α monoclonal antibody) for an average of 10 months. Three patients presented with hypertension, nephritic syndrome and had pauci-immune crescentic glomerulonephritis (GN). Two patients had acute renal failure and had thrombotic microangiopathy (TMA). One patient developed Methicillin-resistant Staphylococcus Aureus (MRSA) septic arthritis. The final two patients had a long RA history and reactive amyloidosis.

| Case | Urinalysis | Proteinuria (g/day) | Creatinine (mg/dL) | Hypertension | Serology | Pathology |
|---|---|---|---|---|---|---|
| 1 | hematuria/RBC casts | 2 | 1.49 | yes | pANCA+/cANCA+ | Crescentic GN |
| 2 | hematuria/RBC casts | ND | 3.2 | no | pANCA+ | Crescentic GN |
| 3 | hematuria/RBC casts | 16 | 0.8 | no | ANCA negative | Crescentic GN |
| 4 | hematuria | 0.6 | 1.5 | yes | ANA+/dsDNA+ | Chronic TMA |
| 5 | proteinuria | ND | 3.2 | mild | Phospholipid Ab | TMA |
| 6 | proteinuria | 8 | 4.1 | severe | MRSA sepsis | Post-Infectious GN |
| 7 | proteinuria | 2 | 0.6 | yes | negative | Amyloidosis |
| 8 | proteinuria | 2.7 | 2.3 | mild | not done | Amyloidosis |

Conclusions: Patients with RA taking anti-TNFα therapy have a spectrum of renal complications from development of auto-antibodies to autoimmune diseases with renal involvement, increased susceptibility to infections and post-infectious GN, or unrelated renal disease such as reactive amyloidosis. A thorough serologic work-up, clinical history and kidney biopsy is warranted in these patients for definitive diagnosis and appropriate management.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1346**

**The Role of EM in Repeat Biopsies in the Management of Persistent Proteinuria in Class V Lupus Nephritis Treated with Rituximab, MMF and No Oral Steroids** Marwa A. Al-Riyami, Marie B. Condon, Liz Lightstone, Jeremy B. Levy, Megan Griffith, Tom Cairns, Candice A. Roufosse, H. Terence Cook. *Imperial College Kidney and Transplant Institute, Imperial College Healthcare NHS Trust, London, United Kingdom.*

**Purpose:** To study the role of EM in repeat renal biopsies in the management of patients treated with a Rituximab-based protocol for class V lupus nephritis (LN).

**Methods:** 22 patients with class V LN were treated with Rituximab & MMF but no oral steroids. 12 patients had repeat biopsies either for partial remission (PR, persistent urine PCR >100mg/mmol, n=8), non-response (n=1) or clinical relapse (n=3). All had membranous LN on light microscopy. EM images were available in 21 out of 24 biopsies from this group. The number & stage of subepithelial deposits were evaluated. Absence of stage I/II deposits defined histological remission. Glomerular and tubulointerstitial scarring were evaluated.

**Results:** Eight of 12 patients had PR and were re-biopsied 14 to 42 months after starting treatment. Four had complete histological remission with either no or only stage III/IV subepithelial deposits. They received no further treatment. The other 4 patients showed a decrease in the proportion of stage I/II compared to stage III/IV deposits. Three patients had relapse of proteinuria & were rebiopsied between 16 and 42 months. EM showed persistence of stage I/II deposits. One non-responder was re-biopsied at 16 months. EM showed an increase in percent of stage I/II deposits. All patients with persistent stage I/II deposits were re-treated with Rituximab. Overall, in the re-biopsies, there was a small increase in global glomerulosclerosis (median = 6.5%, range -15-34), glomerular segmental sclerosis (median = 3.5%, range -38-33) but no increase in tubulointerstitial scarring (median = 0%, range -5-30).

**Conclusion:** EM is an important tool in the evaluation of repeat biopsies in patients with class V LN. The lack of new subepithelial deposits on EM in some cases suggests proteinuria persists due to haemodynamic factors or glomerular basement membrane remodelling rather than disease activity, and avoids the need for increased immunosuppression.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1347**

**Rituximab as a Therapeutic Option in Severe Systemic Lupus Erythematosus** Dario Roccatello. *CMID, Ospedale S. Giovanni Bosco, Turin, Italy.*

Systemic Lupus Erythematosus (SLE) is a multisystem autoimmune disease characterized by the production of numerous autoantibodies against a variety of self antigens. B cells play a central role in SLE. Targeting the B cell compartment is therefore an attractive alternative to current available therapies. Rituximab is a human/mouse chimeric monoclonal antibody that specifically reacts with the CD20 antigen, which is restricted to B-cell lineage.

Eight patients, mean age 41.4 years (range 27-65 years), with severe multiorgan involvement including kidney (5 cases, comprising 3 patient with class IV(G) and 2 with class V ISN/RPS glomerulonephritis), skin (6 cases, with necrotizing ulcers in 3), central nervous system (2 cases) having severe polyarthrities (8 cases) and polysierositis (3 cases) were given Rituximab for intolerance or resistance to conventional therapy (6 patients) or as a front line treatment (2 cases).

Rituximab was administered intravenously at a dose of 375 mg/m$^2$ on days 1, 8, 15 and 22. Two more doses were administered 1 and 2 months later. This treatment was combined with two pulses of 750 mg cyclophosphamide and three pulses of 15 mg/kg metylprednisolone followed by oral prednisone, 50 mg quickly tapered until 5 mg in ten weeks. Response was evaluated by assessing the changes in clinical signs and symptoms (SLEDAI score) and laboratory parameters for at least 12 months.

Levels of erythrocyte sedimentation rate, C reactive protein and anti-dsDNA antibodies significantly decreased already at three months, whereas C4 values significantly increased at six ($p<0.05$). Proteinuria improved in all 5 cases presenting with renal involvement decreasing from 4.8 g/die (range 1.5-10.2) to 1.5 (range 0.1-3.7) in 3 months ($p<0.01$). Constitutional symptoms, skin ulcers, arthralgia, weakness, paraesthesia and fever disappeared or improved. SLEDAI score changed from 17.1 (12-28) to 2.8 (1-4) in 12 months ($p<0.01$). No acute or delayed side effects of clinical relevance were shown.

While Rituximab is not recommended for SLE with moderate activity, this therapy maintains a definite role in severe patients found to be refractory or intollerant to conventional treatment.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1348**

**Implications of Protocol Biopsies in Lupus Nephritis** Abdulkareem Alsuwaida, Jamal S. Alwakeel, Mohammed A. Al-Ghonaim, Sufia Husain, Anhar Ullah, Hala M. Kfoury. *King Saud University, Riyadh, Saudi Arabia.*

**Introduction:** The standard clinical and laboratory parameters have limited predictive values especially in discriminating between an active and chronic disease. The main objective of this study is to examine the utility of protocol biopsy in SLE nephritis.

**Methods:**Patients with Lupus Nephritis were advised to undergo a second kidney biopsy at the end of maintenance therapy. Complete remission was defined as serum creatinine <1.4 mg/dl and proteinuria < 0.33 g/d, while partial remission is defined as < 25% increase in baseline creatinine and >50% reduction in baseline proteinuria at the end of maintenance phase of therapy.

**Results:** Seventy three patients were included in the study. Complete and partial remission was achieved in 43.8% and 21.9%, respectively. The majority of the patients with partial or no remission had no histological evidence of active disease, 75% and 52% respectively.

Laboratory and Histological Characteristics of Patients at the time of Second Biopsy.

| | Complete Remission (n=32) | Partial Remission (n=15) | No Remission (n=25) | P |
|---|---|---|---|---|
| **Activity Index** | 1.8 ± 2.6 | 3.1 ± 4.1 | 5.1 ± 4.7 | *P* 0.001 [b] |
| **Chronicity Index** | 4.4 ± 3.0 | 4.9 ± 3.3 | 5.8 ± 2.5 | NS |
| **dsDNA** | 330 ± 493 | 559 ± 703 | 518 ± 608 | NS |
| **Urinalysis Red cells (per HPF)** | 37 ± 116 | 44 ± 70 | 82 ± 142 | NS |
| **Urinalysis White cells (per HPF)** | 25 ± 72 | 10 ± 16 | 32 ± 47 | NS |
| **Serum C3 (g/L)** | 1.3 ± 0.34 | 1.13 ± 0.58 | 0.79 ± 0.41 | 0.0001[b] |
| **Serum C4 (g/L)** | 0.29 ± 0.17 | 0.28 ± 0.18 | 0.24 ± 0.16 | NS |

Data are means ± SD. [a]dsDNA, doudle-stranded DNA; NS, not significant. [b]$P < 0.001$, Complete Remission versus No Remission; $P < 0.01$, Complete Remission versus Partial Remission.

At the most recent follow-up visit, the doubling of the serum creatinine was 25% in patients with partial remission, and 56% of the patients without remission.

**Conclusion:** Protocol biopsy strategy in patients with partial or no remission of lupus nephritis may allow us to avoid the adverse effects of overtreatment with immunosuppressive medications as a significant proportion of these patients do not show histological evidence of active disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1349**

**Regulatory T Cells in Lupus Nephritis** Andrea Kattah, Vivette D. D'Agati, Vidhyalakshmy Vivek, Sumit Mohan, Jai Radhakrishnan. *Departments of Medicine and Pathology, Columbia University College of Physicians and Surgeons, New York, NY.*

Purpose: To quantitate FoxP3 and CD3 expression in renal biopsies of patients with lupus nephritis and evaluate possible prognostic value

Intro: T regulatory lymphocytes (Tregs) have been implicated in downregulating autoimmunity and decreases may have a role in the pathogenesis of SLE. Our goal was to look at FoxP3, the transcription factor for Tregs, in renal biopsies of patients with lupus nephritis and see if the presence of these cells was renal protective.

Methods: FoxP3 and CD3 immunohistochemistry were performed on 17 patients with lupus nephritis (Class III-V). Slides were examined under 40x and the number of cells/hpf was recorded, as were activity and chronicity indices. Disease progression was defined as a doubling of serum creatinine, transplant, initiation of renal replacement therapy (RRT) or death. Data analysis was performed using Stata 10.1.

Results: Patients were 27.6±12 years with a mean follow up of 4±2.1 yrs and serum creatinine of 1.1±0.5 mg/dL. The mean activity index was 8.6±4.8 with a mean chronicity index of 3.2±2.5 while the mean FoxP3 expression was 3.1±4.3 cells/hpf with mean CD3+ cell count of 33.4±18.9 cells/hpf. Among our cohort, 3 (17.6%) patients reached endpoint (all 3 pts had progressive renal disease requiring renal transplantation). These patients were similar to the rest of the cohort except for a significantly higher activity index (7.2±4.1 vs. 14±2.7, $p=0.02$) at the time of biopsy. No patients initiated RRT and there were no deaths. Kaplan Meier Curves for patients with CD3+ cells counts ≤ and > 40cells/hpf were significantly different (log rank $c^2=6.0$, $p=0.014$). Neither FoxP3+ cells nor FoxP3+/CD3+ ratios were significantly correlated with activity and chronicity index at time of biopsy or disease progression.

Conclusions: We found no relationship between FoxP3 positivity and disease activity or progression. The number of CD3+ cells did show an adverse prognostic value in determining who would progress to end-stage renal disease. This study was limited by sample size and the fact that patients were in different stages of disease and treatment at the time of biopsy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1350**

**Neph1 Is Reduced in Primary Focal Segmental Glomerulosclerosis (FSGS) and Minimal Change Nephrotic Syndrome (MCNS)** Jenny Hulkko,[1] Jaakko Patrakka,[2] Karl Tryggvason,[3] Kjell R. Hultenby,[1] Annika Wernerson.[1] *[1]Pathology, Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; [2]Renal Medicine, Clinical Science, Intervention and Technology, Stockholm, Sweden; [3]Matrix Biology, Medical Biochemistry and Biophysics, Stockholm, Sweden.*

**Background**

The transmembrane proteins Neph1 and Nephrin form a complex in the slit diaphragm (SD) of the podocytes. As recent studies indicate the complexes involvement in polymerization of the actin cytoskeleton and proteinuria we wanted to study the subcellular localization of Neph1 in normal human kidney and the expression in the human glomerular diseases Focal Segmental Glomerulosclerosis (FSGS) and Minimal Change Nephrotic Syndrome (MCNS). Both characterized by substantial foot process effacement (FPE) and proteinuria.

**Material and Methods**

Kidney biopsies from patients with primary FSGS (perihilar type) (n=5) and MCNS (n=5) were compared to normal renal tissue (n=5). All patients had proteinuria ranging from 1 to 27 g / 24h at the time of biopsy. Polyclonal antibodies against Neph1 and Nephrin were used for immunoelectron microscopy (iEM). Antibodies were detected by gold-conjugated protein A and semiquantification was performed. The total number of gold makers was calculated in the foot processes and expressed as gold particles /μm$^2$.

**Results**

We localised Neph1 mainly to, and in close proximity, to the SD. Double staining of Neph-1 and Nephrin showed the proteins colocalized in the slit diaphragm. This is, to our knowledge, the first published EM image showing the expression of Neph1 in human kidney.

The total amount of Neph1 in the podocytes was significantly reduced in FSGS 0,3 (0,1) and in MCNS 0,5 (0,1) compared to controls 1,5 (0,2)**.**

Interestingly, the reduction of Neph1 is also seen in areas without FPE in both diseases. Even more intriguing is the fact that the expression of ¨Nephrin is unchanged in these FSGS patients compared to controls. The complex may therefore be interrupted in this disease, in contrast to MCNS, where Nephrin is reduced in a similar pattern.

**Conclusion**

Our results show that Neph1 is expressed in close proximity to the SD and co-localize with Nephrin. The protein may have a role in the pathogenesis of FSGS and MCNS.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1351

**Improvements in Glomerular Hyperfiltration Are Associated with Weight Loss after Roux-en-Y Bypass for Obesity** John C. Lieske, Maria L. Collazo-Clavell, Ellen Olson, Xujian Li, Eric J. Bergstralh, Zachary C. Ryan, Rajiv Kumar. *Mayo Clinic, Rochester, MN.*

Obesity is associated with hyperfiltration, proteinuria and development of chronic kidney disease. It is has been hypothesized that weight loss could reverse harmful effects of obesity on the kidney.

*Results:* Iothalamate clearance (Io-Cl), creatinine clearance (Cr-Cl) and 24-h urine albumin, α1-microglobulin (α1-MG) and retinol binding protein (RBP) excretion were assessed in a cohort of 11 morbidly obese women (BMI 46±4, mean±SD) before and 12 months (m) after Roux-en-Y bypass (RYGB) surgery. Weight loss was 46 kg at 12 m. Io-Cl fell from 121 ml/min at baseline to 89 ml/min at 12 m after surgery (p=0.03). Cr-Cl tended to decrease, but the change was not statistically significant. Excretion of α1-MG declined, but albumin and RBP excretion was unchanged. The decrease in Io-Cl correlated with change in weight (r=-0.49) and 24-hr creatinine excretion (r=-0.51). The change in α1-MG excretion did not correlate with change in weight.

Urine values prior to and following RYGB surgery

| Serum or urine test | Baseline, 0 m, mean±SD | 6 m, mean±SD | 12 m, mean±SD | P-value, paired t-test, 0-6 m | P-value, paired t-test, 0-12 m |
|---|---|---|---|---|---|
| Serum creatinine, mg/dL | 0.8±0.19 | 0.8±0.14 | 0.7±0.10 | 0.58 | 0.22 |
| Urine creatinine, mg/24h | 1341.7±434.4 | 1019.1±213.1 | 1035.1±255.0 | 0.01 | 0.006 |
| Creatinine Clearance, ml/min | 120±64 | 95±30 | 98±27 | 0.06 | 0.07 |
| Creatinine Clearance, ml/min/1.73M² | 89±45 | 84±21 | 91±24 | 0.25 | 0.52 |
| Iothalamate Clearance, ml/min | 121.1±31.9 | 93.0±33.9 | 82±23.7 | 0.2 | 0.03 |
| Iothalamate Clearance, ml/min/1.73M² | 94.9±27.7 | 83.0±23.6 | 84.6±20.9 | 0.6 | 0.38 |
| Urine α1-MG excretion mg/24h | 12.4±5.1 | 11.2±5.59 | 8.5±3.3 | 0.58 | 0.04 |

*Conclusions:* Weight loss after RYGB was associated with a fall in GFR measured by Io-Cl, a reduction in urinary creatinine excretion and tubular proteinuria. Further investigations are needed to determine the mechanism(s) by which GFR changes following weight loss and whether resolution in glomerular hyperfiltration after RYGB leads to beneficial long-term outcomes.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1352

**Corticosteroids Augment Cyclosporine Nephrotoxicity in Childhood Nephrotic Syndrome: Potential Role of Alternatively Activated Macrophages** Hiroya Hasegawa,[1] Yohei Ikezumi,[1] Toshiaki Suzuki,[1] Tamaki Karasawa,[1] Hiroshi Kawachi,[2] David J. Nikolic-Paterson,[3] Makoto Uchiyama.[1] *[1]Department of Pediatrics, Niigata University Medical and Dental Hospital; [2]Depatment of Cell Biology, Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata-City, Japan; [3]Monash University Department of Medicine, Monash Medical Centre, Clayton, Victoria, Australia.*

Aim: Cyclosporine A (CsA) is an effective steroid-sparing agent for patients with steroid-dependent nephrotic syndrome (SDNS) that may, however, cause chronic interstitial damage. We have previously reported that infiltration of macrophages with an alternatively activated phenotype (M2) is closely associated with the pathogenesis of interstitial fibrosis in IgA nephropathy, and that this is augmented by corticosteroid therapy. This study examined the possible involvement of M2-type macrophages in cyclosporine nephrotoxicity in SDNS.

Material and Methods: A total of 12 children diagnosed with SDNS who were treated with CsA for more than 2 years were investigated. Six biopsy specimens from age-matched SDNS children who had not recieved CsA treatment were used as the control. Biopsy sections were stained for α-smooth muscle actin (α-SMA), type I collagen, CD68 (total macrophages), CD163 (M2 marker) and CD86 (B7-2; M1 marker).

Results: The CsA-treated group showed a significant increase in interstitial fibrosis with accumulation of interstitial CD68+ macrophages compared with SDNS control patients (both p<0.01). Dual immunofluorescence staining showed that about 95% of CD68 macrophages expressed CD163, but were negative for CD86, indicating an M2 phenotype. There was a significant correlation between the degree of interstitial fibrosis and the number of interstitial CD163+ cells (p<0.01), and between interstitial fibrosis and the dose of steroid used during CsA treatment (p<0.05), while no correlation was found between the dose of steroid used before CsA treatment and histological changes.

Conclusion: Our findings suggest that M2-type macrophages may participate in the development of interstitial fibrosis induced by CsA. Steroid treatment during CsA treatment might augment CsA nephrotoxicity.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1353

**Focal Segmental Glomerulosclerosis Plays a Major Role in the Progression of IgA Nephropathy** Gary S. Hill,[1] Khalil El Karoui,[1] Alexandre Karras,[2] Christian H. Jacquot,[2] Patrick Bruneval,[1] Dominique Nochy.[1] *[1]Pathology Department, Hôpital Europeen Georges Pompidou; [2]Nephrology Department, Hôpital Europeen Georges Pompidou.*

IgA nephropathy (IgAN) often shows lesions morphologically identical with those of focal segmental glomerulosclerosis (FSGS).

A cohort of 128 patients with IgAN was evaluated (i) according to the Oxford classification of IgAN and (ii) to determine the extent to which IgAN could be interpreted in terms of a FSGS.

We were able to categorize 101 patients (78.9%) as having some form of FSGS, notably FSGS with hyalinosis (46 patients) and collapsing glomerulopathy (11 patients). Eighteen patients (14%) had no glomerular lesions, and 11 (8.6%) had mild lesions not definably FSGS. Patients with FSGS had much worse renal survivals than those without (32.6% vs 95.1% at 80 months, p = .00027). Comparison of pure forms of FSGS (excluding collapsing glomerulopathy) with cases of FSGS with superimposed other glomerular lesions (mesangial hyperplasia, endocapillary hypercellularity, glomerular necroses, extracapillary proliferation), revealed that FSGS with superimposed glomerular lesions did worse than cases of pure FSGS (22.8% versus 49.2% renal survival at 80 months, p = .042). However, the pure cases had relatively poor survival even without superimposed other glomerular lesions. Glomerular capsular adhesions, were studied in our IgAN biopsies, lupus nephritis (100 biopsies), and primary FSGS (26 biopsies). Capsular adhesions with no lesions in the underlying tuft, consistent with podocyte abnormality, were found regularly in FSGS (69%) and IgAN (41%), but infrequently in lupus (8%). Fifteen IgAN biopsies were also studied immunohistochemically. There was early focal loss of podocyte markers (synaptopodin, GLEPP-1, nephrin, VEGF) in glomeruli still histologically normal. Proliferating cells staining as PECs (PAX2+, cytokeratins+), grew inward along the adhesion onto the tuft, forming a monolayer positive for PAX2, WT-1, PCNA, and cytokeratins. All cases showed changes basically similar to those described in FSGS in other studies, and strongly suggest that a podocytopathy similar to that in primary FSGS occurs frequently in IgAN.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1354

**Subclassification of Focal Segmental Glomerulosclerosis in IgA Nephropathy: Is It of Clinical Value? Evidence from the Oxford Classification Cohort** Ian Roberts,[1] Shubha S. Bellur,[1] H. Terence Cook.[2] *[1]John Radcliffe Hospital, Oxford; [2]Imperial College, London, United Kingdom.*

Focal segmental glomerulosclerosis (FSGS) is frequent in IgA nephropathy (IgAN). It may result from fibrosis of proliferative lesions or reflect podocyte injury analogous to primary FSGS. It has been proposed that subclassification of segmental sclerosis in IgAN is of prognostic value. Here we subclassify FSGS in the Oxford Classification patient cohort and correlate histology with clinical variables.

There was segmental sclerosis of at least one glomerulus in 76% biopsies from 265 patients in the cohort; 147 with FSGS had slides available for second review. The slides were reviewed by a single pathologist and segmental sclerosing lesions subclassified.

Sixty-two of 147 biopsies (42%) showed endocapillary hypercellularity, 16 (11%) hyalinosis, 9 (6%) tip lesions, 54 (37%) podocyte hypertrophy and 13 (9%) resorption droplets within podocytes. None of the biopsies showed collapsing FSGS. There was no significant correlation between percentage of glomeruli with endocapillary hypercellularity and extent of segmental sclerosis.

Tip lesions and podocyte hypertrophy were associated with greater initial proteinuria (see table). There was no correlation between any of the above features and initial GFR or rate of loss of renal function at follow-up.

| | Absent (-) Present (+) | Initial urine protein (g/24hrs) mean±SD | Follow-up urine protein (g/24hrs) mean±SD | Initial GFR (ml/min) mean±SD | Rate of loss of renal function (ml/min/1.73m2 per year) |
|---|---|---|---|---|---|
| Endocapillary proliferation | - | 2.5±2.3 | 1.9±1.8 | 71±35 | -3.7±5.9 |
| | + | 2.6±1.9 ns | 1.6±1.2 ns | 84±38 ns | -5.4±10.8 ns |
| Tip lesion | - | 2.4±1.9 | 1.7±1.4 | 77±37 | -4.6±8.4 |
| | + | 4.9±3.3 p=<0.02 | 2.9±3.2 ns | 68±21 ns | -2.4±9.5 ns |
| Podocyte hypertrophy | - | 2.2±1.8 | 1.7±1.5 | 79±37 | -4.2±6.5 |
| | + | 3.1±2.4 p=<0.02 | 2.0±1.7 ns | 73±36 ns | -4.8±10.9 ns |
| Hyalinosis | - | 2.6±2.1 | 1.8±1.6 | 78±37 | -4.6±8.8 |
| | + | 2.2±2.2 ns | 1.9±1.8 ns | 60±28 ns | -3.2±4.3 ns |

We conclude that podocyte hypertrophy and tip lesions in IgAN-associated FSGS are markers of podocyte injury and associated with higher levels of proteinuria. Subclassification of segmental sclerosis is not of prognostic value in this cohort.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1355**

**Use of the Oxford Classification of IgA Nephropathy To Predict Renal Outcome: A Retrospective Analysis** Timothy T. Yau,[1] Stephen M. Korbet,[1] Melvin M. Schwartz,[2] David J. Cimbaluk.[2] *[1]Department of Nephrology, Rush University Medical Center, Chicago, IL; [2]Department of Pathology, Rush University Medical Center, Chicago, IL.*

The Oxford classification of IgA nephropathy (IGAN) assesses 4 histologic features (Mesangial hypercellularity (M)- </>50%, Endocapillary proliferation (E)- N/Y, Segmental scars (S)- N/Y, Tubulointerstitial fibrosis(T)- 0=<25%, 1=26-50%, 2=>50%) and has been reported as having prognostic value. We tested the clinical significance of the classification in a retrospective study of 72 of our adult pts with IGAN. The pts were 42±15 yrs old, with 56% male and 54% white, 21% Asian, 19% Hispanic and 6% black. The baseline SCr was 2.0±1.9 mg/dl, MDRD eGFR 57±29 ml/min (>60 ml/min in 49% and <15 in 11%,6 pts with ESRD) and the UPro was 2.1±1.7 g/d. All pts had microscopic hematuria and in 26% it was macroscopic. Of the 4 histologic features evaluated, only the degree of tubulointerstitial fibrosis (T0, T1, T2) demonstrated a significant correlation to worsening baseline SCr (1.4±1.4, 1.5±0.5, & 3.8±2.4: p<0.0001), eGFR (71±26, 56±19 & 26±17, p <0.0001) and UPro (1.8±1.5, 2.4±2.5 & 2.7±1.4: p<0.05). Follow-up (FU) on 54 pts was 69±56 mo with FU >1 yr in 85% and >5 yrs in 48% of pts. Pts were treated with ACEi/ARBs-78%, fish oil-70% and steroids-32%. At last follow-up, 17% of pts reached the endpoints of doubling SCr (4%) or ESRD (13%). Only the degree of tubulointerstitial fibrosis (T0, T1, T2) was predictive of progressive renal disease with a follow-up SCr of 1.2±0.4, 3.1±2.6 & 4.4±3.0 (p<0.0001) and MDRD eGFR (69±21, 37±25 & 24±17, P<0.0001). This was associated with 3%, 29% and 50% of pts (p<0.001) reaching the composite endpoint and the renal survival at 5 and 10 yrs for T0, T1 & T2 was 100%, 100%, & 34% and 100%, 50%, & 17% (overall, p<0.001). There was no significant difference in follow-up SCr, MDRD eGFR or proportion of pts reaching the composite endpoint based on the M, E or S scores. In conclusion, we find that of the histologic features assessed by the Oxford classification, only the degree of tubulointerstitial fibrosis correlates with renal function and proteinuria at baseline and is predictive of outcome in our IGAN pts.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1356**

**Oxford Classification of IgA Nephropathy: Outcomes Predictors** Lilian P. F. Carmo, Igor Marques, Liliany P. Repizo, Lectícia Jorge, Elerson Costalonga, Denise Mac Malheiros, Rui Toledo Barros, Viktoria Woronik. *Nephrology, University of São Paulo, São Paulo, Brazil.*

IgA nephropathy (IgAN) is the most common glomerular disease worldwide. There is continuing debate whether pathological parameters contribute additional prognostic information beyond that provided by clinical features. The Oxford Classification proposed some pathological features that predict risk of progression of renal disease in IgAN. We reviewed biopsies according to this new classification and performed a retrospective study using clinical data to access the factors that determine adverse outcomes.We analyzed 165 patients with biopsy-proven IgAN at our center from 1999 to 2009, 62 patients meet inclusion criteria of age > 18 years, biopsies containing at least 8 glomeruli and follow up longer than 2 years.The primary endpoint was reduction of at least 50% of the initial eGFR (MDRD) and/or ESRD.The multivariate analysis was designed to address which of the select pathology variables (mesangial hypercellularity, segmental glomeruloesclerosis, endocapillary hypercellularity, crescents, tubular atrophy/interstitial fibrosis and artery lesions) were independent predictors of outcome.

Baseline features

| | |
|---|---|
| Age (years) | 37±13,6 |
| Female | 53% |
| Hypertension | 56% |
| eGFR (ml/min/1,73m²) | 57.8 ±34,8 |
| Proteinuria (g/d) | 2.9±2.6 |
| Duration of follow-up (years) | 4.7 ± 2,2 |
| Glomerulus numbers | 12.6 ± 4,3 |
| Mesangial hypercellularity | 79% |
| Endocapillary hypercellularity | 25% |
| Artery lesions | 54.8% |
| Crescents | 35.5% |

In the multivariate linear regression model, we observed that the rate of renal function decline correlated with segmental glomerulosclerosis (p=0,042) and vascular lesions (p=0,006) after adjustments for hypertension, initial eGFR and proteinuria. At multivariate logistic regression analysis, the presence of fibrosis > 50% was an independent factor for the primary endpoint after adjusting for initial eGFR (p=0.028; OR=7.2; CI 1.7-41.7). Histopathologic features of segmental glomeruloesclerosis, fibrosis > 50% and artery lesions correlated with patients who are at high risk for progressive loss of kidney function. These pathological features were independent of clinical parameters in predicting the outcome in IgAN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1357**

**The Oxford Scoring System for IgAN Has Excellent Interobserver Consistency in a Population of Canadian Children** Maury N. Pinsk,[1] Chantal Bernard,[4] Janusz Feber,[6] Ian W. Gibson,[2] Aviva M. Goldberg,[2] Christoph Licht,[7] Aicha Merouani,[5] Andrew W. Wade,[3] Tom D. Blydt-Hansen.[2] *[1]University of Alberta, Edmonton, AB, Canada; [2]University of Manitoba, Winnipeg, MB, Canada; [3]University of Calgary, Calgary, AB, Canada; [4]McGill University, Montreal, QC, Canada; [5]Universite de Montreal, Montreal, QC, Canada; [6]University of Ottawa, Ottawa, ON, Canada; [7]University of Toronto, Toronto, ON, Canada.*

The Oxford classification of IgAN is s scoring system with proven reliability and validity in a mixed international population of adult and pediatric patients. A subanalysis of the pediatric cohort used in the initial study suggested the validity of the scoring system in children. However, in Canada, unique populations such as First Nations/ Aboriginals are known to develop IgAN, but were not represented in the original Oxford classification. Purpose: Using a national Renal Disease Database for the Canadian Pediatric Population (REDDCAPP), we sought to confirm the interobserver consistency of the Oxford scoring system in an independent population Canadian children with biopsy proven IgAN/HSPN. Method: 12 children enrolled in the registry had tissue pathology examined by two independent renal pathologists in a blinded fashion. Scores from each pathologist were analyzed using Intraclass correlation coefficients to determine interobserver consistency. Results: Overall consistency between rating pathologist was excellent. Scores for mesangial proliferation, fibrinoid tuft necrosis, extracapillary proliferation, glomerulosclerosis, tubular inflammation, fibrosis and atrophy were all very good to excellent (ICC>0.7). Similar to the Oxford Classification data, assessment of adhesions to Bowman's capsule and endocapillary proliferation was only poor to good. (ICC < 0.3). Conclusions: When applied to a unique cohort of Canadian children with IgA nephropathy, the Oxford Scoring System has overall excellent interobserver consistency.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1358**

**Regulatory T Cells (Treg) and Interleukin 17 Producing T Cells (TH17) in IgA Nephropathy** Roberta Camilla,[1] Valentina Daprà,[1] Elisa Loiacono,[1] Licia Peruzzi,[1] Cristiana Rollino,[2] Giulietta Beltrame,[2] Michela Ferro,[2] A. Amore,[1] Rosanna Coppo.[1] *[1]Nephrology, Dialysis and Transplant, Regina Margherita Hospital, Turin, Italy; [2]Nephrology and Dialysis, G.Bosco Hospital, Turin, Italy.*

**INTRODUCTION AND AIMS:** IgA nephropathy (IgAN) presents with a dysregulation of the immune system leading to abnormal immune response to mucosal antigens. In these patients, we recently reported an increased expression of Toll-like receptor-4 (TLR4) in peripheral blood mononuclear cells (PBMC). Interest has been recently focused on the balance between interleukin (IL)-17 producing T cells (TH17) and regulatory T (Treg) cells in immune-mediated diseases. We aimed at investigating Treg/TH-17 producing cells in patients with IgAN, looking at correlations with TLRs expression, innate immunity marker.

**METHODS:** PBMC were isolated from 28 patients with IgAN (median age 39.6, IQ range 19-65 years), e-GFR 92.6 ± 46.5 ml/min, proteinuria 0.2 g/day (IQ range 0.10-0.40), and from healthy controls (HC).

The research protocol included the measurement in Taqman of mRNA expression of Treg regulation-associated genes (Foxp3), TH17-related factors (IL-17 and retinoid orphan nuclear receptor RORc), and TFG-beta1 which modulates the differentiation of TH17. Similarly, mRNAs encoding for TLR 2, 4 and 4 were measured.

**RESULTS:** The transcriptional level of Foxp3 was significantly lower in patients with IgAN versus HC (0.82 ± 0.30 vs 1.05 ± 0.37, p= 0.041), while those of IL-17 and of its regulatory factor RORc were slightly, but not significantly increased (IL-17 1.17 ± 1.07 vs 1.05 ± 0.41 in HC, RORc 1.25 ± 0.85 vs 1.14 ± 0.71 in HC). A significant correlation was found between IL-17 and RORc mRNAs values (p<0.0001). Transcriptional levels of TGF beta1 were similar to controls and were directly correlated with RORc (p=0.0015) and IL-17 (p=0.031) mRNAs in patients with IgAN. ROR-C level were inversely correlated with e-GFR values (p=0.06). Foxp3 and TLR4 expression were inversely correlated (p= 0.022).

**CONCLUSIONS:** In patients with IgAN there is a functional defect in Tregs correlated with signs of hyperactive innate immunity. TGF beta 1 correlates with the expansion of TH17 cells.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1359**

**Role of Innate Immunity (Toll-Like Receptors) in the Pathogenesis of Henoch-Schoenlein Purpura** Roberta Camilla, Valentina Daprà, Elisa Loiacono, Licia Peruzzi, A. Amore, Rosanna Coppo. *Nephrology, Dialysis and Transplant, Regina Margherita Hospital, Turin, Italy.*

**INTRODUCTION** Toll Like Receptors (TLRs) trigger innate and adaptive immune response. We previously demonstrated (*Clin Exp Immunol* 2009, **159:** 73–81) an up-regulation of TLR4 in peripheral lymphomonocytes (PBMC) of patients with primary IgA nephropathy (IgAN). Since Henoch-Schoenlein purpura (HSP) is a vasculitis which can present with renal involvement and IgAN, we aimed at investigating TLRs in PBMC of patients with HSP.

**METHODS** We studied 15 children (8 males, age 7.4 ±1.8 y) with purpuric rash flare due to HSP before treatment was started; 7 had renal involvement (hematuria, in 4 cases also >0.5 urinary proteine/creatinine excretion).

TLR 3, 4 and 9 was quantified in PBMC both as mRNA (using real-time PCR Taqman, normalizing results on Abelson gene) and as receptor expression with cytofluorimetric method (mean fluorescence intensity, MFI). We then analyzed the switch from proteasome to immuneproteasome (which was activated in IgAN patients and correlated with TRLs expression), studying the ratio between LMP2/β1, MECL1/β2, LMP7/β5 subunits in real-time PCR. Results were compared with a previously studied cohort of 47 patients with primary IgAN.

**RESULTS** We observed a significant up-regulation of TLR4 in HSP patients with respect to healthy controls (HC) (mRNA 3.84 ± 2.88 U vs1.46 ± 1.43 U, p=0.0001, MFI: 2.39 ± 0.75 U vs 1.60 ± 0.44 U, p<0.0001); TLR 9 were also upregulated, but the difference was less statistically significant (mRNA 3.07 ± 2.02 U vs 1.41 ± 1.59 U, p= 0.0125; MFI 1.4 ± 0.2 U vs 1.57 ± 0.26 U, p=ns). The expression of TLR3 did not differ in HSP patients and HC. Results were similar to those observed in primary IgAN.

We failed to show, as opposed to what we observed in primary IgAN, a switch from proteasome to immune-proteasome (LMP2/β1 0.8 ± 0.3 in HSP vs 0.9 ± 0.4 in HC, MECL1/β2 0.7 ± 0.2 vs 1.0 ± 0.3 , LMP7/ β5 1.0 ± 0.2 vs 1.1 ± 0.3).

**CONCLUSIONS** In HSP purpura, as in primary IgAN, we found an activation of innate immunity, with an upregulation of TLRs, especially TLR4, in peripheral lymphomonocytes which, in acute purpuric flare, was independent from the renal involvement.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1360

**Thrombotic Microangiopathy in IgA Nephropathy** Khalil El Karoui,[1] Gary S. Hill,[1] Alexandre Karras,[2] Patrick Bruneval,[1] Christian H. Jacquot,[2] Dominique Nochy.[1] *[1]Pathology, Hopital Georges Pompidou, Paris; [2]Nephrology, Hopital Georges Pompidou, Paris.*

The clinical and histologic features of IgA nephropathy (IgAN)are quite varied. Thrombotic microangiopathy (TMA) is a histologic lesion characterized initially by platelet aggregates and fibrin thrombi, with subsequent organization and luminal narrowing. Cases of TMA associated with IgAN have been described, usually in association with severe hypertension. The purpose of this study was to define the frequency and clinical and histologic features of IgAN-associated TMA.

This series is comprised of 132 adults patients (92% Caucasian, 73.5% male, mean age 38.66 ±13.5 ), with a mean follow-up of 41.6±25 months.

In our series, 53% of patients presented lesions of TMA, acute or organized, in arteries and/or arterioles. There were no age or sex differences between those with and without TMA. Hypertension was present in 90.3% of patients, with 23.4% of them showing malignant hypertension. However, 9.7% of patients with TMA were normotensive. Those with TMA had greater proteinuria and worse renal function than those without TMA. Histologically, the group with TMA had greater glomerulosclerosis (53 ± 27% vs 15 ± 17%, p <0.0001) and worse tubulointerstitial fibrosis (47 ± 25% vs 14 ± 15%, p = 0.0001) than the group without TMA. However, 11.6% of patients showed near-normal histology. TMA was associated with a poor prognosis with doubling of SCr or need for dialysis in 50.7% versus 14% in the group without TMA (p=0.0001). On multivariate analysis it sorted as an independent risk factor for poor prognosis.

Lesions of TMA are frequent in IgAN, sometimes quite early. They may appear in normotensive patients with near-normal renal histology. Their prognosis appears to be independent of that due to other histologic lesions and the Oxford criteria. The pathophysiologic mechanisms involved remain undetermined, but the present study rules out severe hypertension as a sole cause.

The frequency and severity of TMA in IgAN suggests that TMA should be sought systematically in IgAN in order that this aspect can be treated appropriately.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1361

**Renal Thrombotic Microangiopathy Associated with Graft Versus Host Disease after Hematopoietic Stem Cell Transplantation** Akiko Mii, Megumi Fukui, Emiko Fujita, Akira Shimizu. *Nippon Medical School.*

Thrombotic microangiopathy (TMA) is one of the main complications after hematopoietic stem cell transplantation (HSCT). The pathogenesis of HSCT-associated TMA is controversial but considered to involve various factors such as total body irradiation, use of calcineurin inhibitors for prophylaxis against graft versus host disease (GVHD), viral infection, and GVHD.

In the present study, we examined the clinical and pathologic features of two renal biopsy and five autopsy cases with pathological TMA after HSCT, in order to clarify the association between GVHD and HSCT-associated TMA.

In our cases, the median interval of renal biopsy or autopsy after HSCT was 14.3 months (range 1-42 months). Acute GVHD was seen in all of seven patients. Chronic GVHD occurred in one biopsy and three autopsy cases. The clinical evidence of TMA was detected in two autopsy cases. All cases showed renal impairment.

The predominant histological findings were diffuse glomerular endothelial cell injury such as diffuse glomerular capillary collapse with pericapsular fibrosis, double contour of the glomerular basement membrane (GBM) with enlarged subendothelial space, and post-mesangiolytic sclerosis with reticular mesangial matrix. Marked hyalynosis was notable in small arteries. In addition, all our cases showed glomerulitis, renal tubulitis, and peritubular capillalitis (PTCitis) with CD3+ cells infiltration. These findings indicate that GVHD is involved in the development of TMA. Furthermore, C4d deposition on diffuse glomerular capillaries and patchy PTCs was evident in two biopsy and one autopsy cases, indicating that some cases of TMA may be mediated by the antibody deposition and complement activation in chronic GVHD.

In conclusion, renal GVHD occurred in our cases of the development of TMA after HSCT. Some cases of TMA were probably associated with the antibody-mediated renal endothelial cell injury in chronic GVHD. Kidney is one of the target organs of acute and chronic GVHD that may be associated with the development of TMA after HSCT.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1362

**Contaminated Cocaine and Drug-Induced Vasculitis** Martina M. McGrath,[1] Tamara Isakova,[1] Ann M. Mottola,[2] Karen Laliberte,[1] John Niles.[1,2] *[1]Department of Medicine, Renal Unit, Massachusetts General Hospital, Boston, MA; [2]Department of Pathology, ANCA Laboratory, Massachusetts General Hospital, Boston, MA.*

**Purpose:** We have noted a possible association between cocaine use and ANCA positive vasculitis of the drug-induced pattern.

**Methods**: To address this uncommon presentation we restrospectively reviewed the medical records and laboratory results of individuals seropositive with MPO, PR3 or both. From November 2009 through May 2010 at the Massachusetts General Hospital ANCA laboratory we identified 11 such patients, all of whom had a history of cocaine use. Charts were reviewed for clinical presentation, laboratory and other diagnostic evaluation, including skin biopsies when available. Cocaine exposure was identified from the patient history in all cases.

**Results:** Five patients in this series tested positive for both MPO and PR3, a pattern virtually never found except in rare patients with drug induced vasculitis. Based on the currently available data, 8 out of 11 patients presented with a vasculitic rash, of whom 3 had a biopsy proven diagnosis of leukocytoclastic vasculitis. Three patients had renal involvement (1 with biopsy proven necrotizing and crescentic glomerulonephritis). Additional clinical features included arthritis and fever.

**Conclusion:** Recent reports have indicated that illicit cocaine may be cut with levamisole. Levamisole was formerly used as an antihelminthic and chemotherapy agent, but is no longer sold for use in humans due to its side effect and efficacy profile. Levamisole has previously been linked to neutropenia and cutaneous vasculitis. Given the recent increase in levamisole usage as a cutting agent, and the long exposure time present in most cases of drug induced ANCA vasculitis, it is possible that a rapid increase in cases of cocaine / levamisole induced vasculitis may be developing. Additional testing and review is needed to confirm and extend our findings based on these and future patients.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1363

**Pauci-Immune Crescentic Glomerulonephritis in Rheumatoid Arthritis with Serologic Heterogeniety: A Clinico-Pathological Study** Ryuji Ohashi, Steven P. Salvatore, Surya V. Seshan. *Pathology, Weill Cornell Medical College, New York, NY.*

Purpose: A spectrum of renal diseases (immune-mediated with or without immune complexes, secondary to disease modifying agents-drugs, amyloidosis) are encountered in rheumatoid arthritis (RA), though not as common as in systemic lupus erythematosus. This study presents a cohort of 18 patients with pauci-immune crescentic glomerulonephritis (CrGN).

Methods: A retrospective review of the renal pathology database in our center was conducted for a 25 year period where 120 cases of RA with kidney biopsies were identified, 18 of which showed acute/chronic CrGN. The clinico-pathological findings and serology are described.

Results: The age of the patients ranged from 33-80 yrs, M:F is 4:14 presenting with Cr 1.2-10 mg/dl in 14 pts and <1.5 mg/dl in 4 pts. Six pts had nephrotic range proteinuria, and all others had 1-2 gm/24hrs, and hematuria was noted in 8 pts. Evidence of small vessel vasculitis was observed as skin rash showing leukocytoclastic vasculitis in 5 pts, pulmonary hemorrhage in 2 pts and renal vasculitis in one pt. Other associated autoimmune diseases include ulcerative colitis in 2 pts, Crohn's disease in one pt, amyloidosis in one pt, chronic pulmonary disease in one pt, and psoriasis in one pt. Serology: P-ANCA+ in 3 pts, anti-MPO+ in 3 pts, C-ANCA+ in 1 pt, P&C ANCA+ in 1 pt, negative ANCA in 6 pts, and not known in 4 pts. Positive ANA ranged from 1:40-1:640 in 3 pts, and anti-dsDNA was negative and complement levels were normal in all except one pt. Anti-TNFα therapy was given to 3 pts (2 ANCA+, 1 ANCA-). Renal biopsy findings are as follows: acute CrGN in 6 pts, acute & chronic CrGN in 8 pts and chronic GN in 4 pts. One pt also showed acute interstitial nephritis in the latter group.

Conclusion: CrGN contributes to significant renal insufficiency in RA pts, often associated with renal and extrarenal small vessel vasculitides. CrGN in the setting of RA may be ANCA negative or secondary to coincidental predominantly P-ANCA or anti-MPO ANCA. CrGN in RA pts with anti-TNFα therapy may be associated with positive or negative ANCA serology.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1364**

**Assessment of Lamp2 Autoantibodies in Patients with ANCA Disease** Aleeza J. Roth, Michael C. Brown, Anshul K. Badhwar, Hyun Chul Chung, Donna O. Bunch, JulieAnne G. McGregor, Susan L. Hogan, Yichun Hu, J. Charles Jennette, Gloria A. Preston, Ronald J. Falk. *UNC-Kidney Center, Chapel Hill, NC.*

Kain et al. have reported (Nat Med,2008) that 93% of patients with pauci-immune glomerulonephritis have autoantibodies directed against LAMP-2, a heavily glycosylated type 1 membrane protein expressed by many cell types. Most of these patients also have anti-neutrophil cytoplasmic autoantibodies (ANCA) specific for MPO or PR3. To test the validity of this report, sera from patients with MPO- or PR3-ANCA disease were tested for the presence of anti-LAMP-2 compared to controls. LAMP-2 antigens used for screening included recombinant protein produced in a human cell line (HEK293), protein expressed in a glycosylation-deficient hamster cell line (CHO LDL-D), and a synthetic peptide containing the suggested LAMP-2/FimH-like epitope. The pathogenicity of LAMP-2 antibodies raised to the peptide epitope was also tested in rats.

By ELISA, 17% of ANCA-positive sera (n=104) reacted with LAMP-2 protein purified from the human cell line, and 3% reacted with peptide (2 SD above mean of normal controls). Only two patient sera reacted with both the recombinant protein and the peptide. Similar to ANCA patients, 12% of sera from the general population with FimH positive E. coli urinary tract infections (n=105) reacted to recombinant protein and 4% to peptide. By indirect immunofluorescence assay, no patient sera produced positive staining of cells regardless of cell type or glycosylation state (N- plus O- glycans, N-glycans only, or no glycosylation). Samples deemed positive by ELISA were screened by western blot and thus far 0/3 were confirmed as positive. To explore the pathogenic role of anti-LAMP-2 antibodies, high-titer anti-LAMP-2 rabbit IgG or normal rabbit IgG was injected i.v. into WKY rats. None of the rats developed lesions, including no glomerular crescents or necrosis. These data do not support a high frequency of LAMP-2 autoantibodies in patients with MPO-ANCA or PR3-ANCA disease, and do not confirm the pathogenicity of anti-LAMP-2 in rats. The basis for the discrepancy between our data and the studies by Kain et al. is unknown.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1365**

**The Role of In Situ-Immune Complex (MPO-Anti MPO Antibody) in the Pathogenesis of MPO-ANCA Associated Glomerulonephritis** Soko Kawashima,[1] Katuko Sano,[1] Kazuhito Fukuoka,[1] Yoshinori Komagata,[1] Shinya Kaname,[1] Yoshihiro Arimura,[1] Akira Yamada,[1] Akihiko Kudo,[2] Hayato Kawakami.[2] *[1]First Department of Internal Medicine, Kyorin University School of Medicine, Mitaka, Tokyo, Japan; [2]Department of Anatomy, Kyorin University School of Medicine, Mitaka, Tokyo, Japan.*

Myeloperoxidase anti-neutrophil cytoplasmic antibody (MPO-ANCA)-associated glomerulonephritis (GN) is characterized by pauci-immune necrotizing crescentic glomerulonephritis (NCGN). Although MPO-ANCA has been thought to be involved in the activation of neutrophils in the pathogenesis of NCGN, recent studies suggest a different role of immunoglobulins and complements in the formation of glomerular capillary injury in a rat model for NCGN. Here we investigated the possible roles of MPO and IgG in the patients with MPO-ANCA-associated GN. Nineteen patients were analyzed for glomerular infiltration of MPO-positive cells, deposition of extracellular MPO and endothelial cell injury especially in an early stage of the diseases. Immunohistochemistry using enzyme-labeled antibody or immunofluorescence of MPO, IgG and CD34 were performed for the samples of renal biopsies. Sixteen out of 19 patients showed a weak but significantly positive staining for IgG (pauci-immune GN), which was accompanied by MPO deposits in the glomerular capillary walls. Interestingly, the CD34 staining clearly decreased around the area where MPO and IgG were detected, suggesting that the endothelial injury occurs resultantly. These findings suggest that MPO, IgG and the immune complexes composed of MPO-anti MPO antibody may play some direct roles in the pathogenesis of glomerular capillary injury in human MPO-ANCA associated GN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1366**

**Glomerular Endothelial Injuries Contributing to the Formation of Segmental Glomerular Sclerosis in Idiopathic Membranous Nephropathy** Megumi Fukui,[1] Akiko Mii,[1] Akira Shimizu,[2] Emiko Fujita.[2] *[1]Internal Medicine (Division of Neurology, Nephrology, and Reumatology), Nippon Medical School, Tokyo, Japan; [2]Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.*

Membranous nephropathy (MN) with focal segmental sclerosis is associated with a poorer prognosis than MN without these lesions. The mechanism of the development of segmental sclerosis is still uncertain. Vascular endothelial growth factor (VEGF), produced mainly by glomerular podocytes has a crucial role to promote endothelial cell survival and differentiation. We selected renal biopsy cases of idiopathic MN cases with segmental sclerosis (n=27/ total 250), and examined the pathological characterizations. We assessed the formation of segmental sclerosis, focusing on the glomerular endothelial cell injuries, thickening of GBM, and VEGF expression in podocytes. The average age of these cases is 62.6±1.87 years. About 68% (n=19) of these cases developed nephrotic syndrome. eGFR (mean 55.86±3.93 mg/dl) was significantly lower compared to MN without segmental sclerosis. In histopathology, all segmental sclerotic lesions in MN showed the obliteration and loss of glomerular capillaries with disappearance of CD34+ endothelial cells. Interestingly, in all cases with segmental sclerosis, narrowing of glomerular capillaries with ultrastructually endothelial cell injuries was evident even in non-sclerotic areas. No difference of VEGF expression on podocytes was detected between MN cases with and without segmental sclerosis (1.3/0-4 semiquantitative score in MN with sclerosis vs. 1.2/0-4 scores in MN without sclerosis, p=0.82). However, significant difference of the thickness of GBM was evident between MN cases with and without segmental sclerosis (1390nm in MN with sclerosis vs. 955nm in MN without sclerosis, P<0.05). There were no significant differences of the percentage of global sclerotic glomeruli, the degree of interstitial fibrosis and arteriosclerosis were noted between MN cases with and without segmental sclerosis. In conclusion, the glomerular capillary and endothelial cell injuries may be induced by GBM organization and thickening that contributed to the formation of segmental sclerosis in MN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1367**

**The Value of IgG Subclass Staining in Renal Biopsies with Membranous Glomerulonephritis (MGN)** Cheng C. Huang,[1] Sergey V. Brodsky,[1] Alia S. Albawardi,[1] Anjali A. Satoskar,[1] Gyongyi Nadasdy,[1] Lee A. Hebert,[2] Brad H. Rovin,[2] Tibor Nadasdy.[1] *[1]Pathology, The Ohio State University, Columbus, OH; [2]Medicine, The Ohio State University, Columbus, OH.*

A recent study suggested that the antigen responsible for idiopathic MGN is M-type phospholipase A2. However, differentiating idiopathic from secondary MGN in a kidney biopsy is difficult with the currently available methodologies. Previous studies indicate that the glomerular capillary deposits in idiopathic MGN contain primarily IgG4. The goal of our study was to examine the usefulness of IgG subclass staining in MGN.

We perform immunofluorescence staining for the IgG subclasses in every renal biopsy with IgG pre or codominant immune complex glomerulonephritis, including MGN. Between January 2007 and February 2010, 159 consecutive MGN cases were stained for IgG subclasses. Determining the underlying etiology in MGN can be difficult at the time of the biopsy because associated conditions may not be obvious at that time. Follow-up studies to explore additional underlying conditions in these patients are ongoing. At this stage, we have documented an underlying etiology in 30 of the 159 biopsies: systemic lupus erythematosus (n=14), Hepatitis C virus infection (n=5) hepatitis B virus infection (n=4) and malignancy (n=7). Of the 129 remaining (idiopathic?) MGN cases 84 (65%) were either IgG4 predominant (n=57) or codominant (n=27). In contrast, of the 30 secondary forms of MGN, only 13 (43%) were IgG4 predominant (n=8) or codominant (n=5).

Our findings indicate that based on IgG subclass staining, idiopathic and secondary forms of MGN cannot be differentiated reliably. However, in the absence of IgG4 predominance or codominance, an underlying secondary etiology should be suspected. The finding that in several "idiopathic" MGN cases an IgG subclass other than IgG4 is predominant indicates a heterogenous IgG response to different pathogenic antignens in this disease. Correlating IgG subclass staining with phospholipase A2 receptor and other potential antigens in MGN may be helpful in developing a new, more accurate etiologic classification of MGN

Disclosure of Financial Relationships: nothing to disclose

**F-PO1368**

**IgG1 Is the Predominant Immunoglobulin Subclass in Deposits in Hepatitis C Virus Infection Associated Membranous Glomerulonephritis (MGN)** Alia S. Albawardi,[1] Sergey V. Brodsky,[1] Anjali A. Satoskar,[1] Cheng C. Huang,[1] Gyongyi Nadasdy,[1] Lee A. Hebert,[2] Brad H. Rovin,[2] Tibor Nadasdy.[1] *[1]Pathology, The Ohio State University, Columbus, OH; [2]Medicine, The Ohio State University, Columbus, OH.*

The most common glomerular disease associated with Hepatitis C virus infection is a type of membranoproliferative glomerulonephritis with or without cryoglobulin deposits. Rarely, hepatitis C infection may also be associated with fibrillary glomerulonephritis and MGN but reports on Hepatitis C virus associated MGN are scant. The goal of our study was to see if hepatitis C virus associated MGN can be differentiated from idiopathic MGN based on renal biopsy findings.

We reviewed our renal biopsy files between January 2003 and June 2010 and identified 10 cases of MGN in Hepatitis C virus infected patients. All patients had variable degree of proteinuria and microscopic hematuria. Seven patients had increased serum creatinine levels. Most patients had normal serum complement levels and negative cryoglobulin tests. By routine light microscopy, immunofluorescence and electron microscopy, hepatitis C virus associated MGN could not be distinguished from idiopathic MGN. We performed immunofluorescence with antibodies to the IgG subclasses in all 10 biopsies. In 8 of the 10 biopsies, IgG1 was the predominant (in 5) or codominant (in 3) subclass in the glomerular capillary deposits. IgG4 was predominant in only one case and codominant with IgG1 in two cases. One biopsy was IgG3 dominant, and an additional one was IgG3 and IgG1 codominant. Interestingly, in one biopsy, fibrillary GN and MGN coexisted.

In our and others' experience, in idiopathic MGN, the glomerular capillary deposits are usually IgG4 predominant. The finding that in Hepatitis C infection related MGN the deposits are IgG1 predominant indicates that the pathogenetic antigen (probably a protein related to the virus) induces a different type of immune response than the antigen (e.g., phospholipase A2 receptor) in idiopathic MGN, despite the very similar morphology. Therefore, IgG subclass staining is helpful in differentiating idiopathic MGN from secondary forms, including Hepatitis C infection related MGN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1369**

**Class V Lupus Nephritis – At the Interface between Membranous Glomerulonephritis and Lupus Nephritis – Can IgG Subclass Staining Help?** Anjali A. Satoskar,[1] Cheng C. Huang,[1] Lee A. Hebert,[2] Brad H. Rovin,[2] Tibor Nadasdy.[1] *[1]Pathology, Ohio State University, Columbus, OH; [2]Nephrology, Ohio State University, Columbus, OH.*

From a histologic perspective, Class V (membranous) lupus nephritis overlaps with two major disease categories – idiopathic and other secondary forms of membranous glomerulonephritis (MGN); and proliferative lupus nephritis. It can be the presenting manifestation in systemic lupus erythematosus (SLE) in the absence of proliferative glomerular lesions. Therefore correctly recognizing it as a class of lupus nephritis and differentiating it from idiopathic and other causes of secondary MGN becomes important. The goals of our study are two fold: 1. look for differences in pre/co-dominant IgG subclass staining between lupus MGN and other forms of MGN; 2. see whether IgG subclass pre/co-dominance is similar in membranous lupus nephritis and proliferative lupus nephritides.

We examined 87 cases of lupus nephritis (including Class II, III, IV, V and combined) and 145 cases of MGN, (idiopathic n=129; other secondary forms n=16). We considered the predominant or co-dominant IgG subclass staining in each case for this study. The percentage of cases with pre/co-dominant staining for each IgG subclass in the examined disease categories are shown below.

Table 1. Percentage of cases with respective IgG subclass predominance

| Diagnosis | IgG1 | IgG2 | IgG3 | IgG4 |
|---|---|---|---|---|
| Lupus V | 75 | 20 | 30 | 40 |
| Lupus III/IV and V | 82 | 34 | 39 | 8.6 |
| Lupus III or IV | 84 | 22 | 52 | 4.5 |
| Secondary MGN | 56 | 0 | 12 | 50 |
| Idiopathic MGN | 47 | 2.3 | 19.3 | 65 |

IgG4 pre/co-dominance is highest in idiopathic MGN; it is also common in lupus MGN and other secondary forms of MGN but not in proliferative lupus nephritis, suggesting some association between IgG4 subclass response and subepithelial (membranous pattern) immune complex formation. IgG2 predominance is overall uncommon, but if present, favors lupus nephritis. IgG1 and IgG3 predominance makes idiopathic MGN less likely but cannot differentiate lupus from other secondary forms of MGN. IgG subclass staining in MGN and lupus nephritis is helpful but it should only be interpreted in the context of clinical, serological and morphologic findings.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1370**

**Prognosis and Risk Factors of Idiopathic Membranous Nephropathy in China** Ke Zuo, Yongchun Ge, Yan Wu, Sha-Lin Zou, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China.*

Idiopathic membranous nephropathy (IMN) is a representative form of refractory nephrotic syndrome in China. Although IMN is thought to run a more benign course in Asia than in the Caucasian population, there was no convincing study to show the long-term prognosis and risk factors of IMN in Chinese population. We enrolled all patients admitted to Nanjing Institution of Nephrology from January 1990 to December 2005 with biopsy proven IMN and at least 24 months follow-up in this retrospective chart review. The clinical and histopathologic parameters were recorded. At last a total of 217 patients were included in the study, age from 19 to 83, with male ratio 58.5%. The overall renal survival rate was 96.8%, 93.0% and 85.5% at 5, 10 and 15 years after renal biopsy, respectively. When clinical and histopathologic features of biopsy were evaluated, patients with hypertension (p=0.012) and elevated serum creatinine (Scr, $p<0.001$) had worse prognosis. Cox univariate analysis showed hypertension (HR=4.85, $p=0.022$), age section (one decade as a section, HR=1.65, $p=0.037$) and elevated Scr (HR=13.627, $p<0.001$) were clinical risk factors, and tubulointestinal chronic change stage (defined as fibrosis area percentage of cortical tubulointestinal tissue, every 25 percent as a stage, HR=3.627, $p<0.001$) was the pathological risk factor for end stage renal function (ESRF). Cox multivariate analysis showed elevated Scr (HR=11.0, $p=0.001$) and tubulointestinal chronic change stages (HR= 6.93, $p=0.002$) were independent risk factors for ESRF. During follow-up, proteinuria remission (including complete remission and partial remission) occurred in 174 patients (80.2%), and those patients have got a better prognosis than Non-remission (NR) patients ($p<0.001$). In conclusion, IMN is a disease with a comparatively good prognosis in Chinese population and elevated Scr, tubulointestinal chronic change and NR of proteinuria are independent risk factors of ESRF.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1371**

**Thin Basement Membrane Nephropathy Associated with Idiopathic Membranous Nephropathy: A Clinico-Pathological Study of Seven Cases** Su-Xia Wang, You-Kang Zhang, Lin-Chang Liu, Gang Liu, Zhi-Yong Cai. *Renal Division, Peking University First Hospital, Beijing, China.*

Thin basement membrane nephropathy (TBMN), the leading cause of microscopic hematuria in children and adults, is characterized by a diffusely thinned glomerular basement membrane (GBM). Previous studies indicated that TBMN might present with other glomerulopathies. However coexistence of TBMN with membranous nephropathy (MN) was rarely reported. In this study we investigated the prevalence and clinico-pathological features of subjects with TBMN and MN. Four hundred and seventy one subjects (Jan 2003-Dec 2009) diagnosed as stage IMN by renal biopsy were included in this study. All the specimens were re-evaluated by electron microscope. Seven (1.49%) of the 471 patients were diagnosed as stageIMN accompanied by diffuse thinning GBM (TBMN-MN). Thickness of the GBM was measured as 205.96±45.94nm. Thirteen patients with stageIMN and normal GBM thickness and 14 patients with only TBMN were taken as controls. The mean age and gender ratio were matched in three groups (Table 1). Patients with TBMN-MN had more prominent hematuria (p=0.015) and less proteinuria (p=0.019) compared with patients with stageIMN, and had more prominant proteinuria than TBMN group ($P=0.001$). After a follow-up by 22.3±16.4 months, the rate of remission (proteinuria <0.15g/24h) in TBMN-MN group was higher than in stage I MN group ($P=0.017$). So we conclude that TBMN could coexist with stage IMN. Patients with TBMN-MN presented less proteinuria and might have a better prognosis than patients with only stageIMN.

The clinical features of patients with TBMN-MN, stageIMN and TBMN

| | TBMN-MN (n=7) | Stage IMN(n=13) | TBMN(n=14) |
|---|---|---|---|
| Sex(male:female) | 1:6 | 6:7 | 3:11 |
| Mean age(years) | 43.0±12.2 | 48.5±13.8 | 41.4±9.8 |
| Duration (months) | 2.7±2.1 | 2.3±2.6 | 52.7±108.4 |
| Hematuria (>3 RBC/HP) | 7(100% )* | 5(38.46%) | 14(100%) |
| Proteinuria (g/24h) | 2.09±0.78*# | 7.19±5.14 | 0.30±0.25 |
| Serum albumin (g/L) | 37.17±3.64* | 30.51±6.74 | 40.05±3.73 |
| Creatinine(μmol/L) | 65.30±14.09 | 72.90±13.68 | 68.28±12.79 |

*p<0.05, TBMN-MN vs stage IMN; # P<0.05, TBMN-MN vs TBMN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1372**

**Effect of Fenofibrate on Symmetric Dimethylarginine and Asymmetric Dimethylarginine in Dyslipidemic Patients with Type 2 Diabetes** Jean-Claude Ansquer,[1] Paul E. Valensi,[2] R. Neil Dalton,[3] Isabelle M. Gottlieb,[1] Sylvie M. Le Mouhaer,[1] Michel C. Conte.[1] *[1]Clinical Research and Development, Cardiometabolic Unit, Laboratoires Fournier SA, Solvay Pharmaceuticals Now Part of Abbott, Daix, France; [2]Hôpital Jean Verdier, Bondy, France; [3]King's College London, St Thomas' Hospital, London, United Kingdom.*

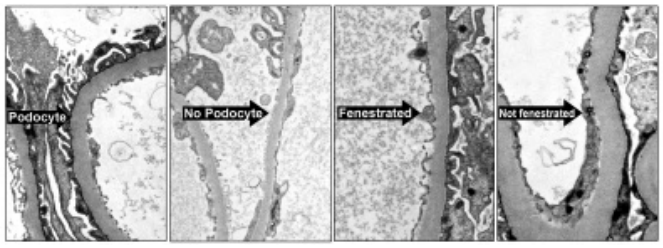
Background and purpose of study: Symmetric and asymmetric dimethylarginine (SDMA and ADMA) are proposed as endogenous markers of glomerular filtration and endothelial dysfunction, respectively. It is known that fenofibrate may induce an increase in creatinine as well as homocysteine levels. The relationships between SDMA and creatinine, ADMA and homocysteine were thus explored. Methods: We studied the effect of a 24-week fenofibrate treatment on SDMA and ADMA in a random sample of 118 patients from a double-blind, randomized, 2-arm study in patients with type 2 diabetes previously treated with metformin (HbA1c ≤8.5%) with dyslipidemia not appropriately controlled with a statin (triglycerides ≥1.69 mmol/L and LDL-C ≤3.35 mmol/L). The study compared fenofibrate 160mg and a stable dose of metformin (FM) daily versus placebo and metformin (M), in addition to statin therapy. Results: Creatinine increased by 7.0 μmol/L at end of treatment in the FM group and decreased by 0.6 μmol/L in the M group, from an overall mean baseline value of 75.9 μmol/L. Homocysteine increased by 4.7 μmol/L in the FM group and by 0.8 μmol/L in the M group, from an overall mean baseline value of 11.9 μmol/L. For both parameters the between group difference for absolute changes was significant (p<0.001). Mean values at baseline were 0.46 μmol/L for SDMA and 0.48 μmol/L for ADMA; these parameters were unchanged at end of treatment with FM or M (0.00 to 0.01 μmol/L). In both groups a minimal linear correlation between creatinine and SDMA and no linear correlation between homocysteine and ADMA were observed for changes from baseline. Conclusion: while not definitive, these results, using SDMA and ADMA as surrogate biomarkers, support the hypothesis that moderate increases in creatinine and homocysteine with fenofibrate treatment are independent of glomerular filtration and endothelial dysfunction, respectively.

Disclosure of Financial Relationships: Employer: Laboratoires Fournier S.A., Solvay Pharmaceuticals in now Abbott.

**F-PO1373**

**Podocyte Detachment and Reduced Endothelial Cell Fenestration in Type 2 Diabetic Nephropathy** E. Jennifer Weil,[1] Clinton C. Mason,[1] Berne Yee,[2] Bryan D. Myers,[3] Kevin V. Lemley,[4] Robert G. Nelson.[1] *[1]NIDDK, AZ; [2]Southwest Kidney Institute, AZ; [3]Stanford University, CA; [4]USC, CA.*

The quantitative relationships of podocyte detachment and reduced endothelial cell fenestration with the classic functional and structural changes of diabetic nephropathy and their appearance on microscopy are described in type 1 but not in type 2 diabetes (T2DM). Percent glomerular basement membrane (GBM) denuded of podocytes, percent of endothelium without normal fenestration, and the classic structural changes of diabetic glomerular injury were assessed morphometrically in 37 Pima Indians with T2DM; 11 with normoalbuminuria (albumin/creatinine ratio (ACR) <30 mg/g), 16 with microalbuminuria (ACR=30-299 mg/g), and 10 with macroalbuminuria (ACR≥300 mg/g). Glomerular filtration rate (GFR) was measured by iothalamate clearance. The mean proportion of GBM denuded of podocytes was higher in macroalbuminuric subjects than in those with normo- or microalbuminuria (1.4% vs. 0.3%; p=0.008) and correlated positively with ACR (r=0.36, p=0.030) and negatively with podocyte number per glomerulus (r=-0.40, p=0.015) and GFR (r=-0.33, p=0.047). The mean proportion of non-fenestrated endothelium was also higher in the macroalbuminuric group (81% vs. 73%; p=0.001) and correlated positively with GBM thickness (r=0.39, p=0.017), ACR (r=0.46, p=0.003), and fractional mesangial area (r=0.57, p<0.001) and negatively with GFR (r=-0.47, p=0.004). Percent GBM denuded of podocytes and percent of filtration surface not covered by fenestrated endothelial cells were not correlated (r=0.26, p=0.125).




Podocyte detachment and diminished endothelial cell fenestration are related to classic lesions of diabetic nephropathy and to kidney function in T2DM. The contribution of these factors to impairment of glomerular permselectivity and GFR remains to be determined.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1374

**Altered Expression Profile of Cadherins in Human Diabetic Nephropathy**
Nileshkumar Shah,[1] Jan Willem Leeuwis,[2] Dionne Van der Giezen,[2] Amelie Dendooven,[2] Tri Q. Nguyen,[2] Roel Goldschmeding,[2] Sarah Yates,[1] Mark Edward Dockrell.[1] *[1]SW Thames Institute for Renal Research, London, United Kingdom; [2]Pathology, University Medical Center Urtecht, Utrecht, Netherlands.*

Cadherins (CDH) are transmembrane proteins that maintain epithelial integrity and cell phenotype. CDH1 (E-cadherin) loss in proximal tubule epithelial cells (PTECs) has been studied extensively in the context of renal fibrosis. In this study we investigated CDH6 (K-cadherin) and CDH2 (N-cadherin) along with CDH1 in human diabetic nephropathy to assess their contribution in tubulointerstitial fibrosis.

We stained archived human kidney tissue (normal n=5 and diabetic nephropathy of various stages n=5 (tubular score 0-2, RPS DN Working Group, JASN 2010;21:556-63)) using antibodies to the extracellular domain of CDH1, CDH2 and CDH6 together with megalin (marker of proximal tubule (PT)) or calbindin D28K (marker of distal tubule (DT)). Localization was studied by confocal and epifluorescence microscopy. In a separate cohort we investigated the appearance of CDH6 in urine from 8 patients with diabetic nephropathy (stage 3&4) compared to healthy controls.

In normal human kidney CDH2 and CDH6 co-localized with megalin (PT), while CDH1 co-localized with calbindin D28K (DT). CDH6 and CDH2 staining showed a basolateral distribution in normal renal proximal tubule.

In early diabetic nephropathy lesions, however, expression of CDH6 and CDH2 on the basal side was hazy and discontinuous. In advanced lesions with marked tubulo-intersitial fibrosis, CDH6 and CDH2 expression was lost. 6/8 patients with CKD had detectable urinary full length CDH6; no full length CDH6 was detectable in control urine.

Our data confirm that CDH 2 & 6, but not CDH1 are present in human proximal tubules. In addition, CDH2 & 6 are depleted early in diabetic nephropathy. CDH6 is detectable in urine from diabetic patients with CKD. This loss of CDH6 in renal fibrosis correlates with our *in vitro* work on human proximal tubule cell models (submitted to this meeting in a separate abstract). This reduction may result in reduced cell cell contact, possible epithelial-to-mesenchymal transition or cell shedding.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1375

**Better Steroid Response in IgG4-Positive Tubulointerstitial Nephritis (TIN)**
Tae Young Kim,[1] Jung-Sik Park,[1] Kyoung Min Kim,[1] Soon Bae Kim,[1] Young Mee Cho,[2] Sung Hee Kang.[2] *[1]Nephrology, Asan Medical Center, Seoul, Korea; [2]Pathology, Asan Medical Center, Seoul, Korea.*

**Purpose**This study was performed to evaluate clinical manifestations of IgG4-positive TIN.**Methods** Out of 5,174 renal biopsies performed during January1996 to February2010,were 82 patients had TIN.TIN was defined as tubulointerstitial infiltration of inflammatory cells and fibrosis with atrophy and dilatation.Thirty six patients were excluded because of combined kideny disease.Biopsy tissues were lost in 2 patients.Immunperoxidase staining with IgG4 were performed in remaining 44 patients."Improve"was defined as"increase of eGFR more than25%".**Results**The median numbers of IgG4-positive plasma cells were 8(range 1-90)/HPF.The number of IgG4-positive plasma cell was significantly associated with the degree of proteinuria($r$=0.471, $p$=0.018) and age($r$=0.529, $p$=0.007)in 25 patients.Eighteen patients out of 25 IgG4 positive patients received steroid treatment and all of them improved,however thirteen patients out of 19 IgG4 negative patients received steroid treatment and only 7 of them improved($p$=0.002).When we analyzed among 3group(No IgG4 cell, 1≤IgG4cells<10, IgG4 cell≥10 ,no parameter was significantly different.

Characteristics

| characteristics | | IgG4 positive cell | |
|---|---|---|---|
| | positive(n=25) | negative(n=19) | p-Value |
| Age(yr) | 47±14 | 51±15 | 0.477 |
| Female gender [n(%)] | 14 (56) | 9(47) | 0.763 |
| Hypertension [n(%)] | 18(72) | 11(58) | 0.34 |
| Kidney size [n(%)] | | | 0.261 |
| normal or increased | 22(88) | 14(74) | |
| decrease | 3(12) | 5(26) | |
| Hepatobiliary disease [n(%)] | 8(32) | 4(21) | 0.431 |
| Sjogren' disease[n(%)] | 1(4) | 2(11) | 0.407 |
| Drug history [n(%)] | 5(20) | 7(37) | 0.223 |
| eGFR(ml/min/1.73m2) | 17.6±16.11 | 27.3±23.0 | 0.197 |
| Proteinuria (mg/day) | 1614±1017 | 1633±1108 | 0.972 |
| ALP(IU/L) | 144+93 | 149±167 | 0.554 |
| Outcome | | | 0.045 |
| improve | 18(72) | 8(42) | |
| aggravate or maintain | 7(28) | 11(58) | |

**Conclusion** IgG4 positive TIN showed better response to steroid treatment than IgG4 negative TIN.IgG4 staining should be routinely performed for TIN without other specific kidney disease.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1376

**Kidney Injury Molecule-1 Identifies High Proximal Tubular Injury as a Prominent Component of Monoclonal Nephropathy**
Ping L. Zhang,[1] Manish Nepal,[2] Michael F. Schultz,[2] Francis Dumler,[1] Joseph V. Bonventre.[3] *[1]Departments of Anatomic Pathology and Nephrology, William Beaumont Hospital, Royal Oak, MI; [2]Department of Nephrology, Geisinger Medical Center, Danville, PA; [3]Renal Division, Brigham and Women's Hospital, Boston, MA.*

The contribution of proximal tubular injury to renal failure has not been well studied in monoclonal nephropathy. The purpose of this study was to characterize proximal tubular injury in variants of monoclonal nephropathy, using kidney injury molecule-1 (KIM-1), a specific and sensitive injury marker for proximal tubules. A group of 20 control nephrectomy sections without injury (removed for renal tumors) and 23 cases of monoclonal nephropathy (including 3 with light chain deposition disease, 13 with cast nephropathy, 6 with AL/AH amyloidosis, and 1 with proximal tubulopathy) were stained for KIM-1 using immunohistochemical techniques (AKG monoclonal antibody). Staining scores in proximal tubules were graded from 0 (no stain) to 1+ - 3 + (mild to strong staining). Control sections stained negatively for KIM-1 (0.15 ± 0.05 arbitrary units). The corresponding serum creatinine (sCr) levels were within the normal range (1.01 ± 0.07 mg/dl). In the monoclonal nephropathy group, values were significantly higher (KIM-1: 2.33 ± 0.21 arbitrary units [p = 0.0001] and sCr: 5.54 ± 0.77 mg/dl [p = 0.0001], using unpaired student t test, respectively). A total of 82.6 % (19/23) of monoclonal nephropathy cases stained moderately or were strongly positive for KIM-1 in the proximal tubules. The KIM-1 staining scores were significantly correlated with sCr levels (r = 0.703; p = 0.0001). Electron microscopy confirmed deposition of monoclonal particles in several variants of monoclonal nephropathy. In summary, toxic monoclonal materials (mainly light chains) affect the proximal tubules in most cases of monoclonal nephropathy, regardless of whether the dominant findings are in glomeruli (light chain deposition disease), distal nephron tubules (cast nephropathy), or monoclonal amyloidosis. We conclude that KIM-1 staining has identified proximal tubular injury as an important component of acute kidney injury in this disease entity.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1377

**CD4+ T Cells Play a Major Role in the Development of Histological Phenotype of Kidney Injury**
Hitoshi Nakashima, Katsuhisa Miyake, Maho Watanabe, Takao Saito. *Div of Nephrolo & Rheumatolo, Dpt of Int Med, Fukuoka University, Fukuoka, Japan.*

In glomerulonephritis (GN), the variable Th1-Th2 predominance of response influences the histologic patterns and severity of GN. In most cases of rapidly progressive GN there is evidence for an important role for cellular immune effectors; Th1 cells, macrophages, and neutrophils. On the other hand the predominant deposition of IgG4 in idiopathic membranous nephropathy (MN) indicates that its presence characterizes the humoral immune response (Th2) of the disease. In tubulointerstitial nephritis (TIN), Mickliz's disease associated TIN, which is one of IgG4-related TIN, shows characteristic serum IgG4 elevation and increased IgG4-positive plasma cells in the renal interstitium. IgG4 is the rarest IgG subclass and is a Th2-dependent isotype. Therefore IgG4-related TIN may be developed in Th2 response. While TINs associated with Sjogren syndrome and with sarcoidosis is supposed to be developed in Th1 responses. CD4+ T cells are supposed to be key components of these kidney injuries.

In this study we evaluated the expressions of various cytokines in the biopsied kidney specimens by real time PCR, and compared of cytokine production pattern between ANCA related GN and idiopathic MN, and between Sjögren's syndrome associated TIN and Mickliz's disease associated TIN, respectively. The expression levels of IL-2, IFN-γ, IL-17 and IL-6 in ANCA related GN were significantly higher than those in MN. On the other hand the expression levels of IL-4, IL-10 and TGFβ in MN were significantly higher than those in ANCA related GN. Additionally the expression of Foxp3, a transcription factor specific for Treg cell, was also increased in MN. Similar contrastive results were obtained in the examination between Sjögren's syndrome associated TIN and Mickliz's disease associated TIN.

These findings suggested that Th1 and Th17 cell responses play a central role in pathogenesis in ANCA related GN and Sjögren's syndrome associated TIN. On the contrary in MN and Mickliz's disease associated TIN, Th2 and Treg cell responses play a major role.

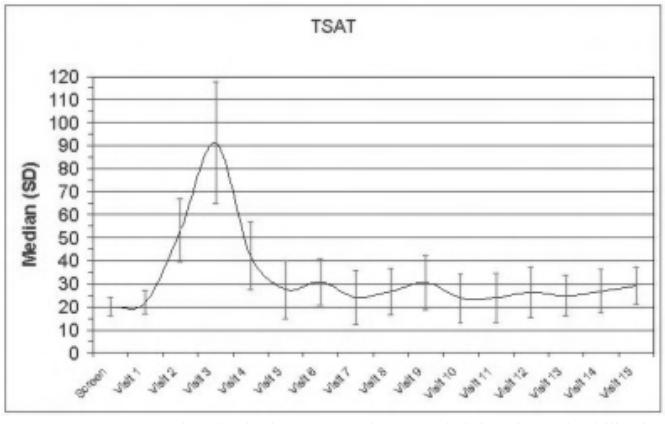
Disclosure of Financial Relationships: nothing to disclose

**F-PO1378**

**Iron Indices and Intravenous (IV) Ferumoxytol: Time to Steady State – A Pilot Study** Myra D. Carreon,[1] Toros Kapoian,[1] Gertrude S. Lefavour,[1] Richard A. Sherman,[1] John A. Walker,[1] Neeta O'Mara,[2] Amelia Gajary.[2] *[1]Med/Neph, UMDNJ-RWJ Med School, New Brunswick, NJ; [2]Dialysis Clinic, Inc., North Brunswick, NJ.*

**BACKGROUND:** Feraheme™ (ferumoxytol), the newest IV iron, is administered as two 510mg doses separated by 3-8 days; however, it is not known when iron saturation (TSAT) and ferritin can be accurately checked after a loading dose. This single center study investigated the time to stabilization of TSAT and ferritin in hemodialysis (HD) patients. **METHODS:** Two 510mg doses of Feraheme™ were administered 3 days apart (on visits 1 and 2) to 15 stable patients on thrice weekly HD (11M, 4F; age 60 ± 14yr; dry weight 80 ± 30kg). Patient selection was as follows: anemia due to iron deficiency, TSAT ≤ 25% or serum ferritin ≤ 200ng/dL; and ferritin <1200ng/dL; hemoglobin 10-13.5g/dL; no IV iron or blood transfusions within 30 days of visit 1. Blood was analyzed on dosing days and preceeding 13 subsequent consecutive treatments. Trends in serum ferritin, TSAT, and high sensitivity CRP were evaluated. **RESULTS:** After loading, TSAT and serum ferritin stabilized 3 and 5 treatments later, respectively. Peak values for TSAT and ferritin occurred 1 and 2 treatments after loading, respectively. No trend in CRP was noted. Three of 15 patients had end-of-study (EOS) visit 15 ferritin > 800ng/mL (2 of 3 had baseline ferritin > 800ng/mL). There were 7 adverse events that occurred during drug dosing visits including headache (3), hypotension (2), nausea (1) and hypertension (1). No events required hospitalization or discontinuation of the study.

| | Baseline | Peak | Stabilization | EOS |
|---|---|---|---|---|
| Ferritin (ng/mL) | 628 | 984 | 713 | 669 |
| TSAT (%) | 20 | 91 | 28 | 29 |
| CRP (mg/dL) | n/a | 5.4 | 5.2 | 5.7 |




**CONCLUSION:** There is a lag between Feraheme™ administration and stabilization of TSAT and ferritin. Iron studies should be drawn no sooner than 2 weeks after completion of a 2-dose load.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1379**

**Weekly (qW) Subcutaneously (SC) Administered Epoetin Alfa (Epo) Reduces Erythropoiesis-Stimulating Agents (ESA) Exposure While Maintaining Hemoglobin (Hb) Concentration** Toros Kapoian,[1] Neeta O'Mara,[2] Amelia Gajary,[2] Kathy Bivens.[2] *[1]Med/Neph, UMDNJ - RWJ Med School, New Brunswick, NJ; [2]Dialysis Clinic, Inc (DCI), North Brunswick, NJ.*

**Background:** In 2007, the FDA issued a warning about complications related to ESA exposure. Following that advisement, we altered our target Hb from 11-13 g/dL to 10-12 g/dL. To further minimize ESA exposure, in late 2008, we developed a SC Epo protocol that was put into effect in Apr/May 2009. We initially transitioned patients from thrice weekly (TIW) intravenous (IV) Epo to TIW SC Epo and then aimed to reduce the frequency to qW as quickly as possible. **Methods:** All DCI New Jersey HD patients who remained in the same facility during the study period from Jan 1, 2009 to Apr 30, 2010 were included. The initial TIW doses of SC Epo were 66-125% of the last IV Epo dose, depending on the baseline Hb. We analyzed the last 3 months (mos) of IV Epo utilization and compared this with SC Epo use during mos 1-3 and 7-9. Data on Epo dose (total Epo given per mo and units/kg/mo), Epo dosing frequency, number of administered (adm) doses and Hb were collected. **Results:** 160 patients (50% male, 56% white, 33% black, 46% diabetes, 24% hypertension) with mean age 66 years (range 26-94 years) and mean dialysis vintage of 5.5 years (range 1-32 years) were evaluated. Overall total monthly Epo utilization was reduced by 15.7%. Two-thirds of patients maintained Hb within the target range.

| | IV Epo | 3-Mo SC | 9-Mo SC | % Change |
|---|---|---|---|---|
| Epo Dose (total/mo) | 32,487,150 | 27,737,550 | 27,390,900 | ↓ 15.7 |
| Epo Dose (units/kg/mo) | 443,392 | 344,812 | 372,577 | ↓ 16.0 |
| Doses Adm | 5152 | 4629 | 2393 | ↓ 53.6 |
| Dosing qW | 14 | 92 | 121 | ↑ 764.3 |
| Dosing BIW | 2 | 8 | 23 | ↑ 1050 |
| Dosing TIW | 144 | 60 | 16 | ↓ 88.9 |
| % Hb < 10 | 5.0 | 13.7 | 15.0 | ↑ 200 |
| % Hb 10-12 | 63.7 | 61.3 | 66.3 | ↑ 4.0 |
| % Hb > 12 | 31.3 | 25.0 | 18.7 | ↓ 40 |

**Conclusions:** Conversion to SC Epo reduces Epo exposure by about 15% and reduces most patients to a weekly Epo dosing schedule while maintaining Hb in the target range.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1380**

**Race Does Not Appear To Influence ESA Use or Time to Hemoglobin Threshold among Elderly Patients Initiating Hemodialysis** Areef Ishani,[1,2,3] Haifeng Guo,[1] Suying Li,[1] Lih-Wen Mau,[1] Tom Arneson,[1] Stephan C. Dunning,[1] David T. Gilbertson,[1] Allan J. Collins.[1,3] *[1]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN; [2]Minneapolis Veterans Affairs Medical Center, Minneapolis, MN; [3]Department of Medicine, University of Minnesota, Minneapolis, MN.*

The Centers for Medicare & Medicaid Services has proposed to change reimbursement for outpatient dialysis to a fixed-payment bundle that will include both outpatient dialysis therapy and injectable medications. The proposal does not include any adjustment for race. We have previously demonstrated that African-Americans (AA) use more ESAs during the first two months after dialysis initiation. For this study we aimed to determine if race influenced the time to a hemoglobin threshold of 11g/dL and total ESA use over the first six months of dialysis.

A cohort was constructed of 12,184 ESA-naïve patients who initiated hemodialysis between 1/2006, and 6/2007, were > 67 years at initiation, had Medicare as the primary payer for 2 years pre-initiation, and received erythropoietin (EPO) therapy (determined from Medicare claims) continuously during the first 6 post-initiation calendar months. Cox regression was conducted to determine the association between race and time to hemoglobin > 11g/dL. Linear regression was performed to determine the association between race and the cumulative EPO dose over six months.

Over the first 6 months of dialysis, AA lagged whites in their time to achieve the threshold hemoglobin value (HR 0.86; 95% CI 0.83-0.90). However, by 6 months the cumulative percent of individuals achieving the threshold hemoglobin value was identical by race (98%). Also, by six months there was no difference in the cumulative EPO dose used between AA and white patients (beta=0.003; p=0.87). These updated results suggest that early after dialysis initiation, AA lag whites in achieving a hemoglobin of 11g/dL, but this difference disappears by 6 months. These similar hemoglobin concentrations are achieved with similar EPO doses. These results suggest that the new Medicare bundle may not disadvantage AA with regard to ESA use within the first six months of dialysis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1381**

**NT-proBNP Levels Are Associated with Severity of Anemia in CAPD Patients without Chronic Heart Failure** Yang Wook Kim,[1] Kyu-Bok Jin,[1] Tae Hee Kim,[2] Hyun Seung Lee.[2] *[1]Nephrology, Internal Medicine, Haeundae Paik Hospital, Inje University, Pusan, Korea; [2]Nephrology, Internal Medicine, Busan Paik Hospital, Inje University, Pusan, Korea.*

**Objective:** Natriuretic peptides are important in the maintenance of body volume homeostasis and elevated in patients with elevated myocardial wall stress. NT-proBNP is as a prognostic marker for risk of cardiovascular events. However, NT-proBNP is almost increased in patients with renal dysfunction. So, It may be limited to clinical application to chronic renal failure and dialysis patients, without symptoms of heart failure,especially.

The aim of this study was to investigate the factors associated with increased NT-proBNP level in asymptomatic patients within 1 month on continous ambulatory peritoneal dialysis(CAPD) without heart failure.

**Methods :** We enrolled 30 patients(14males, 16 females),aged 26-81years, who started to treat with CAPD within 1 month. We measured serum NT-proBNP, Hb, TnI(cardiac troponin I), iPTH, CK-MB and CRP in patients without heart failure through echocardiography during the same period.

**Results:** The mean LV ejection fraction was 62.86%(S.D.: 9.76%) and the mean LVMI was 133.30mg/m2 (S.D.: 30.57 mg/m2). The serum NT-proBNP in all patients was increased by the manufacturer-recommended age-specific cut-off(mean: 8,018 ± 12,119 pg/ml) and all TnI levels were within normal range. The mean hemoglobin was 9.53mg/dl (S.D.: 1.58 mg/dl). In the correlation analysis, the serum NT-proBNP levels showed reverse correlation with hemoglobin(r:-0.48; p <0.05). However, it did not correlate with TnI, CRP, IPTH, and CK-MB.

**Conclusions:** We suggest that elevated levels of NT-proBNP are associated with severity of anemia in CAPD patients without heart failure. The aggressive anemia treatment should be considered for the prevention of cardiovascular events in CAPD patients with elevated NT-proBNT level, not associated with chronic heart failure.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1382**

**IV Iron Replacement Therapy in Patients with ESRD Undergoing Home Dialysis: Experience with Ferumoxytol** Andrea Neitzer, Sheila Doss, Sumi Sun, Brigitte Schiller. *Satellite Healthcare, Mountain View, CA.*

IV iron administration in patients undergoing home dialysis is often challenging due to frequent visit requirements in order to administer sufficient iron. Feraheme (ferumoxytol), a novel iron product, allows for a rapid IV push administration of 510 mg. Ferumoxytol was introduced in an iron maintenance protocol for home dialysis patients in January 2010 following routine monthly blood work in 18 home dialysis clinics. The protocol prescribes 510 mg ferumoxytol to be given if TSAT <30% OR ferritin <500 ng/mL. Contraindications are iron hypersensitivity, ferritin ≥ 800 ng/mL, TSAT ≥50% or Hb > 12.5 g/dL.

A total of 431 incident and prevalent patients treated with peritoneal dialysis or home HD received 598 doses of ferumoxytol until the end of May 2010. 1.6% (7/431) of patients experienced adverse events including allergy, hypotension, and gastrointestinal symptoms resulting in an AE rate of 1.2% (7/598 doses). An SAE occurred in one patient due to an anaphylactoid reaction which resolved completely without sequelae.

A subset of 341 patients treated exclusively with ferumoxytol on this iron maintenance protocol with follow-up data during the 5 months required an average of 1.4 doses (range 1-4). 68% of patients were dosed only once, 26% twice, and 6% required three or more doses. Laboratory results are shown as mean ± SD 5 months prior and during ferumoxytol therapy.

| | Pre-ferumoxytol | Ferumoxytol |
|---|---|---|
| Hb (g/dL) | 11.5 ± 1.0 | 11.2 ± 0.9 |
| TSAT (%) | 32.9 ± 12.5 | 31.9 ± 10.5 |
| Ferritin (ng/mL) | 458 ± 245 | 508 ± 259 |

Clinical performance targets in patients treated solely with ferumoxytol have been reached by 74% of patients for Hb (10-12 g/dL), 85% for TSAT (20-50%), and 75% for ferritin (200-800 ng/mL).

These data indicate that ferumoxytol administered as one time 510 mg IV push in home dialysis patients is well tolerated and achieves excellent iron maintenance results. Its favorable utilization with rapid IV push and less frequent administration and a good safety profile confirming published data make ferumoxytol a valuable alternative for treating iron deficiency in home dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1383**

**Angiotensin Receptor Blocker Treatment Might Affect ESA Response through Disturbing Iron Homeostasis in Hemodialysis Patients** Kanemitsu Yamaya,[1] Yasushi Shimonaka,[2] Hisao Saitoh,[1] Tomihisa Funyu,[1] Hideaki Yamabe,[3] Chikaraa Ohyama.[3] *[1]Oyokyo Kidney Research Institute, Hirosaki, Aomori, Japan; [2]Chugai-pharmaceutical Co., Ltd., Kamakura, Kanagawa, Japan; [3]Hirosaki University, Hirosaki, Aomori, Japan.*

Purpose

Anemia and hypertension are the major complications in hemodialysis (HD) patients. It has been reported that the antihypertensive treatment such as angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are related to erythropoiesis stimulating agents (ESA) hyporesponsiveness in hemodialysis (HD) patients. In the present study, we analyzed the relationship between ARB treatment, ESA hyporesponsiveness and iron homeostasis in HD patients.

Methods

We enrolled 456 HD patients, provided written informed consent in Oyokyo Kidney Research Institute. Patients with infection, severe inflammatory disorders or hematologic disorders were excluded. Anemia and other complications were treated according to national and K/DOQI guidelines. ESA dose was altered biweekly to maintain a target hemoglobin (Hb) level of 10-11g/dL. Clinical parameters and serum levels of hepcidin were measured at 0, 3 and 6 months after started observation.

Results

At the start of the study, 170 patients were without antihypertensive agents. On the other hand, 29 patients were received ACEIs and 242 patients were received ARBs. Fifteen patients were received Ca blockers or other agents. ESA dose and serum levels of hepcidin were significantly higher in patients received antihypertensive agents than in patients without antihypertensive agents. ARB received patients exhibited significantly higher ESA dose and serum levels of hepcidin than patients without antihypertensive agents, although Hb and ferritin were comparable. These results were consistent throughout observation period. Moreover, in 30 patients newly received ARB during observation period, serum levels of hepcidin was significantly elevated.

Conclusions

Present results indicate that antihypertensive treatment using ARB might affect ESA responsiveness through interfering with iron homeostasis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1384**

**High Level of Non Transferrin Bound Iron (NTBI) in the Patients with Maintenance Hemodialysis (MHD) Is Linked to Erythropoietin Hyporesponsiveness** Takahiro Kuragano, Takeshi Nakanishi. *Hyogo College of Medicine, Department of Internal Medicine, Division of Kidney and Dialysis, Nishinomiya, Hyogo, Japan.*

Background/Aim; Serum free iron known as 'non transferrin bound iron' (NTBI) is potentially toxic, possibility leading to free radical generation. To clarify how much free iron in serum in maintenance hemodialysis (MHD) patients without intravenous (IV) iron administration and relationship between free iron and anemia therapy, we evaluated serum levels of NTBI, anemia and iron parameters. Method; 126 MHD patients, who were treated with erythropoietin not receiving IV iron in recent 6 months were recruited in this study. We measured blood level of hemoglobin (Hb), total cholesterol, triglyceride, iron, hepcidin, ferritin, total iron binding capacity (TIBC), transferrin saturation (TSAT), non transferrin bound iron (NTBI), IL-6, TNF-α, and high sensitive (h) CRP. Result; Serum levels of NTBI were significantly (P=0.04, R=0.18) correlated ferritin. There was no significant correlation between serum NTBI level and iron, TIBC, TSAT, transferrin, hepcidin, hCRP, IL-6 and TNF-α. Multivariate analysis was performed to investigate the predictors of NTBI in MHD patients using several variables (ferritin, TSAT, total cholesterol), which had a tendency for significant ($p<0.1$). Only ferritin was selected as a significant determinant of NTBI in MHD patient ($\beta=0.69$, $F=92$, $p<0.0001$). To investigate the relationship between NTBI and iron utilization in MHD patients, we divided into 4 groups according to Hb and ferritin level. Group I (n=37): low Hb (<10g/dL) and low ferritin (<100 ng/dL). Group II (n=20): low Hb and high ferritin (≥100g/dL). Group III (n=45): normal Hb (10≥g/dL) and low ferritin. Group IV (n=26): normal Hb and high ferritin. Serum level of NTBI of group II (0.72± 0.5μM)) was significantly ($p<0.05$) higher than group I (0.57±0.35μM), and III (0.58±0.28μM). Conclusion; In MHD patients, serum NIBI could be associated iron storage. In the patients with low Hb and high ferritin, serum level of NTBI was significantly higher than other groups, which might be relevant to the erythropoietin hyporesponsiveness.

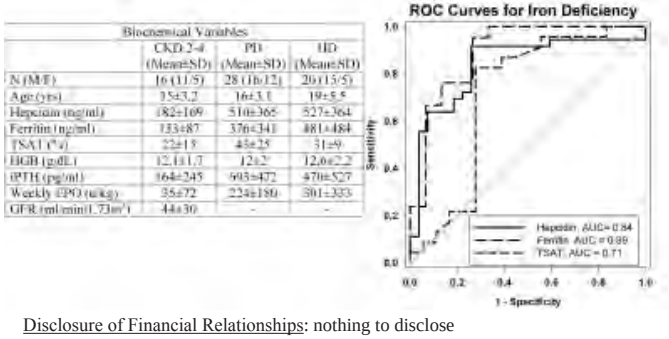
Disclosure of Financial Relationships: nothing to disclose

**F-PO1385**

**Hepcidin, a Novel Diagnostic Marker of Iron Deficiency in CKD** Joshua Zaritsky,[1] Carlos Eduardo Azucena,[1] Brian Y. Young,[2] Barbara Gales,[1] Mark E. Westerman,[3] Tomas Ganz,[2] Isidro B. Salusky,[1] R. C. Pereira.[1] *[1]Pediatrics, UCLA, Los Angeles, CA; [2]Medicine, UCLA, Los Angeles, CA; [3]Intrinsic Life Sciences, La Jolla, CA.*

Hepcidin is a key regulator of iron homeostasis but its diagnostic utility remains untested in the setting of chronic kidney disease (CKD). Thus, we sought to compare serum hepcidin levels to the gold standard of iron status; bone marrow (BM) stained for iron-laden macrophages and erythroid precursors. Serum hepcidin was measured by competitive ELISA along with other iron markers in 64 pediatric patients with CKD stages 2-5D (Table) who underwent iliac crest bone biopsy. BM was Perl stained and iron was quantified blindly by two methods; 1) semi-quantitative: graded from 0 to 4+ (absent to diffuse) staining and 2) quantitative; positive stained area divided by total tissue area. With the reference standard (grade 0 staining), iron deficiency was present in 50% of patients. Using a cutoff of 200 ng/ml, hepcidin was 93% specific, 64% sensitive and had a positive predictive value (ppv) of 90% for the diagnosis of iron deficiency. This was similar to that of ferritin (cutoff of 150 ng/ml, 93% specific, 66% sensitive, 90% ppv) and superior to transferrin saturation (TSAT) (cutoff of 30%, 72% specific, 80% sensitive, 70% ppv) ($p<0.01$)(Figure). Hepcidin levels showed a high degree of correlation with quantitative staining ($r=0.7$, $p<0.001$) similar to that of ferritin ($r=0.7$, $p<0.001$) and TSAT ($r=0.5$, $p<0.002$). These findings suggest that hepcidin levels are as sensitive and specific for the diagnosis of iron deficiency as current day iron markers. Thus hepcidin may be novel and useful biomarker of iron status across the spectrum of pediatric CKD.

| Biochemical Variables | CKD 2-4 (Mean±SD) | PD (Mean±SD) | HD (Mean±SD) |
|---|---|---|---|
| N (M/F) | 16 (11/5) | 28 (16/12) | 26(15/5) |
| Age (yrs) | 15±3.2 | 16±3.1 | 19±5.5 |
| Hepcidin (ng/ml) | 182±109 | 510±365 | 527±364 |
| Ferritin (ng/ml) | 133±87 | 376±341 | 481±484 |
| TSAT (%) | 22±15 | 45±25 | 31±9 |
| HGB (g/dL) | 12.1±1.7 | 12±2 | 12.0±2.2 |
| iPTH (pg/ml) | 164±245 | 593±472 | 478±527 |
| Weekly sPO (u/kg) | 35±72 | 224±180 | 301±333 |
| GFR (ml/min/1.73m²) | 44±30 | - | - |




Disclosure of Financial Relationships: nothing to disclose

**F-PO1386**

**Effect of Intravenous and Oral Ascorbic Acid in Hemodialysis Patients with Anemia and Hyperferritinemia** Ji-Min Jeon,[1] Yong-Ki Park,[1] Ji-Hwan Kim.[2] *[1]Division of Nephrology/Internal Medicine, DongRae BongSeng Hospital, Busan, Korea; [2]Division of Nephrology/Internal Medicine, Good Gang-An Hospital, Busan, Korea.*

Hemodialysis patients with anemia and hyperferritinemia often develop resistance to recombinant human erythropoietin(EPO). Ascorbic acid is believed to improve anemia in hemodialysis patients. We evaluated the efficacy of intravenous and oral ascorbic acid on Epo-hyporesponsive anemia in hemodialysis patients with hyperferritinemia. Forty-seven of 156 hemodialysis patients with Hb < 11g/dL and ferritin levels greater than 300ng/ml were prospectively followed up. Patients were randomly divided into three groups. 16 patients had received standard care(group 1), 17 patients had received standard care and daily oral ascorbic acid at a dose of 500mg/day(group 2) and 14 patients had received standard care and 300mg of intravenous vitamin C with each dialysis session(group 3). Each groups were similar in clinical characteristics. Blood samples for measurement of Hemoglobin, hematocrit, serum iron, ferritin, transferrin saturation and EPO dose were obtained at baseline and after three months of treatment. After 3 months, Hemoglobin and hematocrit and transferrin saturation levels significantly increased in group 2,3($p<0.05$) but not changed in group 1. EPO dosage and ferritin levels decreased in group 2,3($p<0.05$).

table 1. Baseline and 3 month Data summary

| | Group 1 | | Group 2 | | Group 3 | |
|---|---|---|---|---|---|---|
| | baseline | after 3 month | baseline | after 3 month | baseline | after 3 month |
| Hb(g/dL) | 8.9 ± 1.4 | 8.8 ± 1.3 | 8.8 ± 1.3 | 9.5 ± 1.1* | 9.0 ± 1.2 | 9.6 ± 1.2* |
| Fe(μ/L) | 55.84 ± 23 | 58.6 ± 19 | 58.57 ± 25 | 63.7 ± 22* | 61.12 ± 21 | 67.29 ± 19* |
| EPO dose(unit/week) | 14729 | 14538 | 15584 | 12369* | 14986 | 11345* |
| Ferritin(μg/L) | 764 ± 217 | 781 ± 192 | 783 ± 191 | 646 ± 189* | 754 ± 220 | 634 ± 231* |
| T sat(%) | 18 ± 6.4 | 18.2 ± 5.8 | 20.6 ± 5.5 | 26.4 ± 4.1* | 19.8 ± 4.9 | 24.5 ± 3.2* |

* is $p < 0.05$

There was no difference in group 2 and 3. In conclusion, Our study has demonstrated that intravenous or oral ascorbic acid therapy can improve anemia, hyperferritinemia and EPO resistance in hemodialysis patients. The effect of intravenous and oral ascorbic acid are similar. Further studies are needed to determine ascorbic acid dosing optimization.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1387**

**Cumulative Safety Experience in a Cohort of Patients with End Stage Renal Disease (ESRD) on Hemodialysis Receiving Ferumoxytol** Premila Bhat, Joesan A. Gabaldon, Jodumutt Ganesh Bhat. *Atlantic Dialysis Management Services, LLC, Ridgewood, NY.*

Ferumoxytol is a novel intravenous (IV) iron characterized by a carbohydrate-coated superparamagnetic iron oxide particle shown to result in lower free iron levels than other IV iron formulations. Ferumoxytol has been studied in over 1500 Chronic Kidney Disease and ESRD patients with a low occurrence of serious adverse drug reactions. We report here our experience treating a cohort of ESRD patients receiving hemodialysis at a regional chain of hemodialysis centers. An anemia-management protocol using ferumoxytol for IV injection was implemented. Patients were treated with ferumoxytol (510 mg injection delivered at a rate of up to 30 mg/sec) if they had Hemoglobin <13 g/dL; Transferrin Saturation <30% or Ferritin <500ng/mL; and no documented allergies or intolerance to other intravenous irons. Adverse drug events were prospectively monitored and reported. 609 patients received at least one dose of ferumoxytol in the three months following implementation of the ferumoxytol-based strategy (total 1379 doses, mean 2.26 doses/ patient). Eight injection-related adverse drug events were recorded. Six of 8 events were mild and included itching/rash (n=2), nausea (n=2), burning sensation (n=1), and a single hypotensive event wherein the patient transiently lost consciousness. This patient's hypotension and unconsciousness resolved upon completion of ferumoxytol injection. No other interventions were performed, and the patient did not require hospitalization. There were two (n=2) anaphylactoid reactions treated with IV corticosteroids and antihistamines. Both patients were hospitalized but neither required mechanical ventilation or intensive care. Anaphylactoid reactions occurred with the patients' first and second doses of ferumoxytol, respectively. In conclusion, ferumoxytol appears to be generally well tolerated when used to treat iron deficiency anemia in dialysis patients. Most reactions were mild, and occurrence of injection-related anaphylaxis was 0.15%, consistent with the incidence of such events in clinical trials to date.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1388**

**Tissue Distribution of $^{14}$C-Peginesatide Following IV Administration in Albino and Pigmented Rats** Kathryn W. Woodburn,[1] Yoshihiko Tagawa,[2] Yuu Moriya,[2] Christopher P. Holmes.[1] *[1]Affymax Inc, Palo Alto, CA; [2]Takeda Pharmaceutical Company, Ltd, Yodogawa-ku, Osaka, Japan.*

**Background:** Hematide™/peginesatide is a PEGylated, investigational, peptide-based erythropoiesis-stimulating agent that was designed and engineered to stimulate specifically the erythropoietin receptor dimer that governs erythropoiesis. This study was conducted to evaluate the tissue distribution of a single IV dose of $^{14}$C-peginesatide in both albino and pigmented rats. **Methods:** Male Sprague-Dawley rats (albino) were administered a single dose of 5 mg/kg $^{14}$C-peginesatide and evaluated (3 animals per time point) at 0.25, 6, 24, 168, 336, 720 and 1440 h following dosing for the evaluation of tissue radioactivity. The same procedure was following for male Long Evans rats (pigmented), with plasma, skin and eyes being evaluated. **Results:** In albino rats, the plasma concentration of radioactivity was 130.7 μg equiv./mL at 0.25 h following dosing, which decreased to 64.1 μg equiv./mL by 24 h. The concentrations in the other tissues were much lower than in the plasma at these time points, suggesting limited distribution beyond the vascular compartment. At 336 h after administration to albino rats, localization of radioactivity was observed in the bone marrow (6.3 μg equiv./g) and sites of extramedullary hematopoiesis including the spleen (23.6 μg equiv./g), adrenal glands (9.2 μg equiv./g), liver (8.5 μg equiv./g), and kidney (4.5 μg equiv./g) compared to brain (0.1 μg equiv./g), spinal cord (0.07 μg equiv./g), and muscle (0.4 g equiv./g). Elimination of $^{14}$C-peginesatide derived radioactivity was prolonged as was present up to 1440 h postdose. In pigmented rats, the concentrations of radioactivity in the plasma, eyes and skin were comparable to those in the albino rats, indicating that $^{14}$C-peginesatide and related compounds have little affinity for melanin. **Conclusions:** The elimination of $^{14}$C-peginesatide-derived radioactivity was prolonged, and biodistribution occurred in the target site (bone marrow) and sites of extramedullary hematopoiesis.

Disclosure of Financial Relationships: Employer: Affymax Inc; Ownership: Affymax Inc.

**F-PO1389**

**Inflammation and Other Modifiable Patient Characteristics Associated with ESA Dose: Results from the DOPPS** James P. Smith,[1] Jinyao Zhang,[2] Yun Li,[1] Hal Morgenstern,[1] Tadao Akizawa,[3] Joan Fort,[4] Friedrich K. Port,[1,2] Bruce M. Robinson.[1,2] *[1]University of Michigan, Ann Arbor, MI; [2]Arbor Research Collaborative for Health, Ann Arbor, MI; [3]Showa University, Tokyo, Japan; [4]University Hospital Vall d'Hebron, Barcelona, Spain.*

Background: A better understanding of potentially modifiable patient characteristics that influence ESA dose may inform strategies to optimize ESA usage. Inflammation is a putative cause of higher ESA requirements, but this association largely derives from small cross-sectional and retrospective studies.

Methods: We investigated predictors of ESA dose in DOPPS 3 (2005-2008), an international, prospective cohort study. A linear mixed model was used to determine associations of mean weekly ESA dose (updated monthly) with CRP and other lab values from the prior month, adjusted for Hgb level, demographics, comorbidities, and dialysis facility (to account for discretionary dosing practices). Many facilities in DOPPS countries measure CRP by routine.

Results: The analysis included 2,535 pts (21,903 pt-months) from 7 European countries (85%), and Australia/NZ (15%). We excluded USA and Canada because of infrequent CRP measurement and Japan because of notably different ESA dosing practices. The median ESA dose was 9900 U/wk (IQR: 5250, 15250). Across increasing quartiles of CRP, the median ESA dose was 8250, 9000, 9900, and 13000 U/wk ($P<0.001$, test of trend accounting for country). In the adjusted model, higher ESA dose was predicted by younger age, higher weight, lower Hgb, lower TSAT, higher log-CRP, higher ferritin, and lower WBC (all $P \leq 0.01$). Neither albumin, creatinine, nor PTH was appreciably associated with ESA dose.

Conclusion: Inflammation was positively associated with ESA dose in this representative multinational sample of HD patients. Albumin, creatinine, and PTH do not predict ESA dose after controlling for confounders, however, suggesting that nutritional status and hyperparathyroidism may not contribute substantially to ESA dose requirements. Interventions to prevent or treat inflammation may reduce ESA requirements.

Disclosure of Financial Relationships: Research Funding: The DOPPS is administered by Arbor Research Collaborative for Health and is supported by scientific research grants from Amgen (since 1996), Kyowa Hakko Kirin (since 1999, in Japan), Genzyme (since 2009), and Abbott (since 2009), without restrictions on publications.

**F-PO1390**

**The Effect of Intravenous Ascorbic Acid in Continuous Hemodialysis Patients with Anemia** Hyun Lee Kim,[1] Byung Chul Shin,[2] Jung Hoon Chung.[1] *[1]Internal Medicine, Chosun University Hospital, Gwangju, Republic of Korea; [2]Internal Medicine, Seonam University Hospital, Gwangju, Republic of Korea.*

Hemodialysis patients with functional iron deficiency often develop resistance to recombinant human erythropoietin (EPO). Recent studies suggest that intravenous ascorbic acid administration could override EPO resistance in hemodialysis patients. This study was undertaken to test the effect of intravenous ascorbic acid in patients with hemodialysis accompanied with EPO-hyporesponsive anemia. A total of 35 patients (16 men, 19 women) with ESRD on hemodialysis were included in this study. The patients received 500 mg of intravenous Vitamin C with each dialysis session. The effects of Vitamin C on EPO were assessed during the study periods of 4months, and additional 3months follow up after the end of the study. The mean age was 54.6±12.3 years, and the mean duration of hemodialysis was 5.7±4.2 years. The main cause of renal disease was diabetic nephropathy. The end of the vitamine replacement, Hb levels significantly increased from 9.4 to 10.6 g/dL. Hct (27.5 to 31.6%), RBC mass (3.0 to 3.3×103 ug/L), TSAT (30.9 to 38.0%) also significantly increased. EPO dose (136.5 to 72.9 IU/kg/week) is significantly decreased. The 3months after the end of the study, no change in Hb (10.6 to 10.3 g/dL), EPO dose (72.9 to 101.2 IU/kg/week) was noted. But, Hct (31.6 to 29.4%), RBC mass (3.3 to 3.1×103 ug/L), serum iron (81.6 to 67.0 ug/dL), TSAT (38.0 to 30.9%) significantly decreased. There were no change in TIBC, ferritin, calcium, phosphorus, intact parathyroid hormone levels, Kt/V, CRP, aluminum and Vitamin B12. Intravenous ascorbic acid can be a potent and effective adjuvant therapy for hemodialysis patients with EPO-resistant anemia. And ascorbic acid can reduce the dosage of EPO for anemia correction.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1391**

**Cinacalcet Hydrochloride Reduced the ESA Dose by Improving Inflammation on Dialysis Patients** Sato Megumi. *Nephrology, Satojunnkannkikanaika, Matsuyama City, Ehime, Japan.*

[Introduction)

High level of parathyroid hormone (PTH) is well known as a mortality risk factor independently from serum levels of calcium and phosphorus, however, one more important aspect of PTH is a cause of refractoriness to erithropoiesis stimulating agent (ESA). We evaluated the effects of cinacalcet hydrochloride (CH), a new medication for 2HPT, on the responsiveness to ESA.

[Methods)

Nineteen out of 157 chronic dialysis patients in our facility have been followed under the treatment of CH for 1 year based on our therapeutic policy for 2HPT (CH group). The rest 138 patients have been also followed as a control (Cont group). Serum levels of intact- PTH (i-PTH), albumin (Alb), high-sensitive CRP (hs-CRP) and the dosages of darbepoietin-alpha (DA; microgram/week) have been monitored before and 1 year after CH. The patients in each group were divided in to 3 subgroups by the pre-/post ratio in hs-CRP; subgroup 1(-10% >), subgroup 2(-10%˜+10%), subgroup 3(+10% <).

[Results)

In the CH group, the serum level of i-PTH was significantly reduced in each subgroup, whereas the dosage of DA was clearly decreased only in subgroup 1. The levels of Hb, Alb didn't change significantly in each subgroup (table 1) . In the Cont group, there were not significant changes in i-PTH, Hb and dosages of DA in each subgroup. Bone density, the iron saturation level and volume of parathyroid in the CH group did not change(Data not shown).

table 1 Parameters in CH group

| | n | i-PTH(pre) (pg/ml) | i-PTH(post) (pg/ml) | Hb(pre) (g/dl) | Hb(post) (g/dl) | DA(pre) (mcg/w) | DA(post) (mcg/w) |
|---|---|---|---|---|---|---|---|
| subgroup 1 | 5 | 575.0±424.0 | 150.0±59.9* | 11.7±1.8 | 12.0±1.8 | 19.0±12.5 | 6.0±5.5*[1] |
| 2 | 10 | 475.1±238.5 | 143.2±41.0*[2] | 10.9±2.5 | 10.8±3.5 | 8.3±7.5 | 9.8±10.0 |
| 3 | 4 | 432.5±184.7 | 79.0±29.8*[3] | 11.4±0.4 | 11.2±0.9 | 3.3±2.4 | 10.0±8.2 |

*˜*[3]:p<0.05

[Conclusions)

The reduction of ESA dosage in the relation to the improvement of inflammation was only observed in the CH group. It suggests that CH improves responsiveness to ESA by improving the systemic inflammatory status.

Disclosure of Financial Relationships: Employer: Satojunnkannkikkanaika Dr.Yuzuru Sato PhD.

**F-PO1392**

**Reduced ESA Requirement by Continuous Delivery of Soluble Ferric Pyrophosphate (SFP) Via Dialysate in CKD-HD Patients** Ajay Gupta,[1] Richard C. Yocum,[1] Carrie D. Guss,[1] Rosemary Ouseph,[2] Rajiv Agarwal.[3] *[1]Rockwell Medical, Wixom, MI; [2]University of Louisville, Louisville, KY; [3]Indiana U, Indianapolis, IN.*

BACKGROUND: SFP is an investigational, water-soluble, non-carbohydrate, tightly complexed salt of iron (Fe) (III) electrostatically bonded to pyrophosphate for continuous maintenance Fe therapy. A double-blinded Phase II study randomized CKD-HD patients to placebo (standard dialysate) or dialysate with SFP given at each dialysis session for up to 26 wks, while prohibiting ESA dose changes and IV/oral Fe.

METHODS: Sufficient protocol-violation ESA dose changes allowed post hoc analyses comparing ESA in placebo group versus combined SFP dose groups for (1) relative ESA (rESA), the ESA dose at any time/baseline ESA dose (baseline was mean ESA dose over 2 wks prior to study), and (2) proportion of events when ESA dose changes ≥25% of the baseline dose.

RESULTS: SFP was well tolerated. Patients were Fe-replete as suggested by maintenance of Hgb in the placebo group despite withholding all Fe supplements. A pooled, longitudinal analysis of rESA as a continuous variable showed a 13% decrease in ESA dose for SFP relative to placebo at Week 24, ignoring dropouts ($p<0.05$). For all ESA dosing events on study, 72% were unchanged from baseline dose, 17% were dose decreases, and 11% were increases, with a trend favoring lower doses for SFP vs. placebo. After Week 8, rESA dose was decreased ≥25% from baseline in 21% of dosing events for placebo vs. 25% for SFP, and increased ≥25% in 8% for placebo vs. 4% for SFP ($p<0.05$). In the optimal SFP dose group (10 µg/dL, 1.8 µM), the estimated Hgb increase was 0.2 gm/dL/month ($p< 0.05$), even as rESA was reduced compared to placebo.

CONCLUSIONS: SFP administered in dialysate to Fe-replete CKD-HD patients decreased ESA dose requirements despite the protocol prohibition on ESA dose change. The Hgb increase and ESA sparing suggest SFP's physiologic mode of action, allowing Fe transfer directly to apo-transferrin, corrects functional Fe deficiency, thereby enhancing ESA responsiveness. SFP's full ESA-sparing effect will be assessed in a study allowing ESA dose change and is anticipated to be larger than observed in this study.

Disclosure of Financial Relationships: Employer: Rockwell Medical, Wixom, MIResearch Funding: Amgen Inc.

Affymax Inc.

Genzyme Inc.; Honoraria: Fresenius NA; Patent: Administration of soluble ferric pyrophosphate via the dialysis solutions; Other Relationship: CEO and CSO, Applied Medical Technologies, LLC.

**F-PO1393**

**Anaemia Management (AM) in Incident Hemodialysis (HD) Patients (pts): Impact of Predialysis Care** Jacques B. Rottembourg, A. Kadri, A. Dansaert. *Hemodialysis Unit, Diaverum Group, Paris, France.*

**Purpose:**AM in incident HD pts, and the effect of predialysis AM, merits attention. The aims of this retrospective study were to evaluate (1)AM in incident HD pts from their first dialysis (M0) and (2)the impact of predialysis care on AM, both during year 1 of dialysis.**Methods:**Hb, ESA dose and IV iron dose were (1)measured in year 1 of dialysis (2)compared between pts given IV iron therapy(IT) & ESA predialysis vs only oral IT & ESA.**Results:**60 pts received M0 in the unit in the last 6 years: mean(SD) age 60.1(16.8) years; 32% female; ESRD due to diabetes (29%), hypertension (23%), glomerulonephritis (20%), other (20%) and PKD (8%);96% had native arteriovenous fistula for M0.Pts received darbepoetin alfa (DA) once/2 weeks (Q2W) and IV IT weekly (QW).Of these 60 pts, 15 (25%) received IV IT predialysis (300mg iron as iron sucrose 2-4 times in the year predialysis):12 males, mean age 56.2(18.7) years at start. At start of dialysis, Hb was 10.1(1.5)g/dL vs 11.1(1.4) for pts without or with IV IT, respectively ($p<0.003$).

| n=60 Mean(SD) | M0 | M3 | M6 | M12 | p M6 vs M3 | p M12 vs M6 |
|---|---|---|---|---|---|---|
| Hb,g/dL | 10.3(1.5) | 11.5(1.3) | 12.1(1.1) | 11.9(0.8) | 0.0006 | NS |
| TSAT,% | 30.9(14.7) | 37.7(12.5) | 42.4(14.3) | 43(9.8) | 0.06 | 0.9 |
| Ferritin,µg/L | 175(160) | 212(148) | 351(295) | 535(295) | 0.004 | 0.0009 |
| DA,Q2W,µg | - | 100(47) | 76(44) | 68(45) | 0.0002 | 0.0001 |
| IV iron,QW,mg | - | 90(0.4) | 90(1.3) | 80.2 | NS | 0.0001 |
| | Hb,g/dL | | DA,Q2W,µg | | IV IT,QW,mg | |
| M3:No IV IT(n=45) | 11.1(1.2) | | 105(48) | | 90(1.12) | |
| M3:IV IT(n=15) | 12.0(1.0) | | 76.6(32.2) | | 88.2(1.13) | |
| p | 0.006 | | 0.01 | | 0.002 | |
| M12:No IV IT(n=45) | 11.6(0.6) | | 106(51) | | 88.8(3.1) | |
| M12:IV IT(n=15) | 12.5(0.6) | | 59(28) | | 82.1(8.8) | |
| p | 0.0002 | | 0.0005 | | 0.04 | |

**Conclusion:** Incident HD pts typically have low Hb, requiring >6 months to reach Hb target with high doses of ESA and IV IT. Pts starting dialysis after predialysis DA and IV IT require lower doses of ESA and IV IT during year 1 of dialysis. Prospective studies are required to confirm these data.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1394**

**Effect of Vitamin B12 and Folic Acid Supplementation on Erythropoitin Requirements To Maintain Haemoglobin Levels** John P. Killen. *Renal Medicine, Cairns Base Hospital, Cairns, QLD, Australia.*

INTRODUCTION : Vitamin B12 (B12) is an important co-enzyme for DNA synthesis. Normally plasma levels are 150 to 650 pmol/L representing only 0.02 – 0.05% of total body B12. B12 has been used as a marker for haemodialysis middle molecule clearance with modern dialysis membranes and can be effectively removed with high flux membranes. B12 deficiency is known to cause erythropoietin resistance in haemodialysis patients. Large amounts of folic acid supplementation can mask the damaging effects of vitamin B12 deficiency.

AIM : This prospective study assessed the effect of intramuscular B12 and oral folate supplementation on erythropoietin requirements to maintain heamoglobin levels.

METHODS: All 135 haemodialysis patients in our unit were screened for eligibility in this prospective observational study. Initial levels of B12 and were assessed in January 2009 with routine blood examinations. 60 haemodialysis patients with a B12 level of less than 300 pmol/L were offered intramuscular injections of 1000 micrograms hydroxocobalamin (NeoB12) weekly for 3 weeks. Serum B12 and haemoglobin levels and erythropoietin requirements were observed over the following 15 months. No change in intravenous iron protocols occurred during the study period.

RESULTS: Initially, the 60 eligible haemodialysis patients had a mean B12 level of 222 pmol/L (range 88 – 299 pmol/L) and mean red cell folate of 793 nmol/L requiring a corresponding mean erythropoietin dose of 9900 units/week to maintain a mean haemoglobin of 120 g/L. At the end of the study period the mean B12 levels rose to 462 pmol/L and the average folate rose to 1418 nmol/L. The average erythropoietin requirement reduced to 6200 units/week ($p < 0.0005$) to maintain the average haemoglobin of 117 g/L ($p = 0.35$) with no change in the average transferrin saturation at 32% and 33% respectively ($p = 0.77$) during the study.

CONCLUSIONS: In this small prospective observational study, implementation of intramuscular B12 supplementation for haemodialysis patients with serum B12 levels less than 300 pmol/L with oral folic acid supplementation resulted in a significant reduction in erythropoietin requirements, whilst maintaining average haemoglobin concentrations.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1395**

**IV Iron Therapy Leads to Iron Overload in CKD-HD Patients** Ajay K. Singh,[1] Richard C. Yocum,[2] Carrie D. Guss,[2] Mark T. Smith,[3] Ajay Gupta.[2] *[1]Brigham & Women's Hosp., Newton, MA; [2]Rockwell Medical, Wixom, MI; [3]Nephrology Associates, Augusta, GA.*

BACKGROUND: Iron (Fe) loss from hemodialysis is 5-7 mg/session (~1 gm/yr). Most CKD-HD patients receive regular IV Fe in excess of actual iron loss, median dose 4.2 gm/yr (USRDS 2006). IV Fe stimulates hepcidin >20X, induces reticuloendothelial (RE) block limiting Fe availability, causing progressive buildup of Fe stores. Soluble ferric pyrophosphate (SFP) is an investigational, physiologic, soluble, non-carbohydrate, Fe(III) salt donating Fe directly to transferrin via dialysate, bypassing the RE system.

OBJECTIVE: Assess if withholding Fe in CKD-HD patients up to 26 weeks while ESA dose held nearly constant (mean increase 8.7%) led to Fe-restricted erythropoiesis in a double-blinded, randomized Phase II study of placebo dialysate vs. SFP dialysate.

METHODS: Parameters of erythropoietic activity and Fe status at study end compared to baseline in subset of patients randomized to placebo (N=26).

RESULTS: Patients at baseline were similar to the U.S. CKD-HD population based on mean values: age 58 yrs, epoetin 12,510 U/wk (N=21), darbepoetin 59 µg/wk (N=5), IV Fe over prior 2 months 466 mg, albumin 3.9 g/dL, and pre-dialysis lab values (table). Over an average 15.7 wks (median 11.2, range 0.1 -25.4 wks), ferritin decreased 22% ($p< 0.05$) suggesting mobilization of Fe from stores. No significant change in TSAT, CHr or Hgb. This suggests stored Fe was adequate to maintain erythropoiesis.

| | Hgb g/dL | CHr pg | Ferritin µg/L | Iron µg/dL | TIBC µg/dL | UIBC µg/dL | TSAT % |
|---|---|---|---|---|---|---|---|
| Baseline | 11.0 | 32.6 | 503 | 59 | 221 | 162 | 27 |
| Study End | 10.8 | 31.9 | 394 | 52 | 220 | 169 | 24 |
| Change | -0.2 | -0.7 | -109* | -7 | -1 | 7 | -3 |

*Denotes $p<0.05$ for change from baseline.

CONCLUSIONS: The absence of Fe-restricted erythropoiesis despite Fe withholding over 16 wks suggests CKD-HD patients receive excessive IV Fe that might contribute to Fe overload and Fe toxicity in the long-term. Future trials will evaluate SFP's ability to maintain Fe available for erythropoeisis without leading to Fe overload or exacerbating inflammation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1396**

**Current Status of Erythropoietin Immunogenicity in Korean Dialysis Patients** Sung Jin Moon,[1] Sung-Kyu Ha,[1] Tae Won Lee,[2] Yang Wook Kim,[3] Seung-Ok Choi,[4] Tae-Hyun Yoo,[5] Kang Wook Lee,[6] Young-Il Jo,[7] Jin Kuk Kim,[8] Hyeong Cheon Park,[1] Eawha Kang,[9] Sug Kyun Shin.[9] *[1]Internal Medicine, Gangnam Severance Hospital, Korea; [2]Internal Medicine, Kyung Hee University Hospital, Korea; [3]Internal Medicine, Pusan Paik Hospital, Korea; [4]Internal Medicine, Wonju Christian Hospital, Korea; [5]Internal Medicine, Severance Hospital, Korea; [6]Internal Medicine, Chungnam National University Hospital, Korea; [7]Internal Medicine, Konkuk University Hospital, Korea; [8]Internal Medicine, Soon Chun Hyang University Hospital, Buchoen, Korea; [9]Internal Medicine, Ilsan Hospital, National Health Insurance Cooperation, Korea.*

Anti-erythropoietin (EPO) antibody positivity, clinically presented by pure-red cell aplasia is a rare disease. We published the new bridging ELISA method for detecting anti-EPO antibody, this year. Therefore, we aimed to examine the prevalence, clinical characteristics and risk factors of anti-EPO antibody positive ESRD patients. Eight hundred fourteen ESRD patients who used rHu-EPO for more that 6 months were recruited at 12 university based hospital in Korea. Serum, clinical and biochemical data of the patients were collected. The mean age was 57.6±13.3 years and sex ratio was 1:1. The causes of ESRD were DM (46.2%), HTN (36.5%), GN (7.4%). The mean dialysis duration was 54.0±46.4 months, and the number of hemodialysis (HD) patients was 576 (70.8%). The types of EPO were epo-alfa 67.9%, epo-beta 4.2%, dabepoietin-alfa 27.8%, respectively. Eight out of the 814 patients had anti-EPO antibody, the prevalence of EPO immunogenicity was 0.98%. Mean age of the patients was 64.3±5.4 years and sex ratio was 1:1. The number of HD patients was 7 and mean dialysis duration was 65 months. Six patients used epo-alfa. These patients had lower hemoglobin (9.6±1.4g/dL) levels and reticulocyte counts (1.3±0.8%).

We could identify 8 patients with anti-EPO antibody from 814 ones. Mainly positive was shown in patients with epo-alfa (75%). Risk factors for antibody formation have to be evaluated in further studies with larger scale.

Disclosure of Financial Relationships: nothing to disclose

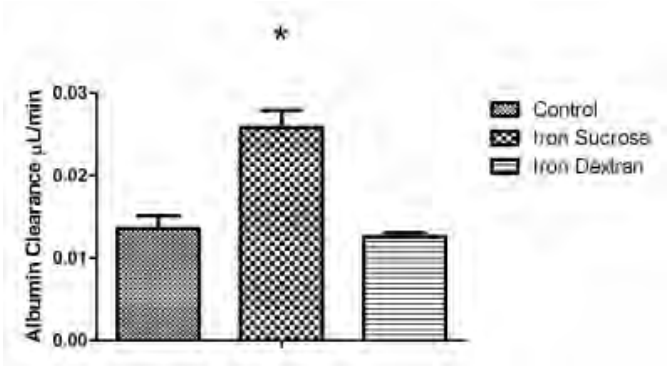
**F-PO1397**

**Role of Intravenous Iron Compounds in Pathogenesis of Pulmonary Edema** Amy B. Pai, Arnold Johnson. *Albany College of Health of Health Sciences, Albany, NY.*

Pulmonary edema is a major complication associated with significant morbidity, mortality and hospitalization costs in hemodialysis patients. IV iron compounds induce ROS generation, which has been implicated in lung endothelial barrier dysfunction. It is unknown if these widely used agents affect lung permeability.

Rat lung microvessel endothelial cell (RLMEC) monolayers were cultured and treated with iron sucrose (IS) or low molecular weight iron dextran (ID) (0.05 mg/mL) for 24 hours. Clearance rate of Evans Blue-labeled albumin between 10 and 60 mins was assessed to determine permeability. ROS were quantitated in RLMEC treated with IS or ID (as above) for 30 minutes incubated with dihydroethidium (DHE) (10 µM, 0.5 h at 37°C). DHE fluorescence is reported as mean fluorescence intensity (MFI). Endothelial nitric oxide synthase (eNOS) activation was determined in lysate by immunochemistry and analyzed using the ratio of anti-p-eNOS-ser$^{1177}$ (an activation site) and p-eNOS-thr$^{495}$ (an inactivation site). Results are mean values ± SEM of $n > 3$ experiments.

Results: Incubation of RLMECM with IS induced significant endothelial barrier dysfunction compared to ID and untreated controls. (*$p<0.05$ for both comparisons, see Figure).




IS but not ID was associated with eNOS activation (mean ± SEM relative density units; 1.6 ± 0.31 and 0.88 ± 0.18 vs. control 0.83 ± 0.21, respectively, $p < 0.05$ IS vs. control and ID). Similarly, IS was associated with significant ROS generation, with an 85% increase in DHE fluorescence vs. untreated controls ($p < 0.05$, IS vs. controls). DHE fluorescence after ID treatment was not significantly different from untreated controls.

Iron sucrose but not iron dextran increased endothelial permeability to albumin, eNOS activation and ROS generation. These data indicate that IV iron has the potential to differentially induce lung injury via an eNOS/ROS mediated pathway.

Disclosure of Financial Relationships: Research Funding: Abbott Laboratories.

**F-PO1398**

**Reduction of Erythropoesis Stimulating Agent Requirements When Converting Patients from Epoetin beta (NeoRecormon) and Darbepoetin alpha (Aranesp) to Mircera in Haemodialysis Patients** Reena A. Popat,[1] Christopher J. Kirwan,[2] Martin J. Raftery,[2] Magdi Yaqoob.[2] *[1]Pharmacy & Medicines Management, Barts and The London NHS trust, United Kingdom; [2]Nephrology and Transplantation, Barts and The London NHS trust, United Kingdom.*

**Introduction:** Two studies (MAXIMA and PROTUS) have shown that patients on dialysis can successfully be converted from shorter acting ESA preperations (e.g. Aranesp and NeoRecormon) to Mircera whilst maintaining stable Hb. We report our experience of switching patients from NeoRecormon and Aranesp to Mircera in our haemodialysis (HD) unit.

**Method:** Patients were included in the study if maintained on the same ESA preparation and HD for the 6 months pre and post conversion to Mircera. Hb, PTH, ferritin, CRP and ESA dose (converted to mcg) were calculated for each patient pre and post conversion . Percentage of total Hb measurements greater than 10.5g.dL was also compared pre and post conversion.

**Results:** 130 patients had been converted to Mircera of which 107 met the inclusion criteria.

53 patients (33male), mean age 58.6 years, were converted from NeoRecormon to Mircera. There was no statistical difference in mean patient Hb pre and post conversion (10.8g.dL and 10.8g.dL respectively). The percentage of all Hb measurements greater than 10.5g.dL was not significantly different pre and post conversion (63.3% and 60.3%; $p>0.05$). However the mean ESA requirements did reduce significantly after conversion to Mircera (180mcg pre vs. 148mcg post; $p <0.001$).

54 patients (35 male), mean age 57.7 years, were converted from Aranesp to Mircera. There was no statistical difference in Hb pre and post conversion (10.7g.dl and 10.5 g.dL respectively). The percentage of all Hb measurements greater than 10.5g.dL was not significantly different pre and post conversion (61% and 52%; $p>0.05$). However the mean ESA requirements significantly reduced after conversion to Mircera (167mcg pre vs. 139mcg post; $p <0.001$).

Note: PTH, ferritin and CRP was not statistically different pre and post conversion to Mircera or between both groups.

**Conclusion:** Switching patients from shorter acting ESAs to Mircera in our HD population maintains haemoglobin control whilst reducing ESA requirements.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1399**

**EPO Treatment at the Start of Hemodialysis Is More Beneficial Than at the End of Hemodialysis** Noritomo Itami,[1] Yasushi Shimonaka,[2] Taishi Nakashima,[1] Kazushi Tsuneyama.[1] *[1]Kidney Center, Nikko Memorial Hospital, Muroran, Hokkaido, Japan; [2]Product Research Dept., Chugai Pharmaceutical Co. Ltd., Japan.*

**Background and Aims**

Although erythroid progenitors require iron for their maturation after EPO stimulation, EPO is usually injected at the end of hemodialysis(HD) for renal anemia treatment as clinical guidelines suggest because EPO given at the start HD is supposed to be absorbed in the HD circuit decreasing its efficacy.

Previously, we have shown that EPO treatment at the start of HD could improve iron utilization and reduce EPO dose, while hemoglobin(Hb) level and iron treatment frequency were unaffected in a short pilot study. Here, we performed a longer study to confirm the beneficial impact of EPO treatment at the start of HD.

**Method**

Thirty six HD patients providing written informed consent were enrolled. At the beginning, EPO treatment was changed from the end of HD (Period I) to the start (Period II). Anemia treatment was carried out as usual. EPO dose was altered biweekly to maintain a target Hb level of 11-12g/dL. After 12 and 25 weeks, average EPO dose, clinical parameters and rate of operational incidents were examined.

**Results**

At the start of the study, mean Hb level was 10.99±0.85g/dL and was unchanged at week 12 and 25 (11.22±0.83g/dL and 11.05±0.83, p=0.16 and p=0.89). Frequency of iron treatment (28%, 33% and 17%, p=0.69 and p=0.40) and serum ferritin were also unchanged (174±113ng/mL, 190±130ng/mL and 176±109, p=0.46 and p=0.76). Average EPO dose in 2 weeks was significantly reduced from 4771±2528 IU/week in Period I to 3949±2140 IU/week at week 12 (p=0.048). At week 25, EPO dose remained reduced at 4412±2583IU/week, but not significantly (p=0.34). There were no operational incidents such as neglecting EPO injection in Period II.

**Conclusions**

Results indicate EPO treatment at the start of HD was more beneficial and effective than EPO treatment at the end of HD. It was suggested that improved iron utilization reduces EPO dose, although Hb level and iron treatment were unaffected. Moreover, more reliable EPO injection can be achieved because incidents at the start of HD were rare. Further study should provide information to establish an efficient EPO treatment.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1400

### Erythropoietin Dose and Peritoneal Membrane Transport Status: A Dose Response Relationship

Sharad K. Ratanjee, James E. Jackson. *Renal Medicine, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia.*

Aim: To determine if the dose of erythropoietin (Epo) used correlates with peritoneal transport status (TS) in peritoneal dialysis (PD) patients.

Background: Epo has demonstrated angiogenic activity, both via the Epo receptor and via upregulation of vascular endothelial growth factor VEGF/VEGF receptor and hence may contribute to peritoneal vasculopathy. Epo may also stimulate the epithelial-to-mesenchymal transition of peritoneal cells. These changes may predispose PD patients to the development of peritoneal membrane changes, including fibrosis, and effective increases in vascular peritoneal surface area leading to rapid TS and ultimately membrane failure.

Methods: A cross-sectional analysis was performed of 88 patients established on PD. The weekly equivalent Epo dose per kilogram body mass (WEED) was calculated. Peritoneal transport status was determined using the peritoneal equilibration test (PET) as popularised by Twardowski. Analysis of variance was performed with the following factors related to TS: body-mass index (BMI), haemoglobin (Hb), number of previous peritonitis episodes (PPE), CRP, serum albumin (alb), days on dialysis, age and WEED.

Results: On multivariate analysis five of the variables were unimportant (BMI, Hb, PPE, Alb, days on dialysis). Age and CRP were important as confounders of relationship of WEED to TS. A significant relationship between WEED and TS was shown on multivariate analysis (p=0.034). Similarly for age and TS (p=0.012). Each additional unit of WEED corresponded to an increase in TS by 0.029 (95% CI 0.002 – 0.057).

Conclusion: There is a significant correlation between the WEED and TS in PD patients in this cross-sectional study. Larger prospective longitudinal studies are needed to further evaluate this relationship.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1401

### Impact of Hemodialysis with Polysulfone Dialyzers on Red Blood Cell Lifespan in Chronic Hemodialysis Patients

Georges Ouellet,[1,2] Anja Kruse,[1,2] Garry J. Handelman,[3] Stephan Thijssen,[1,2] Peter Kotanko,[1,2] Nathan W. Levin.[1,2] *[1]Renal Research Institute; [2]Beth Israel Medical Center, New York, NY; [3]University of Massachusetts, Lowell, MA.*

**Background**

Red blood cell lifespan (RBCLS) is usually markedly reduced in uremia. RBCLS can be estimated by measuring endogenous carbon monoxide (ECO) production, which largely reflects the rate of heme degradation. A 20% acute intradialytic increase in ECO (ascribed to a reduction in RBCLS) has been reported with cellulose acetate membranes (Medina, 1994). We aimed to determine if hemodialysis (HD) with polysulfone membranes influences RBCLS.

**Methods**

We recruited non-smoking patients without inflammatory pulmonary disease receiving maintenance bicarbonate HD with Optiflux polysulfone dialyzers (Fresenius Medical Care, Waltham, MA). Hemoglobin (Hb) was measured pre-HD. Pre- and post-HD alveolar air samples were collected and analyzed for CO with a 910 Buck Scientific gas chromatograph (East Norwalk, CT). ECO production was computed by subtraction of environmental CO concentrations (home and dialysis unit) from alveolar CO levels. RBCLS was calculated from ECO levels (Strocchi, 1992). Intradialytic changes in ECO and RBCLS were assessed by paired samples t test. Data are shown as mean±SD.

**Results**

Nineteen patients (median age 69.2 years [range 32.6-82.5]; 68% male; 68% Black; blood flow 406±53 mL/min) were studied. Mean Hb was 11.4±1.0 g/dL. ECO levels and RBCLS did not change during HD (Table 1). Mean pre-HD RBCLS was not significantly different from that reported by Medina *et al* (P=0.6).

Table 1. Pre- and post-HD ECO and RBCLS

| | Pre-HD | Post-HD | P value |
|---|---|---|---|
| ECO (ppm) | 2.63±0.99 | 2.29±0.86 | 0.09 |
| RBCLS (days) | 75.3±27.7 | 77.1±25.8 | 0.67 |

**Conclusions**

In a group of 19 chronic HD patients, we found no significant changes in ECO levels and RBCLS from pre- to post-HD, suggesting that turnover of erythrocytes is not affected by HD. Improvements in dialysis technologies, especially progress in water quality and the use of more biocompatible membranes causing little complement activation, might explain the difference between our results and older reports.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1402

### Switch from the Original Intravenous Iron Sucrose to an Iron Sucrose Similar Is Associated with Lower Hemoglobin (Hb) Levels in a Hemodialysis (HD) Population

Jacques B. Rottembourg,[1] A. Kadri,[1] E. Leonard,[1] A. Dansaert,[1] A. Lafuma.[2] *[1]Groupe Diavarum, Centre Suzanne Levy, Paris, France; [2]CEMKA/Eval, Bourg La Reine, France.*

**Purpose:** HD patients (pts) received ESAs and IV iron sucrose Venofer® (V) at our center with satisfactory results for 6 years. In June 2009, a switch was made to the IV iron sucrose similar FerMylan® (FM). Hb target was 11.5-12g/dL. **Methods:** The analysis compared two 27-week periods; Period 1 (P1): Dec 1, 2008-June 7, 2009 when pts received V, and Period 2 (P2): Jun 29, 2009-Jan 3, 2010 when they received FM. Inclusion criteria were ≥60 dialysis sessions per P and ≥1 IV iron treatment. Hb was assessed every two weeks, and serum ferritin, transferrin saturation (TSAT), CRP and Kt/V every three months. IV darbepoetin-a (DA) was given every two weeks and IV iron every week (25-100 mg iron). **Results:** 75 pts were analyzed (male 69%), mean age 63.415.2 years, mean dialysis duration 55 months.

| Values | P1 | P2 | p-value |
|---|---|---|---|
| Hb mean (SD) g/dL | 11.78 (0.99) | 11.48 (0.98) | 0.01 |
| Serum ferritin mean (SD) µg/L | 534 (328) | 495 (280) | 0.25 |
| TSAT mean (SD) % | 49.3 (11) | 24.5 (9) | <0.0001 |
| CRP mean (SD) mg/L | 6.4 (6.8) | 8.4 (10.5) | 0.15 |
| Kt/V mean | 1.41 (0.25) | 1.42 (0.24) | 0.80 |
| DA total dose per Period µg | 73510 | 82750 | |
| DAµg/kg/week per pt (SD) | 0.58 (0.52) | 0.66 (0.64) | 0.13 |
| Iron (ampoules/Period) | 923.0 | 1242.5 | 0.001 |
| Iron mean dose/pt per Period (SD) mg | 1231 (897) | 1657 (836) | 0.001 |

Hb levels were significantly lower with FM (P2) vs V (P1). To maintain Hb target, an increase of 12.6% in total DA dose and ~35% in total iron dose was required in P2. **Conclusions:** This analysis suggests that V and FM may not be therapeutically equivalent. Previously well-controlled patients required higher doses to maintain target Hb levels after switch from V to FM, with a resultant ~12% increase in total drug costs. In addition these findings have important implications related to the Medicare bundled payment rate for medications, and the Medicare quality incentive payment program for iron/anemia management in HD patients to be enacted in the near future.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1403

### Correction of Anemia and Iron Metabolism in Chronic Renal Insufficiency. Are Cardio/Cerebrovascular Risks (CC-Risks) Avoidable?

Hannelore B. Hampl. *Nephrology, University, Berlin, Germany.*

Renal cardiac insufficiency is accompanied by anemia due to erythropoeitin (EPO)-iron deficiency. A suffered heart needs increased oxygen to improve. Therefore, full anemia correction with EPO/iron (iv) was added to adequate cardiac therapy with β-blockers, ACE-inhibitors, ARBs to investigate changes in leftventricular mass index(LVMI-regression?) and to investigate amounts of EPO/iron **on the way** to full anemia correction. We conducted this retrospective survey in 350 outdoor HD pats (age>68years)with this therapy(A). As controls served HD pats(N=350;age>67years) from different centers with low cardiac therapy and Hb of 12g/dl (B) and parameters of iron metabolism given by guidelines. **Results-A/B: Hb**(g/dl):11.5/**14.1p<0.001**; 11.6/12.2 n.s.; **LVMI**(g/m²)159.3/**131.2p<0.001**; 162.5/159.3n.s.; **LVEF**(%)58.9/**68.5p<0.001**; 59.3/59.3n.s.; **TSAT**(%):27.2/**46.5**;19.8/22.1n.s.; **Transferrin** (mg/dl):167.6/148.5 p<0.05; 165.6/147.1p<0.05; **Iron**(µ/dl):52.6/**75.2p<0.001**;40.5/5 0.2p<0.05; **Ferritin**(µg/L):500.7/**1537.0p<;0.001**; 200.8/550.1 p<0.05; **Thrombocyte counts**(mm³):310 000/**187 522p<0.001**; 319 000/ 309 000n.s. **In contrast, in B**: **iron was deficient, thrombocyte counts unchanged.** The applicated amount of EPO (Epoeitin-beta) was over 3 times higher (18 000IE pat/week) despite a lower Hb (12g/dl) than in A(5 500 IU pat/week; Hb14g/dl). The average observation time of 10years showed a low mortality ratio(A):16% by non cardiac reasons,10% by cardiac reasons in contrast to high mortality ratio in B. **Conclusion:** Full anemia correction did not increase risks, provided that effective cardiac medication and iron therapy (iv) is optimally applied during EPO therapy(A) to prevent iron deficiency which is well known for inducing **activated thrombocytosis being responsible for severe CC-risks**. Thrombocyte counts >than 300 000/mm³( B ) have a fourfold higher event ratio. Iron- values lower than 60 µg/dl are accompanied with a significantly increase in hazard ratio. The best survival is given by lowest EPO amounts. **A functional iron deficiency with activated thrombocytosis on the way of full anemia correction with EPO is a risk factor not taken in consideration until now!**

Disclosure of Financial Relationships: nothing to disclose

**F-PO1404**

**Chronic Oral Iron Supplementation Reduces Darbepoetin α Doses in Maintenance Hemodialysis Patients** Shinichi Suga,[1] Hironobu Kawai,[1] Tokuyuki Kitahara,[2] Yoshito Tsukada,[3] Kazue Ueki,[4] Tatsuhiko Sakamoto,[5] Keiju Hiromura,[6] Yoshihisa Nojima.[6] *[1]Nephrology, Saiseikai Maebashi Hosp., Gunma, Japan; [2]Medinice, Shibukawa Central Hosp., Gunma, Japan; [3]Medicine, Fujioka General Hosp., Gunma, Japan; [4]Nephrology, Toho Hosp., Gunma, Japan; [5]Medicine, Usui Hosp., Gunma, Japan; [6]Med. & Clin. Sci., Gunma Univ., Grad. Sch. Med., Gunma, Japan.*

The preferred rout of iron administration is intravenous in hemodialysis patients, partially because of their impaired iron absorption. Since iron absorption has been reported to be up-regulated in iron-deficient hemodialysis patients treated with recombinant human erythropoietin (NDT 13:82, 1998), we hypothesized that chronic oral iron supplementation is effective in iron-deficient hemodialysis patients with erythropoiesis-stimulating agents. We compared the efficacy of oral to intravenous iron for the chronic management of anemia in hemodialysis patients treated with darbepoetin α. Fifty-one hemodialysis patients with initial serum ferritin < 100 ng/ml and transferrin saturation (TSAT) < 30% were randomly assigned to one of two groups: those receiving oral iron therapy (40~50 mg/day, n = 32) and those receiving intravenous iron (100~105 mg once weekly, n = 19). At the end of 3 months, the mean Hgb showed no significant difference between the oral group and the intravenous group (11.3 ± 0.2 g/dl vs 11.0 ± 0.2 g/dl, respectively). However, the mean darbepoetin α dose was 32% lower in the oral group than in the intravenous group (14.6 ± 2.0 μg/wk vs 21.5 ± 4.0 μg/wk; P < 0.05). Although the mean serum ferritin was significantly lower in the oral group than in the intravenous group (57 ± 5 ng/ml vs 124 ± 10 ng/ml; P < 0.05), the mean TSAT and the mean serum iron were significantly higher in the oral group than in the intravenous group (TSAT: 34 ± 2 % vs 28 ± 2 %; P < 0.05) (iron: 80 ± 4 μg/dl vs 59 ± 4 μg/dl; P < 0.05). There was no significant difference in the withdrawal rate from the study due to drug-related adverse effects between two groups. We found that administering iron orally for chronic maintenance iron supplementation could result in effective erythropoiesis, at least in iron-deficient hemodialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1405**

**Comparison of Hemoglobin Variability between Erythropoiesis Stimulating Agents in Hemodialysis Patients** Tai Yeon Koo,[1] Jung-Sik Park,[1] Gheun-Ho Kim.[2] *[1]Internal Medicine, Division of Nephrology, Asan Medical Center, Seoul, Korea; [2]Internal Medicine, Division of Nephrology, Asan Medical Center, Seoul, Korea; [3]Internal Medicine, Division of Nephrology, Hanyang University College of Medicine, Seoul, Korea.*

**Background:** In hemodialysis patients, treatment of anemia with erythropoiesis-stimulating agents(ESAs) accompanies recurrent cyclic fluctuations in hemoglobin levels which may be associated with increased morbidity and mortality. It is conceivable that hemoglobin variability may be deferential according to different pharmacodynamic and pharmacokinetic properties in each ESA. This study was undertaken to examine whether differences exist in the hemoglobin variability according to the types of ESA. **Methods**: Clinical data were retrospectively analyzed from 72 patients on hemodialysis without acute illness. As parameters of hemoglobin variability, hemoglobin cycling, variance and SD/mean of hemoglobin were analyzed. Hemoglobin cycling was defined as the presence of cycles with amplitude >1.5 g/dL and lasting more than 2 months. **Results:** Hemoglobin cycling was present in 53 out of 72 hemodialysis patients. The number of hemoglobin cycling was 1.4±0.9 times/year. The amplitude and duration of hemoglobin cycling were 2.4±1.6 g/dL and 4.1±3.1 months, respectively. The number of ESA dose change including withdrawal of ESA was 3.7 ± 1.4 times/year, and the number of ESA withdrawal was 1.5±0.7 times/year. The frequency, amplitude, and velocity of hemoglobin cycling for patients receiving darbepoetin alfa (n=27) were greater than those for patients receiving epoetin beta (n=27). The variance and the SD/mean of hemoglobin for patients receiving darbepoetin alfa was greater than those for patients receiving epoetin beta. The hemoglobin cycling including frequency, amplitude and velocity, the variance of hemoglobin and the SD/mean of hemoglobin were not significantly different between patients receiving darbepoetin alfa and epoetin alpha.**Conclusions:** Hemoglobin variability is common in hemodialysis patients receiving ESAs. It may be deferential according to various ESAs, and epoetin beta may have less hemoglobin variability compared with darbepoetin alpha and epoetin alpha.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1406**

**Long-Term Analysis of Once-Monthly C.E.R.A. Maintainance in 293 Hemodialysis (HD) Patients within an Italian Setting** Francesco Locatelli. *Alessandro Manzoni Hospital, Lecco, Italy.*

**Purpose:** CARISMA is a Phase IIIB single-arm study providing long-term analysis of hemoglobin (Hb) maintenance following once-monthly (Q4W) administration of C.E.R.A. in HD patients (pts) with chronic kidney disease (CKD) converted from shorter-acting erythropoiesis-stimulating agents (ESAs) in a real-life clinical setting.

**Methods:** Adult HD pts receiving different schedules of epoetin alfa, beta, or darbepoetin alfa as maintenance therapy were converted to C.E.R.A. Q4W over a 16-week titration period prior to an 8-week evaluation period. Hb stability was defined by the following criteria A, B, and C, throughout the study: (A) average Hb levels within target range (10.0-12.0 g/dL); (B) average Hb levels ±1 g/dL from baseline. The proportion of pts attaining (A) and (B) was defined as a challenging end point; (A) or (B) was defined as overall stability; (C) was defined as fulfilling overall stability without Hb levels ≥0.5 g/dL outside of 10.0-12.0 g/dL. Safety and tolerability were examined throughout the study, including a long-term safety follow-up of 28 weeks.

**Results:** Pts (n=293) had a mean age of 66.4 (19-92) years with 41% having hypertension and 18% having diabetes at baseline; epoetin was received by 63.8% of pts and the remainder received darbepoetin alfa. Mean Hb levels during titration and evaluation were 11.4 g/dL and 11.1 g/dL, respectively. The most challenging end point (A+B) was achieved by 56% of pts, overall stability (A or B) by 82% of pts, and criterion C by 80% of pts, with mean Hb fluctuations of 0.26 g/dL at titration and 0.24 g/dL at evaluation. Pts received a median dose of 120 μg throughout the 24-week period; with an average of 2.3 dose changes per pt. C.E.R.A. Q4W was well tolerated with only 58% of pts reporting one adverse event.

**Conclusion:** These data show that conversion from different schedules of shorter-acting ESAs to C.E.R.A. Q4W is safe and effective in maintaining stable Hb levels in patients with CKD in a real-life clinical setting. Hb stability was achieved with fewer dose changes and pts demonstrated a safety profile similar to that observed with traditional ESAs.

Disclosure of Financial Relationships: Consultancy: I'm a member of an advisory board of Abbott, Affimax,Amgen,Genzyme,Merck,Shire, Takeda, Roche, GlaxoSmithKline and member of a safety committee of Sandoz.

**F-PO1407**

**Lower Erythropoietin Dose Utilization in Paricalcitol-Treated Patients Compared with Doxercalciferol-Treated Patients** Steven E. Marx, Samina Khan, Utpaul Audhya, Beverly A. Johns. *Abbott, Abbott Park, IL.*

OBJECTIVE: Erythropoietin incurs a significant cost in hemodialysis patients. We assessed erythropoietin utilization among hemodialysis patient receiving paricalcitol versus doxercalciferol.

METHODS: A historical cohort between 2004 and 2009 was used from a large dialysis organization. To enter the study cohort, hemodialysis patients were required to have a minimum of 10 doses of paricalcitol or doxercalciferol within 90 days, age >17, minimum 90 days pre-index, survive first 90 days after the index date, and have consistent records. Index date was the first dose of either paricalcitol or doxercalciferol. A total of 16,770 patients were assessed using a multivariate analysis adjusted for (1) age, gender, race, diabetes status, duration of dialysis, hemoglobin; (2) plus ferritin, and (3) plus study entry period. Additionally, 1,321 propensity matched patients using age, gender, race, and baseline hemoglobin were assessed as above. Additional subanalyses were performed replacing ferritin with iron saturation as well as adding baseline iPTH levels to propensity match.

RESULTS: Both multivariate and propensity matched analyses demonstrated statistically significant lower erythropoietin utilization.

Results

| Paricalcitol vs. Doxercalciferol | Adjusted 1 | Adjusted 2 | Adjusted 3 |
|---|---|---|---|
| Multivariate Analysis | n=15,449 vs. 1,321 | n=15,271 vs. 1,301 | n=15,271 vs. 1,301 |
| Parameter Estimates | -0.05 p<0.05 | -0.05 p<0.05 | -0.06 p<0.05 |
| Propensity Matched Analysis | n=1,321 vs. 1,321 | n=1,285 vs. 1,285 | n=1,285 vs. 1,285 |
| Parameter Estimates | -0.07 p<0.05 | -0.07 p<0.05 | -0.08 p<0.05 |

All subanalyses revealed similar results.

CONCLUSION: In this single dialysis provider organization, patients who received paricalcitol were associated with lower erythropoietin utilization compared to those on doxercalciferol. This is the first study to compare erythropoietin utilization among hemodialysis patients receiving paricalcitol versus doxercalciferol. Further prospective studies are required to confirm these results.

Disclosure of Financial Relationships: Employer: Abbott; Ownership: Abbott Stock.

**F-PO1408**

**Prospective Multicentre Study of HX575 (Human Recombinant Epoetin alfa) Treatment in Patients with Chronic Kidney Disease (CKD) Applying a Target Hemoglobin (Hb) of 10-12 g/dL** Karsten Roth,[1] Francesco Locatelli,[2] Walter H. Hoerl.[3] *[1]Sandoz Biopharmaceuticals, Hexal AG, Holzkirchen, Germany; [2]Nephrology, Dialysis and Renal Transplant, Ospedale "Alessandro Manzoni", Lecco, Italy; [3]Medicine III, Division of Nephrology and Dialysis, University of Vienna, Vienna, Austria.*

The objective of this post-authorization study was to extend the safety database on HX575. Monitoring intravenous HX575 administrations in clinical practice covering 750 patient years should allow for the assessment of rare drug-related adverse events. HX575 (marketed as Binocrit®, Epoetin alfa Hexal®, and Abseamed®) was the first human recombinant epoetin alfa approved according to European biosimilar regulations in 2007.

**Methods:** This open, 6-month study was conducted at 114 sites in 10 European countries with a target enrolment of approximately 1,500 patients with CKD. HX575 dosing was according to label and medical practice with an Hb target concentration of 10-12 g/dL. An independent data safety monitoring board (DSMB) ensured accurate review and assessment of adverse events.

**Results:** 1695 patients who provided written informed consent were enrolled and received study medication; 1393 (82.2%) patients completed the study. The mean age at baseline was 61.8 years, 57% of patients were male. Most frequent primary underlying diseases were chronic glomerulonephritis (24.2%), diabetes mellitus (20.3%), and hypertension (14.0%).

Thrombotic vascular events were reported in 11.9% of patients (0.2612 per patient year). Tumor incidence was 1.4% (0.0299 per patient year). Two Suspected Unexpected Serious Adverse Reactions (SUSARs) were recorded: myelodysplastic syndrome with transformation into acute myeloid leukemia, a causal relationship to erythropoiesis-

stimulating agent (ESA) treatment was not suspected; and uncontrolled hypertension resulting in fatal cerebral hemorrhage, which was assessed as possibly related to ESA treatment. No subject developed anti-epoetin antibodies during the study.

**Conclusions:** No concerning safety findings were reported over a period covering 769 patient years. Intravenous ESA treatment with HX575 in anemic CKD patients was well tolerated and no signs of immunogenicity were observed.

Disclosure of Financial Relationships: Employer: Hexal AG, a Sandoz company (sponsor of the study).

### F-PO1409

**Toward Physiologic EPO and IV Iron Dosing in HD Patients: Patient-Centered and Data-Based** Jonathan Lorch,[1] Victor E. Pollak.[2] *[1]Rogosin Institute, Weill-Cornell Medical College, New York, NY; [2]MIQS Inc. & Department of Medicine, University of Colorado HSC, Denver, CO.*

EPO dosing has been driven by guidelines and changing Medicare regulations rather than patient physiologic responses. Recent US EPO dosing is high (17,996 units/week); Medicare changes now encourage reduced EPO doses. Long term HD patient survival was shown to be best with Hb >12 g/dl, TSAT >25%, moderate IV iron, relatively low EPO (BMC Nephrology 2009, 10:6). Retrospective analysis of prospectively collected individual patient data from 3 dialysis units (The Rogosin Institute, New York, NY) in a patient-centered EMR (MIQS, Inc., Boulder CO) showed that expected Hb change lagged EPO dose changes by many weeks. Over short (4-12 weeks) and long periods (8-12 years) EPO given to individual patients varied widely, sometimes within weeks, with no obvious reasons, and with high Hb variance. 25% of patients were iron insufficient (TSAT ≤25%). In unit A (265 patients) a new dosing model was initiated in February 2010; units B and C using the traditional dosing model served as controls. Objectives: Hb and iron sufficiency (TSAT persistently ≥25% and serum ferritin ≥300 µg/L), low Hb variance; modest/low EPO dosing. Method: evaluate EPO and iron status not more frequently than 4-8 weekly using a reporting tool for each patient that aggregates hematologic data and hematinic medications monthly for the prior 18 months. Actions: replete iron IV as needed; maintain, adjust downward, but do not stop EPO. Results: Unit A: in the first 18 weeks, Hb increased 10% from 11.1 to 12.2 g/dl. Hb variance decreased from 1.21 to 0.68 $g^2/dl^2$. IV iron given doubled from 14 to 28 mg/HD, and TSAT increased from 29.8 to 31.3% while EPO decreased from 17,300 to 15,200 units/week. Units B and C: EPO doses decreased by 13% and 14% to 19,000 and 17,600 units, but Hb was unchanged in unit B and decreased by 2% to 10.81 g/dl in unit C. Conclusion: The new dosing model facilitated orderly EPO reduction, increased Hb and iron sufficiency. The small EPO decrease in the first 18 study weeks is expected as EPO dose was changed at long intervals; a further decrease is anticipated with continued study and contained Hb swings.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1410

**Total and HMW Adiponectin Is Independently Associated with Brain Natriuric Peptide and Anemia in Chronic Hemodialysis Patients** Yasuhiro Abe, Kenji Ito, Maho Watanabe, Hitoshi Nakashima, Takao Saito. *Division of Nephrology and Rheumatology, Fukuoka University School of Medicine, Fukuoka, Japan.*

**Background**: A number of vasculo-protective role for adiponectin have been reported, including the promotion of angiogenesis, inhibition of atherosclerosis and ventricular hypertrophy. In contrast, higher, rather than lower, plasma adiponectin levels are associated with an increased risk of cardiovascular disease and mortality in patients with end-stage renal disease (ESRD). It is not yet elucidated the mechanism why high total adiponectin levels were associated with adverse outcome.

**Methods**: We measured the level of total and HMW adiponectins in 72 patients with HD patients with and without diabetes mellitus (age: 65+-9 years, time on HD: 94+-10 months, male/ female: 30/42), and examined the association between adiponectin and metabolic parameters.

**Results**: Female patients had significantly higher total, HMW adiponectin levels and HMW to total adiponectin ratio than those male patients did. The level of total and HMW adiponectins were positively correlated with those of HDL-C and brain natriuretic peptide (BNP) levels, and negatively associated with BMI, TG, high sensitive-CRP and hematocrit levels. In stepwise regression analyses, HMW adiponectin showed independent association with BMI (β=-0.244, P=0.006), HDL-C (β=0.372, P<0.001), hematocrit (β=-0.217 P=0.009) and BNP (β=0.247, P=0.002) as total adiponectin did.

**Conclusions**: Anemia and BNP level had independent influence on total and HMW adiponectin levels in chronic HD patients. This finding may partly be explained the 'adiponectin paradox' in ESRD.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1411

**Effect of Ergocalciferol on Anemia Management in End Stage Kidney Disease** Neenoo Khosla, Hongyan Du, Nisha N. Patel, L. Tammy Ho, Junine Darby Degraf, Stuart M. Sprague. *Nephrology, Northshore Universty Healthsystem, Evanston Hospital, Evanston, IL.*

Previous studies have demonstrated that lower 25 hydroxyvitamin D (25D) levels are independently associated with lower hemoglobin (Hb) concentrations in chronic kidney disease (CKD) patients. However, the effect of Vitamin D2 (Ergocalciferol) repletion on the anemia of patients with end stage kidney disease (ESKD) is unclear. Therefore, we sought to observe, in three hemodialysis centers, the effects of Ergocalciferol supplementation on Hb levels and erythropoietin (EPO) dosing requirements in patients with ESKD.

Patients from three hemodialysis centers were screened with baseline 25D values. In two centers patients with 25D levels <30 ng/ml were prescribed 50,000 units of Ergo weekly, while patients with levels>30 ng/ml were given 50,000 units monthly. In the third unit the patients were given Ergocalciferol 50,000 units weekly. Subjects were then followed prospectively on a monthly basis with quarterly vitamin D levels for six months. Important variables compared from baseline to month 6 included 25D, Hb, EPO dosing and mortality.

The change from month 0 to 6 was calculated from difference of values with assigned rank test to assess significance. Spearman's correlation coefficient was used to assess correlation.

Of the 132 patients, 25D levels increased significantly from an average of 23.2 ± 11.9 to 35.8 ± 15.6 (p= .0001). The absolute change in 25D was an average of 10.7 (median 9.0) (P= .0001). Total EPO dose significantly decreased at an average of 52,498 units monthly (median: 23,000, p = 0.001). Average Hb increased significantly by a mean value of 0.2 mg/dl (p=0.024). There was no significant change in albumin , ferritin or intravenous iron dosing during this same time period. Change in 25D level was not associated with change in mortality.

In a 6 month prospective study, administration of Ergocalciferol was associated with patients requiring lower doses of EPO with a corresponding increase in the achieved Hb.

Disclosure of Financial Relationships: Research Funding: Shire.

### F-PO1412

**Clinical Evaluation of an Acetate-Free Dialysis Fluid Containing Citrate Used as a Dialysis Buffer – Central Dialysis Fluid Delivery System Used in 860 Patients** Junji Uchino, Toyohiko Yoshida. *Mihama Hospital, Chiba, Japan.*

Background: After being approved in Japan in 2007, acetate-free dialysis fluids have become popular, but reports on their use in central dialysis fluid-delivery systems are rare.

Objective: We aimed to evaluate the usefulness of an acetate-free dialysis fluid by assessing the changes induced by replacing the acetate-containing dialysis fluids (Kindaly AF2P and 2E, both containing 8 mEq/L acetate) with an acetate-free dialysis fluid (Carbostar-P [CP]) in 6 dialysis units of our hospital.

Patients and Methods: Subjects were 860 patients on maintenance dialysis, followed up for 7 to 14 months. AF2P and 2E were sequentially switched to CP, and changes in the quality of life (short form (SF)-36 score), treatment of anemia (amounts of erythropoiesis-stimulating agents (ESAs) and iron preparations used), and bone metabolism after switching were noted.

Results: Assessment of laboratory data after the switch revealed increased pH levels and HCO3 concentration. The levels of hemoglobin, total protein, and intact parathyroid hormone (i-PTH) increased after about 4 months of dialysis with CP. The SF-36 scores showed inhibition of the yearly decreases in values. ESAs were used in similar amounts, while the usage of iron preparations decreased markedly (25% to less than 10%). Among the 30 patients who had low i-PTH (< 60 pg/mL) levels for several months, 15 reached normal values.

Conclusions: The results showed that CP sufficiently decreased the degree of acidosis and had some other effects. In the future, the standards for the use of CP, including guidelines for the treatment of anemia, may require revision, and patients may require long-term monitoring.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1413

**Comparison of Different Equations by Bioimpedance Method for Detection of Body Cell Mass** Li Zuo, Yanna Dou, Li Liu. *Renal Division, Peking University First Hospital, Beijing, China.*

**Introduction**

Body cell mass (BCM) encompasses the intracellular water (ICW) compartment. In fact, ICW are used as a close approximation of BCM. BCM has been deemed a key parameter for assessing nutrition status.

There are two equations by multi-frequency bioimpedance measurement for evaluation of ICW: Xitron second generation ICW volume equation (eq. 1) and BCS equation (eq. 2) $V_{ICW}=V_{ECW}*\{[\rho TBW*(R_E+R_I)/\rho ECW*R_I]^{2/3}-1\}$ (eq. 1) $ICW_{BCS}=K_{ICW}[H2*(BW^{1/2})/R_I]^{2/3}$. (eq. 2). To evaluate the accuracy of different equations, we hypothesized that ICW will not change during one hemodialysis session and a better equation can detect ICW accurately.

**Methods**

A total of 50 stable hemodialysis patients from the dialysis center of Peking University First Hospital were included in this study. Bioimpedance measurements were performed in HD patients before and after HD session by the same operator. ICW were calculated by XE and BCSE, respectively. Paired t-test was used to compare changes in ICW calculated using different equations before and after HD session.

**Results**

In total 50 HD patients (23 males and 27 females) were observed with 115 bioimpedance measurements. The average age was 54.6±13.9 years. The mean height was 165.4±8.8cm. ICW results are shown in Table 1. There is a significant difference between XE and BCSE in pre-HD and post-HD . And ICW calculated by XE increased significantly after HD. However, there is no significant change in ICW calculated by BCSE after HD session.

ICW Comparison between Xitron equation and BCS equation

| | ICW By XE | ICW By BCSE | p Value |
|---|---|---|---|
| Pre-HD | 15.61±5.17 | 17.55±4.42 | <0.01 |
| Post-HD | 16.25±5.40 | 17.61±4.40 | <0.01 |
| p Value | p <0.05 | NS | |

**Conclusion**

BCS equation is more stable than Xitron second generation equation in estimation of body cell mass estimation.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1414

**Alignment of the Dialysate Sodium Concentration with the Serum Sodium: Role of Plasma Water Fraction and Donnan Effects** E. Lars Penne,[1,2] Jochen G. Raimann,[1,2] Len A. Usvyat,[1] Stephan Thijssen,[1,2] Nathan W. Levin,[1,2] Peter Kotanko.[1,2] *[1]Renal Research Institute, New York; [2]Beth Israel Medical Center, New York.*

Low dialysate sodium (dNa) concentrations have been associated with intradialytic symptoms such as hypotensive episodes. High dNa concentrations may lead to intradialytic sodium loading, thirst and subsequent increased interdialytic weight gain and hypertension. Alignment of dNa with pre-dialysis serum sodium (sNa) has been proposed to minimize dialytic morbidity. The aim of the current study was to evaluate whether such alignment strategy would result in a stable sNa concentration over the hemodialysis (HD) treatment.

The relation between the change in sNa from the beginning to the end of the dialysis treatment (Post-Pre Sodium Difference; PPSD) and the sodium gradient (grNa = dNa – $sNa_{pre}$) was evaluated in 150 chronic HD patients (mean age 61 ± 16 [±SD], 56% male, 80% black and 53% diabetic) with and without correcting the sodium level for Donnan effects ($\alpha = 1.007 – 0.009 \times TP$) and plasma water fraction ($f_{pw} = 0.989 – 0.0047 \times TP$), where TP = total protein in g/dL (Gotch FA et al. Proc Clin Dial Trans Forum; 1980). Intradialytic diffusive sodium flux was considered to be zero if PPSD = 0 mEq/L. Sodium was measured on a mid-week dialysis session by indirect potentiometry.

The relation between PPSD and grNa was linear (PPSD = 0.75 x grNa + 1.2; r = 0.80, P<0.001). According to this regression equation PPSD would rise by 1.2 mEq/L (95% CI 0.8 to 1.6) in a patient with grNa=0 mEq/L. Correction of the grNa for Donnan effects and plasma water fraction ($grNa_{corr}$) resulted in a shift of the regression line to the right. Alignment of the dNa based on the $grNa_{corr}$ resulted in a PPSD = -0.3 mEq/L (95% CI -0.7 to 0.2). Additional corrections for intradialytic changes of TP or total ultrafiltration volume did not change the results materially.

These analyses suggest that Donnan effects and plasma water fraction should be taken into account when aligning dNa with sNa. As a clinical rule of a thumb, dNa level should be 1 to 2 mEq/L lower than the pre-dialysis sNa to maintain a stable sNa concentration over the dialysis treatment. These findings need confirmation in a prospective study.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1415

**Relationship between Ionized Calcium Concentration and Activated Clotting Time in Citrate Anticoagulated Blood** Dieter Falkenhagen,[1] Stephan Thijssen,[2] Martin Brandl,[1] Jens Hartmann,[1] Karin Strobl.[1] *[1]Department of Clinical Medicine and Biotechnology, Danube University, Krems, Austria; [2]Renal Research Institute, New York, NY.*

**Background:**

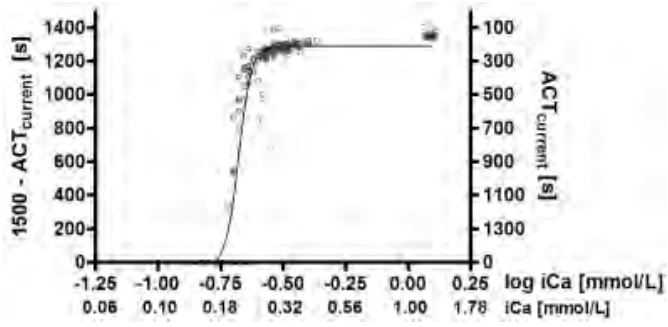
Regional citrate anticoagulation (AC) offers multiple advantages over systemic heparin AC in hemodialysis (HD) patients. An accurate characterization of the relationship between ionized calcium (iCa) level and degree of AC is valuable when modeling citrate AC.

**Methods:**

Whole blood samples were obtained from 10 healthy subjects and citrated with concentrations ranging from 2.75 to 6.5 mmol/L. iCa and activated clotting times (ACT) were measured for each sample. Coagulability was defined as maximum ACT (1500 seconds) minus ACT of current aliquot. The relationship between coagulability and log iCa was displayed, and the data were fitted to a variable-slope sigmoidal dose-response function (Y = bottom + (top-bottom)/(1+10^((logEC50-X)*(hill slope))), where X is the log iCa concentration and Y is the response (coagulability).

**Results:**

Baseline ACT was: min 121s; max 157s; median 148.5s. The relationship between coagulability and log iCa is displayed in Figure 1. The global fit estimates for this relationship are presented in Table 1. Goodness of fit was excellent, with $R^2$=0.93.




Best-fit values for sigmoidal dose-response relationship between blood coagulability [s] and log serum ionized calcium level [mmol/L].

| Parameter | Best-fit value |
|---|---|
| Bottom | 0 |
| Top | 1288 |
| LogEC50 | -0.6776 |
| Hill slope | 17.83 |
| EC50 | 0.2101 |

**Conclusion:**

The relationship between coagulability and iCa levels is non-linear, as previously shown by Falkenhagen et al. [1] and is described by the presented equation with very good accuracy. This characterization is of value for modeling AC during regional citrate AC in HD.

[1] Falkenhagen et al, Renal Diseases & Biotechnology for Blood Purification, proceedings, Chiang Mai, Thailand, 2009.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1416

**Citrasate®: Effects on Hemodialysis Adequacy and Heparin N Requirements** Jeffrey J. Sands,[1] Peter Kotanko,[2] Jonathan H. Segal,[3] Chiang-Hong Ho,[1] Amy Young,[4] Mary Carter,[2] Olga Sergeyeva,[2] Lisa Korth,[4] Eileen Maunsell,[1] Yueping Zhu,[1] Mahesh Krishnan,[4] Jose A. Diaz-Buxo.[1] *[1]Fresenius; [2]RRINY; [3]U Mich; [4]DaVita.*

Citrasate (C) contains citrate instead of acetate as in standard bicarbonate (B) dialysate. C is reported to improve clearance and decrease heparin requirements due to local, non-systemic anticoagulant effects in the dialyzer.

Methods: We prospectively evaluated HD pts over consecutive 2-week periods (P): Baseline-bicarbonate dialysate+standard (100%) dose heparin N (H), P1-Citrasate+100% H, P2-Citrasate+80% H, P3-Citrasate+67% H. Dialyzer clearance (mean KECN) and spKt/V were measured by ionic dialysance and clotting by dialyzer/line replacement or treatment termination. The predefined primary endpoint was non-inferiority of mean KECN between baseline and P2. $\beta$=0.1 at $\alpha$=0.05 required 100 pts to conclude non-inferiority with -8% margin (expected mean -5%, SD=10%).

Results: The cohort comprised 148 subjects: 60% male, 45% white, 49% black, 46% diabetic, 22% on antiplatelets, age 59±15 yrs, vintage 3.7±3.2 yrs, 60% AVF, 22% AVG, 19% catheters.

| | B+100% H | C+100% H | C+80% H | C+67% H | P |
|---|---|---|---|---|---|
| N | 148 | 134 | 117 | 101 | |
| Heparin N (u) | 3707±1423 | 3762±1431 | 3019±1148 | 2528±955 | |
| Heparin N (u/Kg) | 47.2±16.9 | 47.8±16.8 | 38.4±13.6 | 32.2±11.4 | |
| Mean KECN (mL/min) | 248±25.5 | 248±24.4 | 248±24.6 | 250±22.7 | NS |
| % Change Mean KECN | | 0.5 (-0.6, 1.6) | 0.6 (-0.7, 1.9) | 0.4 (-1.0, 1.9) | |
| % Decreased Mean KECN > 8% | | 6.7 (3.1, 12.4) | 5.1 (1.9, 10.8) | 9.9 (4.8, 17.5) | |
| spKt/V | 1.54±.24 | 1.54±.22 | 1.54±.23 | 1.56±.24 | NS |
| % spKt/V < 1.2 | 5.4 (2.4,10.4) | 6.0 (2.6, 11.4) | 6.0 (2.4, 11.9) | 4.0 (1.1, 9.8) | |
| % Clotted Dialyzer/Lines | 2.6 (0.4, 5.8) | 4.3 (1.2, 8.5) | 3.4 (0.9, 8.5) | 4.0 (1.1, 9.8) | |

(95% CI)

There was no difference in study related AEs or post HD time to hemostasis.

Conclusion: Citrasate + 80% heparin N maintained HD adequacy and met the primary endpoint of non-inferiority of mean KECN when compared to bicarbonate dialysis with standard dose heparin N. Dialyzer clearance was similar with 20% to 33% heparin reduction with no significant difference in clotting of dialyzers/lines or AEs.

Disclosure of Financial Relationships: Employer: Fresenius Medical Care NA.

## F-PO1417

**Thrombocytopenia Associated with One Type of Modified Polysulfone Hemodialysis Membrane** Srilatha Nadipineni, Jean Lee, Avrum Gillespie, Jesse M. Goldman. *Section of Nephrology, Temple University School of Medicine, Philadelphia, PA.*

Polysulfone membranes have been noted to activate platelets, depending on their composition and configuration . One case of thrombocytopenia has been linked to the Fresenius Optiflux polysulfone membrane. We wish to report five cases of persistent thrombocytopenia associated with the Fresenius Optiflux dialysis membrane.

table1

| Patient | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| ESRD diagnosis | Hypertension | Diabetes | Diabetes | Hypertension | FSGS |
| Ethnicity | Hispanic | Hispanic | African American | African American | African American |
| Age (yrs) | 66 | 43 | 66 | 63 | 30 |
| Sex | female | male | female | male | female |
| Initial Platelet Count | 218K | 121K | 113K | 186K | 176K |
| Plt s 24 -48 hrs post Fresenius Optiflux | 66K | 46K | 53K | 72K | 66K |
| Plt s 24-48 hrs post second membrane | 205K | 158K | 103K | 135K | 182K |
| Second Membrane | Baxter Xenium | Baxter Xenium | Baxter Xenium | Asahi Rexeed | Asahi Rexeed |
| HIT Antibody | Negative | Negative | Negative x 2 | Negative x 2 | not done |

The patients were on hemodialysis in 3 different units All cases occurred in a two year period .Peripheral blood smears showed decreased platelets without clumping The coagulation profile was normal. Platelet counts were lowest immediately post dialysis. No bleeding or thrombosis was associated with the low platelet count.

Platelet counts did not improve with heparin free dialysis. Medication review did not reveal any platelet lowering drugs. When Patients 1,2,3 were rechallenged with the Fresenius Optiflux membrane, the platelet count fell to less than 60,000. Once back on the Baxter Xenium dialyzer, the platelets counts recovered to greater than 100,000. The Baxter Xenium membrane is polyethersulfone and the Asahi Rexeed is a polysulfone modified to prevent platelet activation.

**Conclusions**: The Fresenius Optiflux membrane was associated with persistent thrombocytopenia in five hemodialysis patients. Platelet counts improved with other polysulfone dialysis membranes.

Further investigation is needed to determine the mechanisms of thrombocytopenia, and possible long term consequences. The thrombocytopenia associated with the Fresenius Optiflux polysulfone membrane may be confused with heparin induced thrombocytopenia or idiopathic thrombocytopenia.

Disclosure of Financial Relationships: nothing to disclose

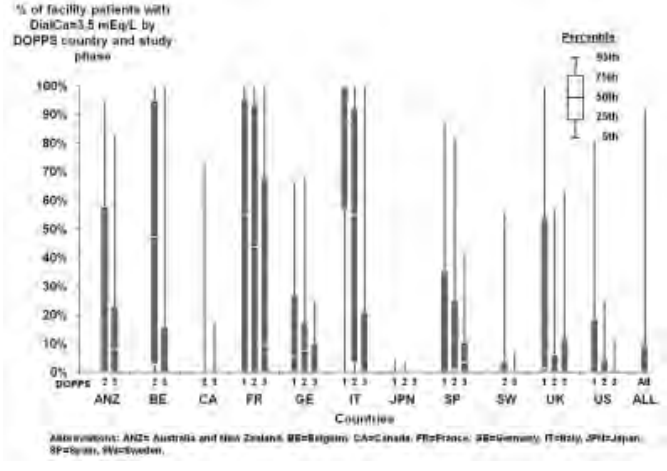
## F-PO1418

**High Dialysate Calcium (DialCa) Is Associated with Mortality in the DOPPS** Francesca Tentori,[1] Lin Tong,[1] Brenda W. Gillespie,[2] Hal Morgenstern,[2] Ronald L. Pisoni,[1] Friedrich K. Port,[1] Bruce M. Robinson,[1,2] Jean Ethier,[3] Michel Y. Jadoul,[4] Takashi Akiba,[5] Juergen Bommer.[6] *[1]Arbor Research; [2]Univ. of M; [3]Center Hosp. de Univ. of Montreal; [4]Univ. of Louvain; [5]Tokyo Women's Medical Univ.; [6]Dialysezentrum Heidelberg.*

Exposure to exogenous calcium (Ca) in the dialysate or from phosphate binders may contribute to vascular calcifications. We postulated that exposure to high levels of Ca may lead to increased mortality.

Data were from 34,575 patients (pts) with DialCa = 2, 2.5, 3, or 3.5 mEq/l from 869 facilities in 12 DOPPS countries. The associations of high DialCa (3.5 mEq/l ) with mortality (all-cause=ACM; cardiovascular=CVM; sudden death=SD) were examined in adjusted Cox models. To reduce bias due to unmeasured confounders, an instrumental variable (IV) approach was also used.

Use of DialCa (mEq/l) was: 2 = 3.4%, 2.5 = 42.2%, 3 = 40.2%, and 3.5 = 3.4% of patients. Facility use of DialCa=3.5 was variable, but fell over time (Figure). Serum Ca and comorbidities were similar across DialCa levels. Compared to lower DialCa, pts on DialCa=3.5 had higher ACM (hazard ratio=1.09 [95% CI=1.01-1.18], p=0.03), CVM (1.12 [1.00-1.26], p=0.05), and SD (1.23 [1.04-1.46], p<0.01). The association of DialCa=3.5 with higher CVM and SD was stronger among pts on ca-based phosphate binders (p<0.01). No interactions were found with low PTH and vitamin D use. DialCa=3.5 was also associated with mortality in IV models (ACM: 1.04 [0.92-1.17], p=0.56; CVM: 1.10 [0.92-1.33], p=0.29; SD:1.32 [1.01-1.73], p=0.04). No difference in mortality were observed for patients on DialCa=2, 2.5 or 3 vs. 3.5 mEq/l.




DialCa=3.5 mEq/l was associated with elevated mortality, especially in pts on Ca-based binders, supporting a detrimental effect of Ca load on outcomes. Exposure over time to lower DialCa levels (i.e. ≤ 3.0 mEq/l) requires additional study.

Disclosure of Financial Relationships: Research Funding: The DOPPS is administered by Arbor Research Collaborative for Health and is supported by scientific research grants from Amgen (since 1996), Kyowa Hakko Kirin (since 1999, in Japan), Genzyme (since 2009), and Abbott (since 2009), without restrictions on publications.

## F-PO1419

**Prediction of Normal Hydration State in Hemodialysis Patients Using Calf Resistivity** Fansan Zhu, Peter Kotanko, Nathan W. Levin. *Renal Research Institute, New York, NY.*

The inability to predict dry weight (DW) makes prescriptions of post dialysis weight difficult and usually subjective. We have previously reported (Zhu et al., Physiol Meas, 2008) the use of calf bioimpedance spectroscopy (cBIS) to estimate DW defined by two criteria: 1) Intradialytic flattening of the calf resistance curve measured continuously during the whole hemodialysis (HD) treatment, and 2) Achieving normalized resistivity (nRho) in the range of healthy subjects. The aim of this study was to model the relationship between differences in nRho and in post HD weights (PWt) between baseline (BL) and at actual DW which was established by the criteria above.

nRho at BL and actual DW were analyzed in chronic HD patients. In randomly selected patients (group 1) a regression model was developed to describe the relationship between nRho and excess fluid volume (ExFV). ExFV is defined as the difference between PWt at BL and actual DW. The regression model was then applied to a different group of HD patients (group 2) to predict DW. Predicted DW was calculated as PWt at any time minus ExFV [eq 1].

We studied 27 patients, 15 in group 1 and 12 in group 2. Regression analysis in group 1 related ExFV to ΔnRho: ExFV=0.504* ΔnRho+0.295 ($R^2$=0.61, p<0.001). Applying this regression model together with [eq 1] in group 2 resulted in an excellent prediction DW as determined by the independent criteria mentioned above ($R^2$=0.989, p<0.0001; Figure 1 (a)). In Bland Altman analysis no systematic bias was observed (p > 0.05; Figure 1(b)).

Using a model relating differences in calf resistivity and post dialysis weight at any time it is possible to predict what appears to be a close approximation to actual DW as determined by an independent method. This information is useful in that it provides objective targets for post dialysis weight prescription over time to achieve dry weight.

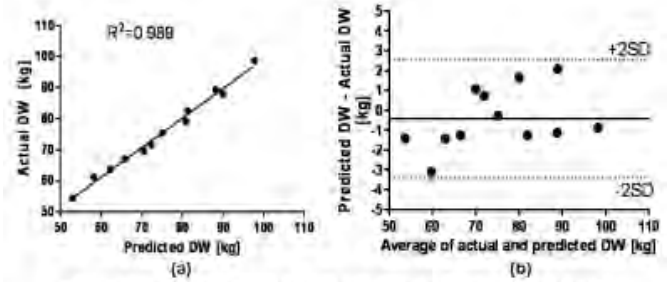



Fig.1 Correlation and Bland-Altman analysis for group2 patient (n=12)

Disclosure of Financial Relationships: nothing to disclose

## F-PO1420

**Improving Hemodialysis Adequacy, Anticoagulation and Dialyzer Efficiency with Streamline Bloodlines** Sharon Lynn Haas, Manohar Ahuja. *Milwaukee Nephrologists, Milwaukee, WI.*

The performance of conventional hemodialysis (HD) bloodlines is typically constrained by traditional technology that involves blood-air contact and arterial pressure (AP) limits caused by circuit turbulence. We evaluated a patented bloodline (Streamline® (SL) from Medisystems®) with reduced circuit turbulence and blood-air contact eliminated. We hypothesized that this newer technology using SL would deliver higher blood flows (Qb) at lower AP, which would enable efficient use of dialyzers while maintaining HD adequacy (Kt/V≥1.4); and lower heparin dosage & cost due to less frequent clotting of system.

Sixty seven patients were measured on lab draw days in 2 periods: 1) Pre-SL period using Fresenius Combiset® bloodlines; and 2) Post-SL period using SL after a 2 month transition. Rx adjustments were made to Qb, dialyzer type and heparin dose (based on rinseback quality). Single pool (sp) Kt/V, delivered Qb, average AP, and dialyzers were recorded for both periods. Dialyzer profile consisted of standard Fresenius and Asahi single-use dialyzers. Other Rx variables such as needle sizes, access types, dialysate rates, and HD times were not modified. Total heparin purchases were tracked over 12 months, and normalized by number of HDs performed/month. The results are shown in Table 1. Statistical significance was calculated using paired t-test where appropriate.

Table 1

| | Pre-SL | Post-SL | % Improvement | P value |
|---|---|---|---|---|
| Average Qb, ml/min | 431 | 453 | 5 | 0.003 |
| % patients with improved Qb | - | 43 | NA | - |
| Average AP, mmHg | -209 | -179 | 16 | <0.001 |
| % patients with improved AP | - | 70 | NA | - |
| Average spKt/V | 1.50 | 1.59 | 6 | 0.024 |
| % patients spKt/V>1.2 | 88 | 93 | 5 | - |
| % patients spKt/V>1.4 | 66 | 76 | 10 | - |
| % patients spKt/V>1.6 | 37 | 51 | 14 | - |
| Dialyzer cost/HD | $12.64 | $10.12 | 20 ($2.52) | <0.001 |
| Heparin cost/HD* | $1.64 | $0.70 | 57 ($0.96) | - |

*3-month rolling average cost based on total heparin purchased. Pre-SL = Mar to Apr 2009; Post-SL = Jan to Mar 2010

*In conclusion* , with SL use, we did increase Qb rates while reducing AP. The resultant improved HD adequacy allowed us to maximize dialyzer efficiency. We also did significantly lower heparin usage and cost/HD.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1421

**Serum Magnesium and Muscle Cramps in Chronic Hemodialysis Patients** Patrick Lynch, Heesuck Suh, Mersema Abate, Nand K. Wadhwa. *Division of Nephrology, SUNY, Stony Brook, NY.*

Muscle cramps are common adverse events among patients receiving chronic hemodialysis (HD). Serum magnesium (MG) levels have been implicated in muscle cramps associated with pregnancy, the elderly, and with restless leg syndrome. We hypothesized that magnesium may play a role in muscle cramps, particularly during HD treatment. The objective of the study was to evaluate prevalence of muscle cramps and its association with serum magnesium levels in HD patients. We conducted a survey of 108 chronic HD patients. 85 Patients (55 males and 30 females) participated in the survey. The mean age of participants was 60.0 ± 16.6 years (range 25-90 years). A single nephrology fellow conducted an in-person questionnaire with each participant. The severity of cramps was evaluated on a 0-10 scale, with 10 rated as maximal severity. Routine monthly pre-dialysis laboratory data were used for analysis. Sixty-two (73%) patients, 41 male and 21 female reported having muscle cramps. The mean age of patients with muscle cramps was 59.2 ± 16.7 years vs. 62.0 ± 16.5 years in patients without cramps. Fifty-seven (92%) patients reported cramps on their dialysis days while 26 (42%) reported cramps on their non-dialysis days. Forty (65%) patients report having muscle cramps during their HD treatment session and 29 (47%) patients had cramps after their session. Only 27% of patients with cramps informed their nephrologist. In contrast, 60% of patients inform a nurse or a dialysis technician. The mean KT/V, pre dialysis serum BUN, creatinine, sodium, potassium, calcium, phosphorus and albumin were not significantly different between the two groups. The mean pre-dialysis serum MG of 1.89 ± 0.34 mg/dl in the group with cramps was not significantly different from 1.84 ± 0.33 mg/dl in the group without cramps. However, the pre-dialysis serum MG level was inversely correlated (r= -0.29; p=0.02) to the severity of muscle cramps. No statistically significant difference in muscle cramps was found in relation to gender, race, diabetes and ESRD etiology. In conclusion, data suggest that serum MG may plays a role in muscle cramps in HD patients.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1422

**Bioimpedance Guided Reduction of Post-Dialysis Target Weight Does Not Lead to Increased Intradialytic Hypotension in Chronic Hemodialysis Patients** Samer Rateb Abbas,[1,2] E. Lars Penne,[1,2] Fansan Zhu,[1] Jochen G. Raimann,[1,2] Li Liu,[1,2] Stephan Thijssen,[1,2] Nathan W. Levin,[1,2] Peter Kotanko.[1,2] *[1]Renal Research Institute, New York; [2]Beth Israel Medical Care, New York.*

Treatment of overhydration is often suboptimal due to the current notion that reducing the post-hemodialysis (HD) target weight may increase the frequency of intradialytic hypotension (IDH). The aim of the present study was to investigate whether a reduction of the post-HD target weight towards dry weight, as assessed by continuous calf bioimpedance spectroscopy (cBIS), increased the risk of IDH.

In stable HD patients the post-HD target weight was gradually reduced over time until dry weight, as defined by cBIS (DWcBIS), was achieved (Zhu, Physiol Meas, 2008). IDH was defined by K/DOQI and EBPG (symptomatic drop of systolic blood pressure ≥20mmHg requiring staff intervention). Treatments were stratified in quartiles of the degree of overhydration (i.e. the difference between post-dialysis weight and DWcBIS). The frequency of IDH was analyzed for each of these quartiles.

We studied 289 HD treatments in 28 patients (57% male, age 54±12). Twenty patients (71%) were overhydrated at baseline and post-HD target weight was gradually decreased over a median period of 5 weeks (interquartile range: 2 to 9) until DWcBIS was reached. Clinical post-HD target weight was 1.2±1.7 L above DWcBIS (P=0.001). IDH occurred in 10% of the treatments, with no significant differences between quartiles of overhydration (<0.4 L above DWcBIS: 11%; 0.4 to 1.1 L above DWcBIS: 7%; 1.2 to 1.9 L above DWcBIS: 13%; > 1.9 L above DWcBIS: 10%, P=0.63).

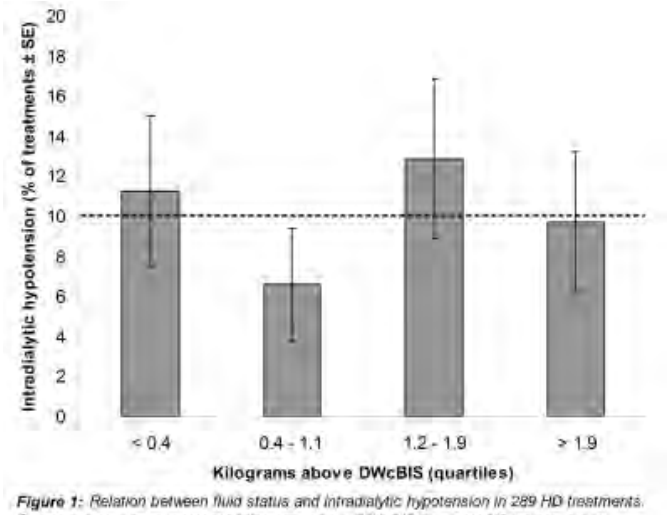



***Figure 1:*** *Relation between fluid status and intradialytic hypotension in 289 HD treatments. Categories on x-axes represent kilograms above DWcBIS (i.e. post-HD weight minus DWcBIS). P=0.63 for differences between groups. Dashed line respresents overall mean.*

The study supports the notion that fluid overload is highly prevalent among chronic HD patients. cBIS is a valuable method to estimate dry weight in HD patients. In overhydrated patients post-HD target weight can be safely lowered to DWcBIS without increasing the risk of IDH.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1423

**Comparison of Measurement of Change in State of Body Hydration in Hemodialysis Patients by Segmental and Whole Body Bioimpedance Methods** Samer Rateb Abbas,[1,2] Fansan Zhu,[1] Laura Rosales,[1] Dayvett A. Ulloa,[1,2] Murat H. Sipahioglu,[1,2] Mary Carter,[1,2] Peter Kotanko,[1,2] Nathan W. Levin.[1,2] *[1]Renal Research Institute, New Nork; [2]Beth Israel Medical Care, New York.*

The aim of this study was to compare calf, chest and whole body bioimpedance techniques in the assessment of changes in hydration state in hemodialysis (HD) patients.

Chronic HD patients were studied pre-HD and post-HD on two occasions in the same week: at the highest (hiWT) and at the lowest pre-HD weight (loWT). In addition, healthy controls (HC) were studied on one occasion. In each subject the following bioimpedance measurements were done to assess changes in fluid volumes: a) Calf bioimpedance spectroscopy (cBIS) to determine calf resistivity (nRho, W $m^3$/kg) (Xitron) in the supine position; b) ZOE fluid monitor (Zo, W) (Noninvasive Medical Technologies) to assess chest fluid volume in the sitting position; c) DF50 (ImpediMed) to measure whole body resistance (R50, W) , reactance (Xc50, W) , and phase angle (PA50, degree) at 50 kHz in the supine position.

We studied 54 HD patients (25f; age 63±16 yrs) and 54 HC (27f; age 35±10 yrs). Calf nRho increased significantly from pre- to post-HD and from hiWT to loWT. Zo increased significantly from pre- to post-HD but did not differ between hiWT and loWT. R50, Xc50 increased significantly from pre- to post- HD both at hiWT and loWT. In HD patients pre-HD R50 did not differ from HC. PA50 increased between pre- and post-HD at loWT..

| | Weight[kg] | Calf nRho[Ωm³/kg] | Zo[Ω] | PA50[°] | R50[Ω] | Xc50[Ω] |
|---|---|---|---|---|---|---|
| Pre-HD hiWT | 74.5±18# | 0.125±0.03*# | 21.4±4.6 | 4.9±6 | 525±84# | 36.7±11*# |
| Post-HD hiWT | 71.7±18# | 0.155±0.04*# | 23±4.3* | 4.9±2.4* | 609±118*# | 50.9±23* |
| Pre-HD loWT | 73.4±18 | 0.14±0.04* | 22.2±3.7 | 4.4±1.4* | 557±94 | 42.8±13* |
| Post-HD loWT | 71±18 | 0.17±0.05 | 23.9±4.3* | 5±1.9* | 650±112* | 57.1±23 |
| Healthy Controls | 75.3±16 | 0.19±0.03 | 22.4±3.7 | 6.2±0.8 | 551±84 | 58.8±7.6 |

# P<0.05 hiWT vs loWT * P<0.05 HD pts vs HC

Calf bioimpedance is more sensitive than ZOE and DF50 for assessing change in hydration and thus appears to be the preferred method for measuring the state of body hydration.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1424

**Cystatin C Reduction Ratio Depends on Normalized Blood Liters Processed and Fluid Removal during Hemodialysis** Shih-Han S. Huang,[1] Guido Filler,[2] Robert M. Lindsay.[1] *[1]Department of Medicine, Division of Nephrology, London Health Sciences Centre, London, ON, Canada; [2]Department of Pediatrics, London Health Sciences Centre, London, ON, Canada.*

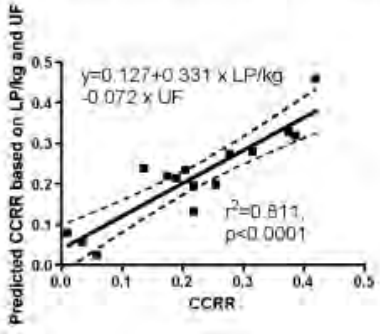
*Purpose:* A negative correlation between the weekly standard Kt/V (urea), an index of dialytic sufficiency, and serum cystatin C level (CysC) in functionally anephric dialysis patients was demonstrated. Our objective was to measure the pre-dialysis CysC reduction ratio (CCRR) and to compare it with other indices of dialytic function.

*Methods:* In a pilot cross-sectional study of 15 functionally anephric patients on conventional high-flux high-efficiency hemodialysis thrice per week, CysC levels were

drawn pre-, mid- and post- dialysis over one week. CCRR was compared with single pool Kt/V (Sp Kt/V) using urea kinetic modeling (UKM), urea reduction ratio (URR), creatinine reduction ratio (CRR), normalized liters processed (LP/kg) and ultrafiltration volume (UF). Normally distributed data (Shapiro-Wilks test) were described as mean one standard deviation; otherwise as median and interquartile range.

*Results:* The mean pre- and post- CysC levels were 6.0±1.0 mg/L and 4.7±1.1 mg/L (p=0.002). The Sp Kt/V and Std Kt/V were 1.5±0.2 and 2.6 (2.2, 2.7). The URR, the CRR and the CCRR were 70.2±9.0 %, 64.5±8.2 % and 26.1±11.8 % (p≤0.002), respectively. There was no correlation between the CCRR and the Sp Kt/V, URR and CRR while CCRR correlated with LP/kg ($r^2$=0.459, p=0.006) and UF ($r^2$=-0.524, p=0.002). Multiple regression analysis with these two parameters provided a model that explained 81% of the variance ($r^2$=0.811, p<0.001).

Figure 3. Correlation Analysis of the measured Cystatin C Reduction Rate (CCRR) and the calculated CCRR based on a model using the ultrafiltration volume (UF [L]) and the normalized Liters processed (LP/kg [L/ kg]). The model explained 81% of the variance




*Conclusion:* Our data suggest that normalized liters processed and ultrafiltration volume explain most of the variance of CCRR. Therefore, CCRR may be an excellent way to monitor dialysis efficiency for low molecular weight proteins.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1425**

**Impacts of Diabetic Foot Checks on Amputations and Hospitalizations in Incident Hemodialysis Patients** Lynn Saunders, Len A. Usvyat, Paul Balter, Lisa Bocklud Harvey, Heather J. Ansede, Nathan W. Levin, Peter Kotanko, Paul M. Zabetakis. *Renal Research Institute, NY, NY.*

Diabetic hemodialysis (HD) patients (pts) experience high incidence of leg or toe amputations. In recent yrs, foot checks in pts with diabetes mellitus (DM) became a routine procedure in RRI clinics. Previous analyses in prevalent DM HD pts have shown that foot checks decrease risk of amputations (Saunders, ERA 2010). This study assesses whether foot checks affect amputations & number of hospitalizations in incident DM HD pts.

We reviewed all RRI DM HD pts who started first dialysis b/n Jan 1, 2001 and Apr 30, 2010. Only pts who survived first 12 mns were included. The number of foot checks done in the first 12 mns was counted ("baseline prd"). Pts' leg and toe amputations after the baseline prd were noted ("follow up prd"). A logistic regression model was built with amputation in the follow up prd as an outcome variable & number of foot checks, race, ethnicity, age & gender as predictors.

N=3057 incident DM pts (53% male, 47% black, 43% white, mean age [SD]: 63.1 [12.5] years). In multivariate logistic regression the number of foot checks (odds ratio 0.87 [95% CI: 0.81-0.94]) and male gender (odds ratio 1.49 [95% CI: 1.05-2.12]) predicted amputations.

Hospital admissions (P<0.05) & hospital days (P<0.05) were lower in pts who received more than 10 foot checks in the first yr of dialysis.

| Number of foot checks in year 1 | Hospital Admissions per pt yr | Hospital Days per pt yr |
|---|---|---|
| ≤10 | 1.50 | 10.73 |
| >10 | 1.32 | 6.73 |

Foot checks in DM HD pts are crucial component of quality care in dialysis clinics. In RRI pts the likelihood of amputation was reduced by 13% per every foot check performed in the first yr of HD. Male pts have notably higher incidence of amputations. While some reduction in hospitalization may be attributed to lower incidence of amputations, overall improvement in hospital admission & days is also notable when monthly foot checks are part of a standard clinic operating procedure.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1426**

**Quantifying and Predicting the Impact of Hemodialysis on Drug Exposure: Application to Saxagliptin** Liping Zhang, David W. Boulton, Marc Pfister. *Research and Development, Bristol-Myers Squibb, Princeton, NJ.*

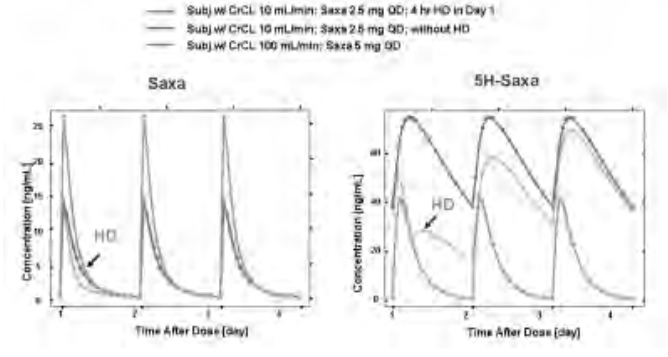
**Background:** The new draft FDA guidance requires PK studies be conducted for renally excreted medicines in subjects under both HD and non-HD conditions to determine the contribution of HD to the elimination of the drug and active metabolites. Yet clinical study designs (often single dose administrated before HD session) do not necessarily represent patient care (multiple doses administrated at varying times relative to HD sessions).

**Objective:** Using saxagliptin (Saxa) and its active metabolite, 5-hydoxy saxagliptin (5H-Saxa) exposure modeling as an example, this work demonstrates how modeling and simulation (M&S) can be used to quantify and predict HD effects on drug exposure in clinical studies and in patient care.

**Methods:** Plasma concentrations of Saxa and 5H-Saxa from 39 subjects with normal renal function, mild, moderate, severe renal impairment, or ESRD undergoing HD were quantified after a single dose of 10-mg Saxa. Nonlinear mixed effect modeling was applied to differentiate the contribution of HD and renal/non-renal clearance of Saxa and 5H-Saxa.

**Results:** The model-estimated mean clearances of Saxa and 5H-saxa contributed by HD are 23.7 (14.9-32.5) and 3.67 (2.96-4.38) L/h, respectively. For an ESRD subject with creatinine clearance of 10 mL/min, clearance of Saxa and 5H-Saxa will increase during HD treatment by 1.7 and 6.5 fold, respectively. The expected concentration profiles of Saxa and 5H-Saxa in subjects receiving 3 HD sessions a week are presented in Figure 1.

**Figure 1. Simulated steady state concentration profiles of Saxa and 5H-Saxa After HD on Day 1**




The total active moiety after Saxa administration in ESRD patients undergoing HD is estimated.

**Conclusion:** Using data collected from a standard single-dose renal impairment study, pharmacometric M&S can provide an efficient way to quantify and predict the impact of HD on drug exposure in the clinical setting.

Disclosure of Financial Relationships: Employer: Employee of Bristol-Myers Squibb Company; Ownership: Stockholder of Bristol-Myers Squibb Company.

**F-PO1427**

**Does Individualized Sodium Concentration of Dialysate Fit All Hemodialysis Patients?** Eun Sook Jung,[1] Ji Yong Jung,[2] Kook-Hwan Oh,[1] Yon Su Kim,[1] Curie Ahn,[1] Jin Suk Han,[1] Suhnggwon Kim,[1] Kwon Wook Joo.[1] [1]*Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea;* [2]*Internal Medicine, Gachon University of Medicine and Science, Incheon, Republic of Korea.*

Most dialysis units adopt a single default dialysate sodium prescription. No single dialysate will provide isonatremic dialysis to all patients and an individualized approach seems to be more physiologic. We investigated the clinical impact and safety of an individualized dialysate sodium prescription. Twenty-six chronic maintenance hemodialysis (HD) patients were enrolled. During the standard period (3 weeks), all the subjects underwent nine consecutive HD sessions with the dialysate sodium concentration of 136 (16 pateints) or 138 mEq/L (10 patients). During the individualized period (3 weeks), they underwent nine HD sessions with the individualized dialysate sodium concentrations set to match the patient's average midweek pre-HD serum sodium concentration during the standard period. The patients were divided into three groups: average midweek pre-HD serum sodium concentration was lower than (group 1, n=7), equal to (group 2, n=6), and higher than (group 3, n=13) the standard dialysate sodium concentration. Pre-HD serum sodium concentration did not change during the entire study period in all groups. In group 1, pre-HD diastolic blood pressures decreased (70.8±11.0 vs. 66.9±8.4 mmHg; P<0.05) and intradialytic hypotensive episodes and nursing interventions increased significantly after dialysate sodium individualization. In group 2, no significant differences were observed in all parameters. In group 3, interdialytic weight gain increased significantly during the individualized period (2.0±0.5 kg vs. 2.3±0.4 kg; P<0.05). Thirst scores in 7 patients whose dialysate sodium was increased by 4 mEq/L were increased (6.4±1.5 vs. 7.6±1.5; p<0.05).

Individualization of dialysate sodium concentration based on patient's serum sodium has little benefit for the chronic stable HD patients with their baseline dialysate sodium concentration of 136-138 mEq/L. An individualized dialysate sodium prescription does not fit all HD patients ant it may be considered only in the selected patients.

Disclosure of Financial Relationships: nothing to disclose

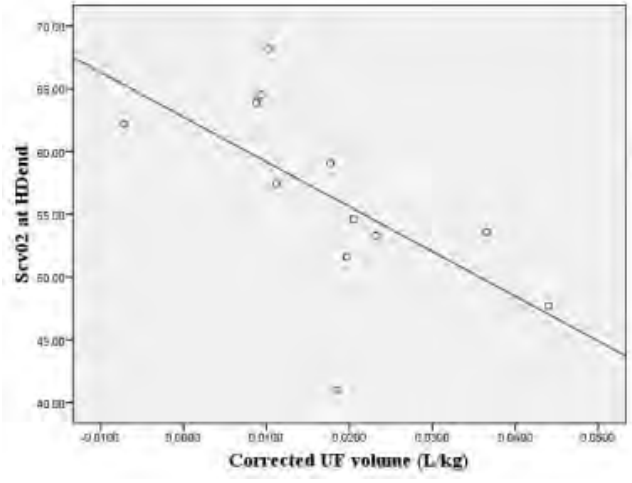
**F-PO1428**

**Central Venous Oxygen Saturation: A Potential New Marker for Circulatory Stress in Haemodialysis Patients** Laura E. A. Harrison,[1] Nicholas M. Selby,[1] Chris W. McIntyre.[1,2] *[1]Department of Renal Medicine, Royal Derby Hospital, United Kingdom; [2]School of Graduate Entry Medicine and Health, University of Nottingham, United Kingdom.*

Haemodialysis (HD) results in significant circulatory effects with subsequent ischaemic end-organ dysfunction. Central venous oxygen saturation ($ScvO_2$), utilised as a composite marker of oxygen delivery/consumption, can predict prognosis in heart failure and septic shock. We aimed to measure $ScvO_2$ during HD and assess its potential in identifying patients experiencing significant circulatory stress.

12 established HD patients utilising tunnelled internal jugular central venous catheters were studied. Central venous samples were drawn from the arterial line in the first and last 10 minutes of HD with immediate blood gas analysis, allowing direct measurement of right atrial $SO_2$. Blood pressure (BP) and heart rate (HR) were measured pre, post and every 15 minutes during HD with weight and ultrafiltration (UF) volumes recorded.

Patient age was 67±16 yrs and dialysis vintage 20 months [IQR 9-24]. At HD initiation, $ScvO_2$ was 63.5±13% (cf quoted normal value of 70%), and during HD it fell further to 56.4 ±8% (p=0.04). UF volume inversely correlated to $ScvO_2$ (r= -0.680, p=0.015). Correcting UF volume for body weight strengthened this association (r= -0.769, p=0.003) (see figure 1). Absolute and $\Delta ScvO_2$ did not correlate with age, pre or post SBP and HR, nor BP reduction during HD (although mean reduction in SBP was only 9±20 mmHg).



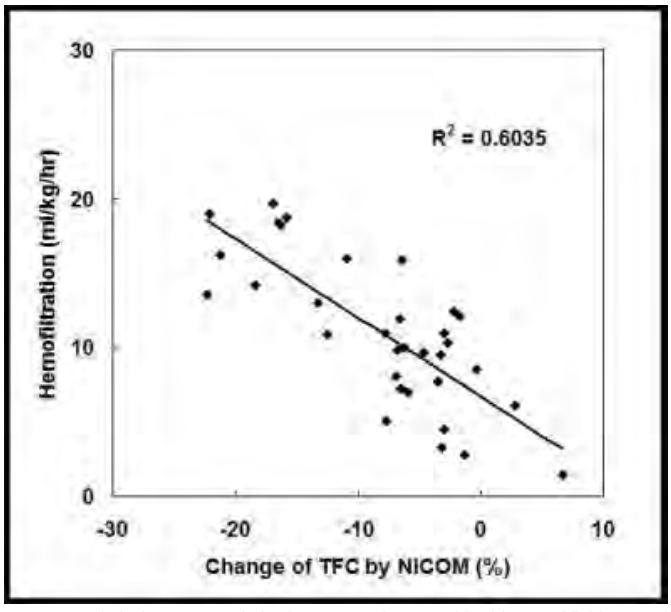

This study demonstrates $ScvO_2$ sampling is practical, with potential clinical utility as an indicator of circulatory stress during HD. Increasing UF volume, previously indicated as the principal driver of cardiac stress in HD, was strongly associated with $ScvO_2$ values at the end of dialysis. Additional study is needed to further define the clinical utility of $ScvO_2$ monitoring in optimising the cardiovascular response to HD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1429**

**Characterization of Cardiovascular Health for Chronic Dialysis Using Continuous Non-Invasive Monitoring** Jose F. Bernardo,[1] Hyung Kook Kim,[2] Michael R. Pinsky.[2] *[1]Medicine/Renal-Electrolyte Division, University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

The principle of fluid removal during intermittent hemodialysis (IHD) is to induce a relative hypovolemic state by ultrafiltration followed by refilling of the vascular space from the interstitial and cellular compartments (plasma refill rate). However, if the ultrafiltration rate (UFR) greatly exceeds the plasma refill rate then hypovolemic cardiovascular collapse may ensue. The objective of this study was to determine if progressive changes in arterial pressure, cardiac output (flow) and related cardiovascular status using the bioreactance non-invasive cardiac output monitor (NICOM)(Cheetah Medical, Tel Aviv) can tract UFR and identify the onset of cardiovascular insufficiency during routine IHD. The NICOM reports CO, stroke volume index, ventricular ejection time, cardiac contractility and TFC. We hypothesized that steady state thoracic fluid content (TFC) and the change in cardiac output (CO) would parallel total volume removal during IHD. These parameters were measured over the course of IHD in 20 chronic dialysis patients on two separate consecutive treatment days corresponding to weekend and weekday interval. Seven runs were lost because of technical reasons. Of the remaining 33 runs, there was no relation between change in CO and the UFR or day of dialysis. However, change in TFC inversely correlated with UFR (r=0.6, p<0.001) but not with total amount of fluid removal.




We conclude that NICOM-derived measures of TFC could guide rate of fluid removal during dialysis whereas measures of flow appear to be insensitive to dialysis-induced fluid removal. A larger prospective clinical trial would be needed to address this issue.

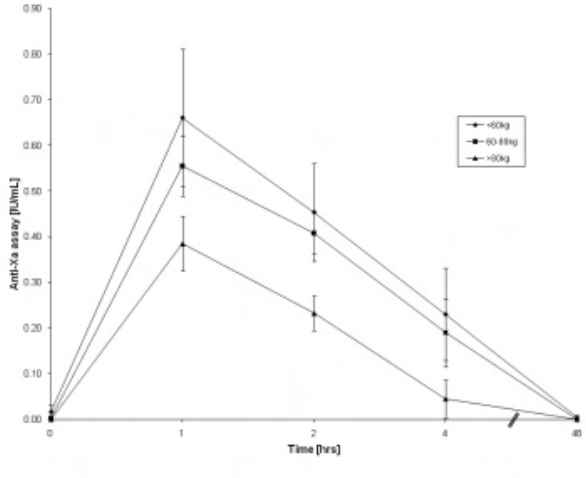
Disclosure of Financial Relationships: nothing to disclose

**F-PO1430**

**Tinzaparin Pharmacokinetics in South Asian Hemodialysis Patients** Albert J. Power,[1] Mike Laffan,[2] Claire Edwards,[1] David Taube,[1] Neill D. Duncan.[1] *[1]Imperial College Kidney & Transplant Institute, West London Renal & Transplant Centre, Hammersmith Hospital, London, United Kingdom; [2]Department of Haematology, Imperial College, London, United Kingdom.*

30% strokes in dialysis patients are hemorrhagic in origin, are associated with worse prognosis and may relate to hemodialysis [HD] circuit anticoagulation. We noted an increase in hemorrhagic stroke at our center on conversion from unfractionated heparin to fixed-dose tinzaparin for circuit anticoagulation [as per European recommendations] which appeared limited to South Asian patients. We therefore examined the influence of ethnicity on the anticoagulant effect of tinzaparin.

We prospectively studied tinzaparin 2500 IU [Innohep®, Leo Pharma, UK] given once via the arterial limb of the HD circuit at initiation of HD. 12 patients dialyzing via central venous catheters grouped according to dry weight [<60, 60-80 & >80kg] had heparin-free HD 72 hrs prior to study with the catheter lumens locked with 46.7% citrate. Coagulation profile [prothrombin time, APTT and fibrinogen], antithrombin-III and anti-Xa assays were performed at 0, 1, 2, 4 and 48hrs from HD start.




Anti-Xa levels peaked at 1 hour reaching 0.66±0.15, 0.56±0.07 & 0.39±0.06 IU/ml in the <60, 60-80 & >80kg groups respectively and with parallel increases in APTT [54±8, 41±6 & 33±4 secs]. Tinzaparin use and initiation of HD had no significant effect on antithrombin-III levels [mean 0.85±0.15 vs 0.86±0.18 IU/l, p=0.7]. There was no significant change in prothrombin time or fibrinogen levels. Tinzaparin exerted no anticoagulant effect extending into the next HD session and no patient experienced catheter dysfunction during the study.

Fixed-dose tinzaparin appears to exert a predictable weight-dependent anticoagulant effect that needs to be considered in patients of higher hemorrhagic risk. White and African-Caribbean patients are currently under evaluation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1431**

**Lowering Dialysate Na Prescription in Patients Undergoing Nocturnal Center HD: Effect on Interdialytic Weight Gain and Blood Pressure** Jair Munoz Mendoza,[1,2] Sumi Sun,[2] Sheila Doss,[2] Brigitte Schiller.[1,2] *[1]Department of Nephrology, Stanford University, Palo Alto, CA; [2]Satellite Healthcare, Mountain View, CA.*

Patients on nocturnal in center hemodialysis (HD) frequently increase interdialytic weight gain (IDWG) over time, supposedly due to more liberalized fluid intake with longer dialysis. We examined whether dialyzing patients against a lower dialysate sodium (Na) concentration closer to the patient's pre-dialysis plasma Na (average of three monthly results) would reduce IDWG.

Fifteen patients were included in this QA project. Patients were told that changes in dialysate prescription would be made, but were blinded to timing to avoid a bias towards over reporting of adverse events (hypotension, cramps). After at least three months observation with "standard" (Na=140mEq/L) therapy, dialysate Na concentration were lowered to 136 or 134mEq/L with gradual biweekly changes. The results from the last two weeks of each prescription were compared by paired t-test.

The mean pre-HD plasma Na during the standard Na phase was 136mEq/L (range 129 to 141mEq/L). IDWG decreased significantly from 4.2 ± 0.8 with standard dialysate Na to 3.6 ± 0.6 with lower dialysate Na concentration ($p<0.004$). Pre-HD MAP and SBP also decreased with lower dialysate Na -6.6 ± 12 ($p=0.05$) and -8.3 ± 15 ($p=0.05$), respectively. Furthermore, a reduction in pre-HD DBP was noted (89.6 ± 11.6 vs 83.9 ± 10.6; $p=0.06$), while no difference in post-HD MAP ($p=0.48$), post-HD SBP ($p=0.60$) and post-HD DBP ($p=0.43$) was found. There was only one episode of intradialytic hypotension requiring saline in each phase. The pre-HD plasma Na was unchanged in both phases ($p=0.99$), while post-HD plasma Na decreased -3.7 ± 1.9mEq/L with lower dialysate Na prescription ($p<0.0001$).

Decreasing dialysate Na from standard 140mEq concentration in extended time center HD resulted in decreased IDWG, reduced post-HD plasma Na and reduction in pre-dialytic BP without increasing intradialytic adverse events. These preliminary data require confirmation in more subjects undergoing conventional thrice weekly HD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1432**

**Extracellular Resistance Changes in the Calf and Whole Body in Different Hydration State during Ultrafiltration** Li Liu, Yanna Dou, Li Zuo. *Renal Division, Peking University First Hospital, Beijing, China.*

**Purpose** Monitoring fluid removal in the calf by bioimpedance technique during ultrafiltration (UF) has been suggested to determine normal hydration status (NH) in hemodialysis (HD) patients [Zhu F, Physiol Meas, 2008]. The aim of the study was to explore the relationship between fluid removal in the calf and whole body (WB) during UF.

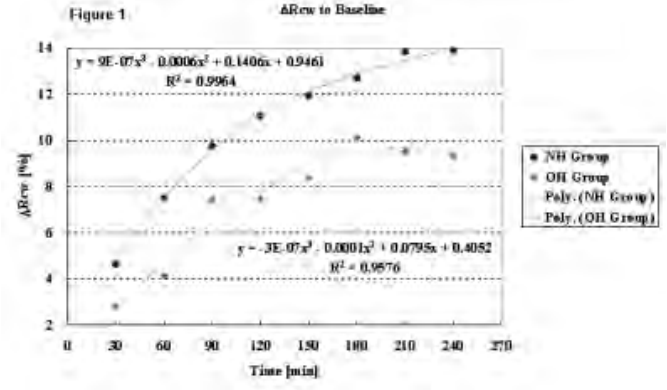
**Methods** Twenty HD patients (11F), aged 56 years, were enrolled. Removal of fluid in WB or calf was represented by change in extracellular resistance ($wR_E$ and $cR_E$) were measured by a Hydra 4200 and modified Hydra device every 30 min during HD respectively. Ratio of $cR_E/wR_E$ was used to indicate fluid distribution between calf and WB. All measurements were divided into two groups according to calf resistivity (nRho<18) defined as over-hydration (OH) and nRho>18 defined as normal hydration (NH). Change in ratio of $cR_E/wR_E$ (ΔRcw) % from baseline (time '0') to time 't' was calculated by formula: $(Rcw_t-Rcw_0)/Rcw_0*100\%$. Average ΔRcw for every 30 minutes in all measurements was calculated in OH and NH respectively. Polynomial regression with 3 orders was performed to distinguish difference in the curve of average ΔRcw between OH and NH during HD.

**Results** Among 25 measurements, 12 were in NH group and 13 in OH group. Both cRe and wRe increased continuously over time during hemodialysis session (Table 1). Figure 1 shows that mean of ΔRcw increased. However, the curve was trend to be flattening during the last hour in OH but increased continuously in NH during UF.

Table 1 Change in Extracellular Resistance in Calf and Whole Body over Time

| | OH Group | | NH Group | |
|---|---|---|---|---|
| Time [min] | $cR_E$ (ohm) | $wR_E$ (ohm) | $cR_E$ (ohm) | $wR_E$ (ohm) |
| 0 | 39.97±7.94 | 536±107 | 43.46±8.12 | 606±77 |
| 30 | 42.35±9.22 | 551±113 | 47.01±8.74 | 624±82 |
| 60 | 44.14±9.51 | 567±116 | 49.82±9.50 | 642±84 |
| 90 | 45.80±10.35 | 575±125 | 52.09±9.80 | 658±84 |
| 120 | 47.39±10.40 | 599±139 | 54.40±10.16 | 680±91 |
| 150 | 49.08±10.94 | 614±145 | 56.76±10.58 | 703±96 |
| 180 | 51.18±11.40 | 634±152 | 58.95±10.37 | 727±103 |
| 210 | 52.85±12.06 | 659±163 | 61.43±11.11 | 749±110 |
| 240 | 54.45±13.03 | 675±168 | 63.17±11.60 | 771±117 |

Figure 1




**Conclusion** Fluid was removed more quickly and continuously from calf than other segments which confirmed that hydration in the calf is relatively higher than the average hydration in whole body. This suggests that normal hydration can be determined more accurately by using calf bioimpedance approach in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1433**

**Effect of Elevating Calcium Concentration of Dialysate To Treat Intra-Dialytic Hypotension: A Cross-Over Self-Control Trial** Zi Li, Ping Fu. *Department of Medicine-Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

**BACKGROUND:** Symptomatic intra-dialysis hypotension (IDH) continues to be a common problem. With the use of low calcium (Ca) concentration dialysate in our dialysis center for 5 years, several patients developed IDH occasionally or frequently. Even after multiple therapeutic strategies were induced, IDH still happened in some patients. In this study, we investigated the changes in haemodynamic parameters induced by calcium concentration of dialysis fluid in IDH patients. **METHODS:** Single-blinded, crossover design was used. Nineteen maintainance hemodialysis patients with IDH were randomized into two groups, beginning by changing the calcium concentration in dialysate either to 1.25mmol/L or 1.75mmol/L. The first 2-week intervention phase was followed by two-week of wash out with calcium concentration in dialysate as 1.5mmol/L, switching round the calcium concentration compared to the initial randomization, by a second 2-week intervention phase. **RESULTS:** Eighteen patients finished the trial except one patient withdrew due to hypercalcemia. Compared to low calcium concentration (1.25mmol/L), medium and high calcium concentration (1.5mmol/L and 1.75mmol/L respectively) elevated intradialytic systolic blood pressure (SBP) and heart rate ($P<0.05$), decrease the maximum decrease of SBP (the difference between predialytic SBP and the lowest intradialytic SBP), decrease the events of symptomatic IDH. Compared to medium concentration, high calcium concentration dialysate only had lower incident of intradialytic intravenous fluid infusion. No sever disorder of electrolyte were found. **CONCLUSION:** Medium calcium concentration dialysate can effectively improve the hemodynamic stability in IDH patients. Clinical trial with larger sample and longer follew-up is needed.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1434**

**Elevated Plasma Brain Natriuretic Peptide (BNP) Levels in Hemo-Dialysis (HD) Patients with Sleep Apnea (SA)** Robert L. Benz,[1,3,6] Rob J. Mathews,[1] Mark R. Pressman,[4,6] Michael M. Chernick,[5] Albert A. Keshgegian.[2,3,6] *[1]Nephrology, Lankenau Hospital, Wynnewood, PA; [2]Pathology, Lankenau Hospital, Wynnewood, PA; [3]Mainline Health, Wynnewood, PA; [4]Pulmonary & Sleep Medicine, Lankenau Hospital, Wynnewood, PA; [5]Biostatistics, Lankenau Institute for Medical Research, Wynnewood, PA; [6]Lankenau Institute for Medical Research, Wynnewood, PA.*

**Background**: BNP is elevated in volume overload states. In HD patients, BNP can be elevated in the absence of overt circulatory overload. SA causes pulmonary hypertension and circulatory patho-physiology which could stimulate BNP from overstretched myocardial tissue. We evaluated BNP levels in HD patients with SA to determine whether SA may contribute to elevated BNP.

**Methods**: 22 SA patients on maintenance high-flux HD without evidence of overt circulatory overload were studied. Volume status was assessed by history, exam, and weight measurements. SA was assessed by prior polysomnography (PSG) and all underwent confirmatory PSG followed by pre/post-dialysis (1st of week) and pre-next dialysis (mid-week) measurements of BNP.

**Results**: 19/22 SA, HD patients had elevated BNP levels. Mean KT/V was 1.66. Mean group post-HD weights matched mean group EDW goal. Mean (1st of week) pre-HD BNP was 846.2pgm/ml; mean post-HD BNP was 895.4 pgm/ml; and mean pre mid-week BNP level was 755.2 pg/ml (nl<100pgm/ml). Mean apnea-hypopnea index (AHI) was 40.94 events/hour. There was no correlation between AHI and BNP [p=0.856, 0.991, and 0.938 (pre/post/pre) respectively].

**Conclusion**: Plasma BNP is elevated in HD patients with SA despite the absence of overt circulatory overload. There was no correlation between BNP levels and AHI, likely eliminating SA as a causative factor of elevated BNP. This data suggests that alternative causes for elevated BNP be investigated in HD patients in the absence of circulatory overload despite the presence of SA.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1435**

**Negative Sodium Gradients Are Associated with Greater Sodium Removal without Adversely Affecting Inter-Dialytic Weight Gain: Support for an Individualisation of Dialysate Sodium** Aghogho Odudu,[1] Maarten W. Taal,[1] Richard J. Fluck,[1] Chris W. McIntyre.[2] *[1]Renal Medicine, Royal Derby Hospital, United Kingdom; [2]School of Graduate Entry Medicine and Health, University of Nottingham, United Kingdom.*

**Background.** Larger inter-dialytic weight gains (IDWG) and higher ultrafiltration volumes and rates are associated with cardiac morbidity and mortality. Control of IDWG is a key therapeutic aim. The sodium set-point hypothesis predicts that the gradient between serum sodium (SNa) and dialysate sodium (DNa) is a major driver of thirst and IDWG. A positive sodium gradient (GNa = DNa - SNa), results in sodium loading and increased IDWG, but the effects of a negative gradient have been subjected to only limited study.

**Methods.** 19 chronic HD patients were studied over four weeks, dialysed against a constant 136 mmol/l dialysate Na concentration. 15 were anuric and 4 had urine output <150 ml/day. We used continuous on-line conductivity monitoring, ion-specific electrodes and a validated sodium kinetic model to calculate sodium gradients and related this to both ionic mass balance (IMB), IDWG, and blood pressure.

**Results.** A full dataset was available for 214 treatment sessions. The mean+/- SD are presented. GNa was -6.1+/-3.8 mmol/l, IMB 347+/- 146 mmol, UF volume 1.9+/-0.9l, IDWG 2.7+/-1.4%, pre-HD systolic BP 142+/-25 and diastolic BP 83+/-17 mmHg. Inter-patient variation in IMB was much greater than intra-patient variation. There was a reasonable correlation of negative gradients with higher ionic mass balance (r =0.59, p<0.01). There was no detectable effect on IDWG or BP.

**Conclusion.** Negative sodium gradients enhance sodium removal without increasing intra-dialytic hypotension. There was no detectable effect on IDWG.

**Relevance.** This study needs to be confirmed in a larger population but suggests that having dialysate sodium set lower than plasma sodium may enhance diffusive removal of sodium without adverse effects. Use of on-line conductivity monitoring makes this operationally feasible.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1436**

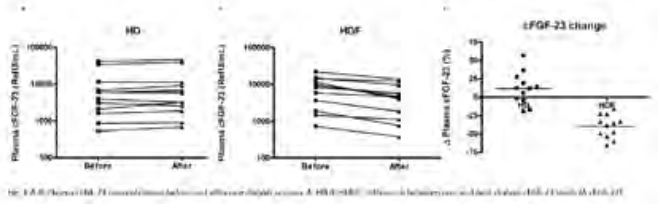
**Fibroblast Growth Factor 23 Is Effectively Removed by Online Hemodiafiltration, but Not by Low-Flux Hemodialysis** Claire H. Den Hoedt,[1,2] Marinus A. Van Den Dorpel,[1] Marc G. Vervloet,[3] Albert H. Mazairac,[2] Neelke C. Van Der Weerd,[3] Muriel Grooteman,[3] E. Lars Penne,[2,3] Michiel Bots,[4] Peter J. Blankestijn,[2] Pieter M. Ter Wee.[3] *[1]Maasstad Hospital, Rotterdam; [2]UMCU, Utrecht; [3]VUmc, Amsterdam; [4]Julius Center, Utrecht, Netherlands.*

**Background.** Fibroblast Growth Factor 23 (FGF-23, ±27 kDa) is strongly associated with increased mortality in dialysis patients. If causality exists, methods to lower FGF23 can have beneficial effects on outcome.

**Purpose.** To investigate whether FGF-23 is more effectively removed by online hemodiafiltration (HDF) than by low-flux hemodialysis (HD).

**Methods.** Blood samples were obtained at the beginning and end of 1 dialysis session in 12 HDF and 12 HD patients. Both full-length FGF-23 (iFGF-23) and C-terminal FGF-23 (cFGF-23, ±12 kDa) were measured. FGF-23 levels are depicted as medians (total ranges). Differences were analyzed by Mann-Whitney tests.

**Results.** cFGF-23 changed from 5030 RefU/mL (531-42319) to 4549 RefU/mL (678-47268) in HD patients and from 8449 RefU/mL (717-22018) to 4540 RefU/mL (378-13635) in HDF. Pre dialysis levels did not differ between the two groups (p=0.5). During HD, cFGF-23 did not change (+12% (-19 to +57)), whereas it decreased during HDF (-37% (-65 to -15)) (p<0.001, for difference in Δ change). iFGF-23 remained stable during HD (+11%) and decreased significantly during HDF (-28%) (p<0.001). Residual kidney function, weight- and calcium change were the same in the 2 groups. Phosphate and PTH levels decreased significantly more in HDF, but did not explain the change in FGF-23.




**Conclusions.** FGF-23 is effectively removed by HDF but not by low flux HD. In view of the possible adverse actions of FGF-23 in the development of cardiovascular disease, removal by HDF may be beneficial. Further studies are needed to elucidate the long-term effects of increased FGF removal.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1437**

**Cytokine Production of Peripheral Blood Mononuclear Cells after Exposure to Endotoxin during High Flux Hemodialysis** Kearkiat Praditpornsilpa, Khajohn Tiranathanagul, Somchai Eiam-Ong. *Division of Nephrology, Chulalongkorn University, Bangkok, Thailand.*

The back filtration of endotoxin across high-flux membranes to blood compartment causes activation of inflammatory cells and inflammation. Question has been raised what endotoxin contamination limit have the least inflammatory cells stimulation. We investigated the effect of dialysate endotoxin contamination by cytokines release from peripheral blood mononuclear cells (PBMCs) after endotoxin exposure.

**Methods:**

Four different levels of endotoxin contaminated hemodialysis (HD) centers where endotoxin in dialysate was monthly monitored by limulus amoebocyte lysate kinetic turbidimetric assay were recruited in this study (0.001 EU/mL in HD center 1, 0.026 EU/mL in HD center 2, 0.558 EU/mL in HD center 3, and 1.960 EU/mL in HD center 4). PBMCs from HD patients who met the inclusion/exclusion criteria were separated and measured the spontaneous cytokines production after HD ( case = 26, 25, 20, and 20 in HD center 1, 2, 3, and 4 respectively). The criteria included patients who had no current infection, had no previous kidney transplantation, had no malignancy or autoimmune disease, and had negative test for HIV Ab, HBsAg and anti-HCV.

**Results:**

There were significant differences of mean IL-6, IL-1beta, and IL-1Ra level spontaneously released from the PBMCs after HD session among patients from HD centers. The higher endotoxin exposure had higher inflammatory cell activation. Cytokines released from the PBMCs were highest in HD center 4 where endotoxin contamintion was highest. There were no statistically significant differences of cytokine release between two ultrapure dialysate (endotoxin level < 0.03 EU/mL) centers (HD center1 and HD center 2).

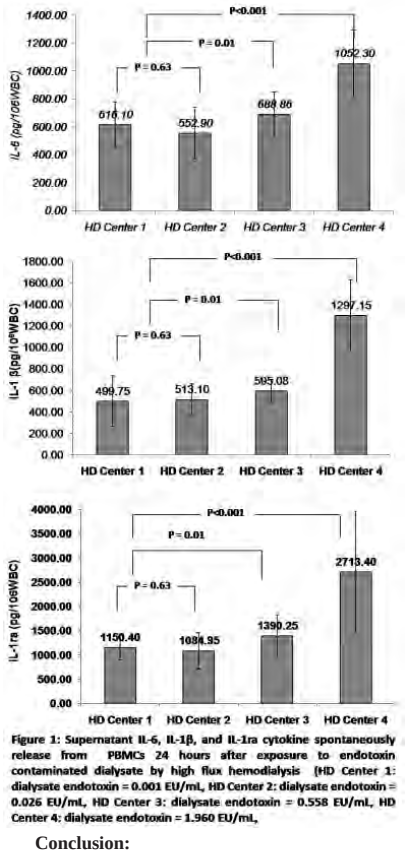



**Figure 1: Supernatant IL-6, IL-1β, and IL-1ra cytokine spontaneously release from PBMCs 24 hours after exposure to endotoxin contaminated dialysate by high flux hemodialysis (HD Center 1: dialysate endotoxin = 0.001 EU/mL, HD Center 2: dialysate endotoxin = 0.026 EU/mL, HD Center 3: dialysate endotoxin = 0.558 EU/mL, HD Center 4: dialysate endotoxin = 1.960 EU/mL,**

**Conclusion:**

Endotoxin < 2 EU/mL as recommended by AAMI can be inadequate. The ultrapure dialysate should be a minimal endotoxin contamination allowed in HD.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1438

**Left Ventricular Torsion Is an Early Marker of Myocardial Dysfunction in Incident Haemodialysis Patients** Aghogho Odudu,[1] Mohamed Tarek Eldehni,[1] Chris W. McIntyre.[2] [1]*Renal Medicine, Royal Derby Hospital, United Kingdom;* [2]*School of Graduate Entry Medicine and Health, University of Nottingham, United Kingdom.*

Contractile coherence is essential for normal ventricular ejection, this can be assessed by measuring ventricular synchrony and the degree of rotational displacement over a cardiac cycle. Such defective ventricular contractile function can exist in the presence of superficially normal systolic function, with preserved ejection fraction (EF). Appreciation of the importance of synchronous ventricular contraction in haemodialysis (HD) patients has been limited to prevalent populations using echocardiography based techniques limited by low resolution and high degrees of operator dependence. We report a pilot study utilising cardiac magnetic resonance (CMR) myocardial tagging of patients new to HD. This utilises a magnetized grid allowing three-dimensional quantification of intra-myocardial motion with high spatial and temporal resolution.

11 HD patients (exposed to HD <90 days) were studied on a post-HD day using a 1.5T MR scanner. Conventional CMR techniques were combined with a 7mm grid tag applied with a spatial modulation of magnetization sequence in 3 short axis slices evenly distributed over the LV. Harmonic phase images were computed using a semi-automated software. Peak absolute rotation was calculated and compared to published values in healthy volunteers.

The mean ±SD of values are presented. Global LV function was normal (EF 69±15%) with only 1 subject with EF<50%. There was a high degree of LVH (LVMI 97.7±25m$^2$) The degree of rotation increased from base to apex whilst basal to mid-ventricular rotation was similar to values in non-uraemic controls(4.1°Vs 3.8° & -4.2° Vs-4.4° respectively). Mean peak apical rotation was significantly lower than non-uraemic controls (6.9° Vs 10.5°, p>0.05).

Conclusion: Incident HD patients are not characterised by global LV dysfunction more typical of patients established on HD. However study of LV torsion reveals subclinical disordered contraction despite preserved EF. The significance of the relationship to HD tolerability and susceptibility to recurrent HD-induced cardiac injury awaits further elucidation.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1439

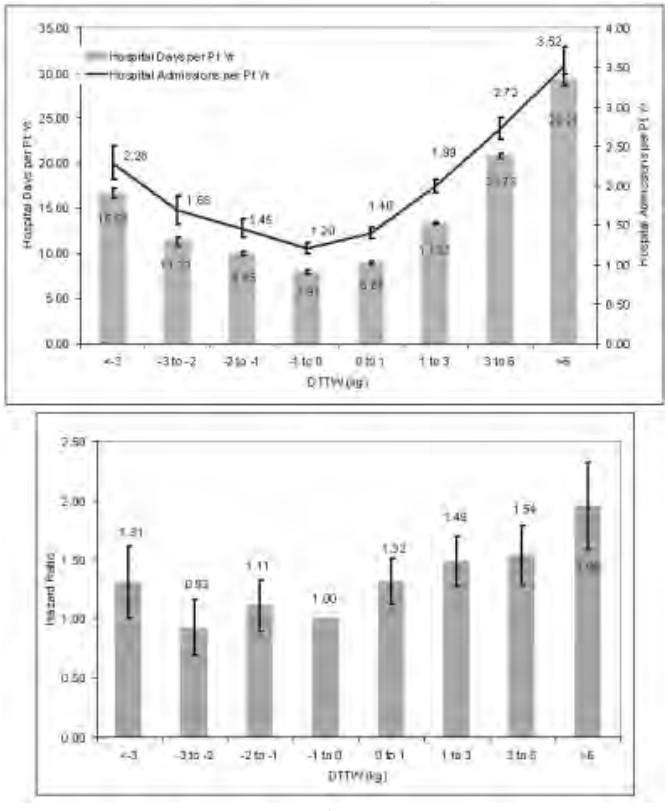
**Failure To Achieve Prescribed Target Weight in Chronic Hemodialysis Patients Is Associated with Poor Outcomes** Len A. Usvyat,[1] Balaji Gandhi,[2] Nathan W. Levin,[1] Peter Kotanko.[1] [1]*Renal Research Institute, NY, NY;* [2]*Fresenius Medical Care North America, Waltham, MA.*

Overhydration is prevalent in the vast majority of chronic hemodialysis (HD) patients (pts). Post-HD target weight (TW) is prescribed by nephrologists primarily based on clinical evaluation. Unfortunately, TW is frequently not achieved. This study evaluates the impact of the difference between achieved post-HD weight and prescribed TW (delta to target weight, DTTW) on hospitalizations and mortality.

We studied HD pts treated in RRI clinics in 2007 ("study prd"). Achieved post-HD weight and laboratory parameters were computed as avg for the study prd. Pts were stratified according to DTTW (in kg): DTTW<-3, DTTW-3 to -2, DTTW-2 to -1, DTTW-1 to 0, DTTW0 to 1, DTTW1 to 3, DTTW3 to 6, DTTW>6.

6,440 pts were studied (4% with DTTW<-3, 4% with DTTW-3 to -2, 8% with DTTW-2 to -1, 19% with DTTW-1 to 0, 23% with DTTW0 to 1, 25% with DTTW1 to 3, 12% with DTTW3 to 6, 5% with DTTW>6). Both number of hospital admissions (ADM) and LOS showed a U-shaped relationship with highest ADM and hospital days in the DTTW>6 group and lowest in the DTTW-1 to 0 cohort. Both ADM and LOS were 3x higher in DTTW>6 compared to DTTW-1 to 0.

Cox survival analysis with adjustment for age, gender, race, albumin, and sodium gradient (dialysate Na - serum Na), also demonstrated a U-shaped relationship with best survival in DTTW-2 to -1, DTTW-1 to 0, and DTTW0 to 1 groups (no statistical differences b/n those groups). HR was nearly 2-times higher in DTTW>6 than in the reference group (DTTW-1 to 0).







This study accentuates the importance of reaching TW. In order to achieve TW in some pts txt time has to be extended. An important consideration is the fact that TW may still be greater than the true "dry weight" in many pts. Means to objectively define dry weight are necessary to elucidate this issue further.

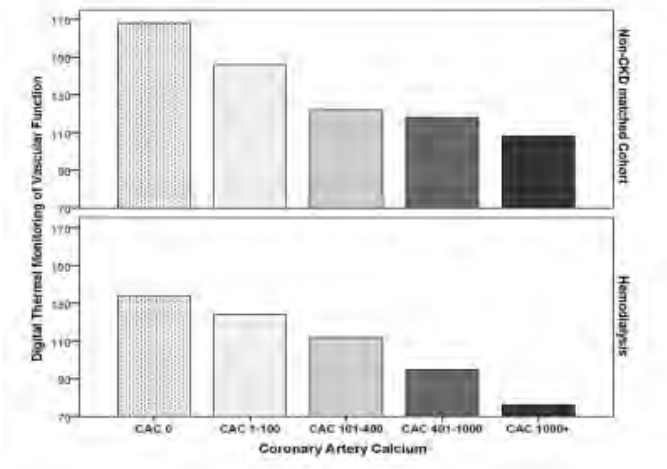
Disclosure of Financial Relationships: nothing to disclose

## F-PO1440

**Vascular Dysfunction and Coronary Artery Calcium Increase with the Presence of End Stage Renal Disease** Irfan Zeb, Naser Ahmadi, Jennie Jing, Ramanath B. Dukkipati, Matthew Jay Budoff, Kamyar Kalantar-Zadeh. *Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA.*

Cardiovascular (CV) morbidity and mortality is comon among maintenance hemodialysis (MHD) patients (pts), which increase with the presence and severity of coronary artery calcium (CAC). We investigated the association of vascular dysfunction measured by digital thermal monitoring (DTM) with severity of CAC in MHD pts compared to a matched cohort without CKD. Study population consisted of 105 MHD pts (age: 57±12, males: 49%) and 105 age, gender and traditional CV risk factor matched non-CKD subjects. All pts underwent CAC and DTM. Area under the temperature curve (TMP-AUC), DTM index of vascular function were assessed through a 5-minute arm-cuff reactive

hyperemia test. TMP-AUC decreased significantly from Non-CKD to MHD pts (143±80 vs. 114±72, p=0.001). CAC (Agatston score) increased from Non-CDK (240±332) to MHD pts (525±425)(p=0.0001). After adjustment for age, gender, DM, HTN, lipid, smoking, ethnicity and race, the risk for each standard deviation decrease in TMP-AUC was 1.46 (95%CI 1.12-1.93,p=0.007) in MHD pts compared to non-CKD. TMP-AUC decreased with increasing CAC (0,1-100,101-400,401-1000 & 1000+) which was significantly higher in MHD pts compared with Non-CKD (P<0.05). The relative risk of increase in CAC from 0 to 1-100, 101-400, 401-1000 and 1000+ was 5.29 (95%CI 1.82-15.38,p=0.002), 7.49 (95%CI 2.45-22.85,p=0.0001), 10.88 (95%CI 3.82-31.03,p=0.0001), and 13.98 (95%CI 4.86-40.14,p=0.0001) in MHD pts compared to Non-CKD, respectively.




In MHD pts, higher rate of vascular dysfunction and coronary atherosclerosis exist compared to non-CKD; and vascular dysfunction increases with the severity of coronary atherosclerosis in MHD pts, independent of conventional risk factors, suggesting the importance of subclinical atherosclerosis assessment in MHD pts.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1441**

**Compliance to Phosphate Binders: An Observational Multi-Centre Study** Monique M. Elseviers, Sofie Alida Marcel Huybrechts, Yoleen Philomena Margueritte Van Camp, Marc E. De Broe. *Nursing Science, Univ. Antwerp, Belgium.*

This study aimed to map compliance to phosphate binders (PBs) in the hemodialysis (HD) population using the electronic Medication Event Monitoring System (MEMS) and to determine patients characteristics related to non-compliance.

Compliance to PBs was evaluated in 3 Belgian and 1 Dutch dialysis centre. All adult patients treated with PBs, in chronic HD for more than 3 months and without mental disorders were asked for participation. Compliance was measured using MEMS-V Track-Cap devices (Aardex Ltd, CH). In addition, a pill count method (adding each week a changing number of extra pills while refilling the medication box) was used to test compliance. Non-compliance was defined as a registered intake <80% of prescribed doses.

A cohort of 216 patients (out of 276 meeting the inclusion criteria) was followed during a period of 14 weeks. Mean age was 67 years (range 21-90), 57% were males, mean HD treatment was 47 months (range 3-189). Patients had a mean pill burden of 14 pills per day (range 2-35) with 33% of patients taking more than one PB. PBs studied were calcium carbonate, sevelamer and lanthanum in 45, 45 and 10% of patients, resp., with a mean intake of 5.4 (range 1-12) PB pills per day. Electronic monitoring revealed a compliance of 68% (range 58-74% per centre) compared to 85% using pill count, both showing a decreasing trend after one month of observation. Compliance was slightly negatively related to mean serum phosphorus (r=-.179; p=.009) and did not show a relationship with mean serum calcium levels (r=0.051; p=0.462). In HD patients, non-compliance was significantly influenced by younger age (OR=1.96), taste complaints of PBs (OR=7.15), nausea related to PB intake (OR 4.19) and a high PB pill burden (OR=2.74).

Only two thirds of HD patients had an acceptable level of compliance to PBs. Main risk factors for non-compliance were associated with complaints related to PB use.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1442**

**Bioimpedance Spectroscopy (BIS): Clinical Application To Evaluate Acute Extracellular Water (ECW) Changes and Overhydration (OH) in Hemodialysis** Mauricio Paredes-Fernandez, Klearly M. Tinoco, Ricardo Correa-Rotter. *Instituto Nacional de Ciencias Médicas y Nutrición.*

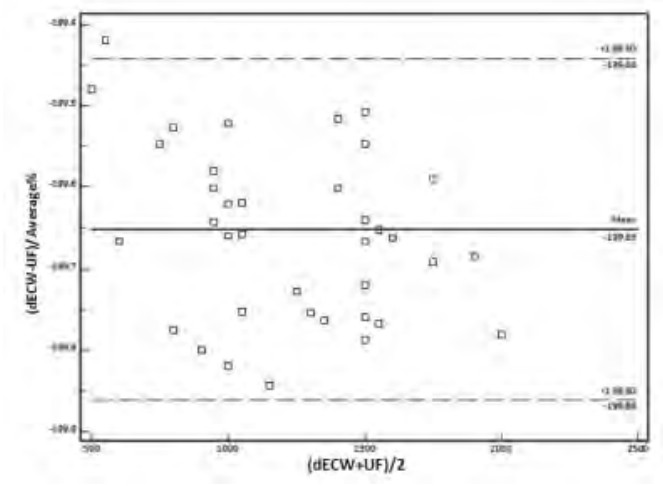
Dry weight estimation is crucial in HD prescription. Multifrequency bioimpedance spectroscopy (BIS) assesses hydration status estimating ECW and OH. For clinical application, BIS must be accurate enough to assess acute variations of volume status, almost exclusive to ECW, without showing artifactual intracellular water (ICW) modifications (as usually happens with other bioimpedance techniques).

Purpose: To estimate OH and determine BIS sensitivity to acute volume variations during HD and to compare hydration status of HD patients with normal controls.

METHODS: We employed a BIS device (Body Composition Monitor (BCM) Fresenius Medical Care) to measure pre and post HD ECW and OH in chronic adult HD patients and compared ECW reduction to net UF. Measured ECW minus calculated ECW (formula for Mexican people= 0.007594+0.03557BSA+0.001229BMI+0.008608gender (0=female,1=male) provides an objective evaluation of ECW expansion (diECW).

RESULTS: We studied 42 HD pts. (age 45.9±16.5 y, 66% males), follow up 41±32 mo. Basal ECW was 0.09±0.01 L/cm (0.07 to 0.16) and significantly greater to that measured in healthy individuals [0.09±0.01 L/cm, (0.06 to 0.13), p=0.03]. UF reduced significantly ECW(16.12±3.8 L preHD vs 14.2±3.6 L postHD, p=0.01) but did not affect ICW (16.5±3.7 L preHD vs 16.6±4.0 L postHD, p=0.91). Basal and final diECW showed good correlation with pre and postHD OH(R=0.88, P<0.001 and r=0.9, p<0.001).

ECW reduction and OH showed good correlations with UF (r=0.7, p<0.001 and r=0.70, p<0.001). ECW reduction and UF had a mean difference of 199.6 mL between them(Bland Altman graph.)




DISCUSSION: BIS accurately estimates acute ECW variations as shown in correlation to net UF. As expected, variations are restricted to ECW. OH or diECW could be used interchangeably to assess fluid status.

Disclosure of Financial Relationships: Employer: Fresenius Medical CareResearch Funding: Fresenius Medical Care.

**F-PO1443**

**Mass Spectroscopy Reveals Numerous Uremic Solutes Produced by Colon Bacteria** Timothy W. Meyer,[1] Pavel A. Aronov,[1] Natalie Plummer,[1] Susan Holmes,[1] Thomas H. Hostetter.[2] *[1]Stanford University & VA Palo Alto, Palo Alto; [2]Albert Einstein College of Medicine, New York.*

Potentially important uremic solutes are produced in the colon by bacteria acting on foodstuffs which escape digestion in the small intestine. This study employed mass spectroscopy (MS) to identify such solutes. Plasma was collected from 7 normal subjects and, prior to dialysis treatment, from 15 ESRD patients of whom 6 had total colectomies and 9 had intact colons. Analysis by UPLC (C18, reverse phase) coupled to high accuracy MS (orbitrap) revealed 1075 chemical features characterized by retention time and mass/charge ratio. Of these, 56 features were absent or found in significantly reduced concentration in patients with colectomies (p<0.05 after adjustment for multiple comparisons). Online databases (HMDB, Madison, Metlin) revealed candidate compounds corresponding to only 27 of these features. The identify of 4 features which accumulate in ESRD patients wth intact colons but are largely absent in those without colons has so far been confirmed by UPLC/MS of chemical standards:

| | Plasma Ratio Colectomy HD vs Intact Colon HD | Plasma Ratio Intact Colon HD vs Normal Control |
|---|---|---|
| p-cresol sulfate | 0.02 | 11 |
| indoxyl sulfate | 0.01 | 45 |
| phenylacetyl-L-glutamine | 0.05 | 77 |
| 5-hydroxyindole | 0.11 | 4 |

This list includes p-cresol sulfate and indoxyl sulfate which have previously been identified as of colonic origin along with an additional indole and a phenyl compound, presumably derived from tryptophan and phenylalanine/tyrosine, respectively. Of note, the plasma concentrations of two other indoles, indole-3-acetic acid and indole-3-lactic acid were not lower in patients with colectomies. These results indicate that colon bacteria produce a remarkably large number of uremic solutes, many of which are not included in current metabolomic databases. Further work is required to elucidate their structures and potential contributions to uremic illness.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1444**

**Phosphate Binders: A Nurse Lead Intervention To Improve Compliance** Monique M. Elseviers, Yoleen Philomena Margueritte Van Camp, Sofie Alida Marcel Huybrechts. *Nursing Sciences, Univ. Antwerp, Belgium.*

This study aims to compare the baseline compliance to phosphate binders (PBs) blindly controlled by the Medication Event Monitoring System (MEMS) to the results obtained by a nurse lead intervention focusing on medication knowledge and personalized support.

In 4 dialysis centres, all adult patients treated with PBs, in chronic HD for more than 3 months and without mental disorders, were asked for participation. Compliance was measured during a period of 14 weeks using MEMS devices. Non-compliance was defined as a registered intake <80% of prescribed doses. An interventional study was set up in an additional Belgian centre using identical inclusion and observation methods. In the latter centre, 4 weeks after the start of the observation period, a nurse visited all participating patients, offering an information chart and an individualized explanation about PBs. Thereafter, she contacted all participants bi-weekly for a personalized talk about compliance.

In the total cohort of 257 patients (including 216 baseline and 41 intervention patients), mean age was 67.5 years (range 21-90), 59% were males, mean HD treatment was 47 months (range 3-267). Patients had a mean pill burden of 14 pills per day (range 2-35) with 33% of patients taking more than one PB. PBs studied were calcium carbonate, sevelamer and lanthanum in 46, 42 and 12% of patients, respectively with a mean intake of 3.9 (range 1-9) PB pills per day. In the baseline cohort, compliance decreased from a mean of 85% in week 1 to 76% in week 14. In contrast, in the intervention group compliance increased from a mean of 82% to 94%. Interrupted time series analysis revealed that the decreasing trend of compliance in the baseline cohort with a slope of -0.782 (95% CI -1.07--0.489) significantly differed from the slope of 0.672 (95% CI -0.13-1.40) in the intervention group. During the intervention study, serum phosphorus levels decreased from 4.8 to 4.6 mg/dL (p=0.110) and knowledge about PBs increased from a mean score of 53 to 75%.

Although non-compliance to PBs was frequently observed in HD patients, a nurse lead program of education and close follow-up of PB intake is worth considering to improve compliance.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1445**

**Dialysate Sodium (Na) 2 to 10 mEq/L Higher Than Plasma Na during Hemodialysis (HD) Increases Interdialytic Na Accumulation** Marcia L. Keen,[1] Frank A. Gotch.[2] *[1]ML Keen & Associates, Moorpark, CA; [2]University of California, San Francisco, CA.*

Sodium (Na) intake in HD patients is assumed to primarily reflect dietary Na intake. Few data are available to determine the relative contribution of dialysate Na ($J_DNa$) to total Na intake ($J_TNa$) in HD patients. It has been reported that individual HD patients may have plasma Na set points ($C_{sp}Na$), which range from 135 to 145 mEq/L (Keen, Int J Artif Organs, 2007). These data have been re-analyzed to determine the relative contributions of $J_DNa$ and $J_{Diet}Na$ to total interdialytic Na accumulation ($J_TNa$).

Methods: Data consisted of monthly pre/post plasma Na ($C_{p0}Na$, $C_{pt}Na$) and urea kinetic modeling for 1 year in 57 HD patients. Dialysate Na was 143 mEq/L, which produced Na gradients of 0 to 8 mEq/L. Twelve sets of values for pre/post urea volumes ($V_0$,$V_t$) and $J_DNa$, $J_{Diet}Na$ were calculated per patient. From known osmotic equilibrium in extracellular and intracellular volumes ($V_e$,$V_i$), anatomical distribution of K in $V_i$ and Na in $V_e$ and $C_{sp}Na=C_{p0}Na$, normal total body osmotically active cation, $TBC=C_{sp}Na*V_t$ was calculated. Mass balance, $J_DNa = (C_{pt}Na-C_{sp}Na)*Vt$ and $J_{Diet}Na=(C_{sp}Na-C_{pt}Na)*V_t+C_{sp}Na*(V_0-V_t)$ as functions of the gradient from dialysate to blood ($\Delta CNa$) could be calculated. A single pool variable volume kinetic model was used to calculate $J_DNa$ and $J_{Diet}Na$ as continuous functions over wide ranges of $\Delta CNa$ and interdialytic weight gain (IDWG).

Results (M±SD): $V_tT=30.6\pm8.7$; $(V_0T-V_tT)=2.2\pm1.0$; $J_{diet}Na=3.0\pm1.1$ and ≠ f($\Delta CNa$); $J_dNa=0.18(\Delta CNa)-0.10$, $r^2 = .62$, $p<0.001$. The results of modeling these data are shown below.

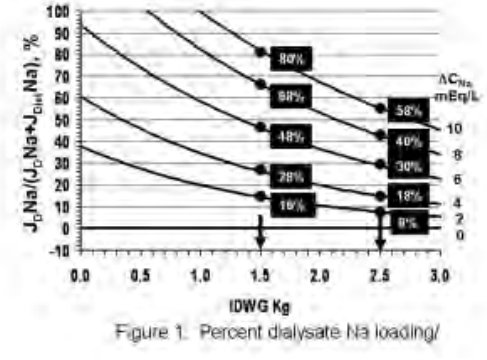



Figure 1. Percent dialysate Na loading/

Conclusion: These data quantify and differentiate the effect of $J_DNa$ and $J_{Diet}Na$ on Na loading in HD. Each 5 mEq/L $\Delta CNa$ was equivalent to a $J_{diet}Na$ of 1 gm/day, supporting the importance of prescribing $\Delta CNa=0$ to avoid chronic Na overload in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1446**

**Relationship of Change in Relative Blood Volume and Hydration State in Hemodialysis Patients** Fansan Zhu, Samer Rateb Abbas, Li Liu, Murat H. Sipahioglu, Jochen G. Raimann, Laura Rosales, Peter Kotanko, Nathan W. Levin. *Renal Research Institute, New York, NY.*

Relative blood volume (RBV) monitoring has been suggested as a simple approach to monitoring hydration state during hemodialysis (HD) treatments. However, high variability in nadir RBV ($RBV_{min}$) and RBV slope ($RBV_{slope}$) during HD complicates its clinical use. This study investigated the relationship between $RBV_{min}$ and $RBV_{slope}$ with ultrafiltration rate (UFR) and hydration state as determined by calf bioimpedance spectroscopy (cBIS).

Post-HD weights were gradually decreased in 40 chronic HD patients with the aim to reach dry weight (DW) as defined by cBIS (Zhu, et al., Physiol Meas, 2008). Continuous RBV monitoring (BVM, Fresenius) and cBIS (Xitron) were done during HD. Calf normalized resistivity (nRho) measured by cBIS was calculated as an indicator of hydration state. Correlations of $RBV_{min}$ and $RBV_{slope}$ with UFR and Pre-, Post-HD weight, and nRho were calculated for each patient. Patients were divided into two groups: DW (n= 23; 248 treatments) and Non-DW (n=17; 260 treatments) who did not reach dry weight.

$RBV_{min}$ (DW: 85.3±6.6; non-DW: 85.8±5.7 %) and $RBV_{slope}$ (DW: 5.1±5.6; non-DW: 4.2±1.4 %/hour) did not differ between DW and non-DW patients. In patients who eventually reached DW, neither $RBV_{min}$ (overhydration:83.8±4.5 ; at DW: 85.3±6.6 %) nor $RBV_{slope}$ (overhydration: 3.9±1.6; at DW: 5.1±5.6 %/hour) differed between the overhydrated state and DW. A significant correlation between a) $RBV_{min}$ and UFR ($R^2$=0.58, $p<0.01$), b) $RBV_{min}$ and UFR together with Pre-HD weight (multiple $R^2$=0.60, $p<0.05$) and c) $RBV_{min}$ and UFR together with Post-HD nRho (multiple $R^2$=0.68, $p<0.05$) was observed in 50 % (a), 50 % (b), and 45 % (c) of patients, respectively. $RBV_{min}$ correlated with Post-HD nRho in only 10% of the patients significantly ($R^2$=0.66). $RBV_{slope}$ correlated with UFR in 17.5%, Pre-HD weight in 5%, Post-HD weight in 22.5% and nRho in 22.5 % of patients significantly.

Changes in RBV are associated with multiple factors such as degree of UFR, variability of hydration state and efficiency of plasma refilling. RBV is not useful for estimating hydration but can indicate appropriate UFR in individual treatments.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1447**

**The Effects of Hemodialysis Circuit on Platelet-Derived Microparticles and Platelet Factor 4 in Hemodialysis Patients** Takanobu Imada,[1] Sanae Kikuchi,[1] Hideki Yamahara,[1] Hiroya Masaki,[2] Mitsushige Nishikawa,[1] Toshiji Iwasaka.[1] *[1]Department of Medicine II, Kansai Medical University, Osaka, Japan; [2]Department of Laboratory Medicine and Clinical Sciences, Kansai Medical University, Osaka, Japan.*

BACKGROUND: Platelet-delived microparticles (PDMP) and platelet factor 4 (PF-4) are released from activated platelet. Activated platelets are thought to play a role in the mediation of platelets-leukocyte interaction for atherosclerosis. Hemodialysis (HD) patient always receives stimulation in extracorporeal circulation. In this study, we investigate that the effect of HD on circulating levels of PDMP and PF-4 in HD patients, and we improvement of HD circuit. METHOD: Nine patients were selected from among patients admitted to our hospital for treatment of HD. The study protocol was approved by our institutional review boad and written informed consent was obtained from each patient prior to the start of the trial. Each patient was measured using four kinds of HD circuits. Blood samples from patients were collected into tubes before and after HD from HD circuit. PDMP levels were measured using an enzyme-linked immunosorbent assay (ELISA). RESULTS: Mean PDMP level was 34.9 U/ml before HD. After HD, PDMP level was 3.05±0.47 times greater than before HD (p=0.004). Mean PF-4 level was 24.6 ng/ml. After HD, PF-4 level was 2.56±1.26 times greater than before HD (p=0.0007). The changing rate of PF-4 level was able to be reduced in predominance by changing the form of air-trap chamber in dialysis circuit into the tornado from perpendicular type (1.58±0.85 in tornado type vs. 3.93±0.73 in perpendicular type, P=0.029). CONCLUSION: Our results clearly indicated that HD activated the patient's platelet. This result suggests that non-physiological HD promotes not only the blood loss by coagulation but atherosclerosis. Interestingly, reform a part of HD circuit has possibility of improvement of this problem.

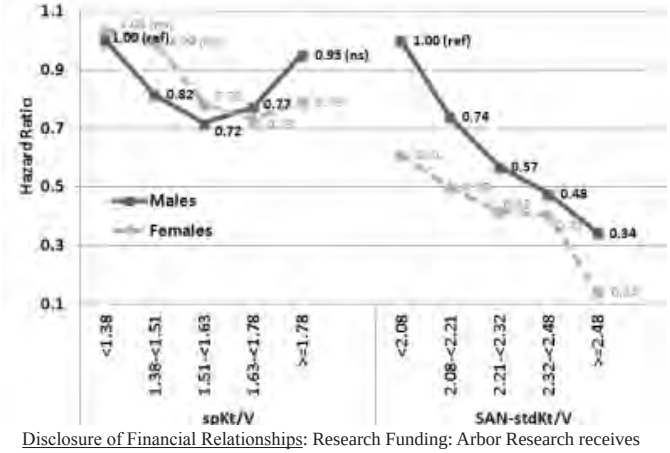
Disclosure of Financial Relationships: nothing to disclose

**F-PO1448**

**Dialysis Dose Scaled to Body Surface Area and Patient Mortality** Sylvia Paz B. Ramirez,[1] Rajiv Saran,[2] Alissa Kapke,[1] Robert A. Wolfe,[1] Jeffrey Pearson,[1] Friedrich K. Port,[1] Richard Hirth,[2] J. M. Messana,[2] John T. Daugirdas.[3] *[1]Arbor Research Collaborative for Health, Ann Arbor, MI; [2]Kidney Epidemiology & Cost Center, University of Michigan, Ann Arbor, MI; [3]University of Illinois College of Medicine, Chicago, IL.*

Previous analyses have shown survival benefit with higher dialysis dose for women but not men. We postulated that dialysis dose normalized for body surface area (S) as opposed to total body water (V) could potentially explain this finding and also be a better predictor of mortality since the ratio V/S differs by sex. A cohort of prevalent thrice-weekly HD patients from the CMS 2008 ESRD CPM Project with at least 6 months since start of ESRD (N=7216) was analyzed. Single pool (sp) Kt/V was calculated using Daugirdas II formula, standardized (std) Kt/V was calculated per Leypoldt (2004), and surface area normalized (SAN) measure (SAN-stdKt/V) was calculated per Daugirdas (2008) by multiplying stdKt/V by Vant/S (Watson/Dubois) and dividing by mean Vant/S (20.5). Patients were grouped into quintiles and Cox regression hazard ratios for mortality during the subsequent year

were calculated censoring at transplant or 12/31/08 (referent=males in quintile 1). Similar to prior studies, the highest quintile of spKt/V appeared to benefit females but not males (stdKt/V results similar, not shown); using SAN-stdKt/V benefits of increasing dialysis dose were more consistent for the sexes. While all measures predicted mortality, the spKt/V model (likelihood ratio chi-square or LR$\chi^2$:23.1, 9df; p=.006) and stdKt/V model (LR$\chi^2$:23.8, 9df; p=.005) were both outperformed by the SAN-stdKt/V model (LR$\chi^2$:108.8, 9df; p<.0001). Scaling dialysis dose to S shows benefits of higher doses for both sexes and better association with mortality risk; hence, measures of dialysis adequacy scaled to S may be superior to those scaled to V.



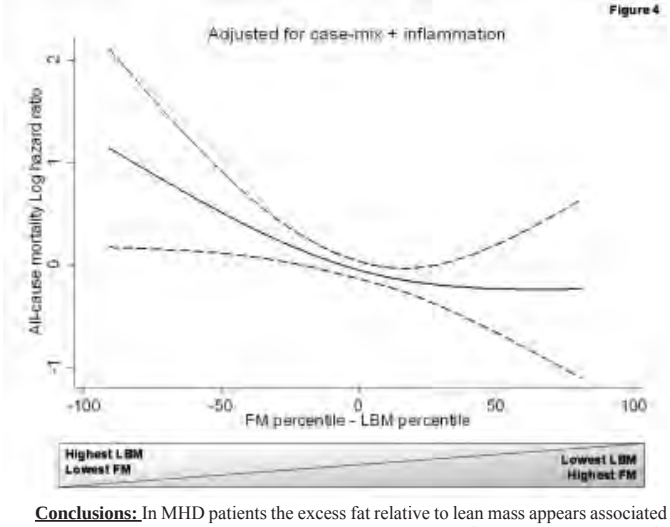

Disclosure of Financial Relationships: Research Funding: Arbor Research receives funding for DOPPS from Amgen, Kyowa Hakko Kirin, Abbot and Genzyme.

**F-PO1449**

**Survival Advantage of Excess Fat Relative to Lean Body Mass in Long-Term Hemodialysis Patients** Nazanin Noori,[1] Csaba P. Kovesdy,[2] Ramanath B. Dukkipati,[1] Uyen Duong,[1] Rachelle Bross,[1] Antigone Oreopoulos,[4] Deborah A. Benner,[3] Joel D. Kopple,[1] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]Salem VA Medical Center, Salem, VA; [3]Davita, Lakewood, CO.*

**Background:** Several studies have suggested that maintenance hemodialysis patients (MHD) with higher body mass index (BMI) enjoy a survival advantage. It is not clear whether lean body mass (LBM) or fat mass (FM) confers this survival benefit. **Methods**: Using near infrared (NIR) technology we measured body compositions in 732 MHD patients and ranked them twice, once according to their absolute FM or then LBM (in kg) and assigned a percentile score to each patient within each gender group, i.e., a number between 0 (lowest) and 100 (highest FM or LBM). The difference between the two percentile scores (FM percentile minus LBM percentile) in each patient yielded a number between –100 (indicating a patient with lowest FM but highest LBM) and + 100 (indicating a patient with highest FM and lowest LBM). The Cox survival regression was modeled using cubic spline for the "FM minus LBM percentiles" after adjustment for case-mix and inflammatory markers. **Results:** A relatively linear and downgoing trend towards greater survival was observed with higher excess fat relative to lean mass (Figure).

Figure 4




**Conclusions:** In MHD patients the excess fat relative to lean mass appears associated with greater survival. Clinical trials to examine the outcomes of interventions that modify body composition in MHD patients are indicated.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1450**

**Elevated Serum Ferritin Level May Determine the Seroconversion Rates to 2009 Influenza A (H1N1) Vaccination in Hemodialysis (HD) Patients** Hyeong Cheon Park,[1] Sang Hun Lee,[1] Areum Kim,[2] Sung Jin Moon,[1] Jung Eun Lee,[3] Jae Myun Lee,[2] Sung-Kyu Ha.[1] *[1]Internal Medicine, Gangnam Severance Hospital, College of Medicine, Yonsei University, Korea; [2]Microbiology and Brain Korea 21 Project for Medical Science, College of Medicine, Yonsei University, Korea; [3]Internal Medicine, Yongin Severance Hospital, Yongin, Korea.*

End stage renal disease patients on HD are at risk for higher case-fatality ratios than healthy subjects. Therefore, HD patients demand more accurate determination of seroconversion rates after vaccination. The aim of the study was to investigate the seroresponsiveness and parameters affecting antibody response after 2009 Influenza A (H1N1) vaccination in HD patients. Clinically stable HD patients (n=102, age 20-80 yrs) were enrolled. Patients received Influenza A vaccine [strain A/ California/ 7/ 2009 NYMC X-179 (H1N1), Green Cross, Seoul, Korea] by intramuscular injection and seroconversion was determined using ELISA method *(FLUAb™* kit, ATGEN, Seoul, Korea). Baseline assessment showed 17 seropositive patients. Other patients (n=85) were assessed for seroconversion rates one month after vaccination. Demographic and other biochemical parameters were also assessed. After Influenza A (H1N1) vaccination, about 1/3 of the patients (n=28) showed positive seroconversion (Group 1) whereas the other patients (n=57, Group 2) showed no significant increase in antibody titers. Both groups showed comparable demographic and biochemical parameters except that Group 1 showed significantly higher serum ferritin level compared to Group2 (257 ± 211 *vs.*183 ± 125 ng/ml, respectively, p=0.008). In binary logistic regression analysis, high serum ferritin level was independent parameter related to better seroresponse rate [Odds ratio (OR) 1.004, 95% Confidence Interval (CI) 1.001-1.008, *p*=0.026]. Patients with high ferritin levels (>400 ng/ml) was strongly related to higher seroresponse rate (OR 12.1, 95% CI, 1.6-92.1, *p*=0.016). In conclusion, our results suggest that elevated serum ferritin levels may predict stronger seroconversion rate after 2009 Influenza A (H1N1) vaccination in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1451**

**Natriuretic Peptides (NP) and Fluid Status in Hemodialysis (HD)** Elena Mancini, Emanuele Mambelli, Antonio Bellasi, Antonio Santoro. *S.Orsola-Malpighi, Bologna, Italy.*

To assess the usefulness of NP as biomarkers of the fluid status in the HD patient, we evaluated the behaviour of adrenomedullin and atrial natriuretic peptide (ADM, ANP) in 50 randomly selected HD patients (28 conventional HD, 23 hemodiafiltration, HDF).

Due to the short half-life of the peptides, we measured their prercusors (medium region pro-ADM and pro-ANP, molecular weight 2,460 and 4,000 daltons; IF assay, Brahms Kriptor) pre- and post a mid-week HD session. Dry body weight had been clinically determined. The interdialytic body weight increase was the measure for fluid overload, the ultrafiltration (UF) volume for fluid removal. Any relationship between the peptide levels or their changes, and fluid overload/removal and blood pressure values was studied.

The basal levels (proADM 3.6±1.8 mmol/L; proANP 836.1±438 pmol/L) did not correlate either each other (R=0.06) or with pre-HD fluid overload (ADM: r=0.11 and ANP: r=0.03) or blood pressure.

Both decreased significantly during HD (proADM: from 3.6±1.8 to 1.8±1.4 mmol/L, p<0.0001; proANP: from 836.1±438 to 599.8 +456 pmol/L, p< 0.0001) but their pre-to-post HD % changes did not correlate with UF.

The % change of both the peptides was significantly lower in HD (low flux membranes) than HDF (high flux membranes) in spite of similar UF. However, the pre-dialysis levels were comparable.

Main results

| | HD | HDF | p |
|---|---|---|---|
| Pre-dialysis ADM ( mol/L) | 3.6 ± 1.5 | 3.7 ± 2.1 | NS |
| Pre-to-post dialysis ADM (%) | -37.4 ± 20.7 | -64.7 ±14.8 | <0.0001 |
| Pre-dialysis ANP (pmol/L) | 890.1 ± 495 | 770.4 ± 357 | NS |
| Pre-to-post dialysis ANP (%) | -15.8 ± 24.6 | -49.13 ± 16 | <0.0001 |
| UF (L) | 1.83 ± 0.9 | 1.9 ± 1.3 | NS |

The correlations between pre or post-HD peptide levels and pre or post-HD blood pressure were not significant.

These data do not support the hypothesis of an association between the fluid status *per se* and NP levels. Moreover, in spite of a greater reduction ratio of the NP levels with the convective-diffusive techniques, pre-dialysis values in HDF patients are not lower than in HD, suggesting a multiple control of their concentration. The use of the NP precursors in the day-by-day clinical setting still needs further studies.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1452**

**Modulation of Von Willebrand Factor, Von Willebrand Factor Propeptide, and ADAMTS-13 in End-Stage Renal Disease** Vinod K. Bansal,[1] Rachael Davis,[2] Evangelos Litinas,[2] Debra Hoppensteadt,[2] Jawed Fareed.[2] *[1]Department of Nephrology, Loyola University Medical Center, Maywood, IL; [2]Department of Pathology, Loyola University Medical Center, Maywood, IL.*

End-Stage Renal Disease (ESRD) is a complex syndrome in which systemic vascular pathophysiologic changes contribute to adverse cardiovascular and cerebrovascular manifestations due to thrombotic activation. Von Willebrand Factor (vWF) plays a well known role in platelet aggregation and has been shown to be upregulated in many

thromboembolic conditions. However in many conditions, the role of von Willebrand Factor Propeptide (vWFpp), a peptide cleaved prior to assembly of active vWF multimers, and ADAMTS-13, the enzyme responsible for disassembly and deactivation of vWF multimers, have not been examined. The purpose of this study is to better characterize the relationship among vWF, vWFpp, and ADAMTS-13 as it relates to ESRD. Plasma samples from 51 patients with ESRD were collected prior to maintenance hemodialysis. A group of 50 normal individuals, both male and female, was included as control. Concentrations of vWF and vWFpp were quantified using the GTI® vWF & Propeptide Assay. A separate assay was used to measure ADAMTS-13 in these samples (GTI®). This data as well as the ratio of vWF:vWFpp were analyzed using the Mann-Whitney U test. Compared to the controls, there was an upregulation of all three analytes in patients with ESRD. Levels of vWF were increased 1.3 fold, (mean 128 ± 69 U/dL, range 9.2 to 308) compared to control (mean 101 ± 60, range 22 to 332). Similarly, there was a 1.6 fold increase in both vWFpp (mean 148 ± 92, range 8.0 to 361) compared to (mean 92 ± 23, range 60 to 163) and ADAMTS-13 (mean 131 ± 27, range 67 to 150) compared to (mean 84 ± 22, range 36 to 128). However, the ratio of vWFpp:vWF (mean 1.2 ± 0.6, range 0.4 to 3.3) was unchanged compared to control (mean 1.2 ± 0.7, range 0.4 to 3.6). This study shows an increase in vWFpp and further validates the upregulation of vWF in ESRD. The normal vWFpp:vWF ratio implies an increased production in vWF in response to endothelial damage without any apparent alteration in vWF degradation or clearance.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1453

### Calf Bioimpedance Ratio Improves Dry Weight Assessment and Blood Pressure Control in Hemodialysis Patients

Yi-Lun Zhou, Jing Liu, Fang Sun, Li-Jie Ma, Bin Han, Yang Shen, T. G. Cui. *Department of Nephrology, Chao-Yang Hospital, Capital Medical University, Beijing, China.*

**Backgroud** Chronic fluid overload due to over-estimation of dry weight (DW) is the major factor in the development of hypertension in hemodialysis (HD) patients. Under physiological conditions, the ratio of extracellular volume to total body water remains tightly regulated, and the ratio of impedance at high frequency to impedance at low frequency is positively related to the ratio of extracellular volume to total body water. The present study was undertaken to investigate whether bioimpedance ratio in the calf (Calf-BR = impedance at 200 kHz / impedance at 5 kHz, as measured by bioimpedance spectroscopy) could be a useful hydration marker for estimation of DW and facilitate better control of blood pressure in HD patients.

**Methods** A multifrequency bioimpedance spectrum analyzer device (BodyStat, UK) was used to measure impedance in right calf . Target range of Calf-BR was derived from 157 healthy Chinese subjects. Post-dialysis Calf-BR was measured in 117 stable, non-edematous HD patients. Those with Calf-BR (s) above target range, had their DW gradually reduced under the guidance of Calf-BR. Home blood pressure was recorded in the mean time.

**Results** The Calf-BR was normally distributed and increased with age, but was independent of BMI and gender in both healthy subjects and dialysis patients. HD patients with Calf-BR above age-stratified target range had significantly higher home blood pressure, in spite of more antihypertensive treatments ($P$=0.058). The patients who reached the target range of Calf-BR by decreasing DW, had their home systolic and diastolic blood pressure significantly decreased ($P$<0.001, and $P$=0.001, respectively), along with reduction in antihypertensive medications ($P$=0.012).

**Conclusions** Modification of bioimpedance technique by the measurement of impedance ratio in the calf (Calf-BR) was a simple and practical method in assessing body fluid status. Recognition and correction of chronic fluid overload based on age-stratified Calf-BR is helpful in hypertension control in Chinese HD patients.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1454

### Smoking and End Stage Kidney Disease: A Systematic Review of Mortality, Cardiovascular Morbidity, and Arteriovenous Access Patency

Scott E. Liebman, Steven P. Lamontagne. *Medicine, University of Rochester Medical Center, Rochester, NY.*

**Background and objectives:** Dialysis patients have a large burden of vascular morbidity and mortality due to both traditional and non-traditional risk factors. Despite its detrimental effects in the general population, smoking has not been well studied in dialysis patients. We systematically reviewed smoking and adverse vascular and arteriovenous (AV) access outcomes in dialysis patients to summarize the current state of knowledge.

**Design, setting, participants, & measurements**: The MEDLINE database was searched for prospective articles examining smoking and vascular disease or AV access in dialysis patients. Of 1397 articles retrieved, 25 were appropriate for inclusion in this review. Three studies specifically examined the association between smoking and adverse vascular outcomes, and one examined smoking and AV fistula failure. In the remainder, smoking was analyzed as a covariate.

**Results:** The three studies examining mortality and vascular outcomes encompassed over 5,000 patients. They all demonstrated a higher all cause mortality in current smokers compared with lifetime non smokers (RR= 1.22-1.37). The risk of former smokers compared with current smokers was less clear. Smoking was also found to increase the risk of new onset heart failure and peripheral vascular disease, but not cerebrovascular or ischemic heart disease. The fourth study, a small, single center report, demonstrated higher rates of fistula failure in smokers.

**Conclusions**- The available data, although scant, support an association between smoking and adverse outcomes in dialysis patients. Dialysis patients should be counseled on smoking cessation. It is not clear how the relative risk of smoking compares with other factors known to impact morbidity and mortality in dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1455

### Risk Factors for Sudden Cardiac Death Using a Competing Risk Approach

Shani Shastri, Navdeep Tangri, Hocine Tighiouart, Alfred K. Cheung, Gerald J. Beck, Gary Eknoyan, Mark J. Sarnak. *Tufts Medical Center.*

**Introduction:** Sudden cardiac death (SCD) accounts for ~60% of cardiac deaths in hemodialysis (HD) patients. There are few data on risk factors for, or prediction equations, to estimate SCD in HD patients.

**Methods:** The Hemodialysis (HEMO) Study was a randomized trial involving 1,846 HD patients testing the effect of dialysis dose and membrane flux on all-cause mortality. For this analysis, deaths were classified into SCD (adjudicated as a secondary outcome), non-sudden cardiac and non-cardiac death. Risk factors were evaluated using cause-specific Cox proportional hazard models and model performance was assessed using Cox models that allowed adjustment for competing risks of death.

**Results:** During a median follow-up of 2.5 years, there were 808 deaths (46.3%). 23%, 61%, and 17% were due to SCD, non-sudden cardiac, and non-cardiac deaths, respectively. Adjusted cause-specific hazard ratios are shown in the Table. The naïve Cox model overestimated the risk of SCD compared to the competing risk model by 10%. The 3-year C-statistic was 0.75 and calibration was adequate ($\chi^2$ 15.02, p 0.09).

**Conclusion:** Risk factors for various causes of mortality differ in HD patients. We present a predictive model for SCD that incorporates easily collected variables with good discrimination and calibration. The model takes into account competing causes of death thus estimating the true risk of SCD. External validation of the model is required.

Multivariable-adjusted cause-specific hazard ratios using competing risk model

| | Hazard ratio (95% CI) | | |
|---|---|---|---|
| **Variables** | **SCD** | **Non-Sudden Cardiac Death** | **Non-Cardiac Deaths** |
| Age* | 1.31 (1.08 1.59) | 1.83 (1.43 2.32) | 1.55 (1.38 1.74) |
| Diabetes | 1.52 (1.11 2.09) | 1.16 (0.82 1.65) | 0.96 (0.80 1.16) |
| Ischemic heart disease | 2.27 (1.65 3.13) | 2.10 (1.46 3.02) | 1.05 (0.87 1.27) |
| Peripheral vascular disease | 1.62 (1.17 2.26) | 1.36 (0.90 2.06) | 1.31 (1.04 1.65) |
| Serum creatinine* | 0.66 (0.55 0.79) | 0.95 (0.78 1.17) | 0.71 (0.64 0.79) |
| Serum alkaline phosphatase* | 1.19 (1.06 1.34) | 1.16 (1.00 1.35) | 1.11 (1.02 1.20) |

* per standard deviation increase

Disclosure of Financial Relationships: nothing to disclose

## F-PO1456

### Different Cardiac Systolic and Diastolic Functions among Patients Undergoing Maintenance Hemodialysis and Peritoneal Dialysis

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**Purpose**-Evaluate systolic and diastolic function in patients undergoing hemodialysis (HD) and peritoneal dialysis (PD) by two-dimensional (2D) speckle tracking echocardiography (STE).

**Methods**- We conducted a prospective study in adult patients (mean age: 62 years) who were receiving long-term dialysis for end-stage renal disease (ESRD). Of these patients, HD patients underwent a thrice weekly maintenance scheme, and PD patients had kept on a regular program. Each subject received conventional echocardiography, TDI, and STE to evaluate cardiac functions. We checked the levels of cardiac troponin T (cTnT), high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), resistin, Cystatin-C, and plasminogen activator inhibitor-1 (PAI-1).

**Results**- Eighty-two patients were enrolled,60 on HD and the remaining undergoing PD. There were no differences in serum levels of cTnT, hs-CRP, IL-6, resistin, cyctatin-C, and left ventricular (LV) systolic functions by conventional echocardiographic and TDI parameters among two groups. With adjustment for age, sex, and co-morbidities by multivariate analysis, HD patients had higher mitral annular E/e' ratio, presenting poor diastolic functions ($p$<0.001). By STE, circumferential strain rate was less negative in PD patients, indicating worse systolic functions ($p$<0.001) despite no detectable difference in LV ejection fraction ($p$=0.993) between groups. Interestingly, patients on PD presented with lower PAI-1($p$=0.001),indicating less severity of cardiac fibrosis. PD patients had higher serum cholesterol and albumin ($p$<0.001).

**Conclusions**-Among ESRD patients, cardiac diastolic functions were better among those undergoing PD with less severity of cardiac fibrosis and lower serum PAI-1 levels. However, HD patients presented with better systolic cardiac contractility by speckle tracking echocardiography. Taken together, no promising dialysis modality would warrant better cardiac functions in patients undergoing renal replacement therapy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1457**

**Predictors and Mortality of Incident Atrial Fibrillation in Hemodialysis Patients** Keiko Nishi, Shouichi Fujimoto, Kazuo Kitamura. *Dialysis Division, University of Miyazaki Hospital, Miyazaki, Japan.*

**Background:** The prevalence and incidence of atrial fibrillation (AF) in hemodialysis (HD) patients are increased. The purpose of this study is to indentify the risk factors including predictive value of electrocardiographic assessment associated with incident AF and to establish the influence of AF on mortality in patients with maintenance HD.

**Method:** A cohort of 299 patients (age, 63.1 ± 14.0 years; men, 59.2%; duration of HD, 80.3 ± 77.7 months) on HD therapy in two centers was retrospectively analyzed between December 2004 and December 2009. An electrocardiogram was scheduled to be carried out on at least a quarterly basis and also at any time that the patient had any cardiac symptoms. Cox proportional hazard analysis was used to determine independent predictors of incident AF after identifying associated variables including clinical, laboratory and electrocardiographic parameters by stepwise procedures. The Kaplan-Meier test was used for analysis of survival.

**Results:** Thirty-seven patients had AF at enrollement. Of the 262 patients who were in sinus rhythm (SR) at enrollement, 45 patients newly developed AF during a follow-up time of five years. In multivaliate analysis, lower hemoglobin level (hazard ratio [HR], 0.72; 95% confidence interval [CI], 0.52 to 0.99) and the presence of P-terminal force > 0.04 mm⁄sec suggesting left atrial enlargement (HR, 2.94; 95% CI, 1.51 to 6.02), premature atrial contractions (HR,7.51; 95 % CI, 3.40 to18.50) and strain pattern (HR, 2.46; 95% CI, 1.19 to 4.84) as electrocardiographic findings were independently associated with new-onset AF in HD patients. The survival curves show a significant difference between the patients with presence or incidence of AF and those who maintained SR. Three year survival rate of patients with incident AF and those maintained SR was 71.4% and 85.6%, respectively.

**Conclusion:** AF is frequent in HD patients and they have poor prognosis compared with those without. Left atrial enlargement, premature atrial contractions and strain pattern as electrocardiographic findings, in addition to lower hemoglobin level, may predict new-onset AF in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1458**

**Increased Plasma S100A12 Level Is Associated with Cardiovascular Diseases in Non-Diabetic Hemodialysis Patients** Yasukiyo Mori,[1] Yayoi Shiotsu,[1] Atsushi Kosaki.[2] *[1]Department of Cardiology and Nephrology, Kyoto Prefectural University of Medicine, Kyoto, Japan; [2]Department of Medicine II, Kansai Medical University, Hirakata, Japan.*

S100A12, also called EN-RAGE, is an endogenous ligand for the receptor for advanced glycation end products (RAGE). Our working hypothesis is that S100A12 protein might contribute to the development of atherosclerosis. Cardiovascular disease (CVD) due to atherosclerosis remains the major cause of morbidity and mortality in the patient with chronic kidney disease (CKD), particularly in the hemodialysis (HD) patients. We have already showed that plasma S100A12 level was more than twice as high in HD patients and the intima-media thickness (IMT) of carotid artery correlated with the plasma S100A12 level, suggesting that plasma S100A12 level is associated with atherosclerosis as a complication of CKD (Mori Y and Kosaki A et al. Am J Nephrol 2009;29:18-24). Moreover, we confirmed the higher level of plasma S100A12 in our larger cross-sectional study to assess the association between plasma S100A12 levels and CVD on 550 hemodialysis (HD) patients including diabetic nephropathy. However, it is well established that the prevalence of CVD in diabetic HD patients remains the highest among all primary diagnoses. In the present study, we focused on the association of plasma S100A12 level with the prevalence of CVD in non-diabetic hemodialysis patients(n=348). The analysis resulted in 1) plasma S100A12 level in non-diabetic hemodialysis patients with CVD (n=97, 32.1±2.96 ng/ml) was significantly higher than those without CVD (n=251, 17.8±0.80 ng/ml) ($p<0.001$), and 2) 10ng/ml increase in plasma S100A12 level (OR, 1.597; 95% CI, 1.343 to 1.898, $p<0.001$) as well as the history of smoking (OR, 2.115; 95% CI, 1.017 to 4.398, $p=0.045$) and age (OR, 1.028; 95% CI, 1.006-1.051, $p=0.011$) were identified as the independent factors associated with the prevalence of CVD in stepwise regression analysis. These results suggest that plasma S100A12 protein may be a novel predictor for CVD events in non-diabetic HD patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1459**

**Serum β2-Microglobulin Levels Predict Cardiovascular and All-Cause Mortality in End-Stage Renal Disease Patients on Chronic Hemodialysis** Hirotake Kasuga,[1] Tetsuya Yamada,[2] Nobumasa Nakamura,[1] Ryo Takahashi,[1] Keiko Kimura,[1] Takanobu Toriyama,[1] Seiichi Matsuo,[3] Hirohisa Kawahara.[1] *[1]Internal Medicine, Division of Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Japan; [2]Seto Kyoritsu Clinic, Seto, Japan; [3]Nagoya University Hospital, Nagoya, Japan.*

**Background:** β2-microglobulin (β2-MG) is a middle-molecule uremic toxin, and the serum levels elevate according with progression of renal insufficiency. In ESRD patients on chronic HD, serum β2-MG concentration is thought to be a surrogate marker which reflects both residual renal function and adequate HD therapy. However, the association of β2-MG levels with clinical outcome has not been fully evaluated in this population. We investigated whether serum β2-MG levels could predict cardiovascular (CV) and all-cause mortality in HD patients.

**Methods:** A total of 363 stable HD patients (male 61%, age 59±12 years, diabetes 25.7%, duration of HD 7.3±6.2 years) were enrolled. All patients were undergoing maintain HD therapy using high-flux membrane dialyzer. Serum β2-MG levels were measured in blood samples before and after HD session. The patients were divided into tertiles according to serum β2-MG levels before HD session; tertile 1 (T1): <31.6mg/L (n=121), T2: 31.6-37.3mg/L (n=121) and T3: >37.3mg/L (n=121), and were followed for 6 years.

**Results:** Baseline variables were comparable among tertiles. Reduction rate of β2-MG during HD session was 28.6%, 19.3% and 17.4% in T1, T2 and T3 groups, respectively ($p<0.0001$). During follow-up period (55±24months), 102 patients (28.1%) died including CV death (12.7%). Six-year event-free survival rates were 93.7%, 87.8% and 80.5% for CV mortality ($p=0.0019$) and 79.7%, 77.3% and 65.7% for all-cause death ($p=0.0019$) in T1, T2 and T3, respectively. After adjustment for gender, age, traditional risk factors, history of CV disease, hemoglobin, albumin and C-reactive protein, elevated serum β2-MG levels were independent predictors for CV mortality [HR 4.96, $p=0.0021$ for T3 vs. T1] and for all-cause death (HR 2.35, $p=0.0040$ for T3 vs. T1), respectively.

**Conclusion:** Elevated serum β2-MG levels could strongly predict both CV and all-cause mortality in ESRD patients on HD.

Disclosure of Financial Relationships: nothing to disclose

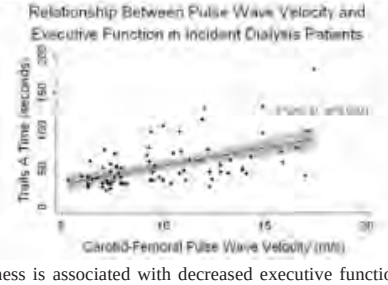
**F-PO1460**

**Arterial Stiffness Is Associated with Impairments in Executive Function in Incident Hemodialysis Patients: The Predictors of Arrhythmic and Cardiovascular Risk in ESRD (PACE) Study** Stephen M. Sozio,[1] Bernard G. Jaar,[1] Tariq Shafi,[1] Lucy A. Meoni,[1] Julia J. Scialla,[1] Wen Hong Linda Kao,[1] Rulan S. Parekh.[2] *[1]Johns Hopkins University; [2]University of Toronto.*

Accelerated arterial calcification and subsequent vascular stiffness is common in patients with ESRD, and likely contributes to clinical cardiovascular events. We hypothesize that vascular stiffness leads to disease in the cerebral vasculature and could manifest subclinically as cognitive impairment. We investigated the cross-sectional association of carotid-femoral pulse wave velocity (PWV), a non-invasive measure of arterial stiffness, with both global cognitive function (Modified Mini-Mental Status Exam [3MSE]) and executive function (Trails A and Trails B) in an incident hemodialysis cohort of the first 176 PACE participants. PWV was measured using the Sphygmocor PVx System; all clinical and cognitive tests were performed in a quiet room on an interdialytic day. Linear and logistic regressions were used to assess the independent associations of PWV with global cognitive function and executive function. Mean age was 55 years with 54% male, 65% African-American, 48% with diabetes, 46% with prior atherosclerotic cardiovascular disease (ASCVD), and mean PWV 10.2 m/s (range 5.2-17.8). A total of 12% of participants had global cognitive impairment by 3MSE. Higher PWV was not associated with global cognitive function but was significantly associated with longer times for executive function in both Trails A and Trails B in unadjusted analyses. After adjusting for CVD risk factors, PWV remained significantly associated with longer Trails A time.

| Executive Function Test | Beta Coefficient (95% CI) for Cognitive Score versus PWV in Incident Dialysis Patients§ | | |
|---|---|---|---|
| | Unadjusted | Model 1* | Model 2** |
| Trails A Time (range 21-181 sec) | **4.80 (2.96, 6.63)‡** | **2.99 (0.92, 5.07)‡** | **2.84 (0.27, 5.42)†** |
| Trails B Time (range 45-300 sec) | **8.68 (2.99, 14.37)‡** | 2.08 (-3.72, 7.88) | 1.63 (-5.72, 8.98) |

§Difference in cognitive score (seconds) per each m/s difference in PWV
*Model 1: Adjusted for age, sex, race
**Model 2: Model 1 plus diabetes mellitus, prior ASCVD, SBP, DBP
†p<0.05
‡p<0.01




Arterial stiffness is associated with decreased executive function among incident dialysis patients, independent of other CVD risk factors including blood pressure. Further studies are warranted to assess interventions to target vascular stiffness.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1461**

**Myeloid-Related Protein 8/14 Predicts All-Cause Mortality in Peritoneal Dialysis Patients** Bonnie Kwan, Peter Yam-Kau Poon, Kai Ming Chow, Philip K. T. Li, Cheuk-Chun Szeto. *Dept of Medicine, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong.*

Background & Aim: Myeloid-related protein (MRP) 8/14 complex, also termed calprotectin, is a marker of phagocyte activation. Studies have found that MRP 8/14 complex is increased systemically and is a potential biomarker for acute coronary syndrome in the general population.

We aim to examine the use of MRP 8/14 complex in the prediction of future cardiovascular events and all-cause mortality among patients on peritoneal dialysis. We aim to study the significance of MRP 8 and MRP 14 on the outcome of patients on peritoneal dialysis (PD).

Method: We retrospectively studied patients commenced on PD from January 2007 – December 2008. Dialysis records, demographic data and past medical history were obtained from medical records, while stored serum were analysed for MRP 8/14 complex levels.

Results: A total of 104 patients were recruited into the study. Fifty-nine were male. Mean age 57.4±11.9 years old. Mean duration of dialysis 7.8±31.0 months. MRP 8/14 level was 4.47±5.77 µg/ml. MRP 8/14 did not differ significantly between males/females, diabetics/non-diabetics, with/without past history of coronary artery disease, cerebrovascular disease or peripheral vascular disease. The subjects were divided into 4 quartiles according to MRP level. Actuarial survival was significantly different among the MRP 8/14 quartiles (p=0.003), while there was no significant difference among the quartiles for technique survival (p=0.255) or cardiovascular survival (p=0.136). MRP 8/14 quartiles was found to be a significant factor in multivariate analysis of actuarial survival (adjusted hazard ratio 3.52, 95% CI 1.203-10.294) after adjusting for residual renal function, KT/V, albumin, subjective global assessment, Charlson's score, age, duration of dialysis and diabetes.

Conclusion: MRP levels were found to be predictive of all-cause mortality in PD patients. In our current study MRP levels were not found to be predictive of cardiovascular mortality. This could be related to confounding effects from the many cardiovascular risk factors in PD patients. Further studies with more subjects are needed.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1462**

**Survival of US Dialysis Patients Receiving Cardiac Valvular Replacement for Bacterial Endocarditis** Charles A. Herzog, David T. Gilbertson. *CVSSC, USRDS, Minneapolis, MN.*

**Background:** Dialysis pts with bacterial endocarditis (BE) have poor long-term survival, with a previously reported one year mortality of 62%. Although cardiac valvular surgery is a key aspect of therapy of left-sided endocarditis, there are no published data on the long-term survival of dialysis pts with BE undergoing heart valve replacement surgery.

We searched the records of the United States Renal Data System database to identify 11,125 dialysis pts hospitalized for BE in 2004-2007, and the subset of 1267 pts receiving cardiac valvular replacement surgery. Long-term survival was estimated by Kaplan-Meier method and independent predictors of death were examined in a Cox model.

**Results:** Valve surgery cohort characteristics: 24% <45yrs, 54% 45-64yrs, 16% 65-74 yrs, 6% 75+ yrs, 50% white, 47% black, 60% male, 36% Diabetic ESRD, 98% hemodialysis, 46% aortic(AVR), 45% mitral (MVR), 9% both (AVR+MVR), 44% tissue valves. In-hospital mortality was 13.7 %. The tables show estimated survival and predictors of death in pts undergoing surgery (age 45-64, male, white, hemodialysis, non-diabetic ESRD, tissue valve, MVR is reference) with hazard ratio (HR).

**Conclusion:** Dialysis patients undergoing left-sided cardiac valvular replacement for bacterial endocarditis have high long term mortality. Selected dialysis pts with BE may potentially benefit from surgery. Survival is not related to valve selection (tissue vs non-tissue).

| | Survival (%) | | |
|---|---|---|---|
| Yrs | Tissue | Non-Tissue | No valve surgery |
| 0.5 | 59.4 | 59.6 | 48.5 |
| 1 | 48.0 | 49.6 | 39.0 |
| 2 | 34.8 | 37.0 | 28.0 |
| 3 | 25.4 | 29.6 | 20.8 |
| | | | |
| **Predictors of Death** | | | |
| **Variable** | **HR (95% CI)** | **P-value** | |
| Age 65-74 | 1.25 (1.05, 1.49) | 0.13 | |
| Age 75+ | 1.60 (1.23, 2.09) | .0004 | |
| Female | 0.97 (0.85, 1.11) | ns | |
| Black | 1.03 (0.89, 1.18) | ns | |
| Peritoneal dialysis | 1.24 (0.82, 1.89) | ns | |
| Diabetic ESRD | 1.49 (1.27, 1.75) | <0.0001 | |
| Non-tissue valve | 0.97 (0.85, 1.11) | ns | |
| AVR (vs MVR) | 0.68 (0.60, 0.79) | <0.0001 | |
| AVR+MVR (vs MVR) | 1.15 (0.91, 1.45) | ns | |

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**F-PO1463**

**Cinacalcet Can Reduce a Drop in Intradialytic Blood Pressure (BP) in Hemodialysis (HD) Patients** Terumi Morita,[1] Akira Furusu,[2] Kinichi Izumikawa,[3] Shigeru Kohno.[4] *[1]Nephrology, Izumikawa Hospital, Minamishimabara, Japan; [2]Nagasaki University, Nagasaki, Japan; [3]Izumikawa Hospital, Minamishimabarashi, Japan; [4]Nagasaki University, Nagasaki, Japan.*

Background; A drop in BP during HD session is reported to be a major risk factor for cardiac death in HD patients. Recent experimental studies have shown the expression of calcium-sensing receptor on heart muscle and vascular cells, and suggest that cinacalcet has cardioprotective effects and stabilizes BP. Purpose; To evaluate the effects of cinacalcet on improving intradialytic BP drop in HD patients. Subjects and Methods; From February 2008 to May 2010, 22 outpatients on maintenance HD who had average intradialytic BP drop > 20% were enrolled in this study after appropriate IC. One year after the start of cinacalcet treatment at dose of 25-75 mg, intradialytic BP profile, parameters for ultrasound cardiographiy, serum PTH and heart rate variability (HRV), a measure of the autonomic nervous system function, were compared. Results; As shown in [Table], the average intradialytic BP drop was drastically improved, whereas and HRV stayed the same, indicating the autonomic nervous system function was not contributing factor. Parameters for cardiac function tended to improve, but not significant. Conclusions; Our data showed that cinacalcet can stabilize intradialytic BP, suggesting it may potentially have cardioprotective effects in HD patients.

Change of intradialytic BP profiles after cinacalcet treatment

| | Before | After | p-value |
|---|---|---|---|
| average BP drop | 28.6±14.7 | 16.8±12.8 | 0.0017 |
| EF | **** | **** | 0.12 |
| LVDd | **** | **** | 0.09 |
| HRV | 2.15 0.97 | 1.99 1.07 | 0.42 |

Disclosure of Financial Relationships: nothing to disclose

**F-PO1464**

**Geographic Variation in Cardioprotective Antihypertensive Medication Usage in Dialysis Patients** James B. Wetmore,[1] Jonathan D. Mahnken,[2] Qingjiang Hou,[2] Edward F. Ellerbeck,[3] Sally K. Rigler,[3] John Spertus,[4] Theresa I. Shireman.[3] *[1]Medicine, Division of Nephrology and Hypertension, University of Kansas Medical Center, Kansas City, KS; [2]Biostatistics, University of Kansas Medical Center, Kansas City, KS; [3]Preventive Medicine and Public Health, University of Kansas Medical Center, Kansas City, KS; [4]St Luke's Mid America Heart Institute, University of Missouri - Kansas City, Kansas City, MO.*

**Introduction:** Antihypertensive agents with cardioprotective properties are commonly prescribed in dialysis patients. However, little is known about clinical and geographic factors associated with their use.

**Methods:** We conducted a national, cross-sectional retrospective analysis of prescription drug claims for dually-eligible (Medicare-Medicaid) chronic dialysis patients diagnosed with hypertension to assess the prevalence of cardioprotective antihypertensive medication use, to examine how the use of these agents was associated with various demographic and clinical factors, and to determine how use varied by state across the US.

**Results:** Of 41,656 dialysis patients with hypertension, 77.7% received at least one angiotensin covenverting enzyme inhibitor / angiotensin receptor blocker (ACEI/ARB), beta-blocker, or calcium channel blocker (CCB) during the period studied. CCBs were the most commonly-used agent (48.9%), followed by ACEI/ARBs (44.8%) and beta-blockers (44.0%). In multivariate analyses, older age, male sex, Caucasian race, high body mass index, and poor functional status were associated with less use of each of the classes of agent studied, as was self-care dialysis. There was substantial state-by-state variation in use of all classes of agents, with roughly 2.5-fold differences in adjusted rate ratios between the highest- and lowest-prescribing states.

**Conclusions:** Relatively modest numbers of hypertensive dialysis patients are treated with antihypertensive drugs having cardioprotective properties. Moreover, substantial variability across states was observed. There appears to be an opportunity to improve the use of cardioprotective anti-hypertensive medications and to support more standardized practice patterns.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1465**

**Novel Insights into Calciphylaxis: The International Calcific Uremic Arteriolopathy (CUA) Registry** Vincent Brandenburg,[1] Jurgen Floege,[2] Markus Ketteler.[3] *[1]Cardiology, RWTH University Hospital Aachen, Aachen, Germany; [2]Nephrology, RWTH University Hospital Aachen, Aachen, Germany; [3]Nephrology, Klinikum Coburg, Coburg, Germany.*

**INTRODUCTION AND AIMS:** Calcific uremic arteriolopathy (CUA, calciphylaxis) is a rare disease associated with high morbidity and mortality. The aim of the International Collaborative Calciphylaxis network (ICCN) was to establish an online registry to collect data about incidence and risk factors for CUA. Thereafter, we tried to gain overview about current treatment strategies. This data collection is intended to be the basis for future prospective treatment trials.

**METHODS:** The internet-based registry allows online notification of all cases of established or suspected CUA (www.calciphylaxie.de). A comprehensive data base including various parameters was established (patient characteristics, laboratory data,

clinical background and presentation as well as therapeutic strategies). Follow-up of the patients is planned by regular queries of long-term outcome.

**RESULTS:** Altogether 110 patients with CUA have been documented until 06/2010: 65 female (59%); 48 diabetics (43%); mean age was 66 +/-14 (20-89) years. N= 52 (57%) were treated with coumadins. Focussing on dialysis pts (n=84, 76%) mean serum total calcium was 2.3+/-0.4 mmol/L, mean serum phosphorus: 2.0+/-0.9 mmol/L. PTH levels varied broadly between undetectably low and > 1200 pg/ml, mean 260 +/- 270 pg/mL. According to KDOQI PTH target levels (150 to 300 pg/mL) 32% were below, 22% within and 46% above target range. Among the most frequently recorded therapeutic procedures were: intensifying dialysis modality, hyperparathyroidism control, i.v. sodium-thiosulfate application, lowering calcium burden by reducing dialysate calcium and / or reducing oral calcium-containing phosphate-binders, systemic antibiotics, and stopping oral anticoagulation.

**CONCLUSIONS:** CUA is a rare complication predominantly occurring in dialysis pts. PTH levels vary substantially and are below 300 pg/ml in 2/3 of cases. The present internet based ICCN registry is a valuable tool to collect data upon CUA cases and may become a basis for prospective systematic evaluations of treatment modalities in the near future.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1466

**Cardiovascular Disease and Cognition in Hemodialysis** Daniel E. Weiner, Lena M. Giang, Brian T. Agganis, Tammy Scott, Hocine Tighiouart, Mark J. Sarnak. *Tufts Medical Center, Boston, MA.*

Cardiovascular disease (CVD) and cognitive impairment are both common in patients with kidney failure. Given the high prevalence of vascular disease in hemodialysis patients and the proposed role of microvascular disease on cognitive function, particularly cognitive domains that incorporate attention, processing and executive functions, we hypothesized that prevalent systemic cardiovascular disease would be associated with worse cognitive performance in hemodialysis patients and identify individuals with a high burden of cognitive impairment.

The Cognition and Dialysis Study is an ongoing investigation of cognitive impairment and its risk factors in 5 Boston area DCI hemodialysis units. After excluding 48 individuals with history of clinical stroke, we evaluated the cross-sectional association between CVD and cognitive functioning. Principle components analysis reduced tests to either processing speed/executive function or memory domains. Multivariable models adjust for age, sex, education, and race as well as any variables with p<0.2 after these adjustments.

Among 200 participants, mean (SD) age was 62 (18) years and 75 (38%) had CVD; of these 13 had peripheral vascular disease only, 34 had coronary disease only and 28 had both. Individuals with CVD were older, more likely to be men, diabetic, and current or former smokers. In adjusted models, individuals with CVD performed 0.46 standard deviations worse (p=0.0003) on tests assessing executive function and processing speed, while there was no difference in performance on tests of memory (Table).

In conclusion, performance on tests of executive function and processing speed is worse in hemodialysis patients with CVD.

Table. Association between CVD and cognitive function

| Cognitive Test/Component | Function Tested | Test Scores | | Multivariable | |
|---|---|---|---|---|---|
| | | No CVD | CVD | Beta | p-value |
| Executive Factor | *Composite of 'executive' tests* | 0.37 ± 0.92 | -0.42 ± 0.85 | -0.46 | 0.0003 |
| Memory Factor | *Composite of memory tests* | 0.15 ± 1.00 | -0.09 ± 1.08 | 0.15 | 0.32 |
| Mini-Mental State Exam | *Screen* | 26.9 ± 2.7 | 26.5 ± 3.0 | 0.3 | 0.46 |
| Short Delayed Recall | *Working memory, recognition, and memory* | 5.4 ± 2.9 | 4.3 ± 3.2 | 0.2 | 0.63 |
| Delayed Recall | | 5.0 ± 2.8 | 4.1 ± 2.9 | 0.3 | 0.54 |
| Recognition | | 21.6 ± 2.6 | 20.3 ± 3.3 | -0.3 | 0.53 |
| Block Design | *Executive function, processing speed and attention* | 30.1 ± 11.2 | 22.1 ± 9.1 | -4.7 | 0.001 |
| Digit Symbol Coding | | 47.0 ± 18.5 | 32.9 ± 12.2 | -4.1 | 0.07 |
| Trails A | | 49.5 ± 26.6 | 70.2 ± 40.1 | 8.4 | 0.08 |
| Trails B (Completers) | | 122.5 ± 62.7 | 164.0 ± 61.9 | 23.5 | 0.10 |
| Trails B (Non-Completion) | | 15.2% | 30.7% | | |

Results are mean ± standard deviation. Individual test results represent number or percent correct except Trails A and B which are reported in seconds required to complete the task. Factor scores by definition have mean of 0 ± 1 for the population, with positive numbers consistent with better performance. Positive beta-coefficients are consistent with worse performance on Trails A and Trails B, while negative beta-coefficients are consistent with worse performance on all other tests, including the factors

Disclosure of Financial Relationships: Research Funding: Covidien.

## F-PO1467

**High Plasma Levels of Fibroblast Growth Factor-23 Are Associated with Left Ventricular Dysfunction in Dialysis Patients** Jessica B. Kendrick,[1] Jacob Joseph,[2] James S. Kaufman,[2] Alfred K. Cheung,[3,4] Tom H. Greene,[4] William L. Roberts,[4] Zahi Rafeq,[2] Gerard John Smits,[1] Michel B. Chonchol.[1] *[1]University of Colorado Denver Health Sciences Center, Denver, CO; [2]VA Boston Healthcare System, Boston, MA; [3]VASLCHCS, Salt Lake City, UT; [4]University of Utah, Salt Lake City, UT.*

Purpose: Fibroblast growth factor-23 (FGF-23) levels are elevated in dialysis patients. FGFs receptors have been implicated in the pathogenesis of atherosclerosis. We tested the hypothesis that high FGF-23 levels are associated with left ventricular dysfunction in dialysis patients.

Methods: The Homocysteine Study was a randomized trial, which evaluated the effects of folic acid and B vitamins on all-cause mortality in dialysis patients with elevated homocysteine levels. We identified 110 dialysis participants in this study who had echocardiograms performed for clinical indications during the study follow-up. FGF-23 levels were measured in stored baseline plasma samples. Multivariate regression analyses were performed to evaluate whether FGF-23 levels were associated with a lower ejection fraction (EF) independently of other covariates that might influence systolic function.

Results: Participants had a mean age of 60±11 years. The median (IQR) FGF-23 level and mean EF at baseline were 4632 [1384-14997] RU/mL and 50±13 %, respectively. Median follow-up time was 1.9 years. Higher FGF-23 levels were associated with lower EF. After adjustment for baseline EF, age, race, sex, hypertension, diabetes, history of cardiovascular disease, body mass index, calcium, phosphorus, 25-hydroxyvitamin D, calcitriol and intact PTH levels, for every log10 increase in FGF-23, EF decreased by 8% (p=0.004). Higher FGF-23 levels were also independently associated with an EF < 40% in multivariate analysis. For every log10 increase in FGF-23, there was a 6-fold increased risk of an EF <40% (odds ratio 6.3, 95% CI 1.04 to 40.1; p=0.04).

Conclusion: Higher plasma FGF-23 level is independently associated with systolic dysfunction in dialysis patients. Findings should be confirmed in studies where echocardiograms are protocol-based rather than selected by clinical indication.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1468

**Brain Natriuretic Peptide and Echocardiographic Evaluation on Patients with End Stage Renal Disease on Hemodialysis: 4-Year's Clinical Follow-Up** Jinil Yoo, Lin N. Lwin, Hugo J. Villanueva, Donjeta Sulaj, Khashayar Sehhat. *Neprology, Montefiore Medical Center, North Division, New York Medical College, Bronx, NY.*

Brain natriuretic peptide (BNP) is considered to be a marker of cardiac dysfunction and its level has shown a predictive value on congestive heart failure (CHF) and cardiac events. The presence of left ventricular hypertrophy (LVH) and LV dysfunction on echocardiogram (ECHO) is also known to be associated with a high cardiac morbidity and mortality.

We obtained pre dialysis plasma BNP levels and post dialysis ECHO at their prescribed dry weight on 45 of 54 patients (pts) in a hospital-based dialysis unit. There were 15 African Americans(33%), 11 Hispanics(24%), 12 non-Hispanic Caribbeans(27%), 4 Caucasians(9%), 3 Asians(7%); avarage age of 63 years, 24 diabetics(53%), 42 hypertensives(93%), 18(40%) with history of coronary artery disease, and avarage on HD of 3.6 years. The following diagram is the summary of our pts mortality rate (MR) based on ejection fraction (EF : %) calculated from ECHO and plasma BNP level, during 4-year's clinical follow up after the BNP and ECHO.

| BNP levels→ | BNP ≤ 100 pg/mL | | BNP ≥ 200 pg/mL | |
|---|---|---|---|---|
| Ejection Fraction(%) ↓ | Pts distribution | Mortality N(%) | Pts distribution | Mortality N(%) |
| EF ≤ 50 | 1/9(11%) | 0 | 8/9(89%) | 6/8(75%) |
| EF > 50 to ≤ 58 | 8/18(44%) | 4/8(50%) | 8/18(44%) | 5/8(63%) |
| EF ≥ 59 | 8/18(44%) | 2/8(25%) | 5/18(28%) | 2/5(40%) |

In this observational study, 26 of 45 pts(58%) had BNP level ≥ 100 pg/mL (higher than the normal reference level) and EF less than 59 in 27 of 45 pts(60%). During the 4-year's observation, the first group of patients with BNP ≥ 200 and EF ≤ 50 had MR of 75%(6/8) while the 3rd group with BNP ≤ 100 and EF ≥ 59 having MR of 25%(2/8). The primary cause of death among the first group was cardiac origin in all, but the cause of death from the 3rd group was non-cardiac origin. All of 3 pts who had no LVH on ECHO had BNP level < 65 pg/mL and all of 4 pts with dilated LVH on ECHO had BNP level > 200 pg/mL. We believe that utility of plasma BNP and ECHO testing may enhance early recognition of asymptomatic cardiac dysfunction and guide us to lessen cardiac events to our pts.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1469

**Impact of Co-Infection with Hepatitis B and C and Human Immuno Deficiency Viruses on Survival in Hemodialysis Patients** Moro O. Salifu,[1] Rahul Jindal,[2] Amarpali Brar,[1] Win Kyaw,[1] Dominic S. Raj.[3] *[1]Medicine, Division of Nephrology, SUNY Downstate Medical Center, Brooklyn, NY; [2]Organ Transplant Program, Walter Reed Army Medical Center, Washington, DC; [3]Division of Renal Disease and Hypertension, The George Washington University, Washington, DC.*

Background: The prevalence of hepatitis viruses and human immunodeficiency virus (HIV) co-infection among maintenance hemodialysis patients and their impact on survival is unknown.

Methods: Data from the United States Renal Data System from January 2000 to December 2001, with at least 3 years of follow-up was analyzed. The study population was categorized based on the viral status at the time of initiation of dialysis. Survival analysis and hazard functions were used to estimate the risk of death over time.

Results: Among the 146,717 incident hemodialysis patients studied, the prevalence of hepatitis B virus (HBV), hepatitis C (HCV), HIV and HCV+HIV were 1.0%, 15.5%, 1.4% and 0.2% respectively. During the observation period 51.1% of the patients died. All cause mortality was higher in patients with HCV (AHR 1.22; 95% CI 1.18-1.27) and HCV+HIV (AHR 1.49; 95% CI 1.17-1.88), but lower in HBV patients (AHR 0.43; 95% CI 0.35-0.52) compared to those without viral disease. The risk for cardiovascular mortality was higher in patients with HCV and compared to other groups (HR 1.47; 1.43-1.50). Death due to liver disease was uncommon, but infection-related mortality was increased in HCV (HR 2.70; 95% CI 2.55-2.78), HIV (HR 2.75; 95% CI 2.47-3.07) and HCV+HIV (HR 3.28; 95% CI 2.50-4.26) patients.

Conclusion: HCV and HBV status have opposing effect on survival among hemodialysis patients. The mortality risk associated with HIV varies with co-existing HBV and HCV. Thus, co-infection status is important for risk assessment as well as for planning therapeutic intervention.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1470**

**Increased Serum ADMA Level Associated with Pulmonary Hypertension in Patients on Hemodialysis** Qianmei Sun,[1] Juan Meng,[2] Zhongxin Li,[1] Wei Jiang,[3] Karl L. Womer.[4] *[1]Departments of Nephrology, Beijing ChaoYang Hospital, Capital Medical University, Beijing, China; [2]Department of Rheumatology, Beijing ChaoYang Hospital, Capital Medical University, Beijing, China; [3]Department of Ultrasonic Medicine, Beijing ChaoYang Hospital, Capital Medical University, Beijing, China; [4]Department of Medicine, Division of Nephrology, Johns Hopkins University School of Medicine, Baltimore, MD.*

**Background** Pulmonary hypertension (PH) in patients with end-stage renal disease (ESRD) on hemodialysis is a newly described entity, with the pathogenesis considered to be ESRD-related endothelial dysfunction. Asymmetrical dimethylarginine (ADMA) is an endogenous nitric oxide (NO) inhibitor. Increased serum concentrations of ADMA may contribute to endothelial dysfunction in patients with pulmonary vascular disease. The present study was undertaken to determine whether a relationship exists between serum ADMA and PH in patients undergoing hemodialysis (HD).

**Methods** The incidence of PH was prospectively estimated by Doppler echocardiography in 69 patients with ESRD receiving long-term HD within 2h of completion of HD. PH was defined as pulmonary artery systolic pressure (sPAP)>35mmHg, as determined by Doppler echocardiography using the modified Bemouli equation. Serum ADMA levels were measured by enzyme-linked immunosorbent assay (ELISA) method. The relationship between ADMA and PAP was then analyzed.

**Results** PH was detected in 31.9% of patients receiving HD. Mean sPAP in the PH group was 40.79±12.32 mmHg compared to 24.81±4.54 mmHg in patients without PH (P<0.001), Patients with PH had significantly higher cardiac output (6.41±0.74 vs 5.08±0.61 L/min/m2; P<0.001 by ANOVA). Serum ADMA levels were significantly elevated in patients with PH compared with those without PH and 25 matched control subjects (1.32±0.15μmol/L vs 0.99±0.19μmol/L vs 0.22±0.05μmol/L; P<0.001). Multiple regression analysis showed that sPAP was independently related to ADMA and arterio-venous fistula (AVF) flow (P<0.01).

**Conclusions** Increased ADMA levels and their positive association with PAP suggests that the NO pathway is involved in the development of pulmonary hypertension in patients on haemodialysis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1471**

**Sudden Cardiac Death in Dialysis Patients from a Meta-Analysis of Cardiac Arrests on Dialysis Units** Darren Green, David I. New, Philip A. Kalra. *Department of Renal Medicine, Salford Royal Hospital, Salford, United Kingdom.*

Sudden cardiac death (SCD) is a major cause of mortality in dialysis patients. The use of implanted cardioverter-defibrillator devices (ICD) in these patients is contentious as studies suggest that survival with ICD is poor relative to other patients. Whether this is because they suffer less shockable rhythms (VF/VT) is not known, as the relative proportion of VF /VT arrests has not been studied in these patients, whereas it is known to be 75-80% in the general population.

Studies have been published on the outcome of cardiac arrests on dialysis units. However, none focus on the arrest rhythm. The purpose of this meta-analysis was to determine the proportion of dialysis-associated cardiac arrests that were of cardiac origin and whether the cardiac arrest rhythm was mostly VF/VT as in the general population.

A pubmed search for "dialysis cardiac arrest" included 9 cohort studies with data quantifying the outcome of cardiac arrests on dialysis units. Of these, 2 were excluded for giving little data about the study population, 1 was excluded as it referred to a cluster of deaths caused by a dialyzer reaction, and 2 referred to the same population. This left 5 study populations. Data was collected of cardiac arrests in chronic haemodialysis patients without a do not resuscitate order.

There were 1325 cardiac arrests covering 24,875,998 dialysis sessions. Where the cause of cardiac arrest was documented, 79.8% (485 of 608) of events were of a cardiac cause. These data equate to an approximate SCD event rate of 61 per 1000 patient years. 57.5% had VF/VT as the arrest rhythm.

This study suggests that SCD may be less likely to be caused a by shockable rhythm in dialysis patients compared to the general population. This may contribute to their poorer survival with ICD. However, if 57% of SCD in dialysis patients is due to VF/VT, the meta-analysis suggests that these events alone will occur at a rate of 35 events per 1000 patient years, which is as much as 20 times greater than the rate in the general population. Dialysis patients are therefore very high risk for SCD which may respond to ICD therapy, making them a worthy target for risk stratification for ICD use.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1472**

**Intravenous Erythropoetin Does Not Influence Intradialytic Hypertension** Albert J. Power, Kakit Chan, Seema Singh, Peter Hill, David Taube, Neill D. Duncan. *Imperial College Kidney & Transplant Institute, West London Renal & Transplant Centre, Hammersmith Hospital, London, United Kingdom.*

Intradialytic hypertension [IDH] confers a 22% increased risk of hospitalization or death per 10mmHg postdialysis increase in systolic blood pressure [BP]. Intravenous [IV] and not subcutaneous erythropoetin [EPO] elevates endothelin-1 levels and mean arterial pressure 30 mins after administration and has been hypothesized to contribute to IDH. However there are no large studies on this in clinical practice.

In this retrospective cohort study of 166 patients established [>90d] on hemodialysis [HD] and receiving protocolised care at 1 dialysis unit at our center Jan-Dec 2009, EPO [darbepoetin] was given IV 1hr into dialysis weekly and adjusted to target hemoglobin [Hb] 10.5-12.5g/dL. The dialysis prescription was tailored to target postdialysis BP≤130/80mmHg with ultrafiltration [UF] to euvolemia and subsequent antihypertensive use. IDH was defined with precedent as ≥15mmHg increase in postdialysis MAP from predialysis levels.

We examined 20407 sessions in all patients [mean age 66.4±14.0 yrs, 57% male, 42% diabetic, 57% hypertensive] using mixed effects models to account for repeat measures. Mean pre & postdialysis BP was 140±24/78±14mmHg & 136±24/75±14mmHg respectively. 6800 EPO doses were given [mean 0.60±0.35mcg/kg] with mean predialysis Hb 12.2±1.0g/dL, ferritin 278±112ng/mL. Overall IDH occurred in 13.7% sessions and clustered with ≥20% prevalence in 21% of the cohort.

IV EPO was not associated with absolute changes in MAP [p=0.2] or odds of IDH [OR 0.9, p=0.3]. UF volume reduced MAP by 1.6mmHg/liter UF [p<0.001] and odds of IDH by 14% per liter. Older age associated with intradialytic rise in MAP [1.4mmHg/10yrs age, p=0.002] and increased odds of IDH by 10% per decade [p=0.03]. Patient ethnicity, major comorbid diagnoses, dry weight and Hb had no significant effect on intradialytic change of MAP or IDH.

This large comprehensive study demonstrates no association of IV EPO use with IDH and MAP changes which are predominantly associated with UF volume and patient age.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1473**

**Association between Left Ventricular Diastolic Dysfunction by Color Tissue Doppler Imaging and Vascular Calcification on Plain Radiographs in Dialysis Patients** Won Suk An,[1] Sung Hyun Son,[2] Young Ki Son,[1] Seong Eun Kim,[1] Ki Hyun Kim.[1] *[1]Internal Medicine, Dong-A University, Busan, Republic of Korea; [2]Internal Medicine, BHS Han Seo Hospital, Busan, Republic of Korea.*

Diastolic dysfunction is frequently associated with left ventricular (LV) hypertrophy, indicating future cardiovascular events and vascular calcification (VC) is known to be associated with coronary artery disease (CAD) in dialysis patients. The aim of the present study was to determine the possible interrelationship between LV diastolic dysfunction by tissue Doppler imaging (TDI) and vascular calcification on plain radiographs in dialysis patients. We recruited 36 hemodialysis (HD) patients and 22 peritoneal dialysis (PD) patients. We checked the plain radiographic films of the feet, hands, pelvis and lateral lumbar spine and evaluated VC scores with previously reported methods. Pulse-wave Doppler examination of mitral inflow and TDI of the lateral mitral annulus was performed. The ratio of the mitral inflow early diastolic filling velocity (E) to the mitral annular early diastolic velocity measured by tissue Doppler (E') was used as an estimation of mean left atrial pressure (E/E'). The mean age of the patients was 55.7±10.4 years, dialysis duration was 40.5±29.5 months and E/E' was 11.0±4.0. The mean age, dialysis duration, the prevalence of VC on plain radiographs, E/E' and cardiac valvular calcifications were not significantly different in HD patients compared to PD patients. Patients who showed the abdominal aortic calcifications (AACs) scores >5 or VC scores of the pelvis and hands >3 had higher E/E' than patients without significant VC in dialysis patients. Positive associations were found between AACs scores >5 (r=0.371, p=0.009) , VC scores of the pelvis and hands >3 (r=0.412, p=0.004), CAD (r=0.305, p=0.037), left atrial volume (r=0.635, p<0.001), CRP (r=0.318, p=0.030) and E/E' in dialysis patients. Positive associations were found between AACs scores >5, E/E' (r=0.547, p=0.028) and cardiac valvular calcification in PD patients.These results suggest that diastolic LV dysfunction may be associated with VC on plain radiographs in dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1474**

**The Relationship of the Course of Depression Symptoms with the Left Ventricular Mass Index and Left Ventricular Filling Pressure in Chronic Hemodialysis Patients** Biro Kim,[1] Yongkyun Kim,[2] Jihee Lim,[3] Minyoung Kim,[3] Cheolwhee Park,[3] Ho-Cheol Song,[2] Yongsoo Kim,[3] Euy-Jin Choi,[2] Yoonsik Chang,[1] Bumsoon Choi.[3] *[1]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea, Yeouido, St. Mary's Hospital, Republic of Korea; [2]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea, Bucheon, St. Mary's Hospital, Republic of Korea; [3]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea, Seoul, St.Mary's Hospital, Republic of Korea.*

*Introduction*: Multiple measurements of depression symptoms were more predictive of cardiovascular mortality than a single time measurement performed at baseline. The aim of this study is to evaluate the association of the course of depression symptoms, based on repeated assessments of depression symptoms over time, with left ventricular mass index (LVMI) and left ventricular filling pressure (LVFP) in patients on hemodialysis (HD).

*Methods*: Sixty-one patients on HD were prospectively assessed the level of depression symptoms using the Beck Depression Inventory (BDI) at baseline and three intervals (5, 10, 15 months). Doppler echocardiographic examinations were performed at the end of follow-up.

*Results*: At the end of follow-up, the patients were divided into three groups according to their course of depression symptoms: the non-depression (n = 21), the intermittent depression (n = 23), and the persistent depression symptoms group (n = 17). LVMI and LVFP were significantly increased in the persistent depression symptoms group compared to those of the non-depression symptoms group and the intermittent depression symptoms group. Persistent depression symptoms was independently associated with LVMI (β-coefficient = 0.347, $P$ = 0.017) and LVFP (β-coefficient = 0.274, $P$ = 0.048) after adjustment for age, gender, systolic blood pressure, diastolic blood pressure, diabetes and interdialytic weight gain.

*Conclusions*: In our study, persistent depression symptoms were associated with LV hypertrophy and diastolic dysfunction. Our data may provide a more complete understanding of cardiovascular risk associated with depression symptoms in the patients on HD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1475**

**Serum Levels of proANP and Albumin Are Independent Predictors of Mortality in the High Risk Patients (Elderly and Diabetics) Treated by Hemodialysis (HD) and Continuous Peritoneal Dialysis (CAPD) in 4 Year Prospective Observation** Madziarska Katarzyna,[1] Waclaw Weyde,[1] Katarzyna Gosek,[2] Waclaw Kopec,[1] Jozef Penar,[1] Magdalena Krajewska,[1] Mariusz Kusztal,[1] Tomasz Golebiowski,[1] Ewa Zukowska-Szczechowska,[2] Marian Klinger.[1] *[1]Nephrology Transplantation Medicine, Wroclaw Medical University, Wroclaw, Poland; [2]Diabetology Nephrology, Medical University of Silesia, Zabrze.*

The undecided issue remains what type of dialysis (HD or CAPD) offers the long-term survival benefit in the highest risk of ESRD patients (pts). The aim of study was to compare survival in groups of 86 pts consisted of diabetics and elderly (>70 yrs) dependent on modality of dialysis (HD–46 pts, CAPD–40 pts; 41F, 45 M). The second goal was to identify the factors independently affecting the survival in 4 yrs prospective observation. The clinical pts characteristics: median age 73.5 yrs, 71% diabetics, 59% elderly, 30% of pts with both risk factors, median time on dialysis 17 months, and residual diuresis 550ml. There were no differences between HD and CAPD pts in these features. However, the distinctive element between HD and CAPD subgroups were lower albumin concentration ($p<0.002$) and higher cholesterol level ($p<0.001$) in CAPD pts. In addition, the following laboratory parameters were determined in all patients: pro-ANP, NT–proBNP, CRP and IL-6. All standard indices of dialysis care (BP, HGB, BMI, Kt/V) were also analysed. In prospective observation 53 pts (62%) survived 20 months, and 27 pts 50 months (last point of the observation). No effect of dialysis modality was discerned. In univariate linear model the survival was significantly associated with elevated CRP ($p= 0.05$), higher pro-ANP ($p= 0.005$), and lower albumin level ($p= 0.013$). The effect of higher pro-ANP ($p= 0.018$), and lower albumin ($p= 0.013$) was left as independent variable in the Cox regression model.

Conclusion: In high risk cohort of prevalent dialysis patients the type of dialysis modality does not influence the poor survival in 4 year prospective observation. The independent negative prognostic factors are reduced albumin level, and as the novel finding higher plasma pro-ANP reflecting volume status and cardiac performance.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1476**

**Prevalence of Chronic Atrial Fibrillation in Dually-Eligible Dialysis Patients** James B. Wetmore,[1] Edward F. Ellerbeck,[2] Sally K. Rigler,[3] Jonathan D. Mahnken,[5] Qingjiang Hou,[5] John Spertus,[4] Theresa I. Shireman.[2] *[1]Medicine, Division of Nephrology, University of Kansas Medical Center, Kansas City, KS; [2]Preventive Medicine and Public Health, University of Kansas Medical Center, Kansas City, KS; [3]Medicine, University of Kansas Medical Center, Kansas City, KS; [4]St Luke's Mid American Heart Institute, University of Missouri - Kansas City, Kansas City, MO; [5]Biostatistics, University of Kansas Medical Center, Kansas City, KS.*

A rigorous claims-based approach for identifcation of chronc atrial fibrillation (CAF) in dually-eligible (Medicare-Medicaid) chronic dialysis patients has never been undertaken. We performed a retrospective cohort analysis of 123,615 dually-eligible patients undergoing chronic dialysis between January 1, 2001 and December 31, 2002. To be classified as having CAF, individuals had to have at least 3 AF Medicare claims (at least 2 of which were outpatient claims) a minimum of 30 days apart. Claims likely to represent transient AF or to be attributable to valvular heart disease or hyperthyroidism were eliminated.

The 2-year period prevalence of CAF was approximately 5.5 %, ranging from 3.6% in females aged 55-59 years to 13.2% in males over age 80. In multivariable analysis, age (adjusted prevalence rate ratio [APRR] 1.57 per decade), male sex (1.21), Caucasian race (approximately 2.0 relative to other groups), coronary artery disease (1.22), and congestive heart failure (1.61) were significantly associated with increased prevalence of CAF (all $p < 0.0001$). Hypertension, however, was associated with significantly lower likelihood of CAF (0.89, $p = 0.006$).

The 2-year period prevalence rate of CAF in dually-eligible dialysis patients is approximately 5.5%. This estimate is lower than typically reported, probably because we employed a strategy to eliminate individuals likely to have transient or secondary causes of AF. Nevertheless, this rate is substantially higher than that of the general population across all strata of age, demonstrating that CAF is a major problem in dialysis patients. The apparent "paradoxical" association of hypertension with a lower rate of CAF must be investigated.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1477**

**Severity of Heart Failure Predicts Outcomes and Health-Related Quality of Life in Maintenance Hemodialysis Patients in the HEMO Study** Kelly V. Liang,[1] Francis Pike,[2] Christos Argyropoulos,[1] Lisa Weissfeld,[2] Jeffrey Teuteberg,[3] Mary Amanda Dew,[4] Mark L. Unruh.[1] *[1]Renal-Electrolyte, University of Pittsburgh, Pittsburgh, PA; [2]Biostatistics, University of Pittsburgh, Pittsburgh, PA; [3]Cardiology, University of Pittsburgh, Pittsburgh, PA; [4]Psychiatry, Psychology, & Epidemiology, University of Pittsburgh, Pittsburgh, PA.*

**Background:** Cardiac disease is the leading cause of death among United States prevalent hemodialysis (HD) patients. We assessed the extent to which severity of congestive heart failure (CHF) influences morbidity and mortality, and health-related quality of life (HRQOL), in HD patients.

**Methods:** Using the database from the HEMO Study, which randomized 1846 HD patients to either high or low dose and flux interventions, we examined the relationship between CHF severity and mortality with Cox proportional hazards models. The relationship between CHF severity and HRQOL scores were modeled via linear regression.

**Results:** CHF was present in 40% of the total HD patients. Increasing severity of CHF was associated with greater age, higher proportion with diabetes, and lower serum albumin levels (all $p <0.0001$). Unadjusted hazard ratios for all-cause mortality were 1.61, 2.05, and 2.73 for mild, moderate, and severe CHF, respectively ($p <0.0001$). All-cause, cardiac, and infectious mortality and cardiac hospitalizations all increased with increasing severity of CHF. Increasing CHF severity was associated with decrements in HRQOL indices, particularly in physical functioning, and sleep quality.

**Conclusions:** Increasing severity of CHF is associated with increased mortality, cardiac hospitalizations, and worse HRQOL, especially in perceived physical limitations. These findings underscore both the need to assess disease severity and the importance of treatment to prevent progression of CHF in the HD population.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1478**

**Differences in Serious Adverse Event Reporting Rates from IV Iron by Country** George R. Bailie,[1] Walter H. Hoerl,[2] Jan-Jaap Verhoef.[3] *[1]Albany College of Pharmacy & Health Sciences, Albany, NY; [2]Medical University of Vienna, Austria; [3]University of Utrecht, Netherlands.*

Adverse event (AE) reporting varies between Europe (Eur) and N. America (NA). We examined serious AE (SAE) by population exposed to IV iron (Fe) products.

Methods: We extracted SAE (anaphylaxis/oid) from IV Fe AE reports in Eur and NA between 1Q03 to 2Q09 from Uppsala Monitoring Center. IMS Health Fe sales data were standardized to 100 mg dose equivalents. Fe use reported as gram of Fe/million population/yr (GFe/mil/y) using census data (www.prb.org). SAE rates derived as total # SAE/GFe/mil/y.

Results: Iron sucrose (IS) was used by all countries, ferric gluconate (FG) by 8, low MW iron dextran (ID) by 12, IS similars (ISS) by 3. For countries using a product, use ranged hugely: for IS from 1 (Ire) to 5349 (Swi); FG from 0.2 (Pol) to 7488 (Ita, where some use of FG is oral); ID from 0.1 (Swi) to 256 (Ire). Many countries under-reported SAE despite

much Fe use (Table). ISS SAE were not separated from IS. SAE rates ranged from $1.7x10^{-3}$ (IS, Swe) to $305x10^{-3}$ (ID, UK). IS and FG had similar SAE rates in Eur and NA but FG rate was much higher in NA. Greatest SAE rates were for ID in Eur and NA.

IVFe use and serious AE

| | IS | | ISS | FG | | ID | |
|---|---|---|---|---|---|---|---|
| Eur | Fe use | SAE # (rate)* | Fe use | Fe use | SAE # (rate) | Fe use | SAE # (rate) |
| Fra | 1870 | 9 (4.8) | 37 | | | | |
| Ger | 231 | 4 (17.3) | | 1990 | 6 (3.0) | 119 | 26 (219.2) |
| Ita | 21 | | | 7488 | 26 (3.5) | 11 | |
| Pol | 478 | | | | | 120 | 1 (8.3) |
| Spa | 1875 | | 334 | 3.0 | | 39 | |
| Swe | 1751 | 3 (1.7) | | | | 51 | 3 (59.0) |
| Swi | 5349 | 53 (9.9) | | | | | |
| Tur | 1572 | 9 (5.7) | 155 | | | 175 | 1 (5.7) |
| UK | 929 | 12 (12.9) | | | | 79 | 24 (305.3) |
| All Eur (16 countries) | 17475 | 92 (5.3) | 526 | 11176 | 34 (3.0) | 866 | 61 (70.4) |
| NA | | | | | | | |
| US | 2351 | 31 (13.2) | | 1313 | 90 (68.6) | 243 | 108 (444.1) |
| Can | 196 | 3 (15.3) | | 130 | 2 (15.4) | 367 | 23 (62.7) |
| All NA | 2547 | 34 (13.3) | | 1443 | 92 (63.8) | 610 | 131 (214.8) |
| **All Eur + NA** | **20022** | **126 (6.3)** | | **12618** | **126 (10.0)** | **1476** | **192 (130.1)** |

*# for total period; rate is $x10^{-3}$

Conclusions: Inter-country SAE reporting rates vary drastically; may be due to IV Fe use in different therapeutic areas or clinical settings, or varied reporting procedures. Generally, SAE rates were highest for ID and lowest for IS.

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### F-PO1479

**Worsening Severity of Peripheral Vascular Disease Associated with Poor Outcomes in Hemodialysis: Findings from the HEMO Study** Patrick Ifesinachi Emelife, Tao Liu, Lindsay J. Proud, Alan J. Rosenbaum, Ryan M. Stephenson, Raymond J. Vergona, Mark L. Unruh. *University of Pittsburgh.*

Background: The contribution of peripheral vascular disease (PVD) to the morbidity and mortality of patients undergoing maintenance hemodialysis remains largely unexplored. This study aimsto describe the distribution of PVD according to its severity and to assess the risk factors associated with increased severity of PVD. Additionally, our study assessed the extent to which PVD was associated with health-related quality of life and long-term survival among a prevalent hemodialysis population.

Methods: Secondary data analysis was performed on 1846 prevalent hemodialysis patients from the HEMOStudy. PVD severity, as defined by the Index of Co-Existent Disease (ICED), was the exposure variable. The relationship between PVD and selected demographic, clinical and laboratory values was examined. Primary outcomes included health-related quality of life as measured by the SF36 and KDQOL Sleep Quality Index as well as all-cause mortality.

Results: The relative frequency of PVD severity was 1372 without PVD (73.6%), 150 with mild PVD (8.1%), 221 with moderate PVD (12.0%), and 103 with severe PVD (6.0%). Factors associated with more severe PVD included older age (P<0.0001), white race (P<0.0001), smoking (P=0.03), drug use (P=0.01), disability (P=0.019), diabetes (P<0.0001), ischemic heart disease (P<0.0001) cerebrovascular disease (P<0.0001), and higher diastolic blood pressure (P<0.0001). The severity of PVD was associated with decreased Physical Function , Role Physical, General Health, Vitality, and Social Function (all p< 0.01). Increased PVD severity was strongly associated with an increased risk of death compared to no PVD; mild PVD HR 1.3; 95% CI (1.03, 1.63), moderate PVD HR 1.35; 95% CI (1.1,1.64), and severe PVD 1.45; 95% CI (1.12,1.87) after adjustment for demographic and clinical variables.

Conclusions: Since severe PVD is a commonly observed in hemodialysis patients, our study identified modifiable risk factors that predisposed patients to PVD. These findings emphasize the need for evaluation of the benefits of more aggressive treatment for PVD in patients on hemodialysis.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1480

**Chronic HCV Infection Independently Increased Mortality Risks for Cardiovascular and Liver Disease-Related Death in Hemodialysis Patients, While Past HCV Infection Did Not** Masaki Ohsawa,[#1] Yosuke Fujishima.[#2] *[1]Hygiene and Preventive Medicine, Iwate Medical University, Morioka, Japan; [2]Department of Urology, Iwate Medical University, Morioka, Japan.*

Whether past hepatitis C virus (HCV) infection or chronic HCV infection contributes to an increase in mortality among hemodialysis patients (HD pts) has not been clarified. A total of 1214 HD pts were enrolled. Numbers of deaths (total death (TD), cardiovascular death (CVD), infectious disease-related death (ID) and liver disease-related death (LD)) and crude and age-adjusted (based on WHO standard population) mortality rates (/1000 patient-years) were estimated in HCV infection status groups (never, HCV antibody negative; past infection, HCV antibody positive and HCV antigen negative; chronic infection, HCV antigen positive), and multivariate-adjusted hazard ratios (HRs) and their 95% confidence intervals (95% CIs) for death attributable to past HCV infection or chronic HCV infection were estimated using Cox's regression model after adjustment for risk factors. The observed patient-years were 4734 patient-years. Mean follow-up period was 3.9 years. A total of 457 patients died during the five-year observation period. Numbers of deaths, crude and age-adjusted mortality rates and HRs (95% CIs) for each cause of death among HD pts are shown in the table.

Number of death, crude and age-adjusted mortality rates and hazard ratios (95%CIs) by groups according to HCV infection status

| Cause of death | HCV status | Number of death | crude mortality | age-adjusted mortality | HR (95%CI) |
|---|---|---|---|---|---|
| TD | never (n=1080) | 393 | 91.4 | 31.2 | 1 |
| | past (n=55) | 25 | 123.0 | 36.1 | 1.73 (1.14-2.64) |
| | chronic (n=79) | 39 | 137.2 | 83.1 | 2.21 (1.56-3.12) |
| CVD | never | 190 | 44.2 | 16.7 | 1 |
| | past | 9 | 44.3 | 12.3 | 1.22 (0.60-2.50) |
| | chronic | 17 | 59.8 | 23.3 | 2.29 (1.16-4.53) |
| ID | never | 103 | 23.9 | 6.4 | 1 |
| | past | 8 | 39.4 | 9.5 | 2.48 (1.17-5.29) |
| | chronic | 10 | 35.2 | 14.4 | 2.29 (1.16-4.53) |
| LD | never | 5 | 1.2 | 0.3 | 1 |
| | past | 1 | 4.9 | 1.0 | 2.77 (0.27-28.9) |
| | chronic | 4 | 14.1 | 32.3 | 11.5 (2.31-56.9) |

Chronic HCV infection independently increased mortality risks for TD, CVD, ID and LD among hemodialysis patients, while past HCV infection did not increase risks for CVD and LD.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1481

**The Relation between Glutathione S-Transferase M1 Polymorphism and the Clinical Outcome of Chinese PD Patients** Peter Yam-Kau Poon,[1] Cheuk-Chun Szeto,[1] Bonnie Kwan,[1] Kai Ming Chow,[2] Philip K. T. Li.[2] *[1]Department of Medicine & Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong; [2]Department of Medicine & Therapeutics, Prince of Wales Hospital, Shatin, Hong Kong.*

Objective To investigate the effect of glutathione S-transferase M1 (GST M1) gene (-)/(+) polymorphism on the survival of patients undergoing peritoneal dialysis (PD). Methods We studied 441 new PD patients (232 males, mean age 56.6 ± 13.5 years). GST M1 gene (-)/(+) genotyping was determined by multiplex polymerase chain reaction (PCR) using primer pairs for both GST M1 gene and β-globulin gene. All patients were then followed for an average of 41.4 ± 18.2 months for survival study. Results The 5-year actuarial survival of patients with GST M1(-) (N = 267) and GST M1(+) (N = 174) genotype are 51.4% and 69.1% respectively (log rank test, p = 0.017). The 5-year technique survival of GST M1(-) and GST M1(+) genotypes were 39.0% and 53.6% respectively (p = 0.051). Multivariate Cox regression analysis showed that only total Kt/V, Charlson's comorbidity index and the presence of pre-existing diabetes are the independent predictors of actuarial survival, while the GST genotype did not appear in the final model. However, for patients ages >70 years, GST M1(+) genotype (N = 30) had significantly better actuarial survival (62.5% vs 26.2%, p = 0.012) and technique survival (55.1% vs 21.9%, p = 0.024) than GST M1(-) genotype (N = 56). Multivariate analysis showed that the GST genotype was an independent predictor of actuarial survival (adjusted hazard ratio [AHR] 0.243, 95% confidence interval [CI] 0.063 – 0.944, p = 0.041) and technique survival (AHR 0.243, 95%CI 0.063 – 0.944, p = 0.041). In patients <70 years, the GST genotype had no effect on actuarial or technique survival. Conclusion GST M1(+) genotype apparently confers a survival advantage to elderly PD patients. Since both natural aging and the process of PD result in the accumulation of oxidative stress, it is possible that the presence of anti-oxidative enzyme GST could have a protective effect on elderly PD patients, but the detailed mechanism deserves further investigation.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1482

**The Relation between Human Oxoguanine-DNA Glycosylase-1 Polymorphism and the Clinical Outcome of Chinese PD Patients** Peter Yam-Kau Poon,[1] Cheuk-Chun Szeto,[1] Bonnie Kwan,[1] Kai Ming Chow,[2] Philip K. T. Li.[2] *[1]Department of Medicine & Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong; [2]Department of Medicine & Therapeutics, Prince of Wales Hospital, Shatin, Hong Kong.*

Objective To investigate the effect of human oxoguanine-DNA glycosylase 1 (hOGG1) gene C1245G polymorphism on the survival of patients undergoing peritoneal dialysis (PD). Methods We studied 441 new PD subjects (232 males, mean age 56.6 ± 13.5 years). hOGG1 C1245G genotyping was determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). All patients were then followed for an average of 41.4 ± 18.2 months for cardiovascular events. Results For the entire cohort, there was no significant difference in the 5-year event-free survival between CC (N = 73) and CG+GG (N = 368) genotypes (42.9% vs 33.2% respectively; log rank test, p = 0.120). However, for patients with baseline serum C-reactive protein (CRP) levels ≤5.0 mg/L, 5-year event-free survival of CC patients (N = 40) were significantly higher than that of CG+GG patients (N = 200) (50.3% vs 31.9% respectively, p = 0.046). Multivariate Cox regression analysis showed that both the hOGG1 genotype (adjusted hazard ratio [AHR] 3.989; 95% confidence interval [CI] 1.445 to 11.013; p = 0.008) and Charlson's comorbidity index (AHR 1.266; 95%CI 1.127 to 1.422; p < 0.001) were independent predictors of the 5-year event-free survival. In contrast, there was no significant difference in 5-year event-free survival between the CC (N = 10) and CG+GG genotypes (N = 49) for patients with baseline CRP >5.0 mg/L (26.7% vs 30.2% respectively, p = 0.9). Conclusions In PD patients with no systemic inflammation, hOGG1 1245CC genotype had a lower risk of cardiovascular event than those with CG or GG genotypes. The protective effect of hOGG1 1245CC genotype,

however, disappeared in PD patients with feature of systemic inflammation. Our results suggest a complex interaction between the enzyme hOGG1, which has a DNA repairing activity, and the systemic inflammatory state in the pathogenesis of cardiovascular disease of PD patients.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1483

**Study of the Relationship between Fluid Overload and Autonomic Response during Hemodyalisis** Manuela Ferrario,[1] Ulrich Moissl,[2] Francesco Garzotto,[3] Maria Gabriella Signorini,[1] Dinna N. Cruz,[3] Flavio Basso,[3] Alessandra Brendolan,[3] Federico Nalesso,[3] Ciro Tetta,[2] Sergio Cerutti,[1] Claudio Ronco.[3] *[1]Politecnico di Milano, Milano, Italy; [2]Fresenius Medical Care Deutschland GmbH, Germany; [3]San Bortolo Hospital, Vicenza, Italy.*

**Background.** Fluid overload (FO) is an important and independent predictor of mortality in chronic hemodialysis (HD) patients [Wizemann V. et al., 2009]. The aim of this study was to investigate the relationship between FO and the autonomic response to the HD, estimated from Heart rate variability (HRV) .

**Methods.** 80 patients were recruited from the dialysis unit of San Bortolo Hospital, Vicenza, Italy. 24 hr ECG Holter recordings were performed once for each patient starting before the HD treatment, immediately after fluid overload ($FO_{pre}$) was assessed with a whole body bioimpedance spectroscopy device (BCM, Fresenius Medical Care, Germany). Heart rate variability (HRV) was analyzed with time domain parameters and frequency domain indices [Task Force, Circulation, 1996]. Mean values of time and frequency domain indices of HRV were calculated for the first 30 minutes and last 30 minutes of HD, and for the entire HD session [Task Force, Circulation, 1996].

**Results.**

Table 1: Pearson Coefficients (HRV indices vs FOpre/ECW%)

| | all pts | diabetic pts | non diabetic pts |
|---|---|---|---|
| **SDANN** | rho= -0.40 Pval=0.002 | rho=-0.16, Pval= 0.552 | rho=-0.51 Pval=0.001 |
| **VLF** | rho=-0.31 Pval=0.020 | rho=-0.28, Pval= 0.302 | rho=-0.39, Pval=0.015 |
| **HF%** | rho= +0.33 Pval=0.012 | rho=+0.56, Pval=0.024 | rho=+0.30, Pval=0.062 |

SDANN estimated over the entire HD was significantly different between overhydrated (FO>2.5l) and non-overhydrated patients (FO<=2.5L) both for D and ND pts (T-test p-value<0.05).

**Conclusion.** These results for the first time demonstrate that there is a link between fluid status and autonomic function. Heart rate variability as a marker of autonomic function significantly changes with rising fluid overload. Diabetic and non-diabetic patients present different correlations between FO and HRV indices.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1484

**The Effects of Long-Term Administration of Cinacalcet on Left Ventricular Function in Hemodialysis (HD) Patients** Jyunichiro Hashiguchi,[1] Miwa Shirahama,[1] Masao Aoyagi,[1] Yoshiaki Lee,[1] Takashi Harada,[1] Satoshi Funakoshi,[1] Mineaki Kitamura,[2] Akira Furusu,[2] Shigeru Kohno.[2] *[1]Dialysis Center, Sakuramachi Clinic, Nagasaki, Japan; [2]Department of Internal Medicine, Nagasaki University Graduate School of Medicine, Nagasaki, Japan; [3]Department of Diabetis, Jikei University, Tokyo, Japan.*

Background; The expression of the calcium-sensing receptor (CaSR) on heart muscle and vascular cells has been well-documented, and calcimimetics have become a potential candidate for cardiovascular intervention. Purpose; To evaluate the effects of cinacalcet on cardiac function when administered over a long term period of 5 years. Subjects and Methods; From April 2004 to March 2010, 12 HD outpatients with secondary hyperparathyroidism were enrolled in this study after appropriate IC, and were monitored using various parameters including yearly examined ultrasound cardiography over > 5 years from the start of cinacalcet administration. There were two groups; Group 1 continuing cinacalcet administration for > 5years (n=7), group 2 stopped cinacalcet administration < 1 year due to poor compliance (n=5). Results; There was no significant difference between the two groups in age, interdialytic weight gain (IWG) or HD vintage. As shown in Table 1, significant suppression was observed in the tei index and the LVDd, while an increase was observed in the LVPW of the group that continued administration of cinacalcet > 5 years.

Change in the left ventricular parameters by cinacalcet treatment in 5 years (%)

| | group 1 | group 2 | p value |
|---|---|---|---|
| average ΔEF | -4.5±4.8 | -5.5±6.1 | NS |
| average Δtei index | -18.5±9.9 | +13.7±12.8 | 0.024 |
| average ΔLVDd | -4.3±3.9 | +8.8±7.9 | 0.041 |
| average ΔLVPW | +26.8±9.0 | +2.8±7.5 | 0.0019 |

Conclusions; Our results indicate the potential effects of cinacalcet on protecting left ventricular function in HD patients.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1485

**Undiagnosed Renal Artery Stenosis in Patients Evaluated for Kidney Transplant** Samer Bani-Hani,[1] Vinaya Rao,[2,4] Min Min Mya,[3] Ahmed I. Al-Absi,[4] Maung Mya.[2,4] *[1]Int. Med, UTHSC, Memphis; [2]Transplant Institute, Methodist University Hospital, Memphis; [3]EMCare, Memphis; [4]Nephrology, University of Tennessee Health Science Center, Memphis.*

Back ground: Renal artery stenosis(RAS) accounts for only 0.8% of primary diagnoses as the cause of prevalent ESRD in 2007. RAS is likely underdiagnosed as physicians are hesitant to use contrast agents in patients(pts) with advanced chronic kidney disease.

Objective: To examine the prevalence of undiagnosed RAS in ESRD pts evaluated for renal transplant.

Method: Retrospective analysis of CT angiogram(CTA) in pts evaluated for renal transplant in a single center.

Results: There were 483 kidney transplant recipients from Jan, 2005 to Oct,2009 who had CTA as part of their pretransplant evaluations in our center. Two pts who had stent placement for RAS before the pretransplant evaluation were excluded from the study. Pts' demographics were: mean age 48 y, male 302, female 179, white 152, African American 317 and other races 12. RAS was present in 14 (2.9%) pts. All pts had hypertension. RAS was significantly associated with age and peripheral vascular disease(PVD). Contrary to our expectation, RAS was not significantly associated with diabetes, hyperlipidemia and history of cerebrovascular accidents(CVA).

| | **RAS**: yes | **RAS**: No | **P** |
|---|---|---|---|
| | N (%) | N(%) | |
| **Total**: 481 | 14 (2.9) | 467 (97.1) | |
| **Age** | 55.85 | 47.82 | 0.004 |
| **Gender**: Male | 8 (57.1) | 294 (63) | 0.658 |
| Female | 6 (42.9) | 173 (37) | |
| **Race**: white | 5 (35.7) | 147 (31.5) | 0.801 |
| Black | 9 (64.3) | 308 (65.9) | |
| other | 0 (0) | 12 (2.6) | |
| **Diabetes**: Yes | 5 (35.7) | 142 (30.4) | 0.671 |
| No | 9 (64.3) | 325 (69.6) | |
| **Hyperlipidemia**: Yes | 6 (42.8) | 158 (33.8) | 0.483 |
| No | 8 (57.2) | 309 (66.2) | |
| **PVD**: Yes | 1 (7.1) | 5 (1.1) | 0.044 |
| No | 13 (92.9) | 462 (98.9) | |
| **CVA**:Yes | 0 (0) | 23 (4.9) | 0.137 |
| No | 14 (100) | 444 (95.1) | |

Conclusion. Undiagnosed RAS was present in 2.9% of pts evaluated for renal transplant. RAS was significantly associated with age and PVD but not with diabetes, hyperlipidemia and history of CVA.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1486

**Effect of Carnitine Supplementation on Carnitine Levels and Left Ventricular Performance in Children on Chronic Hemodialysis** Kristen Sgambat, Ahmad Ellini, Lowell Frank, Craig Sable, Asha Moudgil. *Nephrology and Cardiology, Children National Medical Center, Washington, DC.*

**Background:** Carnitine is essential for transport of fatty acids into mitochondria and plays a key role in beta-oxidation and energy production in the myocardium. Carnitine deficiency may occur in patients on chronic hemodialysis (HD) due to its removal by dialysis and inadequate dietary intake; this may contribute to cardiomyopathy. We assessed carnitine levels and cardiac response to carnitine supplementation by standard echocardiography including more sensitive parameters including left ventricular (LV) strain and strain rate.

**Methods**: The carnitine levels (total and free carnitine and acyl: free carntine ratio) and cardiac function of 8 children (mean age 12.9 years, range 9 – 17 years) on chronic HD were assessed before and after carnitine (20mg/kg/dose) infusion 3 times a week for 6 months. Standard echocardiographic measures of LV size, systolic and diastolic function as well as circumferential, radial, and longitudinal strain and strain rate analysis using speckle tracking were performed.

**Results**: Total (50 ± 36 vs. 267 ± 36 μmol/l) and free carnitine (29 ± 23 vs. 160 ± 23 μmol/l) levels increased significantly ($p < 0.001$), whereas acyl:free ratio remained unchanged (0.72 ± 0.14 vs. 0.69 ± 0.14) after carnitine supplementation. There were no significant changes in standard echocardiographic measures of LV function including end diastolic dimension, mass index, ejection fraction, shortening fraction and mitral E/A and E/E' ratios after carnitine supplementation. However, there was a significant ($p = 0.025$) improvement in longitudinal strain rate (-1.46 ± 0.33 vs. -1.91 ± 0.37) after supplementation. This difference remained significant ($p = 0.013$)after controlling for interdialytic weight gain and blood pressure. There were no significant differences in other strain/ strain rate parameters.

**Conclusion:** Carnitine supplementation improved total and free carnitine levels in children on chronic HD without affecting acyl:free carnitine ratio. LV performance improved after supplementation as assessed by strain rate analysis that was not obvious by standard echocardiographic measures.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1487**

**Ankle Brachial Index, Vascular Calcifications and Mortality in Dialysis Patients** Patricia Quadros Branco,[1] Teresa Adragao,[2] Ana Pires,[3] Rui Castro.[4] *[1]SPD Diaverum, Portugal; [2]NCE Diaverum, Portugal; [3]NC LAV Diaverum, Portugal; [4]Tecsam Vila Real, Portugal.*

The ankle brachial index (ABI) is a non-invasive method to evaluate peripheral artery disease (PAD). ABI<0.9 diagnoses PAD; ABI>1.3 is a false-negative caused by non-compressible arteries. The aim of this study was to evaluate the association between ABI with vascular calcifications (VC) and with mortality, in hemodialysis (HD) pts.

We studied 219 HD pts (60% male; 20% diabetic). At baseline, ABI was evaluated by a doppler device. VC were evaluated by 2 methods: the abdominal aorta calcification score (AACS) in a lateral plain X-ray of the abdominal aorta and the simple vascular CS (SVCS) in plain X-ray of pelvis and hands. VC were also classified by their anatomical localization in main vessels (aorta, iliac-femoral) and in collateral or distal vessels (pelvic , radial or digital) The cut-off values for the different VC scores in relation with ABI were determined by ROC curve analysis. Biochemical parameters were time averaged for the 6 months preceding ABI evaluation.

An ABI <0.9, an ABI >1.3 or a normal ABI were found, respectively in 90 (41%), in 42 (19%) and in 87 (40%) pts. AACS >6 and SVCS >3 were found, respectively, in 98 (45%) and 95 (43%) pts. The adjusted risk for having an ABI<0.9 was 2.9 (p≤0.001) for AACS>6; 3.5 (p≤0.001) for SVCS>3 and 4.4(p<0.001) for iliac-femoral CS>3. The adjusted risk for having an ABI>1.3 was 3.4 (p=0.001) for hands CS >2 and 3.5 (p=0.001) for pelvic CS >2. After a follow-up of 29±7 months, 50 (23%) pts died. Adjusting for age, diabetes and HD duration, an ABI<0.9 and an ABI>1.3 were associated with mortality (HR=3.4; p=0.001 and HR=2.9; p=0.029, respectively).

In conclusion, both low and high ABI were independent predictors of mortality. VC in main arteries were associated with an ABI<0.9. VC in collateral and distal arteries were associated with an ABI>1.3. The hypothesis that the correction of factors associated with development of vascular calcifications might have an impact on PAD outcomes needs to be evaluated.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1488**

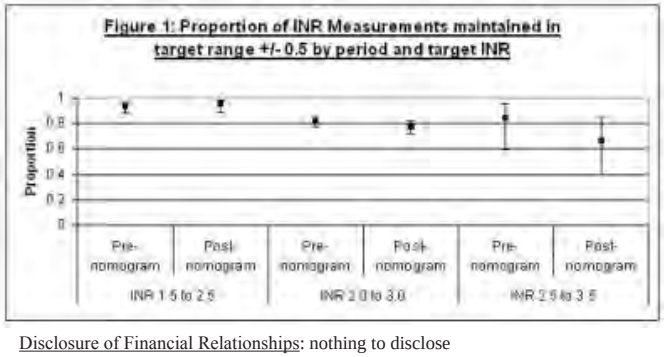
**Evaluation of a Warfarin Nomogram for Anticoagulation in Hemodialysis Patients** Benjamin Ka Thomson, Jianguo Zhang, Jennifer M. MacRae, Megan Alicia Manning, Brenda Hemmelgarn. *Internal Medicine, Division of Nephrology, Department of Internal Medicine, Calgary, AB, Canada.*

Introduction: Outpatient warfarin nomograms to guide warfarin dosing have been shown to improve INR control, compared to physician directed therapy. The effectiveness of these nomograms in the hemodialysis population is unknown. The purpose of this study was to evaluate the adequacy of anticoagulation using an electronic warfarin nomogram administered by nurses in the outpatient hemodialysis population, compared to physician directed therapy.

Methods: The study population included all patients on hemodialysis treated with warfarin in Calgary Alberta. Two five-month time periods were compared: a pre-period prior to implementation of the nomogram and a post-period following implementation of the nurse directed, electronic warfarin nomogram. The primary endpoint was adequacy of anticoagulation (proportion of INR measurements within target 0.5). Secondary outcomes included frequency of INR testing and adverse events.

Results: Overall 67 patients in the pre- period and 55 in the post-period were included (with 40 patients in both periods). Using generalized linear mixed models, the adequacy of INR control was similar in both periods, for all target INR levels: target INR 1.5 to 2.5 (pre 93.6%; post 95.6%, p=0.953); INR 2.0 to 3.0 (pre 82.2%; post 77.4%, p=0.202) and; INR 2.5 to 3.5 (pre 84.3%; post 66.8%, p=0.287).The mean number of INR measurements per patient decreased significantly from pre (30.5) to post (22.3) (p=0.003). There were 3 bleeding events in each of the pre and post periods.

Limitations and Conclusions: Our study was limited by its single centre location and pre-post design. Our results suggest that a nurse directed electronic warfarin anticoagulation nomogram achieves adequacy of INR control similar to that of physician directed therapy among hemodialysis patients.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1489**

**Leptin and Volume Status in Hemodialysis Patients: Preliminary Data** Sejoong Kim,[1] Eun Sook Jung,[2] Hayne C. Park,[2] Hajeong Lee,[2] Jiyoon Sung,[1] Sun Young Na,[1] Ho Jun Chin,[2] Jin Suk Han,[2] Kwon Wook Joo.[2] *[1]Internal Medicine, Gachon University of Medicine and Science, Incheon, Korea; [2]Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.*

*Background:* Adipokines are related to cardiovascular outcome in hemodialysis (HD) patients. Both fluid overload and dehydration are also linked to an increased cardiovascular morbidity in HD patients. However, the association between adipokines and volume status has not been investigated yet.

*Methods:* We enrolled 120 patients who received more than 3-month hemodialysis in major 3 dialysis centers. According to the amount of fluid overload, which was provided by the body composition moniter (BCM, Fresenius Medical Care Korea), we divided into 3 groups: overhydrated group (fluid overload ≥ 1.1L), normohydrated group (-1.1L ≤ fluid overload < 1.1L), and dehydrated group (fluid overload < -1.1L). We also measured the adipokines (leptin, adiponectin, and resistin) and inflammation (interleukin-6, and monocyte chemotactic protein-1).

*Results:* The proportion of the overhydrated group was 36.9% (45/120), and that of the dehydrated group was 18% (22/120). Serum levels of leptin in the dehydrated group was higher, compared to the other groups ($P$ = 0.001). Serum levels of adiponectin, resistin, interleukin-6, and monocyte chemotactic protein-1 were not different among the three groups. Serum leptin levels were negatively correlated with the absolute amount of fluid overoload, controlled for age, gender, and fat contents (coefficient β=-0.427, $P$=0.007).

*Conclusion:*

We found that leptin levels may be strongly associated with volume overload in hemodialysis patients. Further prospective interventional trials could be needed to evaluate serial changes of leptin with volume status.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1490**

**Characteristics of Revascularization Treatment for Arteriosclerosis Obliterans in Patients with and without Hemodialysis** Junichi Hoshino,[1, 4] Keiichi Sumida,[1] Rikako Hiramatsu,[1] Fumi Takemoto,[2] Kunihiro Yamagata,[3] Yoshifumi Ubara.[1] *[1]Nephrology Center, Toranomon Hospital, Minato-ku, Tokyo, Japan; [2]Department of Nephrology, Jichi Medical University, Shimotsuke, Tochigi, Japan; [3]Department of Nephrology, University of Tsukuba, Tsukuba, Ibaraki, Japan; [4]Terasaki Foundation Laboratory, Los Angeles, CA.*

Background: Limb ischemia is a major complication in patients who are receiving dialysis (HD). Here we identified distinctive features and factors affecting the outcome of HD patients with limb ischemia.

Methods and Results: We retrospectively compared 180 consecutive symptomatic limb ischemic patients who were or were not receiving HD and who successfully underwent surgical bypass grafting (bypass, n=75) or endovascular angioplasty (PTA, n=105) at our hospital. The endpoint of this study was amputation of the ischemic leg or death.

Median follow-up was 2.25 years. The amputation-free survivals of HD patients (68.3%, 53.3%, and 40.0% at 1,3 and 5 years) were significantly lower than that of non-HD patients (90.9%, 84.9% and 78.0%, p<0.0001). In bypass group, amputation-free survivals of HD patients (79.3%, 44.3%, and 14.8%) were significantly lower than those of non-HD patients (90.1%, 84.7%, and 80.9%, p=0.0002), whether the graft was patent or not (p=0.32). On the other hand, in PTA group, amputation-free survivals of HD patients (all 62.1%) were lower than those of non-HD patients (91.4%, 85.1%, and 75.2%, p=0.03) with significantly lower patency rate (59.6%, 34.8%, and 23.2%, p=0.0004). Predictors of amputation-free survival differed between HD and non-HD patients; predictors were diabetes mellitus {HR(hazard ratio), 8.34[95%CI, 1.91 to 46.98]} and gender (HR, 0.19[95%CI, 0.06 to 0.63]) in HD patients, while they were Fontaine classification (HR, 6.12[95%CI, 1.99 to 15.75]) and hyperlipidemia (HR, 0.36[95%CI, 0.12 to 0.94)in non-HD patients. Infectious death rate was higher in HD patients than non-HD patients (53% vs 22%, p<0.05)

Conclusions: This study clearly showed poorer prognosis in HD patients than non-HD patients especially after bypass surgery, whether the graft was patent or not.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1491**

**Black Ethnicity Predicts Better Survival on Renal Replacement Therapy Despite Greater Deprivation and Diabetes** Satish Jayawardene, Chris Jones, Nicholas Cole, Andrew Cai, Michael Bedford, Hugh S. Cairns. *Renal Unit, King's College Hospital, London, United Kingdom.*

Background: Black ethnicity is known to carry a survival benefit on renal replacement therapy (RRT). It is unclear if this benefit is affected by deprivation, as it is linked to poorer outcome and assumed to be worse in ethnic minorities.

Aim: To describe survival of black vs white RRT patients and the effect of deprivation and other traditional risk factors for death.

Method: Retrospective analysis of the 1340 patients of white or black race starting RRT in one renal unit between 1996-2008. Deprivation was calculated matching patient postcodes to area scores of the UK Index of Multiple Deprivation 2007. Patient comorbidity and demographics were extracted from the unit database. Survival comparison between groups used Cox's Proportional Hazard models and Hazard Ratios (HR).

Results: During the time period 952 white and 388 black patients started RRT. Black patients were significantly younger than whites at RRT start, though with more diabetes (DM) and worse deprivation scores. Black race was associated with a significant survival benefit even after adjustment for age, sex, deprivation, transplantation and DM (HR 0.51, 95%CI 0.41-0.65, p<0.001).

Demographics of RRT patients (values are means & standard deviations or numbers & percentages)

| | White (952(71%)) | Black (388(29%)) | All RRT (1340(100%)) | P |
|---|---|---|---|---|
| Male Gender | 583(61%) | 229(59%) | 812(61%) | 0.45 |
| Age at RRT start | 62(16) | 54(16) | 60(16) | <0.001 |
| ESRF cause: Diabetes | 248(26%) | 132(34%) | 380(28%) | 0.003 |
| ESRF cause: Hypertension | 69(7%) | 78(20%) | 147(11%) | <0.001 |
| ESRF cause: Ischaemia | 83(9%) | 5(1%) | 88(7%) | <0.001 |
| Deprivation score (higher score = more deprived) | 24(14) | 35(11) | 28(14) | <0.001 |
| Death | 554(58%) | 137(35%) | 691(52%) | <0.001 |
| Transplanted | 170(18%) | 74(19%) | 244(18%) | 0.61 |

Conclusion: In this study the better survival of black patients on RRT outweighed the possible adverse effect of deprivation and DM on survival. More work is needed to assess the cause of this significant benefit.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1492

**Prevalence and Correlates of Orgasmic Function, Intercourse Satisfaction, Sexual Desire and Overall Satisfaction in a Men on Hemodialysis: A Multi-National Cross-Sectional Study** Mariacristina Vecchio,[1] Giorgia De Berardis,[1] Valeria Maria Saglimbene,[1] Marinella Ruospo,[1] David W. Johnson,[2] Jorgen B. A. Hegbrant,[3] Giovanni F. M. Strippoli.[1,3] *[1]Mario Negri Sud Consortium; [2]University of Queensland; [3]Diaverum Medical Scientific Office.*

Although sexual dysfunctions in men include orgasmic problems, intercourse dissatisfaction, sexual desire and overall satisfaction abnormalities, only erectile problems have been generally studied. In this cross-sectional study, we have evaluated the remaining aspects of sexual dysfunction in men receiving hemodialysis.

Patients were identified in 27 hemodialysis clinics selected randomly within a collaborative network. All domains of sexual dysfunction were assessed anonymously with the International Index of Erectile Function (IIEF-15) questionnaire. Multivariate logistic regression was used to determine correlates of the different domains of sexual dysfunction and data are presented as adjusted odds ratio (AOR) and 95% confidence intervals (CI) .

Overall, 1056 (60%) of 1611 eligible men responded. Of these, 773 (73%) men reported orgasmic dysfunction, 927 (88%) sexual desire dysfunction, 957 (91%) intercourse dissatisfaction and 790 (75%) overall dissatisfaction. Results on key correlates of these adverse sexuality outcomes identified by multivariate analyses are reported in the table.

| | Multivariable logistic regression AOR (95% CI) | | | |
|---|---|---|---|---|
| **Characteristic** | **Orgasmic function** | **Sexual desire** | **Intercourse satisfaction** | **Overall satisfaction** |
| **Age (years)** | **1.07 (1.05-1.08)** | **1.06 (1.05-1.08)** | **1.07 (1.05-1.08)** | **1.05 (1.03-1.06)** |
| **Married** | **0.55 (0.40-0.77)** | 0.76(0.56-1.10) | **0.43 (0.31-0.62)** | **0.71 (0.51-0.99)** |
| **Diabetic nephropathy** | **1.47 (1.07-2.04)** | 1.10(0.75-1.64) | 1.12 (0.80-1.57) | 0.96 (0.69-1.33) |
| **Diabetes** | 1.40 (0.96-2.03) | 1.43 (0.98-2.10) | 1.38 (0.93-2.04) | **1.54 (1.05-2.24)** |
| **Depression** | **1.92 (1.45-2.54)** | **1.55 (1.16-2.07)** | **1.90 (1.42-2.55)** | **2.01 (1.52-2.68)** |
| **Hypertension** | 0.70 (0.46-1.07) | **0.69 (0.38-0.93)** | **0.59 (0.38-0.91)** | **0.47 (0.31-0.73)** |
| **Dry weight (Kg)** | | | | |
| <65 | **1.00** | 1.00 | 1.00 | 1.00 |
| 65-76.5 | 0.88 (0.63-1.23) | 0.95 (0.67-1.33) | 0.74 (0.52-1.05) | 0.92 (0.66-1.29) |
| >76.5 | **0.69 (0.49-0.98)** | 0.84 (0.58-1.20) | 0.70 (0.48-1.01) | 0.73 (0.51-1.05) |
| **Emoglobin** | | | | |
| <10 | 1.00 | 1.00 | 1.00 | 1.00 |
| 10-12 | **0.66 (0.46-0.97)** | 0.70 (0.47-1.03) | 0.87 (0.59-1.28) | 1.27 (0.88-1.85) |
| >12 | **0.39 (0.24-0.64)** | 0.62(0.37-1.02) | **0.54 (0.33-0.89)** | 1.07 (0.66-1.73) |
| **Neuroactive conditions** | 1.12 (0.58-2.14) | 1.58 (0.82-3.04) | 1.25 (0.64-2.43) | **2.04 (1.02-4.11)** |
| **Kidney Transplant** | | | | |
| No | 1.00 | 1.00 | 1.00 | 1.00 |
| kidney in place | 0.83 (0.40-1.72) | 1.70 (0.78-3.71) | 1.11 (0.53-2.36) | **2.15 (1.02-4.53)** |
| kidney not in place | 0.53 (0.27-1.04) | 0.72 (0.36-1.44) | 0.90 (0.46-1.75) | 0.69 (0.35-1.37) |
| **Vintage** | | | | |
| ≤19.5 | 1.00 | 1.00 | 1.00 | 1.00 |
| 19.5-53.5 | 0.98 (0.71-1.35) | 1.34 (0.95-1.89) | 1.04 (0.74-1.48) | 0.93 (0.67-1.31) |
| ≥53.5 | 1.29 (0.90-1.85) | 1.17 (0.81-1.69) | 1.08 (0.75-1.56) | **1.46 (1.02-2.09)** |
| **KT/V** | | | | |
| ≤1.3 | 1.00 | 1.00 | 1.00 | 1.00 |
| ≥1.3 | **0.69 (0.49-0.99)** | 0.91 (0.63-1.32) | 0.89(0.62-1.27) | **0.53 (0.37-0.75)** |
| **Calcium** | | | | |
| ≤8.8 | 1.00 | 1.00 | 1.00 | 1.00 |
| 8.8-10 | **1.35 (1.02-1.78)** | **1.51 (1.13-2.02)** | 1.26 (0.96-1.69) | 1.29 (0.97-1.71) |
| ≥10 | 1.7 (0.90-3.20) | **2.02 (1.06-3.86)** | 1.64 (0.84-3.19) | 1.79 (0.94-3.43) |
| **PTH (pg/mL)** | | | | |
| <150 | 1.00 | 1.00 | 1.00 | 1.00 |
| 150-300 | 1.02 (0.71-1.49) | 1.16 (0.78-1.72) | 1.03 (0.70-1.52) | 0.81(0.56-1.19) |
| >300 | 1.17 (0.91-1.69) | **1.03 (1.25-2.69)** | 0.99 (0.68-1.45) | 0.76 (0.53-1.10) |
| **Use of diuretics** | 1.14 (0.79-1.64) | 1.28 (0.86-1.88) | 1.21 (0.82-1.78) | **1.56 (1.07-2.29)** |
| **Use of angiotensin converting enzyme inhibitors** | 1.1 (0.81-1.51) | **1.55 (1.11-2.16)** | 1.29 (0.93-1.78) | **1.59 (1.15-2.19)** |
| **Use of glycosides** | **2.55 (1.00-6.01)** | 1.16 (0.56-2.46) | 2.15 (0.87-5.30) | 1.34 (0.62-2.89) |
| **Use of erythropoietin** | **0.55 (0.35-0.85)** | 0.72 (0.46-1.13) | **0.58 (0.37-0.92)** | 0.71 (0.45-1.11) |

In conclusion, dysfunctions of various aspects of sexuality are highly prevalent in hemodialysis patients. Potentially modifiable risk factors that warrant cautious consideration include hypotension and depression.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1493

**Ankle-Brachial Index Can Predict Mortality among Incident Patients in Hemodialysis** Zaida Noemy Cabrera Jimenez, Joao Egidio Romao, Jr., Benedito J. Pereira, Rodrigo B. Oliveira, Sonia Cristina S. Makida, Rosilene M. Elias. *Nephrology, University of Sao Paulo School of Medicine, Sao Paulo, SP, Brazil.*

**Background and Hypothesis:** Patients in hemodialysis have a high risk of mortality. Ankle-brachial index (ABI) is a non-invasive method reported to be useful in predicting mortality both in general population and prevalent hemodialysis patients (by detecting peripheral arterial disease). However, an analysis of ABI is lacking for incident patients in hemodialysis. We hypothesize that altered ABI can predict risk of death in incident patients on hemodialysis.

**Methods**: We prospectively followed 119 (83 men; age 53.1 ± 18.8 years old.) consecutive incident hemodialysis patients. Patients were prospectively followed until either death or end of period of observation up to 24 months. Patients were stratified into three groups according to ABI (<0.9; 0.9-1.3; >1.3) measured at entry by an oscilometric method.

**Results:** The mean ABI or all patients was 1.17 ± 0.22. There were 34 deaths occurring during the follow-up which mean duration of 12.7 ± 7 months. Table 1 shows characteristics of the three groups, regarding demographic and clinical variables. Highest mortality rate was observed in ABI <0.9 and >1.3. The survival curve analysis revealed that ABI 0.9-1.3 group had better outcomes than ABI <0.9 and ABI >1.3 groups (log-rank test p= 0.0096). There was no difference in survival between ABI <0.9 and >1.3 groups (log-rank test p= 0.694).

Demographic and clinical characteristics of the three groups

| | ABI < 0.9 | ABI 0.9-1.3 | ABI >1.3 | p |
|---|---|---|---|---|
| Age | 65 ± 17 | 53 ± 18 | 49 ± 19 | 0.030 |
| Sex male/female | 9/4 | 47/15 | 27/7 | 0.546 |
| Diabetes yes/no | 8/5 | 29/43 | 13/21 | 0.313 |
| Smoking yes/no | 4/9 | 23/49 | 10/24 | 0.966 |
| Death, n (%) | 7 (53.8) | 13 (18) | 14 (41.2) | 0.004 |

**Conclusion:** Our findings indicate that pathological ABI was a good predictor of risk of mortality among incident patients in hemodialysis. Screening patients by a simple ABI measurement can help to identify a high-risk group for increased mortality.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1494

**Seasonal and Circadian Variability in Body Temperature in Chronic Hemodialysis Patients** Stephan Thijssen,[1] Len A. Usvyat,[1] Frank Van der Sande,[2] Jeroen Kooman,[2] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Renal Research Institute, NY, NY; [2]Maastricht University Medical Centre, Maastricht, Netherlands.*

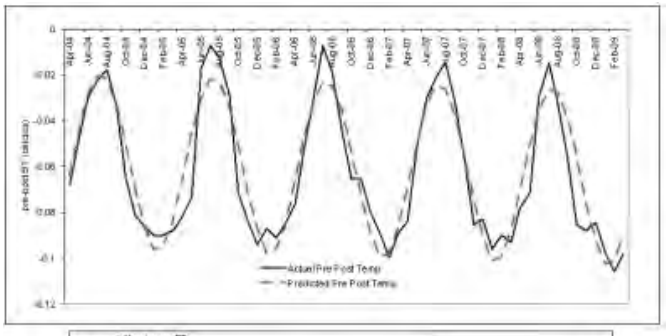
In hemodialysis (HD) patients, body temperature (BT) follows circannual and circadian rhythms. The aim of the present study was to assess seasonal and circadian variations in BT and intradialytic Δ in BT across diverse climatic US regions.

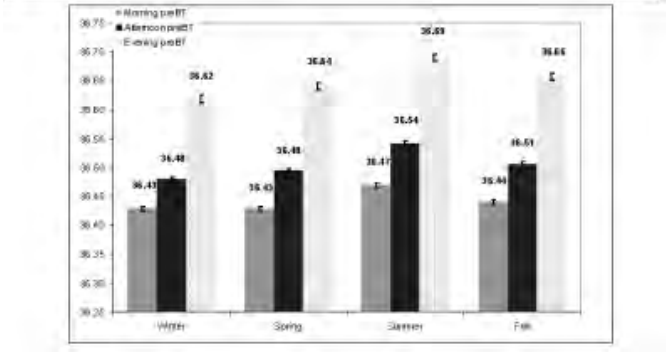
We reviewed data on HD pts treated in RRI clinics b/n Apr 1, 2004 and Mar 31, 2009. Seasons were defined on a calendar basis. Pre-dialysis BT (preBT) and post-dialysis BT (postBT) were recorded per txt. Intradialytic change in BT (BT) = preBT – postBT.

Cosinor analysis was conducted to test for seasonality in preBT and ΔBT. RRI clinics were grouped in distinct climate groups: continental, mediterranean, and subtropical. Consideration was given to the dialysis shift.

10,303 pts were studied (55% male, 49% black, 49% diabetic, avg age [stdev]: 60.5 [15.5] years). Across all climatic zones, preBT (in ºC) was lowest in the winter (mean [95% CI]): continental (36.49 [0.002]), mediterranean (36.37 [0.013]), subtropical (36.08 [0.01]). preBT was consistently highest in summer: continental (36.55 [0.002]), mediterranean (36.46 [0.012]), subtropical (36.08 [0.01]). Notably, in mediterranean & subtropical climates, where the magnitude of difference of outside temp is much smaller across seasons, BT varied suggesting a pattern independent of ambient temp.

Cosinor analysis over a 5 yr period demonstrated a seasonal component of preBT and ΔBT. Increase in ΔBT was largest in winter and smallest in summer.






Analysis of preBT by shift showed that morning pts have the lowest preBT across seasons while evening pts have highest preBT (fig).

This study demonstrates a seasonal influence on preBT and ΔBT. Morning pts in the winter experience the highest intradialytic increase in BT while evening summer pts experience the small ΔBT.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1495**

**Is Smoking a Risk Factor for Cardiovascular Events in HD Patients?** Ioannis (John) E. Droulias, Andreas Soloukides, Stamatina Papakonstantinou, Rainer Fischer, George Zervas, Efi Dampolia, Stylianos Zerefos, Dimitrios Valis, Nicolaos Zerefos. *Renal Unit"Hygeia" Hospital, Athens, Greece.*

**Introduction:** Atherosclerotic cardiovascular disease is a multifactorial condition including genetic factors, hyperlipidemia, hypertension, systemic inflammation and haemodynamic factors. It has already been showed by the Framingham Study and other studies, that the majority of causes of atherosclerotic disease are attributed to varying lifetime habits. Surprisingly, the influence of smoking in HD patients has so far received little attention concerning cardiovascular events in dialysis population, a group at monumental cardiovascular risk.

Aim: We have tried to study associations between smoking, new-onset cardiovascular outcomes and mortality in 120 patients of our Renal Unit who were followed for 5 years (2004-2009)

Methods-Results: Of the participants, 20 patients (16,6%) were lifetime smokers, 43 patients were lifetime nonsmokers (35,9%), 33 patients (27,5%) had quit smoking for more than 1 year, 15 patients (12,5%) were current smokers - new onset less than 6 months, 6 patients (5%) had quit smoking less than 1 year ago while 3 patients (2,5%) were lost in the follow-up. Patients were followed until 31 December 2009. When adjustment was made for baseline age, demographic variables, mode of dialysis therapy, and comorbidity, smoking status was associated with new-onset congestive heart failure (adjusted hazard ratio 1,59, P=0,004), new-onset peripheral vascular disease (adjusted hazard ratio 1,68, P=0,001) and mortality (adjusted hazards ratio 1,37, P<0,001). Former smokers, in contrast, had adjusted event risks similar to lifelong nonsmokers.

Conclusion: Smoking is a major, modifiable, cardiovascular risk factor in patients starting dialysis therapy. Discontinuation of above factor reduces the risk.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1496**

**Relationship of Mitral Annular Calcification and Coronary Artery Calcification in Hemodialysis Subjects** Ronney Sami Shantouf, Naser Ahmadi, Irfan Zeb, Jennie Jing, Matthew Jay Budoff, Kamyar Kalantar-Zadeh. *Medicine, LABiomed at Harbor-UCLA, Torrance, CA.*

Introduction: Vascular calcification is common among maintenance hemodialysis (MHD) subjects. This study aims to look at the relationship of mitral annular calcification (MAC) and coronary artery calcification (CAC) in hemodialysis subjects.

Methods: One hundred and sixty six MHD subjects, age 54±14 years, underwent cardiac CT. Both CAC and MAC scores were calculated. Subjects were categorized into three groups, CAC 0, CAC 1-100, and CAC 100+. Prevalence and extent of MAC was determined for each category. Adjusted odds ratio for the presence of MAC was determined using linear regression analysis.

Results: The prevalence and MAC score increased among CAC categories [CAC 0, MAC 5%, MAC score 1±7; CAC 1-100, MAC 24%, MAC Score 92±242; CAC 100+, MAC 45%, MAC Score 575±1558; prevalence of MAC p=0.002, MAC Score p=0.0001]. CAC 1-100 and CAC 100+ category demonstrated a higher likelihood of MAC with odds ratios of 5.27 [1.16-25.33, p=0.02] and 13.91 [1.76-59.60, p=0.01] when compared to CAC 0 after adjusting for age, gender, diabetes, hypertension, hyperlipidemia, family history of heart disease, smoking, vintage, dialysis dose, and mineral metabolism (table not shown).

Conclusion: The prevalence and likelihood of MAC increases as CAC scores increase in MHD subjects.

MAC Prevalence and Score among CAC categories

| Variable | CAC 0 N=18 | CAC 1-100 N=48 | CAC 100+ N=100 | P value |
|---|---|---|---|---|
| Prevalence of MAC | 5% | 24% | 45% | 0.002 |
| MAC score | 1±7 | 92±242 | 575±1558 | 0.0001 |

Disclosure of Financial Relationships: nothing to disclose

**F-PO1497**

**Ankle-Arm Index (AAI) as Predictor of Mortality on Hemodialysis** Jair Miguel,[1,2] Jorge Strogoff-de-Matos,[1] Luiz T. Naveiro,[1] Claudia M. Miguel,[1,2] Jocemir R. Lugon.[1] *[1]Universidade Federal Fluminense, Niteroi, RJ, Brazil; [2]Clinefron, Padua, RJ, Brazil.*

Aim: to assess the predictive value of AAI to risk of death among patients on maintenance hemodialysis (HD).

Material and methods: 478 patients prevalent on HD, from 6 dialysis facilities, underwent AAI measurement using mercury column sfigmomanometer and portable Doppler (10 MHz, Super Dupplex, Huntleigh Technology, Inc. NJ). Patients were divided into 3 groups, according to AAI (low: <0.9, normal: 0.9 to 1.3, and high: >1.3) and were followed for a 36-month period. Survival rate was assessed by Kaplan-Meier method and comparison between curves by Log-Rank test. Risk of death for each group was calculated using Cox proportional model, with adjustment to age, gender, diabetes status, smoking, and ultra-sensitive C reactive protein, having the normal group as reference.

Results: Participants were 54 (18 to 75) years old, 56% males, and 17% diabetics. The prevalence of low, normal and high AAI were 26.8%, 64.6% and 8.6%, respectively. The 3-year survival rate was lower in the groups with low AAI (60.3%, P <0.0001) and high AAI (69.0%, P= 0.034) than in the group with normal AAI (82.3%). After adjustment to variables, the presence of low AAI persisted to be significantly associated with the risk of death (hazard ratio [HR]= 1.78, 95% confidence interval [CI] 1.14 to 2.79; P= 0.012). High AAI presented a trend toward increasing the risk of death (HR= 1.94, 95% CI 0.99 to 3.80; P= 0.054).

Conclusion: Low AAI was found to be an important risk factor to death among HD patients. Despite the small number of patients with high AAI, that variable seems to have an impact on mortality, but statistical significance was not reached.

Disclosure of Financial Relationships: nothing to disclose

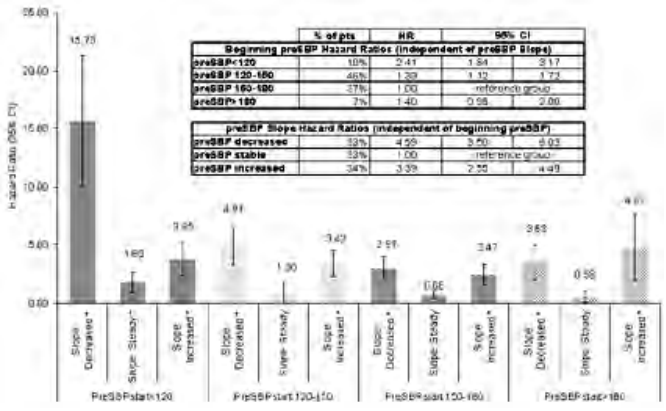
**F-PO1498**

**Patterns of Systolic Blood Pressure Change Affect Patient Outcomes in Incident Hemodialysis Patients** Len A. Usvyat, Peter Kotanko, Nathan W. Levin. *Renal Research Institute, NY, NY.*

The U-shaped curve relating blood pressure (BP) to outcomes in hemodialysis (HD) patients (pts) can be explained by the high mortality with both low systolic BP (Li AJKD 2006) and the hypertension on the left and right sides of the curve, respectively. We attempted to analyze this further.

We reviewed data of all RRI in-center HD pts starting dialysis b/n Jan 2000 and Dec 2009. Pts' pre-dialysis systolic blood pressures (preSBPSTART) were computed per pt as avg of first 30 days; the slope of BP change (preSBPSLOPE) was computed by linear regression using preSBP values in year one. Pts are stratified based on preSBPSTART and preSBPSLOPE (increased, decreased, stable).

N=7077 (56% male, 39% black, 53% white, 53% diabetic, age (SD) 62.2 (15.8)). Initial BPs predicted mortality, with worst survival in preSBPSTART<120 (HR=2.4; 95% CI 1.8-3.2) & preSBPSTART 150-180 with the lowest relative risk (table in fig). In all BP ranges, decreases in BP are associated with increased mortality (table in fig); survival was best in pts with stable BP. When the results of preSBPSTART and preSBPSLOPE are combined, pts with SBP<120 and decreasing slope have the highest HR of 15.7 [10.2-21.7] compared to the ref group of steady preSBPSLOPE and preSBPSTART 120-150 (fig).




Both upward and downward changes in BP have an unfavorable affect on survival. We hypothesize that declines in BP are due to progressive cardiomyopathy. This is reinforced by the lowest BP pts having the highest mortality. The adverse affect of increase in BP, possibly due to persistent overhydration is not surprising. However, the clinical problem presented by these data is that an apparently normal BP (as a result of decease in BP) may be misinterpreted as being satisfactory clinical situation when severe cardiac disease may be underlying the "normal" BP level.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1499**

**Arterial Stiffness in HD Patients Correlates with Early Cognitive Deficits** James B. Post, Kel G. Morin, Dwindally Rosado-Rivera, John P. Handrakis, Mary C. Sano, Ann M. Spungen. *Research & Development, James J. Peters VAMC, Bronx, NY.*

Despite extensive vascular disease and cerebrovascular morbidity in hemodialysis (HD) patients, little is known about the risk of arterial stiffness and its relation to early cognitive deficits. The purpose of our study was to compare arterial stiffness and cognitive function in HD patients with no clinical evidence of cerebrovascular disease (CVD) and appropriate controls matched for age, diabetes, hypertension, BMI, and hyperlipidemia. In 37 HD and 18 control subjects, the MMSE and detailed neuropsychological tests were performed to assess each of the following cognitive domains: Attention & mental processing speed (AMP), Executive function (EXF), Language (LAN), and Memory (MEM). Arterial stiffness was measured as common carotid artery pulsatility index (PI) using a B mode ultrasound and Pulse Wave Doppler portable unit. Composite domain scores were calculated by adding up the T scores of all tests assessed within each domain. Comparisons were made using t-tests, chi-squared tests, and regression models where applicable. There were no significant differences between HD and controls in age 62±10 vs. 63±11 y, years of education, ethnicity, hypertension, BMI, diabetes, or hyperlipidemia. In controls, mean PI was lower than the HD group 1.7±0.3 cm/s vs. 2.1±0.4 cm/s (p=.0005). HD was a significant risk factor for having an elevated PI (>1.8 cm/s) independent of other cardiovascular risk factors (p=.03). HD conferred an 8.8 fold increased risk of having an elevated PI (p=.001). In HD patients, PI correlated with AMP, independent of other risk factors (r=-.58, p=.04), and years on HD (r=.37, p=.02). In controls, there was no correlation between PI and cognitive function. All subjects had a MMSE score ≥26. HD patients had significantly lower composite scores in all domains: AMP 177±30 vs. 215±34 (p=.0001), EXF 114±20 vs. 129±20 (p=.01), LAN 78±12 vs. 99±11 (p<.0001), and MEM 169±27 vs. 189±29 (p=.01). Our results suggest that HD patients without clinical evidence of CVD demonstrate increased arterial stiffness which may be related to early cognitive deficits.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1500**

**Characteristics of Chronic Hemodialysis Patients with Hypertension: Baseline Data of the OCTOPUS Study** Kunitoshi Iseki. *Dialysis Unit, University Hospital of the Ryukyus, Nishihara, Okinawa, Japan.*

Background: Hypertension is one of the culprits of poor survival in chronic hemodialysis (HD) patients, but the benefit of treatment and the target levels are not shown by prospective randomized clinical trial (RCT).

Methods: We, Okinawa Dialysis Study (OKIDS) group, are conducting a multicenter, randomized, parallel study of angiotensin receptor blockade (Olmesartan) in HD patients: Olmesartan Clinical Trial in Okinawan Patients Under OKIDS (OCTOPUS, CRG010600030). OCTOPUS is the first prospective intervention study to examine the benefits, if any, of treatment of hypertension with the target pre-HD blood pressure (BP) levels of 140/90 mm Hg. Patients were randomized into those with the use of renin-angiotensin system (RAS) inhibitors such as ARB and angiotensin converting enzyme inhibitors as RAS (+) and those without RAS inhibitors as RAS (-); the former uses Olmesartan and or other non-RAS antihypertensive drugs and the latter uses conventional antihypertensive drugs but not any RAS inhibitors. Randomization was done using sex and the primary renal disease (diabetes mellitus).

Results: A total of 469 chronic HD patients, N=235 in RAS (+) and N=234 in RAS (-), was registered during June 2006 and June 2008 in Okinawa, Japan. Subjects were 62% men, mean age 59.6 years, HD duration of 88 months, pre-HD systolic BP 159.1 mm Hg, pre-HD diastolic BP 80.7 mm Hg, and pulse rate 77.7 beats per minute. Data of home BP were available for 210 patients. Home and pre-HD systolic and diastolic BP, and pulse rate were significantly lower at home than those obtained at pre-HD state (paired t-test). However, there were no differences between the groups. Follow-up will end on June 2011.

Conclusions: In the OCTOPUS study, the baseline data were similar between those assigned to treat hypertension with RAS (+) and RAS (-). It may prove or disprove the merit of treatment of hypertension and the superiority of ARB as a treatment choice.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1501**

**State-Level Geographic Variation in Statin Exposure among Dually Eligible ESRD Patients** Theresa I. Shireman,[1] James B. Wetmore,[1] Jonathan D. Mahnken,[1] Qingjiang Hou,[1] Sally K. Rigler,[1] John Spertus,[2] Edward F. Ellerbeck.[1] *[1]University of Kansas Medical Center, Kansas City, KS; [2]St. Luke's Mid-American Heart Institute, Kansas City, MO.*

HMG-CoA reductase inhibitors (statins) are a cornerstone of therapy for individuals with cardiovascular disease (CVD). We examined the prevalence of and factors associated with statin use among publicly insured dialysis patients. In a retrospective, cross-sectional sample of dually-eligible (Medicare - Medicaid) dialysis patients, we linked national Medicaid prescription claims data from 2002 with USRDS baseline data, derived from the CMS 2728 Medical Evidence Form, to examine how statin use was associated with various demographic and risk behavior factors, functional status, comorbidity, and dialysis modality. We also explored how use varied across states. Among 49,466 dialysis patients, 23.9% received a statin prescription [56.3%, atorvastatin; 28.7%, simvastatin; 11.2%, pravastatin; 3.9%, fluvastatin or lovastatin]. For persons with diabetes, 31.2% received statins compared to 16.3% of those without (p<0.001). For persons with CAD, 35.5% received statins compared to 21.1% of those without (p<0.001). Persons with higher BMIs had progressively higher rates of statin exposure (p<0.001). Caucasians also had higher exposure rates (28.9%) than African-Americans (20.2%) and Hispanics or other races (25.0% and 25.9%, respectively; p<0.001). In the multivariable analysis, male sex, non-Caucasian race, HF, substance abuse, and poor functional status were associated with less use of statins, while diabetes, CAD, hypertension, and prior CVA were associated with higher exposure. There was also substantial state-by-state variation: when adjusted for patient level factors, the adjusted rate ratios showed a 2.7-fold difference between the highest- and lowest-prescribing states. Statin exposure was generally lower than expected in dialysis patients, given the prevalence of known cardiac risk factors, although use was higher among persons with key risk factors. Noted variations in exposure by race, sex, and state of residence suggest areas for future research.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1502**

**Echocardiographic Evaluation of Left Ventricular Dysfunction and 4-Year's Follow-Up for Patients with End Stage Renal Disease on Hemodialysis** Jinil Yoo, Hugo J. Villanueva, Lin N. Lwin, Yisfalem W. Alamdew, Khashayar Sehhat. *Nephrology, Montefiore Medical Center, North Division, New York Medical College, Bronx, NY.*

A high cardiac morbidity and mortality is known to exist among patients (Pts) with endstage renal disease (ESRD) on hemodialysis (HD), especially with presence of left ventricular hypertrophy (LVH). The echocardiogram (ECHO) was performed at their prescribed dry weight, on 54 of a total 64 Pts who agreed to be enrolled. There were 18 African Americans (33%), 15 Hispanics (28%), 12 non-Hispanic Caribbeans (22%), 4 Caucasians (7%), 5 Asians (9%); average age of 63 years, 29 diabetics (54%), 50 hypertensives (93%), and average HD of 3.8 years. The following diagram is the summary of our Pts' ECHO findings and mortality rates based on LV systolic function (ejection fraction: EF%) and ECHO characteristics of LVH during 4-year's clinical follow-up after the initial ECHO.

| LV systolic function (EF %) | Distribution of Pts | Mortality rate (4 years) | ECHO characterstics | Distribution of Pts | Mortality rate ( 4 years) |
|---|---|---|---|---|---|
| EF ≤50 | 9/54(17%) | 7/9(78%) | Dilated LVH | 4/54(7.5%) | 3/4(75%) |
| EF >50 to <59 | 22/54(41%) | 11/22(50%) | Concentric LVH | 46/54(85%) | 18/46(39%) |
| EF ≥59 | 23/54(43%) | 4/23(17%) | Normal | 4/54(7.5%) | 1/4(25%) |

The initial ECHO study revealed LVH in 50/54 (93%), predominantly of concentric type, significant systolic dysfunction (EF≤50) in 9/54 (17%), EF≥59 in 23/54 (43%) and ECHO-Doppler evidence of diastolic dysfunction in 50/54 (93%). During the following 4 years' observation, the mortality rate (MR) on Pts with EF≤50 reached 78% (7/9) Vs 17% (4/23) on Pts with EF≥59, and MR on Pts with with dilated LVH leading to 75% (3/4) Vs 39% (18/46) on Pts with concentric LVH. The primary cause of death among Pts with EF<59 is cardiac 72% (13/18), CVA 11% (2/18) and malignancies 11% (2/18).

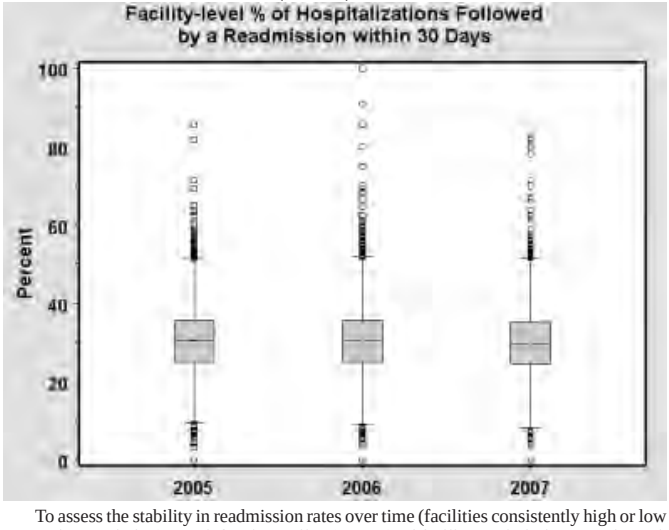
We believe that the majority of patients reaching ESRD develop LVH with significant systolic and/or diastolic dysfunction, which contributes to the extremely high cardiac morbidity and mortality. We feel that initial ECHO evaluation is beneficial to all Pts entering ESRD stage for the prognostic implications and therapeutic endeavor to decrease adverse cardiac events.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1503**

**30-Day Hospital Readmission among Dialysis Patients: Influence of Dialysis Facilities Versus Hospitals** Marc Turenne,[2] S. Hunter,[1] Robert A. Wolfe,[2] T. H. Shearon,[1] Jeffrey Pearson,[2] John Kalbfleisch,[1] Claudia Dahlerus,[2] John R. C. Wheeler,[1] J. M. Messana,[1] Richard Hirth.[1] *[1]Univ of Michigan KECC; [2]Arbor Research.*

Hospital readmission is a relatively common occurrence in the ESRD population. In 2007, 31.7% of hospitalizations among Medicare dialysis patients were followed by a readmission within 30 days. There may be a role for multiple providers, including hospitals and dialysis facilities, in preventing readmissions. Using Medicare claims, we examined inpatient hospitalizations for dialysis patients in 2005-2007. There is substantial variation in readmission rates across facilities (n=5,050).




To assess the stability in readmission rates over time (facilities consistently high or low vs. random variation), we defined quartiles (Q1-Q4) of facility readmission rates in each year. This yielded: 5.4% of facilities remained in Q4 for all 3 years, while 22.2% remained in Q3 or Q4, 4.6% remained in Q1, and 20.8% remained in Q1 or Q2.

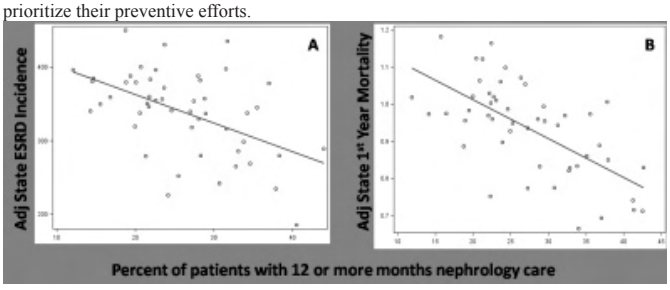
Using 2007 data (n=378,480 claims), we used a generalized linear model with a logit link to model readmission within 30 days, with random effects for facility and hospital. Fixed effects included patient age, race, sex, and diabetes. Facility and hospital random effects were both highly significant based on likelihood ratio tests ($p<0.0001$). The estimated SD in readmission rates was 4.5% across facilities and 4.3% across hospitals. The patient-level adjustments had little impact on the results. The results suggest that dialysis facilities and hospitals provide nearly equal components to the variation in readmissions. The potential influence of dialysis facilities over hospital readmissions may be an opportunity to reduce their high frequency among ESRD patients. Factors that cause readmissions and policies that might encourage facilities or other providers to prevent them should be explored further.

Disclosure of Financial Relationships: Research Funding: The Dialysis Outcomes and Practice Patterns Study (DOPPS) is administered by Arbor Research Collaborative for Health and is supported by scientific research grants from Amgen (since 1996), Kyowa Hakko Kirin (since 1999, in Japan), Genzyme (since 2009), and Abbott (since 2009), without restrictions on publications.

**F-PO1504**

**Longer Nephrologist-Driven Care Associated with Lower Mortality and Lower Incidence of End Stage Renal Disease in the United States** Elizabeth Hedgeman,[1] N. A. Lueth,[1] T. H. Shearon,[1] Brenda W. Gillespie,[1] Laura C. Plantinga,[2] Neil R. Powe,[2] Nilka Rios Burrows,[3] Desmond Williams,[3] Rajiv Saran.[1] *[1]University of Michigan, Ann Arbor, MI; [2]University of California San Francisco, San Francisco, CA; [3]Centers for Disease Control and Prevention, Atlanta, GA.*

The Healthy People guidelines recommend individuals with declining kidney function receive care from a nephrologist a minimum of 12 months prior to the start of ESRD. Using data from the Centers for Medicare and Medicaid Services' Form 2728, we assessed pre-ESRD data in > 260,000 individuals beginning ESRD therapy during 2005-2007. Nationwide, 24.4% of patients had been under the care of a nephrologist for ≥12 months. In most instances, ≥ 12 months of care from a nephrologist was associated with better health and preparedness outcomes than 6-12 months of care (fistula at start, higher albumin, higher hemoglobin, transplant options discussed, all odds ratios were $p<0.05$). Importantly, ≥12 months of nephrology care was associated with a significant reduction of first-year mortality after ESRD onset (adjusted Hazard Ratio=0.58, $p<0.0001$). Wide variation was observed in the duration of nephrology care prior to ESRD onset by state of residence at time of ESRD start. Both incidence rates of ESRD ($R^2=0.25$, $p<0.001$) as well as the first-year standardized mortality ratios ($R^2=0.40$, $p<0.001$) were lower in states with greater proportions of individuals with ≥12 or more months of nephrology care (Figure; panels A and B). Nephrology care for ≥12 months in advance of ESRD has the potential both to reduce the incidence of ESRD in the country as well as mortality after ESRD onset. Further research to investigate factors underlying observed geographic disparities is needed to improve care of patients with CKD and allow public health authorities to prioritize their preventive efforts.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1505**

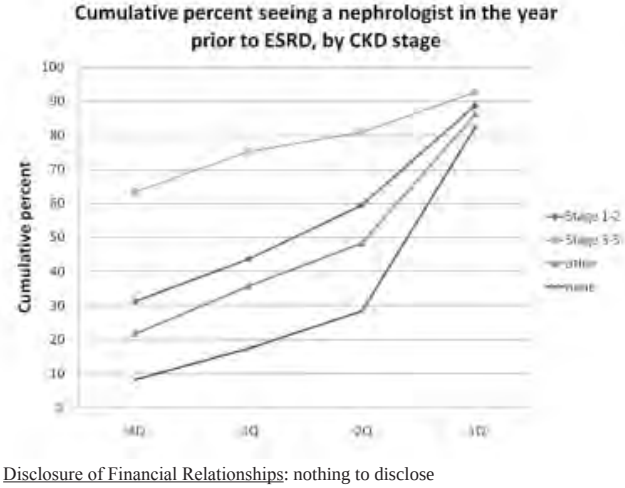
**Frequency of Nephrologist Claims Prior to ESRD Initiation by CKD Stage** David A. Zaun,[1] David T. Gilbertson,[1,2] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

This study investigates the cumulative percent of CKD patients that visit a nephrologist prior to ESRD using Medicare data. Patients included were age 67+ incident ESRD in 2008. Nephrologist claims were studied 12 months prior to ESRD and CKD stage was defined from two years to a year prior to ESRD. CKD stage was grouped into four categories: stage 3-5 CKD (a stage 3, 4, or 5 claim), stage 1-2 CKD (a stage 1 or 2 claim), other (a non stage specific CKD claim); and no CKD (no CKD claims).

Many patients are not recognized to have CKD a year prior to ESRD: 27.6% with no CKD, 1.8% with stage 1-2 CKD, 53.8% with stage 3-5 CKD, and 16.8% with other CKD. Some patients not recognized to have CKD likely had CKD. Many patients are not referred to a nephrologist: 20% with stage 3-5 CKD, 40% with stage 1-2 CKD, 50% with non stage specific CKD, and 70% with no CKD were still not referred to a nephrologist 3 months prior to ESRD.

The percent of patients seeing a nephrologist one year to three quarters prior to ESRD was different across groups: 8.2% with no CKD, 21.8% with non stage specific CKD, 31.3% with stage 1-2 CKD, and 63.5% with stage 3-5 CKD. By the time patients reached ESRD the percent seeing a nephrologist within the year prior to ESRD was similar across the groups: 82.6% with no CKD, 86.4% with non stage specific CKD, 88.9% with stage 1-2 CKD, and 92.7% with stage 3-5 CKD. This is higher than historically seen in the Medical Evidence Form.

There is a large percentage of patients not recognized as having CKD, any stage, 12 to 24 months prior to ESRD. Many patients do not see a nephrologist until the quarter prior to ESRD. Although there is a dramatic increase in the percent of patients with nephrologist claims in the quarter prior to ESRD, there is little time to address CKD care, manage risk factors, and plan for ESRD treatment.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1506**

**Medicare Part D Enrollment and Medication Use and Costs in US Dialysis Patients in 2007** Eric D. Weinhandl,[1] Benjamin L. Howell,[2] Wendy L. St. Peter,[1,3] Christopher Powers,[2] James P. Ebben,[1] Diane L. Frankenfield.[2] *[1]U.S. Renal Data System, MMRF, Minneapolis, MN; [2]ORDI, CMS, Baltimore, MD; [3]Univ. of Minnesota, Minneapolis, MN.*

**Background**: Studies have found that dialysis patients take 11-12 medications at any point in time. The Medicare Part D drug benefit may assist patients in acquiring medication. Predictors of enrollment and descriptors of use are largely unknown. **Methods**: We used data from the United States Renal Data System to assess enrollment in and use of the Part

D benefit in prevalent dialysis patients in 2007. We included dialysis patients alive on Jan 1, 2007, who continued dialysis and carried Medicare Parts A and B during all of 2007 (*N*=196,101). **Results**: Full-year enrollment was 69.2%. From logistic regression, factors positively associated with enrollment included younger age; female gender; black *vs.* white race; hypertension *vs.* diabetes as primary end-stage renal disease [ESRD] cause; shorter ESRD duration; presence of cardiovascular (excepting dysrhythmia and ischemic heart disease), gastrointestinal, and liver disease, along with COPD and diabetes; and absence of cancer and dysrhythmia. There was also geographic variability. In patients with full-year enrollment, 80.0% received low-income subsidy (LIS). Younger age, female gender, and black *vs.* white race were positively associated with LIS receipt. In patients receiving LIS, 95.5% took ≥1 medication, and mean cumulative medications were 13.6 (standard deviation [SD], 7.4). Corresponding estimates in patients not receiving LIS were 93.4% and 11.3 (6.4). Gross drug costs (GDC) per patient per year (PPPY) were higher in patients receiving LIS *vs.* not, although out-of-pocket [OOP] costs PPPY were lower.

| | GDC PPPY† | % with >$2400 GDC | % with >$5451 GDC | OOP costs PPPY† |
|---|---|---|---|---|
| with LIS | $6694 (6186) | 74.9 | 47.6 | $113 (224) |
| without LIS | $4181 (4386) | 60.8 | 15.3 | $1549 (1341) |

†mean (SD)

**Conclusion**: There is high variability in Part D enrollment across patient factors, although most patients enrolled in Part D used the benefit. While high prevalence of LIS may mitigate concern about medication accessibility in the coverage gap, substantial out-of-pocket costs in patients without LIS may preclude appropriate treatment.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1507

### Hemodialysis (HD) Patients Utilizing Kidney Specialty Pharmacy Have Higher Medication Adherence, Fewer Hospitalizations and Lower Healthcare Costs

Harold J. Manley, Steven Wang, Allen R. Nissenson. *DaVita Inc., Denver, CO.*

HD patients' medication adherence and healthcare utilization patterns based upon pharmacy type selection (chain, independent, or specialty) are unknown. In 2004, DaVita Rx (DVA Rx), a kidney specialty pharmacy began operations serving dialysis patients by providing prescription services within the dialysis clinic. We hypothesized that HD patient's medication adherence and healthcare utilization are impacted by pharmacy selection. We evaluated HD patient medication adherence and per member per month (PMPM) healthcare costs and hospitalization rate (per member per thousand days, PMPT) based on pharmacy type (e.g., chain, independent, DVA Rx)

METHODS: We conducted a retrospective review of all pharmacy (Rx) and medical claims for 750 HD patients from 1/06 to 6/09 enrolled in an ESRD Medicare Demonstration project. Patients who filled >70% of all prescriptions using a specific pharmacy type were assigned to a specific group. We calculated medication adherence behavior using medication possession ratio (MPR): MPR = Σ Medication Day's Supply/(# Days between the first fill & the last refill + Day's supply last refill). We used a multivariate analysis to estimate pharmacy type effect on MPR, PMPM costs, and PMPT hospitalization rates after controlling for confounding variables.

RESULTS: 472 HD patients were assigned into a specific pharmacy type (62 DVA Rx; 355 Chain, 55 Independent). DVA Rx patients had more patients with MPR values > 0.8 than either pharmacy type: DVA RX 84.0%, Chain 37.9%, Independent 46.9% ($p<0.003$). Utilizing a 1:1 propensity match, DVA Rx patients had lower PMPT and PMPM costs compared to non-DVA Rx patients (Table).

| | DaVita Rx (N=59) | Non-DaVita (N=59) |
|---|---|---|
| Medical PMPM | $4,477 | $4,791 |
| Medication PMPM | $637 | $745 |
| Total Cost | $5,114 | $5,536 |
| Admits PMPT | 1024 | 1435 |

CONCLUSION: Use of DVA Rx kidney specialty pharmacy by HD patients was associated with higher medication adherence rates, fewer hospitalizations, and lower total healthcare costs compared to those filling their prescriptions at a chain or independent pharmacy.

Note: This is a DaVita analysis of CMS demonstration experience; CMS will conduct an independent evaluation.

Disclosure of Financial Relationships: Employer: DaVita Inc; Ownership: DaVita Inc.

## F-PO1508

### Financial Evaluation of the Centers for Medicare & Medicaid Services (CMS) End-Stage Renal Disease (ESRD) Disease Management (DM) Demonstration

Sylvia Paz B. Ramirez,[1] Sean M. Lyons,[2] W. Pete Welch,[2] Christine Cheu,[1] Jeffrey Pearson,[1] Claudia Dahlerus,[1] Tania Chowdhury,[1] Brett Lantz,[1] Sabrina K. Gomes,[1] Friedrich K. Port.[1] *[1]Arbor Research; [2]Lewin.*

We examined: 1) to what extent did CMS pay more for enrollees in 3 DM Organizations (DMOs) than had they remained in fee-for-service (FFS) and 2) are there savings to DMOs resulting from differences in service utilization.

Propensity score matching identified a comparison FFS population to minimize selection bias; multiple regression adjusted for remaining differences in observed factors, including age, sex, race, Hispanic ethnicity, geography, Medicaid status, new enrollee status, ESRD vintage, comorbidity, and ESRD cause. Costs for DMO enrollees had they remained in FFS were estimated from comparison group claims. Capitated Medicare payments to DMOs were compared to these estimated costs. Estimated savings to DMOs were the product of utilization difference (FFS-DMO) and FFS cost for hospital admissions, skilled nursing facility (SNF) stays, emergency department (ED) visits, and physician (MD) visits.

Mean capitated payments for DMO patients ($6,551 per patient per month) exceeded the estimated cost of remaining in FFS ($5,776), a mean difference of $774 or a 13.4% higher cost to Medicare. Capitated payments were higher: 11.2% in DMO A, 10.9% in DMO B, and 14.7% in DMO C. Estimated savings differed by DMO. DMO C experienced savings primarily due to fewer hospital admissions. For DMO A, excess hospitalizations fell over time.

| | DMO A | | | | DMO B | | | | DMO C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2006 | 2007 | 2008 | 2006-2008 | 2006 | 2007 | 2008 | 2006-2008 | 2006 | 2007 | 2008 | 2006-2008 |
| Hospital Admissions | -$1,951 | -$2,624 | -$391 | -$1,564 | -$3,097 | $769 | -$3,655 | -$1,822 | $1,411 | $490 | $3,238 | $1,575 |
| SNF Stays | $956 | $944 | $506 | $765 | -$73 | $723 | -$102 | $229 | $983 | $1,295 | $1,231 | $1,217 |
| ED Visits | $146 | $50 | $211 | $136 | -$90 | $136 | $269 | $167 | $33 | -$43 | $35 | -$5 |
| MD Visits | $336 | $289 | $119 | $224 | $77 | $104 | $56 | $78 | $96 | $118 | $192 | $135 |
| Est. Total Savings | -$513 | -$1,349 | $445 | -$472 | -$3,183 | $1,732 | -$3,432 | -$1,294 | $2,523 | $1,860 | $4,695 | $2,927 |
| Est. Total Savings % | -9.6% | -17% | 5% | -9% | -45% | 25% | -43% | -20% | 33% | 24% | 59% | 37% |

CMS paid more under Medicare Advantage than it would have had patients remained in FFS consistent with studies of Medicare managed care programs. There was marked variation in cost savings and losses by DMO; one DMO demonstrated consistent reduction in hospitalization admissions which translated to cost savings. DM has potential to reduce costs by reducing service utilization.

Disclosure of Financial Relationships: Research Funding: Arbor Research receives funding for DOPPS from Amgen, Kyowa Hakko Kirin, Abbot and Genzyme.

## F-PO1509

### Utilization of High Cost Services in the Centers for Medicare & Medicaid Services End Stage Renal Disease (ESRD) Disease Management (DM) Demonstration

Sylvia Paz B. Ramirez, Jeffrey Pearson, Christine Cheu, Claudia Dahlerus, Tania Chowdhury, Brett Lantz, Sabrina K. Gomes, Friedrich K. Port. *Arbor Research.*

Hospitalization rates for ESRD patients (pts) have changed little since 1980. ESRD is an ideal target for DM because of comorbidities, fragmented patient (pt) management, high morbidity and mortality, and use of high cost services.

Each DM Organization (DMO) developed a program with clinical interventions and focus areas. DMO in-center hemodialysis pts were compared with similar pts in traditional fee-for-service (FFS) Medicare using propensity score matching on age, sex, race, Hispanic ethnicity, geography, Medicaid status, new enrollee status, ESRD vintage, comorbidity, and ESRD cause. Services evaluated included hospital admissions, readmissions, length of hospital stay (LOS), total hospital days (TD), physician (MD) visits, emergency department (ED) visits, and skilled nursing facility (SNF) stays.

For 2006, 2007, and 2008, respectively, the analyses included 242; 408; and 415 pts in DMO A; 78; 170; and 191 pts in DMO B; and 529; 959; and 612 pts in DMO C. Corresponding numbers of FFS pts were included as controls. Overall there were marginally fewer admissions, MD visits, ED visits, and SNF stays but more readmissions, TD, and longer LOS. DMO C had fewer admissions than FFS: in 2008, 12% (and 19% fewer readmissions). DMO A reduced excess admissions and readmissions by 2008 when rates became very similar to FFS. Admission and readmission rates in DMO B fluctuated suggesting unstable estimates due to fewer pts. All three DMOs had longer LOS and TD than FFS. Generally, DMOs had fewer MD and ED visits and SNF stays than FFS.

| | DMO A | | | | DMO B | | | | DMO C | | | | All | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2006 | 2007 | 2008 | All | 2006 | 2007 | 2008 | All | 2006 | 2007 | 2008 | All | 2006 | 2007 | 2008 | All |
| Hospital Admissions | -8% | -10% | -1% | -6% | -17% | -4% | -17% | -10% | -6% | -2% | -12% | -7% | -2% | -6% | -8% | -6% |
| Total Hospital Days (TD) | +15% | +12% | +24% | +26% | +74% | +17% | +75% | +54% | -8% | +21% | +12% | +15% | +15% | +23% | +25% | +23% |
| Readmissions | -8% | +18% | +12% | +14% | +91% | -4% | +51% | +40% | -9% | -3% | -19% | -10% | -2% | -2% | -1% | +1% |
| LOS | +7% | +20% | +29% | +18% | +48% | +22% | +49% | +40% | +16% | +24% | +28% | +24% | +17% | +19% | +29% | +24% |
| MD Visits | -48% | -37% | -15% | -30% | -18% | -22% | -10% | -14% | -17% | -18% | -27% | -20% | -28% | -23% | -21% | -23% |
| ED Visits | -24% | -7% | -26% | -19% | +9% | -11% | -24% | -14% | -4% | -5% | -3% | -1% | -8% | +6% | -11% | -6% |
| SNF Stays | -41% | -36% | -22% | -32% | +12% | -51% | +7% | -13% | -57% | -57% | -49% | -53% | -47% | -52% | -34% | -44% |

DM may reduce use of costly services. Admission and readmission rates were persistently lower in DMO C. Hospital use was greater in DMOs A and B, though a trend toward reduced admissions over time was seen in DMO A.

Disclosure of Financial Relationships: Research Funding: Arbor Research receives funding for DOPPS from Amgen, Kyowa Hakko Kirin, Abbot and Genzyme.

## F-PO1510

### Characteristics of Dialysis Facilities Losing Income under the CMS Proposed Payment System

Fredric O. Finkelstein, Alan S. Kliger. *Hospital of St. Raphael, Yale.*

**Background**. CMS reported the average dialysis facility will lose an average of 2% Medicare income in 2011 under a new proposed payment system (PPS). Estimates suggest some types of facilities will lose more income than others but variances were not reported. Independent facilities will be more vulnerable to large payment reductions and treatment quality may be jeopardized. Our objective was to analyze the range of payment losses and characteristics of facilities predicted to sustain the greatest losses.

**Method**. We merged the CMS Payment flat file with the 2009 CMS Dialysis Compare file enhanced with US Census data. Amount of predicted loss (PPS income 2007 - Medicare payments in quintiles) was tabulated by type of facility: large dialysis organization (LDO), Regional, Independent, Hospital; number of treatments: <3000,3000-9999,>10,000; census region; urban/suburban/rural; and % minority in zip code population.

**Results**. The 2007 CMS flat file identified 4921 facilities: 60.7% were LDOs, 15.3% Regional chains, 11.2% Independent, 9.6% hospital; 3.3% unknown. In 2009, the CMS provider files indicated that the number of LDO facilities grew by approximately 10% from 2007. The following results are classification current in 2009. The top quintile (N=985) of income losers under the PPS will lose a median average of 10.8% on a median income of $1.65 million. Loses will be higher for LDOs (625, -11.4%) and lower for Independents (102, -7.8%). Top losers were concentrated in the South Atlantic (30.8%) and South Central

(30.6%) census regions. Compared to all Independent facilities those in the high loss quintile were more likely to be rural and performing 3000 – 9999 treatments.

**Conclusions**. Large numbers of facilities will lose far more than an average of 2% Medicare income under the bundled model putting them at risk of failure. Losses were not evenly distributed. Failure of at risk facilities could selectively reduce access to care.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1511

**Integrated Care Management (ICM) Is Associated with Improved Patient Mortality: Results from the CMS ESRD Disease Management Demonstration Project** Stephen D. McMurray, Peter F. Sauer, Robert E. Farrell. *Integrated Care Management, Fresenius Medical Care, Walthan, MA.*

Fresenius Medical Care Health Plan (FMCHP) participated in the CMS ESRD Disease Management Demonstration project. CMS implemented the demonstration to test the effectiveness of an integrated care program & enhanced benefits provided to ESRD patients enrolled in disease specific Medicare Advantage plans. We were able to provide additional benefits (AB) and services to members beyond those available in FFS Medicare with the hypothesis that we could decrease mortality in this vulnerable population. The AB include home tele-health devices, Kidney Tel nurse, nutritional supplements, flexible benefit plan, transportation support and free glucometers and test strips. The results are from data collected from patients enrolled in the program 2006-2009. The mortality results were compared to the most recent USRDS data reports. Table 1 lists the results.

FMCHP Mortality as Compared to USRDS: Deaths per 100/pt yrs

| USRDS | FMCHP 2006 | FMCHP 2007 | FMCHP 2008 | FMCHP 2009 |
|---|---|---|---|---|
| 21.2 | 11.5 | 9.46 | 10.8 | 8.5 |

Note: FMCHP is participating in a CMS demonstration, this is an FMCHP analysis of CMS demonstration experience, and CMS will conduct an independent evaluation

The mortality rate for the FMCHP population has consistently been half the most recent USRDS mortality rate of 21.2 deaths per 100 /pt yrs.

Summary: ICM and the provision of additional benefits reduced mortality in the ESRD Medicare patients as compared to FFS Medicare.

Disclosure of Financial Relationships: Employer: Fresenius Medical Care; Ownership: Fresenius Medical Care.

### F-PO1512

**Pharmacy Management Improves Facility Performance** Richard Mutell, Steven M. Wilson, Carey Colson, Tracy Jack Mayne, Josh Golomb. *DaVita Inc., Denver, CO.*

ESRD patients take an average of 8-11 oral medications per day. Research has shown that pharmacy management can improve patient outcomes, but this has been largely unexplored in ESRD patients. The purpose of this study is to assess the impact of pharmacy management on the facility-level DaVita Quality Index (DQI), a measure previously shown to predict hospitalizations and mortality.

Methods: This was retrospective analysis of 1945 HD dialysis facilities from 1/1/09 to 4/30/10 at a US dialysis organization. Pharmacy management included insurance management, prescription fulfillment, adherence support, and coordination of care with physicians and facility staff. DQI is a composite variable of weighted P, Ca, PTH, albumin, hemoglobin, KT/V, vaccination rates, and vascular access. The mineral and bone disease (MBD) component includes weighted P, PTH and Ca measures. The independent variable in all analyses was the percent of facility patients enrolled in pharmacy management. The primary outcomes were facility-level DQI and MBD scores, and percent of patients with P, PTH and Ca in KDOQI recommended ranges. Analyses were conducted using a Generalized Linear Mixed Model.

Results: Over the course of the study, mean pharmacy management enrollment increased from 11.9%-19.0%. There was a positive and statistically significant relationship between proportion of patients enrolled in pharmacy management and facility-level DQI & MBD scores, and percent of patients in KDOQI range for P and PTH. There was no significant effect on Ca.

| | beta (SE) | p-value |
|---|---|---|
| DQI score | 0.05 (0.007) | <0.001 |
| MBD score | 0.01 (0.003) | <0.001 |
| % P ≤ 5.5 mg/dL | 0.07(0.015) | <0.001 |
| % PTH 150-300 pg/nl | 0.006 (0.018) | <0.001 |
| % Ca<9.5 mg/dL | -0.01 (0.012) | 0.27 |

Conclusions: The burden of treatment, including a high number of medications and daily pill consumption, creates a high potential for inadequate compliance and persistence on important medications. Pharmacy management that focuses on access and adherence can improve important outcomes in dialysis care.

Disclosure of Financial Relationships: Employer: DaVita, Inc.

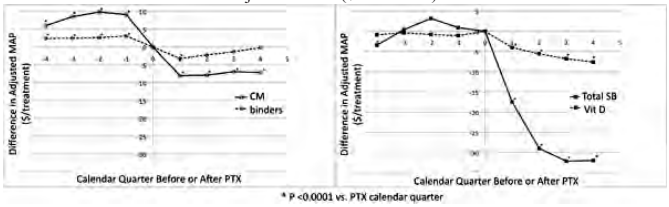
### F-PO1513

**Relationship between Parathyroidectomy (PTX) and Medicare Allowable Payment (MAP) for Separately Billed Services (SB) in Chronic Dialysis Patients** J. M. Messana,[1] Richard Hirth,[1] Marc Turenne,[2] K. Sleeman,[1] Jeffrey Pearson,[1] Jesse L. Roach,[1] John R. C. Wheeler.[1] *[1]Kidney Epidemiology & Cost Center, U Michigan, Ann Arbor, MI; [2]Arbor Research Collaborative for Health, Ann Arbor, MI.*

Given the cost of calcimimetics (CM) and vitamin D analogs (vit D), inclusion of these drugs in an ESRD payment bundle could create financial incentives to perform more PTX in dialysis patients. To define the magnitude of this incentive, we used 2006-08 Medicare Claims data to model total SB and injectable vit D costs and Medicare Part D costs for CM, oral vit D and phosphorus binders (binders) for 4 calendar quarters before and after PTX.

Patients were included in the analysis if Medicare dialysis and Part D claims were available in 4 or more consecutive quarters before and after a quarter with a PTX claim. SB services, including injectable medications, labs and Part D Claims for vit D, CM and binders were calculated for each patient as ($/treatment/quarter). Linear regression models adjusted for age, sex, race and comorbidities were used to estimate MAP for SB and Part D services before and after PTX. (n= 1644; pt quarters= 14895)

Estimated Differences in Adjusted MAP ($/treatment) for SB and Selected Part D




* P <0.0001 vs. PTX calendar quarter

PTX is associated with sustained reduction in SB MAP for at least one year after surgery. Only a fraction of this reduction is explained by vit D use. In addition, CM and binder cost also decreased after PTX. The magnitude and duration of total SB savings will create financial incentives to perform PTX in an expanded bundle, whether or not Part D drugs are included. The combination of cost savings and possible improved survival associated with PTX (Kestenbaum, Kidney Int, 2004) suggests that additional cost-effectiveness analysis is warranted. In addition, PTX rates should be monitored closely after implementation of the expanded payment bundle.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1514

**Disease Management (DM) and Patient-Centered Outcomes in the Centers for Medicare and Medicaid Services (CMS) End Stage Renal Disease (ESRD) DM Demonstration** Claudia Dahlerus,[1] Sylvia Paz B. Ramirez,[1] Brett Lantz,[1] Caitlin C. Oppenheimer,[2] Alycia Infante,[2] Elizabeth Hargrave,[2] Jeffrey Pearson,[1] Christine Cheu,[1] Tania Chowdhury,[1] Sabrina K. Gomes,[1] Friedrich K. Port.[1] *[1]Arbor Research; [2]NORC.*

We evaluated the impact of the DM Demonstration on QoL and patient (pt) satisfaction for pts enrolled in 3 DM organizations (DMOs) 20062008.

The QualityMetric Short Form 12v2 or SF-36 survey collected QoL data. US DOPPS comparison pts completed the Kidney Disease Quality of Life survey. A 5 point change in the Mental Component Summary (MCS) or Physical Component Summary (PCS) composite scores was clinically meaningful. Baseline scores were compared to scores at ≥12 mos. Adjusted changes in scores were assessed with repeated measures linear mixed models controlling for baseline demographic and clinical variables.

Pt satisfaction data were collected from focus groups (2006), follow-up telephone interviews (2007), and a different sample of enrollees in 2008.

Baseline DMO QoL response rates were 17% - 87% for pts completing ≥1 survey; follow-up at 12 mos was 27% - 59%. Adjusted MCS and PCS scores did not change significantly for DMOs A and C, similar to the US DOPPS. DMO B had statistically significant but not clinically meaningful declines in adjusted scores.

Table 1

| | MCS | | PCS | |
|---|---|---|---|---|
| DMO | Change[1] | p-value | Change | p-value |
| A | 0.79 | 0.69 | -0.15 | 0.93 |
| B | -1.47 | <0.01 | -1.09 | <0.01 |
| C | 0.46 | 0.29 | -0.07 | 0.85 |
| All | -0.02 | 0.96 | -0.29 | 0.33 |
| US DOPPS | 0.28 | 0.51 | 0.01 | 0.99 |

[1]on 100-point scale

Results indicate overall pt satisfaction with DMO services (2006 = 4.6, 2007 = 4.6, 2008 = 4.5, on a 5-point scale). Overall satisfaction with DMO A increased by 2008 and decreased slightly with DMO C. Main reasons for disenrollment were misunderstandings about DMO plan services, and cost/billing issues.

Findings for enrollees suggest no clear impact of DM improving QoL. High satisfaction was observed among pts interviewed who remained in the DMOs. Separate data suggest disenrollment may be related to poor health, with sicker enrollees more likely to disenroll.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1515**

**Use and Costs of Anti-Diabetic Medications in U.S. Adult Dialysis Patients with Medicare Part D, 2007**

Wendy L. St. Peter,[1,3] Christopher Powers,[2] Eric D. Weinhandl,[1] Benjamin L. Howell,[2] James P. Ebben,[1] Diane L. Frankenfield.[2]
*[1]USRDS Coordinating Center, MMRF, Mpls, MN; [2]ORDI, CMS, Baltimore, MD; [3]College of Pharmacy, University of MN, Mpls, MN.*

**Background:** Nearly 52% of prevalent dialysis patients (pts) in 2007 had diabetes mellitus (DM) as primary end-stage renal disease (ESRD) cause or a comorbid condition. Metformin and exenatide are contraindicated and other diabetes drugs should be used with caution in severe kidney disease. **Methods:** We used Part D data in Medicare's Chronic Condition Warehouse linked to administrative data from the United States Renal Data System to assess the use and costs of insulin and oral anti-diabetic medications in prevalent adult dialysis patients with DM in the U.S. in 2007. All adult pts alive on Dec 31, 2007 with Part D coverage during all of 2007 were included in the study (n=93,540). **Results:** Insulin was used by 52% of pts, with 16% using rapid, 13% short, 12% intermediate, 24% long-acting and 17% pre-mixed insulin. Use of insulin was higher in younger (< 65 yr), female, White, and Hispanic pts, as well as in those with shorter dialysis duration. Sulfonylureas were the most commonly used oral agents (18%) followed by thiazolidinediones (TZDs) (13%). Metformin was used in 0.7% of pts and exenatide in 0.1% of pts. Medications to be used with caution (chlorpropamide, tolazamide, tolbutamide) were used by < 0.2% of pts. Sulfonylurea use was higher in older (≥ 45 yr), White, Asian/Pacific Islander, and Hispanic pts.; use was lower in those with longer dialysis duration. TZD use was higher in older (≥ 45 yr), American Indian/Alaska Native, Asian/Pacific Islander and Hispanic pts. Across ESRD Networks, there was variation in use of insulin (47-57%), sulfonlyureas (15-26%) and TZDs (9-21%). During 2007, average total costs per patient per year was about $660 for insulin, $88 for sulfonylureas, and $1088 for TZDs. Average out-of-pocket cost per prescription was $9.26 for insulin, $2.49 for sulfonylureas, and $12.67 for TZDs. **Conclusions:** Despite contraindications, metformin and exenatide are being used in some dialysis patients. Variation exists in approaches to anti-diabetic drug treatment in adult Medicare beneficiaries receiving dialysis.

Disclosure of Financial Relationships: Consultancy: Ono PharmaResearch Funding: Chronic Disease Research Group receives research funding from Amgen, Mitsubishi Tanabe Pharma America, Inc.; Honoraria: American College of Clinical Pharmacy, Medical Communications Media, Foundation for Managed Care Pharmacy.

**F-PO1516**

**Calcium-Based Phosphate Binders Are Associated with Better Outcomes in Haemodialysis Patients: Results from the French ARNOS Study**

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**Background:** A short-term favourable effect of phosphate binders (PB) on incident HD has recently been reported (1). The association between the use of calcium-based phosphate binders (CBPB) and risk for vascular calcification remains unclear.

**Objective:** To assess the impact of prescribing PB, using CBPB or sevelamer HCl (SV), on survival in a French HD cohort.

**Methods:** Baseline PB prescription was recorded using a cross-sectional analysis of patients in 25 centres from the regional ARNOS French cohort. A prospective 42-month survival analysis study was performed according to PB, CBPB (Caco3) and SV, using crude and adjusted models.

**Results:** In July 2005, 1347 HD patients (mean age, 67.3 ± 14 years) on dialysis since 63 ± 75 months were included in this study. CBPB was prescribed in 55% of cases, SV was prescribed in 42% of cases, a mixed PB was prescribed in 24% of cases, and 26% of all patients were not prescribed PB. By using crude survival analysis, we found that compared to the group without PB prescription, the group prescribed with PB was associated with less mortality: hazards ratio (HR), 0.69 [0.57–0.89]; in case of CBPB: HR, 0.7 [0.6–0.85], and in the case of SV: HR, 0.8 [0.67–0.96]. Using Cox proportional model adjusted for age, dialysis vintage, gender, diabetes, calcaemia, phosphataemia, PTH, and comorbidities, PB prescription was found to be associated with less mortality (HR, 0.7 [0.5–0.83]), especially in the case of CBPB (HR, 0.64 [0.4–0.78]), but not in the case of SV (HR, 1.13 [0.92–1.3]). A mixed PB prescription was not associated with outcomes (HR, 0.92 [0.7-1.18]).

**Conclusion:** PB prescription is associated with a favourable effect on survival in a French HD population. This favourable impact is mainly associated with the prescription of calcium carbonate. Further studies are needed to confirm these results in dialysis patients.

*1) Isakova T., et al J Am Soc Nephrol 20: 388–396, 2009.*

Disclosure of Financial Relationships: Employer: my wife works for Merk laboratory; Consultancy: fresenius medical care, genzyme; Ownership: noneResearch Funding: Amgen; Honoraria: Shire, Amgen, Fresenius, Genzyme; Patent: none; Scientific Advisor: Fresenius medical care, Amgen, Genzyme; Other Relationship: none.

**F-PO1517**

**Use and Costs of Phosphate Binders in U.S. Dialysis Patients with Medicare Part D in 2007**

Wendy L. St. Peter,[1,3] Christopher Powers,[2] Eric D. Weinhandl,[1] Benjamin L. Howell,[2] James P. Ebben,[1] Diane L. Frankenfield.[2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]ORDI, CMS, Baltimore, MD; [3]College of Pharmacy, University of Minnesota.*

**Background:** Management of hyperphosphatemia in dialysis patients (pts) has been associated with lower mortality risk. Use of phosphate binders is an important component of managing hyperphosphatemia. **Methods:** We assessed use and costs of selected phosphate binders in adult U.S. dialysis pts in 2007 with Part D data from Medicare's Chronic Condition Warehouse linked to administrative data from the United States Renal Data System. All pts alive on December 31, 2007, with Part D coverage during all of 2007, were included (n=169,443). **Results:** Overall, 79% of pts used the following phosphate binders, with 50%, 39% and 13% using sevelamer, calcium acetate (CA), and lanthanum, respectively. Binder use was higher in younger (< 65 yr), female, Asian/Pacific Islander, and Hispanic pts, as well as in those with longer dialysis duration (≥ 2 yr) and pts dialyzing in for-profit facilities. There was also variation in binder use by facility affiliation, with pts dialyzing in the facilities of a large dialysis organization (LDO) having highest binder use. CA use was higher in White, Asian/Pacific Islander, and Hispanic pts, as well as in those dialyzing in DCI facilities. CA use decreased as dialysis vintage increased. For both sevelamer and lanthanum, use was higher in younger (< 65 yr) pts, as well as in those with greater dialysis duration and in pts dialyzing in LDO facilities. Across ESRD Networks, binder use ranged from 68 to 84% (CA 32 to 57%; sevelamer 38 to 64%; lanthanum 9 to 21%). Average total cost per person per year was about $2007 for all phosphate binders, with $420 for CA, $2442 for sevelamer, and $1596 for lanthanum. Average out-of-pocket cost per prescription was $17.39 for all phosphate binders, $11.39 for CA, $20.79 for sevelamer and $21.49 for lanthanum. **Conclusions:** Despite highest total cost per patient per year, sevelamer was the predominant phosphate binder used in 2007. Part D data from 2007 indicate there was variation in use of phosphate binders by geographic region, in addition to some patient and facility characteristics.

Disclosure of Financial Relationships: Consultancy: Ono PharmaResearch Funding: Chronic Disease Research Group receives research funding from Amgen, Mitsubishi Tanabe Pharma America, Inc.; Honoraria: American College of Clinical Pharmacy, Medical Communications Media, Foundation for Managed Care Pharmacy.

**F-PO1518**

**Factors Associated with Cinacalcet Initiation in Hemodialysis (HD) Patients**

Wendy L. St. Peter,[1,2] David A. Zaun,[1] Craig Solid,[1] Ryan D. Kilpatrick,[3] Jiannong Liu,[1] Kimberly M. Nieman,[1] Britt B. Newsome.[4] *[1]Chronic Disease Research Group, MMRF, Minneapolis, MN; [2]U of MN; [3]Amgen, Inc., Thousand Oaks, CA; [4]Denver Nephrologists, P.C., CO.*

**Background:** Many dialysis patients (pts) receive cinacalcet for secondary hyperparathyroidism. Objectives of this study were to describe pt characteristics and predictors associated with cinacalcet prescription to inform case-mix adjustment pertaining to cinacalcet inclusion in proposed end-stage renal disease (ESRD) prospective payment system (PPS). **Methods:** We used a linked DaVita and Centers for Medicare and Medicaid Services dataset with 45,589 prevalent HD pts in August 2004 with follow-up through July 2007. Pts had Medicare as primary payer and used IV vitamin D in baseline period (Aug. 1 to Oct. 31, 2004). Pts with modality change or kidney transplant were excluded. Pts were grouped by parathyroid hormone (PTH) baseline categories (<150, 150-300, >300-600, >600 pg/mL). Adjusted Cox regression was used to predict time to cinacalcet initiation. **Results:** 17,576 non-cinacalcet and 7,674 cinacalcet pts were included. Cinacalcet pts were younger, had longer vintage, were more often African American (AA), used more AV fistulas, had higher median PTH, mean corrected calcium and phosphorus, lower comorbidity burden, slightly higher albumin, higher hemoglobin, lower WBC, higher weekly IV vitamin D and daily phosphate binder doses, more consistent IV vitamin D use, less diabetes as ESRD cause. Characteristics in cinacalcet pts and nonusers varied by PTH category at baseline, e.g. in PTH>600 pg/mL category, consistency of IV vitamin D use was higher in nonusers. Initial doses >30 mg/day were more often prescribed in younger, higher vintage, and AA pts. Lower doses were more often prescribed in pts with diabetes. The strongest predictors of cinacalcet prescription were younger age, female gender, African-American race, vintage 5 years, higher body mass index, AV fistula use, PTH >300 pg/mL, corrected calcium >10.2 mg/dL, phosphorus 5 mg/dL, and sevelamer >7200 mg/day. **Conclusions:** Cinacalcet is preferentially used in AAs. Payment policy for ESRD-related oral medications under PPS should take this into account.

Disclosure of Financial Relationships: Consultancy: Ono PharmaResearch Funding: Chronic Disease Research Group receives research funding from Amgen, Mitsubishi Tanabe Pharma America, Inc.; Honoraria: American College of Clinical Pharmacy, Medical Communications Media, Foundation for Managed Care Pharmacy.

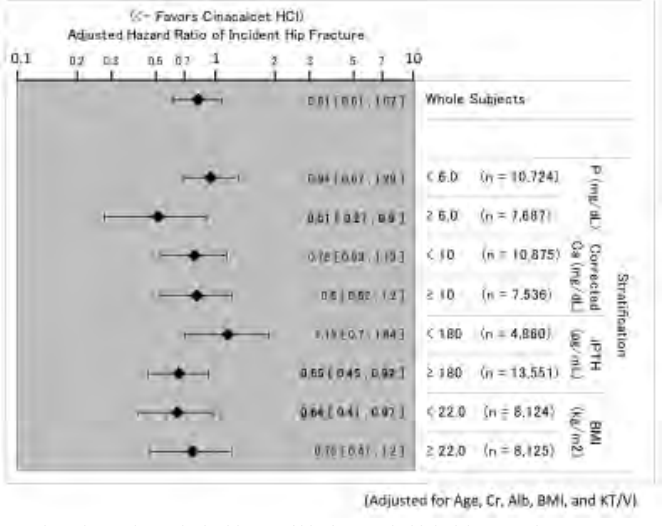
**F-PO1519**

**One-Year Administration of Cinacalcet HCl and Effect on Hazard Ratio of Incident Hip Fracture in Large Japanese Hemodalysis Cohort** Naohiko Fujii, Takayuki Hamano, Masatomo Taniguchi, Takashi Shigematsu, Shigeru Nakai, Kunitoshi Iseki, Yoshiharu Tsubakihara. *Nephrology Department, Committee of Renal Data Registry of the Japanese Society for Dialysis Therapy, Tokyo, Japan.*

Cinacalcet reduces the incidence of bone fracture according to the combined analysis of four randomized clinical trials (KI 2005). It is not clear whether we have similar results in daily practice, or in the real world.

We used the nationwide Japanese Renal Data Registry surveyed at the end of year of 2007 and 2009. The prevalence of hip fracture was studied in these surveys, making it possible to calculate the incidence of hip fracture for 2 years by subtraction in those who had never experienced hip fracture at the time of 2007. Cinacalcet became available at the beginning of 2008 in Japan. The prescription of cinacalcet was retrospectively studied at the end of 2009. Enrolled patients are those who underwent HD 3 times/week and had been dialyzed for at least 1 year. Those who had a history of peritoneal dialysis, kidney transplantation, parathyroidectomy, or PEIT for hyperparathyroidism were excluded from the analysis. As a per-protocol analysis, we treated patients as treated who had been receiving this drug for more than 1 year. We made matched control group of patients who had never received this drug using propensity score based on the data collected at the end of 2007, when cinacalcet was not available.

Cinacalcet Group consists of 6,137 patients and 12,274 control patients were selected (1:2 matching). Cox regression model revealed that hazard ratio (HR) of incident fracture in cinacalcet treated patients was 0.81 (95% C.I. 0.61, 1.07). In subgroup analysis for those with intact PTH>180 pg/mL, or serum phosphate>6.0 mg/dL, and BMI<22.0, the HR was significantly lower than 1.0.




Cinacalcet reduces the incidence of hip fracture in high risk HD patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1520**

**Cinacalcet Hydrochrolide Reduces Mortality in Hemodialysis Patients with Secondary Hyperparathyroidism** Takayuki Hamano, Naohiko Fujii, Masatomo Taniguchi, Takashi Shigematsu, Shigeru Nakai, Kunitoshi Iseki, Yoshiharu Tsubakihara. *Committee of Renal Data Registry of the Japanese Society for Dialysis Therapy, Tokyo, Japan.*

Pooled analysis of four randomized controlled trials revealed that cinacalcet reduces cardiovascular hospitalization in dialysis patients with secondary hyperparathyroidism. However, it is not known whether it reduces mortality in the real world.

We used the nationwide dialysis registry (Japanese Renal Data Registry) surveyed at the end of year of 2007, 2008, and 2009. The number of surveyed patients was 290,675 at the end of 2009. The prescription of cinacalcet was retrospectively studied at the end of 2009. Enrolled patients are those who underwent hemodialysis 3 times/week and had been dialyzed for at least 2 years. Prior to analysis, we excluded those with a history of parathyroidectomy or percutaneous ethanol injection therapy. In intention to treat analysis, we treated these patients as treated who have ever received cinacalcet (Cin Group). Therefore, even patients who had quitted this drug because of adverse effect were included in Cin Group. We made matched control group of patients who had never received this drug using propensity score based on the data collected at the end of 2007, when cinacalcet was not available in Japan. Age, sex, dialysis vintage, diabetic status, serum creatinine, albumin, corrected calcium, phosphate, PTH, CRP, BMI, Kt/V, history of amputation, bone fracture, congestive heart failure, myocardial infarction were matched. Cin Group consists of 5,278 patients and 10,556 control patients were selected (1:2 matching). One-year all-cause mortality (from the end of 2008 to the end of 2009) was compared between control group and Cin Group. Cox proportional hazard model revealed that hazard ratio of cinacalcet was 0.41 (95% CI 0.20, 0.84, P value 0.01). Using Poisson regression model did not alter the result at all.

These results indicate for the first time that cinacalcet reduces mortality in hemodialysis patients with secondary hyperparathyroidism in the real world. Future studies will be needed as to which therapy is more beneficial in reducing mortality, cinacalcet or parathyroidectomy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1521**

**Efficacy and Safety of Paricalcitol Injection (PC) and Maxacalcitol Injection (OCT) in Subjects with Secondary Hyperparathyroidism Receiving Hemodialysis (HD)** Tadao Akizawa,[1] Michael Amdahl,[2] Samina Khan,[2] Utpaul Audhya.[2] *[1]Showa University School of Medicine, Representative of ABT-358 Study Group; [2]Abbott Laboratories.*

**Purpose:** The Japanese Society for Dialysis Therapy recommends an intact parathyroid hormone (iPTH) level between 60 and 180 pg/mL for HD patients. This study compared the efficacy and safety of PC to OCT in Japanese HD subjects with SHPT.

**Methods:**

This was an open-label, multi-center, 12-week study. Eligible subjects (iPTH: ≥300 pg/mL, Ca 8.4 ≤ - <10.2 mg/dL, and P ≤6.5 mg/dL after washout) were allocated to PC or OCT treatment groups in a 1:2 ratio (PC to OCT) based on iPTH at screening. Study drug was injected 3 times a week. The PC group received an initial dose of 2 µg, with dose adjustments in 1 µg increments. The OCT group received an initial dose of 5 µg or 10 µg based on iPTH (<500 pg/mL, and ≥500 pg/mL), with dose adjustments in 2.5 µg increments. The OCT was not held per label for an iPTH level <150 pg/mL in this study. Subjects were titrated to an iPTH level of 60-180 pg/mL. Efficacy was assessed in 45 subjects who completed ≥8 weeks of therapy. Safety was evaluated in all subjects. Efficacy assessments used the mean iPTH value during the last 3 weeks of treatment.

**Results:**

Of the 47 subjects, 14 received PC and 33 received OCT. Three subjects were withdrawn due to adverse events (1 PC and 2 OCT subjects). Efficacy rates in PC group were higher than those in the OCT group in all predefined efficacy endpoints (Table 1). The incidence rates of hypercalcemia (Ca >11.5 mg/dL or 2 consecutive values ≥11.0 mg/dL) in PC and OCT groups were 0% and 9.1%. The incident rates of hyperphosphatemia (2 consecutive values ≥7.0 mg/dl) in PC and OCT groups were 14.3% and 12.1%.

Table: Efficacy of PC Group Compared to OCT Group

| Efficacy endpoints | PC %(n) | OCT %(n) |
|---|---|---|
| Proportion of subjects with ≥ 50% reduction in iPTH | 53.8 (7/13) | 37.5 (12/32) |
| Proportion of subjects with iPTH target range of 60-180 pg/mL | 30.8 (4/13) | 15.6 (5/32) |
| Proportion of subjects with ≥ 50% reduction in iPTH and without hypercalcemia | 53.8 (7/13) | 34.4 (11/32) |
| Proportion of subjects with iPTH target range of 60-180 pg/mL and without hypercalcemia | 30.8 (4/13) | 12.5 (4/32) |

**Conclusion:**

The study suggests that PC has better efficacy and less hypercalcemia compared to OCT. These findings must be confirmed in a larger study.

Disclosure of Financial Relationships: Consultancy: Chugai, Kirin,AbbottResearch Funding: Chugai, Kirin.

**F-PO1522**

**Active Vitamin D3 (VitD) Administration and Insulin Resistance in Chronic Hemodialysis (CHD) Patients** Adriana Hung,[1,2] Mary B. Sundell,[1] Natalia E. Plotnikova,[1] Aihua Bian,[2] Ayumi Shintani,[2] Edward Siew,[1] Charles D. Ellis,[1] Feng Sha,[1] T. Alp Ikizler.[1,2] *[1]Medicine, Vanderbilt University, TN; [2]Biostatistics, Vanderbilt University, TN; [3]Nashville VA, TN.*

Background: Active vitamin D administration in CHD patients has been the mainstay to control secondary hyperparathyroidism. However it has been recently recognized that Vitamin D deficiency is linked to increased cardiovascular risk both in the general population and in CHD patients. One of the postulated metabolic mechanisms implicated in the increased risk include its effects on insulin resistance (IR).

Design: In a pilot double blinded randomized clinical trial, 10 prevalent CHD patients (52.9 years old, 100% African American, 33% females, 33% with history of diabetes, body mass index 34.8 kg/m2) on stable VitD treatment were taken off their VitD for 8 weeks. iPTH levels were kept at baseline levels (within 10%) by administration of Cinacalcet as needed. At the end of 8 weeks, patients were randomly assigned to continue Cinacalcet or to restart an active vitamin D analog. The primary outcome was insulin resistance assessed by glucose disposal rate (GDR) measured by hyperinsulinemic euglycemic clamp (HEGC). Other important measures included other indirect indices of IR and serum adipokines.

Results: PTH levels were not associated with GDR at baseline (p=0.7). There was no detectable change in the GDR after withholding VitD at Week 8; p=0.7). There was also no effect of treatment assignment on GDR (Week 8 to Week 16 period; p=0.8). Similar results were achieved using more practical indices of IR such as HOMA, HOMA AD, leptin-adiponectin ratio (LAR) or QUICKI. Propensity scores were used in all analysis to balance clinical characteristics between groups and prevent selection bias.

Conclusion: This study showed no effect of Vitamin D on IR measured by HEGC or on indirect indices of IR while on a background of Cinacalcet.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1523

**Decreased Epoetin Utilization Associated with Conversion to Ferumoxytol IV Iron Protocol** Amit Sharma,[1] Betsy J. Lahue.[2] *[1]Boise Kidney and Hypertension Institute, Boise, ID; [2]AMAG Pharmaceuticals, Lexington, MA.*

**Purpose**: To evaluate drug utilization and anemia outcomes with conversion to a new IV iron protocol.

**Methods**: A retrospective chart review selected chronic hemodialysis patients receiving ferric gluconate or ferumoxytol from May 2009-May 2010 at two clinics. Demographics, iron indices, hemoglobin values, IV iron and epoetin (EPO) doses were collected; patients with no lab values were excluded. Conversion was defined as the date when >80% of the unit's patients received ferumoxytol; January 2010 for Site 1, October 2009 for Site 2. Descriptive statistics were generated to compare values in pre- and post-conversion months.

**Results**: Review of 167 charts identified 155 patients for study, 85.2% received ≥ 1 IV iron dose. Site 1 had 8 months of pre-conversion data and 5 months post, while site 2 had 5 months pre- and 8 months post data. Compared to site 2, site 1 population was slightly older (63 v. 62 years), had similar dialysis vintage (1.5 median years), had more female patients (51% v. 45%) and had more patients of Caucasian ethnicity (82% v. 66%). Anemia management protocols for EPO and IV iron were identical for site 1 and 2. Comparing pre and post conversion values for all months available, both units used less EPO per patient; the mean monthly EPO (IU) total dose was reduced 19% at Site 1 (41,760 pre v. 33,929 post p=.08) and 26% in Site 2 (75,227 pre v. 55,538 post, p<0.01). Comparing cumulative IV iron utilization for equal time frames (5 months pre and 5 months post), following conversion the total grams consumed decreased by 27% in Site 1 and increased by 40% in site 2. Pooling the 2 sites, 5 month iron utilization increased 18% (123 g pre to 145 g post). Iron indices remained stable post conversion, the mean unit hemoglobins (g/dL) were consistent (11.2 pre to 11.2 post, p=.75) and mean TSATs increased slightly (33% pre to 36% post).

**Conclusions**: In hemodialysis patients, changing IV iron protocol from ferric gluconate maintenance to ferumoxytol bolus was associated with increased total iron use and decreased EPO utilization while maintaining anemia outcome parameters within target ranges.

Disclosure of Financial Relationships: Consultancy: Amgen, AmagResearch Funding: AMAG, AFFYMAX, Luitpold, AMGEN; Honoraria: AMGEN, AMAG; Scientific Advisor: AMAG, AMGEN.

### F-PO1524

**Increased Ferumoxytol Utilization Associated with Decreased Total Anemia Treatment Utilization** Amit Sharma,[1] Betsy J. Lahue,[2] Kellie Y. Becker,[3] Denise Vanvalkenburgh.[3] *[1]Boise Kidney and Hypertension Institute, Boise, ID; [2]AMAG Pharmaceuticals, Lexington, MA; [3]Liberty Dialysis, Mercer Island, WA.*

**Purpose:** To evaluate the impact of implementing a ferumoxytol protocol for treatment of iron deficiency anemia on the utilization of iron, epoetin (EPO) and anemia outcomes.

**Methods:** Anemia treatment utilization was collected across a medium-sized dialysis organization (MDO) from October 2009 through March 2010. Data abstracted was summarized at unit level on monthly and quarterly basis including total patients treated, proportion treated with each anemia drug, total IV doses, total EPO units, and unit hemoglobin labs. Total utilization across MDO sites and mean hemoglobin values per clinic per quarter were compared.

**Results:** From October to December 2009 (Q4), ferumoxtol treatment was 4% of the iron administrations and 22% of total MDO IV iron grams consumed. During Q4 2009, ferric gluconate and iron sucrose comprised 96% of IV iron administrations (47% and 31% of grams, respectively). From January to March 2010 (Q1), ferumoxytol use doubled to 48% of total grams. Comparing Q4 2009 to Q1 2010, the MDO treated more patients (4576 v. 5221) with a higher proportion of patients receiving treatments of iron (53% v. 65%) and EPO (86% to 94%). Total anemia drug utilization decreased for both iron and EPO from Q4 to Q1, with units consumed per treated patient decreasing by 20% for EPO (214,138 to 170,341 IU) and 29% iron (1316 to 933 mg). Hemoglobin values were available for approximately 90% of the patients Q4 and Q1, demonstrating similar proportions of unit population met targets (77% v 78% Hbg 10-12 g/dL, 5% v. 5% Hbg<10 g/dL).

**Conclusions:** As a MDO implemented broader ferumoxytol use and increased patients treated for anemia, treatment utilization decreased and unit populations remained within desired anemia management parameters. Results suggest increased efficiency in anemia treatment protocols, however further research is required to determine ferumoxytol's impact on per patient utilization.

Disclosure of Financial Relationships: Consultancy: Amgen, AmagResearch Funding: AMAG, AFFYMAX, Luitpold, AMGEN; Honoraria: AMGEN, AMAG; Scientific Advisor: AMAG, AMGEN.

### F-PO1525

**Current Status of Dialysis Initiation and Survival in Japan: Effect of Transfer and Facility** Norio Hanafusa, Hiroshi Nishi, Kunihiro Yamagata, Toshio Shinoda, Kunitoshi Iseki, Yoshiharu Tsubakihara. *The Committee of Renal Data Registry, Japanese Society for Dialysis Therapy, Tokyo, Japan.*

**Background:** Little is known about the dynamics and the relationship on survival in Japanese incident hemodialysis (HD) patients.

**Study protocol:** Observational retrospective study using Japanese Society for Dialysis Therapy (JSDT) registry data.

**Methods:** We used a standard analysis file from the JSDT registry database (JRDR-09104). All incident HD patients in 2006 were included (N=34,682). The outcome was examined in 2007. The status of transfer was evaluated as comparing both the HD initiated unit and the year-end HD unit in 2006. We examined relationship between the dynamics of the patients and one-year mortality using logistic analyses adjusted for age, sex and others. We excluded those without pertinent information.

**Results:** A total of 32,398 were included in this study and 86.2% of them started HD at hospital and the rest of them at clinics. The vast majority of the facilities (81.1%) had one or more incident patients. Those who were transferred from the original HD unit during 2006 were 40.8%. The "hospital HD initiation" and "remained at the original HD unit" groups were significantly poor survival, OR 3.84: 95% CI 2.78–5.44 and OR 7.19: 95% CI 4.48–12.5, respectively. Both groups experienced significantly higher rates of death from infection (both p<0.05). Those who began HD at hospitals and remained at the original HD unit showed higher mortality rate at shorter duration of HD. However, in other groups of incident HD patients the causes of death were evenly distributed and lower rate of mortality

**Discussion:** This is the first report on the dynamics of incident HD patients and the relation between the patient dynamics and the survival in Japan. Nursing home residents were reportedly poorest survival after initiating HD [NEJM 361:1539, 2009]. The dynamics of incident HD might warrant further information that covers more socio-economic conditions.

**Conclusions:** Results showed that the dynamics of incident HD patients has impact on survival. Reasons for poorer survival among incident HD patients who did not move remained to be determined.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1526

**White Blood Cell Count Relates to Dialysate Glucose Concentration in Chronic Hemodialysis Patients** Jochen G. Raimann,[1,2] Len A. Usvyat,[1] Stephan Thijssen,[1,2] Nathan W. Levin,[1] Peter Kotanko.[1,2] *[1]RRI, NY; [2]BIMC, NY.*

**Background**

In 2009, over the course of several months, all US hemodialysis (HD) clinics of the Renal Research Institute comprehensively switched dialysates from 200 mg/dL glucose (G200) to 100 mg/dL glucose (G100). Dialysate glucose concentrations above the serum glucose level can lead to diffusive shifts of glucose and raise the serum level (Schneditz, ASAIO 2010). Elevated serum glucose induces oxidative stress and secretion of pro-inflammatory cytokines (e.g. TNF-α, IL-6, IL-8). This is particularly pronounced after acute short-term hyperglycemia (Esposito, Circulation 2002) and may lead to an increase of WBC (van Oostrom, J Lipid Res 2003). The present study investigates the relationship between the switch from G200 to G100 and WBC and neutrophil-lymphocyte ratio (NLR) in chronic HD patients.

**Methods**

WBC and NLR were obtained six months before ($M_{-6}$) and six months after ($M_{+6}$) the change from G200 to G100. Individual differences in WBC and NLR between $M_{+6}$ and $M_{-6}$ were assessed by paired T-test and by multivariate analysis adjusted for age, dialysis vintage, gender, race, diabetes status, change in dialysate to serum sodium gradient, equilibrated normalized protein catabolic rate (enPCR) and post HD weight. A 12 months interval was chosen to account for potential seasonal variability in patient parameters. Data are displayed as mean±SD.

**Results**

We enrolled 1664 patients (age 61±15 yrs, vintage 4±3 yrs, 755 Blacks, 829 diabetic subjects). Univariate analysis showed significant differences of WBC ($M_{-6}$: 6.7±2.1 * $10^3$/$mm^3$ vs. $M_{+6}$: 6.6±2.2 * $10^3$/$mm^3$; P<0.01), but no significant differences of NLR ($M_{-6}$: 3.3±3.3 vs. $M_{+6}$: 3.5±2.4; P=0.14). This drop in WBC remains significant after multivariate adjustment (change in WBC between $M_{-6}$ and $M_{+6}$: -0.11 * $10^3$/$mm^3$ (95% CI -0.12 to -0.10; P<0.001).

**Conclusion**

This study reveals a small but significant decline of WBC following the reduction of dialysate glucose from 200 to 100 mg/dL. We hypothesize that this decline in WBC is related to the reduced prevalence of dialysis-induced hyperglycemia with the use of G100 dialysates.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1527**

**Blood Pressure Prevalence and Control in the General Population and the Incidence of Renal Replacement Therapy (RRT): An Ecological Study of 192 Health Areas** Clare I. Castledine,[1] Julie A. Gilg,[1] Charles Tomson,[2] David Ansell,[1] Fergus J. Caskey.[2,3] *[1]UK Renal Registry, Bristol, United Kingdom; [2]Richard Bright Renal Unit, Southmead Hospital, Bristol, United Kingdom; [3]Clinical Science North Bristol, University of Bristol, United Kingdom.*

**Introduction:** UK healthcare is commissioned via 192 health areas. The UK Renal Registry (UKRR) collects data electronically on all RRT patients and has reported UK-wide variation in RRT incidence. Since 2004 the Quality Outcomes Framework (QOF), a pay-for-performance scheme for primary care physicians, provides data on the prevalence and control of hypertension in the general population.

**Methods:** An ecological design was used, with each health area contributing an observation. RRT incidence per area in 2007-2008 was obtained from the UKRR database and standardised for age and gender. The prevalence of known hypertension in the general population and the percentage of patients achieving BP<150/90 in each health area were obtained from QOF.

**Results:** The 192 health areas had a median population of 255,900 (IQR, 184,000-390,000). There was an average of 6642 new patients starting RRT each year. Each 1% point rise in hypertension prevalence was associated with 2% higher incidence of RRT (incidence rate ratio (IRR) 1.02, 95%CI 1.01-1.03 p<0.0001). After adjustment for area-level ethnicity and socio-economic deprivation the IRR was 1.06, 95%CI 1.05-1.07 p<0.0001 and after adjustment for life expectancy & cancer mortality IRR was 1.08, 95%CI 1.06-1.1 p<0.0001. Each 1% point rise in percentage of hypertensive patients achieving BP<150/90 was associated with reduced RRT risk (IRR 0.94, 95%CI 0.93-0.95 p<0.0001). After adjustment for area-level ethnicity and deprivation each 1% point rise was associated with an IRR of 0.93,95%CI 0.92-0.94 p<0.0001 and after adjustment for life expectancy/cancer mortality IRR was 0.95 ,95%CI 0.94-0.96p<0.0001.

**Conclusions:** In the UK a 1% point increase in hypertension prevalence was associated with up to 8% higher RRT incidence. Furthermore every 1% point increase in the percentage of registered hypertensive patients achieving moderate BP control was associated with 5% fewer patients commencing RRT.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1528**

**Hemodialysis in a Satellite Unit: Clinical Performance Target Attainment and Health-Related Quality of Life** Michael Diamant,[1,2] Ann Young,[1,2] Kerri Gallo,[1] Wang Xi,[3] Rita Suri,[1,3] Amit X. Garg,[1,2,3] Louise M. Moist.[1,2,3] *[1]Division of Nephrology, Department of Medicine, London Health Sciences Centre, London, ON, Canada; [2]Epidemiology & Biostatistics, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada; [3]Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada.*

Background and Objectives: In Canada, patients are increasingly receiving hemodialysis in satellite units, which are closer to their community but further from tertiary care hospitals and their nephrologists. The process of care is different in the satellites with fewer visits from nephrologists and reliance on remote communication. The objective of this study is to compare clinical performance target attainment and health related quality of life (HRQOL) in patients receiving hemodialysis in satellite versus in-centre units.

Design, setting, participants, and measures: The London Health Sciences Centre in London, Ontario, Canada has both tertiary care centre and satellite hemodialysis units. All eligible patients who received dialysis treatment at one of these units as of July 24, 2008 were enrolled into a cross-sectional study (n=522). Patient attainment of hemoglobin, albumin, calcium-phosphate (Ca-P) product, Kt/V, and vascular access targets were compared. Participants were also administered the Kidney Disease Quality of Life Short-FormTM questionnaire.

Results: Patients in both units had similar demographics while in-centre patients had a higher comorbidity index score (5.4 vs. 4.6 [p= 0.005]). Satellite patients were more likely to attain clinical performance targets for albumin (adjusted odds ratio [OR]= 4.87 [2.13, 11.14]), hemoglobin (OR=1.59 [1.08, 2.35]), and Ca-P product (OR=2.02 [1.14, 3.60]), as well as for multiple targets (p < 0.05). HRQOL scores were largely similar between groups.

Conclusions: Patients receiving hemodialysis in a satellite unit were just as likely, or more likely, to demonstrate attainment of clinical performance targets as those dialyzing in-centre, while maintaining a similar HRQOL. This supports the increased use of satellite units to provide care closer to the patient's community.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1529**

**Novel MRSA Reduction Practices in Inpatient Renal Care** Mark G. Parker,[1] August Valenti,[1] Sheila Parker,[1] Rosalie Blenkhorn,[1] Michelle Duval,[1] Curt Lindberg,[2] Mark Munger,[2] Peter Chingos,[1] Kimberly Harvey,[1] Bradley N. Doebbeling.[3] *[1]Maine Medical Center, Portland, ME; [2]Plexus Institute, Bordertown, NJ; [3]Regenstrief Institute, Indianapolis, IN.*

The MRSA Reduction Collaborative is an AHRQ-funded seven hospital Quality Improvement consortium tasked to study the application of systems improvement and positive deviance to reduce MRSA transmission. In July 2009, Maine Medical Center introduced Toyota LEAN methods and social/behavioral change dialogues on the medical renal floor and inpatient dialysis unit to identify effective practices to reduce MRSA transmission. A core group facilitates identification and implementation of prevention strategies determined by frontline personnel. Process and outcome measures include total practice changes, hand hygiene compliance, admission and discharge nasal and peri-rectal MRSA screening rates, and new asymptomatic conversions to MRSA carriage. Since the introduction of the collaborative, at least 90 practice changes have been implemented. Staff compliance with hand hygiene prior to patient encounters has increased from 77% to 90.5% ($p$=.0001). Admission and discharge MRSA screening rates have increased from 61.6% to 90.5% ($p$<.0001) and 68.5% to 77.3% ( $p$=.0043), respectively. Conversions have fallen from 1.49/1000 patient days immediately prior to intervention to 0.80/1000 patient days over the most recent two quarters. Poisson model estimates for MRSA conversions revealed that in the period since the intervention(s), there has been an 11% ( $p$=.570) decrease in the expected number of MRSA conversions, an estimate of about .89 (95% CI .61-1.32) conversions per quarter year compared to about 1.01 (95% CI .72-1.31) prior to the collaborative. A combined LEAN and positive deviance approach for reduced MRSA transmission has resulted in quantifiable process improvement on an acute care nephrology floor and dialysis unit. Though reduction in MRSA transmission is not yet definitively demonstrated in these preliminary analyses, lack of increase in the incidence of new MRSA transmissions in the context of intensified screening suggests the potential efficacy of this strategy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1530**

**Small Solute Clearance in Obese Pediatric HD Patients** Colin T. White,[1] Diane L. Frankenfield,[2] Stuart Goldstein,[3] Cherry Mammen,[1] Alicia M. Neu.[4] *[1]BC Children's Hospital; [2]Centers for Medicaid & Medicare Services; [3]Cincinnati Children's Hospital and Medical Center; [4]Johns Hopkins Medical Institutions.*

CMS' ESRD Clinical Performance Measures Project collected data on all prevalent in-center pediatric (0- <18 yrs) HD pts since 2000. Utilizing 2000-2008 data, we assessed the relationships between body mass index standard deviation score (BMI SDS) and achievement of NKF/KDOQI HD adequacy targets (spKt/V≥1.2). Demographic, clinical & anthropometric characteristics of the population were compared across years. Pts with BMI <85% were compared to overweight (85%< BMI<95%) & obese (BMI >95%) pts. Logistic regression modeling with spKt/V as the outcome was performed with age, race, gender, ethnicity, post dialysis BMI SDS, dialysis vintage, time delivered and access type as covariates. 3399 unique pt had data submitted > 1 study year. Obesity prevalence did not vary by year (9.7-12%). Table 1 describes relevant characteristics of the 3 cohorts. The final regression model excluded all patients with a dialysis vintage <6 months. In the final fully adjusted model, overweight (adjusted odds ratio 0.61, [95% CI 0.41, 0.91]) or obese (0.27, [0.20,0.38]) pts were less likely to achieve spKt/V≥1.2. Male gender (0.57 [0.44,0.75]) and use of catheter as access (0.61 [0.45,0.81]) were associated with a decreased likelihood of achieving a spKt/V≥1.2. High percentages of pediatric HD pts in the US are overweight or obese & less likely to achieve NKF/KDOQI thrice weekly HD adequacy targets for small solute clearance. Our data suggest longer dialysis times will be required to achieve a spKt/V≥1.2 in these populations.

| | BMI Percentile | | | |
|---|---|---|---|---|
| | <85th | 85 to < 95th | ≥95th | p-value |
| | n=4539 | n=496 | n=628 | |
| % of Population | 80.1 | 8.8 | 11.1 | |
| Mean Age, Years (SD) | 13.8 (4.1) | 15.2 (2.0) | 15.2 (2.6) | <0.0001 |
| Mean Dialysis Vintage, years (SD) | 3.2 (3.7) | 2.5 (3.1) | 2.1 (2.7) | <0.0001 |
| % on Dialysis <6mnths | 18 | 22 | 25 | <0.0001 |
| Mean Dialysis Time delivered, min (SD) | 200.6 (32.7) | 205.9 (31.9) | 214.3 (34.5) | <0.0001 |
| % Catheter as Access | 60 | 54 | 58 | 0.037 |
| % spKt/V ≥1.2 | 89 | 84 | 73 | <0.0001 |

Disclosure of Financial Relationships: nothing to disclose

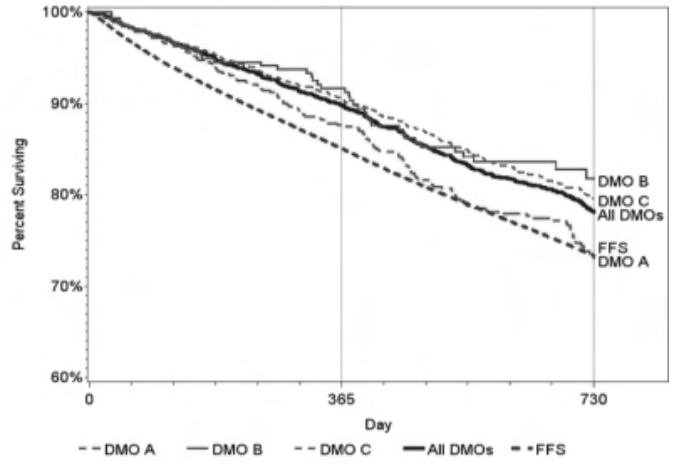
**F-PO1531**

**Hospitalization, Survival and Transplant-Related Outcomes in the Centers for Medicare & Medicaid Services End-Stage Renal Disease (ESRD) Disease Management (DM) Demonstration** Jeffrey Pearson, Sylvia Paz B. Ramirez, Charlotte J. Arrington, Christine Cheu, Claudia Dahlerus, Tania Chowdhury, Brett Lantz, Sabrina K. Gomes, Friedrich K. Port. *Arbor Research.*

ESRD is an ideal target for DM due to complex comorbidities, fragmentation of care, and high morbidity, mortality, and costs. This analysis presents the impact of DM by three DM Organizations (DMOs) on hospitalization, survival and transplant-related outcomes.

Each DMO developed its own DM programs with different clinical interventions and focus areas. Clinical outcomes of patients (pts) enrolled in each DMO between 2006 and 2008 were compared with pts in traditional fee-for-service (FFS) Medicare during the same period and in the same states. Cox models were used for survival, hospitalization and transplantation percentages adjusting for pt age, sex, race, Hispanic ethnicity, cause of ESRD, ESRD vintage, modality, previous failed transplant, state of residence and comorbidity.

There were 2,364 dialysis pts in the Demonstration: 722 pts in DMO A, 268 pts in DMO B, and 1,374 pts in DMO C. We compared these pts with 477,246 dialysis pts in traditional FFS Medicare. More pts in DMOs B and C survived at 1 and 2 yrs compared to FFS.




Fewer pts in DMO C were hospitalized at 1 and 2 yrs compared to FFS with the gap increasing over time. Hospitalizations for DMOs A and B were not significantly different from FFS. Across DMOs, fewer pts were transplanted than in FFS. However, transplantation is not overseen by the DMO, unlike the wait-listing process. DMO A had significantly more pts (11%) wait-listed; DMO C did not have significantly more pts wait-listed; DMO B had significantly fewer pts wait-listed.

While the impact of DM on pt outcomes differed by DMO, the Demonstration provides evidence that DM may improve survival, hospitalization and transplant wait-listing.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1532**

**Validation of an Algorithm for Categorizing the Severity of Emergency Department (ED) Visits in the Medicare Dialysis Population** J. M. Messana,[1] S. Hunter,[1] Robert A. Wolfe,[2] T. H. Shearon,[1] V. B. Ashby,[1] John R. C. Wheeler,[1] John Kalbfleisch,[1] Jeffrey Pearson,[2] Claudia Dahlerus,[2] Richard Hirth,[1] Rajiv Saran.[1] *[1]Univ of Michigan KECC; [2]Arbor Research.*

Differentiation between emergent and non-emergent ED visits may contribute to design of efficient paradigms for utilization of medical services by dialysis patients. The New York University (NYU) algorithm uses primary ICD-9 diagnosis codes to assign severity probabilities to ED visits. Using Medicare claims data, we examined ED visits by Medicare dialysis patients from 1/1/07 to 6/30/08 (1,030,598 visits; 257,581 patients). ED visits are common among the dialysis population (avg 3 ED visits/yr). 92% of all ED visits were categorized as emergent (60%), non-emergent (30%), or intermediate (2%) based on the severity probabilities of the most emergent diagnosis of each visit (Ballard, Medical Care, 2010).

We used logistic regression to examine the relationship between this modified NYU categorization of ED visits and subsequent hospitalization ($\leq$ 1 day) and death ($\leq$ 30 days). Covariates included ED severity group, age, race, sex, ethnicity, ESRD cause, BMI, years on ESRD, and diabetes status. For each group, Table 1 shows the percent of visits followed by a hospitalization or death, and the adjusted odds of subsequent hospitalization or death, compared to non-emergent visits. Emergent and intermediate ED visits were significantly more likely than non-emergent visits to be followed by hospitalization $\leq$ 1 day and by death $\leq$ 30 days.

Table 1

| | Hospitalization <= 1 day of ED visit | | Death <= 30 days of ED visit | |
|---|---|---|---|---|
| ED Visit Severity | % | Adjusted OR | % | Adjusted OR |
| Non-emergent | 29.1 | 1.00 (ref) | 3.8 | 1.00 (ref) |
| Intermediate | 54.1 | 2.50* | 7.0 | 1.38* |
| Emergent | 69.2 | 4.83* | 10.4 | 2.32* |

* p<0.0001

The modified NYU ED severity algorithm is a valid tool for categorization of ED visit severity in this population. Having established a relationship between the severity algorithm and primary outcomes, future research based on this methodology could explore variation in appropriateness of ED utilization by region, facility and patient groups and the impacts of ED utilization on costs of care.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1533**

**Immunogenecity of Single Dose of 2009 Influenza A (H1N1) Monovalent MF59-Adjuvanted Vaccine in Dialysis Patients** Jungmin Son, Soo Bong Lee, Il Young Kim, Harin Rhee, Jung Sub Kim, Sang Heon Song, Eun Young Seong, Ihm Soo Kwak. *Internal Medicine, Pusan National University School of Medicine, Busan, Republic of Korea.*

**Backgrounds :** Governments and public health officials prepared vaccination campaigns against the 2009 influenza A (H1N1) pandemic strain. In a recent study, single dose of monovalent 2009 influenza A (H1N1) MF59-adjuvanted vaccines were highly immunogenic in healthy adults (seroprotection 92%, seroconversion 88%). But the immunogenicity of these vaccines in hemodialysis (HD) and peritoneal dialysis (PD) patients has not been evaluated.

**Methods :** Eighty-two patients (48 HD, 34 PD) were enrolled into the study. Subjects were received single dose of vaccine containing 3.75 μg hemagglutinin of MF59-adjuvanted vaccine. Serum samples were collected immediately before vaccination and 28 days later. Antibody responses were measured by hemagglutination-inhibition assay.

**Results :** Following the single dose of vaccine, seroprotection was observed in 24 of 82 patients (29.3%; 95% CI, 19.2%-39.3%) and seroconversion in 20 of 82 (29.4%; 95% CI, 14.9%-33.9%). Geometric mean fold increase (MFI) was 5.3 (95% CI, 2.9-7.8). Seroprotection rate, seroconversion rate, MFI showed no significant difference between HD and PD group (29.2% vs. 29.4%; 20.8% vs. 26.5%; 5.8 vs. 4.7, respectively). Although both seroprotection rate and seroconversion rate declined when stratified by age (41.7% and 41.7% in age 18-39; 28.6% and 21.4% in age 40-59; 25.0% and 17.9% in age over 60), there was no significant difference between HD and PD group. Sex, dialysis duration, existence of diabetes mellitus, comorbidity, dialysis duration, previous seasonal vaccination, immunosuppressive agent use were not associated with immunogenicity.

**Conclusions :** In dialysis patients, single dose of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine is insufficient to meet the vaccine licensure criteria except geometric mean fold increase. Because the immune system in dialysis patients is severly depressed and antibody response is weaker than that of the normal population, governments and public health officials should consider higher or multiple doses of vaccination.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1534**

**Pandemic (H1N1) 2009 H1N1 Vaccination in Hemodialysis Patients** Philip A. Clayton, Martin P. Gallagher, Julia M. Liang, Roger N. Wyndham, Meg J. Jardine. *Renal Department, Concord Repatriation General Hospital, Sydney, Australia.*

**Aim**: The mortality of pH1N1 infection in hemodialysis patients is around 10 times higher than in the general population, but the efficacy of pandemic (H1N1) 2009 (pH1N1) vaccine in this group is unknown. We assessed the serological response of maintenance hemodialysis patients to a single exposure to pH1N1 vaccine during a period of low influenza activity.

**Methods**: Maintenance hemodialysis patients were vaccinated in two phases. In November 2009 vaccination was offered with 15μg of intramuscular monovalent, non-adjuvanted, split-virus pH1N1 vaccine (Panvax®H1N1, CSL Biotherapies, Melbourne Australia). In March 2010 vaccination was offered with seasonal influenza vaccination containing pH1N1 vaccine. pH1N1-specific hemagglutination inhibition (HAI) titers were measured at baseline and at day 21. Endpoints were derived from FDA criteria and were a) seropositivity (titer ≥40) and b) seroconversion (prevaccination titer of <10 with postvaccination titer of ≥40, or a four-fold or higher increase in titer). Results were compared with FDA licensing criteria specifying seropositivity rates of 70% and 60% and seroconversion rates of 40% and 30% respectively, for people aged 18-64 and 65 and older, as measured by the lower limit of the 95% CI.

**Results**: Vaccination was performed using pH1N1 or the seasonal influenza vaccine containing pH1N1 in 38 and 21 participants respectively. Participants were elderly (median 75 years), predominantly male (66%) and Caucasian (76%). Diabetes was present in 47%. The baseline seroprotection rate was 20% (95% CI:11-33%). At day 21, seroprotection was demonstrated in 64% (95% CI:51-76%) and seroconversion in 68% (95% CI:54-79%) of participants. For participants aged 65 and over seroprotection was 56% (95% CI:40-71%) and seroconversion 60% (95% CI:44-75%).

**Conclusions**: Hemodialysis patients have poorer immune responses to pandemic (H1N1) 2009 vaccination than healthy volunteers following a single exposure to pH1N1 vaccine. These results suggest standard dosing in hemodialysis patients may not meet FDA licensing criteria.

Disclosure of Financial Relationships: Research Funding: My employer has received an unrestricted grant from CSL Biotherapies.

**F-PO1535**

**General Population Diabetes Mellitus and the Incidence of Renal Replacement Therapy in the UK: An Ecological Study of 192 Health Areas** Clare I. Castledine,[1] Julie A. Gilg,[1] David Ansell,[1] Charles Tomson,[2] Fergus J. Caskey.[2,3] *[1]UK Renal Registry, Bristol, United Kingdom; [2]Richard Bright Renal Unit, Southmead Hospital, Bristol, United Kingdom; [3]Clinical Science North Bristol, University of Bristol, United Kingdom.*

**Introduction:**Healthcare is administered via 192 health areas in the UK. The UK Renal Registry (UKRR) collects data electronically on all RRT patients and has reported variation in the incidence rate of RRT between these areas which is only partly explained by the age, ethnicity and socio-economic deprivation profile of each area. The introduction of the Quality Outcomes Framework (QOF), a pay-for-performance scheme for primary care physicians, provides data on the prevalence of known and controlled diabetes for the first time.

**Methods:** The incidence of RRT in each health area for 2007-2008 was obtained from the UKRR database. The prevalence of diabetes mellitus in 2007-2008 and the percentage of these patients achieving HbA1c <7.5% was obtained for each health area from QOF database.

**Results:** There are 192 health areas, median (IQR) population 255,900 (184,000-390,000), with an average of 6642 incident patients per year. Median diabetes prevalence was 3.96%.Each 1% point rise in diabetes prevalence was associated with an 18% higher incidence of RRT (incidence rate ratio (IRR) 1.18(95% CI 1.14-1.22, p<0.0001)). After adjustment for age/gender IRR was 1.23(1.19-1.27, p<0.0001). After adjustment for area level ethnicity and deprivation IRR was 1.22(1.17-1.28, p<0.0001) and after adjustment for life expectancy and cancer mortality IRR was 1.24(1.19-1.30, p<0.0001). Each 1% point

rise in diabetes prevalence was associated with a 32% higher rate of RRT caused by diabetes ($RRT^{DM}$) ($IRR^{DM}$1.32, 1.22-1.43, p<0.0001). After adjustment for age/gender $IRR^{DM}$ was 1.46(1.35-1.58, p<0.0001) and after adjustment for area-level ethnicity/deprivation $IRR^{DM}$ was 1.45(1.32-1.60, p<0.0001). After adjustment for life expectancy/cancer mortality $IRR^{DM}$ was 1.48(1.34-1.63, p<0.0001).

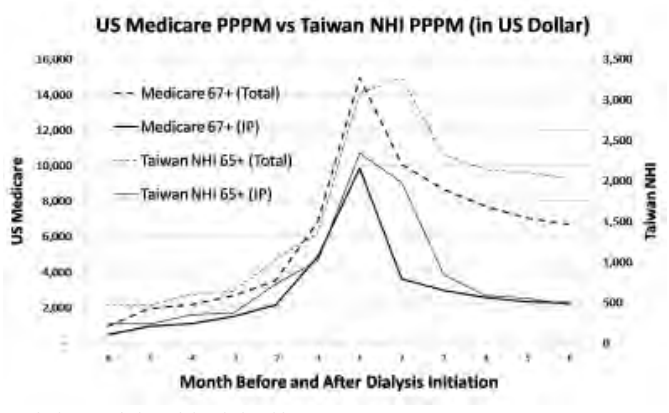
**Conclusions:** In the UK, every 1% point increase in prevalence of diabetes in the general population is independently associated with a 24% higher incidence of RRT and a 48% higher incidence of $RRT^{DM}$.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1536

**A Comparison of Cost Distribution during the Transition to ESRD in the US and Taiwan** Lih-Wen Mau,[1] Xinyue Wang,[1] James P. Ebben,[1] David T. Gilbertson,[1] Herng-Chia Chiu,[2] Allan J. Collins.[1] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Institute of Healthcare Administration, Kaohsiung Medical University, Kaohsiung, Taiwan.*

The burden of ESRD in the US and in Taiwan is among the heaviest in the world, This study aimed to compare the distribution of costs during the 6 months before and after dialysis initiation for older incident ESRD patients in the US and Taiwan. Identified from USRDS ESRD data, patients who initiated dialysis in 2007, were aged ≥ 67 years at initiation, and had Medicare as primary payer for 2 years before initiation were included (n=32,444). A cohort of Taiwanese incident patients from the 1% random sample of the National Health Insurance (NHI) population in Taiwan, aged ≥ 65 years, who initiated dialysis between 2000 and 2008 (n=324) was established for comparison. Taiwan's NHI program uses the same billing format as US Medicare claims. Total costs of Medicare Part A and Part B and of Taiwanese NHI outpatient and inpatient services were calculated by per-patient-per-month (PPPM). PPPM hospitalization costs were also calculated. In the month of dialysis initiation, 75% of the US Medicare and 81% of the Taiwan NHI incident patients were hospitalized; PPPM inpatient costs were highest in the month of dialysis initiation in both study cohorts. However, total PPPM transition cost reached its peak in the first dialysis month for Medicare patients and in the second month for Taiwan NHI patients. The time difference between the peaks in total cost might be due to 48% of Taiwanese patients being continuously hospitalized in the second month after dialysis initiation. Further study is needed to compare differences in use of nephrology care and other health system factors that might explain why the dialysis initiation period seems to be longer for Taiwanese than for US incident ESRD patients.




Disclosure of Financial Relationships: nothing to disclose

## F-PO1537

**Epidemiology and Outcome of Chronic Hemodialysis Patients Requiring Hospital Admissions** Chew Ming Wong,[1] Sridhar Ramanaidu,[1] Soo Kun Lim,[1] Li Ping Tan,[1] Kok Peng Ng,[1] Tee Chau Keng,[1] Wai Yew Kong,[1] Yip-Boon Chong,[1] Si-Yen Tan.[2] *[1]Division of Nephrology, University Malaya Medical Centre, Kuala Lumpur, Malaysia; [2]Nephrology Clinic, Prince Court Medical Centre, Kuala Lumpur, Malaysia.*

Hospital admission among end stage renal disease (ESRD) patients has major implications on healthcare cost and survival of this increasingly prevalent patient population group. Effective identification of the common reasons for admission may help guide strategies targeted at reducing the high rate of morbidity in these patients. We report here an observational study of all admissions involving ESRD patients in University Malaya Medical Centre (UMMC) from 1 January 2009 to 31 Dec 2009. ESRD patients are patients who have undergone maintenance hemodialysis for at least three months.

There were 294 admissions involving 195 patients, 48% were females with mean age 59.6 (range 18-85); 65.6% were diabetics and 19.7% of patients were using non-cuffed dialysis catheter at the time of hospitalization. The reasons for admission were sepsis (29.9%), elective admissions for cataract operations and coronary angiograms (15%), cardiovascular disease (12.2%) and fluid overload (11.6%). Risk factors associated with sepsis related hospitalization were anemia (p=0.026) and hypoalbuminemia (p=0.041). The commonest cause of sepsis was dialysis catheter related sepsis (32.9%) followed by orthopedic related infections (29.5%) and pneumonia (23.8%). The commonest organism grown was methicillin sensitive Staphylococcus aureus. The median length of hospital stay (LOS) was 5 days (IQR 3-8). Sepsis was the strongest predictor for LOS (p=<0.001). The mortality rate was 10.2% and most patients died of septic shock.

In conclusion, although diabetes was the most common cause of ESRD at 54%, there was a higher proportion of diabetic ESRD patients requiring admission. Mortality rate was high with catheter related sepsis being the most common reason for admission. Our findings suggest earlier creation of permanent vascular access can be further optimized to reduce morbidity and in patients dependent on catheters, improvement in catheter care together with use of cuffed catheters may reduce risk of infections.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1538

**Pregnancy Related Polyhydramnios in Chronic Dialysis Patients: A Treatable Pathology, a Hemodialysis Adequacy Tool and an Outcome Predictor** Claudio Luders, Silvia Titan, Lilian P. F. Carmo, Igor Marques, Joao Egidio Romao, Jr. *Nephrology, University of Sao Paulo School of Medicine, Sao Paulo, SP, Brazil.*

Polyhydramnios (PH) is one of the most frequent complication in chronic hemodialysis (HD) pregnant women. Increased fetal urine production secondary to urea osmotic diuresis is probably the etiology of the excess of amniotic fluid. Increasing HD dose may act reducing maternal serum urea and consequently the fetal osmotic diuresis. In a previous report, a non-expected association beetween PH and a better fetal outcome was shown. In this study, we wish to further explore the association between amniotic fluid volume and fetal outcome. In the past 10 years we have followed 44 pregnant women that required HD. Of those, twenty developed PH, amniotic fluid index (AFI) > 25cm or excess of amniotic fluid, AFI > 18 cm. Sixteen patients were treated with half an hour increase in HD time. After treatment, all patients normalized the AFI within 30 days. The AFI and serum urea level before and after the change in HD dose were 24.9 ± 6.2 *vs* 16.6 ± 2.3 cm (p<0.001) and 95.9 ± 26 *vs* 66.8 ± 17 mg/dL (p<0.0001), respectively. The gestational age and fetal weight of the patients with excess of amniotic fluid were significantly higher than the remaining 24 patients (35.2 ± 2.4 *vs* 32.1 ± 3.6 weeks, p=0.002 and 2031 ± 478 *vs* 1349 ± 641g, p=0.0003, respectively). In the multivariate linear regression model, we observed that PH was positively related to birth weight, even after adjusting to predialysis serum urea, standard Kt/V and to the presence of preeclampsia (β=417, 95% CI 98 to 737, p=0.01). At univariate logistic regression analysis, the presence of PH was positively related to the fetal outcome (p=0.04). We speculate that the presence of PH is probably a marker of an adequate placental blood flow in pregnant women on dialysis. This hypothesis is corroborated by the presence of a normal umbilical doppler velocimetry in all patient with elevated AFI. We conclude that PH is a treatable pathology, it can be used as an HD adequacy tool and is related to a better fetal outcome.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1539

**Impact of Telemedicine in Remote Dialysis Units in Northern Quebec** Murray L. Vasilevsky,[1] Claude Sicotte,[2] Khalil Moqadem,[2] Johanne Desrochers,[1] Madeleine St-Gelais.[1] *[1]Nephrology, McGill University Health Centre, Montreal, QC, Canada; [2]Department of Health Administration, University of Montreal, Montreal, QC, Canada.*

This study was designed to assess the impact of telemedicine on the quality of care of prevalent hemodialysis patients receiving treatment in remote settings. Subjects consisted of First Nation Cree patients dialyzed in two remote satellite units (Unit A and Unit B) in the James Bay region of Northern Quebec. All Cree patients older than 18 and dialyzed for a minimum period of 9 months prior to and after introduction of telemedicine were included. Data extracted retrospectively from monthly lab tests and chart review were collected and compared in periods prior to and after introduction of telemedicine and subjected to ANOVA with repeated measures. 28 patients were included in the analysis.

| | Unit A | | Unit B | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| SBP,mm Hg | 155.7 (15.9) | 155.3 (15.7) | 149.6 (19.1) | 151.2 (15.4) |
| DBP, mm Hg | 82.6 (7.8) | 80.3 (9.8) | 78.4 (9.2) | 78.1 (9.2) |
| Hb g/l | 118.1 (7.6) | 110.0 (10.3) | 115.6 (8.5) | 117.2 (9.2) |
| Albumin g/l | 39.2 (3.0) | 39.4 (2.5) | 38.8 (2.8) | 37.9 (2.6) |
| Glucose mM/l | 7.3 (1.5) | 7.4 (1.7) | 8.4 (2.0) | 8.9 (2.1) |
| HbA1C % | 10 (1) | 10 (1) | 10 (1) | 10 (1) |
| KT/V | 1.4 (0.2) | 1.5 (0.2)* | 1.55 (0.1) | 1.6 (0.2)* |
| P mM/l | 1.9 (0.4) | 1.8 (0.5) | 1.7 (0.3) | 1.9 (0.5) |
| PTH pM/l | 44.5 (42.3) | 73.8 (80.1) | 21.4 (21.4) | 31.3 (21.9) |
| Rx changes/mo | 2.5 (1.4) | 1.8 (1.5) | 5.0 (5.8) | 2.7 (2.2) |
| Transfers/month | 1.3 (0.5) | 1.5 (1.1) | 0.7 (1.0) | 1.1 (1.7) |

mean (SD), * p=.034

Pre-dialysis systolic and diastolic BP, serum Hb, serum albumin, phosphate, PTH, glucose and glycosylated hemoglobin were unchanged. There was a statistically but not clinically significant increase in KT/V in each unit (1.5 vs 1.4 and 1.6 vs 1.5, post vs pre, Unit A and Unit B, respectively, p=.034). The number of prescription medication changes and number of transfers to the University center were unchanged. Although no changes in outcome could be demonstrated, telemedicine provides better security and support for patients and staff and may have contributed to maintenance of K/DOQI targets.

Disclosure of Financial Relationships: Honoraria: Astra-Zeneca, Janssen, Amgen, Roche, Shire.

**F-PO1540**

**What Are the Risks Associated with Temporary Haemodialysis Away from Centre?** Richard W. Corbett, Virginia Louise Prout, Deborah Haynes, Claire Edwards, Andrew H. Frankel. *Imperial College Healthcare NHS Trust, London, United Kingdom.*

Temporary haemodialysis away from the patient's 'home' centre is increasing in frequency but there is little data on the effect this change to the patient's dialysis regime has on their health.

Methods: Travel data was collected prospectively over six months on 1179 patients receiving maintenance haemodialysis across our dialysis centres. Biochemical parameters, erythropoietin usage, dialysis access information, positive microbiological investigations, hepatitis serology and antibiotic starts were recorded for twelve weeks prior to and following dialysis away from centre.

Results: 172 individuals, travelled on 200 occasions. 85 trips were made within the UK, while 115 trips were made overseas. 123 had a cuffed central venous catheter [CVC], 48 arterio-venous fistula [AVF] and 1 an arterio-venous graft [AVG].

The blood stream infection [BSI] rate for travellers with CVC was 0.33 vs 0.83/1000 access days (a.d.) [$p<0.05$] in the twelve weeks pre and post travel. The comparable BSI rate for AVF and AVG was identical with a single BSI in this group pre and post travel. Exit site infections were comparable at 0.18 vs 0.30/1000 a.d. [NS]. Parenteral antibiotic starts 1.43 vs 2.32/1000 a.d. and oral antibiotic starts 0.42 vs 1.79/1000 a.d. were both significantly elevated post travel [$p<0.05$] and were mainly instituted for either chest or urinary sepsis.

There was no evidence of hepatitis B or C seroconversion. Haemoglobin levels dropped on return (pre: 12.2 ±0.9 post: 11.9 ±1.3 g/dl, $p<0.05$) but had returned to baseline 8 weeks later. Erythropoietin requirements and phosphate levels were all increased on return to centre.

Conclusion: Travel and dialysis away from a patient's usual haemodialysis unit is associated with anaemia and an increased erythropoietin requirement. The concomitant evidence of an increased risk of bacterial infection, particularly if the patient is dialysing by means of a CVC, may contribute through erythropoietin resistance, as may inadequate dialysis while away from centre. This study provides evidence for the widespread perception that haemodialysis away from centre is associated with increased morbidity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1541**

**Factors Influencing Regional Differences in the Outcome of Dialysis in Japan** Satoshi Ogata, Shinichi Nishi, Kunitoshi Iseki, Yoshiharu Tsubakihara. *Committee of Renal Data Registry, the Japanese Society for Dialysis Therapy, Tokyo, Japan.*

Background: There are regional differences in the outcome of dialysis, but few studies have investigated the causes.

Methods: We stratified 109 factors by gender and 47 prefectures, including the past history and laboratory data, economic and weather conditions, and institutional and nutritional factors, and we investigated factors associated with regional differences in the incidence rate, the survival of incident dialysis patients in 2004-06 (n=102,011), and the number of dialysis specialists using the database of the Japanese Society for Dialysis Therapy (JRDR-09105) by univariate analysis.

Results: A history of leg amputation ($r=0.427$, $p=0.003$), a history of acute myocardial infarction ($r=0.410$, $p=0.004$), the duration between the first visit to hospital and first dialysis ($r=-0.375$, $p=0.009$), intake of fish and shellfish ($r=-0.418$, $p=0.003$), intake of meat ($r=0.597$, $p<0.0001$), the duration of the snow season ($r=-0.477$, $p=0.0007$), and the amount of personal savings ($r=-0.439$, $p=0.020$) were associated with the incidence of starting dialysis. The number of night dialysis institutions ($r=0.484$, $p=0.0006$), blood albumin level ($r=0.316$, $p=0.030$), Kt/V ($r=0.496$, $p=0.0004$), and dialysis time ($r=0.513$, $p=0.0004$) were correlated with the 1-year survival rate. The number of dialysis specialist was associated with the incidence of starting dialysis ($r=0.513$, $p=0.0002$), Kt/V ($r=0.278$, $p=0.05$), the dialysis time ($r=0.290$, $p=0.048$), blood creatinine after dialysis ($r=-0.298$, $p=0.042$), and 1-year survival ($r=0.297$, $p=0.043$).

Conclusions: Many factors, including the number of specialists, were associated with the incidence of starting dialysis and with survival.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1542**

**Quality of End of Life Care: Results from the Pan-Thames Audit Group** Stephen Paul McAdoo,[1] Emma M. Salisbury,[1] Edwina A. Brown,[1] Alistair Chesser,[2] Ken Farrington.[3] *[1]Imperial College Kidney and Transplant Institute, London; [2]Royal London Hospital, London; [3]Lister Hospital, Stevenage, United Kingdom.*

BACKGROUND: Advance discussion and planning of end of life (EoL) care results in patients receiving care in line with their wishes and improved quality of life. The Department of Health document "End of Life Care in Advanced Kidney Disease: a framework for implementation" suggests strategies to achieve high quality end of life (EoL) care for renal patients. To monitor the implementation of these, audit tools are needed to measure quality of EoL management, but currently there is no accepted method of doing this.

AIMS: (1) to develop a proforma to collect information about EoL care; (2) to use the proforma prospectively to collect information about current standards of EoL care; (3) to determine what factors affect quality of EoL care.

DESIGN: We developed a proforma to collect data on EoL care. This was used to prospectively collect data for all deaths in the ESRD population over an 8 week period from 10 centres in London and SE England.

RESULTS: 138 deaths were recorded. Mean age 68 years. 86% on dialysis, remainder transplant or conservative care. 68% of deaths occurred in hospital, remainder at home or hospice. 87% home deaths were sudden. Chosen place of death was known for 22%. Data on inpatient deaths showed that EoL discussion was documented in 30%; dialysis was discontinued in 50%; management was changed to palliative care in 41%; "good quality deat" as judged by form completer was achieved in 51%. Effect of "unexpected" death (37% inpatient deaths) or being non-caucasian (36% total deaths) on EoL care are shown in the Table 1.

Table 1

| | Expected | Unexpected | Caucasian | Non-caucasian |
|---|---|---|---|---|
| Changed to Palliative Care(%) | 67 | 13 | 45 | 32 |
| Dialysis Discontinued(%) | 58 | 18 | 52 | 36 |
| Good Quality Death(%) | 56 | 32 | 51 | 52 |

CONCLUSION: A one-paged proforma completed at the time of death is a useful audit tool for EoL care. Many aspects of EoL care for patients on dialysis are poor. Death being "unexpected" and patient being non-caucasian can result in poorer EoL management. Physicians need to improve their recognition of the EoL phase and to be aware of cultural issues that may affect EoL care.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1543**

**Outcomes in Dialysis and Renal Transplant Patients Admitted Due to Respiratory Diagnoses: Impact of the H1N1 Virus** Odiri Jane Eneje, Neill D. Duncan, Tom Cairns, David Taube, Darren S. Parsons. *Imperial College Kidney and Transplant Institute, Imperial College Healthcare NHS Trust, London, United Kingdom.*

**Background**

Respiratory admissions are a major cause of morbidty/mortality in patients on renal replacement therapy. During the winter of 2009 there was concern of the impact of the H1N1 virus epidemic in haemodialysis (HD) and transplant (TX) patients.

**Methods**

We have retrospectively analysed the data (clinical records, radiology, microbiology) for all cases (HD/TX) admitted with a primary respiratory diagnosis from August 2009 to March 2010 to look at primary cause and the impact of the H1N1 virus. We were additionally interested in the requirements for high dependency respiratory care. All patients with flu like symptoms were screened for H1N1 by PCR.

**Results**

There were a total of 1540 non-elective admissions during this time period (45% TX). A total of 246 primary respiratory admissions were identified (HD 179 and TX 67). The majority had no prior respiratory co-morbidity (65.9%). Primary diagnosis included pneumonia (86, 35%), pulmonary oedema (56, 22.8%), combination septic pulmonary oedema (51, 20.7%), pneumonitis (18, 7.3%), exacerbation of COPD/asthma (8, 3.3%) and pulmonary emboli (7, 2.8%). Pulmonary oedema secondary to poor patient compliance was a major cause of admission in HD (41, 16.6%).

Positive microbiology was found in 46 (18.7%): Candida (12), Pseudomonas aeruginosa (4), Streptococcus pneumoniae (2), Klebsiella pneumoniae (2) and Haemophilus influenzae (1). There were 8 cases of admission related to H1N1 infection.

For cases of pneumonitis, pathogens were identified in 8/18 cases these included Cytomegalovirus (3), H1N1 (2), Pneumocystis (2) and Respiratory Syncitial Virus (1).

Of the acute admissions 104 (42.3%) patients required renal high dependence unit care. Of these 16 required non invasive ventilation and 4 (TX 2, HD 2) were admitted to the intensive care unit for ventilation.

Our overall mortality was 11/246 (4.5%): TX (3, 4.5%) HD (8, 4.5%).

**Conclusion:**

Overall there was little impact of the H1N1 virus on respiratory admissions. Our overall mortality from respiratory diagnosis was 4.5% although this was mainly from standard infections.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1544**

**Surrogate Dialysis: Insured and Uninsured Patients Outcomes in 5 Privates Clinics in Jalisco, Mexico** Karina Renoirte,[1] Guillermo G. Garcia,[1] Laura Cortes Sanabria,[2] Bertha Alicia De la Torre.[1] *[1]Nephrology, Hospital Civil de Gudalajara, Guadalajara, Jalisco, Mexico; [2]Nephrology, CMNO, Guadalajara, Jalisco, Mexico.*

INTRODUCTION/AIMS: End-stage renal disease represents a serious public health problem in Mexico. We reported lower dialysis acceptance rates in the uninsured (UP) than in the insured population (IP) (99 pmp vs 166 pmp). To correct these disparities in 2006 the State Ministry of Health (SMH) implemented the first hemodialysis program in Jalisco that provides surrogate dialysis for the poor free of charge. In 2008 the Federal Guvernment surrogated some of its patients to private hemodialysis clinics. We evaluate the clinical outcomes of UP and IP patients at 5 private hemodialysis clinics in Jalisco.

METHODS: Clinical and laboratory data was collected from March 2007 thru April 2010. Age, gender, etiology, date of first treatment, vascular access, weekly dialysis hours, EPO use, hemoglobin (HB), calcium phosphorus product (CAxPO4), Kt/V were registered. Student's T-test and were utilized. Survival curves were constructed with the Kaplan-Meier method and compared with the log-rank test.

RESULTS: 72 UP patients and 217 IP started dialysis. UP patients were younger (41±21ys vs 48±19ys, p=0.06), and had less DM (33% vs 46%, p=0.009) than the IP ones. Female gender (47% UP and 40% IP) was similar in both groups. A cross-sectional analysis revealed a greater utilization of AV fistulas among UP patients (23.6% vs 19.3% p=< 0.0001). HB (10.1 ±2.2 g/dL vs 9.94 ±1.94, p 0.510 g/dL), and EPO use was similar (85.8% UP vs 85.2% IP, p=0.660). CaxPO4 (44.61 ±18.6 vs 45.37 ±18.4). Average Kt/V was better for UP group (1.21± 0.72 UP and 0.96± 0.23 IP p= < 0.0001). Survival at 12 and 24 months was 87.5% and 83.3 % among UP and 90 and 78% among IP. It was not statistically significant.

CONCLUSIONS: SMH has open a great opportunity for poor chronic renal failure patients to have access to dialysis treatment. A greater utilization of av fistulas and therefore a better dose of dialysis was observed in these patients compared with the insured patients. The results are encouraging, although a longer follow-up will be needed to assess the full impact of this program in Mexico.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1545

**Effects of Icodextrin on Mortality and Technique Failure in Patients Undergoing Peritoneal Dialysis** Eawha Kang,[1] Sang Choel Lee,[2] Seung Hyeok Han,[3] Dae-Suk Han.[3] *[1]Department of Internal Medicine, NHIC Medical Center, Ilsan Hospital, Goyangshi, Gyeonggi-do, Korea; [2]Department of Internal Medicine, Kwandong University College of Medicine, Goyanshi, Gyeonggi-do, Korea; [3]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea.*

**Background**: Many studies have suggested clinical benefits of icodextrin in peritoneal dialysis (PD) patients. However, whether icodextrin can improve patient survival is currently unknown. Therefore, this study aimed to investigate the effects of icodextrin on mortality and technique failure in PD patients.

**Methods**: Study subjects were 2,163 patients from 54 centers in Korea who initiated PD from Jul 2003 to Dec 2006. Outcome data were retrieved retrospectively from the Baxter Korea database. A total of 790 patients were identified as icodextrin group who were maintained on this solution for more than 6 months. We compared all-cause mortality and technique failure between patients with and without icodextrin by using propensity score analysis.

**Results**: Propensity score matching yielded 776 matched pairs of patients. There were no significant differences in age, gender, diabetes, cardiovascular comorbidity, socioeconomic status, biocompatible solution use, or center experience between the two groups. All-cause deaths occurred in 91 (11.7%) patients in icodextrin group compared with 168 (21.6%) in non-icodextrin group (hazard ratio [HR], 0.37; 95% confidence interval [CI], 0.29 to 0.48; P<0.001). In addition, icodextrin use was significantly associated with a reduced risk of technique failure (HR, 0.44; 95% CI, 0.30 to 0.65; P<0.001). Icodextrin group had fewer technique failures due to non-compliance compared with non-icodextrin group whereas peritonitis- or ultrafiltration failure-related technique failure was not different between the two groups.

**Conclusion**: This study suggests that icodextrin solution may improve patient and technique survival in PD patients. To confirm these results, a large randomized prospective study is warranted.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1546

**Facility Influenza Vaccination (IV) Practice: Associations with Mortality and Hospitalizations in the US Dialysis Population** J. M. Messana,[1] K. A. Wisniewski,[1] T. H. Shearon,[1] V. B. Ashby,[1] Sandra E. Callard,[1] Rajiv Saran,[1] Bruce M. Robinson.[2] *[1]Kidney Epidemiology and Cost Center, University of Michigan, Ann Arbor, MI; [2]Arbor Research Collaborative for Health, Ann Arbor, MI.*

Despite public health and regulatory efforts to increase IV in the US dialysis population, IV rates have only increased to 64% over the last decade. A prior study using 1997-99 data (Gilbertson, 2003) showed that patients who received IV had lower mortality and hospitalization in the following year.

Using the national CMS ESRD database for 2007-08, we examined the relationship between facility IV rates and subsequent mortality and hospitalization. Facilities were grouped into quintiles by percentage of patients vaccinated from 9/07-12/07. Poisson regression models, adjusted for demographic factors, were used to determine the relative risk (RR) of mortality and hospital admission in 2008 among the quintiles.

The median facility IV rate was 67%. Facility IV rate was inversely associated with RR of mortality and hospitalization (Table 1). Facilities vaccinating <52% of patients (lowest quintile) had a 22% higher risk of both mortality and hospitalization compared to facilities vaccinating >77% of patients (highest quintile). On average, facilities with a 10% higher IV rate had 4% and 3.5% lower mortality and hospitalization, respectively (p<0.0001).

Although IV rates continue to rise, the quality gap remains, as evidenced by only 2/3 of this patient population receiving IV during the vaccination period. Our analysis demonstrates that mortality and hospitalization rates continue to be lower in dialysis facilities with higher IV rates.

Table 1. RR of Mortality and Hospital Admission for Facilities by Percent of Patients Vaccinated against Influenza, 2007-2008

| Percent of Patients Vaccinated (Quintiles) | RR of Mortality | RR of Hospital Admission |
|---|---|---|
| 0 – 52 | 1.15* | 1.11* |
| 53 – 63 | 1.04* | 1.05* |
| 64 – 70 (Ref) | 1.00 | 1.00 |
| 71 – 76 | 0.97* | 0.96* |
| 77 – 100 | 0.94* | 0.91* |

*p < 0.05

Disclosure of Financial Relationships: nothing to disclose

## F-PO1547

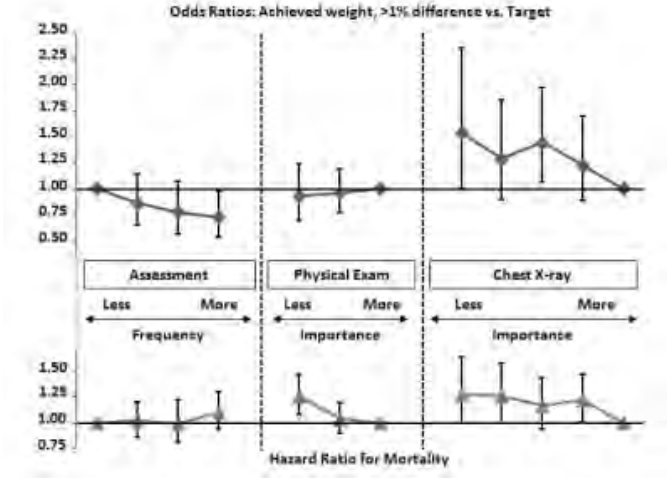
**Dialysis Facility Practices Aimed at Target Weight Achievement and Patient Outcomes in DOPPS** Rajiv Saran,[1] Douglas S. Fuller,[2] Ananda Sen,[1] Stefan H. Jacobson,[3] Raymond C. Vanholder,[4] Tadashi Tomo,[5] Francesca Tentori.[2] *[1]Univ of MI, Ann Arbor, MI; [2]Arbor Res Collab for Hlth, Ann Arbor, MI; [3]Danderyd Hosp, Stockholm, Sweden; [4]UZ Gent, Gent, Belgium; [5]Oita Univ Hosp, Oita, Japan.*

Optimum intradialytic fluid management (IDFM) is critical in dialysis, but current practice guidelines in this area are not fully developed. We sought to link patient outcomes with strategies utilized by dialysis facilities in 12 countries toward achieving target weight (TW).

Medical Directors of DOPPS III facilities (2005-2008, n=240) reported the typical frequency of TW assessment and rated importance of 7 IDFM practices used to assess TW at their unit: physical exam, on-line volume monitoring, chest X-ray, orthostatic BP, intradialytic hypotension, Hb, and symptoms. Adjusted regressions were used to predict patient-level TW achievement and mortality.

Wide variation was noted across countries for the 7 IDFM practices. TW achievement was improved among patients in facilities indicating higher frequency of TW assessment and higher importance of chest X-ray to assess TW (Figure, upper half). Patients in facilities that rated physical examination to have low importance in assessing TW showed significantly higher hazard of mortality (Figure, lower half) compared to patients in facilities rating it to be very important. Patients in facilities that rated chest X-rays to be very important in determining TW also tended to have a lower HR of mortality.

Higher frequency of TW assessment at dialysis facilities and greater emphasis toward physical examination in determining volume status was associated with improved TW achievement and lower mortality in hemodialysis patients. Additional results regarding on-line volume monitoring suggest that reliance on technology alone may not be the best solution in this area compared with clinical assessment. However, this requires further study.




Disclosure of Financial Relationships: nothing to disclose

## F-PO1548

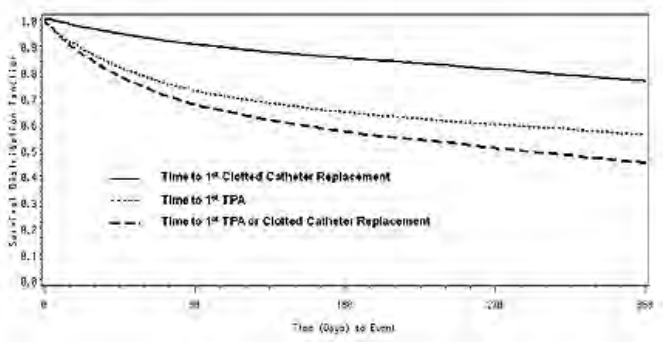
**Central Venous Catheter Dysfunction in Incident Hemodialysis Patients** Eduardo K. Lacson, Weiling Wang, J. Michael Lazarus, Raymond M. Hakim. *Fresenius Medical Care, North America, Waltham, MA.*

**Introduction:** Flow obstruction is a common complication of hemodialysis (HD) catheters. We tracked clotting-related catheter replacement and use of recombinant tissue plasminogen activator (TPA) in a national cohort of incident HD patients.

**Methods:** Among 25,003 incident HD patients admitted in Fresenius Medical Care North America facilities (within 15 days of first dialysis) between January 1 and December 31, 2007, 14,836 patients (59.3%) exclusively used a catheter. These catheter patients were followed for up to one year of catheter use or censored at discharge or upon transition to fistula/graft.. Use of TPA and clotting (non-infection) related catheter replacement(s) were tracked.

**Results**: Mean age was 63.6 ± 15.8 years, 54.0% male, 66.4% white and 29.1% black, with 54.4% having diabetes. Mean follow-up time was 199 ± 129 days. A total of 18,809 TPA administrations and 3,039 non-infection-related catheter replacements occurred during

the study period. Separately, TPA was used by 4,829 patients (32.5%) at a median time of 41 days for the 1st episode while 2,169 catheters present on admission (14.6%) were 1st replaced due to dysfunction at a median time of 75 days. Combined, >50% of patients experienced either intervention, at a median time of 107 days (Figure 1).




**Conclusions**: Flow-related catheter dysfunction requiring TPA or catheter replacement is very common within the first year of HD. This presents a significant cost when bundling starts, and in addition to catheter-related infection, may impact patient outcomes (e.g. hospitalization, quality of life, etc.) and increase utilization of healthcare resources.

Disclosure of Financial Relationships: Employer: I am an employee of Fresenius Medical Care, North America.

## F-PO1549

**Multi-Center Experience of 137 Consecutive HeRO Implants** Shawn Michael Gage,[1] David W. Butterly,[2] Jeffrey Harold Lawson,[1] John R. Ross,[3] Howard E. Katzman,[4] Jason D. Woolard.[5] *[1]Surgery, Duke University Medical Center, Durham, NC; [2]Nephrology, Duke University Medical Center, Durham, NC; [3]Surgery, Bamberg County Hospital, Bamberg, SC; [4]Surgery, University of Miami, Miami, FL; [5]Surgery, Baylor University Medical Center, Dallas, TX.*

**Purpose:** To report a multi-center experience with the novel Hemodialysis Reliable Outflow (HeRO) vascular access device.

**Methods:** Four centers have conducted a retrospective review of patients receiving the HeRO device from implant to last available follow-up. The HeRO is an implantable, completely subcutaneous, hybrid "graft-catheter" device approved for catheter-dependent patients with limited venous outflow. The focus of this evaluation is on HeRO patency and intervention rates, as well as access-related bacteremia. We intend to follow this cohort and update the study data accordingly.

**Results:** Currently, data is available on 137 patients at four institutions with an accumulated 1,346.8 HeRO months. At 6 months, HeRO primary patency is 61.5% (64/104) and secondary patency is 89.4% (93/104). At 12 months, HeRO primary patency is 54.8% (23/42) and secondary patency is 83.3% (35/42). To-date, interventions have been required in 40.1% of patients (55/137) resulting in an intervention rate of 1.8/year. Access-related infections have been reported in 6 patients.

**Conclusions:** To date the HeRO vascular access device has performed comparably to standard AVGs when weighed against the peer review literature. Furthermore, in our experience its performance has proven superior to TDCs in terms of patency, intervention, and infection rates when compared to the peer reviewed literature. The HeRO device has shown great promise as an alternative to catheter dependence as a means for hemodialysis access. Preliminary results are good; however, data from additional prospective randomized trials could make this device a permanent fixture in the vascular access algorithm.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1550

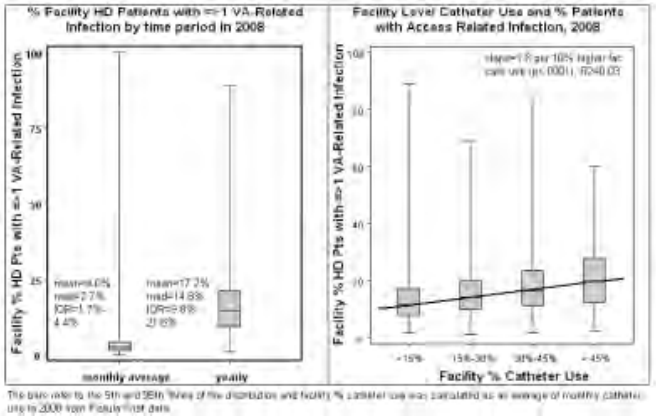
**Hemodialysis (HD) Vascular Access (VA)-Related Infection Rates in US Medicare Patients: Large Variability Not Explained by Facility Catheter Use** N. A. Lueth,[2] J. M. Messana,[2] V. B. Ashby,[2] Sandra E. Callard,[2] Rajiv Saran,[2] Ronald L. Pisoni.[1] *[1]Arbor Research Collaborative for Health; [2]Univ. of Michigan Kidney Epidemiology and Cost Center.*

HD VA-related infections are strongly associated with mortality, hospitalization, and high treatment costs. Clinical trials have demonstrated variability in access-related infection rates among facilities, but comprehensive facility-level national comparisons are currently lacking. We examined facility-level rates of HD VA-related infection using Medicare claims data and its relationship with facility catheter use.

Facility-level monthly rates of VA-related overall infection were calculated among all Medicare patients receiving HD in 2008 (monthly mean n=251,544). Infection events were defined by ICD-9 code 996.62 (n=56,636). Facilities with >10 HD patients were included (n=4,972). Because claims with overlapping or proximate dates made it difficult to determine if 2 or more claims linked to more than 1 infection, these analyses only counted 1 infection per month per patient.

Large variability in monthly rates of VA-related infection was seen across facilities (Figure). On average during a month, the median facility % HD patients with ≥1 VA-related infection was 2.7%. The median facility % HD patients with ≥1 access-related infection in 2008 was 14.6%. Greater facility % catheter use was strongly associated with higher rates of VA-related infection (Figure), but explained only a small fraction of the total variability in VA-related infection across dialysis units ($R^2$=3%, p<.0001).

In summary, highly variable rates of VA-related infection are seen across US dialysis units much of which is not explained by catheter use. These results point to large opportunities for improving infection-control among HD patients, and the need to understand the practices explaining these highly variable VA-related infection rates.




Disclosure of Financial Relationships: nothing to disclose

## F-PO1551

**Location of Biofilm on Tunneled-Cuffed Hemodialysis Catheters in Patients with Catheter-Related Bacteremia** Venkataraman Ramanathan,[1] Sarah Riosa,[2] Atef Al Sharif,[2] Adrian Paul Abreo,[1] Saima Aslam,[2] Rabih Darouiche.[2] *[1]Division of Nephrology, MEDVAMC, Baylor College of Medicine; [2]Infectious Diseases, MEDVAMC, Baylor College of Medicine, Houston, TX.*

**Background:** Biofilm on hemodialysis (HD) catheter surface is difficult to eradicate and is associated with bacteremia. Location of biofilm on tunneled HD catheter surface and origin of bacteremia are unknown.

**Methods:** We identified HD patients who had bacteremia and whose tunneled catheter was removed to treat infection. Patients who had exit site or tunnel infection were excluded. Catheter segment immediately distal to cuff and double-barreled distal tip were defined as "extravascular" and "intravascular" segments respectively. Outer and luminal surfaces of both catheter segments were analyzed and compared using Paired Student's t-test.

**Results:** Seventeen HD patients were identified. Peripheral blood culture grew gram(+) organism in 76% of patients. When examined by confocal laser scanning microscope, biofilm was thicker on outer surface when compared to luminal surface for both extravascular (18.3*vs*14.8 μm, p<0.0001) and intravascular segments (15.3*vs*11.6 μm, p<0.0001). Extravascular segments had thicker biofilm when compared to intravascular segments on outer (18.3*vs*15.3 μm, p<0.001) and luminal surface (14.8*vs*11.6 μm, p<0.001). All patients were on antibiotics at time of catheter removal. Catheter culture was positive in 10 patients. Cultures of blood and extravascular outer surface yielded the same strain of organisms in all 10 patients. Microbiologic yield from extravascular luminal was 20% and from intravascular outer and luminal surfaces was 40% and 0%, respectively. DNA fingerprinting with pulsed-field gel electrophoresis confirmed that the organism retrieved from extravascular outer catheter surface was identical to that from peripheral blood culture in the majority of patients (90%).

**Conclusions:** Biofilm is thickest on outer surface of extravascular segment of an infected HD tunneled catheter and cultures from this segment have the highest microbiologic yield and correlation with peripheral blood cultures. Exit site may be the primary source of infection and this catheter segment should be routinely cultured.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1552

**Relationship of EPO Dose to Vascular Access, Serum Albumin and TSAT** B. Horowitz,[1,2] O. Myers,[1] S. Paine,[1,2] P. Zager.[1,2] *[1]UNM, Abq, NM; [2]DCI, Abq, NM.*

The expanded bundle poses a challenge to the financial stability of dialysis facilities. Reducing the amount of EPO administered will help control costs. We postulated that vascular access type (VA), iron saturation (TSAT) and serum albumin (SA) were associated with differences in EPO dose. We conducted a cross sectional study of 6887 DCI hemodialysis patients with fistulas (55%), grafts (24%) and catheters (21%). All patients had Hgb levels from 10 to 12 g/dl. The relationships of VA, TSAT and SA to EPO dose were assessed using an analysis of covariance, adjusting for age, race, sex and diabetes (Table).

Relative Effect Based on Log Transformed EPO

| Comparison | Estimates (95% CI) |
|---|---|
| Catheter vs Graft | 0.88 (0.82, 0.94) |
| Catheter vs AVF | 0.78 (0.74, 0.83) |
| Graft vs AVF | 0.89 (0.84, 0.94) |
| TSAT <20 vs TSAT 20-29 | 0.71 (0.68, 0.75) |
| TSAT <20 vs TSAT 30+ | 0.58 (0.55, 0.61) |
| TSAT 20-29 vs TSAT 30+ | 0.81 (0.77, 0.86) |
| SA <3.5 vs SA 3.5-3.9 | 0.68 (0.64, 0.72) |
| SA <3.5 vs SA 4.0+ | 0.55 (0.51, 0.58) |
| SA 3.5-3.9 vs SA 4.0+ | 0.80 (0.77, 0.85) |

Median SA (3.6 to 3.8 g/dl) and TSAT (22 to 24%) were similar in patients with fistulas (AVF), grafts and catheters. EPO cost was estimated using the average wholesale price (AWP) of $9.58/1000 U. EPO doses were lower in patients dialyzed via AVF vs. graft or catheter. Adjusted mean weekly EPO doses were 4620 U less in patients with AVF vs. catheters, cost differential of $44/patient/week. TSAT ≥ 30% and SA ≥ 4.0 g/dl were each associated with lower EPO doses vs. lower levels of these variables. EPO doses were 8656 U lower in patients with TSAT ≥30% vs. TSAT < 20% and 11,380 U lower in patients with SA ≥4.0 vs.< 3.5 g/dl, cost differentials of $83 and $109 respectively. EPO dose was inversely related to SA and TSAT, regardless of the type of VA. Adjusted mean EPO dose in patients with AVF, SA ≥ 4.0 g/dl and TSAT ≥ 30% was 10,228 U vs. 29,415 U in patients with catheters, SA <3.5 and TSAT <20%. Increasing AVF use, increasing SA and TSAT may reduce costs and improve both financial performance and patient outcomes under the expanded bundle.

Disclosure of Financial Relationships: nothing to disclose

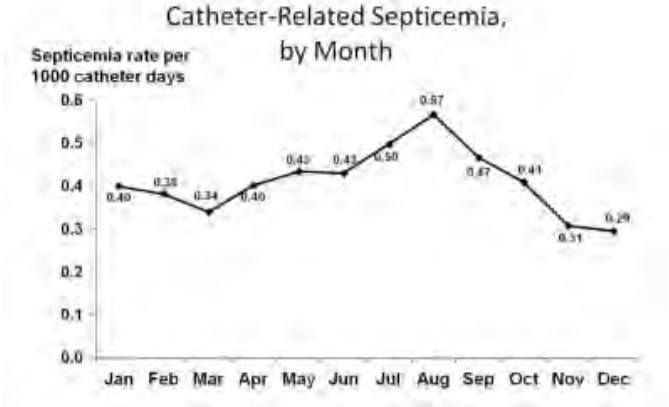
## F-PO1553

**Catheter (CVC) Related Septicemia: Associations with Seasons from the Dialysis Outcomes and Practice Patterns Study (DOPPS)** Charmaine E. Lok,[1] Jyothi R. Thumma,[2] Brenda W. Gillespie,[3] Richard J. Fluck,[4] Mark R. Marshall,[5] Hideki Kawanishi,[6] Bruce M. Robinson,[2,3] Ronald L. Pisoni.[2] *[1]Univ. Hlth. Network-Toronto Gen. Hosp., ON, Canada; [2]Arbor Research, Ann Arbor, MI; [3]Univ. of Michigan, Ann Arbor, MI; [4]Derby City Gen. Hosp., United Kingdom; [5]Middlemore Hosp., Auckland, New Zealand; [6]Tsuchiya Gen. Hosp., Japan.*

While CVC-related sepsis (CRS) contributes to adverse health outcomes in hemodialysis (HD) patients (pts), unexplored risk factors remain, such as season of year and CVC dressing protocols.

8,412 HD pts in 12 countries from DOPPS I and II (1996-2004) were analyzed. CRS was defined as septicemia during or within 15 days after HD CVC use. Catheter time at risk (n=1,754,293 days) and CRS were assigned to 1 of 4 seasons in each country. CRS rates by season and the association of facility vascular access (VA) dressing protocols with hazard ratio (HR) of CRS were determined by Poisson and Cox regression, respectively.

Overall CRS rate was 1.2/month or 0.41/1000 CVC days. In North America, CRS was 0.47/CVC 1000 days. CRS varied by month (Figure) with an adjusted RR for "summer" of 1.42 (95% CI 1.09-1.87) compared to "winter" (Table).




Rate of Catheter-Related Septicemia, by Season

| | Fall | Spring | Summer | Winter |
|---|---|---|---|---|
| septicemia rate /1000 catheter days | 0.34 | 0.38 | 0.49 | 0.34 |
| *Relative rate (RR) of septicemia:* | | | | |
| *Poisson model:* | RR (95% CI) | RR (95% CI) | RR (95% CI) | RR (95% CI) |
| Unadjusted | 0.99 (0.73-1.34) | 1.12 (0.84-1.49) | 1.44 (1.09-1.89)* | 1 (Ref.) |
| Adjusted** | 0.99 (0.73-1.33) | 1.12 (0.84-1.49) | 1.42 (1.09-1.87)* | 1 (Ref.) |

* p-value <0.01
** adjusted for age, gender, race, 13 summary comorbidities, phase and region

CRS was lower with use of betadyne [ adjusted HR=0.81, 95% CI (0.65,0.996)], or chlorhexidine [HR=0.81 (0.59,1.11)] vs. alcohol, with attenuated HRs (0.87-1.06) for combined cleansing agents. VA infection rates varied by personnel type who typically inspects CVC site /changes dressings: nephrologist [HR=0.64 (0.45,0.92)], technician [HR=1.47, 95% CI (0.98,2.2)] compared with nurse.

The higher CRS rate in summer may be due to higher heat, humidity, and perspiration, potentially facilitating bacterial growth and compromising protective measures. Extra vigilance by staff may reduce CRS in this high risk season. Betadyne and chlorhexidine may be more effective than other cleansing agents.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1554

**Comparison of Native Arterio-Venous (A-V) Fistula Needling Using the Button-Hole Versus the Rotation Technique. Evidence for Excess Blood Stream Infections with the Button Hole Method** Rebecca Backenroth, Ruthy Israeli, Dvora Rubinger. *Nephrology, Hadassah University Medical Center, Jerusalem, Israel.*

Native A-V fistulas are considered the preferred access for chronic hemodialysis. However, it is not established which needling technique is optimal. We prospectively compared 2 needling techniques, the rope-ladder or rotation (ROT) and the button-hole (BH) methods, evaluated staff opinions, and reviewed infection rates. The study was done in an ambulatory hemodialysis unit with approximately 70 chronic patients. Blood Stream Infections (BSI) were considered access related only when there was no other plausible cause for infection.

**Results:** In multiple random assessments of 23 BH and 21 ROT patients, mean age 63 and 62 yrs respectively, anxiety level before cannulation, pain, difficulty and complications of cannulation such as bleeding around the needles did not differ between the groups. On termination of dialysis, clotting tended to occur earlier in BH, but this, also, was not statistically significant. Analysis of BSI was performed retrospectively and then prospectively. Interim results are presented in table 1:

table 1

| Group | # of patients | patient months | Unexplained bacteremias |
|---|---|---|---|
| BH, retro | 23 | 1358 | 11* |
| ROT, retro | 21 | 1047 | 1* |
| BH, pros | 41 | 360 | 3** |
| ROT pros | 33 | 234 | 0** |

retro-retrospective; * $X^2$ p<0.05; pros-prospective; **pNS

The fistulas looked and functioned normally in all cases of BSI except 1 where an abscess developed at a BH site. The BSI bacteria were relatively sensitive skin organisms; Staph. aureus was associated with more severe and protracted BSI, and the abscess. Questionnaires revealed that 75% of 16 nurses felt more confident in using the BH method.

**In conclusion**, the BH and ROT cannulation techniques were similar in effectiveness, but BH was preferred by the nurses. Retrospective review of unexplained bacteremias revealed a statistically significant excess of BH associated BSI, with a rate of 1 bacteremia per 123 patient months. Prospective data indicated a similar BH related bacteremia rate, despite enhanced disinfection protocols. Due to these findings, we have suspended BH cannulation when ROT is an option.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1555

**Transvenous Cardiac Device Wires and Vascular Access in Hemodialysis Patients** David A. Drew, Klemens B. Meyer, Daniel E. Weiner. *Tufts Medical Center, Boston, MA.*

Cardiovascular disease is the leading cause of mortality in dialysis patients. Increasingly, cardiac devices such as implantable cardiac defibrillators (ICDs) and pacemakers are being placed for both prevention of sudden cardiac death and for non-lethal arrhythmias; these most often use transvenous pacer wires through the subclavian vein. As these wires may predispose to central vein stenoses, we explored the association between the presence of cardiac devices and vascular access outcomes.

We retrospectively analyzed data from 590 patients who received chronic hemodialysis at DCI Boston since 1995. Cardiac device status, vascular access history and central vein patency were ascertained from review of the DCI and Tufts Medical Center electronic medical record, with radiographs reviewed when available. We recorded the venous access history for each patient, both prior to and after device insertion. The presence of central stenoses was determined by reviewing venogram and duplex results for each patient if available. If imaging was unavailable, stenoses were assumed absent.

From 590 patients, we identified 43 with transvenous cardiac devices; 34 (79%) had imaging and 21 (49%) had documented central vein stenosis. Of these 21, 10 (48%) were catheter dependent after device insertion. In comparison, among 547 patients without devices, 297 (54%) had imaging and 94 (17%) had an identified central stenosis.

In conclusion, hemodialysis patients with cardiac devices have a higher rate of central vein stenoses. This may be related to the physical presence of transvenous pacing wires and has implications for patients' future vascular access options. Careful risk:benefit assessment and consideration of possible alternatives, including use of epicardial leads, should be undertaken prior to transvenous wire insertion in hemodialysis patients as well as late stage CKD patients.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1556

**Bacterial Biofilm in Non-Infected Tunneled Hemodialysis Catheters** Venkataraman Ramanathan,[1] Sarah Riosa,[2] Atef Al Sharif,[2] Adrian Paul Abreo,[1] Saima Aslam,[2] George M. Nassar,[3] Rabih Darouiche.[2] *[1]Division of Nephrology, MEDVAMC, Baylor College of Medicine, Houston, TX; [2]Infectious Diseases, MEDVAMC, Baylor College of Medicine; [3]The Kidney Institute of Houston, .*

**Background:** Biofilm forms on the surface of tunneled hemodialysis (HD) catheter and is often associated with bacteremia. Knowledge about biofilm thickness and location is important, especially in the subset of patients without clinical evidence of infection, to plan preventive anti-biofilm strategies.

**Methods:** We identified patients in whom tunneled HD catheter was removed for non-infective etiologies and who did not have current clinical evidence of infection. The sterile

catheter segment immediately distal to cuff and the double-barreled distal tip were defined as "extravascular" and "intravascular" segments respectively. Outer and luminal surfaces of both catheter segments were analyzed separately. Paired Student's t-test was used.

**Results:** We examined 22 catheters with a confocal laser scanning microscope. Median duration of catheter was 101 days. Biofilm was seen in 17 of them (77%). As shown in the table, biofilm was significantly thicker on the outer surface compared to luminal surface for both extravascular and intravascular segments. Overall, extravascular segments had thicker biofilm compared to intravascular segments on both outer and luminal surfaces.

Biofilm thickness

| | Outer surface | Luminal surface | p-value |
|---|---|---|---|
| Extravascular | 3.1μ | 2.4μ | <0.001 |
| Intravascular | 2.4μ | 1.5μ | <0.0001 |
| p-value | =0.001 | =0.001 | |

Despite absence of clinical infection, 3 catheters grew an organism (14%). All the three patients had significant growth from outer surface of extravascular segment. One patient had growth from outer surface of both extra and intravascular segments. S. epidermidis was the commonest organism (3/3) and one patient had S. aureus (1/3).

**Conclusions:** Biofilm is present on the surface of tunneled HD catheter even in the absence of clinical infection. Outer surface of extravascular catheter segment has the thickest biofilm and harbors bacteria in few patients. It is possible that exit site may be the primary port of entry for bacteria.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1557

**Permanent Dialysis with a Catheter: Fact and Fiction OR One Size Does Not Fit All** Kotagal Shashi Kant, Heather Duncan, Mahmoud T. El-Khatib. *Nephrology, Univ of Cincinnati, Cincinnati, OH.*

**Introduction:** The disadvantages of long term catheter use for hemodialysis vascular access are well known. Mortality, infection as well as other morbidities are all increased by catheter use. Both the KDOQI guidelines and Fistula First Initiative reflect this. However, the increasing co-morbidities of incident and increasing vintage of prevalent patients means that there are a group of patients who now dialyse with permanent catheters. Catheter prevalence in dialysis facilities is likely to be viewed as a marker of negative quality. Despite the well known negatives associated with long term catheters, there are patients who are well served by continuing to use catheters when other modalities are not possible..

**Methods:** All records for HD patients at a single outpatient unit were reviewed and three groups of patients were identified who dialyzed with a single type of access (Catheter, Graft or Fistula) for at least 90% of a 24 month period. Treatment parameters, demographics, labs and prcedures done to maintain the access were analyzed in an effort to identify differences among the three groups.

**Results:** Proportions of males, diabetic and black race were equivalent among C, G and F groups. Time since ESRD diagnosis did not differ. Average Kt/V was higher in G, but was >1.6 in C and F groups. Mean epo dose/kg did not vary, but Alb was lower in C.

Comparison of Several Measures by Access Type

| Group | Alb | Kt/V | HD missed d/t hosp | proc/month | proc cost/yr |
|---|---|---|---|---|---|
| C | 3.64* | 1.61* | .10 | 0.15 | $84 * |
| G | 3.83 | 1.82 | .08 | 0.16 | $7500 |
| F | 3.92 | 1.68† | .08 | 0.04† | $1500† |

*C diff from G, ** C diff from F, † F diff from G

Median # HD treatments missed due to hospitalization were similar in C, G and F. Med # Procedures, defined as activase, cath change, angioplasty, declot, stent or revision were significantly lower in F, but similar for G and C. Using estimates for cost of each of these procedures, the average cost per year for maintenance of an access was substantially higher in G.

**Conclusion:** In this selected group of pts we demonstrated that there were no additional costs or hospitalization associated with dialysis catheter use.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1558

**Risk Factors for Central Vein Stenosis Following Tunneled Dialysis Catheter Insertion** Darren Green,[1] Ganepola Arachhige Chandrkumara Wijesekara,[1] Philip A. Kalra,[1] Babatunde Adeniyi Campbell.[2] *[1]Dept. of Renal Medicine, Salford Royal Hospital, Salford, United Kingdom; [2]Manchester Institute of Nephrology and Transplantation, United Kingdom.*

Using central venous catheters for maintenance haemodialysis is associated with a risk of central vein stenosis and long term compromise of vascular access.

The aim of this study was to evaluate whether pathologies which are known to cause vascular or endothelial injury are associated with risk of central vein stenosis following tunneled dialysis catheter insertion, and whether drugs which improve vascular outcome in other settings reduce the stenosis event rate.

Information was collected from a prospective database of all tunneled dialysis lines inserted at a nephrology unit over 2 years. Medical history and blood results from the time of insertion were recorded. Radiological evidence of ipsilateral stenosis of the subclavian, brachiocephalic or internal jugular vein over the following three year period was noted.

178 Permcath catheters were inserted in 134 patients. There were 38 cases of central vein stenosis. There was correlation between the number of tunneled lines and development of stenosis (mean number of catheters, stenosis vs. no stenosis = 1.66 vs. 1.20, p<0.001). The impact of duration of dialysis via tunneled catheter did not reach statistical significance (weeks dialyzing via catheter = 62.2 vs. 47.3, p=0.053). There was no difference in number of preceding temporary dialysis lines. Patients who developed stenosis suffered more episodes of bacteremia during the catheter life (0.84 vs. 0.35, p=0.004). Age, gender, diabetes mellitus, anti-platelet drugs and statins did not link to developing stenosis. More patients who developed stenosis were taking statins, but this did not reach statistical significance (73.68% vs. 55.91%, p=0.070). Mortality at three years was the same in both groups (52.63% vs. 47.91%, p=0.386).

Recurrent line insertion and infection are significant risk factors for developing central vein stenosis, suggesting an important role of chronic inflammation in its pathophysiology. The higher rate of statin use among patients with stenosis may indicate a causative role of hypercholesterolemia.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1559

**Conventional Short Dwell Alteplase (TPA) Is Less Effective Than Overnight Long Dwell TPA in the Treatment of Hemodialysis Catheter Dysfunction (CD)** Subash Regmi, Muneeb S. Malik, Jesse M. Goldman. *Nephrology, Temple University Hospital, Philadelphia, PA.*

Thrombotic dysfunction of hemodialysis (HD) catheters is common. Though tissue plaminogen activator (TPA) is the most commonly used thrombolytic agent to treat dysfunctional HD catheters it is not FDA approved for this indication. Optimal TPA dwell time is unknown to reestablish HD catheter function. **Methods:** We conducted a review of HD pts receiving intracatheter TPA for CD in our inpatient HD unit: 10/01/08 - 12/31/09. We included pts receiving TPA for AKI & CKD-5D. We included tunnelled and non-tunnelled catheters. 409 TPA administrations were identified using our pharmacy database. Review of records identified 48 pts and 68 TPA administrations that fulfilled eligibility criteria. Mean age: 54.13 yrs. Sex: 24 males and 24 females. Catheter type: 47 tunnelled and 21 non-tunnelled. Catheter location: 46 IJ; 22 femoral. Short Dwell (SD): 40 and Long Dwell (LD): 28. Average SD time: 35 minutes; LD time: 2587.5 min. (1.8 days) Range (905-4375 min). Hemoglobin = 9.5 g/dl (SD), 9.9 g/dl (LD). Eligibility Criteria: 1) Documented CD defined as blood flow ($Q_b$) <300 ml/min or $Q_b$ ≤300 ml/min with arterial pressure (AP) ≤ -250 mmHg. 2) Documented HD session post TPA administration. Exclusion Criteria: 1) TPA administered due to heparin contraindication 2) No clear documentation to support CD. SD: TPA instillation in one or both HD catheter ports with dwell time of ≤ 60 min. LD: TPA instillation in one or both HD catheter ports & dwelled > 60 min (1-3 days). Defined Success: Ability to complete one HD session with improvement $Q_b$≥300 ml/min with AP ≥ -250 mmHg.

Short Dwell success: 25%

Long Dwell success: 61%.

Success Reestablishing Adequate Flow

| Dwell Time | Yes | No | Total |
|---|---|---|---|
| Short | 10 | 30 | 40 |
| Long | 17 | 11 | 28 |
| Total | 28 | 41 | 68 |

Fisher's exact = 0.005 1-sided Fisher's exact = 0.003

Conclusions:

Long TPA dwell is more effective in reestablishing adequate blood flow compared with short dwell in HD catheters. Short dwell is still somewhat effective & may be valuable according to clinical circumstances. A prospective, randomized trial is needed to corroborate these results. It is unknown if TPA is superior to Tenecteplase.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1560

**Impact of Hemodialysis Catheter Dysfunction on Utilization of Medical Services** Robert I. Griffiths,[1] Britt B. Newsome,[2] Geoffrey A. Block,[2] Grace Leung,[3] Mark D. Danese.[1] *[1]Outcomes Insights, Inc., Westlake Village, CA; [2]Denver Nephrologists, P.C., Denver, CO; [3]Genentech, Inc., South San Francisco, CA.*

**Purpose:** To evaluate the impact on medical services of catheter dysfunction, defined by National Kidney Foundation Kidney Disease Outcomes Quality Initiative guidelines as failure to attain an extracorporeal blood flow rate (BFR) above 300 mL/min. **Methods:** We conducted a retrospective cohort study using data from DaVita, Inc. merged with the United States Renal Data System. DaVita data contained records for each dialysis session, including date of service, access type, planned and actual BFR, and length of dialysis session. Records for missed dialysis sessions contained the date and reason for missing the session, including "access problems." Patients were included if they had ≥ 8 consecutive weeks of catheter dialysis between 08/2004 and 12/2006. They were followed from first to last catheter dialysis session. Catheter dysfunction was defined as actual BFR < 300mL/min despite a planned BFR ≥ 300mL/min. Two approaches, multivariate repeated measures and case-crossover, were used to analyze associations between catheter dysfunction and patterns of medical services. **Results:** Of 44,470 patients in the merged data set, 9,707 (22%) met the study criteria. The average age was 62, 53% were female, and 40% were black. The median length of catheter dialysis was 190 days. There were 1,075,701 catheter dialysis sessions, 70,361 (6.5%) met the definition of catheter dysfunction, and 6,331 (65.2%) patients had ≥ 1 session with catheter dysfunction. In multivariate repeated measures analysis, catheter dysfunction was associated with increased odds of a missed session due to access problems (OR 2.50; P < 0.001), receiving an access-related procedure (OR 2.10; P < 0.001), either missed session due to access problems or access-related procedure (OR 2.21; P < 0.001), and all-cause hospitalization (OR 1.10; P = 0.001). It was not associated with length of dialysis. Case-crossover results were similar. **Conclusion:** Catheter dysfunction is associated with increased use of medical services and disruptions in dialysis treatment.

Disclosure of Financial Relationships: Research Funding: Outcomes Insights has received research funding from Genentech and Amgen.

**F-PO1561**

**Burden of Catheter Dysfunction in End-Stage Renal Disease (ESRD)** Robert I. Griffiths,[1] Britt B. Newsome,[2] Geoffrey A. Block,[2] Grace Leung,[3] Mark D. Danese.[1] *[1]Outcomes Insights, Inc., Westlake Village, CA; [2]Denver Nephrologists, P.C., Denver, CO; [3]Genentech, Inc., South San Francisco, CA.*

**Purpose:** To describe the epidemiology of dialysis catheter dysfunction in ESRD.

**Methods:** A retrospective cohort study was conducted using data from DaVita, Inc. and USRDS. Patients were included if they received ≥ 8 weeks of uninterrupted hemodialysis through a catheter between 08/2004 and 12/2006, and either (A) catheter hemodialysis was their first treatment modality following ESRD diagnosis, or (B) catheter hemodialysis immediately followed ≥ 1 month of hemodialysis exclusively through a graft or fistula. They were followed from the beginning to the end of catheter dialysis, death, or end of the observation period, whichever came first. Catheter dysfunction during a dialysis session was defined as actual blood flow rate (BFR) < 300mL/min despite planned BFR ≥ 300 mL/min. Multivariate (MV) Cox regression analysis was performed to identify patient factors associated with catheter dysfunction.

**Results:** There were 3,364 patients in the cohort. The average age was 62, 51% were male, 42% were black, 46% had diabetes and 28% had hypertension reported as the underlying cause of renal failure. The cohort accounted for 268,363 catheter dialysis sessions. The median duration of catheter dialysis was 143 days. There were 19,118 (7.1%) sessions with catheter dysfunction, 64% of patients had ≥ 1 catheter dysfunction session during their catheter treatment history, and approximately 30% had ≥ 1 catheter dysfunction session per month on catheter dialysis. The median time to first session with catheter dysfunction was 95 days. In MV analysis, male gender and black race were associated with lower rates of catheter dysfunction. Patients who had received hemodialysis through a fistula or graft immediately prior to catheter hemodialysis had a higher rate of catheter dysfunction compared to those with catheter hemodialysis as their first modality (Hazard Ratio: 1.13; P=0.04).

**Conclusions:** Catheter dysfunction affects approximately 30% of catheter dialysis patients each month. Patients with prior graft or fistula appear to be at higher risk.

Disclosure of Financial Relationships: Research Funding: Outcomes Insights has received research funding from Genentech and Amgen.

**F-PO1562**

**Comparison of the Palindrome Versus Step Tip Tunneled Hemodialysis Catheter: Prospective Randomized Trial** Hyeonseok Hwang,[1] Seokhui Kang,[1] Byung Ha Chung,[1] Bumsoon Choi,[1] Cheol Whee Park,[1] Chul Woo Yang,[1] Yong-Soo Kim.[1, 2] *[1]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea, Seoul, Republic of Korea; [2]Interventional Nephrology Clinic, Seoul St. Mary's Hospital, Republic of Korea.*

**Background:** The Palindrome tunneled hemodialysis catheter is recently developed vascular access with unique symmetric tip design. The aim of this study was to compare the function and complications of the Palindrome catheter with those of step-tip tunneld hemodialysis catheter.

**Methods:** In this prospective, randomized trial, 22 patients were assigned to the Palindrome catheter group and 23 patients to step-tip catheter group. Interventional nephrologist placed all catheters using ultrasound and fluoroscopic guidance. At baseline and one month, effective flow rate (QbEff) and recirculation was examined at various pump speeds (Qb), and Kt/V of urea was measured at Qb 300 ml/min. The procedure-related data and catheter-related complications were recorded during entire study period.

**Results:** The baseline characteristics, procedure time and placed catheter length were similar between Palindrome and step-tip group (all $p > 0.05$). There were 5 procedure-related complications including tunnel bleeding (n = 1, step-tip) and exit site bleeding (n = 3, Palindrome; n = 1, step-tp) (all p > 0.05). Adjusted means of QbEff at Qb 200, 300, 400 ml/min was 226, 334, 428 ml/min in Palindrome and was 225, 332, 431 ml/min in step-tip group (all $p > 0.05$). The recirculation at each flow, Kt/V of urea (1.45 vs. 1.35) and late complication rate (5% vs. 17%) was similar between two groups (all $p > 0.05$). While the groups did not differ as catheter survival ($p = 0.189$), re-intervention free survival rate was higher in Palindrome group ($p = 0.021$). Of the patients with catheter dysfunction, dialysis lines were reversed in 8 patients (n = 3, Palindrome; n = 5, step-tp) and recirculation tended to be lower in Palindrome than in step-tip group (0.0 vs. 20.3%; $p = 0.124$).

**Conclusion:** The Palindrome catheter was more useful to reduce catheter re-intervention rate than step-tip catheter and might provide minimal recirculation benefit, despite of arteriovenous reversal state.

Disclosure of Financial Relationships: Research Funding: The study was funded by Novartis Pharma.

**F-PO1563**

**Analysis of Exit Site Infections in Facilities Using Electrolytically-Produced Sodium Hypochlorite** Steven M. Wilson, Amy Burdan, Amy Young. *DaVita Inc., Denver, CO.*

**Introduction:** Sodium hypochlorite solution is an effective topical disinfectant and is used to prophylactically reduce the risk of bacterial contamination and skin infections with central venous catheters (CVCs) for dialysis. KDOQI states the average CVC infections for tunneled cuffed catheters is 3.1 per 1000 days at risk. However, little comparative data has been shown about this preparation vs the use of iodine for skin site preparation with ESRD patients. To identify best practices, we surveyed dialysis clinics to document this infection control procedure. **Methods:** 226 dialysis facility administrators (FAs) with a patient census >20 completed the survey. They were asked about their method of skin preparation and patient outcomes. Infection rates in facilities using an electrolytically-produced sodium hypochlorite (ESH; ExSept Plus or Alcavis 50) were compared to those using conventional iodine based antiseptic protocol. The facility proportion of infections was defined as the number of infections found in the study period divided by the number of individuals at risk and their number of days at risk. **Results:** 80% reported using ESH for exit site disinfection. 17% of facilities applied an antibiotic ointment to the exit site following disinfection. Infection rates from 5/09-10/09 showed no significant differences for any month, although in almost all months the observed percentage of infections was lower in facilities using ESH. These facilities had an observed infection rate±SE of 1.99 (±3.26) infections per 1000 patient days with CVC while conventional facilities had an observed infection rate of 2.10 (±7.09) infections per 1000 patient days with CVC (ns). **Conclusions:** ESH use was much higher than expected. This may show a possible change in practice pattern of using ESH in dialysis treatments where both the use of CVCs and infections is common. While the observed proportion of infections was lower in ESH facilities in 5 of 6 months, this difference was not statistically significant. FAs reported anecdotally the perceived benefits of a reduced procedure time to achieve skin disinfection, less aggressive cleaning of the site, no sensitization, and catheter material compatibility.

Disclosure of Financial Relationships: Employer: DaVita, Inc.

**F-PO1564**

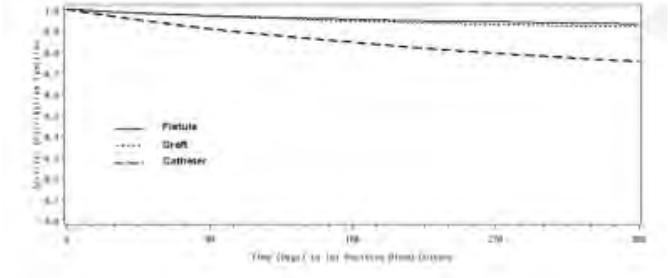
**Hemodialysis Vascular Access Type and Bloodstream Infection Rates** Eduardo K. Lacson, Weiling Wang, J. Michael Lazarus, Raymond M. Hakim. *Fresenius Medical Care, North America, Waltham, MA.*

**Introduction:** We evaluated bloodstream infection (BSI) rates based on positive blood cultures by type of vascular access in a national cohort of incident hemodialysis (HD) patients during their first year.

**Methods:** All HD patients admitted to Fresenius Medical Care North America facilities within 15 days of first-ever dialysis between January 1 and December 31, 2007 with known access type (fistula, graft, or catheter) were included. Use of a catheter was classified under "catheter" despite the presence of another maturing access. Blood culture results from Spectra lab were tracked for up to 1 year or until a change in access type or discharge/death. Age, gender, race, and diagnosis of diabetes were collated on admission.

**Results**: Among 25,003 patients, 78.5% had catheters, 16.6% fistulas, and 4.9% grafts. Mean age was 63.4 ± 15.3 years, 56.1% male, 64.8% white and 30.0% black, with 55.1% DM. Overall, >85% of all blood cultures were captured, with 3,327 (13.3%) patients with at least one positive result during follow-up. The time to 1st BSI by access type, predominantly catheters, is shown in Figure 1.

Figure 1: Kaplan-Meier curves for time to 1st positive blood culture




Median time to presumed catheter-associated BSI was 87 days at a rate of 0.61/1000 catheter-days. The unadjusted hazard ratio for BSI in catheter vs. fistula patients was 3.78 and 3.83 after case-mix adjustment (both p<0.0001). No significant differences existed between fistulas and grafts.

**Conclusions**: Catheter-associated BSI occurs early, within the first 90 days, supporting arteriovenous access placement prior to the need for dialysis or immediate transition towards a permanent access upon HD initiation. The study was limited by incomplete capture of all blood culture results, likely resulting in an underestimate of BSI rates.

Disclosure of Financial Relationships: Employer: I am an employee of Fresenius Medical Care, North America.

**F-PO1565**

**Non Fluoroscopic Insertion of Tunneled Hemodialysis Catheter by Nephrologists** Tee Chau Keng, Wai Yew Kong, Kok Peng Ng, Chew Ming Wong, Yip-Boon Chong, Li Ping Tan, Soo Kun Lim. *Nephrology Unit, University Malaya Medical Center, Malaysia.*

Introduction

Tunneled cuffed catheter (TCC) is the essential lifeline for incident hemodialysis patients without preformed permanent access. Fluoroscopy is mandatory under KDOQI guidelines for TCC insertion but not readily available in many institutions. This invariably results in more usage of non tunneled cuffed catheters (NTCC) which carries unacceptably high risk of infection. We report the role of TCC insertion without fluoroscopy by nephrologists.

Method

We performed a retrospective study on non fluoroscopic TCC insertion by designated nephrologists for incident patients from October 2009 to March 2010. The insertion was via either ultrasound guided cannulation of the deep veins or catheter exchange using the existing NTCC. The procedures were performed using the standard method and guided

by anatomical landmarks, aiming for tip of catheter in the mid right atrium for internal jugular (IJ) catheters or inferior vena cava for femoral catheters. Position of the catheters was verified radiologically post insertion. All patients were discharged to their respective dialysis centers post insertion and followed up by phone calls.

Results

Forty five TCC were inserted in 45 incident patients (24 male and 21 female, mean age 60 years; range 34-89years). Thirty five right IJ, 6 femoral, 3 left IJ, and 1 subclavian TCC were placed. Total length of follow up was 4497 catheter days. The primary patency rates at 30, 60 and 90days were 90.1, 83.3 and 76.9% respectively. The 30, 60 and 90-day secondary patency rates were 95.5, 88.1 and 82.1%. Immediate failure was noted in 2 of the left IJ TCC (4.4%). Catheter dysfunction required intervention was 6.7%. The only complication observed was non life threatening bleeding in 2 patients (4.4%). Infection rates at 30 and 90 days were 4.5 and 18%. Overall infection rate was 0.18 per 100 catheter days. There were 3 deaths but non catheter related.

Conclusion

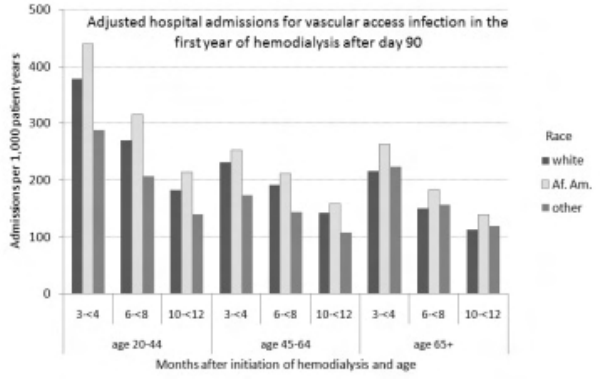
Non fluoroscopic insertion of TCC, excluding left IJV, in the hands of committed nephrologists can be safe with comparable patency rates. This will invariably reduce the dependency on NTCC in institutions with limited access to fluoroscopy.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1566

**Racial Disparity in Hospital Admissions for Vascular Access Infection among Incident Hemodialysis Patients** Tricia L. Roberts,[1] Allan J. Collins,[1,2] David T. Gilbertson.[1] *[1]U.S. Renal Data System Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

While mortality in incident HD patients has been lower among African Americans than whites, little is known about predictive factors behind high first-year vascular access (VA) infectious admission rates. We analyzed predictors of hospital admissions for VA infection in the first year of HD following day 90 after initiation in a cohort of 105,313 U.S. incident patients in 2006 and 2007. To allow data availability, included adult Medicare patients survived the first three months of dialysis and had a Medical Evidence Form indicating access type used on first outpatient dialysis. Admission rates for VA infection were computed by age, race, and months from dialysis initiation, and adjusted for gender, primary diagnosis, and initial VA type. We assessed predictors of first admission for VA infection during months 3 to 12 with Cox proportional hazards regression models.




Admissions for VA infection were highest among African Americans compared to whites and other races within each age and interval. Among race and age combinations, African Americans age 20-44 had the highest percentage (67.9%) with only a catheter as the initial access. Compared to whites, African Americans had higher risk of admission for VA infection (HR=1.12; 95% CI 1.07-1.17) and other races had lower risk (HR=0.88; 0.79-0.97). Other factors associated with VA infection were younger age, diabetes, and catheter as initial access type. Age 20-44 African Americans showed significantly higher adjusted risk (P<0.05) than other age and race groups except age 20-44 white. Despite lower overall mortality rates, African Americans had high first-year admissions for VA infection, and catheter use may be a contributing factor. Results suggest further study of differential racial impact of infections on subsequent mortality.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1567

**Effectiveness of a Shower Washing Method without Antiseptics and Histological Findings at Exit-Site of Tunneled Cuffed Venous Catheters** Hiroshi Shibahara,[1] Nami Shibahara,[2] Susumu Takahashi.[3] *[1]Blood Purification Center, Sagamihara Kyodo Hospital, Sagamihara, Kanagawa, Japan; [2]Hashimoto-Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan; [3]Nihon University Graduate School, Tokyo, Japan.*

**Background** To prevent tunneled cuffed venous catheter (TCC) from exit-site infections, catheter care is essential. We have reported a new care method designed to prevent infection by normalizing skin conditions at the exit-site, which is directly cleaned using a shower without antiseptic agents. We evaluated the efficacy of this care method and examined histological findings of the skin at the TCC exit-site.

**Methods:** The subjects were 109 hemodialysis patients (male/female, 73/36; mean age, 67.2 ± 12.7 years; diabetes mellitus/non-diabetes mellitus, 48/61; mean catheter days, 57.6 ± 46.7 days), using a TCC, who gave informed consent at Sagamihara Kyodo Hospital and Hashimoto-Minami Internal Medicine Clinic between January 2007 and December 2009. Our method involves washing the TCC exit-site with tap water or physiological saline immediately after TCC insertion. Moisture can be wiped away with non-sterile gauze. No antiseptics are applied. TCC is fixed with an appropriate tape. When bathing at home, the exit-site and surrounding skin can be washed using shower water without a water-proof film covering. Then, moisture is wiped away with a clean laundered towel. Shower washing at the exit-site with tap water is continued until TCC removal. In 10 patients, skin at the TCC exit-site was histologically examined at the time of TCC removal.

**Results:** Exit-site infection occurred in 5 patients (4.6%). Neither tunnel nor catheter-related bloodstream infections were observed during this investigation. Histological examination revealed the surrounding epidermis at the exit-site to show downward growth into the deeper dermis, maintaining the normal skin structure.

**Conclusion**: This washing method is effective in managing TCC exit-sites. The skin at exit-sites retained its normal structure, histologically confirming the efficacy and safety of this catheter care method.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1568

**Hemodialysis Catheters Increase Mortality as Compared to Shunts Especially in Elderly Patients** Gurbey Ocak,[1] Nynke Halbesma,[1] Saskia le Cessie,[1] Ellen K. Hoogeveen,[2] Sandra Van Dijk,[3] Jeroen Kooman,[4] Friedo W. Dekker,[1] Raymond T. Krediet,[5] Elisabeth W. Boeschoten,[6] Marion Verduijn.[1] *[1]Clinical Epidemiology, LUMC, Leiden, Netherlands; [2]Internal Medicine, JBH, Den Bosch, Netherlands; [3]Medical Psychology, LUMC, Leiden, Netherlands; [4]Nephrology, UHM, Maastricht, Netherlands; [5]Nephrology, AMC, Amsterdam, Netherlands; [6]HMI, Naarden, Netherlands.*

**Objectives:** Catheter use has been associated with an increased mortality risk in hemodialysis patients. However, differences in the all-cause and cause-specific mortality risk between catheter use and shunt use in young and elderly hemodialysis patients has not yet been investigated.

**Methods:** 1109 incident hemodialysis patients from 38 centers in the Netherlands between 1997 and 2004 were included in this study. Hazard ratios (HRs) with 95% confidence intervals (95% CIs) were calculated for 2-year all-cause, infection-related, and cardiovascular mortality in elderly patients aged ≥65 years with a catheter as compared to elderly patients and young patients aged <65 with a shunt adjusted for age, sex, primary kidney disease, Davies comorbidity score, angina pectoris, myocardial infarction, heart failure, ischemic stroke, claudication, predialysis care, GFR, and serum albumin levels.

**Results:** Of the 1109 patients, 919 had a shunt and 190 had a catheter. The mortality rate was 76 per 1000 person-years in young patients with a shunt, 129 per 1000 person-years in young patients with a catheter, 222 per 1000 person-years in elderly patients with a shunt, and 427 per 1000 person-years in elderly patients with a catheter. The adjusted HRs in elderly patients with a catheter as compared to elderly patients with a shunt was 1.51 (95% CI 1.10-2.06) for all-cause mortality. The adjusted HRs in elderly patients with a catheter as compared to young patients with a shunt were 3.05 (95% CI 2.04-4.56) for all-cause mortality, 3.17 (95% CI 1.00-10.02) for infection-related mortality, and 2.74 (95% CI 1.54-4.90) for cardiovascular mortality.

**Conclusions:** Especially elderly hemodialysis patients with a catheter have an increased all-cause, infection-related, and cardiovascular mortality risk as compared to young patients with a shunt.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1569

**Pattern of Vascular Access in Hemodialysis Patients: Cross-Sectional Study of Clinical Research Center for End Stage Renal Disease in Korea** Ja-Yong Park,[1,2] Young-Deuk Yoon,[1,2] Ji-Young Choi,[1,2] Se-Hee Yoon,[1,2] Sun-Hee Park,[1,2] Chan-Duck Kim,[1,2] Sung-Ho Kim,[2] Jun-Young Do,[2] Seong Eun Kim,[2] Sang Heon Song,[2] Yeong Hoon Kim,[2] Nam Ho Kim,[2] Yon Su Kim,[2] Shin-Wook Kang,[2] Chul Woo Yang,[2] Yong-Lim Kim.[1,2] *[1]Kyungpook National University School of Medicine; [2]Clinical Research Center for End Stage Renal Disease, Korea.*

**Purpose:** To examine vascular access patterns and contributing factors affecting them in hemodialysis(HD) patients.

**Methods:** From April 2009 to March 2010, we conducted a cross-sectional study to identify vascular access patterns and factors that influence the choice of vascular access according to demographics and co-mobidities using the data from 729 HD patients in CRC for ESRD in Korea.

**Results:** Of the 729 HD patients, 465 patients (63.8%) were being dialyzed with a native arterio-venous fistula(AVF), 137 patients (18.8%) with a graft, 69 patients (9.5%) with a tunneled catheter and 58 (8.0%) patients with a non-tunneled catheter. Of the 614 prevalent patients, the proportions of access pattern are 72.5%, 21.2%, 5.9% and 0.5% respectively. And of the remaining 115 incident patients, the proportions are 17.4%, 6.1%, 28.7% and 47.8% respectively. In the 729 patients, the predictors of native AVF versus other access use are body mass index<25kg/m$^2$(adjusted odds ratio(AOR) 1.483, p=0.049), presence of peripheral vascular disease (AOR 0.263, p<0.001), cerebro-vascular disease (AOR 0.377, p<0.001) and arrhythmia (AOR 0.478, p=0.027). In prevalent patients, the predictors are presence of coronary artery disease (AOR 0.551, p=0.006), peripheral vascular disease (AOR 0.193, p<0.001), cerebro-vascular disease (AOR 0.351, p<0.001), and hypertension (AOR 2.031, p<0.001). In incident patients, there are no predictors influencing the choice of access pattern.

**Conclusions:** In incident HD patients of Korea, there are still a very high proportion of patients starting dialysis with a catheter and no patient-specific factors that influence the

choice of vascular access. Comorbid conditions such as obesity, cardiovascular disease, cerebro-vascular disease and arrhythmia could be possible causes for the high proportion of catheter use in HD patients.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1570

**Relationship between Blood Flow Rate in Tunneled HD Catheter and Dialysis Adequacy** Byung Ha Chung, Seokhui Kang, Hyeonseok Hwang, Bumsoon Choi, Cheol Whee Park, Chul Woo Yang, Yong-Soo Kim. *Department of Internal Medicine, Seoul St. Mary's Hospital.*

**Background**: The NKF-DOQI guideline defines central venous catheter (CVC) dysfunction as failure to attain extracorporeal blood flow of 300 ml/min, based on the assumption that a lower blood flow would result in poor dialysis clearance. We observed the relationship between CVC blood flow and dialysis adequacy in Korean hemodialysis patients.

**Methods**: In this prospective interventional study, patients were included when they were on maintenance HD and using a tunneled HD catheter in the right internal jugular vein. Patients underwent dialysis using the FMC 5008 dialysis machine and low flux dialyzer (surface area 1.3 $m^2$).In each patient, urea reduction ratio (URR) and spKT/V were measured at blood flow rate (BFR) of 200, 250 and 300 mL/min.

**Results**: 118 dialysis sessions in 41 patients were analyzed. 30 patients (61.2%) were male. Pre-dialysis BUN, creatinine, hemoglobin, serum protein and albumin levels did not differ among each BFR settings. The mean pre-pump (arterial) pressure during dialysis was significantly lower at BFR of 300 mL/min (-133.0 ± 27.4 mmHg) compared to BFR of 250 mL/min (-99.9 ± 41.0 mmHg) and 200 mL/min (-86.7 ± 12.8 mmHg). The URR and spKT/V at BFR of 200 mL/min (67 ± 6%, 1.36 ± 0.26) was significantly lower than those at BFR of 250 mL/min (71 ± 7%, 1.45 ± 0.24) and 300 mL/min (72 ± 11%, 1.55 ± 0.38) ($P<0.05$, respectively). According to URR, the proportion of inadequate dialysis was significantly higher at BFR of 200 mL/min (13 cases, 34.2%) than at BFR of 250 mL/min (4 cases, 10%) and 300 mL/min (2 cases, 5%) ($P<0.05$). In comparison between the adequate and inadequate dialysis patients, there were significant differences in BFR, sex and body surface area (BSA, $m^2$) and BMI ($kg/m^2$). In multivariate analysis, BFR and BSA were significant predictors for inadequate dialysis. In patients with BSA less than 1.9 $m^2$, adequate dialysis could be attained in more than 90% of patients with BFR of 250 mL/min.

**Conclusion**: Our results suggest that BFR of 250 mL/min may be enough to reach adequate dialysis in Korean hemodialysis patients. The definition of catheter dysfunction needs to be reevaluated.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1571

**Right Atrial Thrombus Associated with Tunneled-Cuffed Hemodialysis Catheters: Usefulness of Transesophageal Echocardiography** Shinji Tanaka, Naobumi Mise, Tokuichiro Sugimoto. *Nephrology, Mitsui Memorial Hospital, Chiyoda-ku, Tokyo, Japan.*

**Purpose:** The aim of this study was to clarify the characteristics of clinically relevant right atrial thrombus (RAT) associated with tunneled-cuffed hemodialysis catheters (TCCs).

**Methods:** This retrospective cohort study comprised 57 consecutive chronic hemodialysis patients (31 male, mean age 72 ± 11 years, mean dialysis vintage 63 ± 95 months) who started to use TCCs between July 1999 and October 2009. All TCCs were inserted via subclavian vein and the catheter tip was located in the right atrium. All patients were followed until the end of 2009 to investigate clinically relevant RAT. Clinically relevant RAT was defined as RAT with refractory fever and/or catheter dysfunction.

**Results:** During the follow-up period of 10 ± 12 catheter-months, clinically relevant RAT (mean size 2.2 ± 0.4 cm) was identified in 5 patients (8.8%), 7.4 ± 7.0 months (range 2-16 months) after TCC insertion. The incidence rate was 0.10 per catheter-year. There was no significant difference in age, dialysis vintage, and diabetic status between patients with and without RAT. RAT was diagnosed by transesophageal echocardiography in 4 patients and enhanced CT in 1, whereas transthoracic echocardiography failed to identify RAT in any of the patients. Bacteremia and septic pulmonary embolism were found in 3 patients. All patients were successfully treated, either by catheter removal in 2 or by catheter salvage with antibiotic and anticoagulant therapy in 3. Four patients died 14 ± 7 months (range 8-25 months) after RAT diagnosis, but no death was attributable to RAT.

**Conclusions:** Clinically relevant RAT was not rare in patients with TCC, and could be formed relatively soon after TCC insertion. Transesophageal echocardiography or enhanced CT is recommended to diagnose RAT.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1572

**A Comparison between Blood Flow Outcomes of Tunneled External Jugular and Internal Jugular Hemodialysis Catheters** Hemender Singh Vats,[1] Micah R. Chan,[1] Henry N. Young,[2] Alexander S. Yevzlin.[1] *[1]Nephrology, University of Wisconsin School of Medicine and Public Health, Madison, WI; [2]Pharmacy, University of Wisconsin School of Pharmacy, Madison, WI.*

**Background**

The right internal jugular (RIJ) vein is the vessel of choice for tunnelled hemodialysis (HD) catheters. The second choice is left internal jugular (LIJ) vein, though use of LIJ potentially jeopardises left arm arteriovenous fistula (AVF) or arteriovenous graft (AVF) placement,given high rates of catheter associated central venous stenosis. The right external jugular (EJ) vein has recently been shown to be a viable alternative to the LIJ, but concerns persist among clinicians about chronic blood flow capacity in EJ catheters. The purpose of this study is to compare the blood flow outcomes in a series of percutaneously placed external jugular (EJ), LIJ and RIJ HD catheters.

**Methods**

Using a prospectively collected database 46 hemodialysis patients referred for tunneled catheter placement were identified over four-year period. We compared the blood flow outcomes of date -matched RIJ, LIJ and EJ catheters . Using ANOVA, the blood flow outcomes of the three tunneled catheter techniques at 30-d and 90-d were compared.

**Results**

The three groups did not differ significantly in demographics or comorbid conditions. The flow data at 30 and 90 days is shown below. Using multiple regression analysis, no covariates (age, gender, race, diabetes) or other identifiable factors were found to be associated with blood flow outcomes at 30-d or 90-d.

Demographics and blood flow (Qb) by tunneled catheter technique

| | Age ( avg, yrs) | Gender (% female) | Race (% African American) | Diabetes Mellitus (%) | Qb at 30 Days | Qb at 90 Days |
|---|---|---|---|---|---|---|
| Right Internal Jugular Vein | 55 | 35.3 | 17.7 | 41.2 | 354.4± 54.48 | 348.5± 56.62 |
| Left Internal Jugular Vein | 60 | 52.9 | 17.6 | 68.8 | 344± 42.14 | 341± 22.42 |
| External Jugular Vein | 52.2 | 43.8 | 31.3 | 58.8 | 380± 55.26 | 365.7± 71.76 |

**Conclusions**

This report demonstrates comparable EJ blood flow outcomes at 30-d and 90-d to both LIJ and RIJ historical data. Further prospective investigation is required to rigorously define the role of EJ CVC placement as another potential long-term access modality.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1573

**Use of Citrate Solution as a Hemodialysis Catheter Lock Does Not Result in Greater tPA Use for Catheter Dysfunction** Abhinai K. Gupta, Andrew I. Chin. *Internal Medicine, Division of Nephrology, University of California at Davis, Sacramento, CA.*

**Purpose:** Hemodialysis (HD) units affiliated with our institution made a switch from the traditional Heparin 5000 units/cc solution (Hep lock) to a Citrate solution (ACD lock) as the standard lock formulation for tunneled catheters (TDC). When TDC dysfunction is noted in the HD unit, our first lines of therapy is the intra-luminal instillation of tissue plasminogen activator (tPA).

**Aim:** We sought to determine whether or not the switch from Hep lock to ACD lock resulted in an increase in TDC dysfunction by comparing the frequency of tPA used before and after this switch.

**Methods:** In this retrospective cohort study, we included only patients who were using a TDC for HD access with Hep lock, at least 2 weeks and up to 3 months before and after the date of switch. Patients were excluded if they were already using ACD locks (for Heparin-Induced Thrombocytopenia). Outcome measures analyzed before and after the switch included: the number of times tPA was used, actual duration of HD treatment, erythropoietin dose, catheter failures requiring exchanges. Statistical testing included comparison of continuous variables by paired T-test, proportions by Chi-test.

**Results:** 67 patients were included in the final analysis.

Comparison before and after switch of TDC locking solution

| | Hep lock | ACD lock | p value |
|---|---|---|---|
| Mean days with TDC | 81 | 83 | |
| Mean HD treatments | 32 | 31 | |
| HD time (min) | 191 | 193 | ns |
| tPA events per 100 HD treatments | 2.4 | 3.8 | ns |
| Erythropoietin (units/HD) | 6916 | 6647 | ns |
| total dose tPA given | 66 | 69 | ns |

Use of tPA did not differ after the switch to ACD lock from Hep lock. The number of catheter failures requiring exchange did not significantly differ in the 2 time periods. No significant adverse effects attributable to either TDC locking solution were noted.

**Conclusion:** We did not find a significantly increased utilization of tPA after switching from Heparin to a Citrate containing TDC lock. Even though the percentage citrate in ACD solution is low (3-4%), tPA use as a surrogate for TDC thrombosis did not change in the 2 time periods. A longer period of observation is needed to confirm the efficacy of ACD for routine TDC use.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1574

**Aboriginal Status and Central Venous Catheter Use in Hemodialysis Patients** Lisa M. Miller, Manish M. Sood, Claudio Rigatto, Joe A. Bueti. *Medicine, Section of Nephrology, University of Manitoba, Winnipeg, MB, Canada.*

Differences in access to health care between different racial groups is described in the literature. In Manitoba, there is a large Aboriginal population that constitutes approximately 30-40% of all hemodialysis (HD) patients. We sought to determine if Aboriginal status is associated with a higher likelihood to commence HD with a central venous catheter(CVC) rather than an arteriovenous fistula (AVF). We conducted a retrospective observational study of patients who started HD between January 1, 2006 and July 1 2009. All data was abstracted by chart review. There were 198 patients included in the study. One hundred sixty seven (84%) started HD with a CVC, 31 (16%) with an AVF, and none with an arteriovenous graft. Sixty-nine (35%) were Aboriginal, 88 (45%) Caucasion, and 38 (20%) other racial

background. Aboriginal patients were more likely to be female (p=0.0079) and have diabetes (p=0.0016) than non-aboriginal people. They were also less likely to live in Winnipeg than non- Aboriginal patients (p< 0.001). However, Aboriginal people were not more likely to start HD with a CVC than non-Aboriginal people. Geographic proximity to Winnipeg was not predictive of starting HD with a CVC. Furthermore, diabetes, hypertension, female gender, coronary artery disease, and peripheral vascular disease were not associated with starting HD with a CVC.

Conclusion: Aboriginal status is not predictive of commencing HD with a CVC.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1575

**Comparison of Macroscopic and Histological Findings at Exit-Site Skin of Tunneled Cuffed Venous Catheters** Nami Shibahara,[1] Hiroshi Shibahara,[2] Susumu Takahashi.[3] *[1]Hashimoto-Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan; [2]Blood Purification Center, Sagamihara Kyodo Hospital, Sagamihara, Kanagawa, Japan; [3]Nihon University Graduate School, Tokyo, Japan.*

**Background:** Exit-site infections of tunneled cuffed venous catheter (TCC) are clinically diagnosed based on inflammatory changes of the skin. We compared macroscopic and histological findings of exit-site skin by scoring both to clarify exit-site infections.

**Methods:** Macroscopic and histological examinations at TCC exit-sites upon removal were performed in 15 dialysis patients (male/female, 12/3; mean age, 68.7 ± 15.7; diabetes mellitus/non-diabetes mellitus, 3/12; mean catheter days, 57.5 ± 31.2 days) who gave informed consent at Hashimoto-minami Internal Medicine Clinic and Sagamihara Kyodo Hospital between January 2008 and December 2009. Macroscopic inflammatory changes of exit-site skin were evaluated by the exit-site scoring system for peritoneal dialysis reported by Piraino et al. (Perit Dial Int 2005; 25: 107-131). The exit-site skin was histologically examined, and the degree of erosion or ulcer in the epidermis was scored 1 point, fibrosis, inflammatory cell infiltration, or neutrophil infiltration in the dermis 0-3 points, and subcutaneous abscess or panniculitis 1 point. Histological inflammatory change was scored by adding the points.

**Results:** Scores for macroscopic inflammatory change corresponded approximately to those for histological inflammatory change. In one case, however, the histological inflammatory change score was 2 despite the macroscopic inflammatory change score being 4.

Table 1 Macroscopic and histological inflammatory change scores

| | | Macroscopic inflammatory change scores | | |
|---|---|---|---|---|
| | | 0-2 | 3 | 4 |
| Histological inflammatory change scores | 0-2 | 8 cases | 0 | 1 case |
| | 3 | 2 cases | 0 | 0 |
| | 4 | 0 | 0 | 1 case |
| | 5 | 0 | 2 cases | 1 case |

**Conclusion:** Macroscopically good condition of exit-site skin corresponded to histological findings, showing normal skin structure. Even if exit-site infection was clinically suspected, skin structure might have been well maintained histologically. Further histological investigation is necessary to correctly diagnose exit-site infections of TCC.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1576

**Catheter Reduction Reduces Hospitalization and Mortality** Renee Jg Arnold, David Madigan, John A. Robertson, Abbe Volz. *DaVita Inc., Denver, CO.*

**Introduction:** Use of central venous catheters (CVC) in hemodialysis patients is associated with increased hospitalization and mortality. Published data have shown that an aggressive catheter reduction initiative (CathAway™) can decrease both catheter incidence and duration. We examined the impact of CathAway™ on hospitalization and mortality in the last 15 months of this program.

**Methods:** This was a retrospective analysis of electronic medical records of patients receiving hemodialysis between 1/1/09 and 3/31/10 (n=175,904) at a large dialysis organization. For hospitalization, we employed a Poisson regression with log total treatment time as an offset, and time on catheter as a primary covariate. For mortality, we fitted a Cox proportional hazards model, using access type (arteriovenous fistula or graft versus CVC) as a time-dependent covariate.

**Results:** In the Cox model analysis, CVC was associated with a more than two-fold risk in mortality, even after controlling for demographic and health variables. Total time on CVC relative to total time on dialysis was also a significant positive predictor of hospitalizations after controlling other factors.

| **Mortality** | **Hazard Ratio** | **95% CI** |
|---|---|---|
| Age | 1.03* | 1.03, 1.03 |
| Charlson | 1.04* | 1.03, 1.06 |
| Diabetes | 0.95* | 0.92, 0.99 |
| CVD | 1.04* | 1.01, 1.08 |
| Male | 1.15* | 1.12, 1.18 |
| CVC | 2.31* | 2.27, 2.45 |
| **Hospitalization** | **Poisson Estimate** | **SE** |
| Age | -0.009* | 0.001 |
| Charlson | 0.075* | 0.004 |
| Diabetes | 0.037* | 0.013 |
| CVD | 0.099* | 0.012 |
| Male | -0.141* | 0.010 |
| Catheter Time | 0.001* | 0.000 |

*p<0.01

**Conclusions:** Both having a CVC, and increased time on a CVC, are associated with a marked increase in hospitalizations and mortality. By decreasing both CVC incidence and time with catheter, the DaVita CathAway™ program is associated with significantly decreased morbidity and mortality. Future research will quantify the economic impact of these benefits.

Disclosure of Financial Relationships: Employer: DaVita Inc.

## F-PO1577

**Effectiveness of a Protocol for the Prevention of Hemodialysis Venous Catheters (CVCs) Related Infections** Carlo Guastoni, Cornacchiari Marina, Heidempergher Marco, Antonella Stasi, Nicoletta Bellotti, Corrado Turri, Lucio Bertoncini, Ambrogio Baroli. *Nephrology Unit, Ospedale di Legnano, Legnano, Milan, Italy.*

**INTRODUCTION**

Infections are a major complication of the use of hemodialysis CVCs. Recently Beathard built 5 degrees of efficiency (from really bad to excellent) based on the incidence of bacteremia ( from >7 to <1/1000 catheter day) (Semin Dial 2008; 21:528).

In our study we evaluated the efficacy of CVCs protocol management adopted in our center through a retrospective analysis of all CVCs inserted in 6 years.

**PATIENTS AND METHODS**

From 2003 to 2008 we placed 73 tunneled CVCs (tCVCs) and 75 non tunneled CVCs (ntCVCs) in 148 patients (mean age 67,6 ± 14 years, dialytic age 23,8 ± 33 months, diabetes 21,6 %, cardiovascular diseases 18%, cancer 13,5%) follow-up 48,5 ± 68 days for ntCVCs and 556,5 ± 551,5 days for tCVCs.

The protocol was the following:

-two operators carrying out sterile connection and disconnection

-soap cleansing of CVCs exit site

-sterile gauze cover and tape (if ntCVCs after 10% sodium hypochlorite pack for 10 minutes)

-CVCs protection terminals and blood lines for 5 minutes with gauze soaked in 50% sodium hypochlorite before connection/disconnection.

**RESULTS**

The isolated germ was: Staphyloccus epidermidis in 46,6% of cases, Staphylococcus aureus in 6,6%, Pseudomonas in 10%, Nocardia in 3,3% and not identified in 33,3%. [Table 1]

| | Total CVCs | ntCVCs | tCVCs |
|---|---|---|---|
| Bacteremia (N) | 16 | 4 | 12 |
| Tunnel or exit site infections (N) | 14 | 4 | 10 |
| Total infections (N) | 30 | 8 | 22 |
| Infection rate for 1000 CVCs days | 0,65 | 1,54 | 0,54 |

**CONCLUSIONS**

The implemented protocol resulted in an incidence of infection that matched the "excellent" grade for tCVCs and "good" grade for ntCVCs taking into account all infections (bacteremia + exit site infections + tunnel infections).

The grade was excellent for both CVCs considering bacteremia only.

Our experience confirms the importance of CVCs management in preventing infections incidence in dialyzed patients with CVCs.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1578

**Malfunction of Tunneled Cuffed Venous Catheter Due to Poor Catheter-Cuff Connection** Hiroshi Shibahara,[1] Nami Shibahara,[2] Susumu Takahashi.[3] *[1]Blood Purification Center, Sagamihara Kyodo Hospital, Sagamihara, Kanagawa, Japan; [2]Hashimoto-Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan; [3]Nihon University Graduate School, Tokyo, Japan.*

**Background:** The cuff of a tunneled cuffed venous catheter (TCC) is firmly fixed to subcutaneous tissues, providing a barrier against infection and preventing unexpected TCC removal. An end-hole type of dual lumen TCC, Soft Cell, is commonly used for dialysis patients. However, we have experienced 15 patients with catheter malfunctions due to a poor connection between the catheter and the cuff since December 2008. We did not experience such malfunctions prior to that date. Therefore, we measured the tensile strength (TS) between the catheter and the cuff of 7 unused Soft Cells.

**Characteristics of 15 patients (18 Soft Cells):** Catheter malfunction with a poor connection between the catheter and the cuff occurred in 15 patients (18 Soft Cells) who had been undergoing dialysis at Sagamihara Kyodo Hospital and Hashimoto-minami Internal Medicine Clinic from December 2008 to December 2009 (male/female, 8/7; mean age, 70.4 ± 10.0 years; diabetes mellitus/non-diabetes mellitus/unknown, 8/6/1; mean catheter days: 40.9 ± 45.1 days). The cuff detached from the catheter in 4 Soft Cells due to slight pulling of the catheter at the time of insertion. The catheter detached from the cuff spontaneously in 8 Soft Cells, and at the time of removal, in 6. All patients were able to undergo dialysis by changing to another TCC or to other forms of vascular access. The cuffs remained under the skin in 14 Soft Cells, which were safely removed surgically.

**Tensile strength of 7 unused Soft Cells:** The TS between the cuff and the catheter of 7 unused Soft Cells was measured using a Universal Testing Machine (IMADA SEISAKUSHO CO., LTD., Aichi, Japan). The average TS was 40.8 ± 22.6 N (range: 19.4 - 80.4N).

**Conclusion:** The TS between the cuff and the catheter of unused Soft Cells varied considerably: the lowest TS was only about one-fourth of the highest TS. This may have been one of the reasons for catheter malfunction in our 15 patients (18 Soft Cells). The causes of malfunction should be promptly investigated and countermeasures taken.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1579**

**Inflammatory Markers during Dialysis in Relation to Vascular Access in Hemodialysis Patients** Benaya Rozen-Zvi, Arie Erman, Asher Korzets, Muhammad Khaskiya, Uzi Gafter. *Nephrology and Hypertension, Rabin Medical Center, Petah-Tikva, Israel.*

Background:

Markers of chronic inflammation are associated with increased mortality and morbidity in hemodialysis (HD) patients. Recent studies suggest that non infected catheters are associated with higher inflammatory markers than arteriovenous fistulas (AVF). Therefore, the effect of vascular access on inflammatory markers changes during dialysis was evaluated.

Methods:

Fifty one HD patients were included in our study. Patients were divided into 2 groups, those with tunneled cuffed catheters (n=23) and those with AVF (n=28). Included patients were at least three month on HD treatment and free of infection, malignancy or active bleeding. Blood samples for high sensitivity C-reactive protein (hsCRP) and Interleukin 6 (IL 6) were collected before and after a 4-hour HD session using high flux polysulfone dialyzer. The primary outcome was a change in the inflammatory markers during the dialysis.

Results:

The baseline values were similar in the catheter and the AVF groups for hsCRP (1.27±1.21 mg/dL and 1.37±1.41 mg/dL, respectively, (p=0.8) and IL 6 (5.82±2.54 ng/mL and 5.44±2.87 ng/mL, respectively (p=0.6)). There was no increase in hsCRP values during the HD session and no difference between the change from baseline values in the catheter and AVF groups (-0.016±0.13 mg/dL and -0.01±0.09 mg/dL, respectively (p=0.9)). IL 6 values increased during HD session in the AVF group (5.44±2.87ng/mL to 6.14±2.51 (p=0.02)) and non-significantly decreased in the catheter group (5.82±2.54 ng/mL to 5.3±2.66 ng/mL (P=0.14)). The change from baseline values for IL-6 was significantly greater in the AVF group compared to the catheter group (0.7±1.44 ng/mL and -0.52±1.66 ng/mL, respectively (p=0.008)).

Conclusion:

There is no increase in inflammatory markers in stable HD patients with tunneled cuff catheters, when compared to patients with AV shunts. The slight increase in IL-6 in patients with AV shunts may be explained by the effect of skin puncture, and the clinical significance of this phenomenon needs further investigation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1580**

**Adjunctive Antibiotic Lock Is an Option for Treating Tunneled Catheter Related Infections** Amit J. Joshi, George Dunea, Peter D. Hart. *Division of Nephrology, Stroger Hospital of Cook County, Chicago, IL.*

**Background:** To assess the efficacy of systemic antibiotics and antibiotic catheter locks in treating tunneled hemodialysis catheter related blood stream infections (CRBSI).

**Methods:** We prospectively examined our experience using antibiotic locks along with systemic antibiotics for tunneled CRBSI without clinical signs of tunnel infection. There were 46 episodes of CRBSI during the study period of 2 years. All patients were treated empirically with systemic antibiotics. Once the organism was identified, systemic antibiotics were tailored accordingly and all patients received an antibiotic catheter lock after each dialysis. Clearance of infection was documented by negative surveillance cultures after completion of the antibiotic course.

**Results:** Out of 46 episodes of CRBSI 16(35%) were due to gram positive organisms, 22(48%) to gram negative organisms, and 8(17%) to polymicrobial (≥2 organisms) infections. 19(41%) cases required removal of dialysis catheter. The antibiotic lock protocol was successful in eradicating the infection in 27 of 46 episodes (59%). Clinical cure was similar in both gram positive and gram negative infections (62% and 63% respectively). One patient developed metastatic infection (endocarditis) with MSSA bacteremia. All patients with MRSA and pseudomonas infection required catheter removal.

Table 1

| Organism | No. of catheter infections | Success rate of catheter lock |
|---|---|---|
| **Gram positive** | | |
| Enterococcus | 2 | 2 (100%) |
| MRSA | 2 | 0 (0%) |
| MSSA | 5 | 2 (40%) |
| MRSE | 1 | 0 (0%) |
| MSSE | 3 | 3 (100%) |
| Others | 3 | 3 (100%) |
| **Gram negative** | | |
| Enterobacter | 12 | 8 (66%) |
| Klebsiella | 2 | 2 (100%) |
| Pseudomonas | 2 | 0 (0%) |
| Serratia | 2 | 1 (50%) |
| Others | 4 | 3 (75%) |
| **Polymicrobial** | **8** | **3 (37%)** |

MRSA, methicillin-resistant S.aureus; MSSA, methicillin-sensitive S.aureus; MRSE, methicillin-resistant S.epidermidis; MSSE, methicillin-sensitive S.epidermidis.

**Conclusion:** An antibiotic lock protocol designed to salvage dialysis access was successful in eradicating the infection in 59% of clinically stable patients with CRBSI. Although 41% required catheter removal, it remains an option in patients with no evidence of metastatic or tunnel infection.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1581**

**Impact of the Use of the HeRO Vascular Access Device vs. Tunneled Dialysis Catheters on Dialysis Provider Economics in an Era of Bundling** Larry Yost,[1] Lesley Dinwiddie.[2] *[1]The Atticus Group, LLC, Portsmouth, NH; [2]Vascular Access Education & Research, Cary, NC.*

**BACKGROUND:** Hospitalization for catheter-related complications and increased utilization of intravenous medications, as a result of catheter use, negatively impact dialysis provider economics. The HeRO vascular access device is an alternative vascular access option which consists of an ePTFE graft attached to an outflow component, comprised of silicone with nitinol reinforcement, designed to traverse central venous stenoses. Clinical studies have demonstrated a reduction in infectious complications with the HeRO device as compared to catheter-related bacteremia rates reported in the literature. **METHODS:** A quantitative model with multiple input parameters was developed to calculate the potential per annum differences in economic outcomes associated with the use of hemodialysis catheters and the HeRO device for dialysis providers under the proposed Medicare bundled payment system. Baseline assumptions utilized to create the model were obtained from the USRDS 2009 Annual Data Report, the 2008 Annual Report for ESRD CPM Project, and from published results of relevant clinical studies. **RESULTS:** Modeled results indicate the use of the HeRO device vs. dialysis catheters in catheter-dependent patients would result in a per patient, annual economic benefit of $2,226, and a total annual economic benefit of $45,340,620 to dialysis providers. Dialysis providers would generate an estimated incremental revenue of $12,986,444, or $637 per patient, when using the HeRO device, as a result of fewer missed dialysis sessions leading due to hospitalizations for catheter-related bacteremias. Dialysis providers would also realize a projected cost savings of $32,353,993 or $1,587 per patient in association with reductions in medications included in the proposed bundled payment. **CONCLUSION:** Our results suggest that the incorporation of the HeRO device into a vascular access algorithm and its preferential use over a catheter in patients who have exhausted their arteriovenous access options will likely be associated with improved dialysis provider economics under a bundled payment system.

Disclosure of Financial Relationships: Consultancy: Hemosphere, Inc.

**F-PO1582**

**Factors Influencing Longevity of Dialysis Catheters** Mahmoud T. El-Khatib, Heather Duncan, Kotagal Shashi Kant. *Nephrology, University of Cincinnati, Cincinnati, OH.*

**Introduction:** USRDS data indicate that more than 30% of hemodialysis patients in the US receive treatment through a tunnelled dialysis catheters, and about 70% of incident patients begin treatment with a tunnelled dialysis catheter. The efforts by the "fistula first initiative" and the KDOQI guidelines have failed so far to reduce the use of catheres to less than 10%. Fewer than 50% of dialysis catheters are functional at six months, lost to infection or flow dysfunction. Despite these odds, we found a minority of patients have a single catheter functioning for more than one year and some for more than two years. Data on factors associated with longevity of dialysis catheters are lacking. The aim of this study to evaluate possible factors associated with longevity of these dialysis catheters.

**Methods:** Records from a single outpatient dialysis unit were examined and identified 32 patients who had a single dialysis catheter in place for more than 1 year (Group "L"). The end dates of these catheters were: started using a graft or fistula, pt transferred from clinic or died while catheter was still in use or catheter was replaced for poor function. A comparison group of 37 patients (Group "S") who had catheters for only 4-8 months was also identified; all of these catheters were ended due to dysfunction such as infection or poor flow. Demographics, treatment parameters, lab results and procedures were analyzed by parametric and non-parametric tests.

**Results:** The average duration of the 32 catheters in group "L" was 567 days. The average duration of the 37 catheters in group "S" was 174 days. Among the demographic characteristics of age, time since ESRD diagnosis, race , sex, weight and diabetic status, there were no differences between the patients in each group. In addition, no differences were found in the measures albumin, ferritin, hemoglobin, URR, Kt/V, Platelets or MCV.

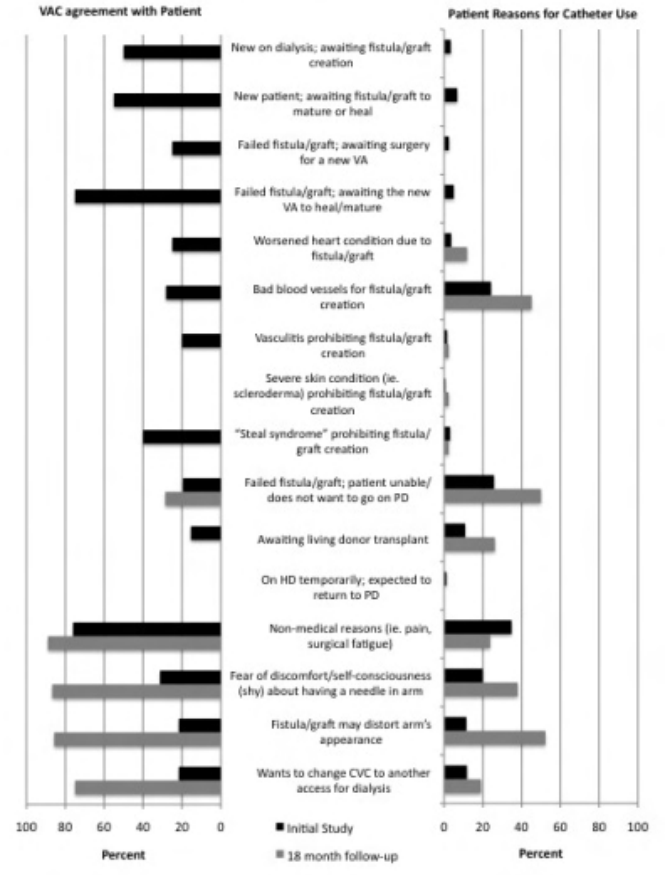
**Conclusions:** Many measures were considered in this analysis and were shown not to be factors in the longevity of tunnelled catheters. The possibility of other characteristics like patient hygiene, catheter locks and the type of catheters may play a role in this difference and need to be studied.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1583**

**Discordant Patient Views on Catheter Use May Impact Catheter Prevalence** Maryum F. Chaudhry,[1] Cynthia B. Bhola,[1] Mohammad Z. Joarder,[1] Deborah Lynn Zimmerman,[2] Patricia A. Quinan,[3] David C. Mendelssohn,[3] Charmaine E. Lok.[1] *[1]Toronto General Hospital and the University of Toronto; [2]The Ottawa Hospital; [3]Humber River Regional Hospital.*

Central venous catheter (CVC) use for hemodialysis is associated with increased adverse health outcomes. Our study aimed to discover why patients prefer using CVCs. A multi-center study was conducted in which 322 patients using CVCs and their vascular access coordinators (VAC) were surveyed to determine why patients use CVCs. Patient responses were compared with the VAC to determine their agreement using multi-rater kappa statistics (K). An 18 month follow-up survey was applied to a subgroup of patients still using their CVCs, and their responses were correlated with the VACs' and patients' previous responses.




Predictive associations for specific reasons for CVC use were explored. Patients indicated non medical reasons (eg. surgical fatigue) (35%; K=0.196), previously failed fistulas/grafts (26%; K=0.226), and fear of disfiguration from fistulas/grafts (12%; K=0.164) as the main reasons for CVC use. The VAC was in agreement with the patient 16.5% of the time, in partial agreement 37.0%, and in disagreement 46.5%. Twelve percent of patients wanted to change their CVC, yet the VAC was unaware of this 78% of the time (K=0.140). In the 18 month follow-up, 39.2% of patients at one site were still using CVCs. Major reasons for persistent CVC use were fear of disfiguration from the fistula (52%; K=0.333) and previously failed fistulas/grafts (50%; K=0.286).The significant discordance between the patients' and VACs' views on why the patient is using a CVC suggests a gap in knowledge, understanding or communication between patients and their VAC. Timely education to address this gap and realistic targets are necessary to reduce CVC prevalence.

Figure 1. Patient reasons for prolonged CVC use and VAC agreement.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1584**

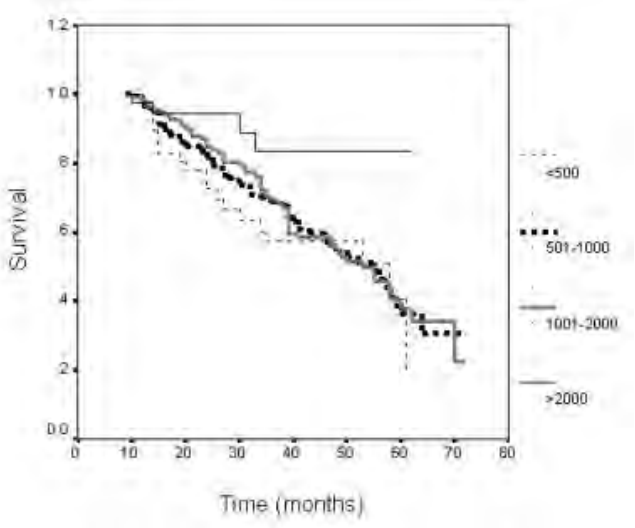
**High Hemodialysis Access Flow Rates Are Not Associated with Increased Mortality** Jesse M. Piceno, Andrew I. Chin. *Internal Medicine, Division of Nephrology, University of California at Davis, Sacramento, CA.*

**Background**: Hemodialysis (HD) arteriovenous shunt flows rates may affect cardiac function. Mortality may be increased in patients with the highest access flow rates.

**Aim**: To determine whether or not high access flow rates, those >2000 cc/min, are associated with greater all-cause mortality.

**Methods**: Retrospective study of thrice weekly HD patients and their first functioning AV shunt. Patients were categorized, based on mean access flow rates measured by ultrasound dilution during the 9-12 month period after insertion of the fistula or graft, into 4 groups: ≤500 cc/min, 501-1000 cc/min, 1001-2000 cc/min, or >2000 cc/min. Unadjusted and adjusted survival models (factors included: age, sex, race, vintage of dialysis, type of access[fistula or graft], weight, and diabetes) were used for analysis up to 5 years after insertion of the access.

**Results**: Unadjusted survival:




Patients with access flow >2000 cc/min (mean flow rate 2754 ± 565 cc/min) had the lowest 5 year mortality on unadjusted analysis compared with all other groups ($p<0.05$). After adjustment for the above mentioned co-factors, the following were associated with increased risk for death: increased age and vintage, Caucasian race, graft use, and shunt flow ≤500 cc/min. High access flow rate >2000 was no longer associated with improved survival after these adjustments. When access flow was analyzed as a continuous factor, higher flows were associated with decreased mortality, but of *very* small effect (HR 0.9996; 0.9993 to 1.0000, p=0.03).

**Conclusion**: In HD patients, an access flow rate of >2000 cc/min, measured at 9-12 months after surgery, was not associated with a higher risk of death. However, the lowest flow, ≤500 cc/min, was associated with a higher risk. Whether or not longer exposure to high shunt flows would eventually contribute to a higher mortality remains to be seen.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1585**

**Distal Convoluted Tubule (DCT) Specific Transcripts Identified Using DCT Large Scale Sampling** Nicolas Picard,[1] R. Lance Miller,[2] Johannes Loffing.[1] *[1]Anatomy Institute, University of Zurich, Zurich, Switzerland; [2]NHLBI/NIH, Bethesda, MD.*

The renal distal convoluted tubule (DCT) is important for the renal control of ion homeostasis and blood pressure. Although several DCT-specific ion transporting proteins have been identified, only little is yet known about the molecular mechanisms that regulate DCT function. We hypothesized that gene products that are specifically expressed in the DCT might be of particular importance for the control of DCT cell function. Here, we used Complex Object Parametric Analysis and Sorting (COPAS) to isolate DCTs in large scale for the identification of a DCT transcriptome. A renal tubule suspension was obtained from transgenic mice expressing EGFP specifically in the DCT. The tubules were then separated by COPAS in three fractions (i.e. all tubules, EGFP-positive DCTs and EGFP-negative non-DCT tubules). Real-time PCR and Western blot analysis confirmed the significant enrichment and derichment of known DCT-specific marker molecules in the EGFP-positive and EGFP-negative samples, respectively. Subsequent microarray analysis (Agilent) revealed about 400 genes that are being more than 20-fold enriched in EGFP-positive tubules compared with EGFP-negative tubules. Aside from the known DCT-specific gene-products parvalbumin, NCC and TRPM6, many novel DCT enriched gene products were identified. Among them, Slc16a7 (MCT2) and its accessory protein GP70 (embigin) sticked out. Immunohistochemistry confirmed the significant expression of these two gene products in the DCT. Thus, transcriptomic analysis of COPAS-sorted renal tubules allows the identification of novel DCT-enriched gene products and may provide a pool of novel candidate genes for DCT-specific functions and diseases.

Disclosure of Financial Relationships: nothing to disclose

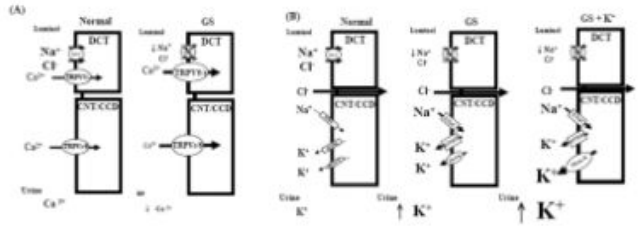
**F-PO1586**

**Generation and Analysis of the Thiazide-Sensitive $Na^+$-$Cl^-$ Cotransporter S707X Knockin Mice as a Model of Gitelman's Syndrome** Sung-Sen Yang,[1] Yu-Juei Hsu,[1] Shinichi Uchida,[2] Sei Sasaki,[2] Shih-Hua P. Lin.[1] *[1]Division of Nephrology, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; [2]Department of Nephrology, Okyo Medical and Dental University, Tokyo, Japan.*

Gitelman's syndrome (GS) is characterized by salt losing, hypomagnesemia, hypokalemic metabolic alkalosis, and hypocalciuria. To better model human GS caused by a specific mutation in the thiazide-sensitive $Na^+$-$Cl^-$ cotransporter (NCC), we generated a nonsense Ncc S707X knockin mice corresponding to human S710X, a recurrent mutation with severe phenotypes in Chinese GS patients. Blood pressure, blood and urine electrolytes and biochemistries, mRNA and relevant protein expression in the kidneys were examined. Compared with wild-type or heterozygous littermates, homozygous (Hom) knockin mice

fully recapitulated the phenotype of human GS. The markedly reduced Ncc mRNA and virtually absent NCC protein expression in kidneys of Hom mice was primarily due to nonsense-mediated mRNA decay (NMD) surveillance mechanisms. Expression of epithelial $Na^+$ channel (Enac), $Ca^{2+}$ channels (Trpv5 and Trpv6), and $K^+$ channels (Romk1 and maxi-k) were significantly increased. High $K^+$ intake could not correct hypokalemia but caused a further increase in maxi-k but not Romk1 expression. Renal tissue from a patient with GS also showed the enhanced Trpv5 and Romk1 expression in distal tubules. We suggest that the upregulation of Trpv5 and Trpv6 and of Romk1 and maxi-k may contribute to hypocalciuria and hypokalemia in the Ncc S707X knockin mice and human GS, respectively.

Figure (1)




Disclosure of Financial Relationships: nothing to disclose

## F-PO1587

**Angiotensin II Regulates the Thiazide-Sensitive Sodium Chloride Cotransporter through SPAK Independently of Aldosterone** Nils van der Lubbe,[1] Christina Lim,[1] Robert A. Fenton,[1] Marcel Meima,[1] Alexander H. Danser,[1] Robert Zietse,[2] Ewout J. Hoorn.[1] *[1]Erasmus Medical Center, Rotterdam, Netherlands; [2]University of Aarhus, Denmark.*

**Background**

Aldosterone and angiotensin II regulate the sodium chloride cotransporter (NCC), but their independent effects are unknown. Here, we use adrenalectomy followed by the selective infusion of aldosterone or angiotensin II as a model to investigate the independent effects of both hormones.

**Methods**

Adrenalectomy was performed in three experimental groups and one control group of male Sprague-Dawley rats (n=5/group). After surgery, the experimental groups received high-physiological aldosterone, a non-pressor (Ang II-NP) or a pressor (Ang II-P) dose of angiotensin II for eight days via osmotic minipump, while controls received vehicle only. Differential centrifugation was used to obtain plasma membrane and intracellular fractions of whole kidney homogenates.

**Results**

The validity of the model was supported by virtually absent plasma aldosterone concentrations in the control group and the development of hypertension in the Ang II-P group only. Aldosterone, Ang II-NP, and Ang II-P caused significantly more sodium retention than vehicle. Thiazides inhibited the sodium retention induced by Ang II-NP. Compared to controls, aldosterone, Ang II-NP and Ang II-P significantly increased the plasma membrane abundance of NCC (3.0-, 4.5- and 3.5-fold). Aldosterone and Ang II-NP increased phosphorylation of NCC at threonine-53 (4.2- and 4.3-fold). Aldosterone and Ang II-NP increased the intracellular abundance of *Ste20*-related proline alanine-rich kinase (SPAK, 3- and 2-fold). No differences were observed in the intracellular abundance of with-no-lysine kinase 4 (WNK4) and oxidative stress responsive protein 1 (OSR1).

**Conclusion**

This is the first controlled *in vivo* study to show that aldosterone and angiotensin II independently increase the abundance and phosphorylation of NCC. The phosphorylation of NCC was likely mediated by SPAK. These results increase the insight in the hormonal control of renal sodium regulation, which also contributes to the pathophysiology of several forms of hypertension.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1588

**Studies on the Relationship between WNK1 and OSR1 Kinases Using Genetic Mouse Models** Jian Xie,[1] Sung-Sen Yang,[2] Shih-Hua P. Lin,[2] Chou-Long Huang.[1] *[1]UT Southwestern Medical Center, Dallas, TX; [2]Tri-Service General Hospital, NDMC, Taipei, Taiwan.*

OSR1 (oxidative stress-responsive kinase-1) and its related SPAK kinase are members of the Ste20 superfamily of MAPK (mitogen-activated protein kinase)-like protein kinase. OSR1 and SPAK, regulate many targets including members of SLC12 family ion transporters that are involved in $Na^+$, $K^+$, and $Cl^-$ transport. Mechanistically, OSR1 and SPAK are phosphorylated and activated by with-no-lysine[K] kinases, WNK1 and WNK4. Activated OSR1 and SPAK, in turn, phosphorylate and activate SLC12 family ion transporters. Mice with homozygous deletion of exons 8-9 of *Osr1*, encoding part of the kinase domain, are embryonic lethal (Yang & Lin, unpublished). Previously, we have reported that deletion of *Wnk1* in mice causes embryonic lethality between E10.5 and E12.5 from cardiovascular (CV) developmental defects. OSR1 is expressed in a wide range of tissues/organs, with high levels in the heart. To test the hypothesis that OSR1 is downstream of WNK1 in embryonic CV development, we compared *Osr1*-knockout embryos with *Wnk1*-knockout embryos. The *Osr1*-knockout embryos are phenotypically identical to wild type littermates up to E9.5, but start to show growth retardation from E10, and die between E10.5 to E13.5 with hemorrhage and pericardial edema. In the yolk sac of *Osr1*-knockout mutant after E10.5, the primitive capillary plexus fails to develop into mature vascular network, leading to defective blood circulation. There is no living *Osr1*-knockout embryo found after E13.5. Thus, OSR1 function is required for CV development in mice. The phenotype of *Osr1* knockout is identical to that of *Wnk1* knockout, except possibly with a slight delay in the onset. This is consistent with the idea that OSR1 is a phosphorylation substrate and downstream effector of WNK1. To further determine OSR1's functions in different organs and its interaction with the WNK1 signaling pathway, a mouse model allowing conditionally expressing constitutive kinase-active mutant form of OSR1 with loxp-cre system is being generated. The effects of constitutive kinase-active OSR1 on various organs and its ability to rescue *Osr1* and *Wnk1* knockout embryos will be investigated.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1589

**WNK3 Is a Component of the WNK Signaling Complex and Is Expressed Predominantly along the Distal Convoluted Tubule** Chao-Ling Yang,[1] Kerim Mutig,[3] Sebastian C. Bachmann,[3] David H. Ellison.[1,2] *[1]Nephrology & Hypertension, Oregon Health & Science University, Portland, OR; [2]Renal Section, Portland VA Medical Center, Portland, OR; [3]Anatomy, Charité Universitätsmedizin, Berlin, Germany.*

Mutations of WNK1 and WNK4, members of the WNK kinase family, cause *Familial Hyperkalemic Hypertension* by stimulating NaCl reabsorption and inhibiting K secretion along the distal convoluted tubule (DCT). We have suggested that WNK kinases form a *signaling complex*, together with SPAK and SGK1, in DCT cells, thereby regulating the thiazide-sensitive Na-Cl cotransporter (NCC). While WNK3 regulates NCC *in vitro*, a role for WNK3 *in vivo* has not been clear, because WNK3 was reported to be expressed predominantly by the proximal tubule (PT) and only minimally along the DCT. Those results were obtained using using a commercial antibody, which was not characterized extensively. To determine whether WNK3 plays a substantial role in the distal nephron, we generated a GST-fusion protein encompassing WNK3 residues 2 to 143, to immunize rabbits. Both immunoblots and immunofluorescence indicated that the anti-WNK3 antibody recognizes WNK3, but not WNK1 or WNK4, when WNKs are expressed in Xenopus oocytes or HEK293 cells. The signal was blocked by the immunizing peptide. In kidney, there was modest paracellular WNK3 in PT, thick ascending limb, and connecting tubule, but much stronger cytoplasmic signal sub-apically along the DCT, where it co-localized with NCC. *In situ* hybridization confirmed high level expression by DCT, but not PT. Transient transfection of HEK293 cells with WNK3 increased N-terminal NCC phosphorylation substantially (presumably via SPAK, as WNK3 does not phosphorylate NCC directly; J Clin Invest. 2007; 117:3403). KS-WNK1, a distal nephron-specific isoform of WNK1, inhibited WNK3 kinase activity *in vitro*. In oocytes, KS-WNK1 also inhibited WNK3's ability to stimulate NCC-mediated sodium uptake. In conclusion, WNK3 is highly expressed along the DCT, where it can increase NCC phosphorylation and activity. WNK3 may be a key component of the WNK signaling complex, where it acts in opposition to KS-WNK1 and WNK4.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1590

**Similar Effect of All WNK3 Isoforms upon SLC12 Cotransporters** Zesergio Melo,[1] Silvia Cruz,[2] Norma Hilda Vázquez,[1] José Ponce-Coria,[1] Herminia Pasantes,[2] Adriana P. Mercado,[1] Gerardo Gamba.[1] *[1]Molecular Physiology Unit, INNSZ-INCICH-IIB, UNAM, Mexico City; [2]IFC-UNAM, Mexico City.*

With-no-lysine kinase 3 (WNK3) is a member of a subfamily of serine/threonine kinases that has been associated with arterial hypertension. We have previously shown that WNK3-18a and its catalytically inactive form (WNK3-DA) modulate the activity of the SLC12 family members in a mirror image: WNK3 activates NKCC1/2 and NCC while inhibits the KCCs, whereas WNK3-DA does the opposite. In doing so, WNK3 bypasses the tonicity requirements for activation/inactivation of these cotransporters. Thus, WNK3 has been proposed as the cell volume sensitive kinase. Four splice variants are potentially generated from WNK3 gene due to the combination of two exons 18 (18a and 18b) and the presence or absence of exon 22. Exon 18b is unique for central nervous system. Thus, this study was designed to define the effect of all WNK3 variants upon all members of the SLC12 family. By RT-PCR from a fetal brain library exons 18b and 22 were separately amplified and subcloned into the original WNK3-18a or WNK3-DA to obtain all four potential combinations, with and without catalytic activity (18a, 18a+22, 18b, and 18b+22). All subcloned segments were sequenced. The basal activity of the cotransporters and the effect of WNK3 isoforms were assessed by measuring $^{22}Na^+$ or $^{86}Rb^+$ uptake in *Xenopus* oocytes co-injected with each cotransporter and WNK3 or WNK3-DA variants cRNAs. In isotonic conditions the activity of NCC and NKCC1/2 was increased by co-injection with any of the WNK3 isoforms. The positive effect occurred even in hypotonic conditions in which basal activity of NKCC1 is completely prevented. Consistent with these observations, when expressed in hypotonic conditions, all KCCs were active, but in the presence of any of the WNK3 variants the activity was completely reduced. All WNK3 isoforms in their catalytically inactive form produced the opposite effect on the cotransporter. That is, NKCC1/2 and NCC were inhibited, even in hypertonic conditions, while KCCs were activated, even in isotonic conditions. Thus, we conclude that all wild-type and catalytically inactive forms of WNK3 have the same effect on all SLC12.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1591**

**Elevated Thiazide Sensitive Sodium Chloride Co-Transporter Activity in PHAII Mutant Mice** Qingshang Yan,[1] Junhui Zhang,[2] Jesse Rinehart,[1] Richard P. Lifton,[2] Gerhard Malnic,[1] Gerhard H. Giebisch,[1] Tong Wang.[1] *[1]Cellular and Molecular Physiology, Yale University, School of Medicine, New Haven, CT; [2]Internal Medicine, Yale University, School of Medicine, New Haven, CT.*

Mutations in WNK4 cause pseudohypoaldosteronism type II (PHAII), a disease featuring increased renal sodium reabsorption and impaired potassium secretion. PHAII mutant mice exhibited phenotypes of higher blood pressure, hyperkalemia, hypercalciuria and marked hyperplasia of the distal convoluted tubule (DCT). To investigate whether hyperplasia of the DCT is accompanied by increased $Na^+$-$Cl^-$-cotransporter (NCC) activity, we examined the effect of hydrochlorothiazide (HCTZ) on sodium and potassium excretion in wild-type and PHAII mutant mice. GFR was measured by inulin clearance and the absolute (ENa, EK) and fractional (FENa, FEK) $Na^+$ and $K^+$ excretions were measured before and after bolus i.v. of HCTZ (30mg/kg). Experimental data show significantly lower urine volume (0.77 vs. 1.45 nl/min) and $Na^+$ excretion, ENa (0.10 vs. 0.35 microEq/min/100g BW) and FENa (0.16 vs. 0.36%), in mutant mice than that in control, consistent with increased $Na^+$ absorption in PHAII mice. HCTZ produced significant diuretic and natriuretic effect in both WT and mutant mice. The sensitivity was much higher in PHAII mutant mice than that in control. HCTZ produced 5-fold increase of urine volume and 16-fold increase in ENa and FENa in mutant mice but only 2-fold increase in urine volume and 7-fold increase in ENa and EK in WT mice. The baseline $K^+$ excretions was slightly lower in mutant mice than WT. HCTZ produced a similar effect on $K^+$ excretions with 1-fold increase in EK and FEK in both mutant and WT mice. Despite such a high degree of $Na^+$ excretion, the $K^+$ excretion was not enhanced proportionally to $Na^+$ loss in the mutant mice. Given the fact that ROMK channel is inhibited by WNK4 mutation and unchanged in HCTZ-increased $K^+$ excretion, a compensatory mechanism of $K^+$ secretion may occur by calcium and flow-activated MaxiK channel activity. These results indicate that NCC activity is elevated and other $K^+$ secretion mechanisms may be upregulated when ROMK channel is downregulated in PHAII mice.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1592**

**WNK4 Inhibits NCC Protein Expression through MAPK ERK1/2 Signaling Pathway** Xiuyan Feng,[1] Yiqian Zhang,[1,2] Bo Zhou,[1] Dexuan Wang,[2] Xuemei Zhang,[3] Dingying Gu,[2] Hui Cai.[1,2] *[1]Medicine/Renal, Emory University School of Medicine, Atlanta, GA; [2]Nephrology, The Second Affiliated Hospital Wenzhou Medical College, Wenzhou, Zhejiang, China; [3]Pharmacology, Fudan University School of Pharmacy, Shanghai, China.*

WNK ((with no lysine (k)) kinase is a subfamily of serine/threonine kinases. Mutations in two members of this kinase family (WNK1 and WNK4) cause pseudohypoaldosteronism type II featuring hypertension, hyperkalemia and metabolic acidosis. WNK1 and WNK4 were shown to stimulate NCC activity through SPAK and OSR1 phosphorylation at T46, T55, and T60 of NCC. Activation of PKC by a phorbol ester inhibits NCC function via activation of ERK1/2 kinase. We have previously shown that WNK4 phosphorylates ERK1/2 in a dose-dependent manner. Knocking down WNK4 expression increases surface NCC expressions in mouse distal convoluted tubular (mDCT) cell. We further examined the effect of knock-down WNK4 on ERK1/2 phosphorylation in mDCT. We found that knock-down WNK4 expression significantly reduced ERK1/2 phosphorylation (0.917 ± 0.17 vs 0.347 ± 0.08, p = 0.006) and knock down ERK1/2 expression increased total NCC expression. To discrete the phosphorylation sites of SPAK/OSR1 from ERK1/2 in WNK4's effect on NCC, we generated serial mutations to these putative SPAK phosphorylation sites on N-terminus of NCC to determine whether WNK4 remains its inhibitory effect on NCC while SPAK phosphorylation sites of NCC are mutated. When Cos-7 cells were transfected with single mutation of NCC T46A, T50A or S91A, WNK4 still inhibits NCC protein expression, whereas Cos-7 cells were trasnfected with single mutation of either NCC T55A or T60A, WNK4 lose its inhibitory effect on NCC protein expression. When Cos-7 cells were transfected with triple mutations of NCC T55A/T60A/S91A, WNK4 retains its inhibitory effect on NCC protein expression. These data suggest that WNK4 inhibits NCC protein expression through a MAPK ERK1/2 signaling pathway and WNK4 down regulates NCC protein expression through ERK1/2-mediated phosphorylation of NCC at the discrete sites other than the SPAK putative sites.

Note: XF and YZ contributed equally to this work.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1593**

**Treatment of Bartter Syndrome Type IV Caused by R8L Barttin Mutation** Naohiro Nomura, Masato Tajima, Eriko Ohta, Akihito Ohta, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan.*

Barttin, a gene product of BSND, is one of four genes responsible for Bartter syndrome. We recently generated barttin knockin mice carrying R8L missense mutation (R8L knockin mice) by gene targeting, and reported the phenotypes in the last ASN meeting. The knockin mice showed Bartter-like phenotypes (hypokalemia, metabolic alkalosis, and decreased NaCl reabsorption in distal tubules) under a low salt diet or a low potassium (K) diet. Plasma membrane localization of both barttin (R8L) and the ClC-K channel were impaired in the knockin mice, and transepithelial chloride transport in the thin ascending limb of Henle's loop as well as thiazide-sensitive chloride clearance, were significantly reduced. Previously, we showed that R8L barttin stably expressed in Madin-Darby Canine Kidney (MDCK) cells was trapped in the endoplasmic reticulum (ER). In this study, we investigated the treatment for R8L mutant barttin in vitro and in vivo. We used curcumin and 17-allylamino-17-demethoxygeldanamycin (17-AAG), which were known to rescue ER-trapped mutant membrane proteins. In R8L mutant barttin stably expressed in MDCK cells, side-specific biotinylation assay revealed that the basolateral expression of R8L mutant barttin was increased after treatment with these agents, whereas the wild-type barttin expression was not altered. In vivo experiment using R8L knockin mice, 17-AAG intraperitoneally administered for a week, and curcumin administered with mouse chow for 9 days, significantly restored the basolateral localization of R8L barttin in the nephron from the thick ascending limb of Henle's loop to cortical collecting ducts. Concomitantly, 17-AAG corrected metabolic alkalosis and hypokalemia, and curcumin restored thiazide-sensitive chloride clearance. These results clearly suggested that the aberrant intracellular localization of R8L barttin is the major cause of this disease, and Batter syndrome type IV caused by R8L mutation could be treated with drugs that release ER-trapped mutant proteins.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1594**

**cAMP Stimulates NKCC2 Recycling in the Thick Ascending Limbs (THALs)** Gustavo R. Ares, Pablo A. Ortiz. *Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Hospital, Detroit, MI.*

The apical Na/K/2Cl cotransporter NKCC2 mediates most NaCl reabsorption by the thick ascending limb (TAL). cAMP increases surface NKCC2 by stimulating the rate of exocytosis. We found that NKCC2 is constitutively retrieved from the apical membrane. However, it is not known whether internalized NKCC2 is reinserted into the apical membrane (recycling), and whether cAMP stimulates this step. We hypothesized that cAMP stimulates NKCC2 recycling in the TAL. We measured surface NKCC2 and the rate of NKCC2 recycling in TAL suspensions by surface biotinylation and Western blot. Surface proteins were biotinylated, allowed to internalize for 30 min at 37°C and then biotin was stripped from proteins remaining at the surface. TALs were warmed to 37°C and recycling of NKCC2 to the surface monitored and expressed as % of the internalized pool. We found that internalized NKCC2 recycles back to the plasma membrane in a constitutive manner (7.5 min = 11.4 ± 2.1%, 15 min = 17.2 ± 3.0 %, 30 min = 25.1± 3.3 %, n = 6). Control experiments indicate that the internalized pool does not loose the biotin label nor is degraded (30 min). To stimulate cAMP, we used Forskolin/IBMX. We found that NKCC2 recycling is stimulated (7.5 min = 22.8 ± 3.6 %, 15 min = 44.6 ± 4.8 %, 30 min = 47.3 ± 1.6 %, n = 6, $p<0.05$), by 160% increase at 15 min. Steady-state surface is maintained by NKCC2 arriving from the Golgi and/or a recycling compartment. To study the contribution of the recycling pool in cAMP-stimulated surface NKCC2 we used Brefeldin A, which blocks delivery of membrane proteins from the Golgi but does not block recycling. TALs were treated with vehicle (control) or brefeldin-A for 60 min and then treated with Forskolin/IBMX for 30 min. In control TALs, Forskolin/IBMX stimulated surface NKCC2 by 32 ± 5.5 % ($p<0.05$) and by 21 ± 1.9 % ($p<0.05$) in Brefeldin A-treated TALs. We conclude that NKCC2 undergoes constitutive recycling, and this process is stimulated by cAMP. Our data suggest that recycling of NKCC2 contributes to cAMP-stimulated steady-state surface NKCC2. These are the first data showing recycling of a renal transporter in native TALs.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1595**

**VAMP2 but Not VAMP3 Mediates cAMP-Stimulated NKCC2 Trafficking in Thick Ascending Limbs** Paulo S. Caceres,[1,2] Mariela Mendez,[1] Pablo A. Ortiz.[1,2] *[1]Internal Medicine-Hypertension & Vascular Research, Henry Ford Hospital, Detroit, MI; [2]Physiology, Wayne State University, Detroit, MI.*

The apical co-transporter NKCC2 is responsible for most NaCl reabsorption by the Thick Ascending Limb (TAL) of Henle's loop. We found that vasopressin increases steady-state surface NKCC2 by enhancing exocytic insertion via cAMP. The vesicle-associated membrane fusion proteins VAMP2 and VAMP3 are expressed in a sub-apical compartment in TALs. Cleavage of VAMP2 and VAMP3 with tetanus toxin blocks cAMP-stimulated surface NKCC2 and NKCC2-mediated NaCl transport. However, whether VAMP2 or VAMP3 mediates cAMP-stimulated NKCC2 trafficking is unknown. We hypothesized that VAMP2, but not VAMP3, mediates cAMP-stimulated NKCC2 surface expression. To inhibit VAMP2 or VAMP3 in rat TALs, in vivo, we used adenoviruses-mediated gene silencing with a specific VAMP2 or VAMP3 shRNA. Control kidneys were injected with adenoviruses carrying a scrambled RNA sequence (scrambled). We then measured surface NKCC2 expression by surface biotinylation in TAL suspensions in the absence or presence of forskolin/IBMX to increase endogenous cAMP. VAMP2-shRNA decreased VAMP2 expression by 69 ± 5% without affecting VAMP3 ($p<0.05$). Similarly, VAMP3-shRNA decreased VAMP3 expression by 68 ± 8% without affecting VAMP2 ($p<0.05$). Silencing VAMP2 blocked the stimulatory effect of cAMP on surface NKCC2 by 40% (scrambled: 80 ± 9 %, VAMP2-shRNA: 49 ± 4% increase from baseline; $p<0.05$). In contrast, silencing VAMP3 did not affect cAMP-stimulated surface NKCC2 (scrambled: 62 ± 14 %, VAMP3-shRNA: 67 ± 9% increase from baseline). Silencing VAMP2 in the TAL did not affect baseline surface NKCC2 (81 ± 7% of scrambled) or the total pool of NKCC2 (93 ± 9% of scrambled). In contrast, silencing VAMP3 decreased baseline surface NKCC2 by 53 ± 13% ($p<0.05$) and also the total pool of NKCC2 by 59 ± 8% ($p<0.05$). We concluded that VAMP2, but not VAMP3, mediates cAMP-stimulated NKCC2 trafficking in TALs. Our data also suggest that VAMP3 may be involved in constitutive NKCC2 trafficking, biosynthesis and protein processing or turnover.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1596**

**Phosphodiesterase 4 (PDE4) Blunts the beta Adrenergic Receptor-Stimulated NKCC2 Trafficking in Rat Thick Ascending Limbs** Mohammed Z. Haque, Pablo A. Ortiz. *Hypertension and Vascular Research, Internal Medicine, Henry Ford Hospital, Detroit, MI.*

The thick ascending limb of the loop of Henle (THAL) reabsorbs ~30% of the filtered NaCl via the apical Na/K/2Cl cotransporter (NKCC2). Acute beta adrenergic receptor stimulation in THALs enhances NaCl reabsorption and increases intracellular cAMP. We found that isoproterenol enhances NKCC2 trafficking to the apical membrane in rat THALs via the cAMP/protein kinase-A pathway. We and others have identified several cAMP-specific phosphodiesterases (PDE) in rat THALs, including PDE1 and PDE4. In other cells PDE4 is primarily responsible for decreasing cAMP generated by beta adrenergic stimulation. We hypothesized that PDE4 decreases beta adrenergic receptor stimulated surface NKCC2 expression in rat THALs. THAL suspensions were obtained from Sprague-Dawley rats and surface NKCC2 expression measured by surface biotinylation and western blot. Data are presented as % of control measured by densitometry. We found that 30 min incubation of THALs with the beta adrenergic receptor agonist, isoproterenol at 0.5 and 1.0 μM increased surface NKCC2 by 14±2 and 29±6% ($p<0.05$) respectively. The effect of 1 μM Isoproterenol was enhanced by nonselective phosphodiesterase inhibition with IBMX (Isoproterenol: 27±6%, IBMX+Isoproterenol: 51±8% of control, $p<0.05$), while IBMX alone had no significant effect (9±5%). To selectively inhibit PDE4 we used 100 μM rolipram, which tended to enhance Isoproterenol-stimulated surface NKCC2 expression from 25±7 to 37±9% of control. However, rolipram (100 μM) alone enhanced surface NKCC2 by 15±3% ($p<0.05$). At a lower concentration (20 μM), rolipram did not affect surface NKCC2 but enhanced the sensitivity to isoproterenol (Control: 100, Isoproterenol 0.2 μM: 100±5, Rolipram 20 μM: 106±6, Rolipram+Isoproterenol: 126±8%; $p<0.05$ *vs* isoproterenol). We concluded that PDE4 decreases beta adrenergic receptor stimulated NKCC2 trafficking in THALs. This may be an important pathway that decreases the stimulatory effect of beta adrenergic agonists on THAL NaCl absorption.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1597**

**Regulation of ROMK along Distal Segments by K Diet** James B. Wade,[1] Liang Fang,[1] Richard A. Coleman,[1] Jie Liu,[1] Tong Wang,[2] Paul A. Welling.[1] *[1]Department of Physiology, University of Maryland School of Medicine, Baltimore, MD; [2]Department of Cell and Molecular Physiology, Yale University, New Haven, CT.*

ROMK (Kir1.1) channels are well known to play a central role in renal K secretion, but the specific nephron segments where these channels are regulated in response to changes in dietary K has remained mysterious. Unreliable specificity or low avidity of available antibodies has made addressing this issue especially challenging. Here, we prepared new antibodies to C-terminal sites of ROMK and screened them by Western blot and immunolocation in ROMK null vs WT mice. Of the six rabbits immunized, only two produced antibodies that specifically competed with antigen and were not reactive with ROMK null kidney. Characterization of segmental ROMK expression with nephron-segment antibodies (NCC, calbindin D28 and AQP2) revealed specific ROMK labeling in distal convoluted tubule regions, DCT1 and DCT2; the connecting tubule (CNT); and cortical collecting duct (CCD). ROMK was found diffusely distributed in intracellular compartments and at the apical membrane of each tubular region. Quantification of cytoplasmic labeling indicated that labeling in DCT1 and DCT2 was not significantly increased by high K diet while CNT and CCD labeling were each increased by 25% and 18%, respectively ($P<0.05$). Apical labeling was significantly increased by high K diet in DCT2 (2 fold), CNT (9 fold) and CCD (5 fold) ($P<0.05$) but not in DCT1. Consistent with these large increases in apical ROMK, Western blots of cortical homogenates from mice on high K diet showed a dramatic increase in the abundance of ROMK with mature glycosylation at about 65 kD with no significant change in the immature band of ROMK at about 37 kD. We conclude: 1) caution should be used when employing ROMK antibodies that have not been screened against ROMK null tissue; 2) High K diet causes a large increase in apical expression of ROMK in DCT2, CNT and CCD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1598**

**The Renal IK Channel Complex Contains ROMK2, Beta1 and CFTR Subunits** Qiang Leng,[1] Tatjana Coric,[1,2] Ximing Zhou,[1] Jerry Feng,[1] Divya Chari,[1] Connie Liu,[1] Qingshang Yan.[1] *[1]Cellular & Molecular Physiology, Yale University, New Haven, CT; [2]Department of Pharmacology & Toxicology, The University of Alabama at Birmingham, Birmingham, AL.*

SK (30 pS small conductance $K^+$ channel) and IK (70 pS intermediate conductance $K^+$ channel) are the two major types of $K^+$ channels responsible for $K^+$ recycling and secretion in the thick ascending limb (TAL) of the kidney. IK channels are partially formed by the ATP-dependent potassium channel ROMK. However, their complete molecular identity of has been elusive. The question why ROMK (SK) is ATP-inhibited *in vivo* but loses its ATP-sensitivity in heterologous expression systems has also been a longstanding puzzle in the field of renal physiology. Such ATP-sensitivity is critical for $K^+$ secretion by the cells of TAL and distal nephron segments responsive to aldosterone. Here we constructed a subtraction library to identify the component(s) required for IK formation in addition to ROMK. We determined that a Beta1 subunit is associated with ROMK2 to form an IK channel in *Xenopus laevis* oocytes. Together with CFTR (cystic fibrosis transmembrane conductance regulator), Beta1 subunit also reconstitutes ATP-sensitivity to both IK and SK. These results implicate Beta1 subunit as the missing component in IK channel formation and lead us to propose a multi-subunit IK channel complex involving ROMK2, CFTR, and Beta1 subunits. Overall, our findings not only provide new insights into the formation and regulation of renal $K^+$ channels, but also potential molecular targets for drug delivery to combat renal hypertension, Bartter's syndrome, and other related hereditary diseases.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1599**

**WNK4 Inhibits $Ca^{2+}$-Activated Big-Conductance Potassium Channels (BK)** WenHui Wang. *Pharmacology, New York Medical College, Valhalla, NY.*

With–No-Lysine Kinase 4 (WNK4) plays an important role in regulating K transport in the aldosterone-sensitive distal nephron (ASDN). Two types of K channels: ROMK and $Ca^{2+}$ –activated big-conductance K channel (BK), are expressed in the ASDN and involved in K secretion. While WNK4-induced inhibition of ROMK channels is well documented, it is not explored whether WNK4 also regulates BK channels. In the present study we used the perforated whole-cell patch-clamp to examine the effect of WNK4 on BK channels in HEK293T cells transfected with Kcnma1 ( α subunit of BK, mSlo) or Kcnma1 +WNK4. Expression of WNK4 decreased the K currents from 536±28 pA to 247±9 pA. Moreover, coexpression of SGK1 abolished the inhibitory effect of WNK4 on BK channels and expression of WNK4S1169D, in which SGK1 phosphorylation site (Ser 1169) was mutated, failed to inhibit BK channels. This suggests that SGK1 modulates the effect of WNK4 on BK through the phosphorylation of WNK4 at the switch-domain. Also, coexpression of dominant negative dynamin K44A abolished the inhibitory effect of WNK4 on BK channels. Expression of WNK4 stimulated the phosphorylation of ERK and p38 MAPK without changing the total expression of ERK and p-38 MAPK. The stimulatory effect of WNK4 on MAPK activity was attenuated by coexpression of SGK1. The role of ERK and p-38 MAPK in inhibiting BK channels had also been supported by the observation that inhibition of ERK with PD098059 and p-38 MAPK with SB202190 completely abolished the inhibitory effect of WNK4 on BK channels and increased K currents from 247±9 pA to 702±11 pA. We concluded that WNK4 inhibits BK channels by a mechanism stimulating ERK and p38 MAPK.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1600**

**Identification and Functional Characterization of Kir2.6 Mutations Associated with Non-Familial Hypokalemic Periodic Paralysis** Chih-Jen Cheng,[1,2] Shih-Hua P. Lin,[1] Sung-Sen Yang,[1] Yu-Juei Hsu,[1] Chou-Long Huang.[2] *[1]Tri-Service General Hospital, NDMC, Taipei, Taiwan; [2]UT Southwestern Medical Center, Dallas, TX.*

Hypokalemic periodic paralysis (hypoKPP) is characterized by episodic muscle weakness and acute hypokalemia during attacks. It may be familial or non-familial. Familial cases are mostly caused by mutations of voltage-gated Na+ or Ca2+ channels. The non-familial hypoKPP includes thyrotoxic periodic paralysis (TPP) and sporadic periodic paralysis (SPP). Recently, mutations of an inwardly rectifying K+ channel, Kir2.6, are reported in some TPP pts and believed to predispose them to attacks. Kir2.6, is exclusively expressed in skeletal muscle and transcriptionally regulated by thyroid hormone. Whether Kir2.6 mutations are present in other hypoKPP is unknown. Here, we studied a large cohort of Taiwanese pts, with 100 TPP and 60 SPP pts, and 100 unrelated healthy subjects. All pts have severe hypokalemia during attacks (K ~ 2.0 mEq/l). TPP and SPP pts have similar clinical features except that TPP have higher T3 and free T4 and suppressed TSH, and lower serum phosphate. Two TPP pts had the same heterozygous V168M mutation in the 2nd transmembrane segment of Kir2.6. Two SPP pts had different heterozygous mutations- R43C in the N- and A200P in the C-terminal cytoplasmic domain. These 3 mutations were not present in control subjects. Wild type (WT) and mutant Kir2.6 were expressed in HEK cells for functional studies. Western blot of cell lysates showed all 3 mutants express full-length protein and have equal expression as WT. Compared to WT, whole-cell current density for R43C and V168M mutants were reduced by 80% and 40%, respectively. No current was for A200P. Furthmore, V168M and A200P, but not R43C, exerted dominant-negative effects on WT channel. Single channel open probability was reduced in both R43C and V168M . Thus, mutations of Kir2.6 are associated with SPP as well as TPP. The regulation of Kir2.6 by thyroid hormone is not central for mutations of Kir2.6 to cause susceptibility to hypoKPP. Hypofunction of Kir2.6 may depolarize the cell membrane potential rendering Na+ channel inactivated and unavailable for firing when action potential arrives.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1601**

**The Kir4.1 Knock Out Mouse Displays Relative Hypokalemia and Increased Urinary $K^+$ Excretion** Daniel A. Gray, Heather L. Connelly, Michael W. Cypress. *Nephrology Unit, University of Rochester, Rochester, NY.*

Mutations in the Kir4.1 potassium channel underlie the SeSAME/EAST syndrome, an autosomal recessive condition characterized by seizures, sensorineural deafness, ataxia and a hypokalemic, hypomagnesemic metabolic alkalosis. To better understand the renal pathophysiology of this syndrome, serum and urine electrolytes have been collected from a generalized Kir4.1 knock out mouse (generated originally by Paulo Kofuji). The knock outs (KO) were smaller than their heterozygous (HET) and wild-type (WT) littermates ($2.12 \pm 0.15$ vs. $2.58 \pm 0.16$ and $2.74 \pm 0.18$ gms respectively). Because the KOs typically die within 2 weeks of age, serum and urine were obtained from 3-5 day old pups. Serum was obtained using a hematocrit tube following decapitation and urine was collected via

bladder aspiration. Serum and urine $K^+$ and $Na^+$ concentrations (in mM) were determined by flame photometry. Serum [$K^+$] was significantly reduced from 6.8 ± 0.5 in WT to 5.1 ± 0.3 in the KO (n=7, p = 0.01). No hemolysis was observed in association with the mild elevation in WT [$K^+$]. WT and HET values did not differ statistically. Although the urine [$K^+$] was similar for all 3 groups, the urine osmolality was significantly lower in the KO (333 ± 4 vs. 403 ± 15 mOsm/kg in WT, p = 0.001). This resulted in a significantly higher transtubular $K^+$ gradient (TTKG) in the KO (13.4 ± 1.0 vs. 8.9 ± 0.7 in WT, p = 0.004). Urinary [$Na^+$] was very low in all groups with a trend toward higher values in the KO (3.6 ± 1.5 vs. 0.7 ± 0.5 in WT, p =0.10). These results suggest that the Kir4.1 knock out displays the $K^+$ wasting phenotype of the SeSAME/EAST syndrome and may provide a useful model for future transport studies.

| | Weight (gms) | Serum [$K^+$] (mM) | Urine [$K^+$] (mM) | Urine [$Na^+$] (mM) | Urine Osm. (mOsm/kg) | TTKG |
|---|---|---|---|---|---|---|
| **WT** | 2.74±0.18 | 6.8 ± 0.5 | 83 ± 3 | 0.7 ± 0.5 | 403 ± 15 | 8.9±0.7 |
| **HET** | 2.58±0.16 | 6.1 ± 0.3 | 93 ± 5 | 0.6 ± 0.3 | 436 ± 22 | 10.0±0.4 |
| **KO** | 2.12±0.15° | 5.1 ± 0.3* | 85 ± 7 | 3.6 ± 1.5 | 333 ± 4† | 13.4±1.0‡ |

Errors represent SEM for n = 7 mice, drawn from 4 litters. °, *, †, ‡ = significant vs. WT with p = 0.02, 0.01, 0.001 and 0.004 respectively.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1602

**NO Reduces Chloride Absorption in Mouse Cortical Collecting Duct** Vladimir Pech, Seongun M. Hong, Susan M. Wall. *Emory University.*

Nitric oxide regulates blood pressure, in part, by modulating transport of $Na^+$ and $Cl^-$ in the kidney. Therefore, we asked if NO regulates net $Cl^-$ flux ($J_{Cl}$) in the CCD and the transporter(s) that mediate NO-sensitive $Cl^-$ absorption. Thus, $Cl^-$ absorption was measured in CCDs taken from aldosterone treated mice. Reducing NO production by inhibiting nitric oxide synthase (L-NAME, 100 mM) increased $J_{Cl}$ from 35.0 ± 6.4 to 49.6 ± 7.4 pmol/mm/min although transepithelial voltage, $V_T$, was unchanged. Conversely, administration of an NO donor (MAHMA NONOate, 10 µM) reduced $J_{Cl}$ from 45 ± 5 to 28 ± 5 pmol/mm/min and reduced $V_T$ from -26 ± 3 to -15 ± 3 mV. These changes in $J_{Cl}$ and $V_T$ were not time-dependent effects. To determine the transport process that mediates NO-sensitive changes in $J_{Cl}$, we examined the effect of NO on $J_{Cl}$ following either genetic ablation or chemical inhibition of various transporters in the CCD. Application of hydrochlorothiazide (100 mM) to the perfusate had no effect on $J_{Cl}$. Moreover, application of hydrochlorothiazide or bafilomycin (5 nM) to the perfusate did not alter NO-sensitive $J_{Cl}$. Thus, NO modulates $J_{Cl}$ independent of the $Na^+$-dependent $Cl/HCO_3^-$ exchanger or the apical $H^+$-ATPase. In contrast both total and NO-sensitive $J_{Cl}$ were nearly abolished either with application of benzamil (3 µM) to the perfusate or with genetic ablation of pendrin (*Slc26a4*). Following the application of benzamil to the lumen, $J_{Cl}$ and $V_T$ were extremely low at baseline and unchanged with NO donor application. In contrast, in CCDs from pendrin null mice, while $J_{Cl}$ was extremely low in the presence or absence of NO, $V_T$ was substantial under basal conditions and markedly reduced with NO application. Thus, NO-sensitive changes in $J_{Cl}$ cannot be explained fully by changes in paracellular $Cl^-$ transport. Instead, total and NO-sensitive $Cl^-$ absorption observed in the CCD of aldosterone-treated mice depends on the presence of the $Cl^-/HCO_3^-$ exchanger, pendrin. We conclude that NO reduces Cl- absorption in the CCD of aldosterone-treated mice through a mechanism that is pendrin dependent. How benzamil reduces total and NO-sensitive $J_{Cl}$ is unexplained, but may occur, at least in part, by modulating the driving force for pendrin-dependent $Cl^-$ transport.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1603

**Pendrin Confers Partial Resistance to the Diuretic Effect of Loop and Thiazide Diuretics in Mice** Hassane Amlal, Sharon L. Barone, Jie Xu, Manoocher Soleimani. *Internal Medicine, University of Cincinnati, Cincinnati, OH.*

Loop diuretics and thiazides are the most commonly used drugs for the treatment of fluid overload in patients with congestive heart failure. It is known that NCC in the distal nephron is activated and partially blocks the diuretic effect of loop diuretics such as furosemide. However, whether pendrin (SLC26A4), which is expressed in the distal nephron, plays any role in enhanced salt absorption in response to loop diuretics or thiazides remains unknown. To address this question, mice with genetic deletion of pendrin (PDS KO) and their wild-type littermates (WT) were treated with furosemide (FURO) or hydrochlorothiazide (HCTZ). Accordingly, mice were placed in metabolic cages with free access to food and water for 3 days. After acclimation, mice were injected subcutaneously with commonly used doses of FURO or HCTZ. Food and water intake, urine volume and chloride excretion were measured for 24 hrs.

The results indicate that PDS KO demonstrated a more robust diuresis to furosemide Vs. WT mice, with urine volume increasing from 2.37 to 4.57 ml/24h, P<0.005 in WT and from 3.08 to 7.62 ml/24h, P<0.002 in PDS KO. The furosemide-induced diuresis was 2-fold higher in PDS KO relative to WT (4.54 ml/24 hrs in KO vs. 2.20 in WT, P<0.02). There was a decrease in food intake in both groups (from 4.5 to 1.32 gm/24h, P<0.0001 in WT and from 5.0 to 1.52 gm/24h, P<0.001 in PDS KO). Despite the reduction in chloride intake subsequent to reduced food intake, PDS KO exhibited a significantly higher chloreuresis as compared to WT mice (0.612 vs. 0.306 mEq/24h, P<0.006). With regards to HCTZ, baseline food intake, urine volume and chloride excretion were similar between WT and PDS KO mice. However, 24h HCTZ caused a significant chloreuresis (0.35 vs. 0.17 mEq/24h, P<0.006) and diuresis (2.86 vs. 1.47 ml/24h, P<0.01) in PDS KO vs. WT mice. Unlike FURO, HCTZ did not significantly alter food intake. We conclude that PDS deletion makes mice susceptible to severe volume depletion in response to furosemide and HCTZ. This clearly suggests that PDS plays an important compensatory role by partially blunting fluid loss of loop and thiazide diuretics in mice kidney.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1604

**Pendrin Regulation by pH and Protein Kinases A and C** Anie Azroyan,[1] Gilles Crambert,[1] Kamel Laghmani,[1] Alain Doucet,[1] Aurelie Edwards.[2] *[1]UMRS 872, ERL 7226, Centre de Recherche des Cordeliers, Paris, France; [2]Chemical and Biological Engineering, Tufts University, Medford, MA.*

Pendrin is expressed in the kidney in type-B intercalated cells of the collecting duct where it contributes to the reabsorption of NaCl and the secretion of bicarbonate, thereby participating in the regulation of blood pressure and acid/base homeostasis. Despite its importance, the intrinsic properties and regulation of pendrin are poorly documented. In this study, we investigated the regulation of pendrin activity by its glycosylation status, extracellular and intracellular pH, and protein kinases A (PKA) and C (PKC) in cultured kidney epithelial cells stably transfected with pendrin cDNA. Pendrin activity was determined by monitoring intracellular pH changes induced by variations in transmembrane anion gradients. Using structural analysis and in situ directed mutagenesis we identified asparagines at position N167 and N172 as pendrin glycosylation sites. Mutation of those sites did not alter its cell surface expression and activity. We also showed, by combining transport measurements and a mathematical model, that pendrin activity is stimulated at both low intracellular and low extracellular pH, suggesting the presence of a proton regulatory site. Finally we found that pendrin activity is significantly increased by the addition of 8Br-AMPc and decreased by phorbol esther (an activator of PKC) treatment. Preliminary measurements of pendrin cell surface expression suggest that the stimulation of PKC, but not that of PKA, reduces pendrin trafficking to the plasma membrane.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1605

**CLIC1 Potentiates Acute Toxic Injury** John C. Edwards,[1] Barbara Ulmasov.[2] *[1]UNC Kidney Center, University of North Carolina, Chapel Hill, NC; [2]Internal Medicine, St. Louis University, St. Louis, MO.*

CLIC1 is a member of the family of "chloride intracellular channels" that is highly expressed in several epithelia including renal proximal tubule and pancreatic duct. CLIC1 has also been implicated as a plasma membrane chloride conductance supporting superoxide production by macrophages. If CLIC1 supports superoxide production, it may contribute to tissue injury during toxic or ischemic insults. The purpose of this study was to determine whether CLIC1 plays a role in acute toxic injury in organs where it is abundantly expressed, using a mouse model in which the gene for CLIC1 has been disrupted. WT and matched Clic1-/- mice were subjected to folic acid (FA) induced acute kidney injury (AKI) or cerulein (CER) induced acute pancreatic injury (API). To induce AKI, mice were given 250 mg/kg injection of FA. Plasma BUN on day 2 was taken to assess severity of AKI. Results: WT day 2 BUN 397 ± 32, n=17; KO day 2 BUN 261 ± 38, n=21; P=0.013. For API, mice were given 6 hourly injections of cerulein (50 µg/kg) and then sacrificed 9 hours after the first injection. The pancreas was removed, weighed, and processed for RNA and TBAR assay (reflecting oxidative injury). Results: Pancreatic weight (mg): WT control 138 ± 10, n=9; WT CER 241 ± 15, n=10; KO control 138 ± 6, n=9; KO CER 185 ± 16, n=10. The difference in extent of edema between CER-treated WT and KO is significant (P<0.01). TBAR values (pmole/mg protein): WT control 126 ± 19, n=9; WT CER 182 ± 17, n=10; KO control 146 ± 12, n=9; KO CER 123 ± 9, n=10. The differences between TBAR levels in WT control vs. WT CER, and between WT CER vs KO CER are significant (P<0.02 and 0.01, respectively) while the difference between WT and KO controls is not significant. In the WT mice, the mRNA level for CLIC1 increased 10.1 fold following cerulein treatment (95% confidence interval 7.9 - 12.9). We conclude that CLIC1 plays a role in acute toxic injury in both kidney and pancreas: CLIC1 mRNA increases following injury in wild type mice, absence of CLIC1 results in attenuated injury of both kidney and pancreas and in pancreas, this correlates with lower level of a marker of lipid peroxidation.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1606

**Altered Ion Currents of ClC-5 by Dent's Disease-Causing Mutations** Teddy Grand,[1,2,3] Jacques Teulon,[1,2,3] Stéphane Lourdel.[1,2,3] *[1]UMR_S 872 Equipe 3, UPMC Université Paris 06, Paris, France; [2]UMR_S 872 Equipe 3, INSERM, Paris, France; [3]ERL 7226, CNRS, Paris, France.*

The $Cl^-/H^+$ antiporter ClC-5, has been linked to Dent's disease, an X-linked renal disease associated with low molecular weight proteinuria, hypercalciuria and nephrolithiasis. CLC-5 is expressed on early endosomes of proximal tubule cells, where it is thought to play a critical role in endosomal function. The impact of Dent's disease-causing mutations on CLC-5 function has not been yet fully investigated. Here, we thought to investigate the functional consequences of three published mutations (E267A, S270G and S270R) in *X. laevis* oocytes and in HEK293 cells. The ClC-5 mutants were synthesized from human wild-type (WT) ClC-5 extracellularly HA tagged subcloned into pTLN expression vector (kindly provided by T. J. Jentsch, FMP/MDC, Berlin, Germany) for expression in *X. laevis* oocytes or into the peGFP expression vector for expression in HEK293 cells. We evaluated electrical activity, subcellular targeting and protein expression of WT and mutant ClC-5 by two-electrode voltage-clamp, immunocytochemistry, chemiluminescence and western blot analysis. The E267A and S270G mutants showed a significant reduction of currents by 50% ($p$<0.001, n=19) and 20% ($p$<0.001, n=24) by comparison to those of WT ClC-5. Currents

recorded with the S270R mutant were not significantly different from non-injected (NI) oocytes ($p<0.001$, n=6). We found no significant difference between these mutants and WT ClC-5 in terms of subcellular targeting and protein expression levels. All mutants trafficked normally to the cell surface and to early endosomes and displayed complex N-glycosylation. In conclusion, this study shows that these three mutants ClC-5 display a reduced electrical activity without alteration of protein expression and subcellular distribution.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1607**

**CFTR from Divergent Species Responds Differently to Channel Inhibitors** Maximallian Stahl,[1,2] Klaus Stahl,[1,2] Marie B. Brubacher,[1,2] John N. Forrest.[1,2] *[1]Nephrology Division, Yale Univ School of Medicine, New Haven, CT; [2]Mt. Desert Island Biological Lab, Salisbury Cove, ME.*

Comparison of diverse species is a powerful tool to study the structure of channel proteins. Ion permeation in ion channels is influenced by charged amino acid side chains around the entrance of the channel pore. These residues attract oppositely charged ions thus increasing their local concentration, while repelling ions of like charge. Site directed mutagenesis has identified positively charged residues in anion channels. We investigated the response of human, killifish, pig, and shark cystic fibrosis transmembrane conductance regulator (hCFTR, kfCFTR, pCFTR, sCFTR) to inhibitors of the channel: CFTRinh-172, glibenclamide, and GlyH-101. From mutagenesis studies each inhibitor is thought to bind to specific positively charged amino acids in CFTR. Using perfusions and primary cultures of the shark rectal gland, and expression studies of each species protein in cRNA microinjected Xenopus laevis oocytes, we observed fundamental differences in the sensitivity to inhibition by these channel blockers. In perfusion studies, stimulation with forskolin and IBMX increased chloride secretion 15-30 fold. However, endogenous sCFTR was insensitive to inhibition by CFTRinh-172 (no inhibitory effect). This was confirmed in cultured rectal gland epithelial cells (2.5 ± 0.15 % maximum inhibition). In oocyte expression studies shark CFTR was again insensitive to CFTRinh-172 (maximum inhibition 8 ± 1.4%), pCFTR was insensitive to glibenclamide (maximum inhibition 12.8 ± 4.2%), whereas all species were sensitive to GlyH-101. The amino acid residues considered responsible for inhibitor binding by previous site directed mutagenesis are entirely conserved in the CFTR isoforms studied. These experiments demonstrate profound differences in the sensitivity of different CFTR proteins to inhibition by CFTR blockers that cannot be explained by the targeted amino acids. Rather, the potency of CFTRinh-172, glibenclamide and Gly-H101 on the CFTR chloride channel is likely dictated by the local environment and the three dimensional structure of residues that form the vestibule and the chloride pore.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1608**

**TGFβ1-Dependent Downregulation of CFTR Is Mediated by DAB2 in Human Epithelial Cells** Agnieszka Swiatecka-Urban.[1] *[1]Nephrology, Children's Hospital of Pittsburgh, Pittsburgh, PA; [2]; [3].*

TGFß1 plays a fundamental role in the pathogenesis of acute and chronic kidney disease and is an important genetic modifier in cystic fibrosis. TGFβ1 inhibits CFTR expression and function by mechanisms that are incompletely understood. Recent work has demonstrated that the multifunctional adaptor protein and an endocytic adaptor, Disabled-2 (Dab2) is integral to TGFβ1 signaling. Our previously published work demonstrated that Dab2 interacts with CFTR. Thus, we hypothesized that Dab2 mediates the TGFβ1-dependent downregulation of CFTR in epithelial cells. The primary differentiated human bronchial epithelial (HBE) cells endogenously expressing wild type (WT)-CFTR and polarized human airway epithelial cells (CFBE41o-) stably expressing WT-CFTR were used as a model of human epithelial cells. Our studies demonstrate that TGFβ1 treatment induced transcriptional responses, including Smad2 phosphorylation and the nuclear translocation of phosphorylated Smad2 in the HBE and CFBE41o- cells. Treatment with TGFβ1 significantly decreased CFTR expression in cell lysates and in the apical plasma membrane in a concentration and time-dependent manner, and the treatment with TGFβ receptor I inhibitors prior to stimulation with TGFβ1 prevented Smad2 phosphorylation. Endogenous Dab2 co-immunoprecipitated with members of the TGFβ1 signaling cascade. Silencing Dab2 expression reduced Smad2 phosphorylation at baseline and after TGFβ1 treatment. Moreover, silencing Dab2 increased CFTR abundance in cell lysates and in the plasma membrane and increased the CFTR mediated chloride secretion. Taken together, our data demonstrate that in human airway epithelial cells, including primary differentiated human bronchial epithelial cells, TGFβ1 downregulates CFTR expression by a mechanism that involves Dab2 mediated signal transduction through the canonical TGFß1 signaling cascade. We anticipate further studies will increase our understanding of the ion transport regulation by TGFß1 and will elucidate the effects of high-producing TGFß1 genotype on the function of ion channels.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1609**

**ATP Inhibits NaCl Absorption Via Basolateral P2X Receptors in Mouse Medullary Thick Ascending Limb (mTAL)** Rita D. Marques,[1] Helle A. Praetorius,[1] Markus Bleich,[2] Jens G. Leipziger.[1] *[1]Physiology and Biophysics, Aarhus University, Aarhus, Denmark; [2]Institut of Physiology, Christian Albrechts University Kiel, Kiel, Germany.*

Extracellular nucleotides regulate epithelial transport via luminal and basolateral P2 receptors. Renal epithelia express multiple P2 receptors, which mediate significant inhibition of solute absorption. Recently, we identified several P2 receptors in the medullary thick ascending limb (mTAL) including luminal and basolateral P2Y2 receptors. In addition we found evidence for a basolateral P2X receptor. It has recently been suggested that extracellular nucleotides influence ion transport in this segment. Objective: Investigate if extracellular ATP influences NaCl absorption in mTAL. Methods: We used isolated, perfused mouse mTAL to electrically measure $Na^+$ absorption. By microelectrodes we determined the transepithelial voltage (Vte) and the transepithelial resistance (Rte) and via these the transepithelial $Na^+$ absorption (equivalent short circuit current, Isc). Results: Non-stimulated mTALs show the following transport characteristics: Vte: +9.03±0.44 mV, (lumen-positive), Rte: 8±1.1 Ω $cm^2$, Isc: 1404±21.4 μA/$cm^2$ (n=16). As expected, luminal furosemide (100 μM) completely blocked transport. Basolateral ATP (100 μM) acutely (within 1 minute) and reversibly reduced the absorptive Isc. After 2 minutes a maximal reduction was measured and amounted to 19.6±2.8% (n=8). In the presence of ATP transport inhibition was sustained. Suramin inhibited the ATP effect. Basolateral UTP, a P2Y2/P2Y4 receptor agonist was without effect as was adenosine. Conclusion: These data define that basolateral ATP exerts a significant inhibition of $Na^+$ absorption in mouse mTAL and point to a P2X receptor-mediated mechanism. Intriguingly, these data add yet another example of P2 receptor mediated inhibition of tubular transport in intact renal epithelium.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1610**

**A Comparison of P2 Receptor mRNA Expression Levels in the Renal Cortical Collecting Duct in Response to Altered Dietary Sodium and in DOCA-Induced Hypertension** Scott S. P. Wildman,[1] Clare M. Turner,[2] Joanne Marks,[3] Claire M. Peppiatt-Wildman,[1] Robert J. Unwin.[3] *[1]Royal Veterinary College, London, United Kingdom; [2]Hammersmith Hospital, London, United Kingdom; [3]UCL Medical School, London, United Kingdom.*

ENaC control Na reabsorption along the CD and help determine systemic blood pressure. Activation of P2 receptor (P2R) subtypes ($P2X_{4\text{ and/or }4/6}$ and $P2Y_{2\text{ and/or }4}$) expressed in CCD can alter ENaC activity[1]. It is known that changes in ENaC activity, following dietary Na restriction, affects the expression levels of some P2Rs[1]. We have proposed that P2R may locally regulate ENaC activity in the CCD and could be involved in the pathogenesis of hypertension.

We have investigated P2R mRNA levels in CCDs from adult Sprague-Dawley rats maintained on 'low' (0.01%), 'normal' (0.5%) or 'high' (4%) Na diets (for 10 days), and in DOCA-salt hypertensive rats (unilateral nephrectomy, 1% NaCl drinking water and DOCA treatment for 5 weeks; SBP = 189±12 $mmHg^{-1}$).

Kidneys were microdissected and CCDs isolated (~15mm). RNA was extracted and reverse transcribed, and the cDNA transcripts used for real time-PCR. A ratio was calculated of the P2R gene of interest ($P2X_{1\text{-}7}$ or $P2Y_{1,\,2,\,4\text{ and }6}$) to a house-keeping gene (HPRT).

We could not detect significant levels (i.e.>0.5 arbitrary units;AU) of $P2X_{2,3,5,7}$ and $P2Y_1$ mRNA under any experimental condition. Significant amounts of mRNA were detected for $P2X_4$ and $P2Y_{2\text{ and }6}$ in CCDs from rats on a 'normal' Na intake. CCDs from rats on a 'low' Na diet showed a significant increase in abundance of $P2X_4$ and $P2Y_6$ mRNA (2-fold); CCDs from rats maintained on a 'high' Na diet showed a significant decrease in abundance of $P2Y_2$ mRNA (to less than 0.5 AU); CCD $P2X_{1\text{ and }6}$ and $P2Y_4$ mRNA levels were not significant on a 'normal' Na diet, but were increased on a 'low' Na diet. There were no differences in mRNA expression between 'normal' Na diet and DOCA-salt hypertensive rats.

P2R mRNA expression levels change in the rat CCD in response to changes in dietary Na but not in DOCA-salt hypertensive rats.From these data it is unlikely that altered CCD P2R function plays a role in the DOCA model of hypertension.

[1] Wildman SS et al(2008) J.Am.Soc.Nephrol. 19:731-42.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1611**

**Decreased $P2X_7$ Receptor mRNA Expression in the CCD of the $P2X_4^{-/-}$ Mouse: A Clue to the Identity of the P2X Luminal Sodium Sensor?** Siobhan N. George,[1] Teresa M. Kennedy-Lydon,[1] Holly B. Callaghan,[1] Eilidh Craigie,[2] David G. Shirley,[2] Frederick W. K. Tam,[3] Claire M. Peppiatt-Wildman,[1] Robert J. Unwin,[2] Scott S. P. Wildman.[1] *[1]Royal Veterinary College, London, United Kingdom; [2]UCL Medical School, London, United Kingdom; [3]Hammersmith Hospital, London, United Kingdom.*

$Na^+$ reabsorption in the CD is controlled by ENaC. It determines the final amount of reabsorbed $Na^+$ and therefore arterial blood pressure. ENaC activity is inhibited by the activation of an apical $P2X_4$ receptor complex when luminal $Na^+$ is high (145 mM), and potentiated when $Na^+$ is lowered (to 50 mM). Consequently we have proposed that $P2X_4$ complexes are luminal $Na^+$ sensors responsible for locally regulating ENaC activity and therefore blood pressure [1]. Currently we are investigating whether $P2X_4$ receptors are significant regulators of ENaC using a $P2X_4^{-/-}$ mouse. We have compared P2X receptor mRNA levels in the CCD of wildtype and $P2X_4^{-/-}$ mice, using real time-PCR.

Kidneys from terminally anaesthetised mice (C57 wildtype and $P2X_4^{-/-}$) were microdissected and CCDs isolated (~15 mm). RNA was extracted and reverse transcribed and the mRNA transcripts used for real time-PCR. A ratio of mRNA expression was calculated of the P2 receptor gene of interest ($P2X_{1-7}$) to a house-keeping gene (β-actin).

We failed to detect significant levels (<20 arbitary units) of $P2X_{1,2}$ and $_3$ mRNA in wildtype and $P2X_4^{-/-}$ mice. In contrast, significant amounts of mRNA were detected for $P2X_{4,5,6}$ and $_7$ receptor subunits in CCD of wildtype mice. CCDs from $P2X_4^{-/-}$ mice showed a significant decrease in abundance of $P2X_4$ and $_7$ mRNA levels.

In conclusion, the $P2X_4^{-/-}$ mouse CCD does not express $P2X_4$ mRNA - thus validating this mouse model for determining the significance of the $P2X_4$ receptor subunit in regulating ENaC activity. In addition, levels of $P2X_7$ mRNA are also significantly decreased in the $P2X_4^{-/-}$ mouse. Interestingly, a functional $P2X_{4/7}$ heteromeric assembly exists [2], and consequently the possibility exists that the $P2X_{4/7}$ receptor is the sodium sensor in the CCD responsible for the local regulation of ENaC.

[1] Wildman et al, (2008) J. Am. Soc. Nephrol. 19:731-42.

[2] Guo et al, (2007) Mol. Pharmacol. 72:1447-56.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1612

**P2Y Receptor Activation Induces Chloride Secretion through Calcium-Activated Chloride Channels in Kidney Inner Medullary Collecting Duct Cells** Madhumitha Rajagopal, Alan C. Pao. *Division of Nephrology, Department of Medicine, Stanford University, Stanford, CA.*

Dysregulation of natriuretic pathways can cause renal salt retention, extracellular fluid volume expansion, and hypertension. Recent studies have shown that urinary ATP increases when rats are placed on a high salt diet. Patch-clamping studies have shown that ATP acts via $P2Y_2$ receptors to inhibit ENaC channels in the cortical collecting duct. $P2Y_2$ knockout mice have increased ENaC activity and develop hypertension, suggesting that this pathway is important for salt homeostasis and blood pressure regulation. We used the mIMCD-K2 cell line as a model system for inner medullary collecting duct epithelium to study other ion transport pathways that might be regulated by P2Y receptors. With an Ussing chamber setup, we observed that ATP acts from both the apical and the basal side of cell sheets to increase chloride ($Cl^-$) secretion. Basal addition of ATP caused a transient increase in short-circuit current ($I_{sc}$), whereas apical addition caused first a transient and then a sustained increase. Both responses were completely inhibited by pre-treatment with $10^{-3}$M FFA, a calcium ($Ca^{2+}$)-activated $Cl^-$ channel blocker. Using specific $P2Y_1$ and $P2Y_2$ receptor agonists, we found that both $P2Y_1$ and $P2Y_2$ receptors at the basal surface mediated the transient $I_{sc}$ increase, and $P2Y_2$ receptors on the apical surface mediated the transient and sustained increases in $I_{sc}$. Furthermore, pre-treatment with a $P2Y_1$ antagonist abolished the effect of the $P2Y_1$ agonist on $I_{sc}$ from the basal side but not of the $P2Y_2$ agonist on $I_{sc}$ from either side. ATP-mediated transient and sustained increases in $I_{sc}$ were also blocked by pre-treatment with phospholipase C (PLC) inhibitor (U73122) and $Ca^{2+}$ chelator (BAPTA-AM), suggesting that ATP signals through PLC and intracellular $Ca^{2+}$ to activate $Ca^{2+}$-activated $Cl^-$ channels. These studies identify two P2Y receptor signaling pathways in inner medullary collecting duct cells that can respond to increases in urinary ATP by stimulating $Cl^-$ secretion. These signaling pathways could represent another mechanism by which urinary ATP can enhance NaCl excretion under conditions of high dietary salt intake.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1613

**The Utility and Accuracy of Four Equations in Predicting Sodium Levels in Dysnatremic Patients** Ramy Magdy Hanna,[1,2] James Wilson.[1,2] *[1]Department of Medicine-Division of Nephrology, Olive view-UCLA Medical Center, Sylmar, CA; [2]David Geffen School of Medicine, University of California Los Angeles (UCLA), Westwood, CA.*

**Introduction:** The Ardogue Madias equation (AM), the Barsoum Levine (BL) equation, the Electrolyte free water clearance based equation (EFWC), and the Nguyen Kurtz (NK) equation are four derived equations based on the empirically derived Edelman equation for predicting Na2 from a known Na1 and fluid/electrolyte input and output.

**Methods:** Our study includes 25 mostly hyponatremic patients and 29 data points. We calculated Na2 based on 5 increasingly precise sets of rules. Sets A-D included all 25 patients and 29 data points. Set E contained 9 patients and 13 data points, and was rigorous to the point of accounting for every ion of sodium/potassium and every ml of fluid intake and output to calculate Na2. All of our data and analysis was reviewed by a statistician.

**Results:** Bland Altmann analysis showed no highly significant trends. Pearson correlation was highly significant (**p<0.00001)* for all 4 equations in sets A-D. In set E,correlations were significant for the NK and EFWC based equations (**P=0.028, and 0.048 respectively).* We note that the correlation observed for a control equation (where Na1=Na2) was also highly significant (**p<0.00001)*. We compared the root mean squared error (RMSE) for all four equations and across the board found it to be between 5.5 and 6.5 meq/L of Na for all sets and 6-7 for both iterations of the control equation Na1=Na2. The average delta Na seen was less than the RMSE (2.55 sets A-D, and 3.23 for set E). Increasingly rigorous analysis did not positively affect accuracy. Up to 20meq/L variability between the predicted and actual Na was noted.

**Conclusion:** Like similar studies in hypernatremic patients (Lindner et.al.), our analysis shows highly significant pearson correlations. We show that the control equation Na2=Na1 is also highly correlated. The lack of correlation seen for the AM and BL equation in set E was due to small sample size. The high variability and RMSE shows that none of these equations are effective for predicting Na2 from Na1 for the range of delta sodium seen in our cohort.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1614

**Responses to Tolvaptan in Regard to Serum Sodium (SNa) and Serum Arginine Vasopressin (AVP) Levels in Patients with Hyponatremia** Rick P. Vaghasiya, Meghana R. Gaiki, Maria V. Devita, Michael F. Michelis. *Division of Nephrology, Lenox Hill Hospital, NY, NY.*

Hyponatremia is the most common electrolyte disorder in hospitalized patients. With the development of agents that increase solute-free water clearance, management of dilutional hyponatremia has been facilitated. The nonselective, intravenous, vasopressin receptor antagonist conivaptan has been used for treating euvolemic and hypervolemic hyponatremia. More recently, tolvaptan, an oral, selective vasopressin V2 receptor blocking agent has also been introduced for this indication. We report experience with tolvaptan at our institution regarding changes in SNa, urine osmolalities and AVP levels pre and post drug administration.

Patients with euvolemic and hypervolemic hyponatremia were selected. Indications for tolvaptan included SNa level ≤ 125 meq/L or symptomatic patients with less marked hyponatremia. Each patient received an initial oral dose of 15 mg tolvaptan. An additional 15 mg dose was given 24 hours after the initial dose if the patient failed to achieve an adequate response. Five patients are reported.

The mean SNa increase was 7.4 meq/L (range 2-11 meq/L) in the first 24 hours post initial tolvaptan dose. Two patients required 1 dose, 2 patients received 2 doses and 1 patient required 3 doses to achieve a SNa level > 130 meq/L. Urine osmolalities decreased a mean of 243 mOsm/kg H2O. In patients that required repeated dosing, the urine osmolalities continued to be suppressed. The mean AVP level pre tolvaptan was 7.6 pg/mL. For 4 patients, AVP levels 24 hours post tolvaptan administration changed by 0, -1, +1.5, and +26.7 pg/mL, respectively. AVP level post treatment was not available for 1 patient. The patient with an initial decrease in AVP, had an increase of 6.7 pg/mL after a second dose of tolvaptan. There were no significant drops in blood pressure associated with tolvaptan administration. Two patients experienced an 8 meq/L increase in SNa level within 12 hours post tolvaptan dose, both of which responded to D5W infusion.

These data indicate that the use of the oral V2 receptor blocking agent, tolvaptan, can successfully treat significant dilutional hyponatremia in hospitalized patients.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1615

**RLY5016: A Novel, Non-Absorbed, Therapeutic Polymer for Serum Potassium Control** I.-Zu Huang,[1] Thomas M. Blok,[2] Michael Burdick,[1] Jamie Cope,[1] Sherin Halfon,[1] Kathleen H. Mellinger,[3] Craig K. Park,[1] James T. Vanderlugt,[2] David A. Bushinsky,[4] Detlef Albrecht.[1] *[1]Relypsa, Santa Clara, CA; [2]Jasper Clinic, Kalamazoo, MI; [3]Battelle, Richland, WA; [4]U. Rochester, NY.*

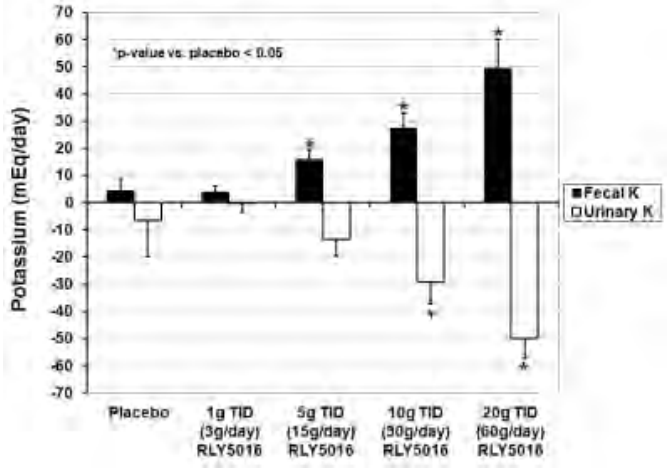
RLY5016 is a novel, high-capacity, non-absorbed, cation exchange polymer being developed for serum potassium (K) control. RLY5016 is administered as a calcium salt and consists of smooth, free-flowing, low-swelling spherical beads (~100um). In two Phase 1 studies, dose-response and different dosing regimens of RLY5016 were evaluated.

In study RLY5016-101, following single-dose and baseline (BL) periods, 31 healthy volunteers (HVs) received 1g, 5g, 10g, or 20g three times a day (TID) of RLY5016 (3, 15, 30, or 60g/d) or placebo orally for 8 days in a double-blind, randomized, parallel-group design. A dose-dependent increase in mean daily fecal K excretion and a corresponding decrease in urinary K excretion compared to BL were observed (Figure). RLY5016 isolated from feces demonstrated that the in vivo K binding capacity of RLY5016 exceeded 1mEq/g. RLY5016 was well tolerated in all dose groups and adverse events (AEs) were mild to moderate in severity.

In study RLY5016-102, 12 HVs received 30g/d RLY5016 orally for 18 days total in a crossover design administered as 10g TID, 15g twice a day (BID), or 30g once a day (QD). No significant differences were observed between the dose regimens in mean daily fecal or urinary K excretion. AEs were mild to moderate in severity, and were reported for 1, 5, and 7 subjects in the TID, BID, and QD periods, respectively.

RLY5016 resulted in a significant dose-dependent increase in mean daily fecal K excretion and decrease in urinary K excretion indicating appreciable intestinal K binding, was effective when administered in several dosing regimens, and was well tolerated.

Figure: Dose-Dependent Effect of RLY5016 on Mean Daily Fecal and Urinary K Excretion




Disclosure of Financial Relationships: Employer: Relypsa, Inc.; Ownership: Relypsa, Inc.
Anthera.

**F-PO1616**

**RLY5016: A New, Effective, Non-Absorbed, Therapeutic Polymer To Control Serum Potassium** Jamie Cope,[1] Vic Ciaravino,[1] Xunxiang Du,[1] Marcus M. Fischer,[1] Lawrence Lee,[1] Jonathan A. Mills,[1] Murray J. Pettitt,[2] Megan Leah Strawford,[2] Jerry Buysse.[1] *[1]Relypsa, Santa Clara, CA; [2]Prairie Swine, Saskatoon, Canada.*

Sodium polystyrene sulfonate (Na-PSS; e.g. Kayexalate, Kionex) has been used for the treatment of hyperkalemia for half a century. Recently the efficacy and safety of Na-PSS, which is commonly co-administered with large amounts of sorbitol as a cathartic, has been called into question. There is a clear clinical need for a safe and effective polymer for reducing total body potassium (K) that does not require sorbitol co-administration.

RLY5016 is a novel cation-exchange polymer that is being developed for long term control of serum K in patients at risk for hyperkalemia. The polymer is free flowing and low swelling and has excellent GI tolerability without added sorbitol.

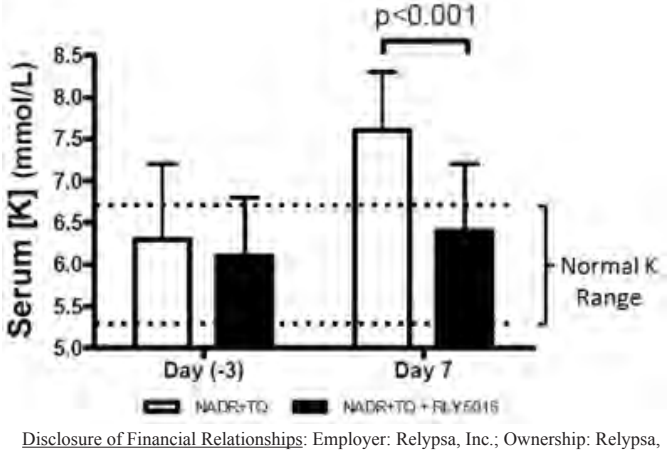
RLY5016 is safe and non-absorbed when dosed in rats and dogs. The polymer was well tolerated and safe at doses of 15g/kg/d when administered for 4 weeks in rats and 3.75g/kg/d when administered for 9 months in dogs. In dogs, 99.9% of the administered 13C-RLY5016 dose was recovered in the feces, <0.1% was detected in the urine and 0.002% in the plasma.

Administration of RLY5016 to rats and pigs with normal renal function increased the fecal excretion of potassium with a concomitant reduction in urinary potassium excretion. RLY5016 isolated from pig feces showed that the in vivo binding was 0.8 mEq K per g polymer in these animals.

In rats with compromised potassium homeostasis, induced by partial nephrectomy and the administration of adriamycin, trimethoprim and quinapril (NADR+TQ), hyperkalemia developed rapidly. Administration of RLY5016 in NADR+TQ rats maintained potassium in the normal range.

RLY5016 increased potassium excretion in the feces and thereby controlled hyperkalemia in animals with renal insufficiency.

The Effect of RLY5016 on Serum K Levels in Hyperkalemic NADR+TQ Rats




Disclosure of Financial Relationships: Employer: Relypsa, Inc.; Ownership: Relypsa, Inc.

**F-PO1617**

**RLY5016: A Novel, Effective, Non-Absorbed, Oral Polymer for the Control of Serum Potassium in Heart Failure and Chronic Kidney Disease** David A. Bushinsky,[1] I.-Zu Huang,[2] Stefan D. Anker,[3] Faiez Zannad,[4] Bertram Pitt.[5] *[1]U. Rochester, Rochester, NY; [2]Relypsa, Santa Clara, CA; [3]Charite, Berlin, Germany; [4]CHU, Nancy, France; [5]U. Michigan, Ann Arbor, MI.*

Both heart failure (HF) and chronic kidney disease (CKD) often coexist. Despite the increased mortality risk among these patients, effective therapies such as inhibitors of the renin-angiotensin-aldosterone system (RAAS) are underutilized due to the high prevalence of life threatening hyperkalemia (HK). In this study (RLY5016-202), 104 chronic HF patients with a serum $K^+$ ($S_K$) of 4.3-5.1 mEq/L clinically indicated to receive spironolactone (SPI) were evaluated. Patients with: 1) CKD (eGFR< 60mL/min) on one or more HF therapies (ACEIs, ARBs, BBs) and/or 2) documented history of HK within last 6 months leading to discontinuation of an AA, ACEI, ARB, or BB, were given SPI 25 mg/d and randomized to either 30 g/d of the oral $K^+$ binding polymer RLY5016 (55 pts) or placebo (PL, 49 pts) for 4 wks. The primary endpoint was a change from baseline (BL) $S_K$ at the end of the double-blind treatment. Secondary endpoints were proportion of patients with HK ($K^+$ >5.5 mEq/L), and whose SPI dose could be increased to 50 mg/d. Baseline characteristics were similar between treatment groups. Starting mean $S_K$ was 4.69 ± 0.06 mEq/L on RLY5016 and 4.65 ± 0.07 mEq/L on PL. RLY5016 significantly decreased mean $S_K$ from BL compared to PL (-0.22 mEq/L vs +0.23 mEq/L, p<0.001), reduced the incidence of HK compared to PL (7% vs 25%, p=0.015), and increased the number of patients whose SPI dose could be increased (91% vs. 74%, p=0.019). Among CKD patients (n=28), there was also a significant decrease in mean $S_K$ change from BL with RLY5016 vs PL (-0.14 mEq/L vs +0.38 mEq/L, p=0.031) and a lower incidence of HK (7% RLY5016 vs 39% PL, p=0.017). RLY5016 was well tolerated. Study withdrawal due to an adverse event (AE) was 7% on RLY5016 vs 6% on PL and there were no drug-related serious AEs. The oral $K^+$ binding polymer RLY5016 effectively controls $K^+$ in patients with HF and CKD given SPI and may allow increased use of RAAS inhibitors in these patients.

Disclosure of Financial Relationships: Employer: University of Rochester School of Medicine; Consultancy: Amgen, Genzyme, Cytochroma, Relypsa; Ownership: Amgen, Relypsa; Honoraria: Amgen, Genzyme, Cytochroma, Relypsa; Scientific Advisor: Amgen, Genzyme, Cytochroma, Relypsa.

**F-PO1618**

**Renal Transplant Recipients with Hyperkalemia and Acidosis: The Thiazide Test** Antje Fürstenberg, Nikhil Johri, Robert J. Unwin, Stephen B. Walsh. *Department of Nephrology and Physiology, Royal Free Hospital, University College London Partners Renal Centre, London, United Kingdom.*

Calcineurin inhibitors (CNIs) are known to provoke hyperkalaemia in many patients: the mechanisms for this are unclear. The precise syndrome that occurs is of hyperkalemia, hypertension, mild metabolic acidosis and hypercalciuria.

This is the same phenotype as pseudohypoaldosteronism type 2 (PHA2, Gordon's syndrome), caused by mutations of WNK kinase 1 or 4, which disinhibit expression of the Na-Cl cotransporter (NCC) in the distal convoluted tubule. We hypothesised that NCC activity is increased in CNI induced hyperkalemia, and that we could use a thiazide test to demonstrate NCC overactivity in the same way that it detects NCC underactivity in Gitelman's syndrome.

The subjects were: 6 patients with CNI induced hyperkalaemia and acidosis (CNI) and 3 healthy controls (CTL). All gave written consent. Baseline blood and urine samples were taken for Na, K, Cl, Mg, Ca and creatinine measurements. 10mg of bendrofluazide was given and bed rest observed for 4 hours, water intake encouraged and urine samples repeated at 30-minute intervals.

The change in FE from baseline to the maximal FE achieved (termed the ΔFE) was used, as previously described for the thiazide test.

Results:

$FE_{Cl}$: The $\Delta FE_{Cl}$ was higher in the CNI group than the CTL group (6.03 ± 4 vs. 2.2 ± 1 p=0.03)

$FE_{Na}$: The $\Delta FE_{Na}$ was also higher in the CNI group (6.1 ± 1 vs. 1.8 ± 0.3 p=0.01)

$FE_{K}$: The $\Delta FE_{K}$ was higher in the CNI group, compared to the CTL group (13.3 ± 2 vs. 5.8 ± 0.4 p=0.03)

$FE_{Mg}$: The $\Delta FE_{Mg}$ was higher in the CNI group than the CTL group (11.1 ± 3 vs. 2.9 ± 1, p=0.01)

$FE_{Ca}$: The $\Delta FE_{Ca}$ was also higher in the CNI group than the CTL group (4.2 ± 0.5 vs. 0.2 ± 0.3, p=0.002)

The results are compatible with an increased expression of NCC in the CNI group, with supramaximal $\Delta FE_{Cl}$, $\Delta FE_{Na}$, $\Delta FE_{K}$ and $\Delta FE_{Mg}$. The $\Delta FE_{Ca}$ was also significantly raised in the CNI group; the hypocalciuria seen with thiazides may relate to volume contraction, and so may not occur acutely. These data point toward a specific mechanism for CNI induced hyperkalaemia and support thiazides as a specific treatment for this phenomenon.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1619**

**Large-Scale Profiling of Protein Half Lives and Translation Rates in Renal Collecting Duct Cells** Dane H. Slentz, Ming-Jiun Yu, Trairak Pisitkun, Jason D. Hoffert, Mark A. Knepper. *NHLBI, NIH.*

In a given cell type, steady-state protein abundance is determined by a balance between rates of protein production and degradation. Long-term exposure of collecting duct principal cells to vasopressin causes an increase in abundance of the water channel

protein aquaporin-2, a process critical to regulation of water excretion. However, whether the abundances of other proteins undergo parallel changes is unknown and mechanisms of abundance changes are unexplored. We carried out large-scale measurements of protein half-lives ($t_{½}$) and relative translation rates for natively expressed proteins in cultured renal mpkCCD(clone 11) cells (±dDAVP, 1 nM) using metabolic labeling with stable isotopes (SILAC) coupled with protein mass spectrometry (LC-MS/MS). A mass-balance model, assuming first-order kinetics of degradation, allowed calculation of $t_{½}$ and the relative translation rate for each identified protein. Initial experiments (n=3) have provided $t_{½}$ measurements for over 1,000 proteins, a small fraction of which (<1%) was found to change significantly in response to vasopressin, including a 66% decrease in the $t_{½}$ for the adhesion protein B-CAM, whose stability is believed to be regulated by SUMOylation via UBC9. The $t_{½}$ of aquaporin-2 was not significantly changed (mean 12.6 hr). Translation-rate measurements show that among all proteins quantified (>500), 55 changed significantly at least 1.5-fold. Among these were two additional proteins involved in cell adhesion and epithelial polarity, viz. afadin and β-catenin-like protein-1. Aquaporin-2 showed the largest increase in translation rate in response to dDAVP (>16-fold). Many of the proteins showing changes in abundance in response to vasopressin did not have corresponding changes in mRNA levels (Affymetrix arrays). Further studies are underway to define the mechanism of mRNA-independent changes in translation rates in response to vasopressin.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1620

**Mice Lacking TRPV4 Show Abnormal Thirst Regulation in Absence of Renal Defects** Sylvie Janas,[1] Sara Terryn,[1] Olivier R. M. Schakman,[2] Johannes Loffing,[3] Philippe Gailly,[2] Olivier Devuyst.[1] *[1]Nephrology, UCL; [2]Cellular Physiology, UCL; [3]Anatomy, University of Zürich.*

TRPV4 is a polymodal cation channel of the transient receptor potential (TRP) family. It is expressed in hypothalamic neurons including the subfornical organ, median preoptic area, and the organum vasculosum of the lamina terminalis, where it could play a role in osmotic sensing. TRPV4 has also been evidenced in the thick ascending limb (TAL) of the rat nephron, where its role remains unclear. To further analyze the role of TRPV4 in osmoregulation, we investigated its distribution pattern and the functional consequences of its disruption in mouse. Using qPCR on microdissected segments and immunohistochemistry, we found that TRPV4 is abundantly expressed in the proximal tubules (apical and basolateral), late distal convoluted tubules and throughout the connecting tubules and collecting ducts (basolateral), including principal and intercalated cells. By contrast, TRPV4 was undetectable in glomeruli and TAL, and weakly abundant in early DCT. Metabolic studies in 12 weeks old TRPV4 knock-out (KO) and wild-type (WT) mice revealed that disruption of TRPV4 does not influence renal function and urinary concentration at baseline. The KO mice did not exhibit anomalies in activity, rearing, food and water intake. Moreover, both WT and KO mice showed a similar renal NaCl and water excretion in response to furosemide injection, acute water loading (100 µl/g BW i.p.), overnight water deprivation, and chronic dietary NaCl restriction (17 days, 0.01% NaCl). However, acute administration of hypertonic saline solution (0.5 M NaCl) resulted in a significantly lower water intake in the TRPV4 KO vs WT mice, suggesting a central defect in osmoregulation. These results demonstrate that TRPV4 is widely expressed in the mouse kidney, but not in the TAL. Disruption of TRPV4 is not reflected by significant alterations in the renal handling of NaCl and water. However, TRPV4 KO mice show a decreased thirst response to hypertonic NaCl, which substantiates the major role of TRPV4 in the central regulation of osmolality.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1621

**Membrane Topology of Mouse Urea Transporter B Defined Using Epitope Tags** Bryce MacIver, John Mathai, Mark L. Zeidel.

Urea fluxes via urea transporters (UTs) help regulate urine osmolality. Hydropathy analysis of many mammalian and bacterial UT sequences show a common motif of a highly hydrophobic region of 5 transmembrane helical passes. UT-B comprises 2 such regions (here called TM1 and TM2) linked by a hydrophilic domain. To assess the validity of the predicted topology map we placed hemagglutinin (HA) tags at various positions in the protein predicted to be intracellular or extracellular loops (fig. 1). We expressed these tagged proteins in *Xenopus* oocytes and assayed for UT function by isotope uptake. We then examined the accessibility of the HA tag to its antibody in whole oocytes. Intracellular tags only become accessible when the oocyte is permeabilized by detergent. All tagged positions in fig. 1 show urea uptake 5-15 times that of control except TM1-1, which appears to be non-functional and TM2-1, which shows ~2 fold increase. To date, antibody localization of TM1-2 and G1 tags corroborate their predicted position, while G2 tag differs in that it appears accessible with detergent permeabilization.

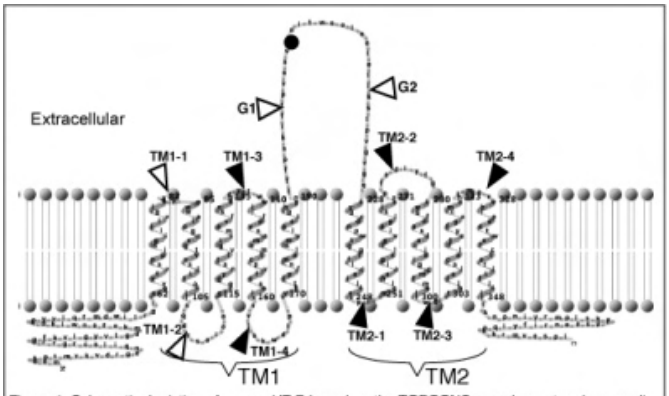



Figure 1. Schematic depiction of mouse UT-B based on the TOPCONS membrane topology prediction algorithm (Bernsel, A. *et al.* Nucleic Acids Research, 2009) and drawn with TMRPres2D (Ioannis, C. *et al.* Bioinformatics, 2004). The topology prediction shows two regions labeled TM1 and TM2 that each comprise 5 transmembrane passes. Open triangles depict positions of HA tag insertions that have been tested for both functionality and location with respect to the membrane while closed triangles are HA tagged insertions tested for function only at this time. The closed circle represents a likely glycosylation site.

Recently a crystal structure of a bacterial UT (dvUT) was reported (Levine *et al*., Nature, 2009). This structure supports the predicted topology showing two hemi-cylindrical domains similar to TM1 and TM2 in fig.1, but adding an additional helix to the 5 transmembrane spans from the topology prediction. However, this additional helix, termed the pore helix, does not span the membrane, instead each pore helix lies at a 50° angle to create a selectivity filter suitable for urea. Interestingly, the G2 tag falls within a region equivalent to a pore helix region of dvUT, yet is functional, but requires detergent for antibody access. In conclusion, our topology mapping corroborates closely with the dvUT structure.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1622

**Dietary Protein Affects Urea Transport across Bladder Epithelium in Rats** David A. Spector, Jie Deng, Kerry J. Stewart. *Divs of Renal Medicine and Cardiology, Johns Hopkins Univ Sch of Med, Baltimore, MD.*

Mammalian bladder epithelia (urothelia) is generally considered impermeable to urinary constituents, but high levels of urea and creatinine and presence of UTB and other transporters in bladder tissue suggest that net urothelial transport (Tx) may occur. We developed an in-vivo rat model to study water and solute Tx across urothelia. To determine the effects of dietary protein on urea Tx we collected 24 hour urines from four groups of rats (n=12) provided with diets differing in protein concentrations (40%, 18%, 6%, 2%- protein deficient ), instilled 0.3 ml urine via urethral catheter into bladders (isolated by ureteral ligation) of anesthetized 225 gm rats, and measured urine volume and concentrations of urea and other solutes (as "controls") in Instilled and Retrieved (after 60 min dwell) urine. Net change (= Tx) of volume and solutes across urothelium were calculated as**:** Retrieved minus Instilled, and differences within and between groups were compared.**Results**: After one hour bladder dwell, there was a mean 2% increase in retrieved urine volume ($p < .0001$) which did not differ between dietary groups. As dietary protein decreased there was a stepwise fall of Instilled UN concentration as well as net urea Tx - reflected by a decrease in change of urine urea concentration (table) and quantity (mg,not shown).

| Dietary Protein | 40% | 18% | 6 % | 2% |
|---|---|---|---|---|
| Instilled UN concentration,mg/dl | 5157* | 2937* | 549 | 247 |
| UN concentration change,mg/dl | -583* | -270 | -141 | -87 |
| percent change | - 11.1 | -11.5 | -25.5* | -23.2* |

* $p < .0001$ vs other diets

In contrast, percent of instilled urea transported was greater in the two low dietary protein groups than in the higher dietary protein groups ($p < .0001$). Regression analysis (not shown) showed a strong direct relationship between initial urine urea concentration and resultant urea Tx. Urine creatinine and K concentrations also fell following one hour bladder dwell, but there were no differences in transport between dietary groups. **Conclusions**: Urea and other solutes are transported across rat urothelia, with magnitude of urea Tx mediated, in part, by dietary protein concentration and consequent urine urea nitrogen concentration.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1623

**Urea Is Important for the Urinary Concentration Capacity of the Isolated Blood Perfused Rabbit Kidney** Paul S. Steels,[1] Jerry Toelsie,[2] Yves Cuypers,[1] Robbert Bipat.[2] *[1]University Hasselt, Belgium; [2]Anton de Kom University, Suriname.*

It is generally accepted that urea plays a critical role in the urine-concentrating mechanism in mammals, but herbivores have a different handling of urea than carnivores and omnivores (Bankir in The Kidney 5th ed. Brenner p. 595). Gunther and Rabinowitz ( KI, 1980,17,205) have reported that urea did not enhance the concentrating ability in calm, unanesthetized, vasopressin-treated rabbits. In this study we used the isolated blood perfused rabbit kidney (Cuypers et al Pfluegers Arch,2000,440,634) to test whether its urinary concentrating activity is indeed independent of urea. Left kidneys of female rabbits

were perfused with 25 ml autologous, heparinized blood at a pressure of 100 mm Hg and at 38°C . The urea concentration was changed via a continuous infusion of 0.1 ml/min in the venous line, to compensate for the urine excretion. [Arg8]-vasopressin (30 IU/l) was added to the perfusion fluid in all experiments. A recovery period of 25 minutes was followed by 4 thirty-minute periods of perfusion during which urine was collected and blood samples were taken . In high urea series (n=8) and low urea series (n=7) the following values were measured from P1 to P4.

| Perfusion period | Pur (mmol/l) | U/Pur | FEur (%) | UF (µL/min.gkw) | Uosm (mOsm/kg) | TCH2O (µL/min.gkw) | TCH2O /UF |
|---|---|---|---|---|---|---|---|
| P1 | 15 | 9 | 18 | 6 | 660 | 6.6 | 1.2 |
| P4 | 18 | 12 | 27 | 5 | 609 | 5.1 | 1.0 |
| P1 | 3.2 | 19 | 43 | 8 | 616 | 8.6 | 1.1 |
| P4 | 1.4 | 12 | 61 | 9 | 387 | 5.0 | 0.6 |

From these results (Uosm and $T_{[sup]C[/sup]H2O}$ /UF) we conclude that urea plays an important role in the urine concentrating mechanism of the rabbit in contrast to the findings of Gunther and Rabinowitz, who applied an osmotic diuresis with mannitol. The high U/P values for urea and the high FEur in the low urea compared to the high urea series, suggest that urea might be actively secreted, although this issue is controversial when studied in isolated rabbit tubular segments (Kawamura, Kokko, JCI,1976,58,604; Knepper,AJP,1983,244,F622).

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Disclosure of Financial Relationships: nothing to disclose

## F-PO1624

**SPAK-Dependent Activation of Thiazide-Sensitive $Na^+$-$Cl^-$-Cotransporter by Vasopressin** Turgay Saritas,[1] Kerim Mutig,[1] David H. Ellison,[2] Eric J. Delpire,[3] Alexander Paliege,[1] Shinichi Uchida,[4] Sebastian C. Bachmann.[1] *[1]Department of Anatomy, Charite University Berlin, Berlin, Germany; [2]Division of Nephrology & Hypertension, Oregon Health & Science University, Portland, OR; [3]Department of Anesthesiology, Vanderbilt University School of Medicine, Nashville, TN; [4]Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan.*

The $Na^+$-$Cl^-$ cotransporter (NCC) of distal convoluted tubule (DCT) effectively contributes to the fine regulation of urine electrolyte composition. Acute activation of NCC by angiotensin II involves luminal trafficking as well as phosphorylation by WNK/SPAK kinases. Vasopressin (AVP) activates NCC by binding to type 2 receptors (V2R), but the kinases involved have not been elucidated. We propose a significant role for SPAK in the AVP-induced activation of NCC.

SPAK-knockout (SPAK-/-, Behav. Brain Res. 2010; 208:377-82.) and wild-type mice (WT) and AVP-deficient Brattleboro rats (DI) were treated with the V2R agonist, dDAVP, or vehicle for 30 min. Kidneys were studied by immunohistochemistry and Western blot.

SPAK and phospho-SPAK were co-expressed with NCC in DCT. dDAVP treatment increased NCC phosphorylation at S71 in WT mice (+87%, $p<0.05$) but less so in SPAK-/- mice (+45%, $p<0.05$). Phosphorylation of NCC at T53 was also significantly increased in WT (+153%, $p<0.05$) whereas SPAK-/- mice showed no change. In DI rats, dDAVP produced increases of SPAK and NCC phosphorylation in 11ß-hydroxysteroid dehydrogenase 2-negative DCT1 (pS373-SPAK: +52%; pS71-NCC: +42%; pT53-NCC: +147%; $p<0.05$) but not in DCT2 of DI rats.

We conclude that V2R stimulation enhances NCC activity via phosphorylation events that depend on SPAK, although other kinases are also involved. Activation of NCC by V2R occurs predominantly along the aldosterone-insensitive DCT1, suggesting that NCC activation by aldosterone is mechanistically distinct.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1625

**Renomedullary Blood Flow and Blood Volume Are Increased during Vasopressin Escape** Fredrik Palm,[1,2] Per Liss,[2] Angelica Fasching,[2] Lars-Ove Magnusson,[2] Petter Quick,[3] Anders Persson,[3] Joseph G. Verbalis.[4] *[1]Nephrology & Hypertension, Georgetown University; [2]Uppsala University, Sweden; [3]CMIV, Linköping University & Hospital, Sweden; [4]Endocrinology & Metabolism, Georgetown University.*

Hyponatremia is a common electrolyte disorder usually caused by inappropriate vasopressin (AVP) levels relative to serum osmolality. The degree of the hyponatremia is limited by "escape" from AVP-induced antidiuresis, characterized by increased urine volume and decreased urine osmolality independently of circulating AVP. The mechanisms mediating escape are not fully understood, but we have hypothesized that increased renomedullary blood flow (BF) contributes to this process. We therefore investigated intrarenal BF and blood volume distribution in rats with and without escape.

Sprague-Dawley rats (n=10) were infused with DDAVP (5 ng/h) to produce maximal antidiuresis. Half were fed a liquid diet (AIN-76) to produce escape; half were fed a solid diet to prevent escape. After 5 days, all rats were anesthetized with Inactin and high resolution images (voxel size 97x97x600 µm) of renal BF were acquired using a Siemens Definition Dual Source CT. Iopromide (0.15 ml/rat) was rapidly injected iv and the contrast over the kidney area was collected during 30s. Data were evaluated by analyzing local renal contrast density utilizing the Siemens Syngo body perfusion tool and correlated to the aorta.

Cortical and medullary BF were 709±41 and 251±50 ml/100ml/min respectively in non-escaped rats. Cortical BF in escaped rats was similar (588±81 ml/100ml/min), but medullary BF was increased compared to non-escaped rats (666±105 ml/100ml/min). Blood volumes were similar in the two groups in cortex (42±1 vs. 49±6 ml/100ml), but elevated in the medulla of escaped rats (70±3 vs 18±3 ml/100ml).

Our results demonstrate that escape is accompanied by markedly elevated renomedullary BF and volume. Elevated BF to the renal medulla results in reduced interstitial osmolality, and may also contribute to the down-regulation of aquarporin-2 water channels known to accompany escape. These results therefore provide a potential mechanistic explanation for the reduced ability to concentrate urine during AVP escape.

Disclosure of Financial Relationships: Honoraria: AstraZeneca.

## F-PO1626

**Characterizing the Roles of eNOS, nNOS, and Angiotensin II in Renal Escape from Antidiuresis** Saad Hussain, Lisa Nicole Linde, Joseph G. Verbalis. *Medicine, Georgetown University, Washington, DC.*

Down-regulation of renal vasopressin (AVP) V2 receptors (V2R) and aquaporin-2 (AQP2) water channels plays a critical role in escape from AVP-induced antidiuresis, but the signaling underlying V2R and AQP2 down-regulation is not known. Previous studies have implicated endothelial and neuronal nitric oxide synthase (eNOS & nNOS), angiotensin II (AngII), and prostaglandins (PG) as molecules involved in modulating V2R activity, and thus possible mediators of escape. We studied the role of these molecules in escape by creating a mouse model of escape and comparing mice with selected gene deletions to C57Bl/6 wild type (WT) mice. For each experiment, 15 mice were divided into 3 groups of n=5: 1) single gene knockout (KO) mice (eNOS, nNOS, or Ang II receptor 1a [atr1a]; Jackson Labs, ME) fed a gel diet and infused with dDAVP; 2) WT mice fed a gel diet and infused with dDAVP; and 3) WT mice fed a solid diet and infused with dDAVP. All experiments were repeated to yield a combined n=10 for each group. Antidiuresis was induced in all groups via dDAVP administration (0.0435 ng/ul/hr/g body weight); groups 1 & 2 were water loaded via the gel diet with high water content, whereas group 3 was not water loaded. Urine volume, osmolality, [Na+] and [K+] as well as ad lib water intake, food intake and weight were measured daily. Escape from antidiuresis was observed over the course of 5 days in groups 1&2; group 3 did not escape antidiuresis. The eNOS KO mice exhibited a near normal pattern of escape from antidiuresis, although the mean urine volume was significantly lower than WT on days 3 & 4. Both the nNOS and atr1a KO mice showed a significantly delayed time course of escape, but they ultimately escaped to the same absolute levels as WT by day 5. Our data demonstrate that each of these signaling molecules likely plays a role in escape from antidiuresis, since no KO mice strain exhibited the same pattern of escape as WT mice. However, each of the KO strains eventually escaped antidiuresis. Consequently, we conclude that eNOS, nNOS, and AngII all participate in the escape mechanism, but none appear to be independently responsible for the phenomenon of renal escape from antidiuresis.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1627

**INSIGHT: Investigation of the Neurocognitive Impact of Sodium Improvement in Geriatric Hyponatremia: Efficacy and Safety of Tolvaptan** Joseph G. Verbalis,[1] Howard S. Ellison,[2] Kianoosh Kaveh,[3] Mary Hobart,[4] John Ouyang,[4] Holly B. Krasa,[4] Frank S. Czerwiec.[4] *[1]Georgetown U., Washington, DC; [2]Rockdale Med. Res. Assoc., Conyers, GA; [3]Coastal Neph. Assoc., Port Charlotte, FL; [4]Otsuka PR&C, Rockville, MD.*

INSIGHT tested tolvaptan effects on neurocognitive function, gait and bone metabolism in elderly patients with sub-clinical hyponatremia.

Fifty-seven subjects ≥50 yrs old with asymptomatic (normal Mini-Mental State Exam), chronic hyponatremia (124-134 mEq/L) were randomized to double-blind treatment (1:1 T=tolvaptan:P=placebo) titrated 15 to 60 mg/d to achieve a target serum [$Na^+$] of 138 mEq/L over 21 d. Mean age was 71yrs in both groups; in each group 30% were taking psycholeptic, and 30% psychoanaleptic drugs.

Serum [$Na^+$] rose from 129 to 136 for T and 130 to 132 for P; +5.15 mEq/L ($p<0.01$), and fell after withdrawal (129 T, 132 P). No overly rapid correction was seen. Cognitive speed, the composite primary endpoint, improved by 0.39 in T and by 0.20 in P (z-score) for an estimated treatment effect of +0.23 ($p=0.08$) at therapy day 21, then fell by -0.17 and -0.05 after withdrawal. The effect was driven by each component (Reaction time +0.20, $p=0.21$; Psychomotor Speed +0.27, $p=0.02$; Processing Speed +0.26, $p=0.21$). Overall Neurocognitive Composite favored T nominally, but not statistically (+0.12, $p=0.16$). The Romberg test improved ($p=0.02$); Postural Sway and the Get-Up-and-Go were divergent, the former favoring P; the later T. Nominally greater serum osteocalcin increases and urine N-telopeptide decreases were seen in T. Correlations with serum [$Na^+$] were significant for Psychomotor Speed; $r=0.319$, $p=0.025$. Thirst, pollakiuria and weight decrease were commonly reported; few (2 T, 1 P) subjects had serious adverse events.

Normal appearing elderly hyponatremic subjects demonstrated improvements in some neurocognitive and gait tests as well as markers of bone metabolism after treatment with tolvaptan. INSIGHT therefore shows that correction of asymptomatic hyponatremia can be safely achieved in elderly adults, supports published data that this can lead to improved neurological functional, and suggests potential reversal of hyponatremia-induced bone loss.

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**F-PO1628**

**Dynamic Changes in Vesicle Movement on the Onset of Hypertonic Challenge** Udo Hasler,[1] Paula Nunes,[1] Richard Bouley,[2] Isabelle Roth,[1] Eric Feraille,[1] Dennis Brown.[2] *[1]Department of Cellular Physiology and Metabolism, University of Geneva, Geneva, Switzerland; [2]Program in Membrane Biology/ Nephrology Division, Harvard University, Boston, MA.*

Cell volume is restored within minutes of hypertonic stimulation via a regulatory volume increase (RVI) mechanism during which the challenged cell undergoes cytoskeletal remodeling and changes in channel/transporter activity. The aim of this study was to investigate changes in vesicle trafficking dynamics that may occur during RVI. We monitored movement of vesicle sub-populations by spinning-disk confocal microscopy performed on live LLC-PK1 cells loaded with FITC-dextran or expressing various fluorescent-tagged proteins (Rab5, 7, 10, 11, ssGFP, AQP2 and V2R). Analysis of movies using ImageJ software of cells exposed to isotonic (290 mOsmol/kg) and then NaCl-hypertonic (500 mOsmol/kg) medium revealed an immediate and dramatic reduction of movement of all vesicle sub-populations upon hypertonic exposure. Movement partly resumed thereafter with different half-maximal recovery times between subpopulations, ranging from 5 min to more than 30 min. In cells loaded with dextran, some vesicles entirely recovered while others remained immobilized during the entire period of analysis (30 min). The effects of hypertonicity was reiterated in two other renal epithelial cell lines (mpkCCDcl4 and mCCDcl1) and in medium in which NaCl was replaced by glucose or mannitol. Confocal microscopy, Western blot and phalloidin fluorescence analysis revealed that hypertonicity induces important effects on actin filament rearrangement that were partly mimicked by jasplakinolide, an actin polymerization agent. Like hypertonicity, jasplakinolide abolished movement of a subset of dextran-loaded vesicles that, unlike hypertonicity, did not recover over time. Recovery of movement of vesicles temporally immobilized by hypertonicity was not affected by jasplakinolide, defining actin remodeling as a factor governing changes in vesicle dynamics by hypertonicity. These observations reveal that hypertonicity 'stalls' vesicle trafficking/sorting events, possibly reflecting cell damage, a situation that must be corrected in order to ensure cell survival.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1629**

**Broad Range Neutral Amino Acid Transporter ($B^0AT1$) Requires Association with Collectrin for Surface Expression in Kidney Cells** Marta Torrente,[1] Lisa Arps,[2] Simone M. R. Camargo,[1] Francois Verrey.[1] *[1]Institute of Physiology and ZIHP, University of Zurich, Zurich, Switzerland; [2]Division of Infectious Diseases, University Hospital Zurich, Zurich, Switzerland.*

The broad range neutral amino acid transporter ($B^0AT1$) is expressed in the brush border of the renal proximal tubules and small intestine and was shown to be crucial for the luminal amino acid uptake and thus for the regulation of amino acid homeostasis. Loss-of-function mutations of $B^0AT1$ result in Hartnup syndrome, an autosomal-recessive inherited disorder characterized by aminoaciduria and, in some cases, pellagra-like rashes, cerebellar ataxia, and psychosis. Recent data indicate that $B^0AT1$ requires the presence of an accessory protein called collectrin for its expression in kidney proximal tubule whereas in small intestine an analogous role is played by angiotensin converting enzyme 2 (ACE2), a protein also expressed in proximal tubules. The mechanism of interaction between collectrin and $B^0AT1$, the stability and the functional role of that interaction, and the reason why collectrin is preferred to ACE2 in kidney cells remain unclear. To address these questions, we utilized Madin-Darby canine kidney (MDCK) cells as expression system. In a first series of experiments, we observed that the constitutive expression of $B^0AT1$ was toxic for the cells. Therefore, using lentiviral transduction and a KRAB repressor system we produced cells that inducibly express either $B^0AT1$ or collectrin or both. Expression of $B^0AT1$ and collectrin was verified at the mRNA and protein levels using RT-PCR and western blot. Immunofluorescence experiments showed that collectrin expressed alone is substantially localised at the luminal cell surface whereas $B^0AT1$ expressed alone remains intracellular. When $B^0AT1$ and collectrin are coexpressed, the quantity of both gene products is decreased but $B^0AT1$ localizes at the apical surface, as does collectrin. In conclusion, we generated cell lines expressing inducible $B^0AT1$ and collectrin in which the surface expression of $B^0AT1$ depends on collectrin coexpression, as in kidney proximal tubule, and thus represent a very good model for testing the role and the mechanism of $B^0AT1$-collectrin interaction.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1630**

**Uremic Toxins Inhibit Transport by Multidrug Resistance Protein 4 and Breast Cancer Resistance Protein** Henricus A. M. Mutsaers,[1,2,3] Lauke Ringens,[1,2] Jeroen Van den Heuvel,[1,2] Lambertus V. Heuvel,[1,4] Joost G. Hoenderop,[1,3] Rosalinde Masereeuw.[1,2] *[1]Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Dept. of Pharmacology & Toxicology; [3]Dept. of Physiology; [4]Dept. of Pediatrics.*

During chronic kidney disease (CKD), there is a progressive accumulation of toxic solutes due to inadequate renal clearance. These uremic toxins can cause a multitude of pathologies, including renal fibrosis, anemia and cardio-vascular disease. The current dialysis strategies are not completely adept to remove uremic toxins, indicating that active transport is required for complete clearance. Therefore, we investigated whether uremic toxins can influence transport activities of two important efflux pumps, *viz.* multidrug resistance protein 4 (MRP4) and breast cancer resistance protein (BCRP). The interaction of several uremic toxins with [$^3$H]methotrexate ([$^3$H]MTX) and [$^3$H]estrone sulfate ([$^3$H]E1S) uptake was studied in membrane vesicles isolated from MRP4- or BCRP-overexpressing human embryonic kidney (HEK293) cells, respectively. Our results show that hippuric acid, indoxyl sulfate and kynurenic acid inhibit MRP4-mediated [$^3$H]MTX uptake (calculated $IC_{50}$ values: 906 μM, 2 mM, 8 μM, respectively) and BCRP-mediated [$^3$H]E1S uptake ($IC_{50}$ values: 1.9 mM, 719 μM and 38 μM, respectively), whereas indole-3-acetic acid and phenylacetic acid only impede [$^3$H]MTX uptake by MRP4 ($IC_{50}$ values: 1.8 mM and 5.8 mM, respectively). In contrast, p-cresol, p-toluenesulfonic acid, putrescine, oxalate and quinolinic acid did not alter transport mediated by MRP4 or BCRP. Furthermore, our results indicate that the tested uremic toxins mostly act as non-competitive inhibitors for MRP4 and BCRP-mediated uptake. Moreover, the $IC_{50}$ values are lower than the maximal concentration of these toxins measured in the plasma of end-stage CKD patients. In conclusion, this study shows that several uremic toxins inhibit active transport by MRP4 (5 out of 10 tested toxins) and BCRP (3/10) at clinically relevant concentrations. Indicating, that uremic toxins hamper kidney function leading to further progression of CKD. Novel strategies should focus on preserving transport activity enabling adequate renal clearance.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1631**

**Inhibitory Effect of Cadmium on Sp1 Binding to *SGLT1* Promoter Is Independent from PKC Activation** Niloofar Tabatabai,[1] Ivan Kamyshko,[2] Rajendra Kishore Kothinti.[2] *[1]Medicine, Medical College of Wisconsin, Milwaukee, WI; [2]Chemistry and Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, WI.*

Cadmium (Cd) exposure causes glucosuria. Glucose reabsorption depends on sodium-glucose cotransporters (SGLTs). Transcription of human SGLT1 gene is regulated by Sp1, whose binding sequence (GC1) is conserved in mouse and pig. Using cultured primary mouse kidney cells, we showed that Cd inhibits sodium (Na)-dependent uptake of glucose, decreases SGLT1 mRNA levels, and inhibits the binding of Sp1 to GC1. Since Sp1 activity can be modulated by phosphorylation, and Cd activates Protein Kinase C (PKC), we examined the role of PKC in inhibition of Sp1 activity by Cd. LLC-$PK_1$ cells were treated either with $CdCl_2$; a PKC agonist, phorbol 12-myristate 13-acetate (PMA); or a specific PKC inhibitor, GF109203X. Glucose uptake was measured using a fluorescent substrate, 2-NBDG. DNA binding activity of nuclear Sp1 was examined by Electrophoretic Mobility Shift Assay (EMSA). To examine PKC activity, expression of c-Fos, which is known to be induced by PKC, was determined by Western blot. To examine mRNA levels of SGLT1, RT and TaqMan real time PCR were performed. Results showed that exposure of cells for 24 hr to 2.5-15 μM Cd decreased Na-dependent uptake of glucose by 22-91%. Compared to controls, SGLT1 mRNA decreased by 70% and c-Fos protein increased by 8 fold in cells exposed to 20 μM Cd for 24 and 2 hours, respectively. Similarly, exposure to 160 nM PMA decreased SGLT1 mRNA by 70% and up-regulated c-Fos expression by 9.5 fold after 6 and 2 hours, respectively. EMSA showed that Cd treatment reduced binding of nuclear Sp1 to GC1 by 40%; however, PMA had no effect on Sp1 activity. GF109203X did not restore Sp1 activity and had no effect on SGLT1 mRNA levels in Cd-treated cells; however, it blocked the inhibitory effect of PMA on SGLT1 mRNA expression. Our results showed that Cd activated PKC, and that both Cd and PMA reduced SGLT1 mRNA levels; only Cd reduced Sp1 binding to GC1, while blockade of PKC activity only protected against the effects of PMA. We conclude that PKC does not play a role in Cd-mediated inhibition of Sp1 binding to *SGLT1* promoter.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1632**

***Drosophila* Prestin Provides an *In Vivo* Model for Oxalate Kidney Stone Formation** Taku Hirata,[1,2] Pablo Cabrero,[3] Julian A. T. Dow,[3] Michael F. Romero.[1,2] *[1]Physiology & Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN; [2]O'Brien Urology Center, Mayo Clinic College of Medicine, Rochester, MN; [3]Biomedical and Life Sciences, University of Glasgow, Glasgow, United Kingdom.*

Kidney stones are major causes of emergency room admissions worldwide. While most stones are of calcium oxalate, our understanding of the convergence of genetic, environmental and metabolic factors that trigger formation, and the dynamics of nucleation and formation, are still patchy. The solute carrier 26 (Slc26) transporters are anion transporters with diverse substrate specificity including $Cl^-$ and oxalate ($ox^{2-}$) exchange. Slc26a6 knockout mice show nephrolithiasis (Ca-oxalate kidney stones). Slc26a6 is expressed in kidney and gut epithelia, playing a central role in secretion and absorption of anions such as chloride, $ox^{2-}$, sulfate ($SO_4^{2-}$), bicarbonate ($HCO_3^-$), The Slc26a6 protein mediates electrogenic $Cl^-/ox^{2-}$, $Cl^-/nHCO_3^-$, $Cl^-/SO_4^{2-}$ exchange. Recently, chicken and zebrafish prestin were shown to mediate electrogenic $Cl^-/ox^{2-}$. We have cloned and localized the *Drosophila melanogaster* Slc26a5 (dPrestin). This dPrestin mRNA is heavily expressed in the gut (adult and larva) and Malpighian tubules ("kidney"). Flies or dissected Malpighian tubules fed oxalate show Ca-oxalate crystal accumulation within hours. *In vivo* dPrestin knockdown reduces crystal formation. Kinetically, dPrestin is similar to mammalian Slc26a6. These results indicate that this system is a *Drosophila* model of kidney stones formation, allowing us study the factors regulating and competing with $ox^{2-}$ transport *in vivo* to understand the genetic influences of kidney stones. Such regulatory genes would be gene candidates for human nephrolithiasis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1633**

**Mechanism of Phosphoenolpyruvate Carboxykinase (PEPCK) mRNA Stabilization during Acidosis** Sachin S. Hajarnis, Lynn Taylor, Norman P. Curthoys. *Biochemistry and Molecular Biology, Colorado State Univ, Fort Collins, CO.*

Onset of metabolic acidosis triggers an essential renal response that includes a pronounced increase in catabolism of plasma glutamine, increased reabsorption and net production of bicarbonate, and increased synthesis and excretion of ammonium ions. A rapid and pronounced increase in PEPCK is a paradigm for this response. This response is effectively modeled in a clonal line of porcine LLC-$PK_1$-$F^+$ cells that exhibit 5-fold increases in PEPCK mRNA and protein when treated for 24 h with acidic medium (pH 6.9, 9 mM $HCO_3^-$). This response is mediated, in part, by a 2-fold increase in the half-life of PEPCK mRNA. HuR is an RNA binding protein that stabilizes mRNAs in response to various stress conditions. siRNA knockdown of HuR decreased basal expression and significantly inhibited the pH-responsive increase in PEPCK protein. Electrophoretic mobility shift assays established that purified recombinant HuR binds with high affinity to multiple sites in the final 92-nucleotides of PEPCK mRNA. Use of deletion and mutated constructs mapped the binding of HuR to a CU-sequence and to two highly conserved AU-rich elements. Segments containing these sites were previously shown to bind recombinant p40-AUF1 and to mediate the rapid turnover of PEPCK mRNA. Selective enrichment of phosphoproteins indicated that treatment with acidic medium results in increased phosphorylation of HuR and AUF1. The pH-responsive stabilization of PEPCK mRNA is reproduced in a chimeric β-globin mRNA that contains the 3'-UTR of PEPCK mRNA. Mutation of a single conserved AU-rich element within the chimeric mRNA resulted in a 2.3-fold increase in half-life and loss of pH-responsive stabilization. Finally, pH-responsive stabilization of the chimeric mRNA is inhibited by SB203580, an inhibitor of p38 MAPK, but not by U0126, an ERK1/2 inhibitor. Therefore, activation of the p38 MAPK pathway may mediate the covalent modification and remodeling of proteins that bind to the pH-response element. This mechanism may stabilize multiple mRNAs and contribute to the pronounced remodeling of the proteome of the renal proximal tubule that occurs during metabolic acidosis.

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**F-PO1634**

**The Diuretic Effect of a Small Molecule Inhibitor of CLC-K1 in Rats** Paru P. Kathpalia,[1] Andrew E. Howery,[2] Lise Bankir,[3] Justin Du Bois,[2] Merritt Maduke,[4] Alan C. Pao.[1] *[1]Division of Nephrology, Stanford University School of Medicine, Palo Alto, CA; [2]Department of Chemistry, Stanford University, Palo Alto, CA; [3]INSERM UMRS 872, Paris, France; [4]Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Palo Alto, CA.*

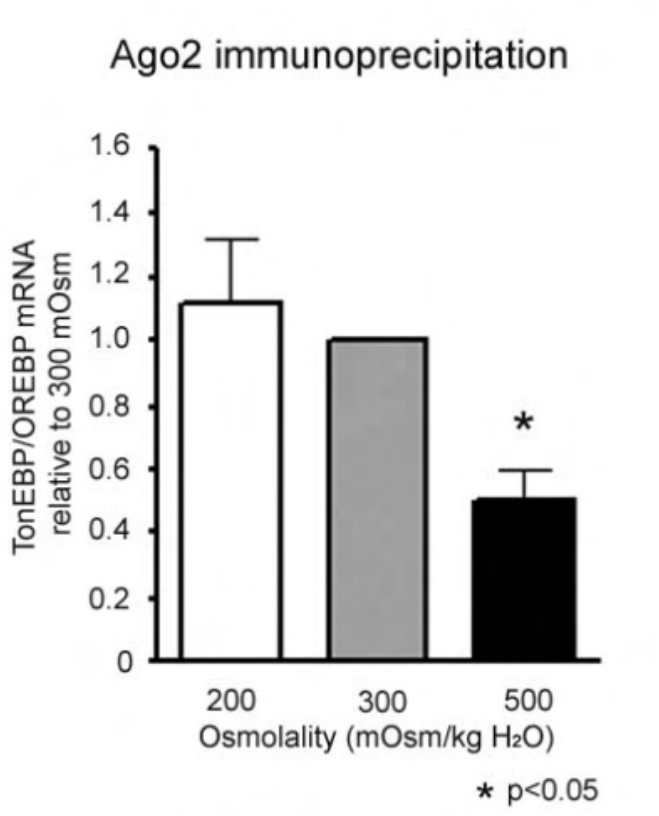
Hyponatremia is the most common electrolyte disorder in the United States and contributes to significant patient morbidity and mortality. Disruption of the renal medullary concentrating gradient by blocking the activity of the chloride channel CLC-Ka (CLC-K1 in rodents) in the thin ascending limb of the loop of Henle represents one strategy to promote aquaresis and treat hyponatremia. We have developed a second-generation small molecule inhibitor, 4,4'-octanamidostilbene-2,2'-disulfonate (OADS), that can specifically inhibit CLC-Ka transport activity. *Xenopus laevis* oocytes expressing CLC-Ka, CLC-Ka-N68D, or CLC-0 were treated with various concentrations of OADS, and chloride currents were measured by two-electrode voltage clamp technique. OADS inhibited CLC-Ka with an apparent affinity of 2 μM and exhibited no measurable effect on the skeletal muscle homolog CLC-0. The affinity of OADS for CLC-Ka decreased by five-fold when an N68D mutation was introduced, suggesting that OADS has substantially less affinity for CLC-Kb. Based upon these findings, we hypothesized that OADS, by specifically blocking CLC-K1 in the kidney, would enhance excretion of water in the whole animal. In conscious rats, intraperitoneal injection of OADS induced a three- to five-fold increase in urine output, with an associated fall in urine osmolality, within the first 4 hours of administration. These findings suggest that OADS may increase water excretion by inhibiting CLC-K1 in the thin ascending limb of Henle's loop and indicate that CLC-Ka/1 may be a new potential drug target for the treatment of hyponatremia.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1635**

**Regulation of TonEBP/OREBP Expression through Ago2 Mediated microRNAs** Yuichiro Izumi, Morgan Gallazzini, Joan D. Ferraris, Maurice B. Burg. *LKEM/NHLBI, National Institutes of Health, Bethesda, MD.*

The osmoprotective transcription factor, TonEBP/OREBP, is important for cell survival under hypertonic conditions, such as exist in the kidney medulla. Hypertonicity is known to increase TonEBP/OREBP mRNA and protein abundance, reaching a peak after several hours, followed by decrease to a lower steady level. The regulatory mechanism of the acute increase is not completely understood. Since the 3'-UTR of TonEBP/OREBP mRNA contains many predicted microRNA binding sites, we investigated the possible involvement of Argonaute 2 (Ago2) mediated microRNA activity on TonEBP/OREBP expression in HEK293 cells. We found that siRNA-mediated knockdown of Ago2 increases TonEBP/OREBP protein abundance. We measured mRNAs that co-immunoprecipitate with HA-tagged-Ago2 (HA-Ago2) by RT-PCR. TonEBP/OREBP mRNA is enriched in the Ago2 immunoprecipitate. As a control, betaine/GABA (BGT1) mRNA, which has few predicted microRNA sites in its 3'-UTR, is not detected in the Ago2 immunoprecipitate. Acutely (1.5 hours) raising osmolality from 300 to 500 mosmol/kg by increasing NaCl decreases the amount of TonEBP/OREBP mRNA in the Ago2 immunoprecipitate, without any change in the total amount of RNA in the immunoprecipitate.




After 24 hours of high NaCl, TonEBP/OREBP mRNA is no longer lower in the Ago2 immunoprecipitate. We suggest that reduced association of TonEBP/OREBP mRNA with the Ago2 microRNA processing complex contributes to the acute high NaCl-induced increase of TonEBP/OREBP expression, resulting in accelerated adaptation to hypertonicity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1636**

**Cultured Human Glomerular Endothelial Cells Display ACE-Mediated Angiotensin-II-Generating Capacity and Limited Angiotensin-II-Degrading Activity** Juan Carlos Q. Velez,[1,2] Michael G. Janech,[1,2] John M. Arthur,[1,2] John R. Raymond.[1,2] *[1]Department of Medicine, Medical University of South Carolina, Charleston, SC; [2]Medical and Research Services, Ralph H. Johnson VA Medical Center, Charleston, SC.*

The intraglomerular renin-angiotensin system (RAS) is linked to the pathogenesis of progressive glomerular diseases. We previously showed that podocytes and mesangial cells play distinct roles in the metabolism of angiotensin (Ang) peptides. However, little is known about the corresponding role of glomerular endothelial cells (GECs). Thus, we explored the RAS enzymatic profile of human GECs (hGECs) in culture. Identity of hGECs was confirmed by expression of platelet/endothelial cell adhesion molecule by immunoblotting. Cells were grown to confluence and serum-starved overnight. Then, cells were exposed to 1 μM Ang-I or Ang-II for up to 8 hours. Cell media samples were collected at various time points and subjected to MALDI-TOF mass spectrometry for Ang peptide analysis. $_{13}C_{,15}N$-valine labeled peptides were used for quantification. During incubation of Ang-I, Ang-II was the most abundant fragment, with lesser amount of Ang-(1-7) detected. Peak for Ang-(2-10) was near the lower limit of detection. When cells were incubated in the presence of captopril (100 μM), Ang-II formation was almost completely abolished (137.8 ± 6.1 nM vs. 5.5 ± 0.5 nM of Ang-II at 4 hours, without and with captopril, respectively; $p<0.001$), suggesting that Ang-II generation is ACE-mediated. The overall rate of disappearance of Ang-I when exposed to hGECs was similar to that observed under exposure to human podocytes (hPODs). The difference resided on the predominant fragments generated by each cell type, i.e., Ang-II for hGECs, and Ang-(2-10) and Ang-(1-7) for hPODs. On the other hand, hGECs metabolized Ang-II at a significantly slower rate compared to hPODs (0.63 vs. 2.20 pmol Ang-II/μg cell protein/hr, respectively; $p<0.001$). These results indicate that hGECs possess prominent ACE activity and limited Ang-II metabolizing activity, in clear contrast to the RAS enzymatic capacity of hPODs. Injury to specific cell types within the glomeruli may lead to distinct effects on the intrarenal RAS balance.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1637**

**Neutrophil Serine Proteases PR3 and Elastase Interact with Glomerular Endothelial Cells Causing PAR-1 Cleavage without Direct PAR Activation**
Samantha Tull,[1] Anne Bevins,[1] Sahithi Josna Panchagnula,[1] Simon C. Satchell,[3] Bahjat Al-Ani,[1] Lorraine Harper,[1] Julie M. Williams,[1] Edward Rainger,[2] Caroline O. S. Savage.[1] *[1]Immunity and Infection; [2]Clinical and Experimental Medicine, University of Birmingham, United Kingdom; [3]University of Bristol, United Kingdom.*

**INTRODUCTION:** Neutrophil proteases, proteinase 3 (PR3) and elastase (HLE) appear to have key roles in the glomerular endothelial cell (GEC) injury/activation that occurs during glomerulonephritis (GN). However, the mechanism by which proteases interact with EC remains unclear. Protease activated receptors (PARs) are activated by specific cleavage and are potential targets of PR3 and HLE. In in-vivo models of GN, both PAR-1 and PAR-2 deficient mice showed reduced crescent formation. **AIM:** To determine if PR3 or HLE, via PARs, caused the alterations in EC phenotype, observed in GN. **METHODS:** EC PAR protein levels were assessed by FACS. Thrombin, trypsin and agonist peptides (ap) for PAR-1 (TFLLR) and PAR-2 (SLIGKV) assessed EC PAR activation. vWF release from viable EC was used as a marker of EC activation. Calcium signalling was detected by fluorimetry and fluorescent microscopy. **RESULTS:** GEC and Human Umbilical Vein ECs (HUVEC) had similar patterns of PAR-1 and PAR-2 expression. PAR-2 mRNA was up-regulated by TNFα while PAR-1 mRNA was largely unaffected. Functional PAR-1&2 were expressed on the EC surface (n=3). Serine proteases caused (time and dose dependent) PAR-1 cleavage, but not receptor activation (n=3). In viable EC, PAR-1-ap induced EC vWF release to the same extent as PR3, after 2hr exposure to either stimuli (n=4). SiRNA knockdown showed that despite the similar effects of PR3 and PAR-1ap on EC vWF release, neither PAR-1 nor PAR-2 activation caused PR3 or HLE-mediated vWF release. GEC calcium signalling indicated that both proteases interacted with and disarmed surface PAR-1, but not PAR-2 receptors. **CONCLUSION:** This study indicates the complex interactions between PAR-1 and proteases. PAR activation and serine protease-induced alterations in EC function may both modulate the inflammation associated with GN; although our data suggests they may act through parallel but independent pathways.

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**F-PO1638**

**Proteomic Mapping of Endothelial Cell Surface Proteins in the Kidney and Glomerulus**
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Endothelial cell (EC) membrane is critically important for their functions, but the characteristics of endothelial cell membrane shave have not well examined in the kidney and glomerulus yet. Our aim was to elucidate protein components of EC membrane proteins of the kidney and glomerulus. In the present study we isolated these membrane fractions and analysed them by mass spectrometry (MS). To isolate rat renal EC membrane specifically, the cationized colloidal silica beads coating method was applied, which is based upon the ionic interaction of cationic colloidal silica with negatively charged EC membranes. Specific labeling of ECs was revealed by light microscopy and transmission electron microscopy. Glomeruli were also isolated for glomerular EC membrane preparation. By homogenization and density ultracentrifugation the membranes with beads were enriched and the enrichment of membranes was confirmed by Western blot analysis using antibody against EC-specific protein caveolin 1. Endothelial membrane proteins from whole kidneys and glomeruli were digested with trypsin and analyzed by MS, respectively. We identified various types of EC proteins and found some difference proteins betweens EC membrane proteins of whole kidneys and glomeruli. We concluded that ECs membranes were isolated from whole kidneys and glomeruli directly, and that mapping of the microvascular EC surface proteome was provided for further understanding of kidney EC membrane pathology and physiology and difference in characteristics of ECs between the kidney and glomerulus.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1639**

**Novel Proteins in Nephrotic Syndrome: Circulating Angiopoietin-Like 4 (Angptl4) Reduces Proteinuria While Inducing Hypertriglyceridemia in Nephrotic Syndrome**
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While investigating a role of podocyte secreted Angptl4 in minimal change disease (MCD) (Clement *et al*, Nature Medicine, in revision), we noted increased circulating Angptl4 levels in human MCD and membranous nephropathy, and in rats with experimental MCD (puromycin nephrosis, PAN). To investigate specific biological effects of circulating Angptl4 in nephrotic syndrome, adipose tissue specific aP2-Angptl4 TG rats were developed. In addition to selective overexpression from the adipose tissue, these rats develop elevated circulating levels of Angptl4 oligomers, but do not develop proteinuria or changes in glomerular morphology. In fact, 18 hours urine albumin excretion was reduced in aP2-Angptl4 TG rats compared to wild type littermates (WT male 367±47 μg/18 hours, TG male 113±12 μg/18 hours;P<0.001). Following induction of PAN, TG rats developed less proteinuria compared to WT littermates (WT 220±30 mg/18 hours, TG 80±25 mg/18 hours, P<0.05). Since Angptl4 is a known inhibitor of lipoprotein lipase (LPL), we assessed plasma triglyceride levels and post-heparin LPL activity. TG rats had higher plasma triglyceride levels and lower LPL activity compared to wild type rats (P<0.05 for both), and these effects were exaggerated following induction of PAN (P<0.01). A longitudinal multiorgan survey in PAN for Angptl4 expression revealed that upregulation of Angptl4 was confined to glomeruli in the early stages (Day 6), but also increased in adipose tissue in later stages (Day 10 onwards) during decline of proteinuria. Using anti-V5 antibodies, we demonstrated binding of Angptl4-V5 secreted by the transgene to glomerular endothelium. Also, recombinant Angptl4 protected cultured glomerular endothelial cells from oxidative injury. In summary, circulating Angptl4 secreted from podocytes and adipose tissue in nephrotic syndrome induces hypertriglyceridemia by binding endothelial LPL, while reducing proteinuria via protective effects on the glomerular endothelium.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1640**

**Severe Glomerular Pathology in Mice Lacking Glomerular Endothelium-Enriched Endocytic Regulator *Ehd3* and the Related Gene *Ehd4***
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Eps15 Homology Domain-containing protein 3 (EHD3), a member of an endocytic recycling regulator family, is highly expressed in glomerular endothelium, but its physiological role is unknown.

We generated *Ehd3* knockout mice that completely lack EHD3. *Ehd3*$^{-/-}$ mice developed normally, were healthy and did not develop proteinuria. Light and electron microscopy showed unaltered glomeruli, and immunofluorescence analyses revealed normal expression/localization of glomerular proteins. Strikingly, *Ehd3*$^{-/-}$ glomerular endothelial cells showed increased levels of EHD4 (an EHD protein normally present in the peritubular vasculature).

We, therefore, generated *Ehd3* and *Ehd4* double-null mice and confirmed the lack of expression of both genes in the kidney. *Ehd3*$^{-/-}$*; Ehd4*$^{-/-}$ mice were small at wean, developed proteinuria and died between 1-7 months of age. Analyses of H&E, PAS, and Jones silver-stained *Ehd3*$^{-/-}$*; Ehd4*$^{-/-}$ kidney sections revealed glomerulomegaly and changes consistent with ongoing endothelial injury, such as duplication of basement membranes, mesangial cell interposition, and RBC fragmentation. Tubular changes included luminal dilatation and protein re-absorption droplets. Abnormal patterns of synaptopodin, nephrin and desmin expression were seen by immunofluorescence. Ultrastructural studies revealed subendothelial widening, loss of glomerular endothelial fenestrations and segmental podocyte foot process effacement.

Our results suggest that EHD3 and EHD4 are critical for glomerular endothelial health. These significant findings are the first report in which deletion of genes expressed in glomerular endothelium results in glomerular endothelial dysfunction and also induces downstream detrimental changes that affect the integrity of the glomerulus as a whole.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1641**

**Factor Responsible for Hematuria in IgA Nephropathy**
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**Introduction** Some patients with IgA nephropathy(IgAN) manifest macroscopic hematuria after tonsillectomy(TXL). The aim of our study is to analyze the mechanism of temporal aggravation of hematuria after TXL. **Methods** The subject of our study is 16 IgAN patients who underwent TXL combined with steroid pulse as a treatment and who gave informed consent. Control patients (n=9) were chronic tonsillitis or sleep apnea patients who underwent TXL. A series of urine samples, obtained from pre-TXL until the next morning, were analyzed by dipstick test. Urinary cytokines such as tumor necrosis factor-α, granulocyte-macrophage colony-stimulating factor(GM-CSF), monocyte chemoattractant protein-1, interleukin-6, macrophage inflammatory protein-1β and interferone-γ were analyzed. We also analyzed cell markers (CD25&CD3, CD8&CD5, CD38&CD20, CD16&CD56, CD45RA&CD4, HLA-DR&CD4 and CD11b&CD8) of peripheral blood before TXL, immediately after TLX, one week after TXL, and after steroid pulse, with flow cytometer(FCM). Next, $CD16^+CD56^+$ cells as well as $CD16^-CD56^+$ and $CD56^-$ cells were sorted from the peripheral blood of patients with IgAN using MACS. Equal number of each cell fraction was transplanted to each nude rat via tail vein and the urine was analyzed with dipstick test. **Results** The aggravation or occurrence of hematuria was temporally seen after TXL in patients with IgAN, but not in control. FCM analysis of the peripheral blood of patients with IgAN indicated $CD16^+CD56^+$ cells, which contain natural killer cells and natural killer T cells, tended to increase immediately after TXL and returned to the basal level in one week, unlike in control. Urinary cytokine profiling revealed that GM-CSF, cytokine secreted from natural killer cells, peaked in the second or third urine sample after TXL. When we transplanted $CD16^+CD56^+$ cells to nude rats, the recipients manifested strong and consistent hematuria, unlike $CD16^-CD56^+$ or $CD56^-$ cells. **Conclusions** $CD16^+CD56^+$ cells in the peripheral blood are the hematuria-inducing factor of IgAN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1642**

**Genetically Determined Regulation of the P2X7 Receptor-Inflammasome Pathway in Macrophages of Glomerulonephritis-Susceptible Rat Strains**
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The P2X7 receptor (P2RX7) is a ligand-gated cation channel that is expressed by a variety of immune cells, including macrophages and lymphocytes. Our previous studies showed that P2X7 receptor antagonists reduce glomerular macrophage infiltration in nephrotoxic nephritis (NTN) in Wistar Kyoto (WKY) rats. We therefore hypothesized that P2RX7-Nlrp3 inflammasome-mediated caspase-1 activation is altered in WKY bone marrow derived macrophages (BMDMs).

We found that P2RX7 mRNA levels were markedly up-regulated in the WKY BMDMs when compared with NTN-resistant Lewis BMDMs. We also observed that the majority of inflammasome genes in WKY rats are up-regulated in both basal and LPS-stimulated macrophages when compared with NTN-resistant Lewis rats. In addition, the expression of IL-18 and IL-33, two cytokines that depend on caspase-1 activation and processing, are markedly increased in LPS-stimulated WKY BMDMs. Interestingly, none of the up-regulated inflammasome genes were controlled by Crgn1 and/or Crgn2, two major NTN QTLs for glomerular crescent formation and proteinuria previously identified in this model.

We then analyzed the secreted IL-1beta levels following BzATP and LL37 (P2X7 agonists) stimulation in LPS-stimulated BMDMs by sandwich ELISA. In both conditions, WKY BMDMs showed increased levels of IL1-beta when compared with Lewis BMDMs, suggesting that P2RX7-mediated IL1-beta secretion is dysregulated in WKY BMDMs, which is likely to be due to up-regulated inflammasome genes. Genetic studies aimed at characterizing the master-regulator controlling the P2X7 receptor-inflammasome pathway are underway, which should help to define this pathway in macrophage-mediated crescentic glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1643**

**Association of Anti-PLA2R IgG Subclasses with Clinical Remission and Relapse in Primary Membranous Nephropathy**
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Background: Our laboratory has recently shown a direct association between levels of circulating antibodies to the phospholipase A2 receptor (PLA2R) and clinical activity in primary membranous nephropathy (MN). The goal of this study was to examine serial levels of anti-PLA2R IgG subclasses from 3 patients in whom clinical remission was followed by subsequent relapse.

Methods: Serum samples and 24-hr proteinuria data were available at 25-30 time points over the course of 3-7 years from three patients with primary MN. Each patient had entered a clinical remission after immunosuppressive therapy, but had subsequently relapsed. Retrospective semi-quantitative analysis of anti-PLA2R levels was performed by immunoblotting native glomerular PLA2R with serum (1:25) followed by antibodies specific for human IgG1, IgG3 or IgG4. Band intensity was quantitated by densitometry.

Results: All three patients were initially positive for anti-PLA2R, but exhibited differences in the levels of anti-PLA2R IgG subclasses during the course of their disease. Patient 1 had a complete clinical remission that followed the disappearance of anti-PLA2R. Re-emergence of IgG1 and IgG3 anti-PLA2R, followed closely by IgG4 anti-PLA2R, preceded the relapse of nephrotic syndrome by months. Patient 2 similarly showed a decline in proteinuria following the disappearance of anti-PLA2R; however IgG1, IgG3, and IgG4 anti-PLA2R all appeared concurrently or slightly following the relapse of proteinuria. Patient 3 exhibited IgG4 anti-PLA2R that persisted at all time points. In contrast, the IgG1 and IgG3 anti-PLA2R subclasses more closely mirrored the changes in proteinuria.

Conclusion: Measurement of anti-PLA2R IgG subclasses provides useful information for monitoring disease course and relapse. IgG1 or IgG3 anti-PLA2R may be better associated with proteinuria than IgG4 anti-PLA2R, but the temporal relationship between the initial re-appearance of anti-PLA2R and the return of proteinuria is variable.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1644**

**MK-2 Inhibition Reduces Albumin-Induced Podocyte Endocytosis and COX-2 Expression**
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Damage to the glomerular filtration barrier results in the passage of serum proteins into the urinary space and increased exposure of podocytes and tubular cells to albumin and IgG, etc. Normally most albumin that passes through the filtration barrier is reabsorbed by the proximal tubules via receptor-mediated endocytosis. However, it has recently been reported that podocytes express the transport receptors FcRn and megalin, and can endocytose albumin and IgG. When exposed to albumin overload, podocytes undergo apoptosis through TGF-β1/p38 MAPK pathways. Additionally, increased COX-2 expression levels enhance susceptibility of podocytes to injury. While COX-2 induction is known to be regulated by p38 MAPK through the transcription factors C/EBP and CREB/ATF, the involvement of MAPKAPK-2 (MK-2), a kinase directly downstream of p38 MAPK, is unclear.

We hypothesized that targeted inhibition of MK-2 in podocytes will decrease albumin-induced cell injury via reduced albumin endocytosis and/or lessened COX-2 expression.

Using conditionally immortalized cultured murine podocytes, we analyzed whether normal serum concentrations of albumin stimulate the p38 MAPK/MK-2 pathway and induce COX-2 expression, and whether the novel MK-2 inhibitor, C23, reduces pathway activation, decreases albumin uptake, or inhibits COX-2 expression.

We found that podocytes endocytosed albumin and that normal serum concentrations of albumin stimulated the p38 MAPK/MK-2 pathway, as measured by both activation of p38 and MK-2, and phosphorylation of MK-2's primary downstream target hspb1 (hsp25). These concentrations of albumin also induced COX-2 mRNA expression. Of greater interest, we found that specific MK-2 inhibition reduced both podocyte albumin uptake and COX-2 mRNA levels.

We conclude that podocyte exposure to serum concentrations of albumin may contribute to cellular injury via stimulation of MK-2 and/or COX-2. Thus targeted MK-2 inhibition may be a promising therapeutic approach to reduce podocyte injury in many glomerular diseases.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1645**

**CD28 Superagonist mAb (JJ316) Ameliorates the Induction of Experimental Autoimmune Glomerulonephritis (EAG) in Rats**
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We have previously shown that EAG in Wistar Kyoto (WKY) rats can be induced by immunization with a 13 amino acid nephritogenic peptide (P13) homologous to the alpha3(IV)NC1 domain resulting in severe glomerulonephritis, immune cell infiltration and proteinuria. A specific T-cell response results in glomerular basement membrane (GBM) damage with epitope spreading and amplification of the disease. Superagonistic anti CD28 mAb (JJ316) induced upregulation of (CD4+CD25+FoxP3+,Tregs) has been reported to prevent or treat several experimentally induced or genetically predetermined autoimmune diseases.

In the present study we examined the protective capacity of JJ316 in prevention of EAG. Two sets of WKY rats (n=15/set) were immunized with P13 in CFAh37RA at day 0. One of the two sets also received JJ316 (tail vein iv) (1mg/dose) on -6,-3, 0 & +3 days. 24 hr urine protein was followed over a course of 8 weeks as the marker for development of EAG. Post experimental kidneys from all the animals were processed for H&E sections and immunofluorescence staining (IgG and fibrinogen) to score the disease severity. Rats in JJ316 group demonstrated overall protection from EAG development over the course of experiment as monitored by 24 hr urine protein and histology. FACS analysis of JJ316 set had a higher percentage of Tregs at +3 day time point compared to control. Histology group average scores (IgG, fibrinogen and H&E) were also significantly lower in the JJ316 rat set. JJ316 group data sets were statistically closer to the age matched control set.

These findings show that in the EAG model JJ316 acts as a protective agent if given prior to disease initiation. JJ316 is probably acting by up regulating Tregs to ameliorate the autoimmune process. Similar strategies might benefit human autoimmune glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1646**

**A Novel Transgenic Mouse Indicates a Role for Toll-Like Receptor Signaling in Type 1 Membranoproliferative Glomerulonephritis**
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Type I Membranoproliferative glomerulonephritis (MPGN) is mediated by subendothelial and mesangial immune complex deposition. The idiopathic form predominates in children, while secondary forms associated with autoimmune diseases, monoclonal gammopathy, and chronic infections occur most commonly in adults. Pathogenesis of MPGN is poorly understood and effective treatment is unproven. Development of an animal model of MPGN would provide an important tool for investigating pathogenesis and treatment. We developed a transgenic mouse that expressed a mutated form of ABIN1 (D485N) lacking the inhibitory function for NF-KB activation. The resulting molecular phenotype of these animals was over-activation of NF-KB. These mice demonstrated enlarged spleens, lymph nodes, and Peyer's Patches; B-cell proliferation leading to enhanced immunoglobulin production; and enhanced cytokine production. Light microscopy of kidney sections from 4 and 6 month old mice showed lobular glomeruli with focal areas of mesangial hypercellularity and matrix expansion, narrowing of the capillary lumens, and diffuse thickening and double contour appearance of basement membranes. The glomerular changes were associated with extensive interstitial cellular infiltration. Electron microscopy showed discrete electron dense deposits in the mesangium and subendothelial space, and immunohistochemistry showed that these deposits contained complement C3. Serum levels of Cystatin C were elevated in the transgenic mice, compared with wild type mice. Crossing ABIN1 (D485N) mice with MyD88 deficient (toll-like receptor (TLR) non-responsive) mice suppressed lymphoproliferation and renal histologic abnormalities. We conclude that chronic TLR

activation produces a model of progressive glomerulonephritis with histologic features of human type 1 MPGN. This model will advance our understanding of the molecular and cellular mechanisms of MPGN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1647**

**Dysregulated Glomerular Stem/Progenitor Cells Participate in Crescent Formation in Munich Wistar Fromter (MWF) Rats, ACEi Modulates Stem Cell Activation and Preserves the Niche** Paola Rizzo,[1] Marina Morigi,[1] Elena Gagliardini,[1] Cinzia Rota,[1] Giuseppe Remuzzi,[1,2] Ariela Benigni.[1] *[1]Mario Negri Institute for Pharmacological Research, Bergamo, Italy; [2]Ospedali Riuniti, Bergamo, Italy.*

MWF rats develop progressive glomerular injury which culminates in glomerulosclerosis. We previously reported that ACEi renoprotection in this model is associated with more parietal podocytes and better preserved glomerular architecture. Here, we studied the time course of the lesions, their cellular components and the effect of the ACEi. Early lesions were glomerular to capsule adhesion - synechiae - (10-25w) followed by extensive extracapillary crescent formation (25-60w). Glomerulosclerosis developed at later stage starting from 40w. Large majority of cells forming synechiae and crescents were claudin+ WT1- parietal epithelial cells (PECs, 60%) and to a lesser extent claudin- WT1+ podocytes (28%). Both PECs and podocytes were in a proliferating state (BrdU+). We next analyzed the cell composition of the Bowman's capsule in control Wistar rats. Ninety % claudin+ PECs expressed NCAM, a marker of metanephric mesenchime that we describe here for the first time in adulthood, suggesting the stem/progenitor nature of this population. In MWF rats, NCAM+ cells were extensively found in synechiae and crescents. ACEi reduced crescents by at least 40% and fully prevented glomerulosclerosis. ACEi also reduced the proliferation of stem/progenitors and restored the ratio between PECs/parietal podocytes in Bowman's capsule. PECs of MWF rats (50-60w) had a remarkable reduction of cEBPδ -a transcription factor known to maintain stem/progenitor cells in a quiescent state- which was normalized by ACEi. When NCAM+ PECs were isolated and cultured from glomeruli of control rats, cEBPδ was expressed at high levels. Angiotensin II ($10^{-7}$ M) reduced the expression of cEBPδ. Thus, chaotic migration and proliferation of stem/progenitor cells of the rat Bowman's capsule pave the way to crescent formation and subsequent sclerosis. ACEi, by moderating stem/progenitor activation, restores the niche, ameliorates glomerular architecture and prevents renal disease progression.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1648**

**Podocyte Dedifferentiation and Autophagy Are Governed by Tissue Transglutaminase That Is Critical to Glomerular Injury in Rapidly Progressive Glomerulonephritis** Cécile Fligny,[1] Marine Milon,[1] Sandra Schordan,[2] Laurent Mesnard,[3] Patrick Bruneval,[4] Pierre-Louis F. Tharaux.[1] *[1]Paris-Cardiovascular Research Center - PARCC, Inserm and Université Paris-Descartes, Paris, France; [2]Institut für Anatomie und Zellbiologie, Ernst-Moritz-Arndt-Universität Greifswald, Greifswald, Germany; [3]UMR 702, Inserm, Paris, France; [4]Service d'Anatomie Pathologique, Hôpital Europeen Georges Pompidou, Assistance Publique-Hôpitaux de Paris, Paris, France.*

We discovered marked early expression of TG2 in podocytes from mice and patients with crescentic rapidly progressive glomerulonephritis (RPGN). Thus, we investigated the role of the TG2 in experimental RPGN, in which immune-mediated endothelial stress leads to podocytes injury. First, outgrowth of podocytes from isolated glomeruli from Podocin-Cre x TG2lox/lox mice revealed that TG2 inactivation specifically in podocytes leads to a strong inhibition of their proliferation. Increased accumulation of LC3 and p62 positive autophagosomes in TG2-deficient podocytes was observed. In the anti-GBM model of RPGN, TG2 invalidation markedly attenuated glomerular injury. Albuminuria to creatinuria ratio was 25-fold lower in TG2 KO mice at day 11 ($0 \pm 0\%$ vs. $21.9 \pm 7.4$, $p<0.001$) that displayed a complete absence of crescent formation ($0 \pm 0\%$ of the glomeruli at day 4 and 30 vs. $25 \pm 6\%$ at day 4 and $45 \pm 6\%$ at day 30, $p<0.001$). Accordingly, TG2 deficiency prevented the development of renal railure with BUN within normal ranges at day 30 ($39.0 \pm 1.6$ mg/dl vs. $76.7 \pm 23.5$ mg/dl, $p<0.001$). TEM analysis of podocytes demonstrated unaffected podocyte ultrastructure in TG2 KO at day 4, compared to TG2 WT mice which already displayed mild to severe foot process effacement.

At last, specific TG2 gene deletion in Podocin-Cre x TG2lox/lox mice also caused significant protection against the development of the RPGN.

We conclude that TG2 in podocytes is critical to RPGN, in part through promotion of proliferation and autophagy. This study suggests that targeting the TG2 pathway would be clinically beneficial for treatment of severe RPGNs.±

Disclosure of Financial Relationships: nothing to disclose

**F-PO1649**

**Ubiquitin C-Terminal Hydrolase-L1 (UCH-L1) Activity Induces Polyubiquitin Accumulation in Podocytes and Increases Proteinuria in Rat Membranous Nephropathy** Catherine Meyer-Schwesinger, Tobias N. Meyer, Elion Hoxha, Friedrich Thaiss, Rolf A. Stahl. *Department of Internal Medicine, Nephrology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.*

UCH-L1 is a key protease in the ubiquitin-proteasome pathway that regulates the intracellular pool of monoubiquitin and is associated with neurodegenerative diseases and cancer. Recently, we described the de novo expression of UCH-L1 in podocytes of patients with membranous nephropathy where UCH-L1 expression correlated with increased ubiquitin content. Here, we investigated the role of UCH-L1 for the ubiquitin homeostasis and proteasomal degradation in a rat model of membranous nephropathy. After disease induction UCH-L1 expression increased in podocytes and coincided with decreased glomerular monoubiquitin content. Following an initial increase of proteasomal activity, the ubiquitin proteasome system (UPS) was impaired. UPS impairment was characterized by decreased proteasomal activity and increased polyubiquitin content in glomerular lysates. Besides an increase of ubiquitin in podocytes, aggregates were observed 1 year after disease induction, as in human membranous nephropathy. Inhibition of UCH-L1 hydrolase function with LDN57444 in membranous nephropathy reduced UPS impairment. Strikingly, proteinuria was also ameliorated by UCH-L1 inhibition. In contrast, inhibition of proteasomal activity with MG132 enhanced UPS impairment resulting in further polyubiquitin accumulation, ubiquitin aggregation in podocytes and increased proteinuria. In additional in vitro experiments, UCH-L1 over-expression resulted in the accumulation of mono- and polyubiquitinated proteins in cultured podocytes. In contrast, stable knock-down of UCH-L1 reduced mono- and polyubiquitinated proteins and significantly increased proteasomal activity, indicating that the observed effects in rat membranous nephropathy also occurred in cultured podocytes. These data show that ubiquitin C-terminal hydrolase L1 activity results in polyubiquitin accumulation, proteasome inhibition and disease aggravation in experimental membranous nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1650**

**Evaluation of Notch Signaling in Rodent Models of HIV-Associated Nephropathy (HIVAN) and in Human HIVAN Patients** Madhulika Sharma,[1] Pravin C. Singhal,[2] Paul E. Klotman,[3] Shilpa Buch,[4] Gregory B. Vanden Heuvel.[1] *[1]Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS; [2]Medicine, North Shore LIJ Health System, Great Neck, NY; [3]Medicine, Mount Sinai School of Medicine, New York, NY; [4]Pharmacology, University of Nebraska, Omaha, NE.*

Notch signaling has emerged as an important pathogenic pathway in a wide range of glomerular diseases. Human Immunodeficiency Virus Associated Nephropathy (HIVAN) presents with sclerosing glomeruli. To determine the role of the Notch pathway in HIVAN, we evaluated Notch signaling in two rodent models of HIVAN and in human HIVAN patients. Using an HIV transgenic rat model (HIV Tg) that expresses seven of the nine HIV genes, we found increased Notch1, Notch3, and Notch4 activation in the glomeruli associated with increased cell proliferation and podocyte dedifferentiation. Using an HIV transgenic mouse model (Tg26) we found an increase in the intracellular domains of Notch1 and Notch4 in the glomeruli, indicating Notch signaling activation. To determine whether Notch signaling was elevated in the podocytes, we evaluated the expression of Notch signaling members in podocyte cell lines obtained from Tg26 mice or control mice. We observed increased expression of the Notch effector protein Hes1 in the Tg26 podocyte mouse line, compared with the expression in wild type cells. These results demonstrate activation of the Notch pathway in the Tg26 mouse line. Finally, we evaluated Notch signaling in renal biopsy samples from HIVAN patients. We found increased levels of the cleaved Notch1, Notch3, and Notch4 in the glomeruli, compared with the expression in normal kidneys. Taken together, our results suggest that activation of the Notch pathway in podocytes contributes to the pathogenesis of HIVAN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1651**

**Elevated Circulating Levels of IGFALS Induce Classic Molecular and Morphologic Changes in Human Non-HIV Collapsing Glomerulopathy** Camille E. Mace,[1] Lionel C. Clement,[1] Hilal Arnouk,[1] Elizabeth Soria-Castro,[2] Maria Carmen Avila-Casado,[2] Sumant S. Chugh.[1] *[1]Medicine, University of Alabama at Birmingham, Birmingham, AL; [2]Pathology, Instituto Nacional de Cardiologia, Mexico City, Mexico.*

Injection of sera from patients with non-HIV collapsing glomerulopathy into disease susceptible rats induces proteinuria and glomerular collapse (Avila-Casado and colleagues, Kidney International 2004). Incubation of these sera with cultured GECs and M15 cells induces molecular changes associated with collapsing glomerulopathy, including downregulation of nuclear WT1 expression, and changes in key cell-cell and cell-matrix contact proteins. We conducted proteomic analysis using DIGE in 24 cm 2D gel format of six patients and control sera depleted of 12 common proteins. Six replicates of each 2D gel (run in 2 different percentages in the second phase) were conducted and analyzed independently by 2 individuals using DeCyder software. Ten most prominent spots that were increased in the patient sera, and seen in at least two of six patient sera, were excised, digested and identified by MALDI-MS/MS and Q-TOF. In vitro screening using

recombinant proteins showed that one of these proteins, Insulin like Growth Factor Acid Labile Subunit or IGFALS, dramatically reduced the expression of WT1 in M15 cells by confocal imaging, in a manner reminiscent of the effect of patient sera on these cells. To test if IGFALS has similar effects in vivo, we conducted pilot studies in Rrm2b -/-, a strain of mice that develop proteinuria and progressive glomerular collapse. Young Rrm2b -/- mice, prior to any morphological evidence of glomerular collapse or significant albuminuria, were injected with purified recombinant IGFALS, and assessed for development of albuminuria. Preliminary studies show a significant increase in albuminuria at 18 hours after injection. The histology of these mice is currently analyzed. In summary, IGFALS is a key circulating factor in human non-HIV collapsing glomerulopathy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1652**

**Deletion of Drosha in Podocytes Results in Collapsing Glomerulopathy** Olga Zhdanova,[1,2,3] Shekhar Srivastava,[1,3] Lie Di,[1,3] Duncan B. Johnstone,[5] Lawrence B. Holzman,[5] Laura M. C. Barisoni,[4] Edward Y. Skolnik.[1,2,3] *[1]Skirball Institute for Biomolecular Medicine, New York University Langone Medical Center, New York, NY; [2]Division of Nephrology, Department of Internal Medicine, New York University Langone Medical Center, New York, NY; [3]Departments of Pharmacology, New York University Langone Medical Center, New York, NY; [4]Department of Pathology, New York University Langone Medical Center, New York, NY; [5]Division of Nephrology, Department of Internal Medicine, University of Pennsylvania, Philadelphia, PA.*

MicroRNAs have been shown to play key roles in both the function and differentiation of a number of different cell types. Drosha and Dicer, two RNAase III enzymes, function in a stepwise manner to generate a mature microRNA. Previous studies have demonstrated that podocyte-specific deletion of Dicer results in a collapsing glomerulopathy. Unlike Dicer, Drosha is thought to play a more specific role in miRNA biogenesis. We now show that a podocyte-specific deletion of Drosha results in a similar phenotype to Dicer mutants confirming that the Dicer mutant phenotype is due to the loss of miRNAs. Drosha mutants developed proteinuria at 2 weeks of age and died of renal failure between 4-8 weeks of age. Drosha mutants developed a collapsing glomerulopathy characterized by folding and wrinkling of the glomerular basement membrane, glomerular sclerosis, pseudocrescent formation, and extensive podocyte foot process effacement. Podocytes displayed a dedifferentiated phenotype with progressive downregulation of mature podocyte markers such as nephrin, podocin, WT-1 and synaptopodin, and upregulation of the injury-related marker desmin. Interestingly, cells in the pseudocrescents were nestin and Ki-67 positive suggesting that pseudocrescents in Drosha mutants were composed of proliferating dedifferentiated podocytes. These findings confirm that microRNAs play a critical role in podocyte biology. Identifying changes in specific miRNAs in various podocyte diseases should provide both new insight into disease pathogenesis and novel therapeutic targets.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1653**

**Complement C5b-9 Upregulates the Function of the Ubiquitin Proteasome System (UPS) in Glomerular Epithelial Cells (GEC)** Thomas M. Kitzler, Joan Papillon, Julie Guillemette, Andrey V. Cybulsky. *Medicine, McGill University, Montreal, QC, Canada.*

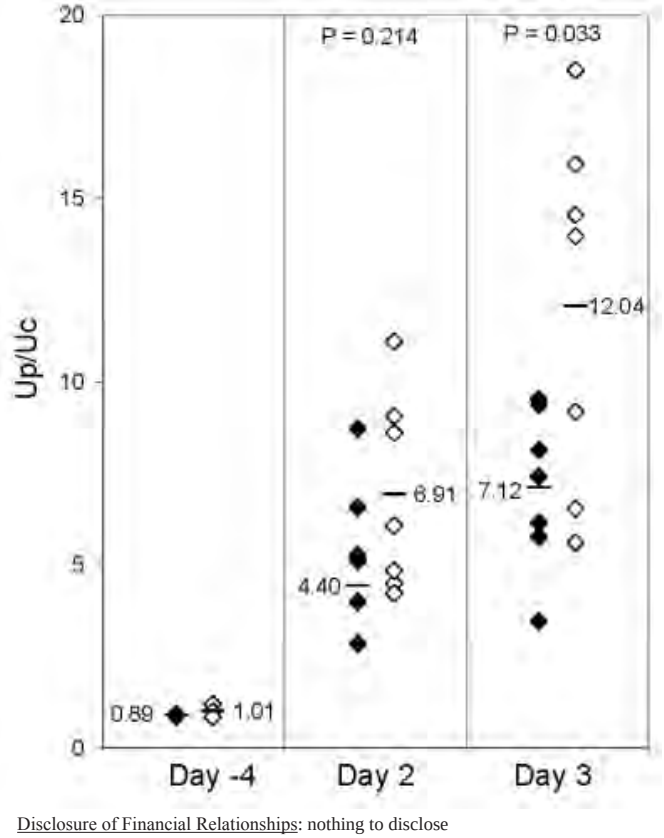
In experimental membranous nephropathy, complement C5b-9 induces sublethal GEC injury and proteinuria. C5b-9 may also trigger mechanisms that restrict injury or facilitate recovery. The UPS selectively degrades damaged or abnormal proteins. Following ubiquitination, proteins are targeted for breakdown by the 26S proteasome. We propose enhanced UPS function as a mechanism to limit complement-induced GEC injury. UPS function was monitored by the degradation of a transfected UPS reporter, $GFP^u$, which consists of a short degron, CL1, fused to the C-terminus of green fluorescent protein (GFP). By immunoblotting, we demonstrated decreased $GFP^u$ levels in GEC after treatment with antibody and sublytic normal serum (NS), compared with GEC treated with antibody and heat-inactivated serum (HIS). In separate experiments, using C8-deficient serum with or without purified C8, cycloheximide (an inhibitor of protein synthesis), and the proteasome inhibitor, MG132, we confirmed that the decrease of $GFP^u$ was due to C5b-9 assembly and enhanced proteasomal degradation of the reporter. $GFP^u$ mRNA levels (RT-qPCR) were unaffected by complement treatment. By analogy to $GFP^u$, in the presence of cycloheximide, complement accelerated degradation of cyclin A, an endogenous substrate for the proteasome. The c-jun N-terminal kinase (JNK)-directed inhibitor, SP600125, attenuated the effect of complement on $GFP^u$ expression, while inhibitors of the extracellular signal-regulated kinase and p38 were not effective. Complement did not affect the level of ubiquitin mRNA in GEC (RT-qPCR), the ubiquitination of $GFP^u$, nor overall ubiquitination of proteins. Proteasome inhibition with MG132 increased the cytotoxic effect of complement in GEC (lactate dehydrogenase cytotoxicity assay), confirming the functional importance of the proteasome in limiting complement cytotoxicity. Treatment of GEC with doxorubicin also resulted in decreased $GFP^u$, suggesting that an increase in UPS function is not limited to complement-mediated injury. Enhanced UPS function, by accelerating removal of damaged proteins may be a novel mechanism to limit complement-induced injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1654**

**Glomerular Epithelial Cell (GEC)-Targeted hHO-1 Expression Reduces Proteinuria in Adriamycin Nephrotoxicity** Pu Duann,[1] Elias A. Lianos,[1] Ling-Mei Chiang.[2] *[1]Medicine Dept / Nephrology Div, Robert Wood Johnson Medical School, New Brunswick, NJ; [2]Pediatrics, Chang-Gung Memorial Hospital, Keelung, Taiwan.*

We previously reported that GEC targeted Heme-Oxygenase (HO-1) expression reduces proteinuria in pNeph-human(h) HO1 transgenic mice following glomerular immune injury. Adriamycin (ADR) causes GEC toxicity via increased oxidative stress. This study used our recently developed line of transgenic mice (pNeph-hHO1 on a FVB background), in which hHO-1 was expressed in GEC (Am J Physiol Renal Physiol. 297[5]:F1476. 2009). FVB control and transgenic mice received 11 mg/kg body weight ADR by a single tail vein injection and 20-h urine samples were collected for urine protein (Up) and creatinine (Uc) analysis. Renal morphology was assessed by light and electron microscopy. ADR treatment induced proteinuria in both pNeph-hHO1 transgenic (Tg) mice and their wild-type (Wt) littermates. Up/Uc values prior to and on days 2 and 3 following ADR were: 0.89, 4.40 and 7.12 in Tg, and 1.01, 6.91 and 12.04 in Wt mice. The reduction of proteinuria was found to be statistically significant in pNeph-hHO1 transgenic mice on day 3 (p value= 0.033). In glomeruli of ADR-treated mice there was foot process effacement. This effect was less pronounced in pNeph-hHO1 mice in which integrity of slit diaphragms was preserved. We conclude that GEC targeted HO-1 expression protects against ADR toxicity by preserving integrity of the glomerular capillary permeability barrier to protein.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1655**

**The Use of Heparin (H) and Aspirin (ASA) To Improve Pregnancy Outcomes in Patients with Early Preeclampsia (PE)** Michelle L. Lubetzky, Orli Etingin, Terri Edersheim, Phyllis August. *Nephrology and Hypertension, New York Presbyterian-Weill Cornell, New York, NY.*

Introduction: Early onset preeclampsia (PE) has a high recurrence rate, and is associated with significant morbidity. The use of heparin (H), aspirin (ASA) or both to prevent early PE in women with and without thrombophilic disorders is controversial.

Methods: We investigated the hypothesis that anticoagulation (H and ASA) prevents recurrent PE and report a case series of 21 women with a history of early (<34 weeks gestation) PE, treated with H and/or ASA in subsequent pregnancies.

Results: Women were 35.7 ± 1 years old (mean± SD), and mostly Caucasian (19/21). Four had pre-existing chronic hypertension. All had early (<34 weeks) PE in one or more untreated pregnancies prior to the treated pregnancy. 20 of 21 had one or more genetic or acquired thrombophilias; 10 women had 2 or more thrombophilias. Treatment started shortly after conception with H and ASA (n=16), ASA alone (n=4) or H alone (n=2) and continued until shortly before delivery.

Figure 1 shows maternal and fetal outcomes in untreated and treated pregnancies. Only one woman had recurrent PE at 37 wks; a recurrence rate (4.7%) significantly less than recurrence rates (about 25%) for early PE reported in the literature (P=0.016).

| | Most Recent Prior, Untreated Pregnancy(n=21) | Treated Pregnancy (n=21) | P value |
|---|---|---|---|
| Preeclampsia | 21/21 | 1/21 | 0.016 |
| Gestational Age at delivery (weeks) | 29.4±4.8 | 37.7±1.2 | 0.001 |
| Fetal loss | 10/21 (47%) | 0 | 0.001 |
| Birth weight (gm) | 1,386±.4 | 2,98±.49 | 0.0001 |
| Growth Restriction | 55 % | 0 | 0.001 |
| Placental Abruption | 4/21 | 0 | ns |

Conclusions: We observed significantly improved pregnancy outcomes in women with a history of early, severe PE who were treated with heparin and/or aspirin in a subsequent pregnancy. Although almost all women had either acquired or genetic thrombophilias, our data do not permit assessment of the relevance of thrombophilias to the pathogenesis of PE. Randomized controlled trials of anticoagulation in women at high risk for recurrent PE are needed.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1656

**Proteinuria in Preeclampsia Correlates Positively with Serum sFlt-1/PlGF Ratio and Negatively with Serum PlGF** Cilene Carlos Pinheiro,[1,3] Cristiane Bitencourt Dias,[3] Gianfranco Zampieri,[2] Patrícia Rayol,[1] Luiz Gozzani,[1] Viktoria Woronik.[3] *[1]Uti Adulto, Hospital e Maternidade Santa Joana, São Paulo, São Paulo, Brazil; [2]Salomão e Zoppi - Medicina Diagnóstica, São Paulo, São Paulo, Brazil; [3]Nefrologia, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil.*

Release of anti-vasogenic factors into maternal circulation seems to be involved in preeclampsia **(PE)**. Maternal outcomes are more benign in later **PE** (≥34th wk) than in earlier **PE** (<34th wk). This study evaluated clinical and biochemical status of thirty-five pregnant with **PE** that were enrolled at their hospital admission. Specimens of urine were obtained to measure 8- isoprostane and placental growth factor (PlGF), and plasma to measure PlGF and sFlt-1 (soluble fms like tyrosine kinase 1). Patients were stratified in groups according to gestational duration. Results table 1.

Clinical parameters of pregnant women with severe (< 34 weeks) and mild (≥ 34weeks) preeclampsia

| | <34 gestational weeks n= 20 | ≥34 gestational weeks n=15 | P |
|---|---|---|---|
| Age (years) | 29.0 ± 3.2 | 31.2 ± 3,87 | ns |
| Gestational age (weeks) | 28.9 ± 4.1 | 36.1 ± 1.5 | 0.03 |
| Systolic BP (mmHg) | 170.0 ± 20.2 | 163.3 ± 23.8 | ns |
| Diastolic BP (mmHg) | 107.8 ± 13.2 | 108.7 ± 15.5 | ns |
| Serum sFlt-1 (pg/mL) | 11781 ± 6304 | 7157 ± 5017 | 0.03 |
| Serum PlGF (pg/mL) | 87.23 ± 110.6 | 159.7 ± 154 | 0.05 |
| Serum sFlt-1/PlGF ratio | 474.7 ± 475.1 | 133.4 ± 151.9 | 0.01 |
| Urinary PlGF (pg/mL) | 8.4 ± 6.8 | 42.6 ± 71.7 | 0.03 |
| Isoprostane urinary (pg/mL) | 1128 ± 1557 | 4527 ± 4474 | 0.04 |
| Proteinuria (g/day) | 1.8 ± 2.4 | 0.4 ± 0.5 | 0.02 |
| Creatinine (mg/dL) | 0.7 ± 0.1 | 0.7 ± 0.17 | ns |

Mean ± SD Isoprostane was measured in 12 and 5 patients, respectively; serum sFlt-1 and PLGF were measured in 20 and 13 patients, respectively.

Proteinuria showed positive correlation with serum sFlt-1/PlGF ratio (r=0.58, p=0.0007) and negative correlation with serum PlGF (r=-0.47, p=0.0085). Severe preeclamptic patients showed lower urinary isoprostane; lower serum and urinary PlGF levels; higher serum sFlt-1/PlGF ratio and protein excretion rate.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1657

**Hypertension in Pregnancy Is Associated with an Increased Risk for Microalbuminuria Later in Life** Vesna D. Garovic, Stephen T. Turner. *Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Women with a history of hypertension in pregnancy, including preeclampsia, are at increased risk for cardiovascular disease (CVD) and end-stage renal disease (ESRD) later in life. As microalbuminuria has been associated with both CVD and progression of renal disease, this study aimed to assess whether a history of hypertension in pregnancy is associated with microalbuminuria later in life. We studied 3031 women, median age 54 years, who participated in the Family Blood Pressure Program (FBPP). The percentage with microalbuminuria, defined as a urine albumin/creatinine ratio ≥ 25 mg/g differed significantly among nulliparous women (n=39, 11%); women with a history of normotensive pregnancies (n=249, 13%), and women with a history of at least one hypertensive pregnancy (n=90, 19%) (*P*=0.002). Multiple logistic regression models were used to compare odds ratios (OR) for microalbuminuria among the groups, after controlling for effects of race, age, education, family history of CVD, diabetes, smoking, current hypertension, body mass index (BMI), and family relationships. There was no significant difference in OR between nulliparous women and those with a history of normotensive pregnancies (*P*=<INS cite=mailto:vdg01 dateTime=2010-06-17T20:58><INS cite=mailto:Stephen> </INS></INS>0.39). In contrast, women with hypertensive pregnancies, when compared to those with normotensive pregnancies, had a significantly greater OR of 1.4 for microalbuminuria later in life (*P* =0.02). In conclusion, a history of hypertension in pregnancy is associated with an estimated 40% greater odds for microalbuminuria later in life, independent of traditional CVD risk factors and BMI. Microalbuminuria may serve as an intermediate biomarker for the increased risks of CVD and ESRD later in life among women with a history of hypertension in pregnancy.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1658

**Hypertension in Pregnancy Is Associated with Decreased Ankle Brachial Indices Later in Life** Iasmina Craici, Steven Wagner, Stephen T. Turner, Vesna D. Garovic. *Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

**Background:** Decreased ankle brachial indices (ABI) have been associated with both cardiovascular disease (CVD) and atherosclerosis. Women with a history of hypertensive pregnancy have also been shown to have an increased risk of future cardiovascular disease and stroke. The aim of this study was to assess if hypertension in pregnancy is associated with changes in ABI later in life, which may be a marker of increased risk for CVD in women with a history of hypertensive pregnancy disorders.

**Methods:** We studied 1698 women who participated in the Genetic Epidemiology Network of Arteriopathy (GENOA) study. Subjects were categorized as nulliparous, i.e., those with no history of pregnancy lasting more than 6 months, (n=145), women with a history of normotensive pregnancies (n=1272), and women with a history of at least one hypertensive pregnancy (n=281). We fit multiple linear regression models to compare mean ABI among the groups. All models included race, age, education, family history of CVD, diabetes, smoking, current hypertension, and dyslipidemia, as adjustment variables and were fit with generalized estimating equations to account for the sibling relationships in the data. As ABI levels may be influenced by body mass index (BMI), the analyses were performed both with and without BMI in the model.

**Results:** There was no significant difference in ABI between nulliparous women and those with a history of normotensive pregnancies either with BMI included (p=0.932) or removed (p=0.960) from the model. In contrast, women with hypertensive pregnancies, compared to those with normotensive pregnancies, had lower ABIs, both when BMI was added (p=0.012) or removed (p=0.047) from the multiple regression model.

**Conclusion:** A history of hypertension in pregnancy is associated with lower ABI levels later in life, independent of traditional CVD risk factors and BMI. A decreased ABI may be reflective of atherosclerotic burden and serve as a marker for increased CVD risk in women with a history of hypertensive pregnancy disorders.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1659

**Meta-Analysis of Pregnancy Outcomes in Patients with Positive Anti-Phospholipid Antibodies and Biopsy Proven Lupus Nephritis** Andrew Smyth, Suzanne Norby, Vesna D. Garovic. *Division of Nephrology & Hypertension, Mayo Clinic, Rochester, MN.*

Introduction

We reported previously that lupus nephritis (LN) was associated with hypertension in pregnancy and premature births. We aimed to review the associations between pregnancy outcomes and biopsy proven lupus nephritis and positive anti-phospolipid antibodies (APA).

Methods

We searched databases from January 1980 to April 2009, reviewed bibliographies and specialty journals. Random-effects methods were used to evaluate complication rates and associations. APA were considered positive in the presence of anti-cardiolipin antibodies and/or, lupus anticoagulant, and/or anti-phospholipid syndrome. Lupus nephritis biopsies were classified using the WHO Classification System.

Results

Twenty-eight papers including 1416 patients and 2225 pregnancies were included. APA were positive in 619 pregnancies and were associated with hypertension (p=0.029), premature birth (p=0.004) and induced abortion rate (p=0.016). No statistically significant associations were observed between histological subclass grouped into proliferative (Class III and IV, n=245) and non-proliferative (Class II and V, n=86) and pregnancy outcomes, including live birth vs. unsuccessful pregnancy (p=0.3853), and maternal complications (p=0.5789).

Conclusion

APA were associated with hypertension, premature birth and increased rate of induced abortion, thereby emphasizing the importance of screening for these antibodies in SLE pregnancies. Stratification of pregnancy outcomes by biopsy classification yielded no significant associations. However, a limited amount of data was available for this analysis, and histological pattern might not have influenced the pregnancy outcomes due to the fact that most of these biopsies were performed years prior to the pregnancies that were analyzed.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1660**

**Vasculature Structure and Function in Women with and without a History of Pregnancy Induced Hypertension** Catherine M. Brown,[1,2] Alice V. Stanton,[2] John J. Walshe.[1] *[1]Department of Nephrology, Beaumont Hospital, Dublin 9, Ireland; [2]Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland.*

**Objective:** Studies have linked a history of gestational hypertension (GH) or preeclampsia (PET) with an increased risk of death from ischaemic heart disease and cerebrovascular events. The aim of this study was to test if such women demonstrate abnormal large vessel structure and/or function at 2 years post partum.

**Methods:** A cross sectional observational study of 114 women with a history of PET (n=40), a history of GH (n=33) and a history of normal BP throughout pregnancy (n=41). Levels of established cardiovascular risk factors (age, body mass index, smoking habit, systolic BP, fasting cholesterol and glucose) were recorded. The assessments of vascular structure and function included; ultrasonic measurement of common carotid intima-media thickness (IMT) (a measure of early atherosclerosis), carotid-to-femoral pulse wave velocity (PWV) (a measure of large artery stiffness), and brachial flow-mediated dilatation (FMD) (a measure of large artery endothelial function).

**Results:**

Results: mean values (± SD) for the three groups

| | Control (n=41) | PET(n=40) | GH (n=33) | ANOVA p-value |
|---|---|---|---|---|
| Common Carotid IMT (mm) | 0.46 ± 0.1 | 0.50 ± 0.1 | 0.50 ± 0.1 | p= 0.001 |
| Carotid to-Femoral | 8.2 ± 0.6 | 8.6 ± 0.7 | 8.7 ± 0.8 | p= 0.002 |
| Brachial FMD (%) | 23 ± 12 | 14 ± 8.8 | 20 ± 11 | p= 0.001 |

After adjustment for established cardiovascular risk factors, common carotid IMT remained significantly greater in both groups of women with a history of pregnancy induced hypertension (p=0.04), whilst brachial FMD remained significantly blunted in women with a history of PET (p=0.003).

**Conclusions:** Women with a history of PIH demonstrate clear abnormalities in vascular structure and function. These differences are independent of established risk factors for cardiovascular events. The greater atherosclerosis and endothelial dysfunction exhibited by these women could contribute to the excess cardiovascular morbidity and mortality experienced by these women in later life.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1661**

**Levels of Systolic BP before 20 Weeks Gestation Are a Predictor of Subsequent Gestational Hypertension** Catherine M. Brown, John J. Walshe. *Department of Nephrology, Beaumont Hospital, Dublin 9, Ireland.*

**Background:**

Blood pressure in women who develop either preeclampsia (PET) or gestational hypertension (GH) is by definition ≤ 140/90 mmHg in the first 20 weeks of pregnancy. Preeclampsia differs from GH in that it is associated with proteinuria (≥ 300mg in a 24hr urine collection). We reviewed early pregnancy data on a group of women to assess differences in their blood pressure at their first booking visit.

**Methods and results:** A retrospective review of data from the bookings visits of women who developed no blood pressure complications, controls (n= 65), women who later developed PET (n=67) and women who later developed GH (n= 66). The following were recorded at their booking visits, the average of 2 blood pressure readings, their weight and age as well as pregnancy outcomes ( weeks gestation at delivery, baby weight, apgar scores, type of delivery and serum urate and total urine protein (TUP) where available).

**Results:**

Results: mean values (± SD)

| | Control (n= 65) | PET (n= 67) | GH (n=66) | ANOVA p value |
|---|---|---|---|---|
| Age at booking (yrs) | 29 ± 4.6 | 31 ± 5.4 | 30 ± 5.2 | p= 0.3 |
| Booking systolic BP mmHg | 114 ± 11 | 119 ± 11 | 123 ± 11 | p<0.001 |
| Booking diastolic BP mmHg | 69 ± 7 | 70 ± 12 | 74 ± 11 | p= 0.01 |
| Booking weight (kg) | 75 ± 12 | 77 ± 13 | 84 ± 1 | p= 0.001 |
| Baby weight (kg) | 3.6 ± 0.4 | 3.0 ± 0.9 | 3.4 ± 0.5 | p< 0.001 |
| Weeks gestation at delivery | 39 ± 1 | 38 ± 3 | 39 ± 2 | p= 0.03 |

Women who developed either PET or GH had significantly higher systolic BP (SBP) at their booking visit. After adjustment for weight and age, booking SBP remained significantly higher for the GH group compared to controls (p= 0.003).

**Conclusions:**

In women who developed gestational hypertension, systolic and diastolic BP at their booking visit was significantly higher when compared to both the PET group and the control group. After adjustment for weight and age, this difference in systolic BP remained significant in the GH group compared to controls. Women who develop GH in pregnancy need regular BP monitoring post partum and follow up screening for cardiovascular risks.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1662**

**Plasma Hemopexin Activity and Preeclampsia: sFlt1 Counteracts Hemopexin Activity and Expression In Vitro** Winston W. Bakker, Judith Muis, Theo Borghuis, Harry Van Goor, Marijke M. Faas. *Pathology, University Medical Center, Groningen, Netherlands.*

**Background:** The soluble VEGF receptor, denoted as s-Flt1, is increased in preeclampsia, causing endothelial dysfunction. Active plasma hemopexin, (Hx) controls vascular responsiveness for angiotensin II by shedding endothelial angiotensin II receptors (AT-1). Hx activity is increased in pregnancy but inhibited in preeclampsia. The question emerged whether Hx activity can be counteracted by sFlt-1, leading to persisting angiotensin II sensitivity in preeclampsia.

**Methods:** We incubated purified active Hx with sflt-1followed by evaluating Hx activity using a standard amidolytic assay. Control incubations were done with sFlt1 supplemented with its ligand VEGF-A. Expression of Hx on endothelial cells was evaluated after overnight incubation with either sFlt alone or sFlt1+ VEGF, using flow cytometry.

**Results**: sFlt-1 showed a dose dependent inhibition of Hx activity [ Hx activity, in arbitrary units: 0.80 ±0.10; Hx+sFl-t1,( 2.5 ng/ml): 0.71±0.20; Hx+sFlt-1 (5.0 ng/ml): 0.51±0.10;Hx+sFlt-1(10.0 ng/ml) 0.49±0.13.(P< 0.05; each +sFlt-1 value vs Hx alone)] Supplementation of VEGF (10ng/ml) + 5.0 ng sFlt-1 to Hx resulted in a significant increase of Hx activity[0.62±0.5 vs 0.51±0.10, P< 0.05] and addition of the same amount of VEGF with 10.0 ng/ml sFlt-1 to Hx resulted in 0.71±0.2 vs 0.49±13.0 (P<0.01). VEGF alone was negative in this test system.

In cultured endothelial cells dose dependent inhibition of Hx expression occurred after incubation with sFlt-1 (2.5 ng/ml): 30%± 10% (P<0.05); 5.0 ng/ml: 42%±6% (p< 0.01); 10.0 ng/ml: 46% ± 10% (p<0.05);(P-values: sFlt-1+ Hx vs Hx expression alone). As VEGF per sé appeared to affect Hx expression, no VEGF/sFlt-1 interactions could be studied using this assay.

**Conclusion**: The activity of plasma Hx as well as its expression on endothelial cells are inhibited by sFlt-1 in vitro. Potential counteraction of plasma Hx activity and /or Hx release from endothelial cells by sFlt-1 in vivo may contribute to a contracted vascular bed and hypertension in patients with preeclampsia.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1663**

**Hypertension in Pediatric Renal Replacement Therapy Patients in Europe, Results from the ESPN/ERA-EDTA Registry** Karlijn J. Van Stralen,[1] Marijn A. M. Kramer,[1] Kitty J. Jager,[1] Franz S. Schaefer,[3] Enrico E. Verrina,[2] Jaap Willem Groothoff.[1] *[1]Academic Medical Center, Amsterdam, Netherlands; [2]G Gaslini Hospital, Genoa, Italy; [3]University of Heidelberg Children's Hospital, Heidelberg, Germany.*

**Objectives**

Determine the prevalence of hypertension (HT) and the distribution of blood pressure (BP) in the European paediatric renal replacement therapy population, for both renal allograft recipients and dialysis patients, and to identify potential determinants associated with hypertension.

**Methods**

Data were derived from the ESPN/ERA-EDTA registry from 2734 children, providing over 10,000 measuements, aged younger than 18 from 1999 to 2009, from 15 European countries. HT was defined as either SBP or DBP ≥95$^{th}$ percentile for age, height and gender. Information on use of antihypertensive medication was available for 54.2% of the patients. Percentages were weighted according to the number of blood pressure measurements available. Linear mixed model analyses were used to determine effect of parameters on HT.

**Results**

HT was present in 40% of haemodialysis, 40% of peritoneal dialysis and 29% of transplant patients. Of those on antihypertensives, 49% had uncontrolled HT (BP ≥90$^{th}$ percentile), while 16% of those not on medication had HT. Having HT was associated with younger age, treatment with haemodialysis and not having CAKUT. There was not difference between males and females. Patients who were hypertensive at start of RRT became less hypertensive in the subsequent years.

**Conclusions**

In this large European cohort fewer patients suffered from HT compared to US studies. Despite the awareness of the possible consequences of HT in the paediatric European RRT population, it is still inadequately treated.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1664**

**Predictors of Hypertension in Children and Adolescents: Role of Perinatal Events** Tetyana L. Vasylyeva, Candace A. Myers, Michael Okogbo. *Department of Pediatrics, Texas Tech University Health Sciences Center, Amarillo, TX.*

**Purpose:** Pre-maturity and low birth weight (LBW) cause hypertension (HTN), ischemic heart disease, and obesity in young adults. The objective of our study was to identify preventable risk factors for the development of HTN in 8-21 years-olds and to assess pediatricians' awareness of the problem.

**Methods:** A retrospective chart review was conducted of 128 children and adolescents born in 1992-2002, at gestational ages (GA) ≤ 37 weeks. Eligible cases had at least five blood pressure recordings during childhood. Familial, neonatal, and postnatal data were collected.

**Results:** Nine percent of the subjects were born at a GA under 28 weeks, 10%: 28-39 weeks, 45%: 31-34 weeks, and 36%: 35-37 weeks. Seven percent of the children born at a GA less than 30 weeks developed HTN *vs.*17% who were born after 30 weeks. We found that 9% of the babies ≤ 1500 g developed HTN compared to 18% of those over 1500 g. Thirteen percent of the babies were small for GA and of these, only 4% developed HTN later in childhood. Of the 7% born large for GA, 20% developed HTN. HTN was noted in 15% of the children born appropriate for GA. The development of HTN was more common in males (56%) than females (12.5%) and a higher prevalence was observed in Hispanics compared to Whites and African-Americans. Among breastfeed babies 25% developed HTN *vs.* 11% of formula-fed babies. Other factors that influenced the development of HTN were maternal age, HTN, and diabetes. In this cohort, the risk of developing HTN by age 18 or 21 was 27% or 33%, respectively (interval regression analysis). The odds ratio of developing HTN was 11.5 if pre-term children became obese and 0.84 if the baby developed respiratory distress syndrome. HTN was diagnosed by the primary care pediatricians in only 32% of the HTN children in our study.

**Conclusion:** Fetal programming of diseases must be considered for the treatment and prevention of HTN among premature/LBW patients. Environmental factors may play a critical role. In our ongoing study we will try to identify preventable risk factors for HTN in patients born preterm/LBW as well as increase pediatricians' awareness of the problem.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1665

**Predictors of Response to Antihypertensive Medication in Children with Primary Hypertension** Hanan K. Tawadrous,[1] Sreevidya Kusuma,[1] Anita Pandey,[2] Svetlana Ten,[2] Amrit Bhangoo,[2] Anil K. Mongia.[1] *[1]Pediatric Nephrology, SUNY Downstate Medical Center, Brooklyn, NY; [2]Pediatric Endocrinology, SUNY Downstate Medical Center, Brooklyn, NY.*

**Background:** Improvement of blood pressure in children is important to prevent target organ damage. Medications along with life style modification are recommended in case of severe HTN. However some patients might be resistant to antihypertensive medication.

This study was done to identify factor that predict response to blood pressure medication in pediatric population with primary HTN.

**Objective:** We studied changes in blood pressure, and cardiovascular disease (CVD) risk factors to determine whether LSM and antihypertensive medications produced improvement in blood pressure and cardiovascular risk factors.

**Design/Methods:** This study included 87 children with new-onset HTN referred from July 2002 to October 2009. The patients after routine work up were advised life style modification and medication were added as per the standard guidelines. Patients with improvement in blood pressure to less than 95% were referred to as responders and rest as non responders.We used paired t-test to evaluate the changes in each group in respect to BP, BMI, Cholesterol, LDL, TG, HDL, and microalbumin compared to the baseline.

**Results:** Mean follow up period was 2.1 years. 58 (66.6%) were boys.73 patients (83.9%) were of AA origin.55 (63%) were overweight. Of 87 patients, 33(37.9%) had stage I hypertension and 38(43.7%) had stage II hypertension. Of these 44 patients were prescribed pharmacologic treatment in addition to lifestyle modification. At baseline pts with or without response to medication did not differ significantly with respect to age, sex ,Race, BMI LDL(P=0.79) ,Cholesterol(P=0.71), TG(P=0.30), HDL(0.39), microalbumin (P=0.24). However at follow up patients with poor response to antihypertensive medication had statistically higher TG(137 vs77mg/dL$p<0,05$), lower HDL(35vs 46mg/dl;p=0.006) and higher free T3(177vs117;p=0.01)

**Conclusion:** Patients with poor response to antihypertensive medication have an unfavorable metabolic profile at follow up. Lifestyle modification might not have been aggressively followed in such patients.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1666

**A Slight Increase in Salt Reduces Endothelial Ecto-Phosphatase (CD 39) In Vitro** Winston W. Bakker,[1] Titia Lely,[2] Theo Borghuis,[1] Harry Van Goor,[1] Marijke M. Faas.[1] *[1]Pathology and Medical Biology, University Medical Center, Groningen, Netherlands; [2]Obstetics and Gynecology, University Medical Center, Groningen, Netherlands.*

**Background**. High dietary sodium intake is associated with cardiovascular and renal risk. Endothelial ectonucleoside triphosphate diphosphohyrolase-1(ENTPD-1) i.e. CD 39, is an important endothelial ecto enzyme, protecting the vessel wall from microthrombus formation and proinflammatory stimuli. The question emerged whether endothelial injury caused by sodium may involve CD39 impairment, leading to increased vessel wall sensitivity.

**Methods**. Confluent human endothelial cells were co-cultured with human peripheral blood mononuclear cells (PBMC; $0.5x10^6$ cells /well) using medium (RPMI 1640) with a standard amount of NaCl (154.0 mMol/L)(low salt, LS), or with "high" NaCl (155.54 mMol/L)(HS), or LS medium supplemented with an $O_2^-$ scavenger superoxide dismutase (SOD,592.0 U/ml) ( LS+SOD), or HS medium supplemented with 592.0 U/ml SOD (HS+SOD). After 16 hrs, cytospins of endothelial cells were immunostained for CD39. Expression was semi-quantitatively scored using an arbitrary scale. In another set of experiments PBMC alone were incubated for 16 hrs at 37°C in LS or HS medium and washed and labelled with dichlorofluorescein diacetate for detection of $O_2^-$ release by flow cytometry.

**Results**. Endothelial cells cultured in high salt medium showed a significant decrease of CD39 expression as compared with cells cultured in LS medium with or without SOD, showing normal CD39 expression. PBMC in high salt medium showed significantly enhanced $O_2^-$ production. as compared with low salt medium. (365.3±73.0 vs 164.8 ± 24.7 in arbitrary units, n=4; $P< 0.01$). No significant CD39 impairment was seen in endothelial mono-cultures in HS versus LS medium. Preliminary data in PBMC from volunteers on high salt versus low salt intake (with approximately 1% difference in plasma NaCl) showed also reduced CD39 expression.

**Conclusion**. A minor increase in sodium affects endothelial CD39 expression in vitro. This form of endothelial injury is mediated through toxic oxygen products produced by PBMC. Further investigation into the role of endothelial CD39 and sodium status is warranted.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1667

**Evaluation of Interactions of Dapagliflozin and Bumetanide** Christopher S. Wilcox,[1] Xiaoni Liu,[2] Sreeneeranj Kasichayanula,[2] Anh Bui,[2] David W. Boulton,[2] Bruce R. Leslie,[2] Steven C. Griffen.[2] *[1]Georgetown University School of Medicine, Washington, DC; [2]Bristol-Myers Squibb, Princeton, NJ.*

Dapagliflozin (DAPA) is a competitive, reversible, selective inhibitor of sodium glucose cotransporter 2 (SGLT2), which mediates most proximal tubule glucose and $Na^+$ reabsorption. DAPA is an investigational diabetes drug whose glucosuric MOA results in diuresis. This study assessed potential pharmacokinetic (PK) and pharmacodynamic interactions of DAPA with a loop diuretic, bumetanide.

METHODS: 42 healthy volunteers (HV) were randomized to 1mg bumetanide, 10mg DAPA, or DAPA plus bumetanide qd for 7 days. On Day 8, all subjects received DAPA plus bumetanide qd for 7 more days. Samples were collected for PK analyses on Days 7 and 14 and for PD analysis daily from Days -1 to 14.

Neither drug meaningfully affected the PK of the other. Adverse events were consistent with the profiles of the individual drugs, with one subject treated for hypokalemia 1 day after completing treatment, and another with orthostatic hypotension and syncope in a subject receiving both drugs together. DAPA, but not bumetanide, increased glucose excretion and urinary osmolality. Urine volumes increased transiently with DAPA, bumetanide, or the combination, and return to baseline was longer (9 days vs. 2 days) when the two drugs were initiated simultaneously vs. singly. Urinary $Na^+$ increased by 22mEq (35%) with DAPA alone, although this response is complicated by the lack of $Na^+$ balance at baseline. However, DAPA added after 7 days of bumetanide increased urinary $Na^+$ by 64mEq [78%; difference 42mEq, 95% CI (23, 61)]. Bumetanide added after 7 days of DAPA resulted in a greater increase (101mEq) in urinary $Na^+$ than when given alone [75mEq; difference 27mEq; 95% CI (8, 46)]. Effects on urinary $Na^+$ were transient (about 2-3 days) for all treatments.

Treatment with DAPA, bumetanide or the combination was safe in this study in HV. Dapagliflozin produces glucosuria and osmotic diuresis as well as a natriuretic effect that was additive with a loop diuretic. DAPA appears to have a low potential to produce serum electrolyte disturbances and does not have clinically significant PK interactions with the loop diuretic, bumetanide.

Disclosure of Financial Relationships: Consultancy: A consultancy agreement with Bristol-Myers Squibb.

## F-PO1668

**Dietary Intake of Amino Acids Influences Blood Pressure in Patients at High Cardiovascular Risk** Katherine R. Tuttle,[1,2] Joan E. Milton,[1] Diane P. Packard,[1] Robert Short.[1] *[1]Providence Medical Research Center, Providence Sacred Heart Medical Center & Children's Hospital, Spokane, WA; [2]Nephrology Division, Department of Medicine, University of Washington School of Medicine, Seattle, WA.*

Diets augmented with plant proteins appear to lower blood pressure (BP), while greater intake of animal proteins may do the opposite. The purpose of this study was to determine if individual amino acids that vary by food source influence BP in patients at high cardiovascular risk defined by a prior myocardial infarction. Participants in a clinical trial of low-fat vs. Mediterranean-style diets (n=92) were randomized to interventions that provided ≥13 individual and group sessions over 2 years. Nutrient intake was serially assessed at baseline, 3 months, and then every 6 months by 3-day food diaries and laboratory studies. Baseline BP was 125±19/73±10 mm Hg (mean±standard devation, SD) in hypertensive participants (n=44) and 114±11/71±9 mm Hg in non-hypertensive participants (n=48), p=0.002. Diuretics were used more commonly in hypertensive than non-hypertensive participants, 23% (10/44) vs. 2% (1/48), p=0.003. Usage of other anti-hypertensive agents, age (59±9 yr), body mass index (29±6 kg/m$^2$) estimated glomerular filtration rate (62±20 ml/min/1.73m$^2$), and urinary albumin/creatinine (14±36 mg/g), sodium (155±76 mmol/day), and potassium (72±28 mmol/day) did not differ by hypertension status. The independent amino acid variables (quartiles of intake) were analyzed by generalized estimating equation models with covariates for the dependent variables of time-varying systolic and diastolic BP (SD units). Intake of methionine ($\beta$=0.24, $p<0.001$; $\beta$=0.20, p=0.006) and alanine ($\beta$=0.15, p=0.005; $\beta$=0.20, p=0.004) were associated with higher systolic and diastolic BP (data shown respectively). Threonine had a reverse effect ($\beta$=-0.17, p=0.008; $\beta$=-0.16, p=0.053). Conclusions: Greater intake of methionine and alanine, amino acids enriched in animal proteins, had consistently positive relationships to higher BP. In contrast, intake of threonine, an amino common in plant proteins, was associated with lower BP.

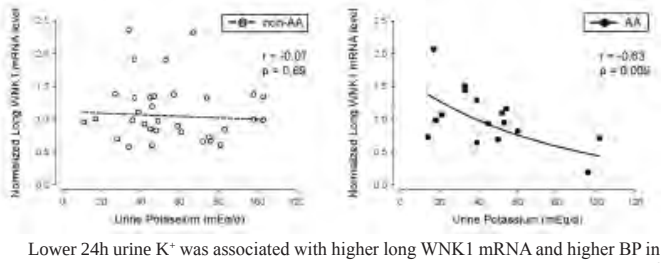
Disclosure of Financial Relationships: Consultancy: Fibrogen Inc., Eli Lilly and Co.Research Funding: NIH/NHLBI.

**F-PO1669**

**Dietary Potassium Deficiency Is Associated with Long WNK1 in African Americans** Susan Hedayati,[1,2] Masoud Afshar,[2] Beverley Adams-Huet,[2] Jian Xie,[2] Orson W. Moe,[2] Chou-Long Huang.[2] *[1]Nephrology, Dallas VA Medical Center; [2]Nephrology and Clinical Sciences, University of Texas Southwestern Medical Center, Dallas, TX.*

Mechanisms that mediate salt-sensitive hypertension are poorly understood. African Americans (AA) have higher salt-sensitivity and may be more affected by dietary $K^+$ deficiency. Dietary $K^+$ restriction in rats increased whole-kidney WNK1 mRNA levels. Upregulation of WNK1 in humans causes hypertension in type 2 pseudohypoaldosteronism.

We sought to investigate whether lower dietary $K^+$ as measured by 24h urine $K^+$ ($U_{K+}V$) is associated with increased human peripheral long WNK1 mRNA expression (using white blood cells as surrogate tissue), and with higher blood pressure (BP), and whether this association was more pronounced in AA. Of the 52 normal subjects with BP <140/90 enrolled, 17 were AA and 35 non-African American (non-AA). Mean age was greater in AA vs. non-AA, 41 ±9 vs. 32 ±11 years, p =0.001. Systolic BP was 111 ±12 in AA and 104 ±12 in non-AA (p =0.05). Diastolic BP was 68 ±7 in AA and 63 ±9 in non-AA, p =0.07. $U_{K+}V$ was not different between AA (47 ±26 meq/d) and non-AA (55 ±24 meq/d), p =0.3. There was a weak and statistically non-significant negative correlation between $U_{K+}V$ and WNK1 in the overall sample (r =-0.25, p =0.07). However, when the analysis was stratified by race, the magnitude of this association was substantially stronger in AA and statistically significant, r =-0.63, p =0.009. There was no correlation in non-AA, r =-0.07, p =0.7. Similarly, there was a negative correlation between $U_{K+}V$ and systolic BP in AA, r =-0.61, p =0.009, but not in non-AA.




Lower 24h urine $K^+$ was associated with higher long WNK1 mRNA and higher BP in AA but not in non-AA. We postulate that WNK1 may play a role in the pathogenesis of salt-sensitivity of blood pressure related to dietary $K^+$ deficiency in African Americans.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1670**

**The Effect of Sodium Intake on Blood Pressure and Kidney Function: Data from Korea National Health and Nutrition Examination Survey (KNHANES)** Seong-Woo Lee,[1] Ho Seok Koo,[1] Ho Jun Chin,[1,2] Ki Young Na,[1,2] Kwon Wook Joo,[1] Yon Su Kim,[1] Dong Wan Chae,[1,2] Curie Ahn,[1] Jin Suk Han,[1] Suhnggwon Kim.[1] *[1]Internal Medicine, Division of Nephrology, Seoul National University College of Medicine, Seoul, Korea; [2]Internal Medicine, Seoul National Bundang Hospital, Seongnam, Korea.*

Background: There have been controversies on the effect of sodium intake on blood pressure and kidney function. Method: Data from the 3rd and 4th KNHANES were used. A total of 13,051 who were aged ≥ 20 years, and not missing data of serum creatinine and sodium intake were included for the analysis. Sodium intake was calculated by using 24 hours' recall method. All values of blood pressure or kidney function were adjusted by age, sex and systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) by using ANCOVA. Result: SBP was not associated with sodium intake (p=0.057). However, DBP revealed substantial association with sodium intake: 75.19 (0.15) mmHg in 1Q of Na, 76.02 (0.13) mmHg in 2Q of Na, 76.08 (0.13) mmHg in 3Q of Na and 75.96 (0.13) mmHg in 4Q of Na (p <0.001). In post-hoc analysis, only 2Q of Na significantly increased DBP compared to 1Q of Na (p=0.001), while no changes were observed in the comparison of the other two adjacent quartiles. In participants with hypertension (HTN), DBP was not affected by the increase of sodium intake. However higher amount of sodium intake increased DBP in participants without HTN. Sodium intake was also related to estimated glomerular filtration rate (eGFR): 80.61 (0.30) ml/min/1.73m$^2$ in 1Q of Na, 80.25 (0.26) ml/min/1.73m$^2$ in 2Q of Na, 79.47 (0.25) ml/min/1.73m$^2$ in 3Q of Na and 79.59 (0.25) ml/min/1.73m$^2$ in 4Q of Na (p=0.008). In post-hoc analysis, however, we could not find statistically significant change in comparison of the two adjacent quartiles. Conclusion: Higher amount of sodium intake increased DBP, but not linearly. Sodium intake was not associated with SBP. The increasing effect of sodium intake on DBP could be found in participants without HTN, but not in participants with HTN. Although sodium intake was associated with eGFR, dose dependent change was not found in this study. Prospective study needs to be followed to confirm our result.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1671**

**Urinary Albumin Excretion and Ambulatory Blood Pressure in a Hypertensive Population** Julian Segura, Manuel Gorostidi, Alejandro De la Sierra, Juan J. De la Cruz, Jose R. Banegas, Luis M. Ruilope. *on Behalf of the, Spanish Society of Hypertension ABPM Registry Investigators.*

**Objective**: The value of ambulatory blood pressure monitoring (ABPM) as the gold standard to investigate the role of blood pressure (BP) in renal disease is now-a-days considered.

**Methods**: We analyzed data from a subsample of 2,551 hypertensive patients from the Spanish Society of Hypertension ABPM Registry in whom quantitative data of urinary albumin excretion was available. Albuminuria was assessed by the albumin/creatinine ratio (ACR) and values were categorized as follows: normoalbuminuria was considered when ACR <10 mg/g in men or <15 mg/g in women, high-normal albuminuria when ACR 10-20 mg/g in men or 15-30 mg/g in women, microalbuminuria when 21-200 mg/g in men or 31-300 mg/g in women, and macroalbuminuria when ACR values were above this thresholds.

**Results**: Mean age was 59 years and 53.5% of patients were men. The distribution of different categories of ACR was: normoalbuminuria 67.4%, high-normal albuminuria 13.1%, microalbuminuria 15.9%, and macroalbuminuria 3.6%. There was an increase in systolic BP levels in all ABPM periods (24-h, daytime, and nighttime) from normo- to macroalbuminuria (p for trend <0.001) despite the use of more antihypertensive drugs (from 1.39 to 2.42 per day). The most pronounced increase in systolic BP was observed during nighttime (119.9, 122.8, 125.3, and 137.3 mmHg respectively in normal, high-normal, micro-, and macroalbuminuria). There was also a progressive increase in the prevalence of the non-dipper pattern, from 50.1% in normoalbuminurics to 72.5% in patients with macroalbuminuria.

**Conclusions**: ABPM characterizes the different stages of albumin excretion in urine and could help to the surveillance of BP control and its influence on albuminuria.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1672**

**Urinary Albumin Excretion within the Normal Range Is Associated with Severity of Coronary Artery Disease** Juan F. Navarro,[1,2] Carmen Mora,[2] Mercedes Muros,[3] Ana Gorgoso,[4] Horacio Perez,[4] Haridian Herrera,[2] Beatriz Meneses.[2] *[1]Nephrology Service, University Hospital Ntra. Sra. Candelaria (HUNSC); [2]Research Unit, HUNSC; [3]Clinical Biochemistry, HUNSC; [4]Cardiology Service, HUNSC, Santa Cruz de Tenerife, Spain.*

Increased urinary albumin excretion (UAE) is associated with a higher risk of coronary artery disease (CAD). However, data about the relationship between normal UAE and CAD are scarce. The aim of this study was to analyze in patients underwent coronary angiography (CA) the relationship between normoalbuminuria and the severity of coronary lesions. Three-hundred and forty seven consecutive patients under elective CA were included. Increased UAE was observed in 101 subjects, whereas 246 patients (146 males, 100 females; mean age 64±12 years) showed normal renal function and normal UAE (albumin/creatinine ratio (A/C) < 30 mg/g). Coronary lesions were observed in 132 normoalbuminuric patients (78 males, 54 females; mean age 64±11 years), whereas CA was normal in the remaining 114 subjects. Prevalence of diabetes, arterial hypertension and dyslipidemia among these subjects were 34%, 61% and 47%, respectively. The mean number of coronary lesions was 2.9. The mean percent occlusion of the left coronary artery (LCA), left anterior descending artery (LDA) and right coronary artery (RCA) were 68%, 70% y 66%, respectively. Patients with coronary lesions showed a higher A/C ratio than subjects with normal coronariography (12.9 vs 8.2 mg/g, p<0.0001). There were no differences regarding the number of coronary lesions when patients were stratified according to UAE. However, subjects in the highest tertile showed a significantly higher severity of coronary occlusion at all locations: LCA (p<0.05), LDA (p<0.001) and RCA (p<0.0001). Multiple regression analysis showed an independent association between A/C and severity of coronary lesions: RCD (r=0.24, p=0.01), LDA (r=0.21, p<0.05), with a trend regarding LCA (r=0.12, p=0.1). In conclusion, UAE within the normal range is independently associated with severity of coronary artery occlusion. Therefore, UAE, even within the normal range, may be a useful clinical tool in the risk stratification of coronary artery lesions.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1673**

**Is There a Relationship between Microalbuminuria and Hypertension in Children?** Mohammed Faizan,[1,2] Atena Asiaii,[1] Siraj Amanullah,[1,2] Sharon W. Su.[1,2] *[1]The Warren Alpert Medical School of Brown University; [2]Department of Pediatrics, Hasbro Children's Hospital, Providence, RI.*

Recently published NHANES data has confirmed the association between microalbuminuria (MA) and hypertension (HTN) in the adult US population. We have previously looked at the relationship between MA and HTN in children as determined by 24 hour ambulatory blood pressure monitoring (ABPM) in a retrospective cohort, but failed to find an association likely due to small sample size. (PAS 2008, *abstract*). **Objective:** To determine an association between MA and HTN as assessed by 24 hour ABPM. **Methods:** Retrospective cohort study from July 1997 to April 2010, on non-diabetic patients between the ages of 0-21 years. All patients had a diagnostic study with 24 hour ABPM in an urban pediatric nephrology clinic. HTN was defined as the 95th percentile of systolic and/or diastolic BP for age, gender and height. MA was defined as an elevated urine microalbumin to creatinine ratio of 19 mg/gm (laboratory definition) on a single random urine sample. **Results:** 229 pts were included. Mean age was 13.7 yrs. 150 (65.5%) were males. 74 pts

(32.3%) met the criteria for HTN. Mean BP in the HTN group was 135/77 vs. 118/65 in the normotensive group. MA was seen in 49 (21.4%) pts. **Microalbuminuria was found to be significantly associated with HTN (OR = 2.2, 95% CI:1.17-4.27).** No statistically significant association was found between hypertension and the following variables*: age, sex, BMI, serum creatinine, glucose, sodium, potassium, family history of hypertension, diabetes, hyperlipidemia, and cardiovascular or renal disease.* **Conclusion:** Viazzi et al have recently reported that microalbuminuria is a predictor of chronic renal insufficiency in non-diabetic hypertensive adults. This relationship is not defined currently in children. Our study shows an association between microalbuminuria and hypertension in children, as determined by 24 hour ABPM. With the explosive rise in the incidence of obesity and metabolic syndrome in children, it is especially relevant to further explore this relationship prospectively, using microalbuminuria as an important screening tool for future risk of morbidity and mortality.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1674

**Control of Hypertension and Other Risk Factors in the Cardiovascular Outcomes in Renal Atherosclerotic Lesions (CORAL) Clinical Trial** Katherine R. Tuttle, Richard A. Dart, Barbara A. Greco, Kenneth A. Jamerson, Alexander M. M. Shepherd, Lance D. Dworkin, William L. Henrich, Donald Cutlip, Timothy P. Murphy, Christopher J. Cooper. *CORAL Trial Study Group, NHLBI, Bethesda, MD.*

The CORAL trial has enrolled patients with atherosclerotic renal artery stenosis (RAS) complicated by hypertension and/or chronic kidney disease (CKD). At entry, participants were randomized to receive stent revascularization or not. All participants receive optimal medical therapy for risk factors. The blood pressure (BP) goal is <140/90, or <130/80, mm Hg in the setting of CKD or diabetes. Renin angiotensin system inhibition is first-line therapy with add-on drugs to achieve BP goals. The LDL cholesterol (LDL-C) goal is <70 mg/dl. Among those with diabetes, the HbA1c goal is <7%. The purpose of this study is to evaluate risk factor control among CORAL participants over time. Age at entry was 69±9 (mean±SD) years with 465/931 (50%) men. Diabetes was present in 297/887 (34%). CKD was common 432/751 (58%), and the estimated glomerular filtration rate was 51±16 ml/min/1.73m$^2$. Bilateral RAS or arterial stenosis in a solitary kidney was identified in 149/931 (16%), while prevalent cardiovascular disease (CVD) occurred in 611/890 (69%).

| BP (mm Hg) | Baseline | 1 year | 2 year | p value |
|---|---|---|---|---|
| <140/90 goal | 248/311 (79%) | 200/213 (94%) | 115/123 (93%) | <0.001 |
| Mean±SD | 151±23/79±14 | 134±20/74±13 | 134±19/74±13 | <0.001 |
| <130/80 goal | 345/574 (60%) | 274/348 (79%) | 131/163 (80%) | <0.001 |
| Mean±SD | 149±23/78±12 | 136±19/73±12 | 134±21/71±12 | 0.001 |
| LDL-C (mg/dl) | | | | |
| <70 goal | 202/699 (29%) | 171/465 (37%) | 106/259 (41%) | 0.013 |
| Mean±SD | 90±33 | 85±33 | 82±39 | 0.100 |
| HbA1c (diabetic) | | | | |
| <7% goal | 168/232 (73%) | 100/163 (61%) | 53/85 (62%) | 0.009 |
| Mean±SD | 6.8±1.4 | 7.2±1.7 | 6.9±1.1 | 0.043 |

**Conclusions:** CORAL participants are at high-risk for CKD and CVD complications. Control of risk factors is approaching goal or improving overall. Whether renal artery stenting adds further benefit on BP, CKD and/or CVD endpoints will be determined as main study outcomes.

Disclosure of Financial Relationships: Consultancy: Fibrogen Inc., Eli Lilly and Co.Research Funding: NIH/NHLBI.

## F-PO1675

**The Effect of Renal Artery Stenting on Left Ventricular Mass: Interim Analysis of the RASCAD Trial** Carmelita Marcantoni,[1] Luca Zanoli,[2] Stefania Rastelli,[2] Giovanni Tripepi,[3] Massimo Matalone,[1] Domenico Di Landro,[1] Corrado Tamburino,[4] Carmine Zoccali,[3] Pietro Castellino.[2] *[1]Nephrology, Cannizzaro Hospital, Catania, Italy; [2]Int Medicine, University of Catania, Catania, Italy; [3]CNR_IBIM, Reggio Calabria, Italy; [4]Cardiology, University of Catania, Catania, Italy.*

The effect of renal revascularization on left ventricular mass (LVM) is not known.

The Stenting of Renal Artery Stenosis in Coronary Artery Disease (RASCAD) study is a RCT designed to evaluate the effect of renal artery stenting+medical therapy (C) vs medical therapy alone (P) on LVM progression (primary endpoint) and CV morbidity and mortality in patients (pts) affected by coronary artery disease (CAD) and renal artery stenosis (RAS).

**Methods**

Pts with RAS>50%≤80% were randomly assigned to undergo C or P therapy. Clinical and echocardiographic studies were performed at baseline and every year. LVM was indexed to BSA (LVMI, g/m2).The interim analysis of enrolled pts who have at least 1yr echocardiographic control is reported.

**Results**

During a 2-year enrolment time, 84 pts were randomized: 43 in C arm and 41 in P arm. At baseline, clinical characteristics were not different between two groups (Male 54% in C vs 65% in P; age 69±8 yrs in C vs 69±9 in P; eGFR 68±24 ml/min in C vs 60±20 in P; LVMI 124±31 g/m2 in C vs 117±27 in P; SBP 133±20 mmHg in C vs 131±16 in P; DBP 73±11mmHg in C vs 74±12 in P). Echocardiographic data at 1yr were available in 38C vs 35P and showed a significant regression of LVM, associated with a better blood pressure control in both groups. However, there were not significant differences between two treatment arms (ΔLVMI -4.9% in C vs -4.1% in P, p=NS; ΔSBP-3.1% in C vs -4.2% in P, p=NS; ΔDBP -2.3% in C vs -6%in P, p=NS) . ΔGFR was also not different between two groups (-1.8ml/minin C vs +1.7ml/min in P, p=NS). The number of major cardiovascular events was similar in the two treatment groups (14 in C vs 19 in P, p=NS).

**Conclusions**

The interim analysis of the RASCAD study showed that the stenting of RAS did not add any advantage to the medical therapy on regression of left ventricular mass in pts affected by CAD. Further, stenting of RAS did not affect CV morbidity and mortality.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1676

**Systolic Blood Pressure and Incident Peripheral Artery Disease in a Community Study** Jennifer A. DeGraauw,[1] Jessica B. Kendrick,[1] Dirk Sander,[2] Michel B. Chonchol.[1] *[1]University of Colorado Denver Health Sciences Center, Denver, CO; [2]Technical University of Munich, Munich, Germany.*

Purpose: Systolic blood pressure (SBP) levels that affects risk for incident peripheral arterial disease (PAD) in the elderly with and without chronic kidney disease (CKD) has been poorly studied.

Methods: Using a community population-based cohort of elderly participants, we evaluated 3354 participants enrolled in Intervention Project on Cerebrovascular Diseases and Dementia over a six year follow-up period. SBP was evaluated in four strata (<120, 120-129, 130-139, and ≥ 140 mmHg). Incident PAD was defined as a new onset of ankle brachial index (ABI) <0.9 assessed at the end of the follow-up period. Chronic kidney disease (CKD) was defined as a Creatinine Clearance (Ccr) < 60 mL/min/1.73 m2 estimated by the Cockroft-Gault equation. The relationship between incident PAD according to SBP level at baseline were tested with Cox proportional regression models. All analyses were adjusted for cardiovascular risk factors.

Results: In those participants with no PAD at baseline (n=2684) their mean (SD) age, SBP, ABI and Ccr were 69±7 years, 139±18 mmHg, 1.08±0.10 and 78±21 mL/min/1.73m2, respectively. During the course of the follow-up period 581 (21%) of patients with no PAD at baseline developed an ABI < 0.9. In this group of patients SBP was directly associated with incident PAD. In fully adjusted models every 10 mmHg increase in SBP had an 8% increase in the risk of developing PAD (HR: 1.08; 95% CI 1.05 to 1.11; p=0.006). SBP at baseline above the reference group (120-129 mmHg) was associated with a higher risk of incident PAD in the 2194 participants with normal kidney function: (adjusted HR and 95% CI) 0.91(0.82, 1.01), 1.0 (REF), 1.29 (1.18,1.41), and 1.51 (1.22, 1.87) for SBP <120, 120-129, 130-139, and ≥ 140 mmHg, respectively after adjusting for confounders. However, there was not a statistical significant relationship between SBP and incident PAD found in the 490 participants with CKD.

Conclusion: Higher SBP levels appear to be an independent risk factor for the development of incident PAD in the elderly with normal kidney function but not in those with underlying kidney disease.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1677

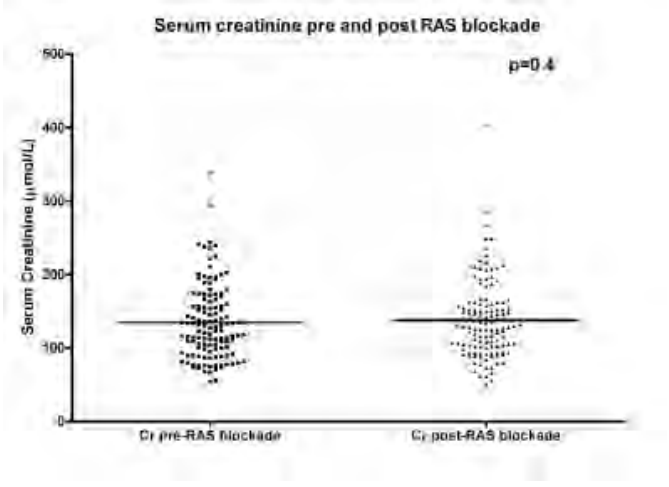
**Renin Angiotensin Blockade Is Safe in Atheromatous Renal Artery Stenosis (ARAS)** Marie Fisk, Jennifer M. Cross. *University College London Partners Kidney Centre, Royal Free Hospital, London, United Kingdom.*

The use of renin angiotensin system (RAS) blockade has historically been contraindicated in the presence of ARAS despite its role in this form of hypertension. HOPE, LIFE and ALLHAT, however, all demonstrate improved survival with RAS blockade in cohorts predicted to have 20-30% occult ARAS.

Aim : To assess whether RAS blockade results in deterioration of renal function in ARAS

Methods : Single centre cohort study of patients with magnetic resonance angiography defined ARAS identified in 2008 and 2009. Degree of stenosis, blood pressure (BP), current medication and plasma creatinine (Cr) were recorded at baseline and 6 monthly intervals. RAS blockade was preferentially prescribed aiming for a BP of 140/80 mmHg. Cr was measured two weeks after drug introduction. Subjects already on RAS blockade were continued and pre drug creatinine sought retrospectively.

Results: 143 patients were identified with a mean study follow up period of 11 months. At baseline 47% had >70% ARAS, 13% bilateral disease and 26% had undergone revasularisation. 64% required > 3 antihypertensive agents. RAS blockade had been commenced in 55% of subjects prior and 28% during the study period. Change in Cr after introduction of treatment (Figure 1), median Cr pre and post-RAS blockade; 129µmols/L and 130µmols/L respectively. At baseline clinic attendance, median Cr =149[SD ±68.33] µmols/L, 6month follow up =157[SD ±68.33]µmols/L. Mean BP at baseline was 142[SD±20.5]/74[SD±13.1]mmHg.




Conclusions: No significant deterioration in renal function was observed following introduction of RAS blockade in this ARAS cohort. Although 6.3% were referred with a label of intolerance to RAS blockade, all were successfully established on treatment. RAS blockade, in combination with other antihypertensive agents, achieved effective blood pressure control with stable renal function over medium term follow up.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1678**

**Effects of Renal Nerve Ablation on Renal Perfusion and Components of the RAAS** Christian Ott,[1] Axel Schmid,[2] Ulrike Raff,[1] Rolf Janka,[2] Michael Uder,[2] Roland E. Schmieder.[1] *[1]Nephrology and Hypertension, University Hospital Erlangen, Erlangen, Germany; [2]Radiological Institute, University Hospital Erlangen, Erlangen, Germany.*

**Purpose of the study:** Renal nerve ablation emerged as new therapeutic approach for treatment resistant hypertension. Measurement of the renal and sympathetic activity revealed a decrease in sympathetic drive to the kidney and small resistance vessels after renal nerve ablation. The precise mechanism how renal nerve ablation exerts its BP-lowering effects are not yet fully understood.

**Methods:** In a pilot study 8 patients (55.4±13 years) with treatment resistant hypertension were included and following assessments were done before (day-1), after (day+1) and again after 3 months of renal nerve ablation. Renal plasma flow (RPF) was measured by magnetic resonance imaging with arterial spin labeling. After 30 min. of complete rest in supine position blood and urine samples were collected for the determination of the individual components of the RAAS.

**Results (median (interquartile range)):** Compared to day-1, there was no change in RPF both day+1 (265 (242-267) vs. 255 (236-89) ml/min/100g, p=0.811) and 3 months (p=0.392) after renal nerve ablation. Plasma renin activity and serum angiotensin II levels did not differ between day-1 and day+1 as well as after 3 months of renal nerve ablation. In contrast, there was a significant acute decrease of aldosterone (day-1: 161 (140-265) vs. day+1: 110 (101-168) pg/ml, p=0.012) and in accordance increased urinary sodium/potassium ratio (day-1: 2.41 (1.17-3.44) versus day+1: 6.02 (4.83-7.92), p=0.028). After 3 months these changes were no longer evident. Urinary angiotensinogen levels, considered as parameter of local renal RAS activity, tended to be reduced at day+1 (p=0.116) and was significantly decreased after 3 months (6.06 (3.02-13.8) vs. 16.6 (8.50-37.0) ng/ml, p=0.046) compared to day-1 levels.

**Conclusion:** Our data indicate that a decrease of aldosterone with the consequence of a greater urinary sodium excretion occurs after the procedure. However, whether this will lead to a long term reduction of total body sodium content needs to be established. Renal perfusion did not appear to be significantly changed.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1679**

**Intradialytic Hypertension Is Associated with Sympathetic Overactivity and with Enhanced Baroreceptor Function** Dvora Rubinger, Rebecca Backenroth, Dan Sapoznikov. *Nephrology and Hypertension Services, Hadassah University Medical Center, Jerusalem, Israel.*

The mechanisms of intradialytic hypertension (HD-Ht) are not well defined. To assess the role of autonomic function during intradialyic hypertensive episodes (Hy), continuous beat to-beat blood pressure and heart rate recordings were performed in non-diabetic patients, 38 with (hypertensive, Ht) and 30 patients without HD-Ht episodes (normotensives, Nt). HD-Ht was defined as an increase in systolic blood pressure of at least 25 mmHg during or immediately after hemodialysis (HD). Systolic (SBP) and mean (MBP) blood pressure, the variability of SBP and interbeat intervals (IBI) in the low frequency (LF) range (LF SBP, LF IBI) and the baroreceptor index LFα were monitored along a whole HD session.

SBP and MBP, similar in Nt and Ht at the beginning (B), were significantly higher in Ht than in Nt at HD end (E). LF indices, representative of sympathetic nervous system activation, were improved after HD in Nt, but not in Ht. SBP, IBI, LF SBP, LF IBI and LFα (median and interquartile range) at HD beginning (B-HD), at the initiation (B-Hy) and during hypertensive episodes (Hy), and at the end (E-HD) of HD are shown in Table1:

Table 1

| Period | SBP (mmHg) | IBI (msec) | LF SBP ($mmHg^2$/Hz) | LF IBI ($msec^2$/Hz) | LFα (msec/mmHg) |
|---|---|---|---|---|---|
| B-HD | 132 (30) | 816 (154) | 80 (33) | 1118 (1515) | 3.40 (2.21) |
| B-Hy | 141 (26) | 842 (144) | 83 (51) | 1367 (1772) | 3.79 (3.55) |
| Hy | 158 (22) | 825 (162) | 76 (40) | 1222 (2002) | 3.55 (2.42) |
| E-HD | 145 (39) | 865 (150) | 71 (44) | 1009 (1546) | 3.57 (2.85) |
| p B-HD vs.B-Hy | 0.006 | 0.013 | NS | 0.010 | 0.024 |
| p B-HD vs.Hy | 0.001 | NS | NS | 0.006 | 0.056 |
| p B-HD vs.E-HD | 0.001 | NS | NS | NS | NS |

These results show that in Ht: 1. HD fails to normalize blood pressure. 2. Initiation of intradialytic hypertensive episodes is associated with increased IBI, LF IBI and LFα. 3. LF IBI and LF α remain significantly increased during Hy. These data point to sympathetic overactivity and enhancement of baroreflex as possible mechanisms of intradialytic hypertension episodes. The causes of sympathetic overactivation remain to be determined.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1680**

**Evaluation of Chronologic Plasma Metanephrine Levels Prior to Diagnosis of Pheochromocytoma** Sylvia C. Yoon, Kevin C. Abbott, Stephen W. Olson. *Nephrology, Walter Reed Army Medical Center, Washington, DC.*

**Introduction**:

Pheochromocytoma is a rare disease with significant morbidity and mortality. Elevated plasma metanephrines are highly sensitive and specific for diagnosis. There is no current knowledge about the progression of plasma metanephrine levels prior to diagnosis. We hypothesize that plasma metanephrines become elevated and continue to rise prior to the diagnosis of pheochromocytoma.

**Methods**:

Thirty-three subjects with biopsy proven pheochromocytoma were identified using military databases. The Department of Defense Serum Repository (DoDSR) identified an age, sex, race, and age of serum matched healthy control for each study subject. Three chronologic serum samples for each subject prior to diagnosis were sent from the DoDSR to Quest diagnostics for measurement of plasma metanephrine levels. Fisher's exact test, T-test, and ROC curve were used for data analysis.

**Results**:

A greater percent of disease subjects had an elevated plasma metanephrine level (>205 pg/mL) prior to diagnosis compared to healthy controls (88% vs. 12%; p=<0.0001). Repeat analysis for subgroups less than 2 years (96% vs. 0%, p<0.0001), 2-8 years (95% vs. 5%, p<0.0001), and greater than 8 years prior to diagnosis (39% vs. 12%, p=0.05) remained significant. An elevated plasma metanephrine level was 95% sensitive and 95% specific for future pheochromocytoma up to 8 years prior to diagnosis. ROC curve demonstrated 100% specificity for plasma metanephrine levels greater than 277 pcg/mL (area under curve = 0.88). Disease subjects had a larger percent of subjects with a greater than 20% average rise in plasma metanephrines per year compared to controls (67% vs. 7%; p<0.0001). A greater than 40% rise in plasma metanephrines per year was 100% specific (50% vs. 0%; p<0.0001).

**Conclusions**:

Elevated and rising plasma metanephrine levels are associated with future pheochromocytoma many years prior to diagnosis. This novel data could provide a future diagnostic and prognostic tool for high risk populations identified by increasingly robust genetic analysis capabilities. A more prompt diagnosis could prevent irreversible outcomes such as end organ damage, metastasis, and peri-operative death.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1681**

**Suppression of Plasma Renin Concentration and Angiotensin II after Inhibition of Systemic Nitric Oxide Synthesis in Healthy Subjects. A Dose-Response Study** Thomas Larsen, Frank H. Christensen, Jesper N. Bech, Erling B. Pedersen. *Departments of Medical Research and Medicine, Holstebro Hospital, Holstebro, Denmark.*

**Purpose**

The relationship between NO and renin has never been investigated in a dose-response study in humans. We wanted to test the hypothesis that systemic NO inhibition causes a dose-dependent suppression of the plasma renin concentration (PRC).

**Methods**

The effect of L-NMMA, an inhibitor of nitric oxide synthase, was investigated in a randomized, placebo-controlled, blinded, cross-over, dose-response study in healthy males (n=12; mean age 20). On four different occasions, subjects received either saline vehicle or one of three different doses of L-NMMA (Bachem) after an overnight fast. L-NMMA was administered as a 3 mg/kg bolus over 3 min, followed by infusion at 2 mg/kg/hr for 60 min, a 4.5 mg/kg bolus + 3 mg/kg/hr infusion, and a 6 mg/kg bolus + 4 mg/kg/hr infusion. Blood samples were obtained at baseline after three hours of supine rest, after 60 min of L-NMMA infusion, and 60 min after cessation of the infusion. PRC and angII were measured using RIAs. Data were analyzed using a general linear model with repeated measures.

**Results**

Mean baseline PRC and angII were 11±4 pg/ml and 9±4 pg/ml, respectively (means±SD), with no difference in baseline values between groups. PRC decreased significantly from baseline during administration of all three L-NMMA doses (dose 1: -20%; dose 2: -30%;

dose 3: -41%), but not during infusion of saline vehicle (9%). Likewise, L-NMMA induced a suppression of angII (dose 1: -15%; dose 2: -22%; dose 3: -33%), whereas saline vehicle did not (4%). Mean arterial pressure (MAP) increased dose-dependently.

**Conclusion**

Systemic inhibition of NO synthesis caused a significant dose-dependent suppression of both PRC and angII in healthy males, which is in good agreement with the role of NO as a stimulator of renin secretion. Thus, the increase in MAP during NO inhibition was *not* mediated by the renin-angiotensin-system. The novel finding that PRC and angII gradually decreased with increasing L-NMMA dose, suggests that basal renin secretion is highly dependent on NO in healthy subjects.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1682

**Shift Work Predicts Disparate Hypertension Risk in African Americans** Sung Ja Lieu, Gary C. Curhan, John P. Forman. *Nephrology, Brigham and Women's Hospital, Boston, MA.*

Background: In the US, the age-adjusted prevalence of hypertension in the non-Hispanic black population is 50% higher than that of the non-Hispanic white population, particularly among black women, and this disparity persists even after controlling for standard hypertension risk factors. Alteration of circadian rhythm, such as with rotating night-shift work or short sleep duration, has been associated with blood pressure elevations in some studies; notably, the adverse physiologic responses to sleep alterations are more pronounced in blacks compared to whites, and thus circadian disruption may be a novel risk factor for hypertension among blacks.

Methods: We prospectively examined the association between rotating night-shift work and the risk of hypertension in 100,484 white and 1,896 black women who participated in the Nurses' Health Study II from 1989-2005. Data on shift work was collected from nurses at baseline and then updated every two years from 1989-2001. Those with hypertension in 1989 were excluded. Associations between rotating night-shift work and incident hypertension were analyzed using Cox proportional hazards regression controlling for age, BMI, physical activity, alcohol intake, DASH score, family history of hypertension, hours of sleep, oral contraceptive use and menopausal status.

Results: Through 2005, we identified 603 incident cases of hypertension in blacks and 19,572 cases in whites. After adjusting for potential confounders, the relative risk for incident hypertension in the blacks was 1.51 (95% confidence interval, 1.11-2.07) and 0.98 (95% confidence interval, 0.93-1.03) in whites. The P value for interaction between shift work and race was 0.001.

Conclusion: Rotating night-shift work is independently associated with hypertension risk in blacks but not in whites. Disruption of circadian rhythms may be a novel risk factor for the development of hypertension in blacks.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1683

**Intradialytic Hypertension Is Associated with Endothelial Cell Dysfunction: Preliminary Results of the Mechanisms and Treatment of Intradialytic Hypertension (MATCH) Study** Jula K. Inrig, Peter N. Van Buren, Bohyun Catherine Kim, Robert D. Toto. *UT Southwestern.*

**Purpose:** Intradialytic hypertension (HTN) is associated with higher hospitalization rates and all-cause mortality, yet the mechanism is uncertain. We hypothesized that intradialytic HTN is associated with endothelial cell dysfunction (ECD), a novel marker of adverse cardiovascular outcomes.

**Methods:** We performed a prospective case-control study including 25 HD patients (pts) without (controls) and 25 HD patients with intradialytic HTN (defined as a systolic BP decrease ≥10 mmHg vs increase ≥10 mmHg during 4/6 consecutive HD sessions). Endothelial cell function was assessed by ultrasonographic measurement of brachial artery flow mediated vasodilation (FMD) and flow cytometric analysis of endothelial progenitor cells (EPCs) from peripheral blood. Wilcoxan rank sum test was used to compare BP, FMD, and EPCs ($ALDH_{br}$+, CD34+/CD133+ cells).

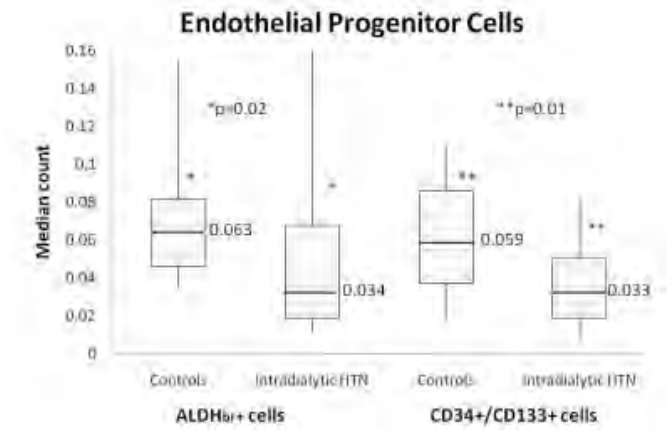
**Results:** Baseline characteristics were similar between groups (Table).

Baseline Characteristics

| | Controls (n=25) | Intradialytic HTN (n=25) |
|---|---|---|
| Age, yrs | 53 | 54 |
| Hispanic | 56% | 64% |
| Black | 40% | 36% |
| Male | 80% | 80% |
| Diabetes mellitus | 80% | 84% |
| Heart failure | 12% | 12% |
| CAD, CVA, or PVD | 36% | 32% |
| PreHD Systolic BP mmHg (2-week average) | 156* | 143* |
| PostHD Systolic BP mmHg (2-week average) | 127* | 159* |

*p<0.01 for between group comparison of pre and postHD SBP

Endothelial cell function was impaired in pts with intradialytic HTN as measured by decreased median $ALDH_{br}$+ cells and decreased median CD34+/133+ cells (Figure). Also, there was a trend toward lower endothelial-dependent brachial artery FMD in pts with intradialytic HTN as compared to controls (2.5±1% vs 3.6±3%, p=0.1).




**Conclusion:** Intradialytic HTN is associated with endothelial cell dysfunction. We speculate that ECD may partially explain the higher hospitalization rates and all-cause mortality observed in these pts.

Disclosure of Financial Relationships: Research Funding: Genzyme.

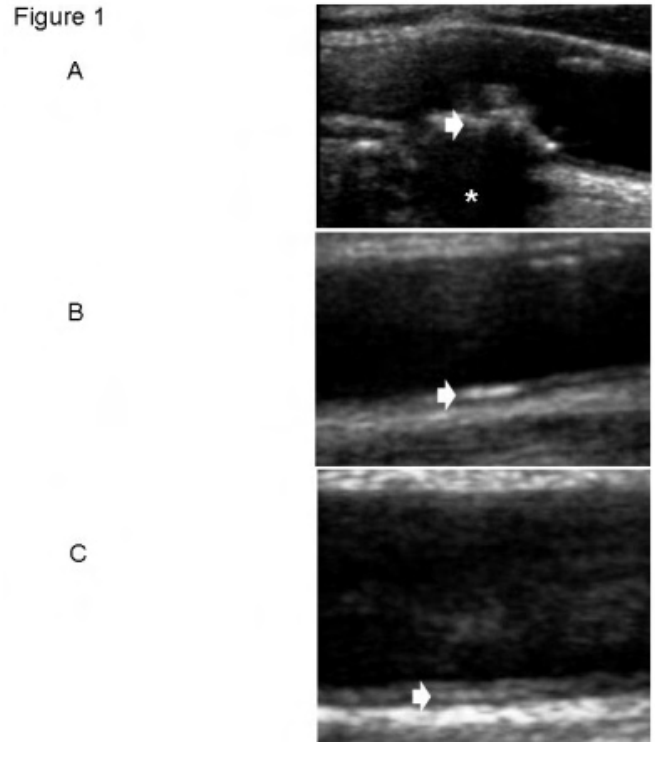
## F-PO1684

**Large Arteries Calcification in Dialysis Patients Is Located in the Intima and Related to Atherosclerosis** Blai Coll,[1] Angels Betriu,[2] M. Vittoria Arcidiacono,[3] Jose M. Valdivielso,[3] Elvira Fernandez.[1] *[1]UDETMA, Hospital Universitari Arnau de Vilanova, Lleida, Spain; [2]UDETMA, Hospital Universitari Arnau de Vilanova, Lleida, Spain; [3]Nephrology Experimental Laboratory, IRB Lleida, Lleida, Spain.*

**Background:** Vascular calcification (VC) has a significant impact in cardiovascular diseases of patients in dialysis. However, VC is assessed with X-ray-based techniques, which do not inform about the calcium localization (intima, media, atherosclerosis-related). The aim of the present work is to study VC using arterial ultrasound to report the exact location of calcium and to study its related factors.

**Design and methods:** observational, cross-sectional, case-control study. Patients under dialysis and age-and-sex matched subjects with normal kidney function were included in the study. Demographic data and laboratory values were collated. Carotid, femoral and brachial ultrasounds were performed to assess VC and atherosclerosis burden using an standardized protocol.

**Results:** 232 patients in dialysis and 208 controls were included in the study. Cardiovascular risk factors were predominantly found in controls, although the burden of atherosclerosis was higher in the dialysis group. VC was significantly more prevalent in the group of dialysis than controls, and in both groups the most prevalent pattern of VC was the linear calcification, located in the intima of the artery wall.



Among the variables significantly and positively related with the linear calcification were age and dialysis. Conversely, the absence of atherosclerosis, low levels of C-reactive protein and phosphorus were significantly protective for the development of linear calcification.

**Conclusions:** vascular calcification in large, conduit arteries is more prevalent in patients in dialysis than controls and is predominantly located in a linear fashion in the intima of the arteries.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1685

**Seasonal and Circadian Variability in Systolic Blood Pressure in Chronic Hemodialysis Patients** Frank Van der Sande,[2] Len A. Usvyat,[1] Jeroen Kooman,[2] Stephan Thijssen,[1] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Renal Research Institute, NY, NY; [2]Maastricht University Medical Centre, Maastricht, Netherlands.*

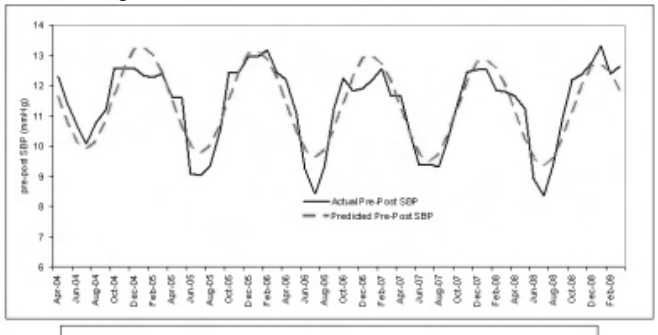
In the hemodialysis (HD) population, systolic blood pressure (SBP) follows circannual and circadian rhythms. The aim of the present study was to assess seasonal and circadian variations in SBP and intradialytic change in SBP across diverse climatic US regions.

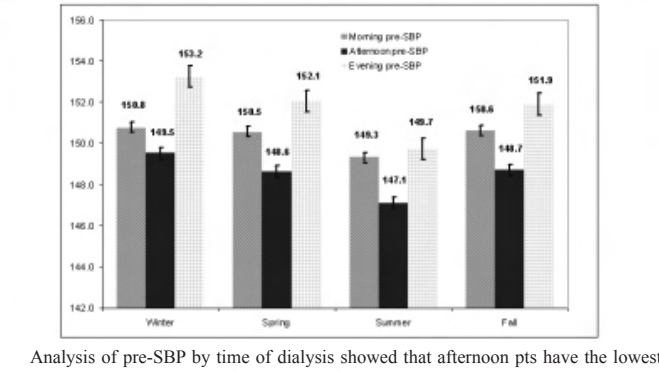
We reviewed records of HD patients (pts) treated in RRI clinics b/n Apr 1, 2004 and Mar 31, 2009. Seasons were defined on calendar basis. Intradialytic change in SBP (ΔSBP) = pre-SBP – post-SBP.

Cosinor analysis was conducted to test for seasonality in pre-SBP and ΔSBP. RRI clinics were grouped in distinct major climate groups: continental, mediterranean, and subtropical. Consideration was given to the dialysis shift (morning, afternoon, evening).

10,303 pts were studied (55% male, 49% black, avg age [stdev]: 60.5 [15.5] yrs). Across all climatic zones, pre-SBP was highest in winter (mean [95% CI]): continental (150.4 [0.15]), mediterranean (146.3 [1.08]), subtropical (152.0 [0.72]). Pre-SBP was consistently lowest in summer: continental (148.4 [0.15]), mediterranean (143.2 [1.04]), subtropical (151.0 [0.74]).

Cosinor analysis demonstrated a seasonal component of pre-SBP and ΔSBP. The drop in ΔSBP was largest in winter and smallest in summer.




Analysis of pre-SBP by time of dialysis showed that afternoon pts have the lowest pre-SBP across seasons & evening pts have highest pre-SBP.

This study demonstrates a significant seasonal influence on pre-SBP and ΔSBP in chronic HD pts with the largest intradialytic SBP drop in winter and the smallest in summer. Evening pts have highest pre-SBP while afternoon pts have lowest pre-SBP. Taken together, evening pts in the winter experience the highest drop in SBP during txt while afternoon summer pts experience the smallest intradialytic drop in SBP.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1686

**Effects of Aliskiren on Blood Pressure and the Predictive Biomarkers for Cardiovascular Disease in Hemodialysis-Dependent Chronic Kidney Disease Patients with Hypertension** Shiho Hanawa, Yoshiyuki Morishita, Junko Chinda, Eiji Kusano. *Nephrology, Jichi Medical University, Shimotsuke, Tochigi, Japan.*

The renin-angiotensin-aldosterone system (RAS) plays pivotal roles in the pathogenesis of hypertension and inflammation both are major risk factors for cardiovascular disease (CVD) in hemodialysis-dependent chronic kidney diseases (HDD-CKD) patients. In this study, we assessed the efficacy of a direct renin inhibitor, aliskiren, on blood pressure (BP) and CVD predictive biomarkers, such as brain natriuretic peptide (BNP), high-sensitivity C-reactive protein (hs-CRP), and diacron-reactive oxygen metabolite (d-ROM) in hypertensive HDD-CKD patients. Thirty hypertensive HDD-CKD patients were assigned to receive aliskiren (150mg) orally once daily with their existing antihypertensives. After 8 weeks, aliskiren treatments reduced systolic blood pressure (SBP) from 169.0±20.1 mmHg to 153.7±19.6 mmHg ($p< 0.001$) and diastolic blood pressure (DBP) from 78.1±12.0 mmHg to 73.0±13.6 mmHg ($p=0.048$), respectively. RAS was suppressed by 8 weeks aliskiren treatment as follows: PRA (from 3.6±4.0ng/ml/hr to 1.0 ±1.5ng/ml/hr ($p=0.004$)), angiotensin I (from 1704.0±2580.9 pg/ml to 233.7±181.0 pg/ml ($p=0.009$)), angiotensin II (from 70.2±121.5 pg/ml to12.4±11.5pg/ml ($p=0.022$)) and aldosterone (from 107.9±148.0 pg/ml to 73.1±34.6pg/ml (N.S)). The biomarkers for CVD were inhibited by 8 weeks aliskiren treatment as follows: BNP (from 362.5±262.1pg/ml to 300.0±232.0pg/ml ($p=0.043$)), hs-CRP (from 6.2±8.1 mg/l to 3.5±3.7 mg/l ($p=0.022$)) and d-ROM (from367.0±89.8 U.CARR to 328.3±70.9 U.CARR ($p=0.022$)). These effects of antihypertensives, blockade of RAS and inhibition of biomarkers for CVD by aliskiren were detected in HDD-CKD patients regardless of the combination with ACEIs and/or ARBs. Furthermore, the inhibition levels of biomarkers for CVD by aliskiren were independent of the decreased levels of SBP and DBP. These results suggested that aliskiren was effective for BP control and may have cardiovascular protective effects independent of its blood-lowering effect in hypertensive HDD-CKD patients.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1687

**Serum Uric Acid Is Associated with Hypertension in Pediatric Dialysis Patients** Douglas M. Silverstein,[1] Poyyapakkam Srivaths,[2] Asha Moudgil,[1] Stuart Goldstein,[3] Daniel I. Feig.[2] *[1]Pediatric Nephrology, Children's National Medical Center, Washington, DC; [2]Pediatric Nephrology, Baylor College of Medicine, Houston, TX; [3]Pediatric Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

**Background:** Elevated serum uric acid (UA) is associated with hypertension (HTN). The role of such an association in children on chronic dialysis and after renal transplant (TX) is not known.

**Methods:** We assessed the association of serum UA with blood pressure (BP) in 46 children receiving maintenance hemodialysis (HD), 17 receiving peritoneal dialysis (PD) and 34 patients with a stable renal TX.

**Results:** UA levels were similar in HD (6.8±0.2) and PD (6.5±0.3 mg/dl) patients, and the levels in both groups were significantly higher compared to TX recipients (5.2±0.2, mg/dL; $p<0.0001$). A negative relationship was observed between UA and dialysis vintage ($r= -0.35$, $p=0.005$) in all dialysis patients and weekly Kt/V in PD patients ($r= -0.52$, $p=0.03$). In TX patients, UA levels were inversely related to GFR ($r= -0.54$, $p=0.0007$) and positively associated with time since TX ($r=0.40$, $p=0.02$). Systolic BP (SBP) percentile was significantly higher in HD compared to TX patients (88.6±2.4 versus 72.4±3.3, $p=0.0004$). Pre-treatment SBP percentile was associated with a high UA level (>6 mg/dl) in HD patients only (91.9±2.3 versus 79.3±5.8, $p=0.01$). SBP and DBP percentiles were 10 and 15% lower, respectively, in PD patients with UA level ≤6.0 mg/dl compared to what those with UA level >6 (p=ns).

**Conclusions:** 1) UA levels are higher in dialysis versus TX patients; 2) UA levels increase with dialysis vintage and time since TX, perhaps related to decreasing residual renal function in dialysis and decreasing GFR in TX patients; 3) UA levels are associated with high BP in HD but not in PD or TX patients.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1688

**The Effect of L-Carnitine Supplementation on the Arterial Stiffness of Patients on Hemodialysis** Botond Csiky,[1,2] Istvan Wittmann.[2] *[1]FMC Dialysis Center, Pecs, Hungary; [2]Nephrological Center and 2nd Dept. of Medicine, University of Pecs, Pecs, Hungary.*

Hypothesis and objectives

The most common cause of morbidity and mortality in patients on chronic hemodialysis is cardiovascular disease. According to data from clinical studies, structural and functional alterations of the large arteries are contributing to the high cardiovascular mortality of these patients. A well accepted way for examining the stiffness of the large arteries is measuring the augmentation index (AIx).

L-carnitine supplementation in hemodialysis patients has beneficial effects on lipid alterations and is improving cardiac function. There is no data on the effect of L-carnitine supplementation on the arterial stiffness.

The aim of the present study was to evaluate the effect of L-carnitine supplementation on the AIx of hemodialysis patients.

Patients and methods

Stable chronic hemodialysis patients (n=22, age=59±13 years, male/female: 14/8) were supplemented with L-carnitine. 11 patients were normotensive, 11 patients were treated hypertensives. The patients medical and dialysis treatment was unchanged during the study period. Renal replacement therapy: hemodiafiltration 3x4 hours weekly using polysulphone membranes. L-carnitine supplementation: 1000 mg iv, after each dialysis treatmet for 9 weeks. Blood pressure measurements were performed with calibrated automatic devices. AIx was measured by applanation tonometry (SphygmoCor, AtCor Inc) prior to the respective dialysis treatment, before L-carnitine supplementation was started and at the end of the 9th week of supplementation.

Results

Predialysis blood pressure measured at the beginning of the carnitine supplementation period and at its end was unchanged (129.3±18.9/76.4±10.9 vs 127.7±12.7/75.2±10.4 mmHg). The AIx was significantly lower at the end of the L-carnitine supplementation period than before it (30.24±14.18% vs 35.86±10.49%; p<0.05).

Conclusions:

L-carnitine supplementation is decreasing the stiffness of the large arteries in chronic hemodialysis patients. Besides its effect on lipid homeostasis and cardiac function, L-carnitine supplementation may decrease the cardiovascular morbidity and mortality of these patients by improving their vascular stiffness.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1689**

**Serum Osteoprotegerin Level and Vascular Stiffness in Hemodialysis Patients** Jung Eun Lee, Sung Jin Moon, Jwa-Kyung Kim, Hye Rim An, Yong Kyu Lee, Sung-Kyu Ha, Hyeong Cheon Park. *Internal Medicine, Yonsei University College of Medicine, Seoul, Korea.*

Vascular calcification and increased vascular stiffness is a frequent finding in uremic patients. Osteoprotegerin (OPG), a soluble decoy receptor of the osteoclast activator, acts as an important regulatory molecule in vascular calcification. Increased OPG levels have been associated with stiffening of the large vessel walls, that may contribute to high cardiovascular mortality observed in hemodialysis (HD) patients. This study was carried out in order to evaluate the risk factors of vascular stiffness and the link between OPG and vascular stiffness in HD patients. We enrolled 97 HD patients. Baseline demographic and biochemical parameters, including calcium phosphate metabolism, were collected prior to a midweek dialysis at the time of arterial stiffness measurements. Arterial stiffness was measured by pulse wave velocity (PWV) using an automated device (VP-2000, Colin Co. Ltd., Japan). Vascular calcification score (VCS) was evaluated using plain radiographs of pelvis and hands (score up to 8) as done by Adragao et.al.(Nephrol Dial Transplant, 2004). Serum OPG level was measured by ELISA kits (R&D systems). We divided two groups according to median value of heart-femoral PWV (1178 cm/s). Higher PWV group was older (59.0±10.8 vs. 54.0±13.2 years, p=0.042), and had more male (67.3 vs. 43.8 %, =0.019) and more DM patients (77.6 vs. 43.8 %, p=0.001), higher DM duration (118.1±124.7 vs. 59.7±107.5 months, p=0.017), lower cholesterol (128±27 vs. 142±37 mg/dL, p=0.033), lower HDL-cholesterol (36±8 vs. 42±14 mg/dL, p=0.011) and more severe VCS patients (41.7 vs. 12.8%, p=0.020). Pulse wave velocity positively correlated with age (r=0.319, p=0.001), DM duration (r=0.329, p=0.001), serum OPG level (r=0.300, p=0.003), and VCS (r=0.343, p=0.001). Multiple linear regression analysis revealed that DM (β=223.9, p=0.007), serum OPG (β=0.081, p=0.012), and moderate-severe VCS (β=155.5, p=0.050) were independently associated with PWV. In onclusion, our data show that increased serum OPG level as well as DM status and severe VCS closely associated with increased vascular stiffness in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1690**

**Evaluating the Utility of Ambulatory Blood Pressure Monitoring in Kidney Transplant Recipients** Kevin C. Wen, Sita Gourishankar. *Department of Medicine, University of Alberta, Edmonton, AB, Canada.*

PURPOSE

Hypertension is a major risk factor for graft failure and cardiovascular disease in kidney transplant recipients(KTX). We aim to assess whether 24 hr ambulatory blood pressure monitoring(ABPM) is superior to clinic blood pressure measurement(CBP) in a cohort of KTX.

METHODS

We performed 24 hr ABPM(Spacelabs) in 244 prevalent KTX, transplanted between 1972 and 2007. CBP values were obtained at the visit closest to day of ABPM. Pearson correlations and linear regression were used to compare CBP and ABPM.

RESULTS

The average clinic SBP was 137.09±19.38mmHg, and DBP was 79.89±10.51mmHg. The ABPM average SBP was 131.34±15.35mmHg and DBP was 75.37±8.8mmHg; daytime ABPM average SBP was 133.54±14.97mmHg and DBP 77.36±9.09mmHg. The correlations between CBP, 24 hr ABPM and daytime ABPM are poor and the range of differences are substantial(Figure 1). CBP measurements overestimate both 24 hr and daytime ABPM by linear regression analysis(p<0.001). 37(15%) patients had increased night-time mean arterial pressure(MAP) and met criteria for "non-dipping". Graft function was not found to be associated with night-time decline in MAP.

CONCLUSION

In this largest study to date in KTX blood pressure monitoring, we found that CBP frequently overestimates ABPM, highlighting the prevalence of white-coat hypertension. The improved accuracy of ABPM will decrease overprescribing of BP medications to avoid hypotension, drug interactions, and non-adherence. In contrast, identifying the nocturnal "non-dippers", not evident by CBP, facilitates appropriate management, as under-treatment of hypertension could lead to accelerated graft dysfunction, cardiovascular disease and mortality. Finally, graft function is not associated with night-time decline in MAP as suggested by previous studies. These data suggest the improved accuracy of 24 hr ABPM is beneficial for BP monitoring in KTX.

| Clinic BP (mmHg) | | ABPM (mmHg) | Correlation | Difference (mmHg) |
|---|---|---|---|---|
| SBP | 137.09±19.38 | Day 133.54±14.97 | 0.555(p<0.001) | -3.56±16.67 (-56 to 57) |
| | | 24 Hour 131.34 ±15.35 | 0.535(p<0.001) | -5.75±17.11 (-64 to 54) |
| DBP | 79.99±10.51 | Day 77.36±9.09 | 0.504(p<0.001) | -2.53±9.24 (-37 to 31) |
| | | 24 Hour 75.37±8.8 | 0.481(p<0.001) | -4.62±9.98 (-37 to 40) |

Disclosure of Financial Relationships: nothing to disclose

**F-PO1691**

**Suppression of the Nitric Oxide Pathway in Metastatic Renal Cell Patients Receiving VEGF-Targeted Therapy** Emily S. Robinson,[1] Eliyahu V. Khankin,[2] Mallika D. Dhawan,[1] Miranda Jo Rogers,[3] Toni K. Choueiri,[3] S. Ananth Karumanchi,[2] Benjamin D. Humphreys.[1] *[1]Brigham and Women's Hospital, Boston, MA; [2]Beth Israel Deaconness Medical Center, Boston, MA; [3]Dana Farber Cancer Institute, Boston, MA.*

**Background**: Hypertension (HTN) and albuminuria are common side effects of vascular endothelial growth factor (VEGF) targeted therapy. The mechanism of HTN in humans receiving these drugs is unknown. We hypothesized that it is caused by suppression of VEGF-mediated vasodilation via the nitric oxide (NO) and prostacyclin pathways.

**Methods**: Urine was collected from 80 patients with metastatic renal cell carcinoma (mRCC) from 2002-2009, 40 at baseline and 40 on VEGF inhibitors. Urinary albumin:creatinine ratio (ACR), nitric oxide (NO) and its downstream effector, cyclic GMP (cGMP), and biomarkers along the prostaglandin pathway: prostaglandin E2 (PGE2), 6-keto PGF 1α, and cyclic AMP (cAMP) were measured and normalized to creatinine. T-tests and Wilcoxon rank sum tests were used to compare linear data.

**Results**: The mean age in both groups was 61.8 years and 76% were male. cGMP/Cr was suppressed in patients on VEGF inhibitors (0.28 pmol/ug vs. 0.39 pmol/ug; p=0.01). There was a trend toward suppression of NO/Cr (0.46 umol/mg vs. 0.62 umol/mg; p=0.09). Both comparisons were strengthened when patients on bevacizumab (a VEGF ligand inhibitor) were excluded and only small molecule tyrosine kinase inhibitors (sunitinib and sorafenib) were analyzed (for cGMP/Cr, p=0.003; for NO/Cr, p=0.01). PGE2, 6-keto PGF1α, and cAMP did not differ between the groups. In patients on VEGF inhibitors, systolic blood pressure rose from 126.7mmHg at baseline to 138.6mmHg on the medications (p=0.0009). The ACR was higher in patients on VEGF inhibitors than those not on VEGF inhibitors (median 18.4mg/g vs. 4.6mg/g; p=0.009).

**Conclusions**: The NO pathway is suppressed in mRCC patients on VEGF targeted therapy suggesting that inhibition of VEGF-mediated eNOS activation may be the primary cause of HTN. These findings support the need for prospective studies exploring these mechanisms and their possible utility as biomarkers of efficacy and toxicity in patients on VEGF inhibitors.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1692**

**Page Kidney: A Forty-Year Single Centre Experience** Andrew Smyth,[1] Bjorg Thorsteinsdottir,[1] Guilherme Oliveira,[2] Garvan Kane,[3] Vesna D. Garovic.[1] *[1]Division of Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [2]Division of Cardiology, MD Anderson, Houston, TX; [3]Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN.*

**Introduction**

Initial clinical description of Page Kidney (PK), a form of renin mediated hypertension, included athletes who became hypertensive after flank trauma causing renal subcapsular hematoma. Subsequently several non-traumatic etiologies were identified. In this study, we review and contrast presenting features, clinical findings, and treatment outcomes among patients with renal subcapsular hematoma leading to hypertension.

**Methods**

We reviewed demographic and clinical data for patients at our institution from 1960-2000 with hypertension attributable to renal hematoma confirmed either by diagnostic imaging or post-operative surgical pathology.

**Results**

Twenty-seven patients with PK and a mean age of 40.8±15.6 years were included. Etiologies included iatrogenic (n=10), spontaneous hemorrhage (n=9) and trauma (n=8). Trauma patients were younger(p=0.017) and hematuria occurred more commonly in spontaneous and iatrogenic cases(p=0.004). GFR at presentation was similar in all groups, as was systolic (SBP) and diastolic (DBP) blood pressures (Table). Surgical treatment was employed in 70.4%(n=19), 42.1%(n=8) of spontaneous, 36.8%(n=7) iatrogenic and 21.1%(n=4) trauma(p=0.231). Post-treatment GFR and DBP were similar in all groups, but SBP was lower in trauma patients (Table).

| Parameter / Etiology | Iatrogenic | Spontaneous | Trauma | p-Value |
|---|---|---|---|---|
| MDRD GFR at presentation (mL/min/1.73.m2) | 31.5 | 37.4 | 63.2 | 0.089 |
| SBP at presentation (mmHg) | 173.2 | 175.2 | 161.8 | 0.277 |
| DBP at presentation (mmHg) | 100.8 | 102.7 | 103.1 | 0.949 |
| MDRD GFR after intervention (mL/min/1.73m2) | 32.8 | 43 | 59.7 | 0.213 |
| SBP after intervention (mmHg) | 160.4 | 167.6 | 134 | 0.049* |
| DBP after intervention (mmHg) | 95.2 | 95 | 87.6 | 0.488 |

**Conclusion**

Among patients with hypertension and subcapsular hematoma, we did not observe significant differences in presenting features, pathological findings, and treatment modalities based on etiology, but those with trauma etiology had improved SBP control after intervention.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1693

**The Clinical Features of Renal Infarction: Single Center, Retrospective Study** Dong-Won Lee, Dong Jun Park, Hyun-Jung Kim, Hyeon Jeong Lee, Jong Woo Seo, Se-Ho Chang. *Internal Medicine, Gyeongsang National University Hospital, Jinju, Republic of Korea.*

Since acute renal infarction is not a common disease that has nonspecific manifestations and laboratory findings, it is often diagnosed late or even missed. Although there are some reports for clinical characteristics of renal infarction, large scale of studies including many patients is rare. To evaluate clinical features and laboratory findings of the patients with renal infarction, we retrospectively reviewed the medical records of ones who were diagnosed as renal infarction from January, 1995 to April, 2010. We enrolled total 80 patients diagnosed as renal infarction by computer tomography and/or ultrasonography. Male to female ratio was 52:28 and the mean age was 58.6±17.2 years old. Underlying diseases were as follows: cardiovascular disease (61.3%), trauma (6.3%), malignancy associated (5%), post-transplantation of kidney (1.3%), iatrogenic (1.3%), intraabdominal mass associated (2.5%), Buerger's disease (1.3%), diabetes or hypertension (11.3%), no underlying disease (10%). The site of renal infarction was right (40.5%), left (36.7%), both (22.8%) and its magnitude was less than 25% (57.7%), 25% ~ 50% (24.4%), more than 50% (17.9%). Initial symptoms were abdominal pain (90%), anorexia (12.5%), nausea (27.5%), vomiting (23.8%), oliguria (1.3%). Initial signs included abdominal tenderness (76.3%), costovertebral angle tenderness (50.0%), fever (10%), and hypertension (32.5%). Initial abnormal laboratory values showed increased WBC (70.0%) and elevation of AST (66.3%), ALT (48.8%), CK (35.3%), LDH (100%), azotemia (serum creatinine ≥1.4 mg/dL, 21.8%). Proteinuria (68.1%) and hematuria (66.7%) was also manifested in renal infarction. The magnitude of renal infarction is positively correlated with presence of azotemia (≥1.4 mg/dL of serum creatinine level, $P < 0.05$). Maximal creatinine level was positively correlated with the magnitude of renal infarction ($P < 0.05$). Because acute renal infarction is usually associated with underlying diseases or conditions, we should intensify our efforts to find underlying disease. In the case of unknown etiology of renal infarction, treatment of plan remains to be determined.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1694

**Beneficial Effects of ARB Olmesartan on Ambulatory Blood Pressure Profile and Renal Function in Hypertensive Patients with CKD** Mai Yanagi,[1] Yoshiyuki Toya,[1] Tetsuya Fujikawa,[2] Hiroyuki Kobori,[3] Satoshi Umemura.[1] *[1]Department of Medical Science and Cardiorenal Medicine, Yokohama City University School of Medicine, Yokohama, Japan; [2]Department of Public Health, Yokohama City University School of Medicine, Yokohama, Japan; [3]Department of Medical and Physiology, Molecular Core in Hypertension and Renal COE, Tulane University Health Sciences Center, New Orleans, LA.*

Objective: In patients with CKD, employing therapeutic strategy to lower blood pressure (BP) and reduce proteinuria is important for a slower progression to kidney failure and a prevention of cardiovascular disease. We examined whether an angiotensin II type I receptor blocker olmesartan would improve ambulatory BP profile in hypertensive patients with CKD. Methods: 46 hypertensive patients with CKD were randomly assigned to the olmesartan treatment group (n=23) or the control treatment group (n=23). At baseline and 4 months after the treatment, 24-h ambulatory BP monitoring and measurements of biochemical parameters were performed. We analyzed the after-treatment/ baseline ratio (A/B ratio) of the ambulatory BP values and renal function parameters in the olmesartan group and the control group respectively, and made a comparison between both groups. Results: There were no significant differences in baseline parameters. The A/B ratio of 24-h ambulatory systolic BP values in the olmesartan group was significantly lower than that in the control group.(0.93±0.08 versus 0.99±0.08; $P$=0.021) There was no significant difference in the A/B ratio of daytime systolic BP values between both groups. However, the A/B ratio of nighttime systolic BP values in the olmesartan group was significantly lower than that in the control group (0.91±0.10 versus 1.00±0.08; $P$=0.006). Furthermore, treatment with olmesartan resulted in a significant decreases in the A/B ratios of proteinuria and urinary type IV collagen excretions. (0.72±0.41 versus 1.45±1.48; $P$=0.036 and 0.87±0.42 versus 1.48±0.87; $P$=0.007) Conclusion: Olmsartan improves diurnal BP profile by preferential lowering of nighttime BP, which is accompanied by effective reduction of proteinuria in hypertensive patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1695

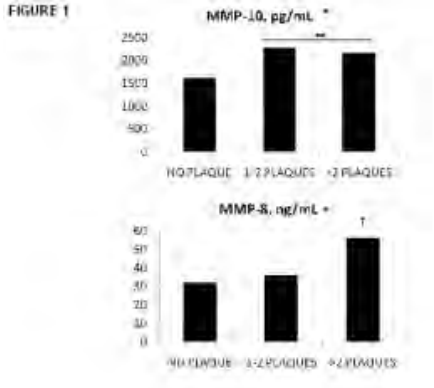
**Serum Matrix Metalloproteinase 10 Is Associated with the Severity of Atherosclerosis in Patients with Chronic Kidney Disease** Blai Coll,[1] Jose A. Rodriguez,[2] Javier Diez,[2] Angels Betriu,[1] Jose M. Valdivielso,[1] Elvira Fernandez,[1] José A. Paramo.[2] *[1]UDETMA, Hospital Arnau de Vilanova, Lleida, Spain; [2]CIMA, University of Navarra, Pamplona, Spain.*

BACKGROUND: Cardiovascular disease is the leading cause of mortality in chronic kidney disease. Matrix metalloproteinases play a major role in atherosclerosis. We hypothesized that alterations in metalloproteinases 8, 10 and their tissue inhibitor 1 can be associated with the severity of atherosclerosis in kidney disease patients.

DESIGN AND METHODS: This is a cross sectional, observational study performed in 111 patients stages I to V, 217 patients on dialysis and 50 healthy controls. The severity of atherosclerosis was estimated with the Atherosclerosis Score, combining the results of ankle-brachial index and carotid ultrasound. Serum levels of metalloproteinases 8, 10 and tissue inhibitor 1 were measured by ELISA.

RESULTS: Metalloproteinases 8,10 and tissue inhibitor 1 levels were significantly ($p<0.001$) increased in patients than controls, being higher in patients on dialysis than in earlier stages of the disease ($p<0.01$). The severity of atherosclerosis score was also more prevalent in the dialysis group (84.7% ), in whom serum metalloproteinases 8, 10 and tissue inhibitor 1 were also significantly ($p<0.05$) higher.

FIGURE 1




After multivariate analysis, metalloproteinase 10, dialysis, C-reactive protein, age and male gender were associated with increased risk of atherosclerosis.

CONCLUSION: Chronic kidney disease patients exhibit elevated levels of circulating metalloproteinase 10, which was independently associated with the severity of atherosclerosis and may represent a new biomarker of atherosclerotic disease.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1696

**Soluble TWEAK as a Novel Biomarker of Atherosclerotic Burden in Subjects with Chronic Kidney Disease** Blai Coll,[1] Jesus Egido,[2] Angels Betriu,[1] M. Vittoria Arcidiacono,[1] Jose M. Valdivielso,[1] Elvira Fernandez.[1] *[1]UDETMA, Hospital Arnau de Vilanova, Lleida, Spain; [2]Vascular Research Laboratory, Fundación Jiménez Diaz, Madrid, Spain.*

BACKGROUND:

Chronic kidney disease (CKD) patients present higher rates of atherosclerosis than subjects with normal kidney function.

Soluble TNF-like weak inducer of apoptosis (sTWEAK) has been inversely related to endothelial function in CKD patients. However, there is no data on the relationship between sTWEAK and atherosclerostic burden in CKD.

DESIGN AND METHODS:

Case-control, cross sectional, observational study. Study population consisted in: 195 patients in dialysis; 58 at CKD stages I-III, 86 at IV-V and 86 controls. Control was defined as normal kidney function, no diabetes and without atherosclerosis. Carotid atherosclerosis was assessed measuring carotid intima-media thickness and identifying carotid plaques. Ankle-brachial index was used to diagnose peripheral atherosclerosis. Plasma sTWEAK concentrations were determined in samples after overnight fasting.

RESULTS:

CKD patients were significantly older than controls (66(13) vs. 54(8), $p<0.001$), and with lower values of body mass index, total, HDL and LDL cholesterol (all $p<0.001$). 126 (37.2%) CKD patients were male and most of them were receiving lowering blood pressure drugs (72.3%) and statins (45%). According to previous reports, sTWEAK levels were significantly reduced in CKD patients compared to controls (315.05 (79) pg/mL vs 401.12 (110); $p<0.05$). Interestingly, patients with more severe atherosclerosis presented a higher reduction in sTWEAK concentrations (311 (79) vs 367 (104); $p<0.001$). In the multiple

linear regression analyses, the variables influencing sTWEAK levels were severity of atherosclerosis (B coefficient -24, p=0.03), previous cardiovascular disease (B coefficient 23, p=0.02) and chronic kidney disease (B coefficients ranging from -93 to -63, p<0.001).

CONCLUSION

A significantly reduction in sTWEAK plasma levels were observed in CKD patients with more severe atherosclerosis. Our results could indicate that sTWEAK is a novel biomarker of atherosclerotic burden in CKD patients

Disclosure of Financial Relationships: nothing to disclose

**F-PO1697**

**Non-Dipper Status and Left Ventricular Hypertrophy (LVH) May Be the Risk Factors for the Development of Chronic Kidney Disease (CKD) in Non-Diabetic Hypertensive Patients** Hye Rim An, Jung-Hwa Ryu, Mina Yu, Seung-Jung Kim, Kyu Bok Choi, Dong-Ryeol Ryu. *Division of Nephrology, Department of Internal Medicine, School of Medicine, Ewha Womans University, Seoul, Korea.*

**Purpose:** In this study, we hypothesized that non-dipper status would be associated with the development of CKD in patients treated with antihypertensive medications.

**Methods:** This study included 102 non-diabetic hypertensive patients with estimated glomerular filtration rate(eGFR)≥60ml/min/1.73m$^2$. Baseline demographic and laboratory data were collected and 24-hr ambulatory blood pressure monitoring(ABPM) and echocardiogram were performed at the beginning of the study, and the levels of serum creatinine were followed up. Non-dippers were defined if their nocturnal systolic BP did not decrease by ≥10% compared to the average daytime BP, and CKD was defined as a sustained decrease in eGFR of <60 ml/min/1.73m$^2$.

**Results:** The average duration of follow-up was 51.7±12.5 months. When participants (mean age 56.0±10.4 years, 39 men, initial eGFR 81.4±16.1ml/min/1.73m$^2$) were divided into two groups as dippers (n=60) and non-dippers(n=42), there were no significant differences in age, duration of hypertension, mean fulltime BP by 24-hr ABPM, urine albumin/creatinine(A/C) ratio, and eGFR between two groups. During the follow-up period, an eGFR < 60ml/min/1.73m$^2$ occurred in 11 patients, and the incidence of CKD was higher in non-dippers compared to dippers [8(7.8%) vs. 3(2.9%) patients, p<0.05]. When comparisons were made between patients who developed CKD and not, patients with CKD had higher baseline urine A/C ratio (52.3±58.6 vs. 17.8±29.3mg/g, p<0.05), higher prevalence of LVH [3(27.3%) vs. 5(5.5%) patients, p<0.05) and lower serum HDL-cholesterol (41.7±8.3 vs. 50.4±12.4mg/dl, p<0.05). In a multiple logistic regression analysis, non-dipper status (OR 6.91), the presence of LVH (OR 35.4), and initial eGFR (OR 0.85) were independent risk factors for the development of CKD.

**Conclusion:** These findings suggest that non-dipper status and LVH may be the therapeutic targets for the prevention of the development of CKD in non-diabetic hypertensive patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1698**

**Ambulatory Blood Pressure Monitoring: Role in Patients with Chronic Kidney Disease and Hypertension** Erdal Sarac. *Internal Medicine, St Elizabeth Hospital, Youngstown, OH.*

Hypertension is present 80-90% patients with CKD with increased risk of cardiovascular events in this population**.** Most CKD patients require multiple BP medications and only 11% of patients reach target BP goal of < 130/80 mmHg. We performed a retrospective study to evaluate the benefits of 24 hour Ambulatory Blood Pressure Monitor (ABPM) in this population.

A retrospective chart review was undertaken of non dialysis (stage 3-5) CKD patients (n=102) with hypertension who underwent 24 ABPM study. The patients were seen at an outpatient CKD clinic and had both office BP measurement and subsequent 24 hour ABPM study. Demographics, past medical history, labs, and number of BP medications were included. Statistical analysis was undertaken using SPSS 17 with *t* test and *chi square*. Office BP was assessed by staff twice with appropriate manual BP cuff in one visit and classified as hypertensive or normotensive with cut off BP of 140/90 mm Hg. 24 hour ABPM was performed, using Welch Allyn 6100*: a)* Average day time, night time and 24h BPs were recorded b) classified as hypertensive ( true HTN, masked HTN) if 24h average BP>135/85 *c)* classified as normotensive (white coat HTN, normotensive) if 24h average BP <135/85 *d)* dippers and non-dippers as per definition.

The results revealed that Office BP misdiagnosed BP in 42% cases and could not identify white coat HTN and masked HTN. As much as 51% of hypertensive patients with Office BP measurement turned out to have white coat HTN (WCH)**.** In addition, 24% normotensive patients with Office BP measurement turned out to have masked HTN with a prevalence of 8%. Non-dipper status prevalence was 58%, concordant with literature and Correlated with advance age (p<0.05).

In summary, office BP measurement in patients with CKD is not sufficient. Combining office BP and ABPM can avoid misclassification of HTN, therefore can avoid under treatment of masked HTN or overtreatment of WCH. Therefore, we recommend use of 24 hour ABPM study in hypertensive CKD patients to achieve target BP and improve cardiovascular risk in this patient population.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1699**

**Modifiable Risk Factors for Incident Hypertension Are Attenuated in Older Women** Lisa J. Cohen, Gary C. Curhan, John P. Forman. *Nephrology, Brigham and Women's Hospital, Boston, MA.*

PURPOSE OF STUDY: Because the pathobiology of hypertension changes with advancing age, we investigated whether diet and lifestyle factors that strongly influence the risk of new-onset hypertension in younger and middle aged women would have similarly robust associations in an older population.

METHODS: We used the Nurses' Health Study I to perform a prospective cohort study in 87,001 women without a baseline history of hypertension**.** Person-time and new cases of hypertension were measured during 24 years of follow-up, and stratified into three age groups (under 50 years, 50-60 years, over 60 years). Cox proportional hazard regression models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI) for six modifiable lifestyle factors. Attributable fractions were calculated to estimate the percent of hypertension that could hypothetically be avoided had all women in a given age group adhered to various combinations of healthy lifestyle factors. We tested appropriate interaction terms to determine whether the HR for individual risk factors and the attributable fractions varied significantly by age group.

RESULTS: During 1,184,602 total person-years of follow-up, 37,348 incident cases of hypertension occurred. We found significant age-related decreases in the associations between BMI, heavy (>30 g/week) alcohol intake, and analgesic use with incident hypertension. For instance, compared to a women with a BMI<25, a woman under age 50 with a BMI>30 had a 5.35-fold greater risk of developing hypertension. For women between age 50 and 60, the HR was 3.17. In women over 60, the HR was 2.14. We also found that attributable fractions for a combination of healthy lifestyle factors became significantly weaker in the older age groups. For example, had all cohort participants had both a normal BMI (<25) and a high degree of physical activity, then 65% of hypertension could hypothetically have been prevented among women <50 years old, but this fraction decreased to 52% for women aged 50-60 years, and to 42% for women older than 60 years.

CONCLUSION: The association between classic risk factors for incident hypertension may be attenuated in older women.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1700**

**Resistant Hypertension: A CKD Clinic Intervention Program To Improve Patient Care** Aashish K. Pandey, Susan P. Steigerwalt, Susan M. Szpunar, Joel Topf, Keith A. Bellovich, Robert Provenzano. *Nephrology and Hypertension, St. John Hospital and Medical Center, Detroit, MI.*

Twenty percent of patients treated in a CKD clinic can be labeled as having resistant hypertension (RH) yet are often overlooked. RH is defined as blood pressure that remains above goal in spite of the concurrent use of three antihypertensive agents of different classes. Having to manage multiple co-morbid conditions in this clinic setting leads to incomplete evaluations for the causes of RH. Checklist reminder systems improve adherence to treatment guidelines. We investigated whether a checklist reminder system and educational interventions improve the rate of completing a thorough evaluation of CKD patients for the causes of resistant hypertension.

After analysis of our database of clinic visits for CKD stages 2-4 from January 2007-December 2009, 130 patients were identified with RH by the above criteria, who had three or more office visits within 18 months, which served as our baseline cohort. The interventions consisted of : a checklist reminder to the provider placed on each patient chart; BPTru™ cuffs were utilized for measuring seated and standing blood pressure for each patient visit; and patient teaching materials on home blood pressure monitoring, physical activity and diet were readily made available in the clinic.The Epworth sleepiness scale was attached to each new patient chart.

A preliminary result 3 months subsequent to these interventions has identified 31 RH patients out of a total of 133 patients (23.3%).

A comparison between baseline data and post intervention are as follows:

Charateristics

| | baseline(n=130) | post intervention (n=31) |
|---|---|---|
| Standing Clinic BP | 8.5% | 80.6% |
| Home BP utilized | 8.8% | 93.5% |
| Meds increased at visit | 23.6% | 64.5% |
| OSA considered | 7.9% | 77.4% |
| Blood aldosterone/renin | 4.6% | 44.8% |
| 24 hour urine Na | 1.5% | 41.9% |
| other secondary evaluation | 35% | 40 % |

As this is an interim analysis, statistical testing will not be performed until the end of the intervention. Our preliminary results show dramatic improvement in these strategies to improve the evaluation and management of resistant hypertension by staff education and provider checklist reminder systems.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1701**

**Decreased eGFR without Evidence of Kidney Damage in Women with a History of Gestational Hypertension (GH)** Catherine M. Brown, John J. Walshe. *Department of Nephrology, Beaumont Hospital, Dublin 9, Ireland.*

**Study Objective:** Preeclampsia has been limked to an increased risk of subsequent ESRD.[1] Our aim was to assess renal function in a group of women 2 years post partum with a previous history of gestational hypertension (GH) in pregnancy and women with uncomplicated pregnancies (controls).

**Methods:** A cross sectional observational study; women with a previous history of GH (n=33) and a group of women with a history of normal BP throughout pregnancy (n=41). Gestational hypertension was defined as a systolic BP of at least 140 mmHg and/or a diastolic BP of at least 90 mmHg on two occasions at least 6 hours apart after the 20th week of gestation, without proteinuria (< 0.3 gm in a 24 hour urine collection). At 2 years post partum, levels of serum creatinine (umol/l), eGFR (using IDMS-traceable MDRD study equation), urinalysis, total cholesterol, BMI and age were recorded. Applanation tonometry was used to record central blood pressure.

**Results:**

Mean values (± SD)

| | Control (n=41) | GH (n=33) | ANOVA p-value |
|---|---|---|---|
| Age (years) | 33 ± 3.7 | 33 ± 4.2 | p= 0.8 |
| BMI (kg/m²) | 24 ± 3.4 | 30 ± 7.2 | p<0.001 |
| Central Systolic BP mm Hg | 109 ± 8.1 | 123 ± 16 | p< 0.001 |
| Central Diastolic BP mm Hg | 71 ± 6.2 | 80 ± 9 | p< 0.001 |
| Creatinine (umol/l) | 67 ± 11 | 74 ± 10 | p = 0.009 |
| eGFR (MDRD) | 92 ± 17 | 82 ± 12 | p= 0.008 |
| Total cholesterol ( mmol/l) | 4.5 ± 0.7 | 5.2 ± 0.8 | p= <0.001 |

Urinalysis was negative in all cases. After adjustment for age, BMI and central systolic BP, differences in serum creatinine remained significant between the GH and control group (p= 0.03) as did eGFR between the two groups (p=0.04).

**Conclusions:** Women with a history of GH have a higher serum creatinine and a lower eGFR compared to controls. Although they have no obvious kidney damage, this group of women with a history of GH, may be at increased risk of future kidney disease and require ongoing monitoring. Long term studies of women with gestational hypertension are warranted.

[1]Vikse BE, Irgens LM, Leivestad T, Skjaerven R, Iversen BM. Preeclampsia and the risk of end-stage renal disease. N Engl J Med. 2008 Aug 21;359(8):800-9.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1702

### Gene Polymorphisms Contributing to Hypertension in IgA Nephropathy

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**Background:** Hypertension (HT), which is one of the major risk factors for chronic kidney disease, has been reported to be affected by multiple genetic and environmental factors. The aim of the present study was to identify genetic factors associated with HT in patients with IgA nephropathy (IgAN), the most common glomerulonephritis in the world, with its onset mainly in younger population.

**Design and setting:** Multicenter cross-sectional study.

**Patients:** 240 patients aged 15-50 years with urinary protein ≥0.25g/day among 1132 patients diagnosed as IgAN by renal biopsy in three major nephrology centers in Osaka, Japan between 1990 and 2005.

**Outcome:** HT defined as ≥140 and/or 90mmHg of systolic and diastolic blood pressure or use of antihypertensives at renal biopsy.

**Independent variables:** 28 polymorphisms with the frequency of minor genotype ≥10% among 100 candidate polymorphisms and clinical characteristics at diagnosis: age, gender, BMI, eGFR, urinary protein, total cholesterol, and uric acid.

**Statistics:** We assessed associations between HT and polymorphisms using $\chi^2$test in dominant and recessive models. We identified polymorphisms associated with HT even after adjustment for clinically relevant factors in multivariate logistic regression models.

**Results:** Baseline characteristics: age 33(24-43) years (median (interquartile range)), male 40.4%, systolic and diastolic blood pressure 122±17/76±14mmHg (mean±SD), HT 36.3%, eGFR 79±27mL/min/1.73m², and urinary protein 0.7(0.4-1.3)g/day. Among 28 polymorphisms, *CD14* (-159CC vs. CT/TT P=0.028) and *ACE* (DD vs. DI/II P=0.034) were significantly associated with HT. Multivariate logistic regression models revealed that *CD14* -159CC (Odds Ratio 3.55[95%CI 1.66-7.61]) and *ACE* DD (4.22[1.72-10.3]) were independently associated with HT.

**Conclusion:** *CD14* C159T and *ACE* I/D contributes to HT in IgAN patients.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1703

### Transvenous Biopsies of Kidneys beyond Renal Artery Stenosis Demonstrate Fibrosis Correlated to Reduced Single Kidney Blood Flow but Not Urinary or Blood TGF-beta Levels

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**Background and Methods:** The relationship between post-stenotic kidney fibrosis and blood flow in human atherosclerotic renal artery stenosis (ARAS) is poorly understood. We examined tissue from transvenous kidney biopsies for percent fibrosis (trichrome sections) and immunohistochemical staining for transforming growth factor beta (TGF-beta) in 9 subjects with measured single kidney blood flow (contrast transit on multi-detector CT) and GFR (iothalamate). Renal vein renin, oxygen, and TGF-beta levels were obtained. Biopsies were compared with age, gender and statin matched nephrectomy specimens from patients with total vascular occlusion.

| | Moderate ARAS with biopsy N=9 kidneys | Severe ARAS with nephrectomy N=9 kidneys | |
|---|---|---|---|
| Age (years) | 64.2±3.3 | 62.2±2.9 | NS |
| Creatinine (mg/dL) | 1.1±0.1 | 1.8±0.2 | p<.01 |
| ACE/ARB Rx | 9 | 7 | NS |
| Statin Rx | 3 | 2 | NS |
| Kidney size (long axe in cm) | 10.3±0.5 | 8.9±0.3 | p<.05 |
| Single kidney GFR (mL/min/kidney) | 29.3±6.6 | | |
| GFR/1.73 (mL/1.73m2) | 67.5±8.5 | 42.5±4.9 | p<.05 |

**Results:** The degree of tissue fibrosis in biopsy samples ranged from 4 to 26%. The semi-quantitative TGF-beta staining score was 2.3 (0=0%, 1 <25%, 2 =25-50%, 3 >50%) and despite higher GFR overlapped the range in kidneys with total occlusion. Kidney was smaller in nephrectomy samples. Percentage of renal blood flow in post-stenotic kidney (RBF) was inversely related to the trichrome estimate of fibrosis (R=.84, p=.0093) and the TGF-beta score (R=.77, p=.038). Urinary and venous measurements of TGF-beta did not correlate with blood flow, TGF-beta staining or kidney function in these studies, nor did they differ from patients with essential hypertension.

**Conclusion:** These data demonstrate parenchymal tissue fibrosis and TGF-beta immunostaining in ARAS biopsies are related to reduced blood flow, are manifest before total occlusion, and are not reflected in peripheral blood or urinary levels of TGF beta.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1704

### Seasonal Variation in Endothelial Function and Migration of CD 34+ Cells May Be Due to Changes in Vitamin D Levels

Rajesh Mohandas, Mark S. Segal. *Department of Medicine, University of Florida, Gainesville, FL.*

Seasonal variation in the incidence of heart disease and stroke has been well documented in several multicenter trials. The exact mechanisms that underlie this are unclear, but changes in temperature, pollution, lipids and other mechanisms have been proposed. Low levels of vitamin D have been associated with increased mortality and cardiovascular events. It has also been shown that there are seasonal variations in serum vitamin D levels, even in people living in warmer weather. However, the effect of vitamin D on endothelial function and angiogenesis is unclear. Recently progenitor cells have been isolated from circulation that might have an important role in maintaining endothelial health. We hypothesized that the seasonal variations in endothelial function are the result of variations in vitamin D levels and its effects on bone marrow derived progenitor cells. **Methods:** We utilized data and specimens that were collected prospectively for a study examining the effects of lowering uric acid on hypertension and endothelial function in African Americans. To better delineate a seasonal effect, data was grouped into those collected end of summer (Aug-Sept) and end of winter (Feb-Mar). Endothelial function was assessed by peripheral arterial plethysmography. CD34+ cells were isolated using immunomagnetic beads coated with antibodies to CD 146. Migration of CD34+ was assessed using a Boyden membrane chamber by migration of CD34+ cells towards recombinant human SDF-1. **Results:** Migration of CD34+ were significantly lower at the end of winter compared to end of summer [(2.3 ± 2.08 n=14) vs. ( 8.2 ± 8.8 n=18) p < 0.05)]. Endothelial function as determined by EndoPAT trended to be improved at the end of summer [2.51 ± 0.68 (n=9)] compared to end of winter [2.14 ± 0.56(n=10) p = 0.22]. Mechanistically, when CD34+ cells were treated with exogenous vitamin D3, they showed enhanced migration towards SDF. **Conclusion :** Seasonal variation in vitamin D levels as well as the direct effects of vitamin D on the migration of CD34+ cells suggests that the seasonal changes in endothelial function, are at least in part, due to the direct effect of vitamin D on CD34+ cells.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1705

### Involvement of Matrix Metalloproteinase-2 in the Development of Aortic Calcification in Uremic Rats

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Background: Vascular calcification is the most important cause of cardiovascular disease in patients with chronic kidney disease (CKD). Medial layer vascular calcification, which is recognized to be an active process (the transformation of vascular smooth muscle cells into osteoblast-like cells), is common in CKD patients, We have recently reported the possibility of interaction between elastin degradation and medial layer vascular calcification *in vitro* (Calcif Tissue Int 2009). Matrix metalloproteinase-2 (MMP-2), which induces the degradation of elastin, has been implicated in the elastic calcification in arteries of CKD patients. However, the precise mechanisms by which elastin degradation interacts with the development of vascular calcification remain to be studied.

Methods: To clarify the mechanisms by which elastin degradation is involved in the development of medial layer vascular calcification in the uremic milieu, we induced aortic medial layer calcification in 5/6 nephrectomized uremic rats (male Sprague-Dawley rats) fed a diet containing high phosphate (1.2%) and lactate (20%). After 8 to 10 weeks, the rats were euthanized for the measurement of serum chemistries and histological analyses.

Results: The uremic rats showed significant increases in blood pressure, serum creatinine, phosphate, and parathyroid hormone levels compared with those of normal rats. Von Kossa staining showed the medial layer aortic calcification in some of uremic rats. In calcified lesions, a decrease in elastin was observed by elastin staining indicating that elastin degradation could occur in the calcified area. In addition, MMP-2 expression determined by immunohistochemistry was observed in the calcified area.

Conclusions: Elastin degradation induced by MMP-2 might be involved in the development of aortic calcification in uremic rats.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1706**

**The Selective P2X$_7$ Receptor Antagonist A438079 Ameliorates Vascular Smooth Muscle Cell Calcification *In Vitro*** John William Booth,[1] Jill T. Norman,[1] David C. Wheeler,[1] Robert J. Unwin,[1] Frederick W. K. Tam.[2] *[1]Centre for Nephrology, UCL Medical School, United Kingdom; [2]Imperial College Kidney and Transplant Institute, Imperial College, United Kingdom.*

**Introduction** Vascular calcification (VC) in chronic kidney disease (CKD) is now recognised to involve an active process in the vessel wall, where vascular smooth muscle cells (VSMCs) adopt an osteoblastic phenotype *in situ.* The P2X$_7$ purinoceptor (P2X$_7$R) is an ATP-sensitive, non-selective cation channel with roles in inflammation and cell death; it is also expressed in osteoblasts, where it contributes to normal bone formation. We hypothesized that P2X$_7$R may have a role in the pathogenesis of VC and tested this using an *in vitro* model.

**Methods** Primary human coronary artery VSMCs (TCS Cellworks) at 50-60% confluence were grown for 7 days in normal medium ($Ca^{2+}$ 1.8mM, $PO_4^{2-}$ 1.0 mM) or calcification medium ($Ca^{2+}$ 2.7mM, $PO_4^{2-}$ 2.0 mM), with or without lipopolysaccharide (LPS) 10µg/ml as an inflammatory stimulus, in the absence or presence of the selective P2X$_7$R antagonist A438079 (1, 3, 10, 20µM; Tocris Bioscience). Calcification was assessed qualitatively by phase contrast microscopy. Calcification at day 7 was quantified colorimetrically by the *o*-cresolphthalein complexone method, corrected for cell protein concentration. Expression of P2X$_7$R and the inflammatory cytokine interleukin (IL)-1β mRNA were measured by quantitative RT-PCR.

**Results** High $Ca^{2+}$ and $PO_4^{2-}$ concentration induced calcification compared with normal medium (0.348 µg calcium/µg protein vs 0.016; $p<0.001$). No significant increase in calcification was seen with LPS. Addition of A438079 (10-20µM) led to a marked reduction in calcification (10µM, 0.085 vs 0.348; $p=0.002$). This effect was sustained in the presence of LPS. Both LPS and calcification media induced expression of IL-1β mRNA. A438079 had no effect on expression of this cytokine. No significant change in P2X$_7$R mRNA expression was seen with high $Ca^{2+}$ and $PO_4^{2-}$ medium or LPS, individually or in combination.

**Conclusion** The P2X$_7$R antagonist A438079 reduces calcification significantly in this *in vitro* model. Hence P2X$_7$R appears to be a promising candidate for investigation as a future treatment target for VC in CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1707**

**Early Compromised Phosphate Handling in Renal Failure Leads to Vascular Calcification In Vitro and in the Adenine Rat Model of Chronic Kidney Disease** Navid Shobeiri,[1] David P. Beseau,[1] Kristin M. McCabe,[1] Michael A. Adams,[1] Rachel M. Holden.[2] *[1]Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada; [2]Medicine, Queen's University, Kingston, ON, Canada.*

Vascular calcification (VC) is markedly accelerated in patients with chronic kidney disease (CKD) and is a major risk factor for mortality in this population. Phosphate (P) is considered to be a key signaling molecule involved in the development of VC. We used the adenine rat model of CKD to assess the time course of VC development and an in vitro organ culture approach to determine aortic predisposition to VC. Using pieces of aorta incubated in DMEM (0.9 to 3.8mM P) for 2 or 4 days we determined that in vitro calcification only occurred at phosphate concentrations above 3.4mM. Rats (14 wks old) were given an adenine diet (0.25%) for 3-11 weeks. This intervention generated mild kidney failure at 3 weeks (serum creatinine 142±12µM vs control 50±5 µM) and more severe failure after 5 weeks (creatinine: 232±30 µM). At 5 weeks these CKD animals had significantly elevated serum phosphate over controls (5.3±0.5 vs 2.4±0.2 mM). At 3 weeks, none of the animals had VC whereas at 5 weeks (17%), 7 weeks (57%) and 11 weeks (78%) there was a progressive increase in the incidence of VC. Compared to control aortas (1.1±0.4 Ca; 2.0±0.7 P µg/mg) incubated in pro-calcification media (3.8mM phosphate) for 2 days, aortas taken from CKD animals pre-calcification (i.e. @ 3 weeks of adenine) had significantly increased tissue calcium (Ca) and phosphate content (3.5±0.8 Ca; 6.3±1.3 P µg/mg) and this process rapidly progressed in all aortas by day 4 (17.6 µg/mg in CKD; 15.3 µg/mg in controls). Furthermore, in our in vivo experiments, CKD animals which eventually calcified had early elevation of serum phosphates (6.0±0.4mM) compared to CKD animals that did not calcify (4.4±0.3mM). Using an organ culture approach, our results reveal, that adenine-induced CKD promotes an increased predisposition of the aorta to phosphate-induced calcification in vitro at a very early stage of the disease. This finding supports the concept that animals with established systemic phosphate elevation early in CKD proceed to develop VC.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1708**

**Mouse Aortic Rings Develop Medial Calcification under Elevated Phosphate Conditions That Is Accelerated by Treatment with Elastase** Cecilia M. Giachelli,[1] Wei Ling Lau,[2] Ashwini S. Pai.[1] *[1]Bioengineering, University of Washington, Seattle, WA; [2]Nephrology, University of Washington, Seattle, WA.*

Hyperphosphatemia has emerged as a non-traditional risk factor for vascular calcification and mortality in the chronic kidney disease population. Recently, elastin degradation by matrix metalloproteinases and elastases has been suggested to play an important role in uremic arterial calcification. We studied the vascular wall changes that occur in mouse aortic rings cultured in elevated phosphate (Pi) medium.

Aortas from C57BL/6 mice were harvested, stripped of perivascular fat and cut into 2-3 mm sections. Rings were cultured in DMEM/5% FBS for 9 days with Pi concentrations varying between 1-3 mM. For time-course analysis, rings were cultured in 2.2 mM Pi for 15 days. Calcium content in non-cultured day 0 mouse aorta was 0.93 ± 0.22 mcg calcium normalized to dry weight (mcg/mg). The aortic rings developed medial calcification that was more severe with increasing phosphate concentration (1.6 ± 0.56 mcg/mg at 1 mM Pi, vs 36.81 ± 6.52 mcg/mg at 1.8 mM Pi, vs 104.15 ± 8.56 mcg/mg at 3 mM Pi). Degree of calcification increased with time in 2.2 mM Pi medium (4.48 ± 1.36 mcg/mg at day 3, vs 42.52 ± 12.54 mcg/mg at day 9, vs 97.8 ± 11.06 mcg/mg at day 15). Vascular smooth muscle cells in the medial layer showed loss of SM22-alpha expression with upregulation of Runx2/Cbfa1 by immunohistochemical analyses, indicating a transition to a bone-forming phenotype.

To determine the effect of elastin degradation on calcification, aortic rings from DBA/2J mice were cultured for 9 days in DMEM/5% FBS containing 3.2 mM Pi. A subset of rings was incubated with elastase for 24 hours prior to the 9-day culture. Calcium content from control aortic rings cultured in standard 1 mM Pi medium was 10.41 ± 2.48 mcg/mg. Rings cultured in 3.2 mM Pi developed calcification in the range of 81.22 ± 4.62 mcg/mg. Elastase pretreatment produced more severe calcification (130.55 ± 20.28 mcg/mg).

In conclusion, mouse aortic rings develop medial calcification that is dependent on phosphate dose and duration of exposure. Elastin degradation accelerates this phosphate-induced vascular calcification.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1709**

**Age-Related Increase in the Predisposition to Vascular Calcification in Adenine-Induced Chronic Kidney Disease** Kristin M. McCabe, Navid Shobeiri, Bonnie W. Y. Shum, Kimberly J. Laverty, David P. Beseau, Michael A. Adams, Rachel M. Holden. *Queen's University, Kingston, ON, Canada.*

The development of vascular calcification (VC) in elderly patients generates significantly increased cardiovascular risk. In patients with chronic kidney disease (CKD) the VC process occurs much more rapidly. There remains a significant gap in knowledge regarding the relevant factors contributing to this accelerated development. In the present study we sought to determine whether age, as well as treatment with warfarin, impacted the time course, magnitude and consequences of CKD in an adenine model. Male Sprague Dawley rats were fed a diet containing either 0.25% adenine (CKD) or 0% adenine (control), both diets containing high phosphate (1%) and low vitamin K (0.2 mg/kg). Animals began the diet at either 9 or 14 weeks of age. After 6-7 weeks on the diet, kidney function was assessed by measuring serum creatinine and VC was assessed using the calcium-O-cresophthalein assay. Creatinine levels were significantly elevated in the CKD group (young, 245.7 ± 43 µmol/L; old, 280 ± 77 µmol/L) compared to controls (67 ± 19 µmol/L). Serum phosphate levels were also elevated in CKD animals (1.5x) and linearly correlated with creatinine ($r^2=0.73$, $p<0.0001$). FGF-23 serum levels were elevated by over 10 fold in CKD animals as compared to control animals and this had a positive correlation with serum creatinine ($r^2=0.70$, $p=0.0001$) and serum phosphate ($r^2=0.35$, $p=0.02$). Warfarin treatment did not alter the levels of creatinine, phosphate and FGF-23. Thus, the dietary adenine model was found to produce equivalent CKD-related outcomes (creatinine, phosphate, FGF-23) in the young and old groups. Despite the similar stimulus vascular calcification was only observed in the older group; i.e. the calcium content of the thoracic aorta of older animals (31 ± 26 ng/mg) was more than 200 fold higher than in young animals (0.12 ± 0.02 ng/mg). Taken together, these results indicate that there is an age-related alteration in the microenvironment of the aorta that predisposes this tissue to a greater risk of vascular calcification. *(Funds: KFOC, KM:OGS).*

Disclosure of Financial Relationships: nothing to disclose

**F-PO1710**

**Involvement of Parathyroid Hormone Related Protein in Vascular Calcification of Patients with End Stage Renal Disease and Its Possible Mechanism Study** Fang Liu, Ping Fu, Songmin Huang, Rong Gou, Youqun Huang, Wenxing Fan. *Division of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

**Objective**: Vascular calcification is the major risk factor for cardiovascular events of ESRD patients. Parathyroid hormone related protein (PTHrP) is a new multifunctional peptide to be considered not only a uremic toxin, but also a cardiovascular regulator. This study was to investigate the role of PTHrP in vascular calcification and its possible mechanism.

**Methods**: (1) the inferior epigastric arteries were obtained from 23 maintenance hemodialysis ESRD patients and 3 cases as normal control. HE staining, elastic fiber staining, the lizarin bordeaux calcium staining, immunohistochemical staining and real-time

quantitative PCR were performed. (2) human aortic smooth muscle cell line was in vitro cultured, PTHrP-siRNA was transfected by liposomes. The cells were divided into seven groups: control group; PTHrP-siRNA-24h; $10^{-8}$mM human recombinant PTHrP (rh-PTHrP)-24h; PTHrP-siRNA+$10^{-8}$mM rh-PTHrP-24h; PTHrP-siRNA-48h; $10^{-8}$ mM rh-PTHrP-48h; PTHrP-siRNA+$10^{-8}$ mM rh-PTHrP-48h. Western Blot and real-time quantitative PCR were used to detect the protein and mRNA expressions of BMP-2/ Cbfa1.

**Results**: (1) Compared with the normal control group, arterial vascular walls were thickened, medial elastic fibers decreased significantly, calcium salt staining increased, and the expressions of PTHrP, BMP-2/Cbfa1 detected by immunohistochemistry significantly increased, and the expressions of PTHrP, PTH1R, BMP-2/Cbfa1 mRNA detected by real-time quantitative PCR were significantly in ESRD patients. Correlation analysis showed that the calcification score was closely related with the expression of PTHrP and BMP-2/ Cbfa1. (2) Western Blot and real-time quantitative PCR show that rh-PTHrP can stimulate the expressions BMP-2/Cbfa1 in non-time-dependent manner; PTHrP-siRNA could partly reduce the expression of BMP-2/ Cbfa1 induced by rh-PTHrP.

**Conclusions**: The expressions of PTHrP, BMP-2/Cbfa1 increased in calcificational arterial vessels of ESRD patients; PTHrP might be involved in the development of vascular calcification by inducing the activation of BMP-2/ Cbfa1 pathway.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1711

**Pulse Pressure, Arterial Stiffness and Malnutrition in Chronic Kidney Disease** Catarina Santos,[1] Ricardo Vizinho,[2] José Diogo Barata.[2] *[1]Nephrology Department, Hospital Amato Lusitano, Castelo Branco, Portugal; [2]Nephrology Department, Hospital Santa Cruz, Carnaxide, Portugal.*

Pulse pressure (PP) is of clinical interest for cardiovascular risk stratification and correlates strongly with arterial stiffness, inflamation and malnutrition. In chronic kidney disease (CKD) the accuracy of peripheral PP (pPP) may be misleading because of vascular stiffening and its prognostic value decreased in this setting. We aimed to evaluate peripheral and central PP in CKD patients and determine its association with arterial stiffness, inflamation and malnutrition. In 40 patients with CKD stages 4 and 5 not on dialysis we determined C- reactive protein (CRP) and albumin and measured pPP and central pulse pressure (cPP), augmentation index (Aix), reflected wave time transit (RWTT), left ventricular ejection time (LVET) and RWTT/LEVT ratio. The central hemodynamic parameters were obtained by radial tonometry (Sphygmocor®) and the statistical analysis was performed using SPSS. Thirty patients were male, average age 62.2 ± 15 years and 10 patients (25%) had type 2 diabetes. The average pPP was 60.8 ± 18.3 mm Hg and cPP 48.2 ± 16.7 mm Hg (p=0.000). pPP and cPP were higher in diabetics (p=0.009 and p=0.05, respectively). Average albumin was 3.6 ± 0.4 g/dL and CRP 0.5 ± 0.4 mg/dL. pPP correlated positively with central systolic arterial pressure (r=0.578; p=0.000) and cPP (r=0.881; p=0.000), inversely with RWTT/LEVT ratio (r=-0.324; p=0.047) but not with Aix (r=0.207; p=0.200), CRP (r=0.324; p=0.123) or albumin (r=-0.173; p=0.418). cPP correlated positively with Aix (r=0.446; p=0.005), CRP (r=0.438; p=0.041) but inversely with RWTT/LEVT ratio (r=-0.433; p=0.007) and albumin (r=-0.378; p=0.043). By logistic regression analysis, predictors of Aix included cPP (β= 0.29; p=0.015), RWTT/LVET ratio (β= -0.56; p=0.000), diastolic arterial pressure (β=0.22; p=0.03) and albumin (β=-0.29; p=0.015). Peripheral PP, however, was not associated with Aix (β= 0.182; p=0.222). We conclude that in CKD pPP is higher than cPP but the two variables correlate positively. However, cPP, rather than pPP, and albumin are better predictors of arterial stiffness in this setting.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1712

**Transplantation of Metanephros Suppresses the Vascular Calcification in Rats with Adenine-Induced Renal Failure** Shinya Yokote,[1,2] Takashi Yokoo,[1,2] Kei Matsumoto,[1,2] Ichiro Ohkido,[1] Tetsuya Kawamura,[1] Tatsuo Hosoya.[1] *[1]Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan; [2]Internal Medicine, Project Laboratory for Kidney Regeneration, Institute of DNA Medicine, Tokyo, Japan.*

*Background.* It has been shown transplanted metanephros may undergo complete nephrogenesis in the host animal, forming a functional kidney. However, metabolic function of transplanted metanephros, in particular for secondary hyperparathyroidism and vascular calcification in chronic renal failure (CRF), remains unknown. To determine this, we studied the therapeutic effect of metanephros transplantation on vascular calcification in rat with CRF.

*Methods.* CRF was induced in 11-week-old male Wistar rats by feeding 0.75% adenine containing diet for 4 weeks, followed by normal diet for another 2 weeks . Time course changes of serum levels of inorganic phosphorus(P), calcium(Ca), parathyroid hormone , 1-α25dihydroxyvitamin D3(1-25$(OH)_2D_3$) were measured. Whole metanephroi from embryonic day 15 rats were transplanted into the omentum and the epidermidic area (Tx groups) at 4weeks. At the end of study, vascular calcification was evaluated by Von Kossa-stained sections, CT scan, and Ca and P content in aorta. Maximal parathyroid gland area was also measured.

*Results.* Aortic calcification and enlarged parathyroid glands were observed in adenine-fed rats, whereas, Tx group (n=12) had significantly reduced vascular Ca and P compared with control CRF group (n=11). Histopathologic examination of the thoracic aorta media (von Kossa staining) also showed Tx suppressed the vascular calcification compared with the control CRF group. RT-PCR showed 1α hydroxylase mRNA was significantly increased in the metanephroi of Tx group compared with native kidney of control CRF group. However, there are no significant differences between these two groups on serum 1-25$(OH)_2D_3$ level and Ca×P product, suggesting its anti-calcification effect can be independent of 1-25$(OH)_2D_3$ activation.

*Conclusions.* These data showed transplantation of metanephroi can contribute to suppress the vascular calcification by independent mechanism of calcium-phosphorus dynamics.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1713

**Eicosapentaenoic Acid Reduces Vascular Calcification In Vitro and In Vivo** Saeko Kanai, Kenta Uto, Chiharu Aoki, Sekiko Taneda, Kazuho Honda, Hideaki Oda. *Department of Pathology, Tokyo Women's Medical University, Tokyo, Japan.*

**Background:** Vascular calcification is associated with elevated cardiovascular disease risk and poor prognosis in patients with chronic kidney disease. Eicosapentaenoic acid (EPA), a major n-3 polyunsaturated fatty acid, has been shown to have protective effects against cardiovascular disease. However, little is known about the effects of EPA on vascular calcification. **Methods and Results:** Vascular calcification was induced by the administration of warfarin (3 mg/g food) and vitamin K1 (1.5 mg/g food) to inhibit γ-carboxylation of matrix Gla protein in Sprague-Dawley rats consisting of 2 groups based on a diet with EPA (1g/kg/day) (EPA group) or without (control group). After 2 weeks, histological evaluation using von Kossa staining revealed a marked reduction of arterial medial calcification in EPA rats**,** compared with that in the control rats (calcification ratio: control vs. EPA = 6.9 ± 4.3% vs. 1.5 ± 0.9%, p<0.05). Immunohistochemical analysis showed that EPA reduced the infiltration of macrophages expressing matrix-metalloproteinase (MMP)-2 or MMP-9 in adventitia around calcification. EPA also reduced MMP-9 activity as well as the mRNA expression of monocyte chemotactic protein-1 in the aorta. Additionally, calcification in A7r5 cells (rat aorta smooth muscle cell line) was induced by the addition of Pi (3 mM) to serum-supplemented medium for 8 days and was evaluated using the o-cresolphthalein complexone method and von Kossa staining. Pre-treatment of the A7r5 cells for 48 h with 10 μM of EPA significantly decreased phosphate-induced calcification. Flow-cytometric analysis using annexin V and TUNEL staining revealed that phosphate-induced apoptosis was markedly reduced by the pre-treatment with EPA. Furthermore, RT-PCR analyses revealed that pre-treatment with EPA suppressed MMP-9 expression in A7r5 cells as well as in RAW264.7cells (murine macrophage cell line) stimulated with TNF-α (10 ng/mL) for 24h. **Conclusion:**[/bold]These observations indicate that EPA reduces vascular calcification through the suppression of MMP-9 activity and adventitial macrophage infiltration in addition to inhibiting the apoptosis of vascular smooth muscle cells.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1714

**Iron Enhances Adhesion of Mononuclear Cells to Human Aortic Endothelial Cells by Increasing Reactive Oxygen Species Generation and Redox-Sensitive Pathways** Ko-Lin Kuo,[1,2] Szu-Chun Hung,[2] Der-Cherng Tarng.[1,3] *[1]Institute of Physiology, National Yang-Ming University, Taipei, Taiwan; [2]Division of Nephrology, Buddhist Tzu Chi General Hospital, Taipei Branch, Xindian, Taiwan; [3]Division of Nephrology, Taipei Veteran General Hospital, Taipei, Taiwan.*

**Aim** Our previous data showed that higher cumulative dose of iron supplementation was associated with increased cardiovascular mortality and events in dialysis patients. Therefore, we test the hypothesis whether iron can exert pro-oxidant property and further induce adhesion of mononuclear cells (MNCs) to human aortic endothelial cells (HAECs), an early sign of atherogenesis.

**Methods and Results** HAECs were incubated with various concentrations of iron sucrose (FeS) and the cell viability were measured by MTT assay. The non-cytotoxic working concentrations of FeS (≤160μg/ml) were used to avoid interference on cell viability in the following tests. Adherence of monocytic cells U937 to activated HAECs by FeS was examined first. There was a significant time-dependent increase of U937 adhesiveness to HAECs at 1, 2, and 4 h compared to the baseline ($P$ <0.05). Dose-dependent effect of FeS on adhesiveness significantly increases at 4 h following incubation with FeS of 40, 80, and 160 mg ($P$<0.05). Co-treatment of HAECs with N-acetylcysteine (NAC, 100 mM), DPI (NADPH oxidase inhibitor; 30μM), pyrrolidine dithiocarbamate (PDTC, an antioxidant and NF-κB inhibitor; 100μM) and U0126 (AP-1 inhibitor; 50μM) significantly inhibited U937 adhesiveness to HAECs treated with FeS of 160μg/ml for 4 h. In addition, an increase of intracellular ROS production on HAECs treated with FeS 160μg/ml for 3 h can be significantly inhibited by NAC, DPI, PDTC and U0126 ($P$ <0.05). Furthermore, HEACs treated with FeS significantly increased endothelial vascular cell adhesion molecule -1 in a time and dose-dependent manner, but the expression of intercellular adhesion molecule-1 was modest.

**Conclusion** FeS can significantly induce endothelial adhesiveness of MNCs by increasing intracellular ROS production, and cell adhesion molecules expression via redox-sensitive pathways. Our results suggest that iron supplementation plays a role in the initiation of atherosclerosis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1715**

**Endothelial Dysfunction Impairs Vascular Integrity and Delays Recovery in Mesenteric Venules of Pkd2 Heterozygous Mice Following Ischaemia-Reperfusion Injury** Sony Prasad,[1] Zoe L. Brookes,[2] Albert C. Ong.[1] *[1]Kidney Genetics Group, Academic Unit of Nephrology, University of Sheffield, Sheffield, S. Yorkshire, United Kingdom; [2]Microcirculation Group, University of Sheffield, Sheffield, S. Yorkshire, United Kingdom.*

Cardiovascular abnormalities are a prominent extrarenal feature of the ADPKD phenotype and are most commonly expressed as an increased prevalence of hypertension. The pathogenesis of hypertension is likely to be multifactorial in ADPKD though abnormalities in both vascular smooth muscle contractility and endothelial dependent vasodilatation have been reported for studies performed on isolated human and murine ADPKD arteries. In a recent study, we reported that mesenteric arterioles from $Pkd2^{+/-}$ mice had a profound baseline defect in acetylcholine (Ach) stimulated vasodilatation *ex vivo* which was endothelial dependent and nitric oxide mediated (Ong et al, JASN 2009). Here we demonstrate that the endothelial dysfunction present in $Pkd2^{+/-}$ mice extends to venular endothelium *in vivo* and moreover is further exaggerated by ischaemia-reperfusion injury (IRI). Wild-type and $Pkd2^{+/-}$ female mice (n=5-7) were subjected to mesenteric IRI by clamping the superior mesenteric artery (15 min) or a sham procedure. Intravital microscopy was employed to study macromolecular leak (FITC-BSA) in the post-capillary venules (20-40μm diameter). No difference in baseline leak was seen in sham operated mice from both genotypes but the extent of the post I/R leak induced in $Pkd2^{+/-}$ mice was significantly higher than in wild-type. To investigate whether this difference was a consequence of loss of endothelial function, the same venules were analysed for Ach induced dilatation. In sham vessels, the Ach response was strikingly reduced in $Pkd2^{+/-}$ compared to wild-type. Following IRI, the Ach response was significantly more impaired in $Pkd2^{+/-}$ venules and was almost absent following IRI. We conclude that endothelial dysfunction is likely to be a widespread feature of both arteries and veins in ADPKD and that this impairs vascular integrity and recovery following injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1716**

**Globotriaosylceramide (Gb3)-Induced Endothelial-to-Mesenchymal Transition as a Novel Mechanism of Vascular Damage in Fabry Disease** Mina Yu,[1] Yang Hee Jang,[1] Eun Sun Ryu,[1] Jin Ok Choi,[2] Sung Cheol Jung,[2] Duk-Hee Kang.[1] *[1]Nephrology, Ewha Womans University, School of Medicine, Seoul, Korea; [2]Biochemistry, Ewha Womans University, School of Medicine, Seoul, Korea.*

The lysosomal storage disorder Fabry disease is characterized by excessive Gb3 accumulation in major organs such as the heart and kidney. Defective lysosomal alpha-galactosidase A (Gla) is responsible for excessive Gb3 accumulation, and vascular endothelium is one of the sensitive cells to the effects of Gb3 accumulation. Although endothelial dysfunction is known to be associated with Fabry disease, it is not known whether it plays any roles in the development of organ damage or whether Gb3 per se is related to endothelial dysfunction. Recent data suggest that endo-MT, which is characterized by the loss of endothelial cell markers and an acquisition of mesenchymal cell markers, is a potential mechanism of endothelial dysfunction. We investigated whether Fabry kidney showed an evidence of endo-MT and whether Gb3 induced endo-MT in cultured HUVEC. Immunostaining with RECA or CD-31 in the kidney of Gla deficient mice, showed a decreased microvascular endothelial staining both in glomerular and peritubular capillaries compared to wild type mice with an appearance of α-SMA (+) endothelial cells. However, a loss of glomerular and peritubular endothelial cells was not demonstrated by electron microscopy, suggesting that not a loss of endothelial cells but a phenotypic transition is present in Fabry mice. Treatment of Fabry mice with of recombinant adeno-associated virus vector encoding alpha-Gal A cDNA (rAAV2/8-hAGA) resulted in the clearance of accumulated Gb3 in kidney with concomitant elevation of alpha-Gal A enzyme activity. rAAV2/8-hAGA therapy also ameliorated endo-MT of glomerular and peritubular capillary endothelial cells. Stimulation of HUVEC with Gb3 (0.1-10 uM) down-regulated the expression of CD31 and VE-cadherin with an up-regulation of α-SMA from 48 hrs in a dose-dependent and time-dependent manner. Gb3 also induced a decrease in nitric oxide production from HUVEC. These finding suggest that Gb3-induced endo-MT is one of the mechanisms of endothelial dysfunction and nephropathy in Fabry disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1717**

**Macrophages and VEGF-C Contribute to Lymphangiogenesis in the Progressive Renal Fibrosis Model** Jung Eun Lee,[1] Aesin Lee,[1] Yujin Jung,[1] Duk Hoon Kim,[1] Ki Dong Lee,[1] Mi Jeong Sung,[2] Kyung Pyo Kang,[1] Sik Lee,[1] Sung Kwang Park,[1] Won Kim.[1] *[1]Internal Medicine, Chonbuk National University Medical School, Jeonju, Republic of Korea; [2]Food Function Reseach Center, Korea Food Research Institute, Songnam, Republic of Korea.*

Lymphatic and lymphangiogenesis are involved in many pathological conditions such as tumor metastasis, wound healing, and lymph edema. A recent study has reported that density of lymphatic vessel was correlated with fibrotic area in human chronic renal disease and idiopathic pulmonary fibrosis model. However, there is a paucity of data regarding the role of macrophages in lymphangiogenesis and progressive renal interstitial fibrosis. We investigated whether there is lymphangiogenesis and lymphatic remodeling in fibrotic area in a unilateral ureteral obstruction (UUO), a progressive renal fibrosis model and evaluated the roles of macrophages and growth factors in obstructed kidney.

Our results demonstrated that the density of LYVE-1-positive lymphatic vessels was increased at 1, 2 and 4 w after ureteral obstruction in the tubulointerstitial area. Density of PECAM-1-positive vasculature was slightly increased at 1 and 2 w after ureteral obstruction. Thereafter, PECAM-1-positive vasculature was gradually decreased at 3 and 4 w after ureteral obstruction. LYVE-1–positive lymphatic endothelial cells were colocalized with podoplanin, Prox-1 and vascular endothelial cell growth factor (VEGF) receptor 3 in fibrotic kidney. The proliferation of LYVE-1-positive lymphatic endothelial cells was increased at 1 w after ureteral obstruction compared to sham-operated mice kidney. VEGF-C expression was colocalized with F4/80(+) macrophages in fibrotic area. VEGF-c mRNA expression was significantly increased after ureteral obstruction compared to the sham-operated mice kidney whereas angiopoietin-2, VEGF-B, and VEGF-D mRNA expression was not changed after ureteral obstruction.

Our results show that F4/80(+) macrophage and VEGF-C have a role in lymphangiogenesis in a kidney fibrosis model.

Disclosure of Financial Relationships: nothing to disclose

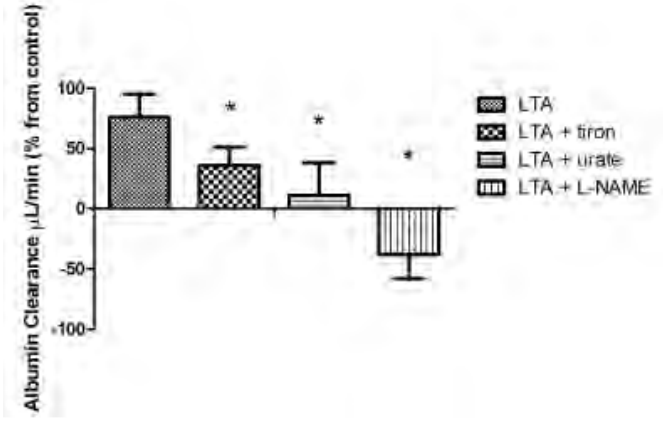
**F-PO1718**

**Lipoteichoic Acid-Induced Lung Endothelial Barrier Dysfunction: Role of Endothelial Nitric Oxide Synthase (eNOS)** Amy B. Pai, Arnold Johnson. *Albany College of Health of Health Sciences, Albany, NY.*

Gram-positive organisms are the leading cause of sepis in hemodialysis patients. We have shown that lipotechoic acid ( LTA, a Gram positive cell wall component) induces increased lung permeability (in vitro and in vivo). Mechanisms for LTA associated endothelial barrier dysfunction have not been investigated.

Rat lung microvessel endothelial cell (RLMEC) monolayers were cultured and treated with ultrapure LTA from *Staphylococcus aureus* 30 μg/mL ± tiron 5mM (reactive oxygen species (ROS) scavenger), urate (reactive nitrogen species (RNS) scavenger) and L-NAME 100 μM (eNOS inhibitor) for 24 hours Clearance rate of Evans Blue-labeled albumin used to determine permeability. ROS were quantitated in RLMEC treated LTA (as above) for 30 minutes incubated with dihydroethidium (DHE) (10 μM, 0.5 h). Values reported as mean fluorescence intensity (MFI). eNOS activation was determined in lysate by immunochemistry and analyzed using the ratio of anti-p-eNOS-$ser^{1177}$ (an activation site) and p-eNOS-$thr^{495}$ (an inactivation site). Results are mean values ± SEM of n > 3 experiments.

Incubation of RLMECM with LTA\ increased endothelial permeability compared to untreated controls that was prevented by tiron, urate and L-NAME. (*p<0.05 vs. LTA alone see Figure)




LTA induced eNOS activation evidenced by an increased p-eNOS-$ser^{1177}$:p-eNOS-$thr^{495}$ (mean ± SEM relative density units; 0.7 ± 0.05 vs. control 0.93 ± 0.06, respectively, p < 0.05 IS vs. control). LTA increased ROS generation, with an 59 ± 18% increase in DHE MFI vs. untreated controls (p < 0.05, LTA vs. controls), that was effectively mitigated by urate and tiron (p<0.05 LTA vs LTA + urate and LTA + tiron)

LTA increased endothelial permeability to albumin, eNOS activation and ROS generation. These data indicate that LTA has the potential to induce lung injury via an eNOS/ROS/RNS mediated pathway.

Disclosure of Financial Relationships: Research Funding: Abbott Laboratories.

**F-PO1719**

**Loss of Glycocalyx Barrier Properties in Dialysis Patients** Carmen A. Vlahu,[1] Marion G. Koopman,[1] Raymond T. Krediet,[1] Hans Vink.[1, 2] *[1]Department of Medicine, Academic Medical Center, Amsterdam, Netherlands; [2]Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht, Netherlands.*

The endothelial glycocalyx exerts a wide range of vasculoprotective effects and is a main regulator of vascular homeostasis. In the present study we investigated whether the microvascular glycocalyx is damaged in dialysis patients as compared to healthy controls.

**Methods**: Investigations were carried out in 7 male HD patients (age 45.9 (27.1-59.9) years; time on renal replacement therapy (RRT) 176.2 (17.4-352.9) months), 9 male PD patients (age 43.7 (18.25-57.0) years; time on RRT 54.2 (2.1-300.8) months) and 9 age and sex-matched healthy controls. Exclusion criteria: diabetes mellitus, use of antioxidants, statins, ACE-inhibitors and ARBs. The status of endothelial glycocalyx in individual blood vessels was assessed by measuring the dimension of the cell poor region (CPR) near the vessel wall. In healthy conditions only the luminal part of the glycocalyx is accessible to red blood cells (RBC). When glycocalyx barrier properties are impaired, the RBC can penetrate further into the glycocalyx, thereby resulting in an increase in the dimension of CPR. This was measured using Sidestream DarkField imaging of the sublingual vasculature with a MicroScan videomicroscope. Measurements were repeated after the administration of 0.4mg of nitroglycerine (NTG) sublingually.

**Results**: Compared to healthy controls, CPR increased in patients by 0.63±0.31 μm (p=0.05) in PD patients and 0.98±0.32 μm (p=0.01) in HD patients. In HD patients the magnitude of glycocalyx barrier loss correlated with total time on RRT (r=0.86, p=0.01). Furthermore, exposure to NTG increased the CPR by 0.3 (-0.2-1.1) μm in healthy controls and 1.3 (0.3-3.4) μm in HD patients indicating increased susceptibility to challenge mediated increases in glycocalyx permeability in HD patients.

**Conclusions**: Dialysis patients have a loss of glycocalyx barrier properties as compared to healthy controls. This alteration is more pronounced in HD patients. Impaired glycocalyx barrier properties may be an early indicator of pathogenic activation of vascular endothelium as a marker of increased cardiovascular risk.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1720**

**Association between Renal Microvascular Sclerosis and Asymmetric Dimethylarginine (ADMA) in Normotensive Patients with Primary Glomerulopathy** Keiji Kono, Kentaro Nakai, Shunsuke Goto, Hideki Fujii, Shinichi Nishi. *Division of Nephrology and Kidney center, Kobe University Graduate School of Medicine, Kobe, Japan.*

Background: Renal microvascular sclerosis is a critical pathological finding, which is related to prognosis of renal function. The etiology, however, remains unclear because this finding can be observed even in the absence of clinical risk factors, such as hypertension and aging. A novel marker of endothelial dysfunction and atherosclerosis, ADMA, has been reported to be a risk factor for the progression to ESRD. We examined the relationship between renovasculopathy associated with primary glomerulopathy and clinical parameters including ADMA.

Methods: 60 patients were performed renal biopsy in our department between March 2009 and December 2009. Of these patients, the patients with the history of hypertension, diabetes mellitus and vasculitis were excluded. 15 normotensive patients (age:41±15 years old) with biopsy-proven primary glomerulopathy were included in this study. The ratio of arterial intimal fibroplasia thickening to outer diameter (R(f)) as well as the proportion of the number of arterioles with hyalinization (Hy) were evaluated in each biopsy specimen. Laboratory tests, including plasma ADMA, were carried out.

Results: The mean value of eGFR, R(f) and Hy in all patients were 77.2±19.4ml/min/1.73m2 ,21.7±15.2% and 6.0±18.4%, respectively. R(f) was significantly correlated with plasma ADMA levels(r=0.691,p<0.005) and age (r=0.521,p<0.05) in all patients. In contrast, Hy was correlated with systolic BP(r=0.593,p<0.05). In multivariate analysis, plasma ADMA levels were independently associated with R(f) (β=0.624, p<0.05). Furthermore, plasma ADMA levels were significantly correlated with R(f) even in the patients who had only mild interstitial fibrosis ( r=0.891, p<0.005).

Conclusion: Our findings suggest that ADMA may affect the progression of early renal microvascular sclerosis producing the interstitial fibrosis independently of hypertension in primary glomerulopathy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1721**

**Endothelial Cell and Pericyte Co-Cultures in an In Vitro Spheroid Model** William Gee Chang, Jordan S. Pober. *Immunobiology, Yale University School of Medicine, New Haven, CT.*

Microvessels are composed of an endothelial cell inner lining that is invested by pericytes. Communication between these two cell types via cell-cell contacts and paracrine signaling are key to vascular formation, remodeling, and stability. In order to better understand these interactions we adapted the Augustin three-dimensional spheroid model to examine cellular interactions between human umbilical vein endothelial cells (EC) and human placental pericytes (PC) in vitro. Both cultured cell types spontaneously form spheroids when suspended in 0.25% methylcellulose in non-adherent 96 well U-bottomed plates, and remain viable for days when embedded in type I collagen gels. EC spheroids do not spontaneously sprout but respond to exogenous VEGF treatment with robust sprout formation. In contrast, PC sprout in the absence of exogenous VEGF. Spheroids formed by mixing EC with PC organize into PC cores coated by EC. Without addition of VEGF, EC/PC mixed spheroids demonstrated PC sprouting alone, while the addition of VEGF led to multiple sprouts consisting of either PC or EC. However, little contact between the EC and PC sprouts was observed. These results suggest that close proximity is not sufficient to foster EC/PC cell-cell contact in vitro.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1722**

**Glomerular Expression of ADAMTS13 in Renal Thrombotic Microangiopathies** Jan U. Becker,[1] Friedrich Modde,[1] Svjetlana Lovric,[2] Mario Schiffer,[2] Anke Schwarz,[2] Verena Broecker,[1] Clemens L. Bockmeyer.[1] *[1]Medizinische Hochschule Hannover, Institut für Pathologie, Hannover, Lower Saxony, Germany; [2]Medizinische Hochschule Hannover, Klinik für Nieren- und Hochdruckerkrankungen, Hannover, Lower Saxony, Germany.*

Introduction:

Diminished ADAMTS13 activity due to inhibitory antibodies or congenital defects plays a critical role in the development of thrombotic microangiopathies (TMA). Glomerular endothelial cells (GECs) and podocytes are known to express ADAMTS13. The hypothesis, that decreased glomerular expression of ADAMTS13 could contribute to the development of renal TMAs is examined in renal biopsies from native kidneys.

Subjects and Methods: 30 kidney biopsies with various acute and chronic forms of TMA (19 idiopathic, 3 ADAMTS13 deficiency, 4 chemotherapy-induced, 1 shiga toxin-associated hemolytic uremic syndrome, 3 calcineurin inhibitor (CNI)-induced) and 6 normal renal control specimen were examined by immunohistochemistry and quantitative real time PCR of microdissected glomeruli for the glomerular expression of ADAMTS13.

Results:

No differences in ADAMTS13 expression between TMA biopsies and control kidneys were found by immunohistochemistry or quantitative real time PCR except for three cases with CNI-induced TMA. These three cases showed significantly reduced mRNA levels and reduced immunostaining of GECs when compared to TMAs of other causes and controls.

Conclusion:

These results from a limited number of renal biopsy proven TMAs show, that the majority of biopsied renal TMAs do not seem to be associated with decreased glomerular expression of ADAMTS13. The exception were the small subgroup of CNI-induced TMA which did show decreased glomerular mRNA levels and GEC immunostaining of antithrombotic ADAMTS13. This potential novel pathomechanism for the development of CNI-induced TMA should be examined in larger cohorts.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1723**

**Racial Differences in the Association of Pentraxin-3 with Kidney Dysfunction: The Multi-Ethnic Study of Atherosclerosis (MESA)** Ruth F. Dubin,[1] Michael Shlipak,[2] Yongmei Li,[2] Joachim H. Ix,[3] Ian H. de Boer,[4] Nancy Jenny,[5] Carmen A. Peralta.[2] *[1]Internal Medicine, Division Nephrology, University of California San Francisco, San Francisco, CA; [2]Internal Medicine, San Francisco VA Medical Center, San Francisco, CA; [3]Internal Medicine, Division Nephrology, University of California San Diego, San Diego Veterans Affairs Healthcare System, San Diego, CA; [4]Kidney Research Institute and Division of Nephrology, University of Washington, Seattle, WA; [5]Laboratory for Clinical Biochemistry Research, University of Vermont College of Medicine, Colchester, VT.*

Pentraxin-3 (PTX3), an inflammatory marker thought to be specific to vascular injury, is elevated in advanced chronic kidney disease (CKD). Whether PTX3 is associated with mild to moderate kidney dysfunction is unknown.

We tested associations of proteins in the pentraxin family (PTX3, C-reactive protein (CRP) and serum amyloid protein (SAP) with estimated glomerular filtration rate by cystatin C (eGFRcys) and microalbuminuria among 2824 participants in MESA. Associations were tested using multivariable linear regression with adjustment for demographics, comorbidities, and systemic inflammation (interleukin-6 (IL-6)).

Among 2824 white, black, Hispanic and Chinese participants, mean eGFRcys was 94 ml/min/1.73m2. Overall, higher PTX3 was significantly associated with lower eGFRcys after full adjustment (β -3.0 ml/min/1.73m2 per unit increase in lnPTX3, p<0.001). CRP and SAP were not associated with eGFRcys after adjustment for comorbidities and inflammation. There was a significant interaction with race/ethnicity (p<0.001) in the association of PTX3 and eGFRcys. After adjustment for demographics, comorbidities and IL-6, this association was significant in blacks (β -6.2 ml/min/1.73m2 per unit increase in lnPTX3, p=0.001) but not in Hispanics, Chinese, or whites. PTX3 and CRP, but not SAP, had correlations with microalbuminuria in unadjusted models, but these were attenuated after full adjustment.

Endovascular inflammation may be an important mechanism associated with early kidney dysfunction, particularly among blacks.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1724**

**Elevated Levels of Plasma Microparticles in Dialysis Patients Are Associated with a Pro-Coagulant State** James O. Burton,[1,2] Hassan A. Hamali,[3] Ruth Stansfield,[2] Nigel J. Brunskill.[1,2] *[1]John Walls Renal Unit, Leicester General Hospital, United Kingdom; [2]Infection, Immunity & Inflammation; [3]Cardiovascular Sciences, University of Leicester, United Kingdom.*

Cardiovascular (CV) death is the largest cause of mortality in dialysis patients but is not fully explained by traditional CV risk models. Plasma microparticles (MPs) are membrane-bound vesicles shed from cells upon activation or apoptosis. Endothelial MPs (EMPs) are

elevated in patients with traditional CV risk factors while platelet MPs (PMPs) are associated with atherosclerosis. This study sought to compare circulating MPs in dialysis patients to matched controls and investigate association with thromboembolic risk.

MPs were isolated from platelet free plasma and their presence confirmed using electron and confocal microscopy. Samples were obtained from HD (n=20) and PD (n=17) patients and matched controls (n=19). Relative concentrations of EMPs (CD144+) and PMPs (CD42b+) were measured by Western blotting. Thrombin generation was measured in plasma before and after filtration (which removes MPs and therefore acts as a negative control) using the continuous automated thrombogram. MPs had the most effect on endogenous thrombin potential (ETP) and peak thrombin (PT) so these data are presented.

EMP and PMP concentrations were higher in dialysis patients than controls (P<0.01). Both dialysis modalities were associated with higher concentrations of EMPs and PMPs (P<0.05). PT was higher in HD and PD patients versus controls (57.1nM vs. 34.8nM; P<0.004 and 76.7nM vs. 34.8nM; P<0.001). Similarly ETP was higher in both patient groups (928nM/min vs. 637nM/min; P<0.02 and 1121nM/min vs. 637nM/min; P<0.001). Following filtration PT and ETP were significantly lower (P<0.0001) and no observed difference remained between patients and controls.

Dialysis patients have higher circulating MP concentrations than matched controls. These MPs are pro-coagulant and promote thrombin generation. Removal of MPs by filtration eliminates this pro-coagulant effect demonstrating that it is not due to soluble mediators or uraemia alone. MPs could be a factor in the increased incidence of cardiovascular events in dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1725**

**Uric Acid as a Predictor of Vascular Function in Healthy Adults** Diana I. Jalal,[1] Kristen L. Jablonski,[2] Kim Mcfann,[1] Michel B. Chonchol,[1] Douglas R. Seals.[2] *[1]Renal Division, University of Colorado Denver, Aurora, CO; [2]Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO.*

High serum uric acid levels are associated with endothelial dysfunction in high risk populations such as patients with congestive heart failure. It is unclear if uric acid and endothelial function are related in healthy adults. We studied 107 healthy adult volunteers from a community based cohort (20-78 years). Baseline characteristics were compared across serum uric acid quartiles defined as: 0-5.2, 5.3-6.1, 6.2-7.0, >7.0mg/dL. Endothelium-dependant dilation (EDD) was assessed using the % change in brachial artery flow mediated dilation. Endothelial independent dilation (EID), a measure of vascular smooth muscle sensitivity to nitric oxide (NO), was defined as % change in brachial artery dilation to sublingual nitroglycerin. The association between serum uric acid levels and EDD and EID was evaluated by linear regression models, first in unadjusted analysis, then in 2 consecutive models: model 1 adjusting for age, gender, and ethnicity, and model 2 adjusting for model 1 + body mass index (BMI), systolic blood pressure, LDL- cholesterol, and fasting glucose levels. In this cohort, 23 subjects (21%) had uric acid levels in the highest quartile. Compared to the lowest quartile of uric acid, these individuals were more likely to be male and had higher BMI and fasting glucose levels. Uric acid levels and EDD were not related in unadjusted or adjusted models in the overall group. There was a tendency for EID to decrease among uric acid quartiles that did not achieve statistical significance. However, there was a significant unadjusted correlation between uric acid and EID in the pooled sample (r= -0.34, p= 0.005). This correlation remained significant after adjusting for covariates in model 1 (p=0.042) and was near significant after adjusting for covariates in model 2 (p=0.071). In conclusion, serum uric acid levels are not associated with EDD among healthy adults, but are related to EID. These results provide the first evidence that uric acid is a determinant of vascular smooth muscle sensitivity to NO in community-dwelling adults free of clinical disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1726**

**Differential Expression of Uric Acid Transporters, GLUT9 and URAT1, in the Blood Vessels of the Normal Rat** Diana I. Jalal,[1] Carlos Alberto Roncal-Jimenez,[1] Wei Chen,[1,2] Chris Altmann,[1] Miguel A. Lanaspa,[1] Christopher J. Rivard,[1] Richard J. Johnson.[1,3] *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Department of Nephrology, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China; [3]Division of Nephrology, Hypertension, and Transplantation, University of Florida, Gainesville, FL.*

Serum uric acid levels have been linked to endothelial dysfunction and cardiovascular disease in several patient populations. We have previously shown that URAT1 (SLC22A12) transports uric acid into the vascular smooth muscle cells. GLUT9 (SLC 2A9) has been shown to transport uric acid from the proximal tubular cells to the circulation. The expression of uric acid transporters, however, has not been characterized in different blood vessels in vivo. Male Sprague Dawley rats were sacrificed and the following arteries were harvested: the carotid arteries, the thoracic aorta, the abdominal aorta, the renal arteries, and the femoral arteries. The expression of URAT1 and GLUT9 was examined by quantitative RT-PCR,western blot, and immunofluorescence (IF). All western blots were normalized for GAPDH. Secondary antibody staining was performed to control for background in IF. We found that URAT1 expression was significantly lower in the thoracic aorta than all the other harvested arteries. The expression of URAT1 was greatest in the abdominal aorta, where it was detected in the vascular smooth muscles and the endothelium. Contrary to URAT1 expression, GLUT9 expression, was greatest in the thoracic aorta. These studies provide the first evidence that URAT1 and GLUT9 are differentially expressed in different arterial segments of the normal rat, and suggest that the vascular effects of uric acid may vary in different vascular beds. Further studies are needed to examine the functional role of uric acid transporters in the different vascular beds.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1727**

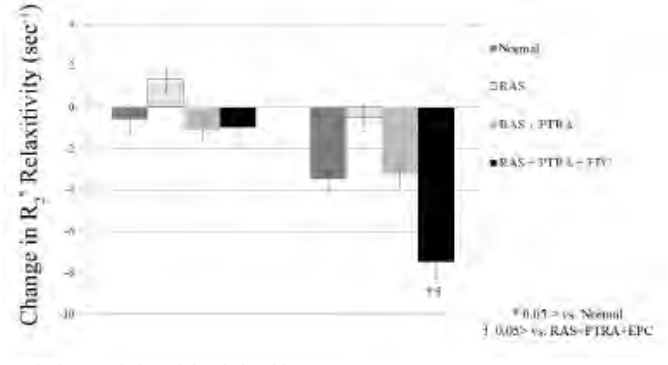
**Adjunct Progenitor Cells Infusion after Revascularization of Renal Artery Stenosis Improves Renal Oxygenation** Behzad Ebrahimi, John R. Woollard, John A. Crane, Lizette Warner, Xiang-Yang Zhu, Stephen C. Textor, Lilach O. Lerman. *Nephrology & Hypertension, Mayo Clinic, Rochester.*

Background: Renal artery stenosis (RAS) may lead to regional renal ischemia, dysfunction, and fibrosis, which are often irreversible after percutaneous transluminal renal angioplasty (PTRA). We have shown that endothelial progenitor cells (EPC) decrease renal injury in untreated RAS. This study tested the hypothesis that EPC would further enhance renal oxygenation after PTRA.

Methods: Pigs were studied after 10 weeks of untreated unilateral RAS (n=4), RAS treated 4 weeks earlier with PTRA alone (n=4), or followed by intra-renal EPC infusion (10^7, n=4). Normal pigs served as controls (n=5). Stenotic kidney blood oxygenation (R2*, reciprocal to blood relaxation) and energy-dependent tubular function were assessed by blood oxygen-level-dependent (BOLD) magnetic resonance imaging (MRI) before and 15 min after the medullary loop diuretic furosemide (0.05 ml/kg IV).

Results: RAS tended to be smaller than normal kidneys (99±6 vs 119±6 cc, p=0.058) but their size was restored after PTRA. Blood pressure was increased in RAS but decreased similarly in RAS+PTRA and RAS+PTRA+EPC (-16 and -13 mmHg). In normal kidneys medullary R2* significantly declined after furosemide (-16.6±3.4%, p<0.05) suggesting decreased O2 consumption. The response to furosemide was blunted in RAS medulla (2.0±5.4%, p>0.05 vs baseline), but improved in pigs treated with PTRA (+13.3±2.5% p<0.05 vs baseline). In RAS+PTRA+EPC, decrease in R2* was significantly greater than in normal or RAS+PTRA (p<0.005 vs both groups). Cortical R2* remained unchanged after furosemide in all groups.

Conclusion: Infusion of EPC immediately after successful PTRA improves recovery of renal oxygenation after revascularization, supporting this adjunct intervention for preservation and improved viability of stenotic kidney.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1728**

**Expression of Senescence Markers in Accelerated Atherogenesis of Uninephrectomized ApoE-/- Mice** Luis Eduardo Becker,[1] Anette Melk,[2] Martin G. Zeier,[1] Marie-Luise Gross.[3] *[1]Nephrology, University of Heidelberg, Heidelberg, Germany; [2]Pediatrics, Hannover Medical School, Hannover, Germany; [3]Pathology, University of Heidelberg, Heidelberg, Germany.*

Atherosclerotic processes are accelerated in patients with chronic kidney disease (CKD), even in children. It is supposed that a premature cellular aging process in CKD functions as a permissive factor for the occurrence of atherosclerosis on the uremic state. In order to evaluate this hypothesis, we investigated the evolution of atherosclerosis by spontaneously atherosclerotic "young" and "old" ApoE-/- mice after Uninephrectomy (UNX) and sham-op. Additionally the expression of the cell cycle inhibitors p53, $p21^{WAF1/CIP1}$ and $p16^{INK4a}$ which are associated to telomere dependent cellular senescence was analyzed.

Methods: Sixty-four ApoE-/- mice receiving normocholesterol diet were divided into 4 groups: UNX and Sham-op, observation period of 16 or 32 weeks (wk) post-operative. UNX was performed at 8 weeks of age. Hearts and aortas were harvested for analysis through stereology and immunohistoschemistry. Aortic p16, p21, p53 and telomerase were accessed through RT-PCR.

Results: Compared to Sham-op (16 and 32wk) aorta and heart remodeling were more pronounced in UNX 32wk animals, which presented a significant increase in wall to lumen ratio, plaque size and a reduced heart capillary length density despite no significant alterations in blood pressure. TGFß expression in the aorta was significantly higher in UNX animals (both 16 and 32wk) compared to sham-op. Plaque total collagen content was overall low and similar between groups. However, collagen I and fibronectin expression in plaques were significantly higher by UNX 32wk animals compared to Sham-op and UNX 16 wk. RT-PCR of aortic material showed a higher expression of p21 in the UNX 32wk animals compared to all other groups (10.4±4.6 vs. 3.1±0.43, 4.5±3 and 6.2±2.3 ratios/HPRT, p<0.05) and a lower telomerase expression compared to Sham-op 16 and 32wk.

Conclusions: UNX promoted significant aortic and cardiac remodeling, which was paralleled by increased expression of p21 and reduced telomerase activity after a 32 week observation period.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1729**

**Endothelial Progenitor Cells (EPCs) in a Human Model of Vascular Hyporeactivity: Relationship with Hypertension and Cardiovascular Remodeling** Lorenzo Calo, Elisa Pagnin, Monica Facco, Elisa Boscaro, Carlo Agostini, Achille Pessina. *Clinical and Experimental Medicine, University of Padova, Padova, Italy.*

Altered endothelial function is critical in the pathophysiology of hypertension and long-term complications (cardiovascular (CV) remodeling). Ang II-induced oxidative stress (OxSt) causes endothelial dysfunction via senescence of EPCs, reduction of NO and secretion of inflammatory cytokines. NO bioavailability and eNOS activation are critical for EPCs activity that is reduced in EPCs of hypertensives and correlates with accelerated EPC senescence while ARBs increase the number of circulating EPCs. In Bartter's/Gitelman's patients (BS/GS) who have increased levels of Ang II yet normo/hypotension, we demonstrated blunted Ang II type 1 receptor (AT1R) signaling (Kidney Int 2006, JCEM 2004, J Hypertens 2007 and 2008), activation of AT2R signaling (J Hypertens 2010) explaining their reduced OxSt (J Hypertens 1998, NDT 2003) increased NO production, high insulin sensitivity, absence of endothelial dysfunction (NDT 2008, Diabetes Care 2006, J Hum Hypertens 2007) and lack of CV remodeling (NDT 2008, JEI 2009) depicting the opposite picture of the Ang II signaling/insulin-glucose relationships of diabetes and hypertension and intrinsecally reproduce the effect of ARBs. There are no data in BS/GS on EPCs number. Peripheral blood progenitor cells were analyzed in 10 genetically characterized BS/GS and 10 normotensive healthy subject (C) for the expression of cell surface antigens via direct three-color analysis using fluorescein isothiocyanate-, phycoerythrin- and allophycocyanin-conjugated monoclonal ABs by flow cytometry analysis. EPCs were defined as CD34+KDR+ or CD133+KDR+ and CD133+CD34+KDR+ cells. In BS/GS CD34+KDR+ cells did not differ from C: 50.00±36.32 vs 29.33±14.38 while both CD133+KDR+ and CD133+CD34+KDR+ cells were higher: 22,55±11.46 vs 12.00±9.54, p=0.049 and 10.33±3.53 vs 3.53±3.00, p=0.0003. These data in a human model opposite of hypertension contribute to clarify mechanisms involved in the ARBs effect on EPCs and support the therapeutic strategy based on stimulation of circulating EPCs proliferation for the prevention of atherosclerotic CV diseases.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1730**

**The Unprecedented Proliferative Changes on Renal Arteriolar Walls Induced by a Long-Term Administration of an Angiotensin II Receptor Blocker in Rats** Yohko Nagai,[1,2] Kazushige Nakanishi,[2] Nobuaki Yamanaka.[1] *[1]Tokyo Kidney Research Institute, Tokyo, Japan; [2]Department of General Medicine and Emergency Care, Toho University, Faculty of Medicine, Tokyo, Japan.*

**Introduction**: Angiotensin II type 1 receptor blockers (ARBs) are widely known for having nephro-protective effects; decreasing intraglomerular pressure in various primary and secondary kidney diseases. However, the histopathological studies on renal arterioles by the ARB treatment have not been reported. We examined the morphological changes on afferent arterioles in rats after a long-term administration of an ARB.

**Method**: Eight 6-week-old male Wistar Kyoto rats (WKY) and 16 age-matched Zucker Fatty Rats (ZFR) were divided into the following four groups: the WKY+ARB group (n=4) and the ZFR+ARB group (n=8) fed a standard diet (0.4%NaCl) containing ARB (olmesartan, 5mg/Kg/day) for 12 weeks, the WKY group (n=4) and the ZFR group (n=8) fed a standard diet without ARB as controls. All of their kidneys were examined by light and electron microscopes and immunohistochemistry using anti-αSMA, SM-1,SM-2 and renin antibodies, especially focusing on the renal vessels.

**Results**: Unprecedented concentric proliferative changes and marked morphological irregularities of smooth muscle cells (SMCs) in afferent arteriolar walls were observed in the WKY+ARB and ZFR+ARB groups (45±5.0%, 77.3±10.3% of observed arterioles, respectively). The changed arteriolar lumens narrowed extremely. The proliferative SMCs were considered to be activated and dedifferentiated. Elastic and collagen fibers could be observed between the multiplying SMCs. In the control WKY and ZFR groups no abnormal findings were shown in the afferent arterioles.

**Conclusion**: It is indicated that the long-term administration of ARB induces unusual proliferative changes in afferent arterioles and has a risk of obliterating of them over a longer period.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1731**

**The Role of Apolipoprotein A1 in Uremic Atherosclerosis** Saleem H. Bharmal, Manish P. Ponda. *Division of Nephrology, Department of Medicine, New York University School of Medicine, New York, NY.*

**Purpose:**

To determine the effect of human ApoA1 over expression in uremic ApoE$^{-/-}$ mice.

**Background:**

Atherosclerosis is accelerated in chronic kidney disease. Studies of the ApoE knockout mouse model corroborate clinical observations. Animal models have shown that the pathogenesis of accelerated atherosclerosis may be due to decrease in rates of plaque regression. Human trials of intervention with statins did not show any benefit in cardiovascular outcomes in end-stage renal disease patients despite significant reductions in LDL cholesterol levels; emphasizing the need for investigating alternate mechanisms and potential treatments. We wanted to further study the importance of reverse cholesterol transport in uremic atherosclerosis by comparing atherosclerotic plaque size in human ApoA1 transgenic/ApoE knockout (hApoA1tg/ApoE-/-) mice that were made uremic to those that had normal renal function.

**Methods:**

hApoA1tg/ApoE-/- mice underwent surgical reduction of renal mass (uremic N=5) or sham operation (controls N=7). The mice were maintained on a Western diet for 16 weeks. Aortic roots were harvested, sectioned, and stained for quantification of plaque size.

**Results:**

Uremic mice had significantly higher serum BUN compared to the controls (72±26 vs. 33±13 mg/dl, P<0.001). The average blood pressures between the two groups were the same (97/58 vs. 102/66). There was a trend towards increase in absolute plaque size in the uremic mice compared to the controls (461,721±37,124 vs. 373,221±130,716 μm2 P=0.06). There was no significant difference in % plaque within an aortic root section (42%±10 uremic vs. 38%±12 controls P=0.29).

**Conclusion:**

Uremic hApoA1tg/ApoE-/- mice showed a trend toward increased aortic root plaque size compared to non-uremic controls. However, the increase was of a smaller magnitude than expected from prior studies of atherosclerosis in uremia. The results suggest that ApoA1, and thus enhanced reverse cholesterol transport, maybe protective against accelerated atherosclerosis seen in uremia.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1732**

**Myogenic Reactivity Is Decreased in Renal Arteries of the Zucker Diabetic Fatty (ZDF) Rat** Peter Vavrinec, Robert H. Henning, Richard P. Van Dokkum, Hendrik Buikema. *Department of Clinical Pharmacology, University Medical Centre Groningen, University of Groningen, Groningen, Netherlands.*

Introduction: On average, one out of six Europeans with up to one in three in some EU countries suffers from a condition referred to as the metabolic syndrome. These individuals are at increased risk for development of type 2 diabetes mellitus (DM-2) and the complications of renal end-organ damage associated herewith. In obese Zucker diabetic fatty (ZDF) rats with DM-2, early metabolic abnormalities confer into structural and functional kidney damage. Myogenic constriction (MC), one of the autoregulatory mechanism that protects the kidney from excessive pressure, was found to be impaired in several models of renal failure, but has not been previously studied in the ZDF rat model of DM-2.

Methods: Renal arteries isolated from 25 weeks old ZDF and lean control rats were transferred to a arteriograph to asses agonist and pressure induced (MC) contractile properties. Furthermore blood glucose, proteinuria and focal glomerulosclerosis FGS were assessed.

Results: Compared to lean controls, ZDF had significantly increased plasma glucose levels (9.6±1 vs 23,9±2 mmol/L, resp.; p<0,0001), and displayed renal failure in terms of proteinuria (15,1±2,1 vs 337,3±39,7 mg/24h resp.; p<0,0001) and FGS (6,3±1,6 vs 27,9±1,3 % resp.; p<0,0001). Renal arteries isolated from ZDF rats showed impaired myogenic constriction (23,6±3,4 vs 14,5±1,9 %(max.), resp.; p<0,05.), whereas sensitivity to the α1 agonist phenylephrine was significantly increased compared to lean controls ( pD2: 6,7±0,03 vs 6,4±0,03; p<0,0001 ).

Conclusion: Impaired myogenic constriction of renal arteries in ZDF rats suggests that deterioration of autoregulatory mechanisms in the kidney might be the cause of renal failure in this model of DM-2. Targeting this problem may be a therapeutic approach for prevention from renal failure in this model.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1733**

**Epidermal Growth Factor Receptor Inhibitor PKI-166 Governs Cardiovascular Protection without Renal Protection in 5/6 Nephrectomized Rats** Peter Vavrinec,[1] Gemma M. Mulder,[2] Richard P. Van Dokkum,[1] Hendrik Buikema,[1] Sjoerd Landheer,[1] Maaike Goris,[1] Harry Van Goor,[2] Hakan Gurdal,[3] Robert H. Henning,[1] Nadir Ulu.[3] *[1]Department of Clinical Pharmacology, University Medical Centre Groningen, University of Groningen, Groningen; [2]Departments of Pathology, University Medical Centre Groningen, University of Groningen, Groningen, Netherlands; [3]Department of Medical Pharmacology, Faculty of Medicine, University of Ankara, Ankara, Turkey.*

Epidermal growth factor receptor (EGFR) signaling has been implicated in hypertension and chronic kidney disease (CKD) via transactivation by angiotensin II type 1 receptor- and/or α1-adrenoceptor. As the therapeutic potential of EGFR inhibition in these conditions is unknown, we studied effect the EGFR kinase inhibition in CKD and associated hypertension in the rat remnant kidney model. Experimental CKD was induced by 5/6 nephrectomy (5/6Nx) and 6 weeks after 5/6Nx rats were treated either with EGFR kinase inhibitor PKI-166 (50mg/kg/day) or lisinopril (5mg/kg/day) or vehicle until week 12. Sham animals received either PKI-166 or vehicle. Blood pressure, parameters of cardiac and renal damage, aortic contractility and myogenic reactivity of small mesenteric artery were investigated. Nephrectomized rats displayed characteristic features of CKD including severe proteinuria, significantly higher plasma creatinine and focal glomerulosclerosis score, increased fluid intake, elevated urine production and renal hypertrophy. PKI-166 treatment prevented the progression of hypertension without any effect on the progression of renal injury. Moreover,

the impaired contraction of isolated thoracic aortic rings to phenylephrine or angiotensin II in vehicle-treated nephrectomized rats was restored by PKI or lisinopril treatment. Small mesenteric arteries of vehicle-treated nephrectomized rats failed to develop myogenic tone which was also completely restored by PKI or lisinopril treatment.In conclusion, blockade of the EGFR pathway displays a therapeutic benefit in kidney disease associated hypertension which was independent of limiting the progression of renal injury. Our findings extend the evidence on EGFR signaling as a target in arterial hypertension.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1734

**The Changes on Renal Vessels Introduced by the Long-Term Administration of an Angiotensin II Receptor Blocker in Zucker Fatty Rats** Kazushige Nakanishi,[1] Yohko Nagai,[1,2] Nobuaki Yamanaka.[2] *[1]Department of General Medicine and Emergency Care, Toho University, Faculty of Medicine, Tokyo, Japan; [2]Tokyo Kidney Research Institute, Japan.*

**Introduction** : The nephro-protective effects of angiotensin II receptor blockers (ARBs) are widely known. However, there are few reports focusing on the renal vessels for long-term effects. We clarified afferent arteriolar changes induced by the long-term administration of an ARB.

**Materials and Methods** :Thirty-two 6-week-old male Zucker Fatty Rats (ZFR) were divided into following four groups (n=8 in each): ZFR Group and ZFR+High Group fed a standard or high-salt diet respectively; ZFR+ARB Group and ZFR+High+ARB Group fed a standard or high-salt diet with ARB (olmesartan, 5mg/Kg/day), respectively. Blood pressure, proteinuria, morphological examinations and glomerular hemodynamics in vivo were studied.

**Results :**Remarkable proliferative changes in the afferent arteriolar smooth muscle cells (SMCs) were frequently observed in the two groups given ARB; in the ZFR+ARB group (77.3±10.3%) compared with the two groups without ARB (1.7%, $p<0.005$; 1.2%, $p<0.0005$).They were 37.4±15.6% in the ZFR+High+ARB group. Proteinuria markedly decreased in the two groups treated ARB. The glomerular erythrocyte velocities showed no differences.

**Conclusions** :It was indicated that long-term ARB administration induced unusual proliferative changes of SMCs in afferent arterioles of ZFR. These changes could narrow arteriolar lumens and reduce intraglomerular pressure, but they could cause irreversible damage on the arterioles.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1735

**Novel, Non-Invasive Vascular Assessment Tools Can Enhance Cardiovascular Risk Profiling at the Bedside in CKD** Gavin Dreyer,[1] Arthur Tucker,[2] Martin J. Raftery,[1] Magdi Yaqoob.[1] *[1]Renal Unit, Royal London Hospital, London, United Kingdom; [2]Microvascular Department, Barts and the London NHS Trust, United Kingdom.*

Introduction

Traditional risk factors for cardiovascular disease in CKD including eGFR, blood pressure, cholesterol, calcium and phosphate do not give a comprehensive assessment of cardiovascular risk in individual patients. Novel clinical assessments of circulatory function are required to enhance risk stratification and therapeutic management in CKD.

Methods

We piloted 3 operator independent techniques to provide bedside measures of circulatory function in patients with CKD – skin auto-fluorescence (skin AF) for advanced glycation end products, aortic pulse wave velocity (aPWV) for arterial stiffness and iontophoresis (IOP) of acetylcholine with laser Doppler monitoring for endothelial function. Thirty eight patients (25 male) with stage 3-4 CKD and 15 healthy volunteers (9 male) underwent two assessments of each technique. Time taken for all assessments was 10-15 minutes per patient.

Results

In the CKD group, the mean creatinine was 192 µmol/L (eGFR 35.7ml/min/1.73m$^2$). aPWV and skin AF were higher in the CKD group. Area under the curve by laser Doppler flowmetry for IOP of acetylcholine was lower in the CKD patients reflecting impaired endothelial function.

Vascular assessments

| | Healthy | CKD | p value |
|---|---|---|---|
| BP (mmHg) | 106/64 | 119/79 | <0.001 |
| aPWV (m/s) | 7.3 | 8.5 | <0.001 |
| Skin AF (AU) | 2.0 | 2.9 | <0.001 |
| AUC IOP | 71335 | 36436 | <0.001 |

AUC IOP = area under curve for iontophoresis of acetylcholine

These results demonstrate significantly impaired vascular function in patients with CKD compared to healthy controls. There is no correlation between methods indicating they each add independent information to the clinical assessment. Coefficient of variance for all techniques demonstrates acceptable reproducibility.

Conclusion

Three simple, rapid and non-invasive bedside tests provide additional information on vascular function in CKD beyond what is normally available in a standard clinical environment. This information can assist with risk stratification and enhance targeted therapies and monitoring in patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1736

**Sex Difference in the Association of Renal Arteriolar Hyalinosis and Hyperuricemia in Chronic Kidney Disease Patients** Kentaro Kohagura,[#1] Masako Kochi,[#1] Yusuke Ohya,[#1] Kunitoshi Iseki.[#2] *[1]Cardiovascular Medicine, Nephrology and Neurology, University of the Ryukyus, Nishihara-cho, Okinawa, Japan; [2]Dialysis Unit, University of the Ryukyus, Nishihara-cho, Okinawa, Japan.*

We have previously reported that hyperuricemia was a risk factor for ESRD only in women among general population in Okinawa. Hyperuricemia causes renal arteriopathy in animals. However, an association of serum uric acid with renal arteriopathy in patients with chronic kidney disease (CKD) and its relationship to sex difference has not been investigated. In the present study, we examined the cross-sectional association between the levels of serum uric acid and renal arteriolar hyalinosis using renal biopsy specimen both in men and women. Arteriolar hyalinosis was assessed by semi quantitative grading for arterioles among 180 patients with CKD (mean age, 43.4 yrs; 99 men and 81 women). The mean age and eGFR were similar in both sexes, but the levels of serum uric acid were significantly higher in men than in women (6.9 vs. 5.8 mg/dL, $P<0.0001$). With increasing tertiles of serum uric acid, the proportions of higher-grade hyalinosis were increased only in women ($P=0.004$ for trend). Multiple logistic analysis adjusted for age and hypertension showed that the 2nd and 3rd tertiles of uric acid were significantly associated with higher risk for the presence of renal arteriolar hyalinosis than the lowest tertile only in women. The adjusted odds ratios (95% CI) were 29.15 (4.64 to 183.17) and 8.62 (1.72 to 43.32), respectively.

In conclusion, significant association between renal arteriolar hyalinosis and increased level of serum uric acid was observed only in women. Such difference in association might explain the sex-associated difference in susceptibility to CKD progression previously shown in epidemiologic studies.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1737

**Kidney Function and Multiple Hemostatic Biomarkers: The Multi-Ethnic Study of Atherosclerosis** Ruth F. Dubin,[1] Mary Cushman,[2] Aaron Folsom,[3] Linda F. Fried,[4] Walter Palmas,[5] Carmen A. Peralta,[6] Christina Wassel,[7] Michael Shlipak.[6] *[1]Internal Medicine, Division Nephrology, University of California San Francisco, San Francisco, CA; [2]Internal Medicine, University of Vermont, Burlington, VT; [3]Epidemiology and Public Health, University of Minnesota, Minneapolis, MN; [4]Medicine, Epidemiology, VA Pittsburgh Healthcare System, Pittsburgh, PA; [5]Medicine, Columbia University, New York, NY; [6]Internal Medicine, University of California San Francisco, San Francisco VA Medical Center, San Francisco, CA; [7]Family and Preventive Medicine, University of California San Diego, San Diego, CA.*

Cystatin C has a strong association with cardiovascular and overall mortality in persons with and without CKD, independent of inflammation. Abnormalities of hemostasis may play an important role in this association.

We tested cross-sectional associations between glomerular filtration rate (eGFR) and multiple hemostatic markers among 6751 participants representing a broad spectrum of kidney function in the Multi-Ethnic Study of Atherosclerosis (MESA). Kidney function was measured using cystatin C (eGFRcys) or creatinine (CKD-EPI). Hemostatic markers included soluble thrombomodulin (sTM), soluble tissue factor (sTF), D-Dimer, plasmin-antiplasmin complex (PAP), tissue factor pathway inhibitor (TFPI), E-selectin and CD40 ligand. Associations were tested using multivariable linear regression with adjustment for demographics and comorbidities.

The biomarkers most strongly associated with eGFR were of small molecular weight. In comparison to the reference group with eGFRcys >90 ml/min/1.73m2, subjects with eGFRcys < 60 ml/min/1.73m2 had adjusted levels of sTM, sTF, D-Dimer and PAP that were respectively 86%, 68%, 44%, and 22% higher. Subjects with eGFRcys 60-90 ml/min/1.73m2 had adjusted levels that were respectively 16%, 14%, 12%, and 6% higher ($p< 0.05$ for all).

Throughout a broad spectrum of kidney function, lower eGFR is associated with elevations in selected hemostatic markers. Dysregulation of hemostasis may be a mechanism by which reduced kidney function leads to higher cardiovascular risk.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1738

**Antigen Specificity and Functionality of CD4$^+$CD28$^-$ T Cells in Pre-Dialysis CKD Patients** Behnam Zal,[2] Nihil Chitalia,[1] Juan Carlos Kaski,[2] Christina Baboonian,[2] Debasish Banerjee.[1] *[1]Renal Medicine, St. George's Hospital, London, United Kingdom; [2]CV Sciences, St. George's University of London, London, United Kingdom.*

**Background**

Cardiovascular disease (CVD) is common in CKD, often in the absence of traditional risk factors with inflammation being implicated in the pathogenesis. An aggressive and unusual CD4$^+$ T cell subpopulation (CD4$^+$CD28$^-$) expand in coronary artery disease (CAD). These cells exhibit significant proinflammatory and cytotoxic functions activated by human heat shock protein 60 (hHSP60; a stress protein upregulated in inflammation). The activating Killer Immunoglobulin-like Receptor 2DS2 (KIR2DS2) mediates the effector function of these T cells.

**Aims**

We investigated the frequency and effector function of $CD4^+CD28^-$ cells in predialysis CKD patients.

**Methods and Results**

14 non-diabetic CKD patients (age 55±13), men 57%, hypertension 93%, eGFR 27±11 ml/min/1.73m$^2$, current smokers 14% and 10 healthy controls were investigated for the presence of $CD4^+CD28^-$ cells using FACS.

Four patients had $CD4^+CD28^-$ cells constituting 8-11% of the $CD4^+$ compartment. None of the controls had these T cells. T cell clones (n=80) established from 2 patients and analyzed by RT-PCR revealed presence of KIR2DS2 in 35 & 38% with co-expression of inhibitory KIR2DL3 in 16 & 18% of cells. We investigated the effector function of these cells upon exposure with autologous hHSP60 pulsed target cells. Only 2DS2+ clones co-expressing the adaptor protein DAP-12 (70%) and lacking KIR2DL3 reacted by releasing perforin. This is significant as our previous study in CAD showed that the inhibitory KIRs had no protective role in $CD4^+CD28^-$ cells.

Presence of these killer cells was lower in these patients (15%) compared to ESRD patients (41%; studied separately) indicating clonal expansion with progressive renal failure.

**Conclusion**

We propose a mechanism of gradual loss of inhibitory role, with acquisition of killer function in $CD4^+CD28^-$ T cells with progressive renal failure. This is the first report on antigen specificity and killing potential of $CD4^+CD28^-$ in CKD and their potential role in the development and progression of CVD in these patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1739**

**Effects of Shock Wave Therapy in Chronic Kidney Diseases** Jean-Jacques Boffa, Pierre-Antoine Michel, Matthieu Colin, Christos Chatziantoniou, Pierre M. Ronco. *U702, INSERM, Paris, France, Metropolitan.*

Neoangiogenesis can be involved in renal repair. Extracorporeal low-energy shock wave therapy (SWT) is a new effective, noninvasive proangiogenic therapy which has been used for ischemic heart and limb diseases in animals and humans. In ischemic tissues, SWT enhances the expression of vascular endothelial growth factor (VEGF) and its receptor FLT-1, the production of NO, the blood flow, the capillary density and the circulating endothelial progenitor cells (EPC). The objective our study was to investigate whether SWT could protect from the development of chronic kidney disease (CKD). To this end, CKD was induced by L-NAME in 13 rats for 4-5 weeks. After reaching the level of proteinuria over creatininuria of 1000 g/mol, SWT was performed in the left kidney 3 times/week for 4 weeks.

SWT did not change hypertension (206 ± 5 before SWT vs 194 ± 5 mmHg after 4 weeks of SWT), nor proteinuria (1004 ± 138 vs 1146 ± 235 g/mol). The renal function (urea and creatininemia) remained unchanged. The treated kidney with SWT did not show an improvement in the rarefaction of peritubular capillaries (8,4 ± 1,1 % LK SWT vs 8,1 ± 1,0% RK CTRL) or an increase of the VEGF and of its receptors compared to the contralateral kidney. SWT did not reduce renal fibrosis, but decreased macrophage infiltration evidenced by CD 68+ staining : 7,3 ± 1,3 in LK SWT vs 12,1 ± 1,9 in RK CTRL, p< 0,005. In addition, SWT decreased cellular proliferation, 11,8 ± 1,8 cells in LK SWT vs 23,8 ± 5,5 in RK CTRL, p< 0,01.

In conclusion, SWT did not enhance the expression of proangiogenic factors in L-NAME treated rats. In contrast we observed an important reduction of inflammation associated with a reduction of renal cell proliferation in the treated kidney suggesting that SWT could be useful against inflammatory renal disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1740**

**Soluble Epoxide Hydrolase Inhibition Decreases Blood Pressure Independent of Nitric Oxide in Mice with Renovascular Hypertension** Libor Kopkan,[1] Bruce D. Hammock,[2] John D. Imig,[3] Ludek Cervenka.[1] *[1]Department for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Department of Entomology and UCD Cancer Center, University of California, Davis, CA; [3]Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI.*

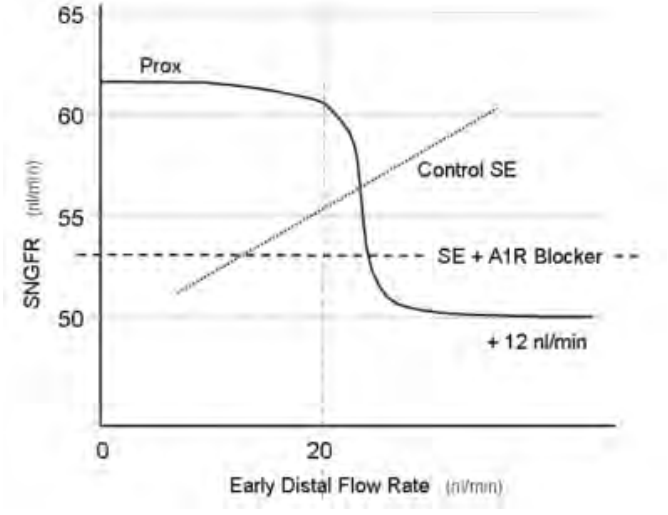
Inhibition of soluble epoxide hydrolase (sEH) induces substantial antihypertensive responses in several hypertensive models. The present study was performed to determine antihypertensive effects of sEH inhibition in mice with renovascular hypertension. Male C57Bl6 (wild type; WT) and mice lacking gene for endothelial nitric oxide synthase (eNOS-KO) were implanted with transmitters to monitor systolic blood pressure (SBP) continuously by radiotelemetry. Renovascular hypertension was induced by placing a silver clip on the right renal artery in these mice (two-kidney, one clip model). After clipping for 25 days, animals were treated with the novel sEH inhibitor, *cis*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*c*-AUCB; 26 mg/L in drinking water) or left untreated for two weeks. Animals were placed into metabolic cages to determine 24-hrs sodium excretion throughout the treatment period. Renal artery clipping caused significant SBP increases in both WT (121±2 to 158±4 mmHg; n=6) and eNOS-KO mice (141±3 to 169±7 mmHg; n=7). However, *c*-AUCB treatment given for two weeks from day 25 significantly reduced SBP in WT (147±3 mmHg, n=6) and also in eNOS-KO mice (154±2 mmHg, n=7). During second day of the treatment, both WT and eNOS-KO animals exhibited transiently higher sodium excretion (0.141±0.008 and 0.127±0.006 mmol/day, respectively) compared to untreated WT and eNOS-KO mice (0.118±0.007 and 0.092±0.005 mmol/day, respectively) but not at the end of the treatment period. These data indicate that inhibition of sEH displayed appreciable blood pressure reductions in a mouse model with established renovascular hypertension and moreover these antihypertensive and transient natriuretic effects seemed to be NO independent as observed responses to c-AUCB were present also in mice lacking eNOS.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1741**

**Tubuloglomerular Feedback (TGF) Is Maintained after Acute Saline Expansion (SE) and Inhibited by Adenosine 1 Receptor (A1R) Blockade** Roland C. Blantz, Prabhleen Singh, Scott C. Thomson, Volker Vallon. *Nephrology, UCSD and VA, La Jolla, CA.*

Previous studies suggest that acute SE suppresses or eliminates TGF, however our current closed-loop analyses reveal that TGF is maintained and reset with a new relationship between nephron GFR (SNGFR) and early distal tubular flow rate (EDFR). After SE (10% body weight) for 1 hour we evaluated TGF by assessing proximal and distal SNGFR in the same nephrons and also changes in distal tubule SNGFR before and after adding 12 nl/min to free flowing late proximal tubule in Fromter-Wistar rats. After SE distal SNGFR was increased and EDFR also increased fourfold to 21 nl/min, but TGF activity was maintained. The proximal-distal SNGFR difference was +7±2 nl/min ($p<0.01$) and the reduction in distal SNGFR after addition of fluid to proximal flow was -8±4 nl/min ($p<0.05$) suggesting normal TGF after SE, but reset at higher values. After blockade of A1R by systemic administration of DPCPX, TGF activity was absent with no proximal-distal SNGFR difference (2±2 nl/min, NS) and no reduction in distal SNGFR after addition of fluid (-3±5 nl/min, NS).




Both distal SNGFR and EDFR (21±1 vs 22±2 nl/min) were not altered by A1R blockade. However whole kidney GFR was higher during SE with A1R blockade compared to control SE (1.6±0.2 vs 2.5±0.3 ml/min, $p<0.02$) as was urine flow rate (77±11 vs 127±15 ul/min, $p<0.02$). This suggests that inhibition of A1R 1) exerts greater effect on SNGFR in deeper nephrons (possibly via TGF) and 2) inhibits Na and water transport. Conclusions: 1) TGF after acute SE adapts and is reset to a new relationship between SNGFR and EDFR. 2) During acute SE, A1R blockade inhibits TGF and increases whole kidney GFR and decreases transport beyond the early distal tubule and/or in deeper nephrons. TGF persists and resets after SE and may act to limit changes in GFR and diuretic response.

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UCSD Nephrology for the Consultant course; Scientific Advisor: Current Opinion in Nephrology-Hypertension (section editor)

International Society of Nephrology Council Member

CJASN, Editorial Board

JASN, Editorial Board, Renal Advisory committee - Boehringer-Ingelheim.

**F-PO1742**

**P2 Receptor-Mediated Changes in Vasa Recta Diameter by In Situ Pericytes: Evidence for Tubular/Vascular Cross-Talk?** Teresa M. Kennedy-Lydon,[1] Holly B. Callaghan,[1] Carla Sprott,[1] Carol Crawford,[1] Robert J. Unwin,[2] Harriet M. Syme,[1] Scott S. P. Wildman,[1] Claire M. Peppiatt-Wildman.[1] *[1]Royal Veterinary College, London, United Kingdom; [2]UCL Medical School, London, United Kingdom.*

We hypothesise that tubular/vascular cross-talk is important in the pericyte-mediated regulation of vasa recta diameter and therefore medullary blood flow. We have developed a live kidney slice model to study interactions between vasa recta pericytes *in situ* and adjacent renal tubules. ATP is released by tubular epithelial cells and ATP-activated P2 receptors (P2R) are expressed on pericytes. We investigated the effect of exogenous extracellular nucleotides on vasa recta pericytes in our live slice model.

Kidney slices (200 μm thick) from adult male Sprague Dawley rats were maintained in PSS, bubbled with 95% $O_2$/5% $CO_2$. Real-time images of vasa recta (~50 μm below the surface of the live tissue) were recorded using video imaging. Vasa recta diameter at pericyte and non-pericyte sites was measured following application of nucleotides+/-antagonist.

ATP (100 μM) evoked a significantly greater vasoconstriction of vasa recta at pericyte sites (12.4±1.3%) than at non-pericyte sites (3.8±1.3). 2meSATP (10 μM giving a maximal effect) and UTP (100 μM giving a maximal effect) also caused a significantly greater constriction of vasa recta at pericyte sites (8.5±0.7% and 12.7±1.3%, respectively) than at non-pericyte sites (1.5±0.4% and 2.9±1.3%, respectively). BzATP (1 mM) caused complete occlusion of the vessel at pericyte sites. The constriction evoked at pericyte sites by ATP (100 μM) was significantly attenuated (~15%) by the P2R antagonist RB2 (100 μM) but not by suramin (100 μM) or PPADS (100 μM). UTP-evoked constriction was not significantly altered by PPADS, suramin or RB2.

Nucleotides acting on P2R expressed on pericytes cause vasoconstriction of *in situ* vasa recta.That nucleotides are released from tubular epithelial cells in close proximity to vasa recta suggests that tubular/vascular cross-talk may regulate medullary blood flow.Our data also goes some way to pharmacologically characterizing the P2R on pericytes and include the UTP-sensitive $P2Y_2R$, the RB2-sensitive $P2Y_4R$, and the BzATP-sensitive $P2X_7R$.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1743**

**PPARγ Is a Master Regulator of Circadian Rhythms of Behavior and Metabolism** Guangrui Yang,[1,2] Toshinori Aoyagi,[1,2] Zhanjun Jia,[1,2] Tianxin Yang.[1,2] *[1]Internal Medicine, University of Utah, Salt Lake City, UT; [2]Veterans Affairs Medical Center, Salt Lake City, UT.*

Both human and animal studies have established a physiological link between the circadian rhythm and metabolism. However, the molecular basis of coordinated control of the circadian clock and the metabolic pathways is not well understood. Here we describe a novel role of PPARγ, a key regulator of energy metabolism, in the control of physiological and behavioral rhythms by analyzing two strains of whole-body PPARγ null mouse models. Systemic inactivation of PPARγ was generated constitutively by using Mox2-Cre mice (Mox PPARγ KO) or inducibly by using the tamoxifen system (TM PPARγ KO). The floxed mice exhibited nocturnally activated rhythms in food and water intake, urine and feces production, locomotor activity, blood pressure and heart rate, oxygen consumption, CO2 production, and heat production. The circadian variations of most of these parameters were nearly abolished in Mox PPARγ KO mice and significantly attenuated in TM PPARγ KO mice. qRT-PCR detected impaired rhythmicity of Bmal1, Per1, Per3 and Per2, rev-erbα in the apididymal fat depot and Bmal1, Per1-3, Cry1-2, and rev-erbα in the liver contrasting unaltered rhythmicity of any of the clock genes in the skeletal muscle and the kidney. PPARγ inactivation in isolated preadipocytes following in vitro exposure to tamoxifen led to a similar blockade of the rhythmicity of the clock gene expression. Both null strains exhibited elevated baseline heart rate and a marked increase in urinary excretion of epinephrine and norepinephrine, which exhibited blunted circadian variations. The baroreflex response during infusion of sodium nitroprusside was blunted but remained intact during infusion of phenylephrine. Together, these results demonstrate that PPARγ integrates circadian rhythms and metabolism likely through a direct interaction with the clock system in the peripheral tissues.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1744**

**Preservation of Renal Vascular Function Despite Increases in Blood Pressure in the Ageing Fawn-Hooded Rat Model of Hypertension-Associated Renal Damage** Richard P. Van Dokkum, Peter Vavrinec, Robert H. Henning. *Clinical Pharmacology, University Medical Center Groningen, Groningen, Netherlands.*

*Background.* In humans, the risk for cardiovascular diseases increases with age. The effect of age on vascular function and the relation between blood pressure and renal damage in a model of hypertension-associated renal damage, the Fawn-Hooded (FH) rat, is unknown. Therefore, we studied myogenic constriction in 52-wks. old FHH and control FHL strains, the latter not developing increased systolic blood pressure (SBP), proteinuria and glomerulosclerosis at a young age.

*Methods.* Small renal (interlobular) and mesenteric resistance arteries isolated from FHH and FHL rats in an established phase of their disease (52-wks. old) were mounted in a perfused vessel set-up. Myogenic reactivity was assessed constructing pressure-diameter curves in the presence and absence of calcium. Blood pressure and proteinuria were measured using the tail-cuff method and nephelometry, respectively.

*Results.* At 52 wks, SBP was similar in both strains (169.7 ± 5.4 vs 166.0 ± 5.4 mmHg in FHH and FHL, respectively). However, FHH rats showed overt proteinuria compared to FHL rats (213.4 ± 20.7 vs. 48.9 ± 5.6 mg/24h), as well as decreased creatinin clearance (9.7 ± 0.7 vs. 13.8 ± 0.4 ml/min) and increased FGS (43.5 ± 3.9 vs. 7.9 ± 1.5 %). Moreover, FHH renal arteries developed significantly lower myogenic tone compared to FHL (5.1 ± 0.6 vs. 10.9 ± 1.4 % of max. MC). Therefore, preserved MC of FHL despite an increase in blood pressure is most likely involved in the mechanism by which the FHL kidney is protected from developing renal failure.

*Conclusion.* The present study shows that preserved MC of renal arteries from FHL in the presence of hypertension protects the kidneys in renal end-organ damage in a spontaneous model of hypertension-associated renal damage.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1745**

**Lutheran/BCAM Controls Glomerular Permeselectivity and Glomerulosclerosis during Hypertension** Jin Huang,[1] Cécile Rahuel,[2] Anne Filipe,[2] Yves Colin,[2] Pierre-Louis F. Tharaux.[1] *[1]Paris-Cardiovascular Research Center - PARCC, Inserm and Université Paris-Descartes, Paris, France; [2]Institut National de la Transfusion Sanguine -INTS, Inserm and INTS, Paris, France.*

The Lutheran (Lu) blood group/ basal cell adhesion molecule (BCAM) antigen is highly expressed in endothelial cells and has been recognized as the receptor for the a5 chain of laminins 10/11. Lu -/- mice display mild abnormalities of the glomerular basement membrane with normal proteinuria, renal function and blood pressure. We investigated whether Lu/BCAM could contribute to glomerular permselectivity in hypertensive conditions. Methods: Hypertension and glomerulosclerosis were induced by chronic infusion of Angiotensin II (1 μg/kg/d) for 28 days. Lu-/- mice and their wild-type (WT) male littermates were compared (n=11-13/group). Because dysregulation of nitric oxide (NO) may favour weak glomerular permselectivity, we also challenged Lu (-/-) mice with the NO synthase inhibitor L-NAME for 16 weeks (n=9 /group) in a distinct set of experiments.

Results: High level of Lu/BCAM was found in the glomerular endothelium and to a milder extent in podocytes. Both group developed similar degree of systolic hypertension throughout the course of the study (mean SBP:172±9 vs. 186±6 mmHg on day 28 in Lu -/- and WT, respectively). Upon AngII infusion, Lu -/- mice, exhibited a larger increase in albuminuria (541±177 g/mol creatinine on day 14 and 1722±601 g/mol on day 21) than WT mice (163± 43 g/mol creatinine on day 14 and 461± 179 g/mol on day 21, $p<0.01$). L-NAME for 16 weeks promoted a similar rise in systolic pressure in Lu (-/-) than in WT animals but also induced unusually high albumin urinary output ($p<0.01$) in the absence of Lu/BCAM. Interestingly, whereas hypertensive Lu/BCAM (-/-) animals displayed major albuminuria, the development of glomerulosclerosis was markedly alleviated in both models of hypertension, indicating that albuminuria per se is not a major factor of glomerulosclerosis. We conclude that Lu/BCAM that is highly expressed in the renal microvascular endothelium did play a critical role in limiting glomerular permselectivity under hypertensive condition in a NO-independent fashion.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1746**

**Albuminuria Associated with Genetically-Induced Podocyte Defects Is Not Accompanied by Changes in the Plasma Elimination of Dextran Mol wt 70,000 or Albumin** Wayne Comper,[1] Leileata M. Russo.[1] *[1]Exosome Diagnostics Inc, New York, NY; [2]Pathology and Immunology, Washington University School of Medicine, St Lous, MO.*

It has previously been established that the increase in plasma elimination (PE) of albumin in nephrotic states is due to the increase in urinary excretion of albumin (Kaysen et al 1982). In nephrotic rats the PE rate of albumin increases by >160%. Therefore in order to test the hypothesis that a genetically-induced podocyte defect results in nephrotic-like changes in plasma albumin elimination we examined the PE of fluorescently labeled albumin in CD2AP ko mice. PE of dextran mol wt 70,000 was also measured to estimate changes in glomerular permeability. 5 week old CD2AP ko mice exhibited significant albuminuria (>20-fold by electrophoresis) and hypoalbuminemia but normo-proteinemia. The percentage of material lost 3h post iv injection for albumin was 28.2±3.9 for WT and 25.6±5.6 for KO ($P$=0.545, n=3) and for dextran 68.1±6.6 for WT and 55.1±7.9 for KO ($P$=0.095, n=3). Urinary total protein was 35.8±6.0 (WT) and 35.2±2.8 (KO) ($P$=0.882,n=3). Similar dextran and albumin PE results were obtained in an earlier series of experiments (n=2). The lack of change in the dextran PE demonstrates that the increase in albuminuria due to the genetic podocyte defect is not directly due to changes in glomerular permeability as these changes were small. The albumin PE results demonstrate that albumin processing pre-urinary excretion is identical in both WT and KO. These results are consistent with the fact that albuminuria n CD2AP KO is primarily the result of the inhibition of the proximal tubular cell (PTC) albumin degradation pathway due to the loss of CD2AP from the PTC.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1747**

**Glutamine Supplementation Alleviates Vasculopathy and Alters the Profile of Plasma Metabolome in an In Vivo Model of Endothelial Cell Dysfunction (ECD)** Francesco Addabbo,[1] Qun Chen,[2] Brian B. Ratliff,[3] Tammer Ghaly,[3] Dhara P. Patel,[3] Michael S. Wolin,[3] Steven S. Gross,[2] Michael S. Goligorsky.[3] *[1]University of Bari, Bari, Italy; [2]Weill Cornell Medical College, NY, NY; [3]New York Medical College, Valhalla, NY.*

Using a mouse model of ECD, triggered by continual sub-pressor dosing with L-NG-methylarginine (L-NMMA), we previously demonstrated that the renal microvasculature displays an abnormal protein profile, including diminished expression of two key enzymes of the Krebs cycle (aconitase-2 and enoyl-CoA-hydratase-1) and consequently a Warburg-type suppression of mitochondrial metabolism. We hypothesized that supplementation with metabolites such as glutamine (GLN), that enters the Krebs cycle downstream of this enzymatic blockade, would normalize vascular function and alleviate mitochondrial dysfunction. Mice with chronic L-NMMA treatment-induced ECD were co-treated with GLN. L-NMMA-treatment resulted in defect in acetylcholine-induced relaxation of aortic rings that was dose-dependently prevented by GLN supplementation. Using an LC/MS platform for broad untargeted metabolite profiling, we detected 4,098 plasma molecules with 100% frequency in mice from at least one treatment group. Among these molecules, 372 were found to be differentially expressed in a 4-way comparison of Control vs. L-NMMA

vs. GLN vs. GLN+L-NMMA groups ($p < 0.05$). In isolated renal microvasculature, 1360 metabolites were detected with 100% frequency in at least one group, 19 of them overlapping on Venn's diagram and 20 and 45 being highly specific for L-NMMA and L-NMMA+GLN groups, respectively. Paradoxically, hippuric acid, an established uremic toxin, was found to be significantly increased in these non-uremic mice receiving L-NMMA, and normalized by the concomitant treatment with GLN. In conclusion, functional and metabolic profiling of animals with early ECD reveal that the truncation of Krebs cycle, characteristic of this condition, is associated with the development of macrovasculopathy and that supplementation of these mice with the intermediary of Krebs cycle, downstream of the enzymatic block, is capable of significantly improving vasculopathy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1748**

**Losartan Concentration-Dependently Attenuates Angiotensin-II-Evoked Constriction of Vasa Recta by *In Situ* Pericytes: Studies Using a Novel Live Kidney Slice Model** Carol Crawford,[1] Scott S. P. Wildman,[1] Robert J. Unwin,[2] Claire M. Peppiatt-Wildman.[1] *[1]Royal Veterinary College, London, United Kingdom; [2]UCL Medical School, London, United Kingdom.*

Angiotensin-II (Ang-II; 10 nM) causes pericyte-mediated vasoconstriction (~40% constriction) of isolated descending vasa recta (1). We have proposed that tubular/vascular cross-talk is important in the regulation of vasa recta diameter and therefore medullary blood flow. We have developed a live kidney slice model to investigate the effects of vasoactive substances on vasa recta pericytes *in situ*. Here we have investigated the effects of Ang-II in our live slice model.

Kidney slices (200 μm thick) were obtained from adult (~300 g), male Sprague Dawley rats and maintained in PSS, bubbled with 95% $O_2$/5% $CO_2$. Real-time images of vasa recta (~50 μm below the surface of the live tissue) were recorded using video imaging. Vasa recta diameter at pericyte and non-pericyte sites was measured following application of Ang-II+/-losartan.

Superfusion of kidney slices with Ang-II (10 - 100 nM) significantly constricted vasa recta at pericyte sites (by ~15%), but not at non-pericyte sites. In contrast, the $AT_1$ receptor antagonist losartan (10 nM and 100 nM) did not alter vasa recta diameter at either pericyte or non-pericyte sites. Losartan (10 nM) significantly and reversibly inhibited 100 nM Ang-II-evoked vasoconstriction by ~30%. Higher concentrations of Losartan (100 nM) irreversibly abolished the Ang-II-evoked vasoconstrictrion.

In summary, Ang-II evokes vasoconstriction of vasa recta specifically at pericyte sites, in our live slice model. Vasoconstriction was less than previously reported using isolated vasa recta. The inhibition of Ang-II-evoked vasoconstriction by losartan indicates that vasoconstriction is mediated by $AT_1$ receptors (presumably expressed on pericytes). Furthermore, given that vasa recta diameter is unaltered by losartan application it suggests that Ang-II is not required for maintenance of vasa recta tone.

(1) Zhang Z et al (2001) Am J Physiol Regul Integr Comp Physiol 280:1878-86.

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**F-PO1749**

**Identification and Characterisation of *In Situ* Vasa Recta Pericytes** Carol Crawford, Carla Sprott, Teresa M. Kennedy-Lydon, Scott S. P. Wildman, Claire M. Peppiatt-Wildman. *Royal Veterinary College, London, United Kingdom.*

Pericytes have been identified along isolated vasa recta capillaries of the renal medulla and have been shown to regulate vessel diameter of isolated descending vasa recta (1). Here we have identified pericytes along vasa recta in kidney slices using an anti-NG2 (neural-glial-2) antibody and present morphological characterisation of *in situ* vasa recta pericytes in the inner and outer medullary regions.

Kidney slices (~200 μm thick) were obtained from adult, male Sprague Dawley rats (250-300 g). Pericytes were labelled with an anti-NG2 antibody and probed with an alexa 555-conjugated secondary antibody; and vasa recta capillaries labelled with an Alex 488-conjugated isolectin $B_4$. Fluorescent images of both inner and outer medulla were taken with a Zeiss LSM 510 confocal microscope, and used to calculate the density of pericytes and measure pericyte cell body length, and pericyte process length in the inner and outer medulla.

The density, per 100 $\mu m^2$, of pericytes in the outer medulla was 12±1, which was significantly greater than in the inner medulla, 9±1. The average distance between pericyte cell bodies was 15.1±1.1 μm and 16.0±1.2 μm in the outer and inner medulla, respectively. Mean pericyte cell body length in the outer medulla was 9.0±0.2 μm, which was not significantly different to that in the outer medulla 8.7±0.3 μm. Similarly, mean pericyte process length in the outer medulla was 8.6±0.5 μm, which was not significantly different to that in the inner medulla 9.3±0.6 μm. Process length ranged from 2 μm to 18 μm, with some processes running along the vasa recta before wrapping around the vessel, and others wrapping around the vessel directly from the cell body.

Given the distance between cell bodies, and mean process length, it is likely that pericytes form a complex network, intertwined around the entire length of the vasa recta. These physical characteristics, together with their ability to regulate capillary diameter, further emphasise the importance of pericytes in the regulation of blood flow in the renal medulla.

(1) Pallone TL & Silldorff EP *Experimental Nephrology* 2001 9:165-170.

Disclosure of Financial Relationships: nothing to disclose

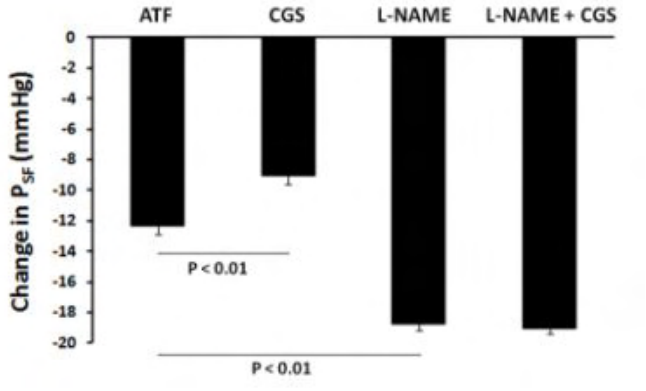
**F-PO1750**

**Adenosine $A_2$ Receptors Attenuate Tubuloglomerular Feedback Response Via Stimulation of Nitric Oxide** Mattias Carlstrom, Christopher S. Wilcox, William J. Welch. *Department of Medicine, Division of Nephrology and Hypertension, Georgetown University Medical Center, Washington, DC.*

**Objective:** Adenosine can mediate the tubuloglomerular (TGF) response via activation of $A_1$ receptors on the afferent arteriole. We have recently shown that adenosine $A_2$ receptors modulate the TGF response by counteracting the effects of adenosine $A_1$ receptors. We tested the hypothesis that $A_2$ receptor mediated dilatation is linked to release of nitric oxide (NO).

**Methods:** Maximal TGF responses were measured in male Sprague-Dawley rats as changes in proximal stop-flow pressure ($\Delta P_{SF}$) in response to increased perfusion of loop of Henle (0 to 40 nl/min) with artificial tubular fluid (ATF). The maximal TGF response was studied after 5 min intratubular perfusion (10 nl/min) with ATF alone, or with ATF plus the $A_{2A}$ receptor agonist (CGS 21680; $10^{-7}$ mol/l), the nitric oxide synthase (NOS) inhibitor (L-NAME; $10^{-3}$ mol/l), or with a combination of L-NAME ($10^{-3}$ mol/l) and CGS 21680 ($10^{-7}$ mol/l).

**Results:** Blood pressure, heart rate, urine flow, $P_{FF}$ and $P_{SF}$ (at 0 nl/min) were similar among the groups. The maximal TGF response ($\Delta P_{SF}$) with ATF alone was 12.3 ± 0.6 mmHg. Specific $A_2$ stimulation attenuated the maximal TGF response (9.0 ± 0.6 mmHg). L-NAME enhanced the response ($\Delta P_{SF}$: 18.7 ± 0.5 mmHg). Stimulation of $A_2$ receptors, during simultaneous NOS inhibition, did not attenuate the maximal response ($\Delta P_{SF}$: 19.0 ± 0.4 mmHg).




**Conclusion:** Adenosine $A_2$ receptors modulate the $A_1$ mediated TGF response via stimulation of NOS-derived NO in the juxtaglomerular apparatus.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1751**

**Large Increases in Glomerular Permeability Following Atrial Natriuretic Peptide (ANP) Infusion in Rats** Josefin Axelsson, Anna Rippe, Bengt Rippe. *Department of Nephrology, Lund University, Lund, Sweden.*

Plasma volume overload, in conjunction with e.g. congestive heart failure (HF), is associated with an increased release of atrial natriuretic peptide (ANP), which may be partially responsible for the microalbuminuria seen in HF. The present study was performed to investigate the effects of systemic ANP infusion on the glomerular permeability to macromolecules in rats. In anaesthetized Wistar rats (250-280g) the left urether was cannulated for urine collection while simultaneously blood access was achieved. Rats were continuously infused i.v. with ANP, 30 ng/min/kg (Lo-ANP; n=8) or 800 ng/min/kg (Hi-ANP; n=10) or 0.9% NaCl (SHAM; n=16), respectively, and with polydisperse fluorescein isothiocyanate (FITC)-Ficoll-70/400 (mol.radius 13-90Å) and $^{51}$Cr-EDTA for 2 h. Plasma and urine samples were taken at 5, 15, 30, 60 and 120 min of ANP infusion, and analyzed by high performance size exclusion chromatography (HPLC) for determination of glomerular sieving coefficients (θ) for Ficoll. GFR was also assessed ($^{51}$Cr-EDTA). In Hi-ANP there was a rapid (within 5 min), but bimodal, increase in glomerular permeability. θ to high MW Ficoll thus reached a maximum at 15 min, after which θ returned to near control at 30 min, to again increase moderately at 60 and 120 min. Max. θ increase for $Ficoll_{70Å}$ was from $3.48 \cdot 10^{-5} \pm 1.01 \cdot 10^{-5}$ to $3.88 \cdot 10^{-4} \pm 7.63 \cdot 10^{-5}$ (Hi-ANP). In Lo-ANP there was also a rapid, reversible increase in glomerular θ, returning to near control at 30 min, followed by just a tendency of a sustained increase in permeability, but with a significant increase in "large pore" radius. In conclusion, in Hi-ANP there was a rapid increase in glomerular permeability, with an early, partly reversible permeability peak, followed by a (moderate) sustained increase in permeability. In Lo-ANP animals, only the initial permeability peak was evident. For pharmacological ANP-doses (Hi-ANP), the glomerular sieving pattern observed was found to mainly reflect an increase in the number and radius of "large pores" in the glomerular filter, while for physiological doses, increases in large pore radius dominated, particularly during the last 60 min of the ANP infusion.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1752**

**GDF15, a TGF-beta Family Member, Impairs Vascular Responsiveness in Wild Type and GDF15 Knockout Mice** Magdalena Mazagova, Robert H. Henning, Leo E. Deelman. *Clinical Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands.*

Background: Alterations in hemodynamics and (intrarenal) vascular dysfunction contribute to the development of cardiovascular and diabetic kidney disease. Growth differentiation factor 15 (GDF15), a member of the TGF-beta superfamily, is an early response gene upregulated after induction of renal injury. Furthermore, increased plasma GDF-15 levels have been associated with cardiovascular dysfunction in patients suffering from type I and II diabetes. In this study, we investigated whether GDF15 modulates vascular function in wild type and GDF15 knockout mice.

Methods: Aortic rings were obtained from healthy C57BL/6 (wt) and GDF15 k.o and measured in a wired myograph (Danish Myo Technology). Aortic rings were preincubated with GDF15 (50ng/ml) or TGF-β (3ng/ml). Contraction to phenylefrine (PE) and relaxations to acetylcholine (Ach) were assessed in all groups. To evaluate the contributions of different relaxant pathways, Ach-mediated responsiveness was also studied in the presence of indometacine and L-NMMA.

Results: Contractions to PE and relaxations to ACh were not significant different between wt and GDF15 k.o mice. Preincubation with GDF15 impaired the contraction to PE (6.29±1.19 AUC in wt mice and 5.9±1.03 AUC in k.o mice with control treatment, 3.68±0.81 AUC in wt mice and 3.04±0.64 AUC in k.o mice with GDF15 treatment). Denudation restored PE responses to control levels, suggesting that the GDF15 mediated impaired contraction is endothelium-dependent. Preincubation with TGF-beta did not affect vasoconstriction and vasodilatation. Further, GDF15 treatment impaired total Ach (141±13.59 AUC in wt mice and 142±36.5 AUC in k.o mice in control treatment, 90±20.7 AUC in wt mice and 63±27.5 in k.o mice with GDF15 treatment).

Conclusion: We demonstrated for the first time that GDF15 plays an important role in inhibition of vascular contraction and relaxation in both wildtype and GDF15 knockout mice.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1753**

**Globally-Altered Permeability in Rat Models of Proteinuria** Andy Salmon,[1,2] Joanne Ferguson.[1] *[1]Physiology and Pharmacology, Univeristy of Bristol, Bristol, United Kingdom; [2]Clinical Science @ North Bristol, University of Bristol, Bristol, United Kingdom.*

Widespread vascular dysfunction accompanies albuminuria in both diabetic- and non-diabetic nephropathies. One facet of this widespread dysfunction is excessive leak from blood vessels throughout the body. In the kidney this is manifest as albuminuria, but leak also occurs from extra-renal blood vessels (e.g. excessive sodium fluorescein leak from skin microvessels in diabetic patients).

Increased capillary pressure and increased capillary wall permeability will both cause excessive leak. I have measured capillary wall permeability coefficients directly, in structurally distinct capillary beds (fenestrated capillary: glomeruli, and continuous capillary: gut mesentery) in two rat models of proteinuria: Munich-Wistar-Fromter (MWF) rats (that spontaneously develop focal segmental glomerulsosclerosis) and streptozotocin-induced diabetes mellitus, to test the hypothesis that altered capillary permeability occurs in diverse microvessels in proteinuria.

Adult male MWF rats were examined at 15-24 weeks, and adult male Sprague-Dawley rats were examined 7 days after streptozotocin (45 mg/kg iv). Animals were anaesthetised, the gut mesentery exteriorised, single microvessels cannulated and perfused with 4% bovine serum albumin (BSA) at known hydrostatic pressure, and hydraulic conductivity ($L_p$: $10^{-7}$ $cm.s^{-1}.cmH_2O^{-1}$) calculated. For glomerular experiments, single glomeruli were harvested by sieving, and exposed to an oncotic pressure gradient in a flow-controlled observation chamber, allowing calculation of hydraulic conductivity-area product ($L_pA$: $nl.min^{-1}.mmHg^{-1}$), adjusted for glomerular volume ($L_pA/V_i$).

Mesenteric $L_p$ (MWF: 6.4±4.6; Wistar: 2.8±0.4; $p<0.05$, unpaired t-test) and glomerular $L_pA/V_i$ (MWF: 1.5±0.1; Wistar: 1.0±0.1; $p<0.05$, unpaired t-test) were both increased in proteinuric MWF rats. Mesenteric $L_p$ (2.56±0.15 vs 2.13±0.11; $p<0.05$, unpaired t-test) and glomerular $L_pA/V_i$ (1.56±0.29 vs 0.93±0.07; $p<0.05$, unpaired t-test) were also both increased in proteinuric diabetic rats.

Widespread alterations in permeability occur early in these proteinuric animals. Future work will examine the structural correlates of these functional changes.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1754**

**Healthy Intraglomerular Arteriolar Function Predicts Doxorubicin-Induced Proteinuria, Which Can Be Reduced Using Angiotensin-II** Richard P. Van Dokkum, Peter Vavrinec, Robert H. Henning. *Clinical Pharmacology, University Medical Center Groningen, Groningen, Netherlands.*

*Background.* Susceptibility to renal injury varies among individuals. Previously, it was shown that individuals with pronounced baseline endothelial dilatory ability (measured *in vitro*) developed more renal damage after doxorubicin (doxo) injection. We tested whether constrictive ability of afferent (aff.) and efferent (eff.) glomerular arterioles in response to angiotensin-II (AII) predicts and whether AII limits the development of proteinuria after doxo injection.

*Methods.* Using an intravital microscopy set-up to envision *in vivo* glomerular arteriolar responses, three doses of AII were infused in an experimental group (n=15) and only saline throughout the entire protocol in a control group (n=16). Thereafter, proteinuria was induced in both groups by a doxo injection. During the intravital protocol, blood pressure (BP), heart rate (HR) and renal blood flow (RBF) were measured. Images of glomeruli were recorded for measurements of changes in glomerular arteriolar diameter. BP and proteinuria were followed for 6 wks. using the tail-cuff method and nephelometry, respectively.

*Results.* AII infusion significantly changed all measured hemodynamic parameters from baseline except HR (BP $p<0.05$; RBF $p<0.001$) and similarly when compared to the control group and induced significant contraction of afferent and efferent glomerular arterioles ($p<0.001$). Regression analysis on the change in aff. and eff. glomerular arteriolar diameter upon AII infusion and proteinuria 6 wks. after doxo injection showed a significant positive correlation ($p=0.04$ and $p=0.03$, respectively), which was also found between RBF at the time of injection and proteinuria 6 wks. thereafter. The group that received AII prior to the injection with doxo developed significantly less proteinuria than the saline treated group.

*Conclusion.* We conclude that glomerular arteriolar constrictive ability in response to AII predicts the development of doxo-induced proteinuria. Moreover, limiting blood supply to the kidney by infusion of AII diminishes the development of proteinuria, creating opportunities in reducing anti-cancer drug induced side effects.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1755**

**Effects of Chronic SDMA Infusion on Hemodynamics, Myocardial Function, GFR and Microvascular Fibrosis in C57 Black 6/J-Mice** Hendrik Veldink,[1] Robert Faulhaber-Walter,[1] Jens Martens-Lobenhoffer,[3] Arash Haghikia,[2] Harald Schuett,[2] Denise Hilfiker-Kleiner,[2] Joon-Keun Park,[1] Hermann G. Haller,[1] Jan T. Kielstein.[1] *[1]Department of Nephrology and Hypertension, Medical School Hannover, Hannover, Germany; [2]Department of Cardiolology and Angiology, Medical School Hannover, Hannover, Germany; [3]Institute of Clinical Pharmacology, Otto-von-Guericke-University, Mageburg, Germany.*

Background: Symmetrical dimethylarginine (SDMA), the structural isomer of the NOS inhibitor ADMA has long been regarded as an inert substance. Recent preclinical and epidemiological data suggest that it might be involved in the pathophysiology or renal and cardiovascular disease. Therefore we aimed to investigate the effect of chronic SDMA infusion on renal and cardiac function in mice. Methods: Vehicle controlled, infusion of SDMA at 250 micromol/kg/d for 28d using osmotic minipumps in 8 week old male C57Bl/6 mice (n=10 / group). Following parameters were monitored: GFR (FITC-inulin excretion kinetic), cardiac function (echocardiography), blood pressure (tail cuff). Blood samples for SDMA determination were obtained at baseline, 2 and 4 weeks. Mice were euthanized at 4 to obtain tissue for renal histology.Results: Chronic SDMA infusion lead to a significant increase of SDMA levels from 0.264±0.100 to 3.495±1.655 µmol/l ($p<0.001$) at 4 weeks. Despite this SDMA increase GFR did not change (1224±351 vs. 1017±345 ml/min/g bw, n.s.) at 4 weeks, as compared to baseline. We did not find histological changes, especially no effect on fibrosis or NOS expression. There was neither an effect of SDMA on blood pressure (106±12 vs. 111±18 mmHg, n.s.) nor on ejection fraction (54.2±1.7 vs. 58.4±1.9%, n.s.). Conclusion: SDMA exposure over 4 weeks in mice did not result in major changes in renal and cardiac function. Future studies have to clarify whether SDMA might have an effect in the presence of other cardiovascular risk factors.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1756**

**mPGES-1-Derived PGE2 Mediates Thiazolidinedione-Induced Vascular Hyperpermeability in the Adipose Tissue** Zhanjun Jia,[1,2] Toshinori Aoyagi,[1,2] Tianxin Yang.[1,2] *[1]Internal Medicine, University of Utah, Salt Lake City, UT; [2]Veteran Affairs Medical Center, Salt Lake City, UT.*

Adipose-specific increases capillary permeability contributes to thiazolidinedione (TZD)-induced fluid retention. We examined the role of microsomal prostaglandin E synthase-1 (mPGES-1) in this phenomenon. RGZ-induced body weight gain in WT mice was 1.72 ± 0.24 gm on day 7 and 1.62 ± 0.53 gm on day 14. In contrast, the BWG in mPGES-1 KO mice was absent on day 7 and reduced by 60% on day 14. RGZ-treated WT mice had a 60% increase in apidydimal water content, which was significantly attenuated in the KO mice. In parallel, the apididymal capillary perbeability (ACP), as assessed by Evans blue leakage assay, exhibited a 60% increase in WT mice after RGZ treatment. The KO mice had 60% and 62.5% reductions of ACP at baseline and after RGZ treatment, respectively. Interestingly, neither water content nor capillary permeability in the skeletal muscle was affected by RGZ or the genotype. In separate experiments, we examined time-dependent effects of RGZ on expression of key enzymes in the PGE2 synthesis pathway along with PGE2 production in the adidydimal fat depot (AFD). Adipose COX-2 induction was detected at day 1 of RGZ treatment and peaked at day 2 and declined at day 14. On day 2, qRT-PCR detected an 104-fold increase in COX-2 mRNA and immunoblotting a 98-fold increase in COX-2 protein, in parallel with a 2.5-fold increase in mPGES-1 mRNA and a 64-fold increase in PGE2 production in the AFD. At baseline, adipose mPGES-1 protein expression was suppressed by endothelial but not smooth muscle deletion of PPARγ. RGZ upregulated mRNA of apididymal VEGFB and VEGF receptor B, which was blocked by mPGES-1 deletion. In cultured endothelial cells, 1 µM RGZ induced increases in mPGES-1 mRNA and PGE2 release. mPGES-1 KO mice were intolerant to alive oil loading in term of the increased plasma levels of triglyceride, free fatty acid, and glycerol, a phenotype almost analogous to endothelial PPARγ KO mice. This study has elucidated PPARγ/COX-2/mPGES-1/PGE2/VEGFB pathway as a key determinant of adipose capillary permeability that influences transport of fluid as well as nutrients for metabolism.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1757**

**The Angiotensin II Receptor Type 2 Polymorphism Influences Hemodynamic Function and Circulating RAS Mediators in Normotensive Humans** David Cherney, Judith A. Miller, James W. Scholey, Heather N. Reich. *Medicine, University Health Network, Toronto, ON, Canada.*

**Background:** The hemodynamic responses to angiotensin II type 1 receptor blockade may be mediated by interactions between angiotensin II and the angiotensin II type 2 receptor (AT2R). An AT2R G1675A gene polymorphism has been described, but the functional effects of this polymorphism are unknown.

**Methods:** Hemodynamic function, circulating renin angiotensin system mediators and norepinephrine were measured in healthy subjects at baseline and at 2 and 4 weeks after treatment with irbesartan (75 mg daily). Subjects were divided into 2 groups on the basis of the AT2R G1675A gene polymorphism: AA/GA subjects (n=22) and GG subjects (n=12).

**Results:** AA/AG subjects exhibited hypotensive (mean arterial pressure decline from 86±3 to 81±3 mmHg, p<0.05) and renal vasodilatory responses to irbesartan at 4 weeks but GG subjects did not . In accord with hemodynamic effects, circulating aldosterone levels were suppressed in AA/AG subjects (89±10 to 59±19 pmol/L, p<0.05), while circulating norepinephrine levels were augmented only in GG subjects. In contrast, increases in circulating renin, angiotensin II and plasma renin activity after irbesartan were exaggerated in AA/AG subjects (p<0.05 vs. GG subjects).

Table 1: Renal hemodynamic function responses to irbesartan in subjects with and without the AT2R G1675A gene polymorphism (mean±SD)

| | Baseline | 4 weeks |
|---|---|---|
| | AA/AG group | |
| ERPF (ml•min -1•1.73 m-2) | 697±45 | 749±53* |
| GFR (ml•min -1•1.73 m-2) | 118±6 | 112±5 |
| FF | 0.18±0.01 | 0.15±0.01* |
| RBF (ml•min -1•1.73 m-2) | 1097±68 | 1215±82 |
| RVR (ml•l -1•min -1) | 0.083±0.006 | 0.070±0.005* |
| | GG group | |
| ERPF (ml•min -1•1.73 m-2) | 719±37 | 750±42 |
| GFR (ml•min -1•1.73 m-2) | 120±7 | 120±7 |
| FF | 0.17±0.10 | 0.16±0.01 |
| RBF (ml•min -1•1.73 m-2) | 1214±59 | 1221±67 |
| RVR (ml•l -1•min -1) | 0.072±0.005 | 0.069±0.005 |

* p<0.05 for effect of irbesartan

**Conclusions:** The AT2 G1675A polymorphism is a determinant of hemodynamic responses to AT1 receptor blockade, an effect that may be due to influences on aldosterone escape.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1758**

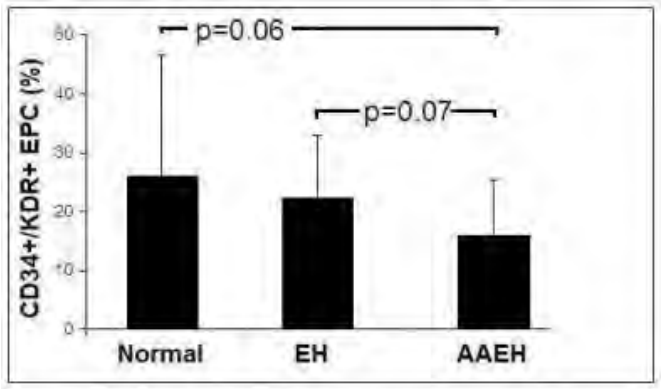
**Ethnic Differences in the Number of Endothelial Progenitor Cells and Inflammatory Biomarkers in Patients with Essential Hypertension** Alfonso Eirin,[1] Monika L. Gloviczki,[1] Mario Gossl,[2] Hui Tang,[1] Kyra L. Jordan,[1] Amir Lerman,[2] Stephen C. Textor,[1] Lilach O. Lerman.[1] *[1]Division of Nephrology and Hypertension, Mayo Clinic; [2]Cardiovascular Diseases, Mayo Clinic, Rochester, MN.*

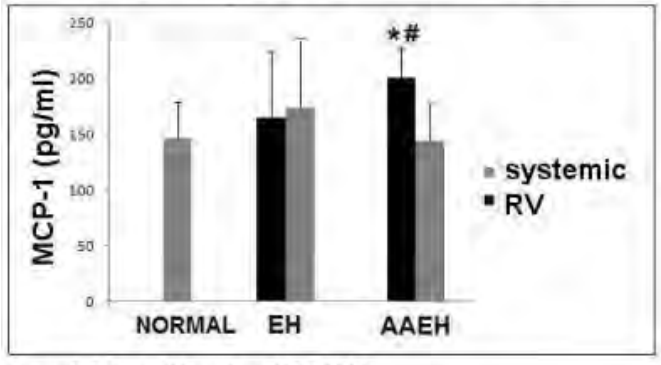
**Introduction:** Morbidity and mortality associated with hypertension are higher in African American (AAEH) compared to Caucasian (EH) patients, possibly related to increased inflammation. Because circulating endothelial progenitor cells (EPC) promote vascular repair after injury and might thereby curtail target organ damage, we hypothesized that plasma EPC levels would be lower and inflammatory biomarkers higher in AAEH compared to EH patients.

**Methods:** Hypertensive subjects treated with blockade of the renin-angiotensin system were studied during 150 mEq sodium intake . We measured renal vein (RV) and inferior vena cava (IVC) levels of tumor necrosis factor alpha (TNF-), interleukin (IL)-6, IL-10, monocyte chemoattractant protein-1 (MCP-1), and vascular endothelial growth factor (VEGF), as well as circulating CD34+/KDR+ EPC, in treated EH (n=17), AAEH (n=13), and normotensive control (n=7) subjects.

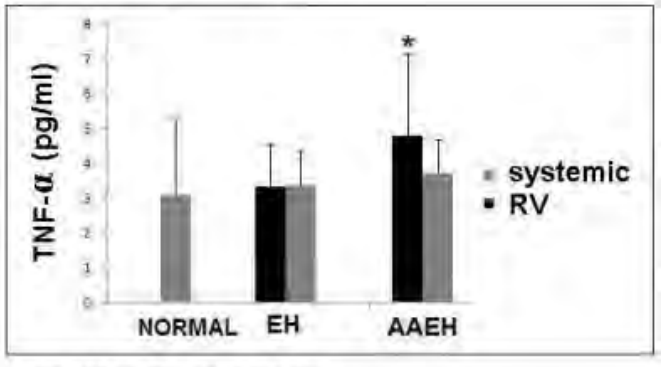
**Results:** Mean arterial pressure, serum creatinine, eGFR, cholesterol fractions, and antihypertensive medications did not differ between EH and AAEH patients. Renal vein levels of MCP-1 and TNF-were higher in AAEH compared to normal and EH patients (p<0.05). These differences were eliminated in the IVC. The number of circulating CD34+/KDR+ EPC tended to be lower in AAEH (p=0.06 vs. normal, p=0.07 vs. EH) (Figure).

**Conclusion:** Lower circulating levels of CD34+/KDR+ cells were associated with increased levels of inflammatory biomarkers in AAEH patients from the renal vein, suggesting greater potential for inflammatory kidney injury and impaired repair mechanisms. These mechanisms may contribute to the poorer outcomes of hypertension in AAEH compared to Caucasian EH patients.




* p<0.05 vs EH and vs NORMAL
\# p<0.05 vs systemic AAEH




* p<0.05 vs EH and vs NORMAL

Disclosure of Financial Relationships: nothing to disclose

**F-PO1759**

**Normal Values for Arterial Stiffness in a Large Cohort of Healthy Children and Adolescents** Daniela Kracht,[1] Anke Doyon,[3] Christoph Jacobi,[1] Franz S. Schaefer,[3] Bernhard M. W. Schmidt,[2] Sajoscha A. Sorrentino,[2] Elke Wuehl,[3] Anette Melk.[1] *[1]Pediatric Nephrology, Medical School, Hannover, Germany; [2]Nephrology, Medical School, Hannover, Germany; [3]Pediatric Nephrology, University Hospital, Heidelberg, Germany.*

Study purpose: Aortic pulse wave velocity (aPWV), an indicator of arterial stiffness predicts cardiovascular mortality in adults. Arterial stiffening advances with age and is accelerated in specific diseases. In childhood aPWV has not been investigated in larger cohorts. The aim of this study is to provide normal values and prove the suspected increase with age.

Methods: Pulse waves were captured by oscillometry simultaneously on the right carotid and femoral artery (Vicorder) in 405 healthy school children aged 6 to 18 years. Intima-media thickness (IMT) and elasticity on both carotid arteries by B-and M-mode ultrasound was measured.

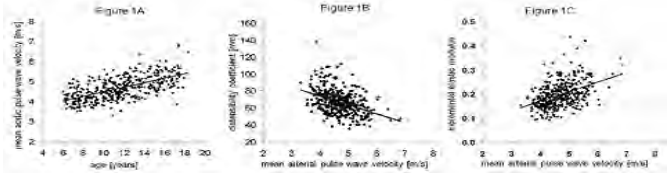
Results: aPWV significantly increased with age: 6-8 year olds (n=97): 4.2±0.4 m/s; 9-11 year olds (n=135): 4.5±0.4 m/s; 12-14 year olds (n=97): 4.9±0.5 m/s; 15-18 year olds (n=76): 5.2±0.5 m/s (p<0.0001). aPWV significantly correlated with age (r=0.63, p<0.0001, fig.1A). Further significant correlations existed for weight, height, mean systolic and diastolic blood pressure. We found no correlation of aPWV with IMT, but significant correlations with elasticity markers: distensibility coefficient (r=-0.42, p<0.0001, fig.1B)

and incremental elastic modulus (r=0.43, p<0.0001, fig.1C). Independent predictors for aPWV in multiple regression analysis were found for age, gender, diastolic blood pressure and elasticity parameters.

Conclusions: This study defines aPWV normal values in children and adolescents using a new non-invasive

oscillometric method. Even in healthy children correlations to cardiovascular risk factors can be seen.

Interestingly, a connection of aPWV to functional parameters of arterial elasticity was observed.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1760**

**Apical Lumen Formation Is Disrupted by Rosiglitazone in MDCK Cells through Inhibition of Cdc42 Activation** Zhiguo Mao,[1,2] Andrew J. Streets,[1] Albert C. Ong.[1] *[1]Academic Unit of Nephrology, Medical School, University of Sheffield, Sheffield, United Kingdom; [2]Division of Nephrology, Kidney Institute of CPLA, Changzheng Hospital, Second Military Medical University, Shanghai, China.*

Thiazolidinediones have been reported to retard cystic disease in rodent models by uncertain mechanisms. To clarify potential mechanisms of action, we investigated the effect of the highly selective PPAR gamma agonist, rosiglitazone, in the well-established MDCK model of cyst formation. The inhibitory effect of rosiglitazone on overall cyst expansion was accompanied by a reduction in basal cell proliferation and an increase in apoptosis. In addition, we observed a striking early abnormality in apical lumen formation resulting in a characteristic multiple lumen or loss of lumen phenotype in treated cells at doses which did not inhibit cell proliferation. These changes were preceded by mislocalisation of the apical marker protein gp135, misorientation of the mitotic spindle and retardation in centrosome reorientation. We observed later changes in primary cilia length and mislocalisation of E-cadherin in treated cysts. Cdc42 activation was profoundly inhibited by rosiglitazone and its cellular localization was randomised in parallel with gp135. We conclude that rosiglitazone influences MDCK cyst growth by multiple mechanisms involving dosage-dependent effects on proliferation, apoptosis, spindle orientation, centrosome migration and apical lumen formation. The loss of Cdc42 activation and its spatial mislocalisation is the earliest defect that underlies the observed abnormality in apical lumen formation. These changes could underlie the inhibitory effect of these compounds on cystic disease in vivo.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1761**

***In Vivo* Phosphorylation Status of Polycysytin-2 Correlates to the Formation of Polycystin-2 Associated Complexes in Kidney Tissue** Yiqiang Cai, Xin Tian, Seung H. Lee, Xiaoli Xie, Ming Ma, Stefan Somlo. *Internal Medicine, Yale University School of Medicine, New Haven, CT.*

Loss of polycystin-2 (PC2), a cation channel, results in autosomal dominant polycystic kidney disease (ADPKD). PC2 has been localized to cilia and is abundantly expressed in the endoplasmic reticulum. Loss of signaling by the PC1/PC2 complex underlies the pathogenesis of ADPKD. Our previous studies have shown that PC2 is constitutively phosphorylated at residue $Ser^{812}$, and that loss of phosphorylation at $Ser^{812}$ results in a 10-fold decrease in sensitivity to $Ca^{2+}$ activation of PC2 but has no effect on PC2 localization at the ciliary membrane. We recently employed a screen using phosphoproteomic analysis of TiO2 enriched immunoprecipitated PC2, and identified two novel phosphorylation sites in cultured epithelial cells. To gain further understanding of the functional role of PC2 phosphorylation in native tissues, we have now carried out a phosphoproteomic analysis of PC2 in mouse kidney. PC2 immunoprecipitated from mouse kidney yielded two PC2-positive bands with respective apparent molecular masses of ~110 kDa and ~200 kDa. Phosphoproteomic analysis of these bands revealed that the 200-kDa containing PC2 was not phosphorylated, whereas the 110-kDa PC2 had two residues that were phosphorylated. One of the two identified phosphorylation sites was the previously described $Ser^{812}$, whereas the other site is novel and was not detected in the cell culture-based screen. Taken together with the previous studies, these data suggest several conclusions. First, $Ser^{812}$ is constitutively phosphorylated in native kidney tissues. Second, phosphorylation of PC2 in cultured cells is different from that in the native kidney. Third, PC2 migrating as a monomer is phosphorylated, whereas multimeric PC2 appears to be dephosphorylated. Discovery of the mechanisms controlling PC2 phosphorylation in kidney tissues will provide novel insights into functional regulation of PC2 *in vivo*.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1762**

**Ciliary Localization of Retinitis Pigmentosa 2 (RP2) Is Regulated by Importin Beta-2** Shuling Fan, Toby W. Hurd, Benjamin L. Margolis. *Internal Medicine, University of Michigan Medical School, Ann Arbor, MI.*

Ciliopathies represent a newly emerging group of human diseases that share a common etiology, dysfunction of cilium or centrosome. We have been examining the RP2 protein which is mutated in X-linked Retinitis Pigmentosa. It has been demonstrated that RP2 localizes to primary cilia and this requires the dual acylation of the amino-terminus, but the precise mechanism by which RP2 is trafficked to the cilia is unknown. Here we have characterized a novel interaction between RP2 and importin beta-2 (transportin-1), a member of the importin beta family that regulates nuclear-cytoplasmic shuttling. We demonstrate that importin beta-2 is necessary for localization of RP2 to primary cilia as ablation of importin beta-2 by shRNA blocks entry both of endogenous and exogenous RP2 to the cilium. Furthermore, we identify two distinct binding sites of RP2 which interact independently with importin beta-2. One binding site is a Nuclear Localization Signal (NLS) like sequence that is located at the amino-terminus of RP2 and another is an M9-like sequence within the Tubulin Folding Cofactor C (TBCC) domain. We find that the NLS like sequence is not essential for RP2 ciliary targeting, since mutation of NLS like consensus sequence does not abolish the localization of RP2 to cilia. Interestingly, we find that several missense mutations that cause human disease fall within the M9-like sequence of RP2 and these mutations block entry of RP2 to the cilium as well as its interaction with importin beta-2. Together this work demonstrates a novel role of importin beta-2 that regulates entry of RP2 and likely other proteins into the ciliary compartment and this process may be analogous to the shuttling of proteins between the cytosol and nucleus.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1763**

**Inversin Modulates the Cortical Actin Network during Mitosis** Robert L. Bacallao,[1] Heather H. Ward,[1] Vincent H. Gattone,[2] Michael Werner.[1] *[1]Medicine, Indiana University and Richard Roudebush VAMC, Indianapolis, IN; [2]Anatomy and Cell Biology, Indiana University, Indianapolis, IN.*

Mutations in inversin cause Nephronopthisis type II, an autosomal recessive infantile form of polycystic kidney disease associated with situs inversus, dilatation of renal tubules and kidney cyst formation. Inversin regulates Wnt signaling by targeting Dishevelled for APC/C mediated degradation; however the underlying mechanisms by which inversin prevents cyst formation are unknown. Since cyst formation may represent a planar polarity defect we investigated whether inversin plays a role during cell division. Depletion of inversin by RNAi in mammalian tissue culture cells leads to an increase in bi- or multinucleated cells. While spindle assembly, contractile ring formation or furrow ingression appear normal in the absence of inversin, mitotic cell rounding and the underlying rearrangement of the cortical actin cytoskeleton are perturbed. In particular we find that loss of inversin causes the formation of extensive filopodia in both interphase and mitotic cells. Failure to properly round up in metaphase and ensuing spindle positioning defects lead to frequent misalignment of the division plane and unequal cell division. Similarly we observe an increase in binuclear cells, an increase in overall cell area and spindle positioning defects in nephritic tubules from inv-/- mouse embryos. Together these data suggest that inversin is a regulator of the cortical actin cytoskeleton that is required for proper cell rounding and spindle positioning during mitosis. By participating in mitotic spindle control, inversin contributes to the integrity of tubular epithelium during kidney development.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1764**

**Loss of microRNAs Reduces Polycystic Kidney Disease Severity in an ADPKD Mouse Model** Carol G. Carlton,[1] Klaus B. Piontek,[2] Gregory G. Germino,[3] Gregory B. Vanden Heuvel.[1] *[1]Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS; [2]Medicine, Johns Hopkins University, Baltimore, MD; [3]NIDDK, Bethesda, MD.*

The processing enzyme Dicer1 is essential for the production of microRNAs (miRNAs). Complete deletion of Dicer1 is embryologically lethal prior to gastrulation indicating the importance of this protein in development. To determine the role of miRNAs in kidney development, we used a conditional allele of Dicer1 to specifically delete miRNA processing in the ureteric bud derivatives. These mice, called $Dicer^{CD}$, exhibited hydronephrosis and cortical cysts, demonstrating the importance of miRNAs in kidney development. Recent studies have shown that Polycystin-2 is regulated by the miRNA miR-17 (Development 137:1107-1116, 2010). To begin to determine whether miRNAs interact with Polycystin-1, we deleted both Dicer and Pkd1 in the ureteric bud derivatives. Mice carrying floxed alleles of both Dicer and Pkd1 were crossed with Hoxb7cre mice to generate $Dicer^{CD}$/$Pkd1^{CD}$ mice. $Dicer^{CD}$/$Pkd1^{CD}$ mice were compared with mice carrying only the $Dicer^{CD}$ or the $Pkd1^{CD}$ mutations at postnatal day 0 (P0), P7, and P14. At all stages, $Dicer^{CD}$/$Pkd1^{CD}$ mice showed fewer renal cysts with an overall reduction in kidney size than $Pkd1^{CD}$ mice. Moreover, while Pkd1CD mice died between P14 and P17, $Dicer^{CD}$/$Pkd1^{CD}$ mice survived until P31. Taken together, these results suggest that miRNAs are involved in polycystic kidney disease progression.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1765**

**The Role of Fibrocystin in Post-Developmental Renal Homeostasis and Repair of Injury** Rachel Gallagher,[1] Seung H. Lee,[1] Sorin V. Fedeles,[1] Stefan Somlo.[1, 2] *[1]Internal Medicine, Yale School of Medicine, New Haven, CT; [2]of Genetics, Yale School of Medicine, New Haven, CT.*

Human autosomal recessive polycystic kidney disease (ARPKD) is largely a developmental disorder that results from mutations in a single gene locus, *PKHD1*. The *PKHD1* protein product, fibrocystin/polyductin (FPC) has been implicated in mitotic spindle orientation during postnatal kidney tubule elongation, a process thought to be governed by planar cell polarity (PCP) pathways. The predominant kidney presentation in ARPKD is severe perinatal or juvenile-onset collecting tubule dilation which coincide with *in utero* development and postnatal growth of the kidneys, respectively. These are periods when developmental PCP processes are active. Studies of *Pkhd1* have focused on its pathogenic role in ARPKD, however its role in adult kidney homeostasis independent of any polycystic disease has yet to be elucidated. In the current study, we sought to examine whether loss of FPC plays a role in post-developmental recovery from kidney injury. *Pkhd1*$^{del4/del4}$ mice and littermate controls were subjected to acute kidney injury (AKI) by 3 days of unilateral ureteral obstruction (UUO) followed by recovery periods of 4 days and 14 days after release of UUO. The *Pkhd1*$^{del4/del4}$ mice had comparable injury to their wild type littermates following 3 days of UUO but had impaired recovery with sustained tubule dilation in collecting duct segments following release of UUO. Histochemical staining and quantitative PCR analysis show increased fibrosis in the *Pkhd1* mutant mice 14 days after release of UUO. In addition there were significantly increased levels of inflammatory infiltrates including myofibroblasts and macrophages in the injured *Pkhd1* mutant kidneys. In aggregate, the data demonstrate aberrant repair following 3 day injury by UUO in the absence of FPC and therefore implicate FPC in normal renal homeostasis and repair following AKI. Further elucidation of the pathways involved will lend insight into the function of FPC and the mechanisms of repair after injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1766**

**Constitutive Up-Regulation of IL-8 in ADPKD by AP-1 and β-Catenin Activation** Ram Singh,[1] Wei Wei,[2] Elsa Bello-Reuss.[1] *[1]Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, TX; [2]Internal Medicine, UTMB, Galveston, TX.*

Angiogenesis is prominent in ADPKD and a major factor for cyst growth. We studied the mechanisms of activation of the angiogenic cytokine IL-8 in ADPKD kidneys, cyst-cell primary cultures (CC) and cell lines with defined genotypes: normal human kidney (UCL93/3) and ADPKD (SKI-001 and OX161). We found constitutive up-regulation of IL-8 and IL-8 receptor-B in cysts of ADPKD kidneys. IL-8 mRNA was ~100% higher in CC than in normal tubule cells (NTC, $P<0.001$) and, by ELISA, the secretion of IL-8 was increased 3-4 fold ($P<0.001$ *vs.* NTC). IL-8 promoter activity (luciferase assay) increased 4-fold in MDCK cells transfected with polycystin-1 (PC1) *vs.* control cells ($P< 0.001$) as well as after transfection with a truncated PC1 that, in humans, causes ADPKD. Immunostaining with anti-PC1 C-terminus antibody 46-2 (CTab) showed increased expression of PC1 in cysts of ADPKD kidneys and no expression in normal kidneys. PC1 C-terminus (CT) activates the IL-8 promoter containing multiple AP1 binding sites. Expression of β-catenin (β-cat) in normal kidney cells also activates the IL-8 promoter. Western blots showed that the CTab detects full length PC1 in UCL93/3 and in SKI-001 cells but not in OX161 cells. CT fragments were present in nuclear extracts of normal and ADPKD cell lines. Transcriptional activity of β-cat, measured by lenti-TCF/LEF luminescence assay, was increased in ADPKD cell lines ($P<0.001$ *vs.* control). Co-immunoprecipitation showed β-cat pulldown by PC1 in lysates of normal cells but not in lysates of ADPKD cell lines. In cyst cells, silencing IL-8 inhibited cyst growth; silencing β-cat decreased expression of β-cat and IL-8 and inhibited cyst growth. In summary: 1. IL-8 is increased in ADPKD. 2. CT fragments activate AP-1 binding sites of the IL-8 promoter 3. β-cat is activated in ADPKD cyst cells and does not co-precipitate with PC1. 4. Transactivation of the TCF/LEF site by β-cat activates the IL-8 promoter. The inhibition of β-cat activation by CT, found in normal cells, is absent in ADPKD cyst cells. 5. Silencing IL-8 or β-cat inhibits *in-vitro* cyst growth.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1767**

**Cystogenesis of ARPKD Results from Increased Apoptosis in Collecting Duct Epithelial Cells of *Pkhd1* Mutant Kidneys** Bo Hu, Ao Li, Dan Liang, Guanqing Wu. *Medicine and Cell & Developmental Biology, Vanderbilt University, Nashville, TN.*

Mutations of *PKHD1* result in autosomal recessive polycystic kidney disease (ARPKD) in humans. To determine the molecular mechanism of cystogenesis in ARPKD, we have recently generated a mouse model for ARPKD which carries a targeted mutation on the mouse orthologue of human *PKHD1* gene (*JASN 2008:19;455*). The homozygous mutant mice display hepatorenal cysts whose phenotypes are similar to human ARPKD patients. Using littermates of this mouse model, we developed a pair of immortalized renal collecting duct cell lines with or without *Pkhd1*. Under nonpermissive culture conditions, *Pkhd1*$^{-/-}$ renal cells display aberrant cell-cell contact, ciliogenesis, and tubulomorphogenesis compared to wildtype littermate cells. We also found significant reduction in cell proliferation and elevated apoptosis when *Pkhd1* is absent. To determine which factor was the primary cause of ARPKD phenotypes, cell proliferation or apoptosis, we analyzed multiple putative signaling regulators affected by *Pkhd1* absence. We demonstrated that loss of *Pkhd1* disrupts focal adhesion kinase (FAK) phosphorylation, which inhibits the Ras/C-Raf pathways, and causes suppressed MEK/ERK activity, ultimately contributing to reduced cell proliferation. These observations indicate that cell proliferation may not be a necessary factor to induce cystogenesis in ARPKD. We therefore assume that apoptosis may act as a major player to induce cyst formation in ARPKD. Compared to wildtype cells, *Pkhd1*-deficient cell lines showed significantly increased apoptosis via inhibition of PDK1/AKT and upregulation of Bax/caspase-9/caspase-3. To validate this finding *in vivo*, we examined proliferation and apoptosis between the kidneys of *Pkhd1*$^{-/-}$ and their wildtype littermates. Using proliferation (PCNA and Histone-3) and apoptosis (TUNEL and caspase-3) staining makers, we also found significantly increased apoptosis and decreased proliferation in the *Pkhd1*$^{-/-}$ kidneys. The finding indicates that apoptosis may act as a major player for cyst formation in ARPKD, which can guide us to establish new therapeutic strategies for ARPKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1768**

**Overexpression of Polycystin-1 Alters Morphogenetic Rearrangements of Cultured Renal Tubular Cells** Valerie Leroy,[1] Udo Hasler,[1] Pierre-Yves F. Martin,[2] Eric Feraille.[1] *[1]Cell Physiology and Metabolism, University of Geneva, Geneva, Switzerland; [2]Internal Medicine, University Hospital of Geneva, Geneva, Switzerland.*

Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations of polycystin (PC)-encoding genes PKD1 or PKD2 that upset a genetically defined program that controls the size and geometry of renal tubules. Cysts which frequently emanate from the collecting duct (CD) result from cell proliferation and fluid secretion. Either invalidation or overexpression of PKD1 results in polycystic kidney disease in transgenic mouse models, suggesting a major role of imbalanced PC1/PC2 expression in ADPKD. To test this hypothesis, we used mCCDcl1 cells, a differentiated mouse CD principal cell line, to generate cells conditionally overexpressing the full-length PC1 in the presence of doxycyclin (Dox). Microscopy analysis of cells grown in collagen/matrigel matrix showed that while PC1 overexpression is not sufficient to induce cyst formation, it deeply alters morphogenetic rearrangement. We observed an inhibition of the initial elongation of cells rods, that resulted in decreased number and length of tubular structures, a delay of tubular lumen development and a decreased number of tubular branching events. To investigate the underlying mechanisms of altered tubulogenesis, we assessed the effects of PC1 overexpression on cell proliferation, migration and terminal differentiation. PC1 overexpression reduced density-dependent growth, promoted cell adhesion on collagen and stimulated cell migration in a wound-healing assay. Neither total nor cell surface abundance of key ion and water transporters nor the polarized distribution of Na,K-ATPase was altered. However, PC1 overexpression increased transepithelial resistance under basal conditions and promoted its recovery in calcium switch experiments. These effects were associated with increased E-cadherin protein expression and lateral membrane localization. These results do not support the idea that imbalanced expression of PC1/PC2 per se is responsible for cyst development by collecting duct cells, but rather shed light on the important role of PC1 in branched tubular morphogenesis and intercellular junctions dynamics.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1769**

**Role of Integrin-β1 in the Cystogenesis and Hyperproliferation of ADPKD Cells** Kim Lee, Lorenzo Battini, Carles Martinez-Romero, Lin Geng, G. Luca Gusella. *Division of Renal Medicine, Mount Sinai School of Medicine, New York, NY.*

Dysregulation of the PKD1 gene, encoding polycystin-1 (PC-1), leads to autosomal polycystic kidney disease (ADPKD), a disease characterized by formation and progressive expansion of renal cysts, and renal failure. Cystic renal epithelia are hyperproliferative and characterized by increased expression of integrin-α2β1. We have previously shown that integrin-α2β1 mediate the resistance of PC1 knockdown cells to anoikis. The purpose of this study is to determine the role of integrin-β1 in the cystogenic process and hyperproliferation of ADPKD cells.

As a model system of ADPKD, we used the inner medullary collecting duct cell line, IMCD3, and the canine Madine-Darby collecting duct cell line, MDCK, in which the expression of PC1 is constitutively knocked down following the transduction with a lentivirus carrying a specific anti-*PKD1* siRNA. This model system recapitulates features that characterize ADPKD, such as hyperproliferation and cystic growth, centrosome amplification, and increased apoptosis. We show that the increased integrin-β1 expression in PC1 knockdown cells heightens their sensitivity to stimulation with collagen type I. In addition, the knockdown of PC1 expression is associated with the increased extracellular deposition of fibronectin (FN), a ligand of integrin-β1 heterodimers. Interference with the expression of FN lead to the reduction of the proliferation of PC1 knockdown cells, indicating that a positive feed-back signaling mechanism between FN and integrins contributes to the stimulation of cystic growth in ADPKD.

We also show that the siRNA-mediated inhibition of integrin-β1 reverted the proliferation, adherence, centrosome integrity, and migratory function of PC1 knockdown cells to the levels of control parental cells. No adverse effects on the cell viability were observed during integrin-β1 suppression. Overall, these observations support the essential role of integrin-β1 and extracellular matrix components in a new mechanism of cystic cells proliferation. Furthermore, our findings suggest that the targeting the integrin-β1 signaling pathway may be an effective approach to slow down ADPKD progression.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1770**

**A Single Amino Acid Residue Constitutes the Third Dimerization Domain Essential for the Assembly and Function of the Polycystin-2 (TRPP2) Channel** Shuang Feng,[1] Lise Rodat-Despoix,[2] Patrick Delmas,[2] Albert C. Ong.[1] *[1]Kidney Genetics Group, Academic Nephrology Unit, University of Sheffield Medical School, Sheffield, South Yorkshire, United Kingdom; [2]Centre de Recherche en Neurophysiologie et Neurobiologie de Marseille, CNRS, Université de la Méditerranée, Marseille, France.*

Autosomal dominant polycystic kidney disease (ADPKD), the most common inherited cause of kidney failure, is caused by mutations in either *PKD1* (85%) or *PKD2* (15%). The PKD2 protein, polycystin-2 (PC2 or TRPP2), is a member of the transient receptor potential (TRP) superfamily and functions as a non-selective calcium channel. PC2 has been found to form oligomers in native tissues suggesting that similar to other TRP channels, it may form functional homo- or heterotetramers with other subunits. We have recently demonstrated that the homodimerisation of PC2 is mediated by both N-terminal and C-terminal domains and it is known that PC2 can heterodimerise with PC1, TRPC1 and TRPV4.

In this paper, we report that a single cysteine residue, C632, mutated in a known PKD2 pedigree, constitutes the third dimerisation domain for PC2. PC2 truncation mutants lacking both N- and C-termini could still dimerise under non-reducing conditions. Mutation of C632 alone completely abolished dimerisation in these mutants indicating that it was the critical residue mediating disulphide-bond formation between PC2 monomers. Full-length PC2 channels with a C632A mutation showed diminished ATP-sensitive ER $Ca^{2+}$ release in HEK293 and in combination with mutations in the C-terminal coiled coil domain (4M) completely abolished channel activity. However, unlike the 4M mutation, a C632A mutant could still heterodimerise with polycystin-1 (PC1). Our results indicate that PC2 homodimerisation is regulated by three distinct domains and that these events regulate formation of the tetrameric PC2 channel.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1771**

**The Polycystin-1 PLAT Domain Acts as a Binding Scaffold for Phospholipids and Targets Polycystin-1 to the Basolateral Membrane of Epithelial Cells** Yaoxian Xu, Andrew J. Needham, Albert C. Ong. *Kidney Genetics Group, Academic Unit of Nephrology, University of Sheffield Medical School, Sheffield, S. Yorkshire, United Kingdom.*

The PLAT (Polycystin-1, Lipoxygenase and Alpha Toxin) or LH2 (Lipoxygenase Homology 2) domain is a signature domain of polycystin-1 (PC1), the protein product of *PKD1*, the gene mutated in 90% of patients with autosomal dominant polycystic kidney disease (ADPKD). It is highly conserved in the polycystin protein family, being present in all PC1 homologues (PKD1L1, PKD1L2, PKD1L3, PKDREJ) and shows evolutionary conservation in orthologues down to nematodes. PLAT is predicted to have a β-sandwich fold but its precise structure has not been elucidated. In addition, little is known about the function of mammalian PLAT though the PLAT domain of the *C. elegans* PC1 orthologue, *LOV1*, is reported to bind to the β-subunit of CK2 and to ATP-2, the β-subunit of ATP synthase. Here, we report the first experimental evidence that PC1 PLAT can act as a lipid binding scaffold. Recombinant PLAT proteins bound to two plasma membrane phospholipids, phosphatidylserine (PtdSer) and phosphoinositol-4-phosphate (PI-4P) but not to PI-4,5P2 or PI-3,4,5P3 on lipid arrays and this was verified by liposome sedimentation assays and lipid bead pull-down. Mutation of two predicted charged residues abolished binding to PtdSer but not to PI-4P. A PLAT-YFP fusion showed basolateral plasma membrane localisation in stably transfected MDCK cells which overlapped most closely with desmoplakin but did not localise to primary cilia. Mutation of the PtdSer binding residues alone did not alter basolateral expression of the PLAT-YFP fusion. We conclude that PC1 PLAT can function both as a membrane associated scaffold for phospholipid signalling and provides independent targeting information for PC1 localisation to the basolateral membrane via discrete lipid-protein and protein-protein interactions.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1772**

**PCP Proteins Vangl2 and Dvl Regulate a Novel Apical Localization of Polycystin-1** I.-Chun Tsai,[1] Zheng D. Lan,[1] Feng Qian,[1] Gregory G. Germino.[1,2] *[1]Division of Nephrology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; [2]NIDDK, NIH, Bethesda, MD.*

Recent studies suggest that the planar cell polarity pathway (PCP) may play an important role in regulating renal tubule diameter. This pathway is activated by a subset of Wnts and Frizzled receptors and is mediated by membrane and intracellular proteins, including Vangl2 and Dishevelled (Dvl). Downstream of Vangl2 and Dvl, small GTPases such as Rho and Rac then join to facilitate cytoskeleton rearrangement. PCP signaling is thought to produce oriented tubular extension during the tubular growth phase of renal development. There also is emerging evidence indicating that disruption of PCP signaling within the kidney can result in renal cystic disease. As a first step in determining the role of PC1 in this process, we developed an MDCK cell culture system with inducible expression of EGFP-tagged PC1 and used this system to identify a previously undescribed apical pool of PC1 that co-localizes with Dishevelled (Dvl) and Vangl2. We confirmed this pattern for endogenous PC1 in IMCD cells. Disruption of PCP activity by over-expressing dominant negative Dvl, silencing Vangl2 or inhibiting aPKC prevents PC1 from localizing to this apical pattern. Importantly, this localization appears to be essential for proper tubule formation since a PC1 mutant that does not localize apically is also defective in forming tubules in vitro. In addition, PC1 cell fails to form tubule when Vangl2 expression is knocked down. Finally, we investigated PC1 signaling downstream of PC1 and found enhanced RhoA activation, localized to the apical membrane near PC1. Our findings directly link PC1 to canonical PCP proteins and suggest that PC1 is one of the effector molecules downstream of PCP signaling.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1773**

**Morpholino-Mediated Knockdown of a Novel RP2-Interacting Centrosomal Protein, OSTF1, Results in Cystic Kidneys and Microphthalmia in Zebrafish** Toby W. Hurd,[1] Weibin Zhou,[1] Benjamin L. Margolis,[2] Friedhelm Hildebrandt.[1,3,4] *[1]Department of Pediatrics, University of Michigan, Ann Arbor, MI; [2]Department of Internal Medicine, University of Michigan; [3]Department of Human Genetics, University of Michigan; [4]Howard Hughes Medical Institute.*

Cilia dysfunction has been linked to an ever expanding group of human diseases that have been termed ciliopathies. Within the kidney primary cilia protrude from the apical side of renal epithelia into the tubular lumen. Abnormal function or formation of these cilia may underlie the cystic kidney disease phenotype as found in a number of different disorders including both autosomal recessive and autosomal dominant polycystic kidney disease (ARPKD and ADPKD, respectively) and nephronophthisis. In many ciliopathies, cystic kidneys are accompanied by extra-renal manifestations such as retinal degeneration, polydactyly and mental retardation. We have been examining the function of the Retinitis Pigmentosa 2 (RP2) protein in renal cilia. We have previously demonstrated that RP2 localizes to renal cilia and forms a complex with Polycystin 2. Loss of RP2 results in accumulation of Polycystin 2 within the cilia and gives rise to cystic kidneys in Zebrafish. Here we identify a novel RP2-interacting protein, OSTF1, that localizes to centrosomes but not cilia. Interestingly, a pathogenic missense mutation in RP2, L253R, abolishes this interaction. Similar to RP2, knockdown of OSTF1 by shRNA results in golgi fragmentation but does not perturb ciliogenesis in cell culture. Finally we demonstrate that in zebrafish loss of OSTF1 gives rise to pronephric cysts and retinal degeneration. These data indicate that OSTF1 and RP2 represent an important complex that may regulate cilia function in both kidney and eye. Further understanding of these proteins may provide unique insight into the etiology of retinitis pigmentosa and cystic kidney disease especially in syndromic ciliopathies such as Senor Loken syndrome (SLS) where there is involvement of both organs.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1774**

**In Vivo Analysis of the Drosophila PKD2 (TRPP2) Homolog** Michael Kottgen,[1] Alexis Hofherr,[1] Weizhe Li,[2] Kristy Chu,[2] Terry J. Watnick.[2] *[1]Nephrology, University Hospital Freiburg, Freiburg, Germany; [2]Nephrology, Johns Hopkins School of Medicine, Baltimore, MD.*

Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in either one of two genes, PKD1 or PKD2, which encode for Polycystin-1 and TRPP2, respectively. TRPP2 functions as a non-selective cation channel in the primary cilium. Activation of TRPP2 triggers cytosolic Ca2+ signals, but its physiological function in vivo remains unknown. The Drosophila melanogaster homolog of TRPP2 is a testis-specific protein that is localized at the tip of the sperm tail. We used Drosophila as a model organism to study TRPP2 function.

In order to test the function of PKD2 patient mutations in vivo we expressed wild-type (wt) and mutant TRPP2 in TRPP2 knock-out flies. Wt and mutant sperm were studied using multiple approaches, including fertility tests, immunofluorescence and in vivo confocal live imaging.

Male TRPP2 deficient flies are sterile although they produce fully differentiated, motile sperm, which are effectively transferred to the female upon mating. Sterility is caused by impaired directed movement of mutant sperm to the female sperm storage organs. TRPP2 mutant sperm were found to lack the up-regulation of tail beating frequency and sperm speed in the uterus that we observed in the wt sperm.

Furthermore, we identified a patient mutation that is expressed normally in the endoplasmic reticulum (ER) of spermatocytes but fails to traffic to the sperm tail. Absence or mis-localisation of the TRPP2 protein equally impaired fertility in Drosophila melanogaster, suggesting that the localization in cilia rather than in the ER is critical for TRPP2 function.

In conclusion, we have established versatile model system to investigate polycystin proteins in vivo. TRPP2 is required for directed movement and regulation of sperm speed in the female reproductive tract of Drosophila melanogaster. Real time analysis of TRPP2 mutant sperm provided new insights into sperm chemotaxis and TRPP2 signalling. These results provide indirect evidence for the existence of yet to be identified ligands regulating the polycystin complex.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1775**

**Protein Kinase D Mediated Phosphorylation of Polycystin-2 (TRPP2) Is Essential for Its Effects on Cell Growth and Channel Activity** Andrew J. Streets, Andrew J. Needham, Albert C. Ong. *Academic Unit of Nephrology, Sheffield Kidney Institute, Kidney Genetics Group, University of Sheffield, Sheffield, United Kingdom.*

PKD2 is mutated in 15% of patients with autosomal dominant polycystic kidney disease (ADPKD). The PKD2 protein, polycystin-2 or TRPP2, is a non-selective Ca2+-permeable cation channel which has been shown to function at several locations including primary cilia, basolateral membrane and at the ER. Nevertheless, the factors that regulate the channel activity of polycystin-2 are not well understood. Reversible protein phosphorylation has been shown to regulate the structure and function of many other members of the TRP family of ion channels. Accordingly, polycystin-2 has been shown to be regulated by phosphorylation at two serine residues (Ser812 and Ser76) with distinct functional consequences.

We noted however that mutation of both Ser76 and Ser812 residues did not abolish polycystin-2 phospholabelling in vivo. This led us to seek other potential phosphorylation sites and to define their functional significance. Using a combination of site-directed mutagenesis and phospho-antibody mapping we have identified a previously unrecognised phosphorylation site within the polycystin-2 C-terminus (Ser801) and have demonstrated that it can be phosphorylated by Protein Kinase D. Phosphorylation at this site was detectable in serum starved cells but was significantly increased in response to serum and EGF stimulation.

In order to ivestigate the functional significance of phosphorylation at this site we generated a series of inducible MDCK cell lines expressing polycystin-2 or Ser801 phosphomutants. Using these cell lines we show that inducible expression of polycystin-2 inhibited cell proliferation and resulted in a significant increase in ATP-stimulated Ca2+ release from ER stores. Mutagenesis at Ser801 abolished these effects of polycystin-2 on cell growth and also significantly reduced ATP-stimulated Ca2+ release from ER stores. Our results suggest that growth factor stimulated, Protein Kinase D mediated phosphorylation of ER polycystin-2 channels is essential for its function and links extracellular stimuli to intracellular calcium regulation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1776**

**Co-Ordinated Regulation of Rho-GTPase and PKHD-1 in Ureteric Bud-Mediated Morphogenesis** Patricia D. Wilson,[1] Nadezhda N. Zheleznova.[2] *[1]Medicine, University College London (UCL), London, United Kingdom; [2]Physiology, Medical College Wisconsin, Milwaukee, WI; [3].*

The cystic proteins polycystin (PC)-1,-2 and PKHD1-encoded Fibrocystin(FC)-1 are developmentally regulated, highly expressed in the developing mammalian ureteric bud epithelial cell membranes and primary cilia where they form multi-molecular, mechanosensory complexes with integrin and focal adhesion(FA) proteins. Inhibition of PC-1, -2, or FC-1 due to endogenous mutation, or transfection with dominant negative or si/shRNA constructs leads to increased ureteric bud-derived epithelial cell-matrix (ECM) adhesion, decreased growth factor-mediated migration, and cystic tubulogenesis in 3-dimensional collagen gels and fetal mouse kidneys in organ culture and in vivo. Rho-GTPases regulate the actin cytoskeleton via integrin/FA kinase (FAK) ineractions. In vitro and ex vivo studies were conducted using the specific RhoA inhibitor Y-27632. In mouse inner medullary collecting duct (mIMCD) cells, RhoA inhibition significantly altered cell shape, decreased cell-spreading, cell-cell attachment, proliferation and wound-healing. Quantitative Western blot analysis showed time-dependent decreases in FAK and pY397-FAK after 30min and 4 hr of ECM attachment as well as decreases in paxillin and pY31-paxillin after 30 min of attachment. In ex vivo mouse embryonic day (E) 12.5-13.5 kidneys in organ culture Rho-GTPase inhibition led to cystic disruption of organogenesis. Transfection with pMyr-EGFP-PKHD1-CTD induced increased levels of expression of full length (>460kDa) FC-1 and but decreased levels of β1-integrin, FAK, c-src and cleaved (<20kDa) FC-1. FACS-sorted PKHD1-over-expressing cells showed increased ECM adhesion at 4hr, decreased growth-factor-induced migration in Boyden chamber (Fuoroblock) assays and increased tubulogenesis in 3D collagen gels. All of these effects were reversed by specific inhibition of Rho-GTPase by Y-27632. We conclude that coordinated regulation of Rho-GTPase activity and FC-1 are required for normal ureteric bud branching morphogenesis and differentiation of the mammalian kidney

Disclosure of Financial Relationships: nothing to disclose

**F-PO1777**

**Depletion of Macrophages in Pkhd1-Cre-Mediated Pkd1 Null Mice Results in Reduced Cyst Load and Improved Renal Function** Anil K. Karihaloo,[1] Farrukh M. Koraishy,[1] Stefan Somlo,[1] Lloyd G. Cantley.[1] *[1]Internal Medicine, Section of Nephrology, Yale University School of Medicine, New Haven, CT; [2]Department of Pathology, Yale University School of Medicine, New Haven, CT; [3]Deapartment of Biomedical Engineering, Yale University, New Haven, CT.*

Polycystic kidney disease (Pkd) is characterized by progressive growth of multiple cysts that eventually replace the renal parenchyma that may lead to loss of renal function. Cell proliferation plays an important role in the progression of this debilitating genetic disorder. Several studies in the recent past have indicated an existence of inflammatory component in Pkd. Our laboratory has recently determined that macrophages contribute to the tubular cell proliferation following an ischemic injury. We thus hypothesized that macrophages in a polycystic kidney may contribute to the proliferation of the cyst-lining cells and that depletion of macrophages should impede the cyst growth. To test this we used mice in which Pkhd1-Cre mediated deletion of Pkd1 results in large cystic kidneys with end stage cystic disease by ~ 4 weeks of age.

Pkd1 null mice, received liposomal clodronate (LC) or liposomal vehicle (LV) i.p. from post-natal day10 until day 24. Mice were sacrificed on day 24 and kidney sections stained with either H&E, the macrophage marker F4/80, TUNEL or Ki-67 for cell proliferation. The cystic index was determined using metamorph. LC treatment led to better preservation of renal parenchyma and 40% drop in the cystic index. This translated to a modest but statistically significant improvement in renal function as was determined by lower BUN values (151 v 109, $p<0.05$). F4/80 staining revealed an average of 50% fewer macrophages in the kidneys from LC treated mice. Further we determined that LC treatment led to a significant decrease in the proliferation of the cyst-lining cells as identified by Ki-67 and dolichos-biflorus lectin (DBA) co-staining. There was no appreciable change in the number of apoptotic cells. Taken together, these data demonstrate that depleting macrophages leads to better preservation of renal parenchyma, reduced cyst load and improved renal function.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1778**

**ADPKD-Associated F4145V Mutation in Polycystin-1 Disrupts Its Dephosphorylation by Protein Phosphatase-1** Stephen C. Parnell,[1] Sanjeev Puri,[2] Lance C. Brandenburgh,[1] Darren P. Wallace,[3] James P. Calvet.[1] *[1]Department of Biochemistry and Molecular Biology and the Kidney Institute, University of Kansas Medical Center, Kansas City, KS; [2]Biotechnology Institute; University Institute of Engineering and Technology, Panjab University, Chandigarh, India; [3]Department of Medicine and the Kidney Institute, University of Kansas Medical Center, Kansas City, KS.*

The C-terminal cytosolic tail of polycystin-1 (PC1) contains a highly conserved, putative protein phosphatase-1 (PP1) binding motif (R/KVxF; KVRF between K4142 and F4145 in mouse PC1) located in close proximity to a cAMP-dependent protein kinase (PKA) phosphorylation site 14 residues downstream at S4159. PP1 is a broadly expressed serine/threonine phosphatase that regulates numerous cellular functions including ion channel activity, cytoskeletal organization, cell cycle progression, and gene transcription. PP1 activity is regulated by its interactions with a diverse set of regulatory protein binding partners that form unique holoenzyme complexes with PP1. We had previously demonstrated that PC1 interacts with and is dephosphorylated by PP1 and that alanine-mutagenesis of critical hydrophobic residues in the R/KVxF motif (V4143A; KARF and F4145A; KVRA) disrupted the ability of PP1 to dephosphorylate PKA-phosphorylated PC1. We now show that an ADPKD-associated mutation corresponding to mouse F4145V (KVRV; Tan YC *et al.* 2009, Hum Mutat 30:264-273) also blocks the ability of PP1 to dephosphorylate PC1, and that KVRF mutations increase the ability of PC1 to activate nuclear factor of activated T-cell (NFAT) transcriptional activity. Our results suggest that a critical function of PC1 is to regulate the enzymatic activity of PC1-associated PP1, and in particular that PP1 antagonizes the ability of PC1 to activate NFAT. These results suggest that perturbation of PC1-PP1 holoenzyme activity may be sufficient to cause ADPKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1779**

**Wnt5a Is Upregulated in ADPKD Cyst Epithelial Cells and Stimulates cAMP Production and In Vitro Cyst Growth** Katherine Swenson-Fields,[1] Darren P. Wallace,[1] Carolyn J. Vivian,[1] Cibele S. Pinto,[1] Gail Reif,[1] Brenda S. Magenheimer,[1] James P. Calvet,[1] Timothy A. Fields.[1] *[1]The Kidney Institute, University of Kansas Medical Center, Kansas City, KS; [2].*

Gene expression profiling of cyst epithelial cells from human ADPKD kidneys showed upregulation of Wnt5a transcripts compared to normal human kidney cells. Increased Wnt5a message was confirmed by qRT-PCR, and immunohistochemistry demonstrated that Wnt5a protein was present in cyst-lining epithelial cells. Wnt5a is a non-canonical member of the Wnt family of secreted glycoproteins that influences morphogenesis and can regulate both cell proliferation and migration in adult tissues. Wnt5a has been shown to activate a number of different signaling pathways depending on the cell type and cell surface receptor repertoire, including those that elevate intracellular Ca2+ and cAMP levels. Since these second messengers are known to affect cyst growth in ADPKD, we sought to determine the affect of Wnt5a on ADPKD cells. ADPKD cyst cells were seeded within polymerized collagen gels and treated with either Wnt5a or forskolin, an activator of adenylate cyclase leading to increased levels of cAMP. cAMP is known to stimulate both ADPKD cell proliferation and transepithelial fluid secretion to promote cyst enlargement. Notably, Wnt5a treatment alone stimulated cyst growth. While the total number of cysts in the Wnt5a-treated cells was lower relative to forskolin-treated cells (56 vs. 116), the total cyst area in the Wnt5a-treated cells was greater than that achieved by forskolin (1.17 ± 0.11 vs 0.77 ± 0.08 cm2/well; $p=0.014$). To investigate the mechanism underlying the Wnt5a effect on cyst growth, we directly measured cAMP levels in ADPKD epithelial cells after treatment with purified recombinant Wnt5a. Strikingly, Wnt5a stimulated a 2.5-fold increase in cellular cAMP relative to vehicle. Thus, Wnt5a can promote ADPKD cell cyst enlargement in vitro, and the effect may be related to its ability to promote cAMP production. Since Wnt5a is upregulated in ADPKD cells, these data suggest the intriguing possibility that expression of Wnt5a in ADPKD cyst cells promotes cystogenesis and disease progression.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1780**

**Role of Integrin Signaling in Cystic Kidney Diseases** Balaji Karthick Subramanian,[1,2] David Kaplan.[2] *[1]Biomedical Engineering and Biotechnology, University of Massachusetts, Lowell, MA; [2]Biomedcial Engineering, Tufts University, Medford, MA.*

Cystic kidney diseases are a heterogeneous group of problems leading to kidney failure. Studies with animal models have attributed kidney failure to the expansion of cysts and extensive interstitial fibrosis. Aberrant integrin expressions were observed in cystic kidney tissues and have been hypothesized to play a major role in cyst progression. Though many attempts have been made to understand the role of integrin signaling in cyst initiation and progression, the lack of relevant models has left the mechanisms elusive. The purpose of this study was to advance the understanding of integrin signaling in cystogenesis using an engineered tissue model. The model was developed using tissue engineering principles by culturing normal and polycystin-1 silenced mouse kidney epithelial cells in extracellular matrix molecules infused into porous silk scaffolds in perfusion bioreactor systems. Silk biomaterial scaffolds are used due to the slow degradation, bio-compatibility and demonstrated utility in 3D tissue model systems, able to maintain transport and function in perfusion bioreactor systems for sustained time periods. Structural and functional relevancies of the cystic structures developed were evaluated by polarity marker distribution and transport assays. In addition, abnormal expression of beta 1 integrin and its intracellular mediator Integrin Linked Kinase (ILK) were observed in the engineered 3D cystic tissues developed from silenced cells, unlike in the normal cell systems, similar to in vivo cystic kidney tissues. Further modulation of integrin signaling to understand the role in cystogenesis is achieved by either inducible silencing or over-expression of ILK in cystic structures to evaluate outcomes.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1781**

**Gli3 Repressor Inhibits Collecting System Development in a Murine Model of Pallister-Hall Syndrome** Josh Blake,[1,2] Jason Cain,[1] Norman D. Rosenblum.[1,2,3] *[1]Prog Dev & Stem Cell Biol, Hosp Sick Child, Canada; [2]Dept Physiology, U Toronto, Canada; [3]Div Neph, Dept Peds, U Toronto, Toronto, Canada.*

Pallister-Hall Syndrome (PHS), characterized by renal hypodysplasia and hydronephrosis, is caused by truncating mutations in *GLI3*. Full-length *Gli3* mRNA encodes a transcriptional activator (GLI3A) that is proteolytically processed to form the transcriptional repressor GLI3R to an extent dependent on Hedgehog (HH) signaling activity. Gli3R controls renal development in a temporal, spatial and lineage-specific manner, inhibiting early inductive tissue interactions (Hu *et al.*, 2006) but promoting *Wnt11* expression in ureteric tip cells (Cain *et al.*, 2009). Here, we define Gli3R functions during collecting system development in mice in which the WT *Gli3* allele is replaced by a $Gli3^{\Delta 699}$ allele (Bose *et al.*, 2002) that produces a truncated GLI3 protein as in PHS. Whereas $Ptc1^{lacZ}$ expression in the periureteric mesenchyme, medullary stroma and ureteric bud (UB) cells indicated robust HH activity in WT mice, $Ptc1^{lacZ}$ expression was markedly reduced in $Gli3^{\Delta 699/\Delta 699}$ (mutant) kidneys. Mutant mice died shortly after birth with severe hydronephrosis. Phenotypic analysis prior to birth revealed nonobstructive hydronephrosis, defined by intrapelvic dye injection at E18.5. A double collecting system was observed in 43% of mutants at E15.5 with a loss of periureteric alpha-smooth muscle actin expression in all mutants but with varying degrees of severity. Examination of nephrogenesis at E15.5, prior to the onset of hydronephrosis, revealed simple hypoplasia (4/7 mice) characterized by a 55% decrease in kidney volume ($p<0.001$) with a 49% decrease in glomerular number ($p<0.05$). While gene expression in metanephric mesenchyme (WT1, Cited1) and stroma (*Raldh2*, *Foxd1*) was comparable in mutant and WT kidney tissue, renal hypoplasia was preceded by a 46% reduction in ureteric branching at E12.5 ($p<0.0001$). Remarkably, the mutant phenotype was completely rescued in $Gli3^{\Delta 699/-}$ and $Gli3^{\Delta 699/+}$ mice. We conclude that decreased ureteric branching and delayed smooth muscle differentiation during collecting system development are caused by increased Gli3R and not by decreased Gli3A in a murine model of PHS.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1782**

**Epithelial-to-Mesenchymal Transition (EMT) in *cpk* Mouse, a Model of ARPKD** Hiroko Togawa,[1] Koichi Nakanishi,[1] Hironobu Mukaiyama,[1] Taketsugu Hama,[1] Yuko Shima,[1] Masayasu Miyajima,[2] Kandai Nozu,[3] Hisahide Takahashi,[4] Shizuko Nagao,[4] Kazumoto Iijima,[3] Norishige Yoshikawa.[1] *[1]Department of Pediatrics, Wakayama Medical University; [2]Laboratory Animal Center, Wakayama Medical University, Wakayama; [3]Department of Pediatrics, Kobe University, Hyogo; [4]Education and Research Center of Animal Model for Human Disease, Fujita Health University, Aichi, Japan.*

The cyst-lining epithelial cell pathophysiology in PKD is characterized by dedifferentiation and perturbations of the polarized phenotype with consequent renal cyst formation and progressive enlargement. Some of these features resemble an early developmental epithelial cell phenotype.

The PCK rat, an orthologous model of human ARPKD is characterized by slowly progressive cyst formation and fibrosis. Previously, we showed that epithelial cells in cysts acquired mesenchymal features in response to cyst enlargement and demonstrated pathological significance of tubular EMT in PCK rat. It's not clear whether EMT is consistently present in PKD or a secondary phenomenon associated with fibrosis. Therefore, we examined the role of EMT in *cpk* mouse, another ARPDK model with a rapid progression.

Formaldehyde fixed, paraffin embedded kidneys from 5 male *cpk* and control mice(day 0,7,14,21) were sectioned and stained with antibodies to epithelial markers, mesenchymal markers and Snail1, a transcriptional repressor of E-cadherin. We also evaluated the involvement of TGF-β and Smad3, which were thought to be key mediators of EMT, using real-time PCR methods. E-cadherin and β-catenin in cyst were attenuated and localized to lateral cell-cell contact according to cyst enlargement in *cpk*. Vimentin and fibronectin were de novo expressed in cystic epithelial cells. Snail1 was predominantly expressed in the nuclei of tubular epithelial cells in *cpk*, while there was no significant staining in controls. Smad3 was also increased in the nuclei in *cpk*. The mRNA levels of TGF-β and Smad3 were up-regulated, which suggested the activation of TGF-β/Smad3 pathway. In conclusions, these findings suggested that EMT is also involved in *cpk*, and EMT may be a common key pathophysiology in PKD and a target for a disease-specific intervention.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1783**

**Abl Interactor-1 Induces Cytoskeleton Reorganization, MT1-MMP Re-Localization and Tubular Structure Formation of ADPKD Cells** Yunxia Tao, Zonghan Dai, Chenghai Li. *Internal Medicine, Texas Tech University Health Sciences Center, Amarillo, TX.*

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is caused by mutations in *PKD1* and *PKD2* genes, which encode polycystin 1 and polycystin 2 proteins, respectively. Both polycystins have been shown to interact with actin cytoskeleton, localize to lamellipodia, and induce cell scattering and motility. These data suggest that dysregulation of cytoskeleton remodeling and lamellipodia formation may contribute to cyst formation. The present study was aimed to determine the effect of overexpression of Abl Interactor-1 (Abi1), a key regulator of actin cytoskeleton remodeling and lamellipodia formation, on cyst and tubule formation of ADPKD cells cultured in three-dimensional (3D) collagen gel.

An Abi1 expression vector was stably transfected into WT 9-7 cells, a cell line derived from human ADPKD cyst. The effect of Abi1 expression on actin cytoskeleton reorganization was studied by staining the cells with rhodomine-coupled phalloidin. We show that Abi1 significantly induced cell scattering and increased the number of lamellipodia compared to control cells. Both control and Abi1 transfected WT9-7 cells were cultured in 3D collagen gels for 6 days to allow cyst and tubule formation. Under this condition we found that the control WT 9-7 cells forms mainly cysts (72% of cyst and 28% tubule), whereas Abi1 WT 9-7 cells forms mainly tubules (2% cyst and 98% of tubule). Because MT1-MMP has been shown to localize at lamellipodia and play a critical role in regulating cell migration and morphogenesis; we examined the effect of Abil overexpression on subcellular localization of MT1-MMP by immunostaining cyst and tubule structures. Significantly, we observed that in cysts formed by control WT 9-7 cells, MT1-MMP is polarized and localized to the basal membrane of the cyst cells, however, in tubules formed by Abi1 WT9-7 cells, MT1-MMP was found in both basal and lateral membrane as well as in cytoplasm of the tubule cells.

These studies suggest that the dysregulated MT1-MMP localization resulted from the disorganization of cytoskeleton may be implicated in the pathogenesis of ADPKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1784**

***AHI1* and *CC2D2A* Genetically Interact – Zebrafish Models of Nephronophthisis and Joubert Syndrome** Roslyn Jane Simms, Lorraine Eley, Ann Marie Hynes, Colin Miles, Bill Chaudhry, John A. Sayer. *Institute of Human Genetics, Newcastle University, Newcastle-upon-Tyne, United Kingdom.*

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease (CKD) and is a common cause of childhood renal failure. It is clinically heterogenous with extra-renal manifestations that include the cerebello-oculo-renal disorder Joubert Syndrome (JS). NPHP and JS are ciliopathies, since all genes implicated in these syndrome express their protein products in the primary cilium / basal body complex. Mutations in *AHI1* and *CC2D2A* are the leading causes of JS. These genes are highly conserved throughout evolution, including zebrafish (ZF). Using gene knockdown in ZF, we evaluated the role and potential genetic interactions of *AHI1* in the pathogenesis of NPHP and JS.

Antisense morpholino oligonucleotides (MOs) directed towards splice donor site exon 8 or 5'UTR blocking the translation of *AHI1* (Gene Tools) were injected into 1-4 cell stage wild type, claudin-b-GFP (transgenic) and *sentinel* (*CC2D2A* mutant) ZF embryos. mRNA rescue was performed by co-injecting capped mouse *AHI1* mRNA (mMessage Machine, Ambion). Embryos were incubated at 28.5ºC and phenotyped using light microscopy at 72 hours post fertilisation (hpf). Using mRNA from injected ZF, RT-PCR and direct sequencing was performed. Immunofluorescent (IF) microscopy and histology were used to evaluate pronephric cysts.

*AHI1* knockdown with both splice site and translation blocking MOs created a phenotype with a spectrum of dysmorphology including curly tail, cardiac oedema, hydrocephalus and pronephric cysts. Claudin-b-GFP embryos facilitated imaging of pronephric cysts. RT-PCR and sequencing confirmed an in-frame deletion of exon 8 from *AHI1*. Co-injection of *AHI1* MO and mouse *AHI1* mRNA rescued the mutant phenotype. Histology identified pronephric duct dilatation. IF demonstrated cloacal cysts in *AHI1* MO injected ZF. *AHI1* knockdown in *sentinel* ZF resulted in synergy of the mutant phenotype.

*AHI1* knockdown in ZF recapitulates the human cystic kidney disease NPHP and JS. Synergy of the mutant phenotype in *sentinel* ZF implicates a novel genetic interaction between *AHI1* and *CC2D2A*.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1785**

***MKS3* Knockdown in Zebrafish Models the Ciliopathy Meckel Gruber Syndrome** Roslyn Jane Simms,[1] Lorraine Eley,[1] Ann Marie Hynes,[1] Colin Miles,[1] Matthew Adams,[2] Bill Chaudhry,[1] Colin A. Johnson,[2] John A. Sayer.[1] *[1]Institute of Human Genetics, Newcastle University, Newcastle-upon-Tyne, United Kingdom; [2]Ophthalmology and Neurosciences, Medical and Molecular Genetics, Leeds Institute of Molecular Medicine, St James's University Hospital, Leeds, United Kingdom.*

Meckel-Gruber syndrome (MKS) is an autosomal recessive disorder characterised by occipital encephalocele, cystic renal dysplasia and congenital hepatic fibrosis. MKS is clinically and genetically heterogenous and defined as a ciliopathy because all mutated genes express their protein products in the primary cilium/basal body complex. Mutations in *MKS3* frequently cause MKS, its protein product Meckelin also colocalises at the actin cytoskeleton, where it interacts with the actin binding protein Filamin A. Using gene knockdown in zebrafish (ZF), we aimed to create a model of MKS and evaluate genetic interactions between *MKS3* and *Filamin A*.

Antisense morpholino oligonucleotides (MOs) were designed to splice selected exons in ZF *MKS3* and *Filamin A* (Gene Tools). MOs were injected alone and in combination, into wild type and claudin-b-GFP (transgenic) ZF embryos. mRNA rescue was performed by co-injecting capped human *MKS3* mRNA (mMessage Machine, Ambion). Embryos were phenotyped using light microscopy at 72 hours post fertilisation. Using mRNA from *MKS3* injected ZF, RT-PCR and direct sequencing were performed.

*MKS3* knockdown created a mutant phenotype: curly tail, hydrocephalus, meningocele, notochord malformation and pronephric cysts. RT-PCR and sequencing of *MKS3* MO injected ZF embryos identifed inclusion of an intron in MKS3 mRNA, leading to a premature stop codon. Co-injection of *MKS3* MO with human *MKS3* mRNA rescued the mutant phenotype. An increased frequency of mutant characteristics occurred after co-injection of *MKS3* and *Filamin A* MOs.

*MKS3* MO knockdown in ZF creates a novel model of the human ciliopathy MKS. Synergy of the MKS mutant phenotype by co-injection with *Filamin A* supports a genetic interaction and suggests that mutations in actin binding proteins may contribute to the pathogenesis of ciliopathies. These ZF models may be useful to evaluate oligogenicity and triallelism in the clinical disorder MKS.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1786**

**VIP17: A Modulator of Cyst Formation and Ciliogenesis** Vinita Takiar, Kavita Mistry, Monica Carmosino, Michael J. Caplan. *Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT.*

Epithelial cell polarity is a fundamental requirement for vectorial transport in mammalian tissues, which is disrupted in renal cystic diseases such as polycystic kidney disease. Vesicle integral protein of 17 kD (VIP17) is involved in the apical transport and delivery of carrier vesicles. Originally identified in mature T-cells and myelin producing cells, VIP17 overexpression *in vivo* results in renal cysts (originating primarily from distal nephron segments) surrounded by pseudostratified epithelial cells with amplified apical membranes.(Frank, M et al., 2000) The mechanism responsible for this cyst formation is not clear. In the collecting duct, VIP17 co-localizes and interacts with aquaporin-2 (AQP2). VIP17 increases surface expression of AQP2 by decreasing the channel's internalization. Thus, VIP17 overexpression could lead to cyst formation by increasing the potential for AQP2-mediated fluid secretion. However, it is also well-established that renal cystogenesis occurs in association with defects of the primary cilium. To explore the role of VIP17 in renal cystogenesis and ciliogenesis, we examined the polarization and ciliary morphology of wild-type and VIP17-overexpressing cells grown in two-dimensional (2D) culture and three-dimensional (3D) suspension in Matrigel. VIP17-overexpressing MDCK cells display apical localization of VIP17 both in 2D and 3D cyst culture. Interestingly, VIP17-overexpressing cell cysts are more multi-lumen compared to controls ($p<0.01$). We next localized membrane markers including E-cadherin, β-catenin, and zona occludens 1 by immunofluorescence and found that VIP17 expression has no effect on their distribution. Examination of the lumens in 3D cyst culture reveals shortened or absent cilia with VIP17 overexpression. Immunohistochemistry on kidney sections from VIP17 transgenic mice also demonstrates fewer and shortened cilia within dilated lumens ($p<0.01$) Collectively, these studies demonstrate that VIP17 overexpression results in abnormal cyst and cilium development, *in vitro* and *in vivo*, suggesting that VIP17 overexpressing mice may develop cysts secondary to a ciliary defect.

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**F-PO1787**

**c-MET Over-Expression and mTOR Hyperactivation in ADPKD** Shan Qin,[1] Mary E. Taglienti,[1] Jing Zhou,[2] Jordan A. Kreidberg.[1] *[1]Nephrology, Children's Hospital Boston, Harvard Medical School, Boston, MA; [2]Renal Division, Brigham And Women's Hospital, Harvard Medical School, Boston, MA.*

Autosomal Dominant polycystic kidney disease (ADPKD) is a common life-threatening genetic disorder that causes chronic renal failure. mTOR, a major regulator of cell growth and metabolism, has been shown to be hyperactivated in ADPKD. mTOR inhibition via rapamycin is among the emergent therapies for ADPKD currently being tested in clinical trials, but the mechanism of mTOR hyperactivation in ADPKD is not fully understood. We found that the expression of c-MET, a tyrosine kinase receptor for hepatic growth factor (HGF), was increased at the protein level in pkd1 knock out cells (pkd1 -/-). Akt, a kinase downstream of c-MET and known to be an activator of mTOR, is also hyperactivated in our pkd1 -/- cells. Administration of a c-MET inhibitor abrogates hyperactivation of Akt and mTOR in pkd1 -/- cells. Remarkably, in an organ culture model of cystic kidney disease, c-MET inhibitor can also inhibit Akt activity and cyst formation in pkd1 mutant embryonic kidney explants. Our results demonstrate that c-MET can activate mTOR via the Akt pathway. We hypothesize that the c-MET signaling pathways represent a promising therapeutic targets to prevent cyst formation in PKD.

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**F-PO1788**

**Analysis of *Cys40*: A Novel Gene Required for Ciliogenesis in Both Zebrafish and Mice** Lisa M. Swanhart, Neil A. Hukriede. *Developmental Biology, University of Pittsburgh, Pittsburgh, PA.*

Renal abnormalities are common structural birth defects that are observed in as many as 1 in 500 pregnancies and can cause perinatal death or chronic disease. The primary cilium has emerged as a key player in the development of polycystic kidney disease and it is now appreciated that ciliary defects underlie nearly all forms of this disease in humans, mice and zebrafish. A preliminary screen for mice with congenital heart disease identified several mutations that also caused kidney defects. The kidney defects in these animals included polycystic kidney disease, renal agenesis and hypogenesis, hydronephrosis and hydroureter. One such mutant, *Cys40*, displayed evidence of kidney duplication and cyst formation as well as a low incidence of left-right defects. Immunocytochemisty demonstrated that Cys40 protein localized to both the axoneme and the basal body of the primary cilium in IMCD cells, suggesting a role for this gene in the development of this organelle. To further elucidate the function of Cys40 and its role in ciliogenesis, morpholino oligonucleotides were used to knockdown protein levels in zebrafish embryos. Cys40 morphants displayed several phenotypes that are indicative of aberrant ciliary function, including a curved axis, hydrocephaly, pericardial edema, and the development of kidney cysts by 120 hours post-fertilization. In the zebrafish pronephros, the functional larval kidney, cilia appear disorganized and normal motility is compromised. Analysis of Kupffer's vesicle, a structure analogous to the mouse node, demonstrated both a reduction in number and length of the cilia in this organ. These changes in cilia structure may explain why Cys40 morphants display mild left-right defects, such that heart looping and normal positioning of the liver are disrupted. Similar to what was observed in mammalian cell culture, Cys40 protein localizes to both the basal body and the axoneme of the cilia within the pronephros. Taken together, these data suggest that the function of Cys40 is not only conserved among vertebrates but appears critical for ciliogenesis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1789**

**Effects of ER Stress, PERK and eIF2α on Regulation of Polycystin-2** Jungwoo Yang, Zuocheng Wang, Carlos Lara, Xing Z. Chen. *Physiology, University of Alberta, Edmonton, AB, Canada.*

Autosomal dominant polycystic kidney disease (ADPKD) is associated with numerous cellular abnormalities such as cell over-proliferation and apoptosis. Mutations in polycystin-2 (PC2), a Ca permeable non-selective cation channel present in ER membrane, plasma membrane and cilia, account for ~10% of ADPKD cases. How PC2 expression is regulated and how it regulates cell growth are not well understood. We reported that PC2 is regulated by the ER-associated degradation pathway through the ubiquitin–proteasome system (Liang et al, Hum Mol Genet, 17:1109-, 2008) and that PC2 down-regulates cell proliferation and protein synthesis through promoting the phosphorylation of eukaryotic initiation factor 2α (eIF2α) by pancreatic ER eIF2α kinase (PERK) (Liang et al, HMG, 17:3254-, 2008). ER stress is induced by disruptions of ER homeostasis such as alterations in cellular Ca concentrations and ER protein glycosylation, and leads to acumulation of unfolded proteins in the ER lumen. Cells respond to the situation by a number of ways together termed unfolded protein response (UPR). One way of UPR is induction of PERK auto-phosphorylation, which stimulates its kinase activity and phosphorylates eIF2α, thereby shutting down global protein synthesis and regulating cell proliferation and apoptosis. We found that PC2 is up-regulated in HEK and HeLa cells under ER stress induced by thapsigargin or tunicamycin. Using cells with over-expression of WT or mutant PERK, siRNA or knockout, we showed that PC2 expression is up-regulated by PERK kinase activity. In MEF cells with PERK-/-, but not in control MEF cells, ER stress failed to up-regulate PC2. These data together indicate that ER stress up-regulates PC2 expression through increased PERK activity. We are currently examining 1) whether eIF2α activity is sufficient to up-regulate PC2 expression, using eIF2α over-expression, knockdown and induction of eIF2α activity by other eIF2α kinases; and 2) whether ATF4, an activating transcription factor up-regulated by phosphorylated eIF2α and PERK, is sufficient to up-regulate PC2. These studies together will shed light on how PC2 is regulated and its cellular functions in the ER. Supported by KFoC and CIHR (to XZC).

Disclosure of Financial Relationships: nothing to disclose

**F-PO1790**

**mTORC2 Signalling Is Independent of HIF-1α in Polycystic Kidney Diseases** Iram Zafar, Kameswaran Ravichandran, Franck A. Belibi, Charles L. Edelstein. *Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

mTORC2 is a complex of mTOR and Rictor that, unlike mTORC1, is rapamycin-independent. Increased proliferation of cyst epithelial cells plays a crucial role in cyst formation and mTORC2 signaling induces proliferation. First we determined mTORC2 signaling in rat and mouse models of PKD. 112 day PKDWS25/- mice and 8 w old male Han:SPRD rats (Cy/+) with PKD were studied. Phospho-Akt (serine 473) (60 kDa), the functional readout of the mTORC2 activity was measured in kidney. On immunoblot, p-Akt (60 kDa) was not different between ++ and PKDWS25/- mice but was more than 3-fold increased in Cy/+ compared to +/+ rats ($P<0.01$, n=4). Unexpectedly the increase in pAkt was inhibited by rapamycin ($P<0.01$, n=4). Cyst formation in PKD kidneys results in localized hypoxia in the kidney that can activate HIF-1α. The mTORC2 pathway can activate HIF-1α. Thus, we measured HIF-1α by ECL using the ultrasensitive singleplex kit from Meso Scale Discovery (MSD). HIF-1α (units corrected for protein) was 54.8 4.2 in ++, 52.3 3.3 in Cy/+, 88.4 3 in 4 w old homozygous Han rats (Cy/Cy) with very large kidneys ($P<0.001$ vs.+/+, Cy/+). Thus the increase in pAkt in Cy/+ was not associated with an increase in HIF-1. However, HIF-1α was increased in Cy/Cy. To determine whether HIF-1α plays a role in the pathogenesis of cyst formation, Cy/Cy rats were treated with the HIF-1α inhibitor, 2-methoxyestradiol (2ME2) 2mg/kg/d IP from 2-4 weeks of age. Immunoblot of Cy/Cy kidneys demonstrated a decrease in HIF-1α protein in 2ME2-treated rat kidneys. Two kidney to total body weight ratio (%) was 25 in Cy/Cy treated with vehicle and 22 in Cy/Cy treated with 2ME2 (NS). Cyst volume density was 73 in Cy/Cy treated with vehicle and 65 in Cy/Cy treated with 2ME2 (NS). In summary 1) the large increase in pAkt in Cy/+ rats is not associated with an increase in HIF-1α , 2) rapamycin reduced pAkt, 3) despite an increase in HIF-1α in Cy/Cy rats, HIF-1α inhibition in Cy/Cy rats did not reduce kidney or cyst volume. In conclusion, the proliferative mTORC2 pathway merits further study in PKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1791**

**Activation of the Replicative Stress Response as a Possible Therapeutic Target in Triplex DNA-Associated Diseases** Anastasia K. Ketko, Bradley P. Dixon. *Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Polypurine·polypyrimidine tracts can form triplex DNA that can disrupt promoters and silence genes. Such a mechanism has been postulated to regulate expression of the γ-hemoglobin gene. Hydroxyurea (HU) can induce fetal hemoglobin expression and can also cause replication arrest, thus activating replicative stress proteins such as BLM, a RecQ helicase that is known to unwind triplex DNA. We postulated that such induction of replicative stress and DNA damage response proteins may release gene silencing and cause the expression of fetal hemoglobin.

K562 cells, an erythroleukemia cell line which can be induced to express γ-hemoglobin, were exposed to graded concentrations of HU (25μM to 1mM). Single strand DNA breaks representing stalled replication forks were quantified by single cell gel electrophoresis (comet assay) under alkaline lysis conditions. Expression of γ-hemoglobin was measured by western blot of cell lysates and benzidine staining of treated cells. Activation of the DNA damage response and replicative stress pathways were also assessed by western blot analysis.

A dose-dependent increase in γ-hemoglobin expression was identified in K562 cells exposed to HU. Single-strand DNA breaks also increased in a dose-dependent manner following exposure to HU. Activation of the DNA damage response mediated by ATM kinase and apoptosis occurred at high concentrations of HU (400μM to 1mM). However, activation of the replicative stress protein BLM, presumably mediated through the ATR-Chk1 pathway, occurred at lower concentrations of HU (25μM to 200μM).

These results demonstrate that activation of BLM can be modulated by hydroxyurea, and is associated with an increase in γ-hemoglobin expression in K562 cells, suggesting a possible role for the unwinding of triplex DNA by BLM in the promoter region of the γ-hemoglobin gene in its mechanism of action. Implications of this work include the possibility that activation of BLM could reduce somatic mutation in triplex-associated diseases, such as ADPKD, and therefore serve as a novel therapeutic agent for these diseases.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1792**

**TGF-β Is an Inhibitor of ADPKD Cell Cystogenesis** Gerard Elberg, Dorit Elberg, Siddarth Jayaraman, Martin A. Turman. *Pediatrics/Nephrology, University of Oklahoma, Health Sciences Center, Oklahoma City, OK.*

The purpose of this study is to determine a possible role of extracellular factors secreted by epithelial cells in modulating cyst formation in autosomal dominant polycystic kidney disease (ADPKD). To analyze the presence of such factors, we used conditioned media (CM) from monolayer of normal kidney (NK) and ADPKD epithelial cells from human kidneys in primary cultures. The effect of CM was tested in a 3D model of cyst formation using human ADPKD cells. Results indicate that incubation of CM at 80°C induces inhibitory activity on cyst formation. Importantly, this treatment affects the process of cyst formation but has little effect on cyst growth and expansion. The effect of high heat suggests activity of a latent factor, which prompted us to assess the expression of transforming growth factor β (TGF-β) in CM and possible inhibitory function of TGF-β on cystogenesis. We found that high heat induced TGF-β activity in CM as measured by immuno-assay and expression of known TGF-β target genes in 3D cultures of ADPKD cells. Analysis of level of expression of 3 different mammalian TGF-β isoforms indicates that TGF-β2 is mainly secreted in CM of NK and ADPKD cells. The inhibitory effect of heat-activated CM on cyst formation is hampered by TGF-β2 blocking antibody and TGF-β receptor I kinase inhibitor. Recombinant TGF-β1, 2 and 3 have a similar ability to inhibit cyst formation at low concentrations (IC50=15-26 pg/ml). Our data suggest that activation of TGF-β2 secreted by renal epithelial cells regulates cystogenesis. Therefore, the fibrotic process in ADPKD induced by massive secretion of TGF-β is likely to be a failed, excessive mechanism of healing.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1793**

**Curcumin Inhibits cAMP-Mediated Cystic Dilation in Pkd1-Deficient Kidneys in Metanephric Organ Culture** Robin L. Maser, Andreea Chiselita, Brenda S. Magenheimer, James P. Calvet, Donna Ziemer, Melissa Johnson, Dianne Vassmer. *The Kidney Institute, Univ of Kansas Medical Center, Kansas City, KS.*

We have reported that antioxidant gene expression is abnormally reduced in multiple animal models of PKD, suggesting that inadequate antioxidant protection and dysregulated cellular redox homeostasis may influence cellular pathways important in PKD pathology. We tested whether antioxidant treatment could affect cyst pathogenesis. Curcumin is a food spice shown to have antioxidative, antiproliferative, and anti-inflammatory properties and to be an effective chemotherapy against multiple forms of cancer. We chose the metanephric organ culture model as an initial experimental system. Kidneys from embryonic day 15.5 Pkd1 mutant mice were placed in culture in the presence of DMSO or of 20 or 30 μM curcumin. 8-Br-cAMP was added to all cultures to induce cystic dilation. Kidneys were loaded with fluorescein on the last day in culture, photographed, and then fixed in methanol for whole mount staining with DBA- or LTA-FITC. Percent cystic index was determined by measuring the total surface area and the cystic surface area using analySIS software. Cyst-like structures that stained with DBA or LTA were visible in all three genotypes, however, a Pkd1 gene dosage-dependent effect was observed as reported previously. Curcumin treatment significantly reduced tubule dilation in all genotypes and was able to reduce % cystic area of Pkd1-deficient kidneys to DMSO-treated wildtype levels. Confluent mouse embryonic kidney epithelial cell cultures were treated with varying concentrations of curcumin for 24 hrs, and lysed for protein. Western blot analyses demonstrated induction of heme oxygenase-1 and NAD(P)H-oxidoreductase 1 proteins in these cultures. In addition to upregulation of antioxidant defenses, curcumin is reported to have effects on multiple cellular pathways including the cell cycle, Wnt/beta-catenin signaling, the mTOR pathway, and intracellular calcium levels, all processes that are reported to be dysregulated in PKD. These experiments suggest that curcumin or curcumin-like compounds may have beneficial effects in PKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1794**

**Ouabain Activates the Na,K-ATPase Signalosome in Autosomal Dominant Polycystic Kidney Disease Cells** Kyle Jansson, Anh Nguyen, Darren P. Wallace, Gustavo Blanco. *Department Molecular and Integrative Physiology and Kidney Institute, University of Kansas Medical Center, Kansas City, KS.*

The Na,K-ATPase is part of a cell signaling complex (Na,K-ATPase signalosome), which upon activation by the hormone ouabain, stimulates the proliferation of several cell types. Our previous results show that ouabain induces proliferation in epithelial cells derived from renal cysts of patients with autosomal dominant polycystic kidney disease (ADPKD). This effect suggests that ouabain is a factor that can contribute to ADPKD cyst progression. At present, the signaling pathways involved in the response of ADPKD cells to ouabain are unknown. Understanding the mechanisms by which ouabain exerts its action is important. In this study, we investigated the signaling pathways responsible for mediating ouabain's effect on ADPKD cell proliferation. Ouabain concentrations such as those found in blood (3nM), stimulated phosphorylation and increased the activity of the kinase Src. In addition, ouabain stimulated phosphorylation of the epidermal growth factor receptor (EGFR) in the cells. Accordingly, tyrphostin AG1478 and PP2, inhibitors of EGFR and Src respectively, blocked the proliferative effects of ouabain. Treatment of ADPKD cells with ouabain also caused phosphorylation of the caveolar protein caveolin-1, and disruption of cell caveolae with methyl-β-cyclodextrin prevented ouabain-induced proliferation of the cells. This suggests that ouabain's effect requires the integrity of the plasma membrane caveolae. Downstream effects of ouabain in ADPKD cells included phosphorylation of the kinase B-Raf, activation of MEK and of the extracellular regulated kinase ERK. Finally, ouabain reduced the expression of the cyclin-dependent kinase inhibitors p21 and p27, which are suppressors of cell proliferation. In conclusion, this study shows that ouabain uses the Na,K-ATPase signalosome to stimulate proliferation of ADPKD cells and identifies intracellular mediators of ouabain dependent ADPKD proliferation. [Supported by NIH grant DK081431].

Disclosure of Financial Relationships: nothing to disclose

**F-PO1795**

**Role of Phosphodiesterases on cAMP-Dependent ERK Activation and Cyst Formation in PKD** Cibele S. Pinto, Brenda S. Magenheimer, Gail Reif, James P. Calvet, Darren P. Wallace. *The Kidney Institute, University of Kansas Medical Center, Kansas City, KS.*

cAMP plays a central role in the pathogenesis of polycystic kidney disease by promoting cell proliferation and fluid secretion. Intracellular cAMP is regulated by a balance between its production by adenylyl cyclases and its degradation by phosphodiesterases (PDEs). We found that ADPKD cells have a higher basal cAMP level compared to normal human kidney (NHK) cells ($1.17 \pm 0.12$ vs. $0.75 \pm 0.06$ pmol/monolayer, $P < 0.02$). 3-isobutyl-1-methylxanthine (IBMX), a non-specific PDE inhibitor, increased basal cAMP levels in NHK and ADPKD cells, and amplified AVP-induced cAMP accumulation. IBMX alone or in the presence of AVP significantly increased ERK phosphorylation in ADPKD cells, but decreased ERK phosphorylation in NHK cells. In metanephric organ culture, IBMX alone increased the level of phosphorylated ERK and induced cyst-like dilations in $Pkd1^{-/-}$ embryonic kidneys. These results demonstrate that PDE activity plays an important role in regulation of cAMP-dependent ERK activation in PKD. Using microarray analysis, we found that mRNA levels for PDE1, a $Ca^{2+}$-activated PDE, and PDE4 were elevated in human ADPKD cells compared to NHK cells; however, no significant difference in protein levels was observed. Rolipram, a selective PDE4 inhibitor, caused a higher increase in basal cAMP and a greater effect on AVP-mediated cAMP accumulation compared to vinpocetine, a PDE1 inhibitor. On the other hand, vinpocetine had a significantly greater effect on AVP-induced ERK activation compared to either IBMX or rolipram, suggesting that PDE1 may be involved in the cross talk between the cAMP and MAPK pathways. These data show that PDE inhibition alone increases cAMP accumulation and ERK activation in cyst derived epithelial cells, and induces cyst-like dilations in embryonic $Pkd1^{-/-}$ kidneys. These data support the hypothesis that decreased activity of PDE1 due to lower intracellular $Ca^{2+}$ levels may partially account for higher intracellular cAMP and a greater effect of AVP on cAMP accumulation and ERK activation in ADPKD cells.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1796**

**Metabolite of Sir2 Reaction Modulates Polycystin-2 Ion Channel Activity** Lucy X. Fan,[1] Tengis S. Pavlov,[2] Alexander Staruschenko,[2] Xiaogang Li.[1] *[1]Department of Pediatrics and Physiology, Medical College of Wisconsin, Milwaukee, WI; [2]Department of Physiology, Medical College of Wisconsin, Milwaukee, WI.*

Autosomal dominant polycystic kidney disease (ADPKD) is an age-related genetic disorder characterized by renal cyst formation and progression to end stage renal failure. Our microarray analysis has shown that Sirt1 and Sirt2, the members of class III histone deacetylases (HDACs) and age-related regulators, are upregulated in *Pkd1*$^{null/null}$ mouse embryonic kidney (MEK) cells, which has also been confirmed by Western blot analysis. Sirt1 and Sirt2 have been found to modify and silence the transcription of integrated reporter genes by histone deacetylation. They also deacetylate nonhistone proteins such as some transcriptional factors including Rb, E2F1, p53 to regulate cell cycle and cytoskeleton proteins, such as α-tubulin, to regulate mitotic exit. In addition, it has been reported that O-acetyl-ADP-ribose (OAADPr), a metabolite of the Sir2 reaction which is formed when Sir2 removes an acetyl group from a protein target and transfers the moiety to NAD, acts as a secondary messenger or a cofactor to regulate the activation of TRPM2, a nonselective cation channel. We found that Sirt1 formed a complex with Rb and E2F1 and Sirt2 formed a complex with HDAC6 in MEK cells. Since Sirt1 and Sirt2 were upregulated in Pkd1 mutant MEK cells, thus, more OAADPr may be produced through deacetylating Rb, E2F1, and a-tubulin by Sirt1 and Sirt2 in these cells. To test the effect of OAADPr on polycystin-2 (PC2) channel, we reconstituted PC2 channel either alone or together with polycystin-1 (PC1) in chinese hamster ovary (CHO) cells and performed whole cell patch clamp analysis when 2'-NAADPr or 3'-NAADPr, analogs of 2'-OAADPr and 3'-OAADPr, were included in the pipette solution. We demonstrated that rather 2'-NAADPr or 3'-NAADPr activated PC2 currents in CHO cells when PC2 alone or PC1/PC2 heterologously expressing these channels. In addition, ADPr, the hydrolyzed product of OAADPr, has the similar effects on the PC2 channel activation as 2' or 3'-NAADPr does. These results suggest that Pkd1 mutation may be through OAADPr to regulate PC2 channel activity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1797**

**Stable Suppression of Glis2/Nephrocystin-7 Results in Aberrant Centrosome Duplication and Nuclear Division in Kidney Tubular Cells In Vitro** Binghua Li,[1] Alysha Rauhauser,[1] Massimo Attanasio.[1,2] *[1]Department of Internal Medicine; [2]Eugene McDermott Center for Growth and Development, UT Southwestern Medical Center at Dallas, Dallas, TX.*

Nephronophthisis (NPHP), an autosomal recessive cystic kidney disease, is the most frequent genetic cause of chronic renal insufficiency in the first three decades of life (1). Glis2/Nephrocystin-7 loss of function causes NPHP type 7 in humans and an alike cystic kidney phenotype in mice (2). We have previously reported that Glis2 is localized at the centrosomes, the mitotic spindle and the midbody of kidney epithelial cells in vitro with a cell cycle dependent pattern, suggestive of a possible role of this protein in controlling centrosome duplication and cell division.

To test this hypothesis, we generated IMCD3 cells stably knocked-down for Glis2 by lentiviral delivered shRNAs. High knockdown efficiency (more than 70%) was verified by quantitative real-time PCR and was obtained with two different targeting constructs. Centrosomes were labeled with antibodies against the specific centrosomal marker γ-tubulin and evaluated by immunofluorescence confocal microscopy; nuclei were counterstained with DAPI. Cells were grown in standard conditions (37 °C, 5% CO2 in DMEM/F-12 plus 10% FBS).

Stable suppression of Glis2 in IMCD3 cells resulted in the aberrant generation of multiple centrosomes that became more evident after several passages. Nuclei appeared to be either multilobulated or abnormally fused in a single megakarion, indicating an impairment of the nuclear division. The aberrant centrosomes conserved the ability of nucleating microtubules, as demonstrated by the concomitance of multiple associated spindles.

Our results indicate that Glis2 is necessary for the correct centrosomal and nuclear duplication in kidney tubular cells in vitro. Analogous centrosome amplification has been recently associated with the loss of function of another NPHP protein, MKS3/NPHP11, suggesting that defects of centrosome duplication can be implicated in the pathogenesis of cystic kidney diseases.

(1) Hildebrandt F et al. J Am Soc Nephrol 20:23, 2009
(2) Attanasio M et al. Nat Genet 39: 1018, 2007

Disclosure of Financial Relationships: nothing to disclose

**F-PO1798**

**Aberrant Regulation of Planar Cell Polarity Pathway in Human and Mouse Polycystic Kidney Disease** Annouck Luyten, Xuefeng Su, Sarah Lynn Gondela, Ying Chen, Ayumi Takakura, Jing Zhou. *Medicine, Renal Division, Brigham and Women's Hospital, Boston, MA.*

Autosomal dominant polycystic kidney disease (ADPKD), characterized by the formation of fluid-filled cysts in the kidney, is a major cause of end stage renal disease in adults. Mutations in the PKD1 gene, which encodes polycystin-1 (PC1), contribute to more than 85% of ADPKD cases. The planar cell polarity (PCP) pathway has recently been implicated to be necessary for convergent extension and oriented cell division in the establishment and maintenance of kidney tubule diameter. Here, we show that inactivation of Pkd1 in postnatal developing kidney leads to a defect in oriented cell division in pre-cystic kidney tubules. This defect is also seen in pre-cystic Pkd1 inactivated mature kidneys after receiving ischemic reperfusion injury (IRI) as a "third-hit". Frizzled proteins play a critical role in the regulation of PCP. We find striking upregulation and activation of Frizzled 3 (Fz3) and its downstream effector CDC42 in cystic kidneys. Fz3 is expressed on the cilia in cystic kidneys although it is barely detectable on the cilia of normal kidneys. We also show that PC1 and Fz3 antagonize each other to control CDC42 expression and cell migration rate in HEK293T cells. Our data suggest that PC1 controls oriented cell division and that aberrant PCP signaling contributes to cystogenesis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1799**

**E2F Family Transcription Factors Regulate the Polycystic Kidney Disease-1 Gene Promoter through an ETS Binding Element** Anuj Gupta,[1] Veena Puri,[3] James P. Calvet,[2] Sanjeev Puri.[1] *[1]Panjab University, Chandigarh, India; [2]Univ. of Kansas Medical Center, Kansas City, KS; [3]DAV College, Chandigarh, India.*

E2F transcription factors are key regulators of cell-cycle progression and function in a wide range of biological processes including DNA replication, mitotic and DNA-damage checkpoints, DNA repair, differentiation, development and apoptosis. They can stimulate or repress target genes. We have analyzed a 3.3 kb 5' upstream region of the human *PKD1* promoter using transient transfection in HEK293T and COS-1 cells to demonstrate that the *PKD1* promoter is a target of E2F family transcription factors. Our studies demonstrated that *PKD1* promoter–luciferase reporter gene expression is downregulated by E2F family transcription factors (E2F1, E2F2, E2F4 and E2F6). A time course study demonstrated highest repression at 24 hrs post-transfection. E2F transcriptional effects are mediated after their release from retinoblastoma (RB). Overexpression of RB, which sequesters E2F, enhanced *PKD1* promoter activity. RT-PCR analysis further corroborated E2F-mediated repression of the endogenous *PKD1* gene in HEK293T cells. Deletion-construct analysis demonstrated that the sequences responding to E2F lie within -200 bp of the proximal *PKD1* promoter, which in previous studies was reported to respond to a number of transcription factors, including Ets, Sp1, RXR, and p53. Sequence analysis suggested the presence of several potential E2F binding elements in a GC rich region (5'-CGCGCCGCGCGG-3' -31 to -42). EMSA supershift assays demonstrated binding of E2F proteins to this region. However, site-directed mutagenesis indicated the involvement of a more distant Ets site at -129 to -133, rather than the candidate E2F binding elements, in regulating the *PKD1* promoter. These results thus suggest a complex regulatory mechanism for the *PKD1* gene that may involve concerted action of several transcription factors. Among several possibilities is that E2F may upregulate an Ets transcription factor which in turn causes transcriptional repression of the *PKD1* gene.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1800**

**Reduced Notch Signaling in Autosomal Dominant Polycystic Kidney Disease** Karen Tamano,[1] Carol G. Carlton,[2] Binu M. Paul,[2] Seth V. Vande Kamp,[2] Madhulika Sharma,[2] Gail Reif,[1] Darren P. Wallace,[1] Gregory B. Vanden Heuvel.[2] *[1]Medicine, University of Kansas Medical Center, Kansas City, KS; [2]Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.*

Recombination signal binding protein for immunoglobulin kappa J region (RBPJ/k) is a transcription factor that interacts with the Notch intracellular domain (Notch-IC) and is required for canonical Notch signaling of all the Notch receptors. Mice carrying a conditional RBPJ/k allele were crossed with Hoxb7/cre mice to specifically delete RBPJ/k in the developing ureteric bud and collecting duct. Newborn RBPJ/$k^{CD}$ mice exhibited cortical and medullary cyst-like dilations, and by postnatal day 14 these mice showed extensive medullary dilations. Preliminary analysis of planar cell polarity using anti-phospho-Histone H3 antibodies showed a disruption of spindle orientation in the collecting ducts of RBPJ/$k^{CD}$ mice. This is consistent with recent studies showing that reduced Notch signaling in developing proximal tubules results in cystic disease (JASN 21:819-832, 2010). To further evaluate the role of Notch signaling pathway in polycystic kidney disease, we analyzed Notch signaling in human ADPKD cells. We found decreased Notch3-IC in ADPKD cells, compared to normal human kidney cells. In addition, Hes1, a Notch pathway effector protein, was also downregulated in ADPKD patient samples compared to controls. Taken together, these results suggest that Notch signaling is required for the normal development of the collecting duct system in the kidney, and that reduced Notch signaling may contribute to the development of renal cysts.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1801**

**Disruption of Mouse AQP11 Induces Endoplasmic Reticulum Stress, Followed by Apoptosis and Cellular Proliferation, in the Proximal Tubule Cells of the Kidney** Katsuki Kobayashi,[1] Sei Sasaki.[2] *[1]Division of Molecular Genetics, Clinical Research Center, Chiba-East National Hospital, Chiba City, Chiba, Japan; [2]Department of Nephrology, Tokyo Medical and Dental Universtity, Bunkyo-ku, Tokyo, Japan.*

Aquaporin-11 (AQP11) is established as member of aquaporin water channel family, but its cellular and molecular function remains unclear. In an earlier study on mice harboring ablated coding regions of the AQP11 gene, our group observed vacuolation of the renal proximal tubule cells, followed by the formation of renal cortical cysts originating from the proximal tubule. In the present study we sought to identify the cellular and molecular mechanisms by which renal cysts are generated in AQP11-disrupted mouse. We initially noticed that the proximal tubule cells at the surface to mid cortex became vacuolated and apoptotic, whereas those deeper within the cortex proliferated vigorously and eventually formed cysts. Then by staining kidneys with antibodies against ER-stress-responsive factors, we recognized a strong activation of CHOP expression in the vacuolated and apoptotic proximal tubule cells. This was evidence that the apoptosis was the result of ER stress. In contrast to CHOP, GRP78 expression was augmented exclusively in the deep-cortex proximal tubule cells, a sign of a similar induction of ER stress in these cells. Yet in these cells, the ER stress was followed by cellular proliferation. Moreover, the cystogenesis of AQP11-disrupted mouse kidney was driven by mTOR activation and inhibited by the administration of rapamycin (a potent mTOR inhibitor). Taken together, our data suggested that the deletion of AQP11 elicits ER stress which in turn induces apoptosis and cellular proliferation in different parts of the renal proximal tubule. This PKD mouse model is likely to become a valuable tool for clarifying the complex mechanisms underlying PKD cystogenesis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1802**

**Differential Laminin alpha Chain Expression in an ADPKD Mouse Model** Binu M. Paul,[1] Patricia St. John,[1] Klaus B. Piontek,[2] Gregory G. Germino,[3] Dale R. Abrahamson,[1] Gregory B. Vanden Heuvel.[1] *[1]Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS; [2]Medicine, Johns Hopkins University, Baltimore, MD; [3]NIDDK, Bethesda, MD.*

Alterations in the tubular basement membrane (TBM) including thickening, splitting, fraying, and multilayering have been described previously in human polycystic kidney disease (PKD). Cystic kidneys from several animal models of PKD also showed basement membrane abnormalities including thickened and laminated basement membranes as well as increased expression of α1 type IV collagen and the laminin β1 and β2 chains. Moreover, mice carrying a hypomorphic mutation in the laminin α5 chain develop PKD (JASN 17:1913-1922, 2006). We used chain specific antibodies to analyze the expression of laminin in a collecting duct specific Pkd1 knockout mouse model (Pkd1$^{CD}$) during early and late stages of cystogenesis. During normal kidney development we observed a reduction in expression of the laminin α1 chain during collecting duct maturation, with minimal expression in mature collecting ducts. In contrast, we observed a complete loss of the laminin α1 chain in the collecting duct derived cysts. Moreover, the rapidly expanding cysts showed intense labeling for the laminin α5 chain. In contrast to laminin α chain expression, no differences were observed in the expression of laminin β1 or the α1 or α2 chains of type IV collagen between cystic and wild type mice. Taken together, these results suggest that laminin α chain expression is disrupted in polycystic kidney disease, which may result from an accelerated switching of laminin isoforms in the cystic collecting ducts.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1803**

**Differential Expression of Ras-GTPase Isoforms in an Orthologous Model of Autosomal Dominant Polycystic Kidney Disease** Ayesha Irtiza-Ali,[1] Richard N. Sandford,[2] Dorien J. M. Peters,[3] Claire C. Sharpe,[1] Bruce M. Hendry.[1] *[1]Renal Medicine, King's College London, United Kingdom; [2]Cambridge Institute of Medical Research; [3]Leiden Univ Medical Center.*

Previous work by our group demonstrates that distinct patterns of Ras GTPase isoform expression occur in different kidney cell types, and inhibition of specific Ras isoforms has marked anti-proliferative actions in both *in vitro* and in *in vivo* models of renal disease. We have most recently shown this *in vivo* in the rat unilateral ureteric obstruction model, where antisense targeting Kirsten Ras had dramatic anti-fibrotic effect. Aberrant renal tubular epithelial cell proliferation is critical to cystogenesis in autosomal dominant polycystic kidney disease (ADPKD). The role of Ras isoforms in ADPKD has not previously been defined.

In this work we demonstrate differential expression of Ras GTPase isoforms occurs in the orthologous *PKD1$^{nl/nl}$* mouse model. On a C57/Bl6 CD1 genetic background, renal cystic disease progresses from postnatal weeks 3 with development of grossly enlarged kidneys by weeks 6-8. On qPCR and western blot analysis, *PKD1$^{nl/nl}$* cystic kidneys at postnatal weeks 4 to 6 predominantly express Kirsten(Ki) and Neural(N) Ras, with up to 5 and 3.5 fold increases in Ki- and N-Ras mRNA respectively in comparison to wild type kidneys. Activated Ras, as detected by Ras-GTP assay, is strongly upregulated in cystic kidneys compared to wild type. Further studies in mouse collecting duct cells (mIMCD3) show predominant expression of Ki- and N-Ras isoforms, correlating with findings in cystic kidneys. These cells were treated for up to 48 hours with farnesylthiosalicylic acid (FTS), a pan-Ras antagonist. FTS inhibited cell proliferation, as measured by MTS assay, in a dose dependent manner indicating proliferation to be Ras-dependent.

These findings suggest Ras GTPases are important in renal tubular epithelial cell proliferative responses, with roles specifically for Ki- and N-Ras isoforms in cystic kidney disease pathogenesis in an orthologous *PKD1* mouse model. The precise actions of Ras GTPase isoforms in the abnormal proliferative responses that underlie cystogenesis in ADPKD is under our further investigation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1804**

**Characterization of a Hypomorphic *Pkd1* Mouse Model of ADPKD** William E. Sweeney, Jr.,[1,2] Kandai Nozu,[1,2] Dorien J. M. Peters,[3] Ellis D. Avner.[1,2] *[1]Pediatrics, Medical College of Wisconsin, Wauwatosa, WI; [2]Children's Research Institute, Childrens Hospital Health System, Wauwatosa, WI; [3]Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands.*

Purpose: A *Pkd1* hypomorphic mouse model of ADPKD was phenotypically characterized to assess its utility for testing potential therapeutic interventions for human ADPKD.

Methods: A recently described *Pkd1* hypomorphic mouse model of ADPKD (J of Path 216: 120, 2008) was backcrossed onto C57BL/6J strain for > 10 generations in order to develop a pure inbred background to control for any phenotypic variability from strain effects. As the C57BL/6J strain is fully sequenced strain, the resultant congenic *Pkd1* mouse will permit future high resolution interrogration by genetic and proteomic chips.

Genotypically, proven wildtype (WT), heterozygous (HET) and homozygous *Pkd1* null (CY) mice, were weighed and kidney, liver, spleen and heart were harvested from each at seven day intervals from PN-0 to PN-63. Kidney and liver were assessed by Western analysis for expression of EGFR, p-EGFR, ERK1/2, p-ERK 1/2, b-Raf and p-b-Raf. Kidneys were examined by immunohistochemistry for cyst localization with nephron specific markers, proliferation (Ki-67 and BrdU), and polarity with EGFR, and NaK ATPase. Trichrome staining was used to assess renal fibrosis.

Results: There were no differences found between WT and HET mice in any parameter assessed up to PN-63. Small proximal tubule (PT) cystic lesions along with small collecting tubule (CT) cystic lesions were present in PN-0 CY kidneys that were 60% larger (KW/BW ratio=1.99±.23) than age matched WT kidneys. PN-7 CY kidneys showed decreased PT cysts and increasing size and number of CT cysts. The size of the kidneys and the KW/BW ratio increased steadily to PN-35 due to increasing CT cyst size and number, reaching a KW/BW ratio of 22.85±2.0 ($p<0.01$ vs control). Thereafter a steady, significant decline in KW/BW and kidney size occurred due to increasing renal fibrosis. Through PN-35, IHC and Western analysis demonstrated abnormalities consistent with human ADPKD thereby making this orthologous *Pkd1* model uniquely suited for preclinical therapeutic testing.

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**F-PO1805**

**Metabolomic Profiling of Pkd1 Mutant Cells Reveals a Switch to Anaerobic Glycolysis in PKD** Isaline Rowe,[1] Valeria Mannella,[1] Silvia Mari,[1] Marco Chiaravalli,[1] Musco Giovanna,[1] Alessandra Boletta.[1] *[1]Division of Genetics and Cell Biology, Dibit-San Raffaele Hospital, Milano, Italy; [2]Biomolecular NMR, Dibit-San Raffaele Hospital, Milano, Italy.*

Polycystin-1 (PC-1), the product of the PKD1 gene, mutated in the majority of cases of Autosomal Dominant Polycystic Kidney Disease (ADPKD), is a very large plasma membrane receptor. In previous studies we have shown that PC-1 regulates the mTOR pathway, a major actor in cellular metabolism regulation.

During routine culturing of Pkd1-/- mouse embryonic fibroblasts (MEF) we noticed that the culture medium tended to acidify faster than the one from wild-type (wt) MEF. Furthermore, overexpression of the PKD1 gene resulted in the opposite phenotype suggesting a role for PC-1 in regulating the cellular metabolism. To test this possibility, we performed metabolomic profiling of the extracellular medium (metabolic footprinting) of mutant and wt MEFs. 1H-NMR experiments have been done using a 600-MHz Bruker Biospin spectrometer. 2D 1H-NMR spectra were acquired in order to further assign peaks to metabolites in the 1H-NMR spectra using either databases available. Preliminary data had shown different profiles of metabolites. We observed a greater uptake of glucose and increased production of lactate in Pkd1-/- MEF compared to the controls. Next, we confirmed a significant increase in the production of lactate in several independent Pkd1-/- MEFs. Conversely, cells overexpressing PC1 showed reduced lactate production. This effect correlated with the amount of intracellular ATP detected. In fact, the Pkd1-/- MEFs had increased ATP content. Next, we looked at mitochondrial potential using two different techniques and found that this is not altered in mutant versus wt cells. These results suggest cells lacking a functional PC-1 have enhanced glucose consumption and ATP production, due to a switch towards the anaerobic glycolysis. Preliminary data performed in a PKD mouse model suggest that a similar switch might occur in vivo. Our data uncover a previously unrecognized role for the PKD1 gene in regulation of cellular energy balance and might help identifying biomarkers that could be used to define the ADPKD phenotype.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1806**

**Over-Expression of Mxi1 Represses Renal Epithelial Tubular Growth by Transcriptional Regulation of MMP9** Jong Hoon Park, Seon Ah Song, Kyung Hyun Yoo. *Department of Biological Sciences, Sookmyung Women's University, Seoul, Korea.*

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease. The major characteristic of ADPKD is the cyst formation along the renal epithelial tubular cells, and this renal cyst leads to abnormal kidney architectures and renal insufficiency. Renal tubules are fundamental unit of architecture, so controls of tubular growth and formation are important for renal function. To find out new ways of treatment and diagnosis for ADPKD, recently mechanisms of tubulogenesis are being actively studied. Mxi1 is a member of MAD family proteins functioning in terminal differentiation, inhibition of cell cycle progression and tumor suppression. While Myc protein, antagonized by Mxi1, is known to cause the renal cystogenesis. Based on these molecular relationships between Mxi1 and Myc proteins, we confirmed that Mxi1 has an effect to ADPKD through lack of Mxi1 gene expression could cause cyst formation in Mxi1-deficient mice. And then to determine whether Mxi1 affects to renal epithelial cell tubulogenesis, we performed three-dimensional cultures using mIMCD-3 cells and stably Mxi1 over-expressed mIMCD-3 cells. As a result, over-expression of Mxi1 gene suppresses renal tubulogenesis by regulating several genes playing a key role in renal epithelial branching tubulogenesis, such as MMP9, integrins, E-cadherin and so on, in mIMCD-3 cells. Also we verified that MMP9 is tightly affected by expression level of Mxi1. Taken together, we concluded that stably Mxi1 over-expressed mIMCD-3 cells fail to renal epithelial tubule formation because of abnormally reduced expression of MMP9.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1807**

**Genotype-Phenotype Correlation in 368 Individuals with NPHP-Related Ciliopathies** Moumita Chaki, Julia Hoefele, Susan J. Allen, Gokul Ramaswami, Sabine Janssen, Edgar Otto, Friedhelm Hildebrandt. *Pediatric Nephrology, University of Michigan, Ann Arbor, MI.*

Nephronophthisis (NPHP), an autosomal recessive cystic kidney disease, is the most frequent genetic cause for end-stage renal failure in the first 3 decades of life. Mutations in thirteen genes (*NPHP1-11*, *AHI1*, *CC2D2A*) have been identified to cause NPHP. Recently, it became evident that in some NPHP-related ciliopathies (NPHP-RC), the nature of the mutations determines the disease severity (Delous *et al*., *Nat Genet* 2007): whereas truncating mutations cause severe, early-onset, dysplastic, multiorgan phenotypes, missense mutations result in milder, late-onset, degenerative phenotypes with limited organ involvement. Besides multiple allelism, the broad spectrum of organ involvement in NPHP could also be explained by the broad heterogeneity in NPHP.

Our aim was to evaluate genotype-phenotype correlation in 368 families with NPHP-RC, in which both disease-causing alleles were detected in: *NPHP1* (235 families), *NPHP2* (12), *NPHP3* (8), *NPHP4* (23), *NPHP5* (26), *NPHP6* (18), *NPHP7* (1), *NPHP8* (8), *NPHP9* (1), *NPHP10* (8), *NPHP11* (20), *AHI1* (5) and *CC2D2A* (3). Phenotypes were ranked in the order of severity from mild/degenerative [NPHP, Senior-Loken syndrome (SLSN)] to degenerative/dysplastic [Joubert syndrome (JBTS)] to severe/dysplastic [Meckel-Gruber syndrome (MKS)].A genotype of 2 null alleles caused a range of phenotypes in the following ascending order of increasing severity [phenotype in brackets]: *NPHP1* [NPHP, SLSN], *NPHP4* [NPHP, SLSN], *NPHP5* [early-onset SLSN], *NPHP2* [infantile NPHP], *NPHP10* [SLSN with other organ involvement], *NPHP6* [SLSN and JBTS] and *AHI1* [JBTS]. For *NPHP8* and *NPHP11*, we never found patients with 2 truncating mutations. In *NPHP6, NPHP8, NPHP11, AHI1* and *CC2D2A*, allelic influences on the phenotypes were compatible with the literature i.e. the presence of 2 truncating mutations caused dysplastic phenotypes (JBTS/MKS), whereas at least one missense allele "rescued" the phenotype to a milder degenerative form (NPHP/SLSN/JBTS). These data have important implications for genetic counseling and management of renal replacement therapy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1808**

**Deep Sequencing of the *PKD1* and *PKD2* Genes by Bar-Coded and Multiplexed Illumina Next-Generation Sequencing** Sandro Rossetti,[1] Jamie L. Sundsbak,[1] Bruce W. Eckloff,[2] Robert A. Sikkink,[2] Yean Kit Lee,[2] Vicente E. Torres,[1] Peter C. Harris.[1] *[1]Nephrology Research, Mayo Clinic, Rochester, MN; [2]Genome Center, Mayo Clinic, Rochester, MN.*

Autosomal Dominant Polycystic Kidney Disease is caused by mutations in the *PKD1* and *PKD2* genes, with high allelic heterogeneity and genomic duplication of *PKD1*. Next-generation sequencing is potentially well suited to analyze these genes in large populations, for diagnostic or research purposes, but the cost and labour associated with it require the development of more efficient frameworks. We have developed a framework for next-generation sequencing of the *ADPKD* genes by using degenerate bar-codes (indexes) and the Illumina GA platform. The genomic regions of both genes were amplified as 14 long-PCR amplicons (8 for *PKD1* and 6 for *PKD2*, 1.2 to 12.9 Kb long) for a total of 76 Kb of sequence (all coding exons and most introns). After amplicon equimolar assembly, different levels of pooling and multiplexing were tested. To test pooling, 2, 4, 6, and 8 samples were pooled in the same indexed library per individual flow cell lane; to test multiplexing, 2, 3, and 4 indexed libraries of 4 samples each were run per individual flow cell lane. The system was validated using 16 known controls carrying 283 *PKD1/2* sequence variants (range 5-30 per sample, 33-66 per flow cell lane), including single nucleotide changes and small indels. Paired-end, 51 bp-long reads were obtained that were mined using the package NextGENe, and filtered using as cut-off the lowest score of a known variant for each library. An average of twenty-six million effective reads were realigned for each lane, with an average of 14,000 reads for each of the known variants (range 397-72467). All the known variants were detected, with a sensitivity of 100%. A low level of false positives was detected, particularly when pooling 6 or 8 samples in the same indexed library (precision 90 and 76%). This experiment suggests that 4 samples may be pooled per indexed library, but that at least 12 such libraries may be run per lane (384 samples total). We are now using this approach to characterize a cohort of 249 uncharacterized and mutation-negative ADPKD samples.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1809**

**Mutation Screening of HALT PKD Population** Jamie L. Sundsbak,[1] Christina M. Heyer,[1] Robert W. Schrier,[2] Arlene B. Chapman,[3] Vicente E. Torres,[1] Ronald D. Perrone,[4] Jared J. Grantham,[5] Theodore I. Steinman,[6] William E. Braun,[7] Kyong Tae Bae,[8] Kaleab Z. Abebe,[8] James E. Bost,[8] Peter C. Harris.[1] *[1]Mayo Clinic; [2]U Colorado; [3]Emory U; [4]Tufts U; [5]Kansas U; [6]Beth Israel Deaconess Med Ctr; [7]Cleveland Clinic; [8]U Pittsburgh Med Ctr.*

Genetic characterization of the HALT PKD population is required to help understand the role that RAAS and hypertension may play in disease progression. Once complete, it will be >4X larger than any previous genetic study of ADPKD (1018 patients) and hence the most complete view of the genetics of this disorder.

Using conventional sequencing of the exonic and flanking intronic region of *PKD1* and *PKD2*, we have identified mutations in 614 subjects from 551 families (84% *PKD1*, 16% *PKD2*). A total of 434 different mutations were identified (85% *PKD1*, 15% *PKD2*); 22% were recurrent within the study and comparison with the ADPKD Database showed that 60% were novel. Overall, 399 families had mutations predicted to truncate the protein (318 *PKD1*, 81 *PKD2*), while 152 (143 *PKD1*, 9 *PKD2*) were non-truncating. Mutations were classified as frameshifting insertions/deletions (33% *PKD1*, 29% *PKD2*), in-frame insertions/deletions (6% *PKD1*, 0% *PKD2*), missense (27% *PKD1*, 10% *PKD2*), nonsense (27% *PKD1*, 45% *PKD2*), or splicing (8% *PKD1*, 16% *PKD2*). Study A (18-50y, with a GFR > 60ml/min/1.73m$^2$) and Study B (18-60y with a GFR 25-60ml/min/1.73m$^2$) did not significantly differ in the level of *PKD1* and *PKD2* cases. eGFR was significantly greater ($p<0.001$) and total kidney volume smaller (62.5 ml/min/1.73m$^2$ $p<0.001$) in the *PKD2* group. In *PKD1*, truncating mutations were associated with a lower eGFR than non-truncating changes (69.5 ml/min/1.73m$^2$ $p<0.001$).

Some interesting cases noted included one with two *PKD1* nonsense mutations in cis and a family with a *PKD2* nonsense mutation and in-frame deletion (2 amino acids) in *PKD1*. No likely mutations were identified in 59 (8.7%) families that will now be screened for larger deletions/insertions. The characterization of the mutations in this clinically well defined population will greatly aid molecular diagnostics of ADPKD and other genetic studies of this disorder. [22]

Disclosure of Financial Relationships: nothing to disclose

**F-PO1810**

**The *Pkd1* Hypomorphic Allele, R3277C, Modifies the *Pkd1*$^{+/-}$ Phenotype to Early Onset Disease, Suggesting a Dose Dependent Pathogenesis of ADPKD** Katharina Hopp,[1] Christopher James Ward,[2] Sandro Rossetti,[2] Cynthia J. Hommerding,[2] Vicente E. Torres,[2] Peter C. Harris.[2] *[1]BMB, Mayo Clinic; [2]Nephrology Research, Mayo Clinic, Rochester, MN.*

ADPKD develops in patients with a single inactivating mutation to *PKD1* or *PKD2*. Recently, a role has been suggested for hypomorphic *PKD1* alleles that may result in very mild PKD in heterozygotes but modify the typical adult disease presentation to severe, *in utero* onset disease when found *in trans* with a truncating mutation. To prove the significance of hypomorphic alleles in ADPKD, we have made a knock-in mouse model carrying the *PKD1* R3277C mutation. After removal of the selection cassette, detailed analysis of *Pkd1*$^{R3277C/+}$ ES cells at the RNA level showed no abnormal splicing due to the mutation or

the residual loxP site. We crossed our outbred *Pkd1*$^{R3277C/+}$ model with a well established *Pkd1* null model, *Pkd1*$^{del2/+}$, to further characterize the pathological effects of the incomplete penetrant allele. In the first two litters although five compound heterozygotes (*Pkd1*$^{R3277C/del2}$) were born, only one pup survived past perinatal (P) day six. At P18 the surviving pup had a visibly enlarged abdomen and dissection showed grossly enlarged cystic kidneys with a 10-fold weight increase compared to WT, *Pkd1*$^{+/-}$ and *Pkd1*$^{R3277C/+}$ mice. This phenotype mimics precisely the *in utero* onset cases and shows the pathogenic but hypomorphic nature of the R3277C allele. Analysis is underway of the phenotype in homozygous R3277C animals. This model provides strong evidence for a dosage dependent disease mechanism in ADPKD where the level of functional polycystin-1 is the key determinant of whether cysts develop in the kidneys. It seems certain in this model, at least, that no second-hit mutation is required for cystogenesis.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1811

**Mutations in *Pkhd1* Splice Enhancer Motifs Impact *Pkhd1* Alternative Splicing: A Potential Mechanism for ARPKD Pathogenesis** Ravindra Boddu,[1] Gregory G. Germino,[2] Luiz Fernando Onuchic,[3] Lisa M. Guay-Woodford.[1] *[1]Genetics, UAB, Birmingham, AL; [2]NIDDK, NIH, Bethesda, MD; [3]Medicine, Univ of Sao Paulo, Sao Paulo, SP, Brazil.*

*PKHD1*, the gene disrupted in ARPKD, and its mouse orthologue are transcriptionally complex. In previous studies, we catalogued exon usage in 22 unique *Pkhd1* transcripts from a kidney-specific plasmid library. Informatic analysis revealed: 1) several uniquely positioned exon splice enhancers (ESE), including a 3' SC35 motif in exon 6 and a 5' SRp40 motif in exons 49, 51 and 52; and 2) fewer intron splice enhancers (ISE) in IVS6 than in IVS48, IVS50 and IVS51. In the current study, we sought to validate the predicted alternative splice variants in mouse kidney, characterize the *Pkhd1* enhancer motifs, and evaluate human *PKHD1* missense variants that disrupt these motifs. **Methods/Results:** RT-PCR analysis of membrane-bound polysome fractions prepared from whole kidney lysates identified the expected exon 6-7 junction, as well as the predicted novel junctions 6-49, 6-51 and 6-52, indicating that these splice variants are likely to be translated. Rat monoclonal antibodies were generated against a peptide encoded by exons 3/4/5/6. In initial characterization, these immunoreagents each recognize a unique pattern of peptides in IMCD cell lysates. Minigene assays confirmed canonical splicing between exons 6-7, as well as alternative splicing between exons 6-49, 6-51 and 6-52. In assays where site directed mutagenesis disrupted the target ESE and ISE motifs, alternative splicing was abolished. Finally, we evaluated 311 *PKHD1* missense variants (ARPKD Mutation Database; www.humgen.rwth-aachen.de) and identified two variants of indeterminant pathogenicity that disrupt SRp40 and SC35 motifs: R760H and P805L. We are currently examining these missense variants using minigene assays. **Conclusions:** Our data: 1) add to the cumulative evidence that *Pkhd1* is transcriptionally complex; and 2) demonstrate that *Pkhd1* ESE and ISE motifs direct alternative exon usage. We are evaluating the impact of naturally-occurring human *PKHD1* missense variants on alternative splicing. We propose that altered *PKHD1* splicing may contribute to ARPKD pathogenesis.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1812

**Proteomic Signature Profiles in Autosomal Dominant Polycystic Kidney Disease** Nikolay Bukanov,[1] Sarah E. Moreno,[1] Xuewen Song,[2] Steven R. Ledbetter,[1] Oxana Beskrovnaya,[1] York P. Pei.[2] *[1]Department of Cell Biology, Genzyme Corporation, Framingham, MA; [2]University Health Network, Toronto, ON, Canada.*

As the number of clinical trials for ADPKD increases, there is a need to monitor renal disease progression in response to therapy. Magnetic resonance imaging (MRI) is an adequate method to track the progression of ADPKD. However, MRI evaluations are expensive and require specialized facilities for analysis. Our goal was to establish a set of anonymous biomarkers that are robust, reproducible, and correlate with progression of ADPKD. As a source of biomarkers, we used urine and plasma samples which can be conveniently collected by non-invasive methods.

We applied a three-step approach to achieve our goal. First, we used SELDI-TOP technology for proteome profiling of urine and plasma samples from ADPKD patients with known MRI kidney values. Second, we performed comparative analysis of spectra from ADPKD samples and normal controls to identify anonymous biomarker signatures specific to diseased individuals. Finally, we performed statistical analysis to identify a subset of anonymous markers which are in correlation with disease progression.

In total, we generated a set of 18 plasma and 6 urinary biomarkers with good correlation ($R^2$>0.5) to the progression of ADPKD. Several markers from this panel are characterized by >8-10-fold difference in expression levels between ADPKD and normal samples. This data is in the same range as MRI measurements, which indicate an 8-fold difference in total kidney volume in patients representing earlier and late stages of ADPKD. Replication of these results in an independent cohort of patients is in progress. In conclusion, the availability of such disease progression biomarkers should improve the ability to monitor efficacy of experimental therapies for ADPKD.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1813

**Comparison of the PKD-ELV Proteome in Normal and ADPKD Individuals** Marie C. Hogan,[1] Cristine Charlesworth,[2] Kenneth L. Johnson,[2] Linda Page,[1] Peter C. Harris,[1] H. Robert Bergen,[2] Christopher James Ward.[1] *[1]Div. Nephrology & Hypertension, Mayo Clinic; [2]Mayo Proteomics Research Center, Mayo Clinic, MN.*

Exosome-Like Vesicles (ELVs), are 50-200nm diameter membrane bound structures found in all biological fluids, including urine and bile. We showed that there are multiple subpopulations in urine, one of which is enriched in polycystin-1 (PC1), polycystin-2 (PC2) and fibrocystin/polyductin -the PKD-ELV. A 5-30% sucrose heavy water ($D_2O$) gradient method allowed the reproducible isolation of of PKD-ELVs. First or 2nd morning urines from 7 normal individuals (Age=28 sd+/-3.3 years) and 9 individuals with ADPKD (Age=27.7 sd+/-6.2 years) were collected, PKD-ELVs isolated and analyzed by label-free proteomics. Four of the ADPKD individuals are known to have PKD1 mutations; we are presently genotyping the remainder. Our initial results show that there are 27 proteins that are differentially regulated between normals and PKD ELVs with p values ranging from 0.0007 to 0.01. Twenty-three out of 27 are underexpressed in ADPKD vs controls, with -1.98 to -6.28 fold differences in mean relative abundance. Four proteins are overexpressed in PKD1 ranging from 2.54 to 16.56 fold. The PKD proteins are consistently under expressed in ADPKD individuals vs controls: PC1 protein 2.2 fold (p=0.0007), PC2 2.2 fold (p=0.008) and fibrocystin 1.98 fold (p=0.004). We have developed a ratiometric western blot assay using a Mab to PC1 (7e12) and a commercially available Mab to a protein which is upregulated in ADPKD. Initial results indicate that the assay can distinguish normal from ADPKD individuals in unpurified urine exosome preparations.

Disclosure of Financial Relationships: Consultancy: Hoffmann-La RocheResearch Funding: Novartis USA.

### F-PO1814

**Urinary Monocyte Chemotactic Protein-1 (MCP1) Predicts Progression in Autosomal Dominant Polycystic Kidney Disease (ADPKD)** Jared J. Grantham,[1] Vicente E. Torres,[2] Arlene B. Chapman,[3] Kyong Tae Bae,[7] Cheng Tao,[7] Lisa M. Guay-Woodford,[4] Peter C. Harris,[2] Michal Mrug,[4] William M. Bennett,[5] Marva M. Moxey-Mims,[6] James E. Bost.[7] *[1]Kansas U; [2]Mayo; [3]Emory; [4]UAB; [5]Legacy Good Samaritan; [6]NIDDK; [7]UPitt.*

The progression to ESRD begins in ADPKD long before changes in GFR are clearly recognizable, complicating prognosis counseling and impeding the implementation of novel treatments targeted to specific molecular pathogenetic mechanisms. Inflammation and fibrosis begin relatively early and have been associated with renal chemokines and cytokines. In ADPKD patients with normal GFR and varying degrees of renal insufficiency, MCP1 was excreted in the urine to a greater extent in those with advanced disease than in those with normal GFR (Zheng, D. et al Kid Int 14:2588, 2003). MCP1 is produced by cyst epithelial cells, moves into the urine and accumulates to high levels within cysts. The Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) has followed 241 subjects (15-46 y.o. and eGFR at entry >70 ml/min) between Jan 5, 2001 and December 1, 2009 in a prospective study including measurements of total kidney volume relative to height (htTKV) by MR and GFR by iothalamate clearance. Urine MCP1 (pg/mg creatinine) was measured at baseline to determine the extent to which the chemokine predicted progression of htTKV increase and GFR decrease over the next 7.3 years. Baseline urine MCP1 in women and men was 687±641 pg/mg, n=138 and 465±403 pg/mg, n=89, respectively (P=0.0016) and GFR was 99±26 and 96±22 ml/min/1.73 $m^2$, respectively (P=0.2792). Urine MCP1 (pg/mg) was greater in PKD1 (655±610, n=175) than in PKD2 (365±283, n=34) (P<0.001). Baseline urine MCP1 correlated positively with baseline htTKV (R=0.436, P<0.0001, n=227) and inversely with baseline GFR (R=-0.184, P=0.0059, n=222). In a multivariable model including baseline htTKV, age, BMI, gender, race, htTKV slope, and GFR, baseline urine MCP1 associated significantly with declining GFR (OR 1.13, P=0.000) and K/DOQI Stage 1-4 (OR 1.11, P<0.001). On the basis of these findings we conclude that urinary MCP1 predicts disease progression in patients with ADPKD.

Disclosure of Financial Relationships: Consultancy: Otsuka Corp.

### F-PO1815

**Vascular Endothelial Growth Factor Level Correlates with Disease Severity in Young Patients with Autosomal Dominant Polycystic Kidney Disease** Berenice Y. Gitomer, Melissa A. Cadnapaphornchai, Wei Wang, Amirali Masoumi, Kim Mcfann, Xiang-Dong Yan, Robert W. Schrier. *Department of Medicine, University of Colorado Denver, Aurora, CO.*

Renal cysts are often evident on ultrasound imaging in children with autosomal dominant polycystic kidney disease (ADPKD), who may become symptomatic in young adulthood. However, we have previously shown that hypertensive (95th percentile for blood pressure) and borderline hypertensive (75th -95th percentile for blood pressure) ADPKD children have a significantly higher left ventricular mass index (LVMI) than children with ADPKD and normal blood pressure. Moreover, hypertensive children with ADPKD have larger renal volumes than children with lower blood pressure. Therefore it is apparent that vascular changes including expansion and remodeling must occur in order to support the renal and cardiac structural changes in ADPKD patients As vascular endothelial growth factor (VEGF) is a potent regulator of angiogenesis and has been demonstrated in cyst fluid from human ADPKD kidney, we hypothesized that up-regulated expression of VEGF might be related to disease severity. Thus, serum levels of VEGF were measured in 71

young ADPKD patients (mean age 16 ± 4 years) and correlated with renal and cardiac structure (measured by magnetic resonance imaging) and renal function. Because of the skewed distribution, renal and cyst volume, left ventricular mass index and serum VEGF were reported as $\log_{10}$. Serum $\log_{10}$ VEGF was positively correlated with total renal ($P = 0.007$) and cyst volumes ($P = 0.003$) and LVMI ($P < 0.0001$) and negatively correlated with creatinine clearance ($P = 0.022$). In conclusion, the correlation between serum VEGF and both renal and cardiac disease severity reflects a possible role for angiogenesis in early progression of renal and cardiovascular disease in ADPKD.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1816

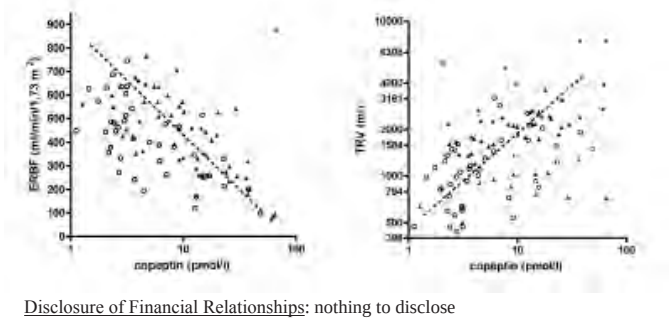
**Copeptin, a Surrogate Marker of Vasopressin, Is Associated with Disease Severity in Autosomal Dominant Polycystic Kidney Disease** Esther Meijer,[1] Stephan J. L. Bakker,[1] Gerjan Navis,[1] Joachim Struck,[2] Paul E. de Jong,[1] Ron T. Gansevoort.[1] *[1]UMCG, Netherlands; [2]BRAHMS, Germany.*

Experimental studies suggest a detrimental role for vasopressin (VP) in the pathogenesis of Autosomal Dominant Polycystic Kidney Disease (ADPKD). It is, however, unknown whether endogenous VP concentration is associated with disease severity in patients with ADPKD.

Because measurement of VP is problematic, we measured plasma copeptin concentration (a marker of endogenous VP levels) in 102 ADPKD patients (Ravine criteria) by immunoassay. Plasma- and urinary osmolarity were also measured. To assess disease severity, we measured glomerular filtration rate (GFR) and effective renal blood flow (ERBF) by continuous infusion of $^{125}$I-Iothalamate and $^{131}$I-Hippuran, resp., total renal volume (TRV) by MRI and 24h urinary albumin excretion (UAE) by nephelometry.

In these ADPKD patients (age 40±11y, 56% male, GFR 77±31 mL/min per 1.73 m$^2$, TRV 1.5 (0.9-2.2)L), median copeptin concentration was 7 (3-15) pmol/L. Copeptin was associated with plasma osmolarity ($R=0.53$, $p<0.001$), but not with 24h urinary volume, 24h urinary osmolarity or fractional urea excretion ($p=0.7$, $p=0.9$, $p=0.3$, resp.). Copeptin was associated with the various markers of disease severity in ADPKD (with TRV $R=0.45$, UAE $R=0.39$, GFR $R=-0.58$ and ERBF, $R=-0.52$ all $p<0.001$, figure). These associations were independent of age, gender and use of diuretics.

In conclusion, plasma osmolarity is associated with copeptin levels in ADPKD, but copeptin is not associated with its normal physiologic effects (24h urinary volume, -osmolarity and fractional urea excretion). Most importantly, in this cross-sectional analysis, copeptin levels are associated with severity of ADPKD. Together with prior experimental studies showing renoprotective properties of VP antagonism in ADPKD, these data support a possible pathogenetic role for VP in ADPKD.




Disclosure of Financial Relationships: nothing to disclose

## F-PO1817

**Copeptin, a Surrogate Marker for Vasopressin, Is Associated with Renal Function Decline in Patients with ADPKD** Wendy E. Boertien,[1] Esther Meijer,[1] Ton J. Rabelink,[2] Joachim Struck,[3] Stephan J. L. Bakker,[1] Dorien J. M. Peters,[2] Paul E. de Jong,[1] Ron T. Gansevoort.[1] *[1]Nephrology, Groningen, Netherlands; [2]Nephrology, Leiden, Netherlands; [3]BRAHMS, Germany.*

Experimental studies suggest a detrimental role for vasopressin in Autosomal Dominant Polycystic Kidney Disease (ADPKD). It is, however, unknown whether endogenous vasopressin concentration is associated with renal function decline in patients with ADPKD.

We measured plasma copeptin (a marker of endogenous vasopressin) by immunoassay in 79 ADPKD patients who participated in a study on renal disease progression during 1994-1999 (1). During this study, GFR was assessed by inulin clearance. Plasma creatinine was measured to determine eGFR (MDRD). After completion of the study, patients were followed and the last available plasma creatinine value was obtained. If applicable, start date of renal replacement therapy (RRT) was reported.

In these patients (57% female, age 36.8 ±10.1 y, GFR 106.6 ±25.9 mL/min) median copeptin level was 2.7 (IQR 1.6 – 5.5) pmol/L. Copeptin was associated with change in GFR (inulin) during the study (median follow-up 3.3 (3.1 - 3.5) y, $R=-0.3$, $p<0.01$) and with change in eGFR (MDRD) during follow-up (median 11.4 (4.5 - 14.3) y, $R=-0.3$, $p<0.01$). On average, patients with a copeptin level of 10 pmol/L had a fall in eGFR of 4.4 ml/min/1.73m$^2$/yr compared to no loss of eGFR in patients with a copeptin level of 1 pmol/L. These associations were independent of age, gender and baseline (e)GFR. Nine patients started with RRT during follow-up. A twofold higher copeptin was associated with a hazard ratio for start of RRT of 2.0 (95%CI 1.2-3.4, $p=0.01$).

These data show that in ADPKD patients a higher vasopressin concentration is associated with renal function decline, suggesting that in these patients blockade of the vasopressin receptor may have a renoprotective effect.

(1) van Dijk, et al: No effect of enalapril on disease progression in ADPKD. NDT 2003;18:2314-20

Disclosure of Financial Relationships: nothing to disclose

## F-PO1818

**Renal Blood Flow (RBF) Is an Underestimated Tool To Monitor the Progression of Autosomal Dominant Polycystic Kidney Disease (ADPKD)** Vicente E. Torres,[1] Arlene B. Chapman,[2] Bernard F. King,[1] Diego R. Martin,[2] Jared J. Grantham,[3] Michal Mrug,[4] Kyong Tae Bae,[5] William M. Bennett,[6] Marva M. Moxey-Mims,[7] Li Jie,[5] James E. Bost,[5] CRISP Consortium.[1-7] *[1]Mayo, Rochester, MN; [2]Emory U, Atlanta, GA; [3]Kansas U, Kansas City, KS; [4]U of Alabama, Birmingham, AL; [5]U of Pittsburgh, Pittsburgh, PA; [6]Legacy Good Samaritan, Portland, OR; [7]NIDDK, Bethesda, MD.*

Polycystic kidneys exhibit media thickening, increased wall-to-lumen ratio, vascular rarefaction and global glomerulosclerosis suggesting ischemia, possibly due to cyst compression, *PKD1* or *PKD2* mutation linked vascular abnormalities, or both. Ischemic damage has been proposed as the final common pathway to ESRD in CKD. Implementation/evaluation of treatments aimed at protecting RBF and preventing hypoxia is hampered by lack of non-invasive methods to monitor RBF in diseased kidneys. Technological innovations allow high-resolution breath-held RBF imaging with improved signal-to-noise ratio and cardiac cycle synchronization. Strict image acquisition and analysis standardization are essential. CRISP seeks to determine whether RBF reduction predicts ADPKD progression. RBF decreased more rapidly than GFR: % changes (*$p<0.05$) from baseline were -4.0*, -10.9*, -8.5*, -33.9*, and -32.6* vs +0.5, +2.0, -3.4*, -18.1*, and -22.0%* at 1 (n 118), 2 (n 106), 3 (107), 6 (n 99) and 8 (n 38) years, respectively. RBF significantly added to the predictive value of total kidney volume (TKV) on rate of GFR decline when controlling for baseline GFR (Table). Higher TKV and lower RBF correlated with GFR decline; higher GFR at baseline associated with steeper GFR slopes during BL-YR3 due to regression to the mean.

| Covariates | GFR slope ($R^2$) | |
|---|---|---|
| | BL-YR3 | BL-YR8 |
| lnTKV0 | 0.15* | 0.22* |
| GFR0 | 0.07* | 0.03 |
| Mean RBF | 0.06* | 0.08* |
| lnTKV0, GFR0 | 0.33* | 0.34* |
| lnTKV, Mean RBF | 0.16* | 0.23* |
| lnTKV, GFR0, Mean RBF | 0.45* | 0.43* |

*significant predictor in the model

Reduction in RBF precedes GFR decline. RBF adds to the predictive value of TKV on rate of GFR decline. Properly standardized measurement of RBF is an underestimated tool to monitor ADPKD and CKD progression.

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Novartis,.

## F-PO1819

**Diagnostic Accuracy of Positron-Emission Computed Tomography in the Diagnosis of Kidney and Liver Cyst Infection in Patients with Autosomal Dominant Polycystic Kidney Disease** Francois Jouret,[1] Renaud Lhommel,[2] Claire Beguin,[3] Olivier Devuyst,[1] Yves A. Pirson,[1] Ziad Hassoun,[4] Nada Kanaan.[1] *[1]Nephrology, Université Catholique de Louvain, Brussels, Belgium; [2]Nuclear Medicine, Université Catholique de Louvain, Brussels, Belgium; [3]Medical Informatics and Statistics, Université Catholique de Louvain, Brussels, Belgium; [4]Gastro-enterology, Université Catholique de Louvain, Brussels, Belgium.*

Cyst infection (CI) remains a challenging issue in patients with autosomal dominant polycystic kidney disease (ADPKD). In most cases, conventional imaging techniques remain inconclusive. Recent observations have suggested that $^{18}$fluorodeoxyglucose ($^{18}$FDG) positron-emission computed tomography (PET-CT) might help detect kidney and liver CI in ADPKD patients. Here, the systematic assessment of databases from 01/2005 to 12/2009 identified 27 PET-CT performed in 24 patients for suspicion of CI. These episodes were further categorized upon the following 4 criteria: temperature >38°C for >3 days, loin or liver tenderness, C-reactive protein plasma level >5 mg/dl, and microbiological documentation. Thirteen infectious events in 11 patients met all criteria for kidney (n=3) or liver (n=10) CI (group A), whereas 14 episodes in 13 patients only encountered 2 to 3 of them (group B). In group A, PET-CT proved CI in 11 cases (84.6%), while CT was contributive in one case. Still, PET-CT overlooked one liver CI in a 74-year-old renal transplant recipient (RTR) and one kidney CI in a 62-year-old patient with stage IV chronic kidney disease. In group B, PET-CT identified the source of abdominal infection in 9 cases (64.3%). Among these, two kidney CI were found in an 81-year-old patient under chronic haemodialysis and a 58-year-old RTR. Conversely, PET-CT showed no abnormal $^{18}$FDG uptake in 5 cases, including 2 renal intracystic bleedings. The median delay between the onset of symptoms and PET-CT procedure was 9 days. The sensitivity and specificity of PET-CT imaging in CI diagnosis reach 86.7% and 100%, respectively. In conclusion, PET-CT nowadays represents the optimal tool to (i) confirm and locate cyst infection, and (ii) identify alternative sources of abdominal infection in ADPKD patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1820**

**A Unique Urine Proteomic Pattern in Autosomal Dominant Polycystic Kidney Disease** Andreas D. Kistler,[1] Harald Mischak,[2] Justyna Siwy,[2] Arlene B. Chapman,[3] James E. Bost,[4] Rudolf P. Wuthrich,[1] Andreas L. Serra.[1] *[1]Division of Nephrology, University Hospital, Zurich, Switzerland; [2]Mosaiques Diagnostics and Therapeutics AG, Hannover, Germany; [3]Renal Division, Emory University School of Medicine, Atlanta; [4]Department of Medicine, University of Pittsburgh.*

Using capillary electrophoresis coupled to mass spectrometry (CE-MS), we have previously found a unique urinary polypeptide profile characteristic for autosomal dominant polycystic kidney disease (ADPKD). Here, we validated these findings from a Swiss cohort in a large North American ADPKD cohort and tested whether urinary biomarkers correlate with total kidney volume (TKV) progression of polycystic kidneys. Spot urine samples of 48 patients from the control arm of the SUISSE ADPKD study, 124 patients from the CRISP cohort (all PKD1) and 86 healthy controls were analyzed. All ADPKD patients were followed with serial magnetic resonance imaging (MRI) based TKV every 6 (SUISSE) or 12 (CRISP) months for 18-36 months. Our previously published diagnostic biomarker model for ADPKD performed well in the CRISP cohort (sensitivity 91.9%, specificity 90.7%, AUC 0.964). A refined diagnostic model based on proteomic data of all 48 analyzed samples from the SUISSE ADPKD study achieved an even higher performance upon validation in the CRISP cohort (sens. 93.5%, spec. 94.2%, AUC 0.974). Using LC-MS/MS we were able to identify 30% of these diagnostic biomarkers. Most of them represent collagen fragments mirroring drastic changes of extracellular matrix turnover in ADPKD. We then used 110 patients from both studies as a training and 62 CRISP patients as a validation cohort to identify prognostic biomarkers. Although a few markers showed significant correlation with annual TKV progression after adjustment for multiple testing, the correlation was weak and not sufficient to yet establish a clinically useful prognostic biomarker model. In conclusion, we have now validated and refined our previously established diagnostic urinary biomarker model for ADPKD in an independent cohort. We continue to evaluate potential prognostic biomarkers, utilizing additional follow up time and other markers of disease severity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1821**

**HALT-PKD Clinical Trials, Analysis of Baseline Parameters** Vicente E. Torres,[1] Robert W. Schrier,[2] Arlene B. Chapman,[3] Ronald D. Perrone,[4] Kyong Tae Bae,[5] Marva M. Moxey-Mims,[6] Theodore I. Steinman,[7] William E. Braun,[8] Franz Winklhofer,[9] Kaleab Z. Abebe,[5] James E. Bost,[5] HALT PKD Study Group.[1-9] *[1]Mayo Clinic; [2]U Colorado; [3]Emory U; [4]Tufts; [5]Pittsburgh U; [6]NIH/NIDDK; [7]Beth Israel; [8]Cleveland Clinic; [9]Kansas U.*

HALT-PKD comprises two randomized trials: A (n=548, eGFR >60 ml/min/1.73 m², 2-by-2 design randomization to ACEi/ARB *vs* ACEi alone and to low *vs* standard BP targets) and B (n=470, eGFR 25-60 ml/min/1.73 m², randomized to ACEi/ARB *vs* ACEi alone). Primary outcomes are % change in kidney volume (TKV) by MR in A and time to 50% eGFR reduction, ESRD, or death in B. All A patients underwent MR measurements of TKV and liver volume (TLV); 268 had MR measurements of renal blood flow (RBF). We sought to identify associations of baseline factors with TKV and TLV in A and with eGFR in both studies. TKV adjusted for height (htTKV) were larger in men (779±415 *vs* 611±368 mL, p<0.0001), whereas liver cyst volumes were greater in women (148±706 vs 343±801 mL, p=0.006). There was no significant correlation between htTKV and htTLV. HtTKV significantly correlated with eGFR (r=-0.31), BSA (0.24), urine albumin excretion (UAlbE, 0.21), RBF (-0.17), systolic BP (0.14), urine sodium excretion (0.11) and age (0.09). Multiple regression showed independent associations of eGFR, BSA, UAlbE and gender with htTKV. eGFR were similar in men and women. eGFR significantly correlated with age (-0.63), RBF (0.44), htTKV (-0.31), serum potassium (-0.22), UAlbE (-0.18), urine aldosterone (0.17), urine sodium/potassium ratio (0.15), office systolic BP (-0.09), weight (-0.09), urine volume (-0.08) and urine potassium excretion (-0.08). Age, htTKV and RBF independently associated (all p<0.001) with eGFR in A. In A and B without htTKV and RBF covariates, age, serum potassium, UAlbE, urine aldosterone and weight independently associated with eGFR.

*Conclusion*: The factors that modulate the severity of PKD are different from those modulating severity of PLD; male gender, large body size, reduced eGFR and increased UAlbE are associated with larger htTKV; older age, larger htTKV, and reduced RBF are independently associated with reduced eGFR in ADPKD.

Disclosure of Financial Relationships: Consultancy: Hoffmann-La RocheResearch Funding: Otsuka
Novartis.

**F-PO1822**

**TEMPO 2/4 Update: Changes in ADPKD Total Kidney Volume and eGFR with 3 Years of Tolvaptan and after Withdrawal** Vicente E. Torres,[1] Arlene B. Chapman,[2] Jared J. Grantham,[3] Terry J. Watnick,[4] John Ouyang,[5] Holly B. Krasa,[5] Frank S. Czerwiec,[5] TEMPO 2/4.[1-5] *[1]Mayo Clinic; [2]Emory U; [3]U of Kansas; [4]The Johns Hopkins Univ School of Med; [5]Otsuka Pharma Devel & Comm, Inc.*

TEMPO 2/4 is an open-label, randomized, dose-ranging study monitoring long-term effects of tolvaptan on patient safety, tolerability and efficacy (renal volume and function). After baseline evaluation; 46 subjects entered a 1-month dose ranging titration phase. They were then randomized 1:1 to open-label, split-dose tolvaptan regimens for 3 years. Tolerability, safety and clinical labs were evaluated every 4 months; renal MRI annually. After dose ranging, 46 subjects were randomized to low (L=45/15mg, N=22) or high (H=60/30mg, N=24) daily dose regimens for 3 years. In the L and H, mean ages were 39 and 43, and 1/3 in each group were male. Hypertension was seen in 82 and 71%, respectively, and ~80% in each group used ACEi/ARB agents. Baseline mean total kidney volume (TKV) and eGFR (MDRD) were 1.57 and 1.67 L and 63 and 57 mL/min/1.73m² for the 46 L and H subjects, respectively. Follow-up data for 35 subjects (L=17, H=18) at 3-7 months after withdrawal are available. Seven subjects discontinued: loss to follow-up (1 each), adverse event (2 and 1, respectively), met withdrawal criteria (1 in H), subject withdrew consent (1 in L). Six subjects down-titrated from H to L. Four subjects did not have withdrawal data.

ADPKD Outcomes in 35 Subjects

| Visit/Treatment | TKV-L | TKV-H | TKV-All | eGFR-L | eGFR-H | eGFR-All |
|---|---|---|---|---|---|---|
| Baseline/Off | 1.48 | 1.54 | 1.51 | 64 | 56 | 60 |
| 2 mo/On | 1.49 (-1.0%) | 1.52 (-1.4%) | 1.51 (-1.2%) | 61 (-3.1) | 56 (-0.3) | 58 (-1.7) |
| 3 yr/On | 1.61 (7.9%) | 1.63 (3.8%) | 1.62 (5.8%) | 60 (-3.7) | 53 (-2.7) | 57 (-3.2) |
| 4 mo/Off | 1.72 (14.1%) | 1.70 (8.4%) | 1.71 (11.2%) | 59 (-4.7) | 55 (-1.2) | 57 (-2.9) |

Mean and change from baseline TKV (L), eGFR (mL/min/1.73M²)

The data suggest that tolvaptan slows ADPKD patients' cyst growth. On withdrawal, TKV growth resumes at expected rates, but overall eGFR appears stable. Other analyses suggest earlier treatment may confer a larger benefit.

Disclosure of Financial Relationships: Consultancy: Hoffmann-La RocheResearch Funding: Otsuka
Novartis.

**F-PO1823**

**Somatostatin Analog Therapy for Severe Polycystic Liver Disease: Results after Two Years** Marie C. Hogan,[1] Tetyana V. Masyuk,[2] Linda Page,[1] David R. Holmes,[3] Xujian Li,[4] Eric J. Bergstralh,[4] Sandro Rossetti,[1] Bohyun Kim,[5] James Glockner,[5] Peter C. Harris,[1] Nicholas Larusso,[2] Vicente E. Torres.[1] *[1]Nephrology Division; [2]GI Research; [3]Biomedial Imaging Core; [4]Biostatistics; [5]Radiology, Mayo Clinic, Rochester, MN.*

In our randomized, double-blind, placebo-controlled trial, Octreotide LAR® reduced kidney and liver growth in patients with severe PLD through 12 months. To substantiate long-term safety & clinical benefits of OctLAR patients we completed an open-label extension through year 2. **Methods:** Primary endpoint was % change in liver volume (TLV) from baseline to Yr 2 (MRI); 2° endpoints were changes in total kidney volume (TKV), GFR, quality of life, safety, vital signs, and laboratory tests. 40 patients (26 OctLAR; 14 placebo) received OctLAR during Yr2 (max dose 40 mg). Patients already on OctLAR continued therapy. **Results:** There was a significant reduction in TLV during the first year of OctLAR therapy in both groups. Patients originally randomized to placebo showed substantial reduction in TLV during treatment with OctLAR in Yr 2 (Δ% -7.66 ± 9.69%; p= 0.011). The reduction in TLV in the OctLAR treated group was maintained, but did not increase significantly, during Yr2 (Δ% -5.96 ±8.90%; p =0.002). OctLAR inhibited renal enlargment during Yr1 but not significantly during Yr2. OctLAR treated individuals experienced improvements in QOL. Changes in GFR were similar in both groups. **Conclusions:** Over a two year period OctLAR significantly reduced the rate of TLV & possibly TKV increase. OctLAR improved health perception and had an acceptable side effect profile in patients with PLD.

Mean (SD) TKV & TLVs at Baseline, Y1 &Y2.

| Treatment Yr0,1/Yr1,2 | Baseline | Year 1 | Year 2 | %Δ Yr 0,1 | %Δ Yr 1,2 |
|---|---|---|---|---|---|
| **OctLAR/OctLAR** | | | | | |
| TKV (n=19) | 1152 (869) | 1139 (838) | 1214 (884) | 0.42 (7.61) | 6.49 (7.08) |
| TLV (n=26) | 5984 (2961) | 5628 (2720) | 5649 (2870) | -5.23 (6.22) | -0.77 (6.82) |
| **Placebo/OctLAR** | | | | | |
| TKV (n=8) | 803 (269) | 874 (306) | 885 (355) | 8.61 (10.07) | 0.41 (9.45) |
| TLV (n=14) | 5374 (3565) | 5360 (3331) | 4952 (3344) | 0.90 (8.35) | -7.66 (9.69) |

Disclosure of Financial Relationships: Consultancy: Hoffmann-La RocheResearch Funding: Novartis USA.

**F-PO1824**

**Short-Term Effects of Tolvaptan on Renal Function and Volume in Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD)** Maria V. Irazabal-Mira,[1] Vicente E. Torres,[1] Marie C. Hogan,[1] James Glockner,[1] Bernard F. King,[1] Troy G. Ofstie,[1] Holly B. Krasa,[2] John Ouyang,[2] Frank S. Czerwiec.[2] *[1]Mayo, Rochester, MN; [2]Otsuka Pharm. Devel. & Comm., Inc.*

The AVP V2 receptor antagonist tolvaptan slightly and reversibly elevates serum creatinine in clinical subjects with ADPKD. We studied the mechanisms of this effect after daily, split-dose tolvaptan (45/15 mg) for one week in 20 ADPKD patients, 12 with estimated creatinine clearance (eCrCl) >60 ml/min (Group 1) and 8 with eCrCl 30-60 ml/min (Group 2). On Days 0 and 8, subjects underwent iothalamate and para-amino-hippurate (PAH) clearances and renal blood flow (RBF) measurements by MRI. Tolvaptan-induced aquaresis was accompanied by 8.6% GFR (p=0.018) reduction, 13.0% increase in serum uric acid (p=0.002), 26.8% reduction in uric acid clearance (p<0.001), and 4.8% reduction in serum potassium (p<0.001), without change in RBF measured by PAH clearance (-5.9%, NS) or MRI (+1.0%, NS). Underlying mechanisms may include attenuation of urine concentrating activity and tubuloglomerular feedback (V2 receptor blockade); decreased glomerular ultrafiltration (reflex V1 receptor activation); proximal sodium-linked uric acid reabsorption (compensating distal sodium loss); distal potassium loss (increased distal sodium/water delivery). Post-hoc, blinded analysis of renal MRIs showed that tolvaptan

induced a 3.1% reduction (p<0.001) in total kidney volume (TKV), more marked in group 1 (-4.1%, p<0.001) than in group 2 (-1.7%, NS), and in volume of individual cysts ≥10 mL (-4.1%, n= 79, p<0.001) without a significant change in those <10 mL (n=59). Total body water (TBW) decreased by 1.1% (p=0.001). No significant correlation was detected between the change in TKV and TBW. These results are consistent with effects of tolvaptan on glomerular hemodynamics, explaining the increase in serum creatinine, and on fluid secretion into the cysts, explaining the rapid reduction in kidney and cyst volume.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1825

**An Open-Label Long-Term Administration Study of Tolvaptan in Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD) in Japan: Three-Year Results** Eiji Higashihara,[1] Kikuo Nutahara,[1] Shigeo Horie,[2] Fumitake Gejyo,[3] Akira Hishida.[4] *[1]Urology, Kyorin University, Tokyo, Japan; [2]Urology, Teikyo University, Tokyo, Japan; [3]Nephrology, Niigata University, Niigata, Japan; [4]Nephrology, Hamamatsu University, Hamamatsu, Japan.*

**Background:** Tolvaptan was shown to inhibit cyst enlargement in animal models orthologous to human ADPKD. At last year's ASN annual meeting we reported the second-year results of a 3-year administration study of tolvaptan. Here we report on the safety and efficacy of tolvaptan at completion of the third year of the study.

**Methods:** Of 18 patients with ADPKD who participated in a phase 2 study of tolvaptan conducted from December 2004 to May 2005, 17 consented to participate in a 3-year extension study, and tolvaptan was administered at 15 mg twice a day beginning from April 2006.

**Results:** Of the 17 patients enrolled in the study, there were 5 discontinuations: one patient died due to intracranial hemorrhage, 2 withdrew due to renal impairment, and 2 withdrew for non-medical reasons. Baseline values and changes from baseline for serum sodium, serum creatinine, creatinine clearance (estimated by Cockcroft-Gault equation), and renal volume (measured by MRI) are shown below.

Table

| | Baseline | Change from Baseline | | |
|---|---|---|---|---|
| | (n=17) | 48W (n=14) | 96W (n=13) | 156W (n=12) |
| Serum sodium (mEq/L) | 141.2±1.4 | 1.1±1.5 | 1.4±2.1 | 1.3±2.3 |
| Serum creatinine (mg/dL) | 0.966±0.311 | 0.048±0.111 | 0.022±0.083 | 0.094±0.183 |
| Creatinine clearance (mL/min) | 85.7±29.5 | -3.1±8.8 | -0.6±7.7 | -5.0±8.4 |
| | Baseline (n=17) | 52W (n=14) | 104W (n=12) | 156W (n=12) |
| Renal volume (mL) | 1831±1095 | -58±149 | -84±174 | -69±219 |

Common adverse events included nasopharyngitis, thirst, contusion, blood ADH increased, and hypertension, but none of these events were serious enough to discontinue administration.

**Conclusions:** In a 3-year phase 2 extension study in patients with ADPKD, tolvaptan was well tolerated and demonstrated beneficial effects on renal enlargement (ClinicalTrials.gov identifier: NCT00841568; off-label: Tolvaptan for ADPKD).

Disclosure of Financial Relationships: Consultancy: Otsuka Pharmaceutical Co.

## F-PO1826

**Vitamin D Deficiency and Renal Volume in Autosomal Dominant Polycystic Kidney Disease** Berenice Y. Gitomer, Melissa A. Cadnapaphornchai, Amirali Masoumi, Michel B. Chonchol, Kim Mcfann, Wei Wang, Xiang-Dong Yan, Robert W. Schrier. *Department of Medicine, University of Colorado Denver, Aurora, CO.*

**Purpose:** Increased renal volume in autosomal dominant polycystic kidney disease (ADPKD) correlates with early onset and persistent hypertension and ultimately left ventricular hypertrophy and decline in kidney function. Activation of the renin-angiotensin-aldosterone system plays a central role in the hypertension in ADPKD patients and active vitamin D is a well-established potent regulator of renal renin. However, despite emerging evidence on the role of vitamin D deficiency in cardiovascular disease and renal disease progression no studies have addressed the role of vitamin D on renal disease progression in an ADPKD patient population. We hypothesized that lower serum levels of 25(OH)D in ADPKD would be associated with more severe renal disease as assessed by renal volume measured by magnetic resonance imaging (MRI).

**Methods:** Study subjects comprised 30 young adult ADPKD patients (15 men and 15 women) aged 18-24 years. Renal volumes were measured by MRI and serum 25(OH)D levels measured by ELISA assay.

**Results:** Patients mean (SD) age and creatinine clearance were 20.3 (1.6) years and 112 (28) ml/min/1.73m$^2$ respectively. For this analysis the patient population was categorized on the basis of a clinically significant cutoff for 25(OH)D deficiency (less than 30 ng/ml vs. higher). Thus, 14 ADPKD patients whose serum 25(OH)D level was >30 ng/ml were compared with 16 deficient patients with serum 25(OH)D ≤30 ng/ml. The vitamin D deficient subjects had higher total renal volume than those who were 25(OH)D sufficient after adjustment for sex, height, hypertension and season [1044 (765-1427)cm$^3$ vs. 680 (524-882)cm$^3$, p = 0.03] (Reported as geometric mean and 95% CI).

**Conclusion:** These results indicate that vitamin D deficiency is associated with larger kidneys in this ADPKD population with normal renal function. Further studies are needed to determine if correction of vitamin D deficiency significantly impacts renal volume in ADPKD.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1827

**Left Ventricular Hypertrophy in the HALT PKD Study** Ronald D. Perrone,[1] Robert W. Schrier,[2] Arlene B. Chapman,[3] Vicente E. Torres,[4] Theodore I. Steinman,[5] James E. Bost,[6] Kaleab Z. Abebe,[6] William E. Braun,[7] Jared J. Grantham,[8] Diana Kaya,[6] Marva M. Moxey-Mims,[9] Kyong Tae Bae.[6] *[1]Tufts; [2]U Colorado; [3]Emory U; [4]Mayo Clinic; [5]BIDMC; [6]U Pittsburgh; [7]Cleveland Clinic; [8]Kansas U; [9]NIH/NIDDK.*

Left ventricular hypertrophy (LVH) is found in ADPKD children and associates with hypertension (HTN). Prior echocardiographic studies in ADPKD revealed a 41% prevalence of LVH by age 41. The present study focuses on 543 HALT PKD Study A subjects aged 15-49 with eGFR >60mL/min/1.73m$^2$ who underwent baseline magnetic resonance (MR) measurement of total kidney volume and left ventricular mass (LVM). LVM was measured using a semi-automated program from ECG-gated dynamic cardiac MR (true-FISP or FIESTA). LVM and LVM indexed by body surface area (LVMI), height$^2$ (H$^2$), H$^{2.7}$, and a new allometric H-weight (W) index calculated using H, W, and sex (ppLVmass$_{HW}$) (pp % predicted) are shown; the 95$^{th}$ percentile from Int J CV Imaging (26:259,2010) defines the upper limit of normal§. HTN was of similar duration in M (male) (N=279) and F (female) (N=273) (years: M 5.8±6; F 5.7±6). At baseline, 59 (11%) subjects took ACE-I; 171 (31%) took ARB. Office BP was well-controlled (M 127±14/80±11; F 123±15/79±12). M were significantly (P<0.005) younger (M 35±8; F 37±8), taller (cm: M 181±8; F 166±8), and heavier (kg: M 90.2±16.2; F 74.5±17.0) than F; BMI was similar (kg/m$^2$: M 27.6±4.7; F 27.2±10.4).

LMV and LVH

| Gender | LVM (gm) | LVM/BSA (gm/m$^2$) | LVM/H$^2$ (gm/m$^2$) | LVHH$^{2.7}$ (gm/m$^{2.7}$) | ppLVM$_{HW}$ |
|---|---|---|---|---|---|
| M | 153.0+29.9 | 73.0+13.2 | 46.8+8.7 | 31.0+6.0 | 0.9+0.2 |
| F | 105.8+24.3 | 58.3+12.2 | 38.4+9.3 | 27.0+7.5 | 0.9+0.2 |
| 95$^{th}$ percentile (M/F)§ | 203.5/140.3 | 106.2/84.6 | 65.7/53.0 | 45.1/38.0 | 1.31/1.31 |
| **Prevalence of LVH %, (N)** | 6.6 (36) | 1.8 (10) | 4.1 (22) | 3.7 (20) | 1.6 (9) |

LVM expressed as Mean+SD

Indices of LVM that account for body W show a lesser degree of LVH than indices not including weight. The prevalence of LVH in hypertensive ADPKD <50 yo with good BP control, short duration of HTN, and use of ACE-I/ARB is low. Early BP intervention in ADPKD may have decreased LVH and potentially decrease cardiovascular mortality.

Disclosure of Financial Relationships: Research Funding: Otsuka.

## F-PO1828

**Follow-Up of Unruptured Intracranial Aneurysms (UIA) Detected by Presymptomatic Screening in Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD)** Maria V. Irazabal-Mira, John Huston, Vickie J. Kubly, Sandro Rossetti, Marie C. Hogan, Peter C. Harris, Robert D. Brown, Vicente E. Torres. *Mayo Clinic, Rochester, MN.*

Feasibility and success of screening for UIA depends on their rates of growth and rupture. ADPKD patients found to have an UIA by MRA screening at the Mayo Clinic during 1989-2009 were recommended surveillance (except in one patient in whom surgery was deemed indicated), initially at 6 months and yearly, and less often after showing stability. At initial screening, 48 saccular aneurysms were detected in 40 patients from 38 families (16 men, 24 women, 50.7±10.4 y.o.). Genotype was *PKD1* in 10, *PKD2* in 1, not determined in 29 patients. There was a family history of UIA in 5 (12.5%), subarachnoid hemorrhage in 13 (32.5%) and both in 2 (5.0%). Eighteen patients were or had been smokers at the 1$^{st}$ MRA and of those 5 continued to be active smokers. Hypertension was present in 38 (33 treated with ACEI, ARBs or both) and hyperlipidemia in 24 (18 treated with statins). Most UIA were small (mean diameter 3.9 mm, range 1.0 to 10.0 mm); only 2 had a diameter ≥7mm at baseline. The majority (83.3%) were in the anterior circulation. Eight patients (20.0%) had 2 aneurysms on their 1$^{st}$ MRA. During cumulative imaging FU of 238 years, 1 de novo UIA was detected and increased in size from 2 to 4.4 mm over 144 months. An UIA in a second patient grew from 4.5 mm to 5.9 mm after FU of 69 months. Ten patients did not have imaging FU (2 died from unrelated causes shortly after diagnosis, 3 have only clinical FU, 5 recently diagnosed). No change was detected in the remaining 28 patients. During cumulative clinical FU of 315 years, no aneurysm ruptured. Five patients died from unrelated causes and 1 patient was lost to FU after 120 months. Three patients underwent surgical clipping of UIA measuring 10 mm (at Mayo) and 3 and 5 mm (elsewhere). Conclusion: Extended FU of these patients confirms that most UIA detected by presymptomatic screening in ADPKD patients are small, in the anterior circulation, and with risks for growth/ rupture not higher than those of UIA in the general population. These data do not support widespread screening for UIA in ADPKD.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1829

**The Calcimimetic R-568 Inhibits Renal Pathology in Rodent Nephronophthisis (NPHP) and Autosomal Recessive Polycystic Kidney Disease (ARPKD)** Neal X. Chen,[1] Sharon M. Moe,[1,2] Xianming Chen,[1] William Hoffmeyer,[1] Vincent H. Gattone.[1] *[1]Indiana University School of Medicine, Indianapolis, IN; [2]Roudebush Veterans Medical Center, Indianapolis, IN.*

Cystic kidney diseases are common causes of chronic kidney disease (CKD) for which there is no treatment. We have previously demonstrated that calcimimetics, an allosteric activators of the calcium sensing receptor (CaSR, a G-protein linked receptor) inhibits cyst

progression in the Cy/+ rat, likely due to increased intracellular calcium and decreased cAMP. In the present study we sought to test the efficacy of the calcimimetic R-568 in two orthologous rodent models. We treated pcy mice, orthologous with human nephronophthisis (NPHP), from 4 -15 weeks (prevention) or 20 -35 weeks (treatment after disease was established) with the calcimimetic R-568 (0.05% in food). PCK rats, orthologous with human ARPKD, were similarly treated from 8 -18 weeks. Renal pathology and serum biochemistry were evaluated. The results demonstrated that R-568 treatment from 4-15 weeks was efficacious in inhibiting the progression of rodent NPHP by reducing kidney weight as a percent of the total body weight (KW%BW, 3.3±0.8 vs. 4.8±1.1, $p<0.001$); cyst volume density (27.7±10 vs. 40.9±9.1, $P<0.001$) and fibrosis (3.0±0.3 vs. 4.0±0.01, $p<0.002$). Early treatment of pcy mice was more effective that the later treatment, but both stages of the disease responded positively to treatment. In PCK rats, we also found R568 significantly reduced KW%BW (1.1±0.1 vs. 1.3±0.1, $p<0.02$); Cyst volume density (14.5±1.4 vs.19.8±2.7, $p<0.02$) and fibrosis (3.1±0.2 vs. 3.8±0.2, $p<0.05$). In all treatments, R-568 also lowered serum PTH. We further demonstrated that CaSR mRNA is expressed in epithelial cells lining renal cysts in kidney from both the pcy mice and PCK rats. In conclusion, treating renal cystic disease with the calcimimetic R-568 was efficacious in two rodent models of childhood cystic disease, suggesting that calcimimetics may be a potential therapeutic intervention for these conditions.

Disclosure of Financial Relationships: Research Funding: Amgen, Inc; Patent: Licensing agreement with AMGEN.

### F-PO1830

**Inhibition of Angiogenesis Decreases ADPKD Cyst Growth in Nude Mice Xenografts** Wei Wei,[1] Elsa Bello-Reuss.[2] *[1]Internal Medicine, University of Texas Medical Branch, Galveston, TX; [2]Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, TX.*

Primary cultures of Autosomal Dominant Polycystic Kidney Disease (ADPKD) cyst cells were grown *in vitro*, included in a collagen matrix (growth factor reduced (GFR)-Matrigel) and grafted into immunoincompetent (nude) mice. We found that the cells form cysts that develop in about two weeks and that mouse blood vessels invade the implant. We studied the effect of inhibitors of the angiogenic factors IL-8 and VEGF on the formation and growth of the cysts. Systemic administration to the animals of anti-IL-8 antibody (inhibitor of IL-8); bevacizumab (BMab, an anti-VEGF) or dexamethasone, (Dexa, a nonspecific IL-8 inhibitor), decreased the number and size of the cysts in the implants. Cyst volume of treated animals *vs.* control decreased by 65% (anti-IL8), 47% (BMab), 61% (Dexa), 46% (BMab + antiIL-8) and by 62% by BMab + Dexa, all $P<0.001$. The decrease in cyst volume by anti-IL-8 was significantly larger than those elicited by BMab, Dexa and BMab + anti IL-8 ($P < 0.04$, $< 0.02$ and $< 0.04$, respectively) but not for BMab + Dexa. Cyst cell immunostaining in control implants was positive for VEGF, PDGF and IL-8, as well as their receptors. In the cysts still growing in treated animals, expression of VEGF, PDGF, IL-8 and their receptors was not different from control. The presence of red blood cells in the implants' vasculature indicates functional circulation. Vascular invasion decreased by about 50% by antiangiogenic treatments. Summary: 1. Implants containing ADPKD cyst cells develop cysts and functional vasculature. 2. Inhibition of angiogenesis decreases the number and size of the cysts. 3. The viable cysts in treated animals continue expressing angiogenic factors and their receptors. 4. Antiangiogenic treatment significantly decreases implant vascularization. Thus, this system can be used as a model of ADPKD for testing treatments. Our studies confirm the importance of secretion of angiogenic factors by cyst cells in the recruitment of vasculature, creating a positive feedback for ADPKD cyst growth.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1831

**Vasopressin V2 Receptor Antagonism in a Mouse Model for Autosomal Dominant Polycystic Kidney Disease. Optimal Timing and Dosing of the Drug** Esther Meijer,[1] Ron T. Gansevoort,[1] Paul E. de Jong,[1] Wouter N. Leonhard,[2] Anne Marike Van der Wal,[2] Jacob Van den Born,[1] Harry Van Goor,[1] Emile De Heer,[2] Dorien J. M. Peters.[2] *[1]UMCG, Groningen, Netherlands; [2]LUMC, Leiden, Netherlands.*

The renoprotective effect of a vasopressin V2 receptor antagonist (V2RA) presently is tested in a clinical trial in early ADPKD. If efficacious, this warrants life long treatment with V2RA, however with associated physiologic side effects as polydipsia and polyuria. We questioned whether we can modify the side effects without influencing the renoprotective effect by starting the treatment later in the disease, or by lowering drug dose. In addition, we evaluated the time course of the effects.

We administered the V2RA OPC-31260 at high (0.1%) and low (0.05%) dose to a tamoxifen-inducible kidney epithelium-specific *Pkd1*-deletion mouse model (*Pkd1*-gene deletion at post natal day 11) starting treatment at day 21 (early) or 42 (late). After 3 and 6 wks of treatment, we monitored physiologic effects, e.g. water intake and potential renoprotective effects, e.g. kidneyweight and cystogenesis.

Initiation of V2RA treatment late in the disease lacked renoprotective effects. It also had less pronounced physiologic effects. When started early, both after 3 and 6 wks of treatment, the V2RA caused dose-dependent physiologic effects. On high dose, water intake at both timepoints was 2-fold higher than on low dose ($p<0.001$). After 3 wks on high dose, cystratio and kidneyweight were reduced vs. untreated controls (18 vs.25%, $p=0.05$ and 0.33 vs. 0.45g, $p=0.03$, resp.). After 6 wks of treatment however, administration of the V2RA lacked renoprotection, even at high dose (kidneyweight 0.55 vs. 0.66g, $p=0.12$ and cystratio 24 vs. 27%, $p=0.38$, resp.). After 6 wks of treatment, mice receiving high dose also exhibited less polydipsia than after 3 weeks (2-fold vs. 4-fold increase resp. compared to untreated controls, $p=0.001$).

These data suggest that intervention with a V2RA should be instituted early in ADPKD and at high dose, and that it might be necessary to increase the dosage of this drug later in the disease to reach renoprotection, however at the expense of physiologic side effects of the drug.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1832

**Thiazolidinedione Ameliorates Autosomal Recessive Polycystic Kidney and Liver Disease in the PCK Rat, an Orthologous Model of Human ARPKD** Daisuke Yoshihara,[1,2] Hiroki Kurahashi,[2] Miwa Morita,[1] Masanori Kugita,[1] Yoshiyuki Hiki,[3] Harold M. Aukema,[4] Tamio Yamaguchi,[1] James P. Calvet,[5] Darren P. Wallace,[5] Shizuko Nagao.[1] *[1]Education and Research Center of Animal Models for Human Diseases, Fujita Health University, Toyoake, Aichi, Japan; [2]Division of Molecular Genetics, Fujita Health University, Toyoake, Aichi, Japan; [3]School of Health Sciences, Fujita Health University, Toyoake, Aichi, Japan; [4]Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, Canada; [5]The Kidney Institute, University of Kansas Medical Center, Kansas City, KS.*

Polycystic kidney disease (PKD) is characterized by progressive enlargement of numerous fluid-filled cysts and aberrant proliferation of tubule epithelial cells in the kidneys often leading to end-stage renal disease. Liver cysts are the most common extrarenal manifestation in PKD, and can lead to massive liver enlargement and congenital hepatic fibrosis. Peroxisome proliferator-activated receptor gamma (PPAR-gamma), a member of the ligand-dependent nuclear receptor family, is expressed in a variety of tissues, including the kidneys and liver, and plays important roles in cell proliferation, fibrosis and inflammation. In the current study, we determined the effect of thiazolidinedione, a PPAR-gamma agonist on polycystic kidney and liver disease progression in the PCK rat, an orthologous model of human autosomal recessive PKD (ARPKD). Daily treatment with 10 mg/kg pioglitazone, a thiazolidinedione derivative, for 16 weeks significantly decreased kidney weight (% of body weight), renal cystic area, SUN and the number of Ki67-, pERK1/2- and pS6-positive cells in the kidney. There also was a significant decrease in liver weight (% of body weight), liver cystic area, hepatic fibrosis index, and the number of Ki67-, pERK1/2-, pERK5- and TGF-beta-positive cells in the liver tissue. These results demonstrate that pioglitazone inhibits the progression of polycystic kidney and liver disease in a model of ARPKD by inhibiting cell proliferation, fibrosis and inflammation. Current findings suggest that thiazolidinediones may have therapeutic value in the treatment of the renal and hepatic manifestations of ARPKD.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1833

**Proteasome Inhibition Leads to Reduced Cyst Growth in a Mouse Model with Inactivation of the ADPLD Gene, *PRKCSH*** Sorin V. Fedeles,[1] Seung H. Lee,[1] Xin Tian,[1] Craig M. Crews,[2] Stefan Somlo.[1] *[1]Internal Medicine/Nephrology, Yale University, New Haven, CT; [2]Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT.*

Isolated Autosomal Dominant Polycystic Liver Disease (ADPLD) is characterized by the presence of bile-duct cysts spread throughout the liver parenchyma. *PRKCSH*, one of the genes mutated in ADPLD, encodes the β subunit of the ER glucose-trimming enzyme glucosidase II (GIIβ) which functions in the post-translational processing of integral membrane and secreted glycoproteins. We have previously shown absence of GIIβ results in decreased stability and ciliary trafficking of PC1 and that kidney selective inactivation of *Prkcsh* causes polycystic disease that is made worse by polycystin-1 (PC1) haploinsufficiency and improved by PC1 over-expression. Since loss of GIIβ is expected to increase the burden of misfolded proteins, we tested the effects of proteasome inhibitors in cell and animal models of ADPLD. *Prkcsh*$^{-/-}$ cells treated with the proteasome inhibitors MG132 and carfilzomib showed ~3.5-fold increase in rates of apoptosis as compared to wild-type cells. Additionally, levels of PC1 in *Prkcsh*$^{-/-}$ cells increased in the presence of MG132. We used carfilzomib (5 mg/kg) to treat *Prkcsh*$^{flox/flox}$*;Ksp-Cre;Pkd1*$^{+/-}$ mice from P21 until P42. Treatment resulted in significantly decreased kidney/body weight ratio, cystic index and blood-urea nitrogen levels indicating improvement in the polycystic disease progression. The levels of apoptosis in the DBA positive kidney tubule segments was significantly increased (~2-fold) in the carfilzomib treated mice whereas proliferation was decreased (~1.4-fold). These data suggests that *Prkcsh*$^{-/-}$ cyst cells are more sensitive to proteasomal inhibition leading to enhanced apoptosis. Proteasome inhibition also increased steady state levels of PC1, which may be contribute to the reduced proliferation seen in cyst cells. Proteasome inhibitors are effective in reducing cyst growth in orthologous models of ADPLD and may define a potential target for therapy in human ADPLD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1834**

**Therapeutic Potential of Combination of the Novel Dual PI 3-Kinase/mTOR Inhibitor and MAPK Inhibitor in the Han:SPRD Rat Model of PKD** Yang Liu,[1] Rudolf P. Wuthrich,[1] Andreas L. Serra.[1] *[1]Institute of Physiology, University of Zürich, Zürich, Switzerland; [2]Experimental Infectious Diseases and Cancer Research, University Children's Hospital, Zürich, Switzerland.*

The mTOR and Akt pathway are aberrantly activated in PKD. NVP-BEZ235 is a novel dual PI3K/mTOR inhibitor. We analyzed the the mechanism of NVP-BEZ235 on proliferation and apoptosis in renal primary tubular epithelial cells derived from Han:SPRD rats (Cy/+ TECs).

The effect of BEZ (c= 0.01nM to 1000nM) alone or combination with UO126, a mitogen-activated protein kinases (MAPK) inhibitor (c= 5μM to 25μM), on Cy/+ TECs proliferation, apoptosis, mTORC1, mTORC2 and MAPK signaling pathway was assessed.

BEZ reduced total cell numbers, cell viability and DNA synthesis of Cy/+ TECs without inducing complete inhibition at 1μM, which is the highest concentration tested in these studies. Cleavage fragments of caspase 3 could not be detected and the proportion of PI–/Annexin V+ and PI+/Annexin V+ cells treated with BEZ remained substantially unchanged. The phosphorylation of the downstream effectors of mTORC1 (4EBPThr37/46, S6KThr421/Ser424, S6Ser235/236) were prevented at a concentration lower than the one blocking upstream regulators of mTORC1 (such as AktThr308). BEZ suppressed phosphorylation of Akt at Ser473 site, the readout of mTORC2 activity. Phosphorylation of the MAPK increased (indicating activation) upon BEZ treatment in a dose-dependent way. Combined BEZ with UO126 completely decreased proliferation, inhibited the activation of downstream effectors of mTORC1 and down-regulated the phosphorylation of the MAPK which triggered by BEZ treatment.

BEZ inhibited the proliferation of Cy/+ TECs incompletely and apoptosis was not observed in vitro. A partial lack of sensitive of mTORC1 upstream regulators, as well as an activation of the MAPK signaling pathway occurs in parallel with these effects. Combination treatment of BEZ and UO126 significantly blocked the proliferation of Cy/+ TECs due to inhibit the activation of mTORC1 downstream effectors and MAPK signaling pathway. Our data suggest that combined dual PI3K/mTOR inhibitor with MAPK inhibitor could have a synergistic effect on Cy/+ TECs.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1835**

**Progression of Polycystic Kidney Disease (PKD) in *jck* Mice in the Absence of Cyclin-Dependant Kinase 2 (Cdk2)** Jane S. Burgess,[1] Philipp Kaldis,[2] David C. Harris,[1] Gopala K. Rangan.[1] *[1]Centre for Transplant and Renal Research, Westmead Millennium Institute and The University of Sydney, Sydney, NSW, Australia; [2]Institute of Molecular and Cell Biology, Singapore.*

**Background:** Loss of polycystin function promotes Cdk2 activity and this may mediate the increased proliferation and cyst enlargement in PKD. Here, we tested the hypothesis that the progression of cystic renal disease is reduced by Cdk2 deficiency.

**Methods:** *jck* mice are homozygous for a single point mutation in *Nek8* (a mitotic kinase downstream of polycystin-dependent cell cycle activation) and develop PKD. Compound mice deficient in both $Nek8^{jck}$ and *Cdk2* were compared with $Nek8^{jck/jck}/Cdk2^{+/+}$ mice. $Cdk2^{-/-}$ mice are sterile due to a defect in gonad meiosis, and therefore heterozygous $Nek8^{+/jck}$C57/BL6 mice were crossed with $Cdk2^{+/-}$C57/BL6 mice. Progeny that were $Nek8^{+/jck}/Cdk2^{+/-}$ were then crossed to produce $Nek^{jck/jck}/Cdk2^{-/-}$ and $Nek8^{jck/jck}/Cdk2^{+/+}$ mice. Data from $Nek8^{jck/jck}/Cdk2^{-/-}$(n=6), $Nek8^{jck/jck}/Cdk2^{+/+}$(n=6), and $Nek8^{+/+}/Cdk2^{+/+}$(n=8) was collected at week 12.

**Results:** $Nek8^{jck/jck}/Cdk2^{-/-}$ mice were viable and indistinguishable from their littermates. As expected, ovaries and testes were markedly atrophic in Cdk2 deficient mice. Renal function, as assessed by serum creatinine (Table 1), was worse in $Nek^{jck/jck}/Cdk2^{-/-}$ compared to $Nek8^{jck/jck}/Cdk2^{+/+}$ mice. Kidneys from all $Nek8^{jck/jck}$ groups exhibited PKD. However, the pattern was different, and cysts were more numerous in $Nek8^{jck/jck}/Cdk2^{-/-}$ mice and almost all were round in shape, in contrast to the typical rectangular cystic dilatation present in $Nek8^{jck/jck}/Cdk2^{+/+}$. The %cyst area was higher in the $Nek8^{jck/jck}/Cdk2^{-/-}$ group compared to $Nek8^{jck/jck}/Cdk2^{+/+}$, but this did not reach statistical significance (p=0.14).

Table 1.

| | jck (Cdk2 null) | jck (Cdk2+/+) | Wild-type |
|---|---|---|---|
| BW(g) | 20.5±3.2 | 21.9±4.1 | 22.5±3.2 |
| KW/BW (%) | 4.8 ±3.6* | 3.3 ±3.0* | 0.7±0.1 |
| Serum Cr (μmol/L) | 40.0±27.1# | 17.6±6.1 | 16.7±4.9 |
| Cyst Area (%) | 52.1±13.7 | 40.4± 9.9 | - |

Mean+/-SD; *P<0.05 compared to wild-type; #P<0.05 compared to jck (Cdk2+/+)

**Conclusion:** Cdk2 deficiency unexpectedly exacerbates renal function and also alters the nephron-segment specificity of cyst burden in *jck* mice.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1836**

**Silencing of RAGE Suppresses Cystogenesis in Polycystic Kidney Disease** Jong Hoon Park, Bo Hye Kim, Eun Young Park. *Department of Biological Science, Sookmyung Women's University, Seoul, Korea.*

Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary and chronic kidney disease characterized by cyst formation. ADPKD is developed due to the malfunction of PKD1/2. PKD1 encodes a GPCR-like receptor, and PKD2 produces a transient receptor potential (TRP)-like calcium channel. They are localized on the primary cilia of the renal epithelial cells and form a complex to regulate intracellular [$Ca^{2+}$]. By the fluid flow, they are activated and initiate intracellular signal pathway. In general, ADPKD is caused by loss of function of PC1/2. However, overexpression of PKD1 or PKD2 gives rise to cystogenesis in the kidney. In our laboratory, we generated PKD2 overexpression transgenic mice. These TG mice developed the cyst formation since one year.

To find candidate genes responsible for cyst development in PKD2 TG mice, microarray was performed and S100A8/A9 and RAGE (receptor for advanced glycation end product) were chosen as the interesting genes. In our previous study, qRT-PCR and western blot results said that expression level of RAGE and S100A8/A9 was elevated. Also, immunohistochemistry (IHC) showed strong expression of RAGE and S100A8/A9 on the renal cyst epithelial cells. S100A8/A9-RAGE signaling was involved in inflammation around the cyst, indicating inflammation was the one of factors aggravating ADPKD. On the basis of previous result that treatment of anti-RAGE siRNA and adenovirus to mIMCD cells reduced p-ERK level and suppressed cyst formation on 3D culture, we tried to apply anti-RAGE adenovirus to mice. We have PKD2 knockout mice which were rescued by overexpression of hPKD2. These rescued mice can survive until one month in spite of bearing cyst-filled kidneys. To these mice, anti-RAGE adenoviruses were intravenously injected. After that, improved renal function and reduction in cyst development were detected. Furthermore, both MAPK and NF-kB signaling were silenced.

These data showed that silencing of RAGE suppresses cystogenesis in ADPKD, indicating the value of RAGE as the new therapeutic target of ADPKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1837**

**Lonidamine Derivatives Have Properties Expected of Effective Drugs for Treating ADPKD** Brenda S. Magenheimer,[1] Shirin Sundar,[1] Dar B. Shah,[1] Sumanth Mulamalla,[1] Gail Reif,[1] Darren P. Wallace,[1] Joseph S. Tash,[1] Gunda I. Georg,[2] James P. Calvet.[1] *[1]Univ. of Kansas Medical Center, Kansas City, KS; [2]Univ. of Minnesota, Minneapolis, MN.*

A lead compound for the treatment of ADPKD should effectively block both abnormal cell proliferation and cyst-filling fluid secretion in addition to having favorable safety/toxicity and biodistribution profiles. Previously, we showed that the novel lonidamine derivative JWS 1-190 inhibits cell proliferation and fluid secretion. In this study, we further examined JWS 1-190 and eleven other lonidamine compounds to generate comparative data profiles and to find new compounds to inhibit cyst initiation and/or progression in ADPKD, using cell-based and metanephric organ culture assays. The MTT assay was used to generate dose response curves with primary human ADPKD cells. Nine candidate compounds showed an IC50 at 1-15 μM (IC50 for lonidamine = 5.7 μM). Mouse embryonic organ cultures made use of kidneys at E15.5 from Pkd1m1Bei heterozygous crosses. When E15.5 cultures were treated with 8-Bromo-cAMP in the presence of the compounds for 4 days, several drugs strongly inhibited cyst formation. The compounds that had a low μM IC50 in ADPKD cells were the most effective at inhibiting cyst growth in metanephric kidneys. Fluorescein competition in embryonic kidneys indicated that the drugs may be transported by organic anion transporters and thus may accumulate to high concentrations in kidneys. Short circuit current experiments were conducted on M-1 cell monolayers to determine whether the candidate compounds inhibit CFTR channel activity, which is required for cyst-filling fluid secretion. The bioavailability of JWS 1-190 was determined by administration of 100 mg/kg by i.p. injection, and heart, lungs, kidneys, urine, serum, and testes were harvested at 6 and 24 hrs for mass spectrometry, which showed high amounts of the drug in the kidney at both time points. JWS 1-190 was also shown to significantly ($p < 0.01$) inhibit wound closure in a cell motility scratch assay at 1 μM and 5 μM. We conclude that JWS 1-190 and several other lonidamine derivatives have properties expected of effective therapeutic drugs for ADPKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1838**

**TCF/β-Catenin Activity Is Suppressed Normally in Two Independent Models of ADPKD** Michelle Miller,[1] Diana Iglesias,[2] Stefan Somlo,[3] Gregory G. Germino,[4] Paul R. Goodyer.[1] *[1]Human Genetics, McGill University, Montreal, QC, Canada; [2]Pediatrics, McGill University Health Center, Montreal, QC, Canada; [3]Internal Medicine, Yale University, New Haven, CT; [4]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.*

Autosomal dominant polycystic kidney disease (ADPKD) results from mutations in either the *PKD1* or *PKD2* genes, which encode the cilial proteins PC1 and PC2. In ADPKD, it has been proposed that during kidney development a subset of cells acquire second "hits" to the wildtype allele and cysts are thought to arise when mechanosensory stimuli fail to initiate cilial signalling that normally suppresses the canonical WNT/β-catenin signalling pathway. This hypothesis predicts that cystogenesis is driven by aberrant WNT/β-catenin activity. To test this theory, we crossed a TCF/β-catenin reporter mouse to mice with mutations in *Pkd1* or *Pkd2*. We then assessed the onset of cyst formation relative to the ontogeny of primary cilia and the normal downregulation of TCF/ β-catenin reporter activity.

By E15.5, the primary cilia were present and ureters had connected with the bladder, allowing the onset of flow in normal mice. In *Pkd1* null mice, kidney cysts appeared at E16 but suppression of the TCF/β-catenin reporter was normal. Since the *Pkd1* null mice die at E18, we also studied the *Pkd2/WS25* mice, which carry one null and one hypomorphic allele of the *Pkd2* gene. The *WS25* allele undergoes somatic recombination to form the second null allele, permitting perinatal survival and delaying cyst formation until roughly 2 weeks of age. By 8 weeks, renal cysts are widespread but no activity of the TCF/β-catenin reporter was seen.

In conclusion, suppression of the canonical WNT signalling pathway appears normal in both the *Pkd1* null and *Pkd2/WS25* mouse models. We speculate that the pathogenesis of ADPKD may be driven by a failure to activate non-canonical WNT signalling pathways, however the developmental regulation of WNT/β-catenin signalling activity remains intact.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1839**

**PPARγ Agonists Decrease CFTR Expression in the Apical Membranes of Liver Cysts from the PCK Rat Model of Polycystic Kidney Disease (PKD)** Bonnie L. Blazer-Yost,[1, 3] Jey-Hsin Chen,[2] Vincent H. Gattone.[3] *[1]Biology, Indiana University - Purdue University, Indianapolis, IN; [2]Pathology & Laboratory Medicine, IUSM, Indianapolis, IN; [3]Anatomy and Cell Biology, IUSM, Indianapolis, IN.*

In PKD, cyst growth involves secretion of electrolytes and fluid by the cyst-lining epithelial cells. One mediator of electrolyte/fluid secretion is the $Cl^-$ channel, cystic fibrosis transmembrane-conductance regulator (CFTR). We previously found that PPARγ agonists decrease renal CFTR mRNA and inhibit vasopressin-stimulated $Cl^-$ secretion in MDCK-C7 renal principal cells. In the current studies, we used immunohistochemical techniques to examine expression of CFTR in apical membranes of biliary cysts from PCK rats, an orthologous model of autosomal recessive (AR) PKD. Control (untreated) animals were compared with littermates that had been treated with 20 mg/kg body weight of pioglitazone (Pio), a PPARγ agonist, from weaning (4 weeks) to 18 weeks. We previously showed that this treatment resulted in a decrease in cystic burden in both kidney and liver. To elucidate a mechanism of action of pioglitazone inhibition of cyst growth, apical membrane expression of CFTR in cholangiocytes lining liver cysts were stained, using the monoclonal CFTR antibody, 596, on formalin-fixed paraffin-embedded tissue. In comparison to control animals, the staining intensity for CFTR in apical membranes was diminished in Pio-treated rats. To provide a better quantitative approach, gold-labeled immuno-electron microscopy was used to assess the density of CFTR expression at the apical plasma membrane of cyst cholangiocytes. Sections were subjected to analysis by counting the number of gold particles bound to the surface of the apical membrane and expressed as number per linear μm of apical surface. Cholangiocytes from Pio-treated rats had a statistically significant decrease ($p = 0.009$) in apically-localized immunogold labeled CFTR. The biliary cysts from animals on control diet had an average 1 gold particle per 0.23 μm of apical surface versus 1 per 6.04 μm in Pio-treated rats. Based on these studies, we suggest that PPARγ agonists may be useful for treating PKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1840**

**Kidney-Specific Inactivation of the Tsc1 Gene Reveals a Time Disconnect between mTORC1 Upregulation and Renal Cystogenesis** Monika Pema, Claas Wodarczyk, Marco Chiaravalli, Alessandra Boletta. *Dibit-San Raffaele Hospital, Milan, Italy.*

ADPKD is a genetic disorder characterized by bilateral renal cyst formation, caused by mutations in the PKD1 or PKD2 genes. Their products (the polycystins) function as a complex to prevent cyst formation. Previous studies have suggested that the mTORC1 cascade might play an important role in renal cyst formation and in line with this the mTOR inhibitor rapamycin has beneficial effects in rodent models of PKD. We have recently shown that Polycystin-1 (PC-1) inhibits mTORC1 and its downstream effectors in an ERKs-TSC2 dependent, but Akt-independent manner (Distefano et al,MCB.29:2359,2009).

To better understand the role of mTORC1 activity and the TSC genes in renal cystogenesis, we crossed mice carrying a kidney-specific Cre (Ksp-Cre) with mice harbouring either a Tsc1 floxed or a Pkd1 floxed allele and compared the resulting models (Tsc1kKO and Pkd1kKO, respectively). As previously reported, Pkd1kKO mice develop massive renal cystogenesis starting at P1 and leading to death by P14. Biochemical analysis showed upregulation of mTORC1 and IHC revealed that the majority of cyst-lining epithelia stain positive for P-S6Rp. However, a considerable number of cysts (10-20%) stained negative for P-S6Rp, suggesting that upregulation of mTORC1 might not be necessary for cysts to form.

Interestingly, Tsc1kKO mice survive much longer than the Pkd1kKO (>50 days). Biochemical analysis revealed an upregulation of mTORC1 at P9, P14, P26, P40. Surprisingly, mTORC1 hyperactivation did not correlate with cyst formation or tubular dilatation, since neither can be observed at P9. By P14 a strong expansion of the renal medulla can be appreciated, with only small cortical tubular dilatations appearing. Medullary expansion can be observed also at P56 when massive cystogenesis is also present. Notably, we observed major morphological differences both of whole kidneys and the cystic epithelia between the Pkd1kKO and Tsc1kKO. These data suggest that there is a disconnect between the time of mTORC1 upregulation and renal cystogenesis and highlight a more complex interplay between the Pkd and Tsc genes than anticipated.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1841**

**Effect of Angiotensin Converting Enzyme (ACE) Inhibition and Angiotensin Receptor Blockade on Urinary pH in ADPKD** Amirali Masoumi,[1] Berenice Y. Gitomer,[1] Robert W. Schrier,[1] Wei Wang,[1] Arlene B. Chapman,[2] Ronald D. Perrone,[3] Vicente E. Torres,[4] Theodore I. Steinman,[5] Dana C. Miskulin.[3] *[1]U. Colorado Denver, Aurora, CO; [2]Emory U.; [3]Tufts; [4]Myao Clinic; [5]Beth Israel.*

Autosomal dominant polycystic kidney disease (ADPKD) affects an estimated 600,000 Americans. The prevalence of nephrolithiasis is approximately 5-fold higher in ADPKD patients than in the general population. Approximately 50% of the stone burden is attributed to uric acid stones. The major risk factor for uric acid stone formation in ADPKD appears to be low urine pH (especially urine pH<pH 5.5). Recent studies have shown that angiotensin II stimulates the vacuolar $H^+$-ATPase (V-ATPase) in renal type-A acid secretory intercalated cells. We thus hypothesized that ADPKD patients who were treated with ACE inhibitors (ACEI) or ACEI plus angiotensin receptor blockers (ARB) may have increased urinary pH and thus decreased risk for uric acid formation. The HALT-PKD clinical trial assesses the effect of ACEI vs ACEI + ARB therapy on slowing the progression of renal disease in patients with ADPKD. We measured urine pH in 106 subjects who were enrolled in the HALT-PKD study at the University of Colorado at baseline and at 12 months after initiation of therapy. Among those 106 patients, 20 patients (18.8%) had a urine pH<5.5 (pKa for uric acid) at baseline and 6 patients had previously had a kidney stone. In these 20 patients treatment with ACEI or ACEI + ARB resulted in a significant increase in urine pH (baseline 5.2±0.1 vs 12 month 5.9±0.1, $p<0.0001$). A similar trend was seen for 22 patients with baseline pH between 5.5 and 6.0 (baseline pH 5.7±0.1, 12 month 5.9±0.4, $p=0.06$). However, in patients with baseline pH≥6.0 no effect with therapy was observed (baseline pH 6.5±0.4 vs 12 month 6.2±0.5). There was no significant difference in urine pH between patients whose estimated glomerular filtration rate (eGFR) was>60 ml/min/1.73 $m^2$ and those with eGFR<60 ml/min/1.73 $m^2$. Thus patients with low urinary pH<5.5 may benefit from ACE inhibitor and/or ARB therapy by reduction in risk for uric acid stone formation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1842**

**ADPKD Is Associated with a Central and Peripheral Defect in Osmoregulation** Thien Anh Ho, Yves A. Pirson, Olivier Devuyst. *Nephrology, Universite Catholique de Louvain Medical School, Brussels, Belgium.*

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is associated with an impaired urine concentrating ability (Umax, maximal urine osmolality after water deprivation), attributed to cystic alteration of the renal parenchyma. The existence of a central defect in vasopressin (AVP) release has also been suggested by studies in Pkd1 mouse. In this study we investigated the central and nephrogenic components of osmoregulation in ADPKD patients.

Patients and methods

We enrolled 10 ADPKD adults (44 ± 4 yr) and 10 ADPKD children (11 ± 1 yr) with normal renal function, and 20 age- and sex-matched controls (CTRL). Plasma and urine parameters were determined at baseline and after a 12-hour water deprivation. Total kidney volume was assessed by MRI in ADPKD adults.

Results

At baseline, renal function, plasma and urine osmolality and plasma AVP levels were similar in adult ADPKD and CTRL. After water deprivation, Umax was significantly lower in ADPKD than in CTRL (710 ± 68 vs. 920 ± 47 mOsm/kg $H_2O$). This difference reflects the significant increase (+37%) of plasmatic AVP level in the CTRL group, contrasting with an attenuated variation (+19%, non significant) in the ADPKD group, despite of a significantly higher maximal plasma osmolality (304 ± 1 vs. 300 ± 1 mOsm/kg $H_2O$, ADPKD vs CTRL). ADPKD patients showed a significantly lower Umax than CTRL for a same range of plasma AVP and urinary cyclic adenosine monophosphate (cAMP) levels. There was a significant inverse correlation between Umax and kidney volume. The urine concentrating defect was confirmed in ADPKD children.

Discussion

These results confirm the presence of a urine concentrating defect in early ADPKD. In adults, this defect is proportional to the kidney volume, and is distal to AVP secretion and cAMP production, possibly related to inefficient translocation of aquaporin-2. Furthermore, ADPKD patients show a central defect in osmoregulation, as revealed by the significantly attenuated reactivity of AVP to plasma osmolality.

These alterations of osmoregulation associated to ADPKD have important clinic and therapeutic implications, particularly for the use of aquaretic molecules.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1843**

**Capecitabine Can Be Used in Patients with Severe Renal Impairment (GFR <30CC/Min) and in Patients with End Stage Renal Disease on Hemodialysis**

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Capecitabine use is contraindicated in patients with severe renal impairment (GFR<30ml/min) based on a single Phase II trial, which included only 4 such patients. Retrospective chart review over a 5 year period identified 12 patients with a GFR<30ml/min, including 2 patients on hemodialysis, who received capecitabine.

The usual recommended dose is 1250mg/m$^2$ twice daily, administered orally for 2 weeks, every 21 days. The starting dose used ranged from 225-1100mg/m2 twice daily at varying intervals. In response to reports of AEs, dose reductions of 25- 50% of the starting dose were made, and occurred after completion of the first or second treatment cycle. Grade 2 diarrhea was the most common cause for dose reduction (25%), followed by grade 2 hand and foot syndrome (12%). One patient reported grade 3 diarrhea and one patient died while on treatment with capecitabine. Progression of disease was the most common reason for discontinuation of capecitabine (83%). Of the adverse events reported, fatigue and diarrhea were reported at equal frequencies, and when present, was generally reported as grade 1 in severity. Fifty percent of patients reported hand and foot syndrome; of these, 66% reported grade 1 symptoms.

Serum tumor marker levels and follow up imaging studies were available on 9 patients. Response to capecitabine was documented in 4 patients; stable disease in 2; and disease progression in 3. On average, patients tolerated capecitabine for 8.3 months (range 1-26 months) before requiring therapy change for progression of disease (POD). The 2 patients on HD received capecitabine for 16 and 20 months and had documented response to treatment.

The labeling prohibiting use of the drug in patients with a GFR<30ml/min may be unsupported. With close monitoring of their clinical and chemical data, capecitabine can be safely and administered be effective therapy at dose reductions of 50-80% of the usual starting dose, for patients moderate and severe renal impairment, including patients on hemodialysis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1844**

**The Rasburicase Pilot Study for the Prevention of Acute Kidney Injury in CV Surgery**

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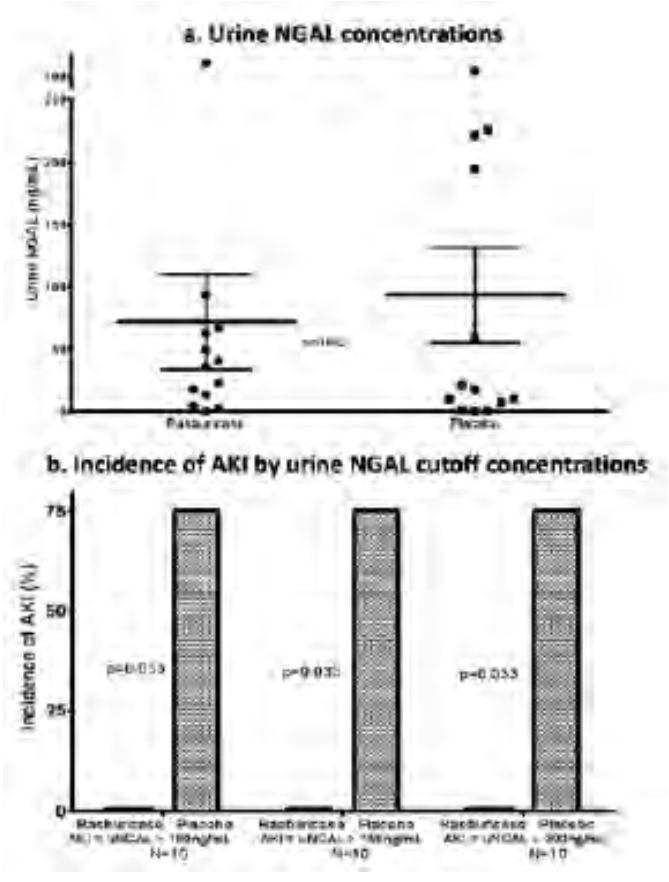
**Background:** Uric acid is an independent risk factor for acute kidney injury (AKI) and adversely affects renal blood flow autoregulation, glomerular filtration rate, inflammation and angiogenesis. The aim of the pilot study was to investigate the effect of lowering uric acid on AKI.

**Method:** In this prospective, double blind, placebo-controlled, randomized pilot trial, participants were randomized to receive rasburicase or placebo in the preoperative period.

**Results:** A convenience sample of 26 patients who underwent cardiac surgery was studied. Rasburicase was associated with a trend towards decreased AKI by urine neutrophil- associated lipocalin (uNGAL) concentrations (Fig.a; rasburicase 72.1±38.1 vs. placebo 93.8±38.1, p=0.692), and this trend persisted in those with higher serum uric acid levels, more severe renal (baseline GFR≤ 45mL/min/1.73m$^2$) and cardiac impairments (left ventricular ejection fraction ≤45%). The use of rasburicase was also associated with a trend towards a decreased incidence of AKI (rasburicase 7.7% vs. placebo 30.8%, p=0.322) by uNGAL cutoff concentrations. In the subset of patients with severely impaired renal function (GFR≤45ml/min/1.73m$^2$), rasburicase was associated with a dramatic 75% reduction in AKI (Fig b; rasburicase 0% vs., placebo 75%, p=0.033).




**Conclusion:** Rasburicase therapy was associated with a trend towards decreased AKI (defined by uNGAL excretion) in high-risk cardiac surgery patients which was significant in patients with GFR≤ 45mL/min/1.73m$^2$.

Disclosure of Financial Relationships: Research Funding: Study supported by an investigator-initiated research grant from Sanofi-Aventis; Patent: I have a patent application related to uric acid lowering therapy in acute kidney injury.

**F-PO1845**

**Prognostic Indicators of Renal Disease Progression in Adults with Fabry Disease: Natural History Data from the Fabry Registry**

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Renal involvement is an important manifestation of Fabry disease, an X-linked metabolic disorder caused by deficiency of a-galactosidase A activity. Data from the Fabry Registry from 462 untreated adults (121 males and 341 females age ≥18 years as of July 2009) who had 2 or more estimated glomerular filtration rate (eGFR) values over a span of ≥12 months before starting enzyme replacement therapy were included in these analyses. Most males (86 of 121, 71%) had more rapid loss of kidney function than the normal adult population (loss of eGFR > -1 mL/min/1.73m$^2$/year), whereas fewer females (133 of 341, 39%) had rapid loss of kidney function. Patients with rapid progression had significantly higher mean averaged urinary protein to urinary creatinine ratios (UP/Cr) than patients with slower progression (1.5 versus 0.2 for males; 1.4 versus 0.5 for females; p<0.0001). Patients were grouped into quartiles based on averaged UP/Cr; renal function in males declined more rapidly with higher UP/Cr, with the steepest declines observed in the quartile of males with UP/Cr >1.5 (mean eGFR slope -5.6 mL/min/1.73m$^2$/year, n=30). eGFR slope declined more slowly in females, with the steepest declines observed in the quartile of females with UP/Cr >1.2 (mean eGFR slope -1.3 mL/min/1.73m$^2$/year, n=85). Regression models of eGFR slope indicated that UP/Cr is the most important indicator of renal disease progression in adult Fabry patients. In addition to UP/Cr, lower baseline eGFR levels and age at baseline were associated with faster renal disease progression in female patients. Cardiac and cerebrovascular events were associated with increased UP/Cr in males but not females. Females who had clinical events had more rapid loss of kidney function. We acknowledge the members of the Fabry Registry Renal Outcomes Workgroup, who made important contributions to this work.

Disclosure of Financial Relationships: Research Funding: Grant from Genzyme lysosomal storage disease to institution

Grant from Amgen to Institution; Honoraria: Genzyme, Mitsubishi, Vifor, Astellas,; Scientific Advisor: European Fabry Registry Board of Advisors, Novartis,.

**F-PO1846**

**Predictors of Mortality in Clostridium Difficile Infected Hospitalized Patients (Pts)** Venkata A. Suda, Lalathaksha Murthy Kumbar, Ashwini M. Shadakshari, Parampreet S. Ghuman, Sara Asadi, Paul A. Fein, Jyotiprakas Chattopadhyay, Morrell M. Avram. *Avram Division of Nephrology, Long Island College Hospital, Brooklyn, NY.*

There is increasing recognition of the importance of Clostridium Difficile (CDI) infection in hospitalized pts though predictors of mortality with this condition are not well studied. The objective of this study was to investigate the various factors predictive of mortality in pts with CDI. We retrospectively collected demographics, clinical, laboratory and outcome data of 142 pts with CDI. Eighteen percent (n=25) of the pts expired. The pts who expired were older than those who survived (77 vs. 61 years, p<0.0001). Admission albumin was lower (p<0.0001) and peak creatinine (p=0.029) was higher in pts who expired. By univariate logistic analysis, age (p=0.001), presence of hypertension (p=0.047), hypercholesterolemia (p=0.03), cardiovascular disease (CVD) (p=0.001), acute kidney injury (AKI) (p=0.004), hypotension (p=0.09), diuretics treatment (p=0.006), pressor treatment (p<0.0001), aminoglycoside treatment (p=0.034), peak creatinine (p=0.064) were predictors of mortality. By multivariate logistic regression analysis controlling for other variables, serum albumin (odds ratio=0.36, p=0.028), AKI (odds ratio=28.3, p=0.011), pressor treatment (odds ratio=9.39, p=0.001) were significant predictors of mortality.

Predictors of mortality: Multivariate logistic regression analysis

| Variables | Odds Ratio | p |
|---|---|---|
| Age (years) | 1.028 | 0.20 |
| Hypertension (Yes vs. No) | 1.64 | 0.60 |
| Hypercholesterolemia (Yes vs. No) | 1.32 | 0.72 |
| CVD (Yes vs. No) | 1.74 | 0.45 |
| AKI (Yes vs. No) | 28.3 | 0.011 |
| Albumin (g/dL) | 0.36 | 0.028 |
| Aminoglycoside (Yes vs. No) | 2.0 | 0.462 |
| Diuretics (Yes vs. No) | 3.31 | 0.088 |
| Hypotension (Yes vs. No) | 1.065 | 0.93 |
| Pressors (Yes vs. No) | 9.39 | 0.001 |

In conclusion, CDI in hospitalized pts is increasingly becoming a challenge. Low albumin, acute kidney injury and severe hemodynamic instability are significant predictors of mortality in hospitalized pts with CDI. Further studies are needed to address earlier recognition and intervention of these risk factors.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1847**

**Screening and Enrolling Children with Vesicoureteral Reflux: Preliminary Data from the RIVUR Trial** Tej K. Mattoo,[1] Myra A. Carpenter,[2] Russell W. Chesney,[3] Marva M. Moxey-Mims.[4] *[1]Children's Hospital of Michigan, Detroit, MI; [2]University of North Carolina, Chapel Hill, NC; [3]University of Tennessee, Memphis, TN; [4]NIDDK/NIH, Bethesda, MD.*

RIVUR is a double-blind placebo-controlled trial of TMP/SMZ prophylaxis in children with vesicoureteral reflux (VUR). Patient enrollment, which started in June 2007, is ongoing at 19 sites in the U.S. Through May 2010, 472 patients (93% female) with a mean age of 20 months (SD: 18; range: 2-71) have been randomized in the study.

Data collection on patient screening started later. Since November 2007, 7134 patients have been screened for the study, of which 365 (5%) have been randomized; 107 additional enrolled patients are not represented on screening logs due to incomplete data collection. The primary referral sources for patient screening are primary care practices (27%), radiology (25%) and urology (24%). Of 6769 patients screened but not randomized, 6245 (92%) were ineligible and 524 (8%) were eligible but did not consent for the study. Failure to meet RIVUR urinary tract infection (UTI) criteria (53%) and/or VUR criteria (67%) were the predominant reasons for ineligibility. Only 15% of screened patients had a non-UTI/VCUG-related reason for exclusion. Among those ineligible for VUR reasons, 56% had no VUR, 41% did not have a VCUG result available, 2% had VUR grade V and 2% failed to meet a VCUG timing criterion. In conclusion, RIVUR investigators are successfully identifying children with VUR and enrolling them following a first or second UTI. Preliminary data through May 2010 indicate that an average of 19.5 children have been screened for each patient recruited, which highlights the challenges faced in patient recruitment for the RIVUR study.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1848**

**Clostridium Difficile Infection in Hospitalized Patients with Chronic Kidney Disease** Muhammad Ali,[1] Nilay Kumar,[1] Gagan Kumar,[1] Shahryar Ahmad,[1] Khurram Nazeer.[2] *[1]MCW, Milwaukee, WI; [2]Bluegrass Kidney, Louisville, KY.*

**Purpose of the Study**: Risk factors for Clostridium Difficile Associated Diarrhea(CDAD) include antibiotic use, age & gastric acid suppression. Studies have indicated End Stage Renal Disease(ESRD) as a risk factor for CDAD. Purpose of our study was to determine association of CDAD with chronic kidney disease(CKD) & ESRD in hospitalized patients using a national database & to determine impact of CDAD on mortality in hospitalized patients with CKD and ESRD.

**Methods**: Retrospective cross sectional design was utilized to analyze the Nationwide Inpatient Sample from year 2007. Patients were identified using ICD-9-CM codes. Patients with a discharge diagnosis of C Diff formed the controls & patients with a discharge diagnosis of either CKD or ESRD along with C Diff formed the cases. Chi Square and Logistic Regression were used for comparing variables & calculating Odds ratios. $\alpha$ was set at 0.05.

**Results**: C. Diff infection was more prevalent in hospitalized patients with CKD (2.2% vs 0.83%) & ESRD (3% vs 0.83%) as compared to controls. Patients with C. Diff & CKD were more likely to fall in the >65 age group (84% vs 67%), whereas C. Diff & ESRD patients were less likely to fall in the >65 age group (59% vs 67%) as compared to controls. Patients with C Diff & ESRD were more likely to be admitted at a teaching institution (56% vs 47% for controls and CKD). Patients with CKD and ESRD were more likely to be males (49% vs 40%). Mortality was higher in C Diff patients with CKD (12% vs 8.2%) and ESRD (16% vs 8.3%). Patients with C Diff and CKD were more likely to die as compared to controls (OR 1.30, 95% CI 1.20-1.41). Hospitalized patients with C Diff and ESRD had higher odds of dying (OR 2.36,95% CI 2.12-2.63) vs. the controls.

**Conclusion**: The prevalence of C Diff is higher in hospitalized patient with CKD and ESRD. Among hospitalized patients who develop C Diff, the odds of dying are higher in patients with CKD and ESRD. Further studies need to be conducted looking at CKD and ESRD as independent risk factors for developing C Diff infection. We recommend vigilance in diagnosis & treatment of C Diff infection among hospitalized patients with CKD and ESRD.

Disclosure of Financial Relationships: nothing to disclose

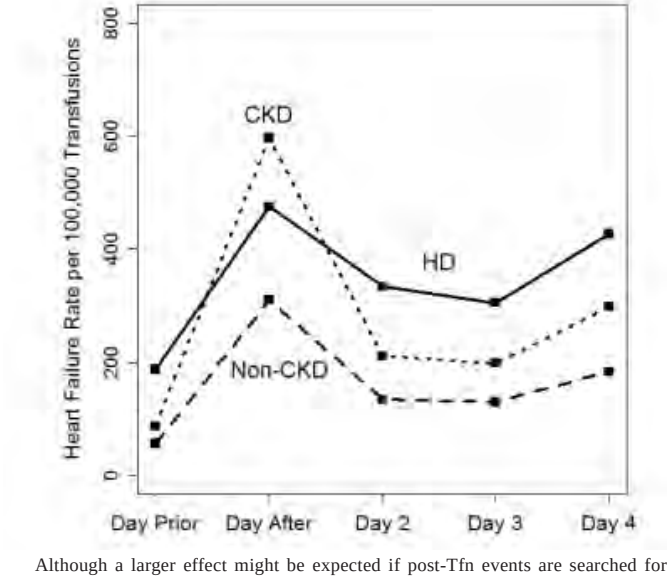
**F-PO1849**

**Transfusion-Related Complications in Kidney Patients** David T. Gilbertson, Haifeng Guo, Tom Arneson, Stephan C. Dunning, Robert N. Foley, Allan J. Collins. *Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN.*

Transfusion (Tfn) avoidance is a major indication for ESA treatment in the dialysis population due to risk of iron overload, immunologic sensitization, fluid overload and hyperkalemia. Little is known about Tfn patterns in the CKD population and the types of complications that may occur beyond blood-borne infections. These data may be important when considering the potential risks and benefits of anemia treatment in the kidney disease population.

Using Medicare data on dialysis, CKD not on dialysis, and non-CKD patients, we searched for outpatient (OP) Tfns and possible adverse events during 2004-2008, including all-cause hospitalizations or ER visits, and cause-specific hospitalizations and ER visits for heart failure, fluid overload, pulmonary edema, and hyperkalemia.

During 2004-2008, there were 45,879, 8,049, and 65,360 OP Tfn events in dialysis, CKD non-dialysis, and non-CKD patients, respectively. The figure shows the heart failure hospitalization or ER visit rate per 100,000 outpatient Tfns the day prior, and days following the Tfn, for dialysis, CKD, and non-CKD Medicare pts. Lower rates of heart failure in HD pts compared to CKD pts may reflect the ongoing use of chronic dialysis in the HD population vs. diuretic use in the CKD population. Other complications also showed patterns of increased risk the day following the Tfn event.



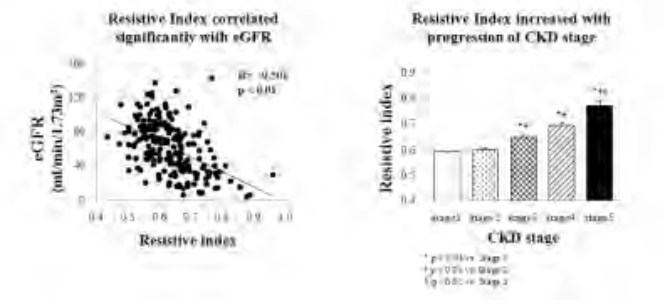

Although a larger effect might be expected if post-Tfn events are searched for immediately after the Tfn, the temporal sequence of Tfns and presumed outcomes on the same day is not available. Thus, the results are most likely a conservative estimate of actual risk. Possible increased risk of adverse events following Tfn suggests that anemia treatment in CKD patients should take into consideration the risks and benefits of Tfns vs. other forms of anemia management.

Disclosure of Financial Relationships: Consultancy: AmgenResearch Funding: NIH, CDC, National Kidney Foundation, Amgen, AMAG, Bristol-Myers Squibb, DaVita, Fresenius, NxStage, Sigma Tau, Genzyme, Takeda.

**F-PO1850**

**Resistive Index Predicts Histological Damage and eGFR in CKD Patients**
Kikuno Hanamura, Akihiro Tojo, Satoshi Kinugasa, Kensuke Asaba, Toshiro Fujita. *Division of Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan.*

**Purpose:** We evaluated whether renal function and histological changes could be predicted non-invasively by ultrasonography in chronic kidney disease (CKD) patients. **Method:** 202 CKD patients diagnosed with renal biopsy from 1999 to 2010 in our department were examined, including 81 patients with IgA nephropathy, 26 with FSGS, 24 with membranous nephropathy, 24 with MCNS, and 10 with diabetic nephropathy. Renal length, cortex area, volume, and resistive index were measured with ultrasonography. Renal biopsy samples were evaluated for the severity of glomerulosclerosis, arteriosclerosis and tubulointerstitial damages, using glomerulosclerosis (GS) score, arteriosclerosis (AS) score and tubulointerstitial damage (TI) score, a five-level scoring system. **Results:** Renal length, cortex area, and volume did not change until CKD stage 5, showing poor correlation with morphological scores. Renal function correlated better with TI score (R= -0.607 p<0.01) than with GS (R= -0.521 p<0.01) and AS scores (R= -0.528 p<0.01). Resistive index by Doppler ultrasonography showed a significant correlation with renal function (R= -0.501 p<0.01), and it increased with progression of CKD stage. Resistive index also correlated better with TI score than with GS and AS scores. **Conclusion:** Resistive index by Doppler ultrasonography could be a useful non-invasive tool to predict renal function and histological lesions in accordance with CKD stage.




Disclosure of Financial Relationships: nothing to disclose

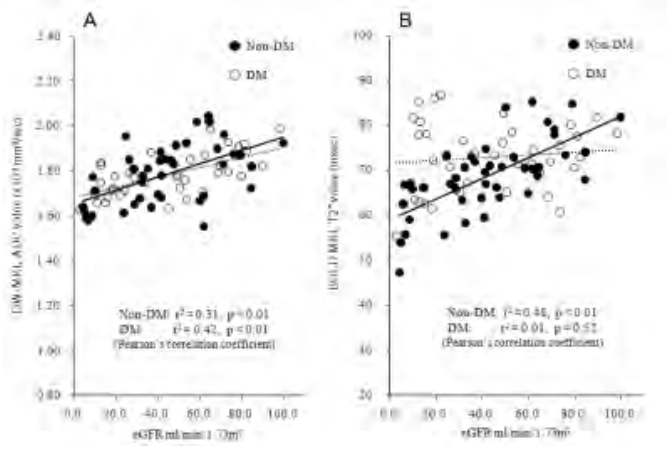
**F-PO1851**

**Different Contribution Ratio of Hypoxia to Renal Insufficiency, Comparison between Diabetic and Non-Diabetic Nephropathy**
Tsutomu Inoue,[1] Eito Kozawa,[2] Hirokazu Okada,[1] Kouichi Inukai,[3] Tsuneo Takenaka,[1] Yusuke Watanabe,[1] Hiromichi Suzuki.[1] *[1]Nephrology, Saitama Medical University, Iruma-gun, Saitama, Japan; [2]Diagnostic Radiology, Saitama Medical University International Medical Center, Hidaka, Saitama, Japan; [3]Endocrinology and Diabetes, Saitama Medical University, Iruma-gun, Saitama, Japan.*

Evaluating the degree of renal parenchymal fibrosis and hypoxia in patients with chronic kidney disease (CKD) has remained challenging. Recent advances in magnetic resonance imaging (MRI), however, have made it possible to visualize these processes in situ. In the present study, we investigated the feasibility of using two different MRI techniques, namely, diffusion-weighted (DW)-MRI and blood oxygen level-dependent (BOLD)-MRI, to evaluate kidney function and reveal renal parenchymal hypoxia and tubulointerstitial fibrosis, respectively.

A total of 84 patients (54 males and 30 females) presenting with CKD, and a control group of 10 healthy volunteers (5 males and 5 females), were recruited. All subjects were subjected to MRI, and laboratory tests were performed. The eGFR was calculated using the modification of diet in renal disease (MDRD) formula.

The decreased eGFR was accompanied by reduced ADC (apparent diffusion coefficients) values of DW-MRI (graph A). BOLD-MRI demonstrated hypoxic conditions, as decreased T2* values, in patients with advanced renal dysfunction in non-diabetic nephropathy patients, but not in patients with diabetic nephropathy (graph B).




Our results demonstrated that eGFR was reflected by the ADC values from DW-MRI, independent of the primary disease. The BOLD-MRI T2* values revealed a hypoxic condition in the kidney associated with advanced renal dysfunction in patients with non-diabetic nephropathy. Functional MRI could contribute to a multilateral assessment of CKD in vivo, non-invasively and repeatedly.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1852**

**Factors Associated with Publication of Randomized Controlled Trials Presented at a Nephrology Conference**
Ranjani N. Moorthi,[1] Navdeep Tangri,[2] Martin Wagner,[3] Ahsan Alam.[4] *[1]Indiana University School of Medicine; [2]Tufts Medical Center; [3]University Hospital of Würzburg, Germany; [4]McGill University, Canada.*

Randomized controlled trials (RCTs) remain the gold standard for interventions in medicine; however there are fewer RCTs in Nephrology than in any other subspecialty of Internal Medicine. Given that evidence for many interventions in Nephrology is lacking, it would be important to determine publication rates of RCT data presented at national conferences and the characteristics of studies that are associated with their publication.

Methods: All abstracts submitted to the American Society of Nephrology (ASN) 2005 meeting reporting RCT data were included. Exclusion criteria were interim data and secondary safety endpoint analyses. Univariate logistic regression was used to compare characteristics between published RCTs and those not published.

Results: Seventy three completed RCTs were presented out of 4280 abstracts. Fifty three % of these were published; median time to publication (start date June 2005) was 24 months (IQR 13,36). Oral presentations were published more frequently (OR 3.66, p=0.01) as well as those with stated industry funding (OR 2.92, p=0.03) and larger sample sizes (OR2.1, p=0.01). Blinded RCTs had approximately 4 times the odds of being published. Only 45% of abstracts reported blinding and more than one third did not clearly state a primary outcome.

Conclusions: There were major differences in quality of abstract reporting for RCTs presented at the ASN 2005 meeting. Almost 50% of RCTs were not published up to 5 years later. Performing an RCT is often expensive and difficult, but if well-conducted they enhance the body of evidence needed to care for patients. It is important to identify characteristics associated with non-publication, to help us perform and report our studies better.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1853**

**Are Quarterly Serum Aluminum Levels Useful?**
David N. Djebali, Steven D. Smith, Germaine Z. Chan. *Division of Nephrology, St. Luke's-Roosevelt Hospital Center, New York, NY.*

**Background**: The incidence of abnormal aluminum levels in dialysis patients in prior studies has been reported to be as low as 2.1%. This calls into question the value of quarterly serum aluminum measurement as recommended by the KDOQI guidelines. However, there is concern that drugs such as iron dextrose, which contains small amount of aluminum, may be a cause of aluminum burden in the dialysis population.

**Methods**: We retrospectively analyzed the serum aluminum levels of all patients at our dialysis center in New York City between 01/01/2002 and 12/31/2009. Beginning on 01/01/2009, the intravenous iron supplementation agent used at our center was changed from sodium ferric gluconate to iron sucrose. 122 patients received 100mg iron sucrose load (1630 µg aluminum per load) in 2009.

**Results:** The table 1 illustrates the total number of aluminum levels checked per year and the number (percentage) of abnormal levels in 589 patients. Of the total 5674 aluminum levels, 32 levels (0.5%) were equal or above 20 µg/L in 25 patients.

These abnormal levels were sustained - 2 and 3 consecutive levels - for 4 and 1 patients respectively. The other elevated levels were isolated.

After a review of the medical records of the 25 patients, we identified a cause in only two patients (aluminum antacids).

None of the 122 patients (53.7 % of our patient population in 2009) who received iron sucrose load had an elevated aluminum level.

**Conclusion**: Our data show that the number of abnormal serum aluminum levels is exceedingly low. The routine quarterly measurement of serum aluminum level may no longer be necessary.

Results of the study

| Year | Number of Aluminum levels | Number of abnormal Aluminum level (%) |
|---|---|---|
| 2002 | 506 | 9 (1.7%) |
| 2003 | 780 | 6 (0.7%) |
| 2004 | 748 | 6 (0.8%) |
| 2005 | 577 | 4 (0.6%) |
| 2006 | 778 | 0 |
| 2007 | 766 | 6 (0.7%) |
| 2008 | 766 | 1 (0.1%) |
| 2009 | 753 | 0 |

Disclosure of Financial Relationships: nothing to disclose

## F-PO1854

### Declining Mortality in Children with Diarrhea Associated Hemolytic Uremic Syndrome – A Single Centre Experience

Melissa Morgunov, Lorraine A. Hamiwka, Susan M. Samuel, Silviu Grisaru. *Pediatric Nephrology, Alberta Children's Hospital, University of Calgary, Calgary, AB, Canada.*

Diarrhea associated hemolytic uremic syndrome (D+HUS) is a leading cause of childhood acute renal failure and is frequently associated with severe morbidity involving other systems. Reported mortality rates associated with this disease declined over the years since it was first described currently ranging from 2.5% to 5%. Central nervous system (CNS) involvement is reported in approximately 30% of cases and is considered the leading cause of mortality. Our objective was to define the mortality rate and outcome of CNS complications associated with D+HUS in our centre.

We identified and confirmed by chart review 123 cases of childhood D+HUS managed at our centre from April 1994 onwards. During this period, patients with D+HUS were offered only supportive therapy including primarily peritoneal dialysis.

There was one death recorded among the 123 reviewed cases ( mortality rate 0.8%) while in a previously published cohort from our centre describing 104 children with HUS presenting between 1980 and 1992 there were 4 deaths (mortality rate 3.8%). Among the reviewed 123 cases of D+HUS, 17(14%) had CNS complications ranging from severe lethargy to seizures, stroke and coma. Five of these cases had a more severe course requiring intubation and admission to the intensive care unit; two developed hemiparesis. All five had evidence of diffuse brain lesions resembling micro-infarcts, particularly in the basal ganglia. Sixteen of these patients including the severe cases eventually recovered with favorable neurological outcomes. The single case of death occurred in a patient who had seizures however the death was attributed to bowel necrosis and sepsis.

We conclude that despite the lack of specific therapy for D+HUS, in our centre the mortality has declined over the last 30 years to the lowest rate reported so far. In addition, in our experience CNS complications secondary to D+HUS had good outcomes and were not the major cause of mortality.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1855

### Hemodynamic Factors Associated with Proteinuria in the Chronic Renal Insufficiency Cohort (CRIC) Study Population

Matthew R. Weir,[1] Raymond R. Townsend,[2] Jeffrey C. Fink,[1] Valerie L. Teal,[2] Marshall M. Joffe.[2] *[1]University of Maryland School of Medicine; [2]University of Pennsylvania.*

**Background:** Proteinuria is associated with the risk of adverse renal and cardiovascular outcomes in patients with chronic kidney disease (CKD). Brachial artery blood pressure has been shown to be a determinant of proteinuria. The relationship between proteinuria and measures of vascular stiffness or central aortic pressure is not known.

**Study Design:** Cross-sectional

**Setting and Participants:** Baseline data from the Chronic Renal Insufficiency Cohort (CRIC) Study was analyzed in 2,129 patients with estimated GFR (eGFR) 20-70 ml/min/1.73m2 at study entry.

**Outcomes:** 24 hour log transformed proteinuria among persons with (DM) and without (non-DM) diabetes.

**Measurements:** Baseline characteristics including demographics, body mass index, waistline, weight, fat-free mass, smoking history, use of ACE/ARB, and systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), along with central measures of vascular stiffness (compliance) including central systolic blood pressure(CSBP), central pulse pressure (CPP), and pulse wave velocity (PWV).

**Methods:** A step-wise multivariate regression model of the log transformed 24 hour urine protein was utilized. The final model included: age, gender, ethnicity, eGFR, smoking, waistline, SBP, PWV and CSBP. Data in the table are described as a beta coefficient.

**Results:** Increasing eGFR was associated with less proteinuria across all participants. Brachial SBP was associated with more proteinuria, especially in non-DM. However, in DM, increasing vascular stiffness (PWV) was associated with more proteinuria in contradistinction to non-DM. CSBP was associated with (p=.053) increased proteinuria in DM, unlike non-DM.

| | Non-DM | DM |
|---|---|---|
| Estimated GFR/10 ml/min/1.73m2 | -.38 /< .0001 | -.38 /< .0001 |
| SBP/10 mmHg | .13 /< .0001 | .11 / .04 |
| PWV/m2 | .02 / NS | .08 /< .0001 |
| CSBP/10 mmHg | .01 / NS | .11 / .053 |

**Conclusions:** In patients with CKD and DM, vascular stiffness and CSBP correlated with the level of proteinuria. This pattern was not evident in non-DM.

Disclosure of Financial Relationships: Honoraria: Less than $10,000/year for each listed industry entity; Scientific Advisor: Amgen, Nicox, Novartis, Daichi-Sankyo.

## F-PO1856

### Effect of Renin Angiotensin Aldosterone System Blockade Therapy on Incidence of Contrast Induced Nephropathy

Christin M. Spatz,[1] Apurva Lapsiwala,[1] Amin Parhizgar,[2] Lawand A. Saadulla,[1] Umar Farooq,[1] Nasrollah Ghahramani.[1] *[1]Nephrology, Hershey Medical Center, Penn State, Hershey, PA; [2]Internal Medicine, Hershey Medical Center, Penn State, Hershey, PA.*

BACKGROUND: Contrast induced nephropathy (CIN) is an iatrogenic disorder resulting from exposure to contrast media. The renal hemodynamic and direct cytotoxic effects are evident in its pathogenesis, whereas other mechanisms are still poorly understood. The incidence of CIN ranges between 10 – 50% depending on the presence of risk factors, with CKD being a major risk factor. It is not clear whether medications that block Renin Angiotensin Aldosterone System (RAAS) have any impact on CIN.

METHODS: We performed a retrospective study looking at the incidence of CIN in patients with CKD Stage 3 or 4 (January 1, 2007 – December 31, 2008) who were either on or off RAAS blockade therapy at the time of coronary angiogram. Serum creatinines were followed for five days post procedure.

RESULTS: 398 patients with CKD Stage 3 or 4 had a coronary angiogram between January 1, 2007 and December 31, 2008. 220 patients were excluded due to lack of sufficient follow up data. 178 patients were included in the study. 62 patients (35%) were on ACE Inhibitors, 12 patients (7%) were on ARB and 2 patients (1%) were on combination of ACE Inhibitors and ARB. Mean age 71.3 ± 10.7, estimated GFR 44 ± 11.5 and DM 108 (61%). The 5 day relative risk of acute renal failure with ACE inhibitors was 1.17 (95% CI: 0.57 to 2.42; p=0.66) and 0.50 (95% CI: 0.07 to 3.37; p=0.48) with ARB.

CONCLUSION: Patients treated with RAAS blockade therapy before contrast exposure did not have increased incidence of CIN.

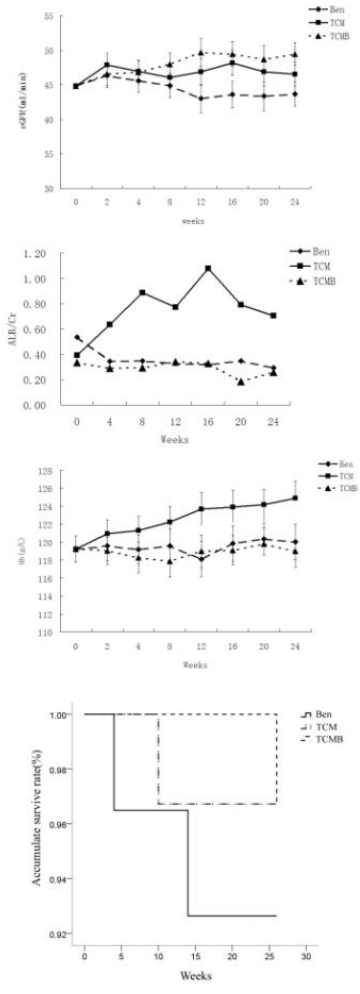
Disclosure of Financial Relationships: nothing to disclose

## F-PO1857

### Effects of Optimized Project of Traditional Chinese Medicine by Differentiation of Symptoms in Treating Chronic Glomerulonephritis in CKD Stage 3: A Randomized Double Blinded Controlled Trials

Yongjun Wang, Ying Lu, Bin Zhu, Xiao Tu, Hongyu Chen. *Department of Nephrology, Guang Xing Hospital, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China.*

**Objective.** To study the effects and safety of optimized project of traditional chinese medicine(TCM) by differentiation of symptoms in treating primary chronic glomerulonephritis in CKD stage 3 using an randomized double blinded controlled (RCT) method. **Methods.** 96 patients with CKD 3 stage primary glomerulonephritis were randomly assigned to three groups: Patients received TCM (TCM group), 10mg/d benazepril (Ben group), traditional Chinese medicine combined with 10mg/d benazepril (TCMB group). The differentiation of Chinese medicine was divided into four patterns assessed using a TCM measuring scale. eGFR, UAlb/Cr, Hb were measured. The primary endpoint was time to the composite of 50% increased of serum creatinine. **Results:** At week 24, eGFR in TCM group (46.56±1.70ml/min) was increased as compared with Ben group (43.65±1.76ml/min)(P>0.05). eGFR in TCMB group (49.48±1.67ml/min) was higher than TCM group (P>0.05) and Ben group(P<0.05). UAlb/Cr in Ben group(median:0.30 g/gcr)and TCMB group(median:0.26g/gcr) was lower than TCM group(median:0.70g/gcr) (P<0.05). UAlb/Cr on TCMB group was lower than Ben group (P>0.05). Hb in TCM group (127.49±1.21 g/L) was increased significantly compared with Ben(124.00±1.19 g/L)and TCMB group(123.78±1.13 g/L)(P<0.05). The accumulative survive rate in TCMB group was higher than the other 2 groups. Patients with hyperkalemia in TCMB group was more than the other 2 groups(P<0.05). **Conclusion:** This was the first RCT evaluating the effects of TCM in CKD stage 3 patients. TCM can improve eGFR and Hb. TCM integrated with benazepril can improve eGFR and decrease proteinuria significantly as compared with either TCM or Ben group. RCT registration code: ChiCTR-TRC-00000204.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1858**

**Osmotic Nephrosis-Like Lesions after Hydroxyethylstarch (HES 130/0,4) Administration: Detection of HES in Renal Tubules by Raman Microscopy** Vincent Vuiblet,[1] Olivier Piot,[2] Alain Wynckel,[1] Tran Nguyen,[2] Michel Manfait,[2] Philippe Birembaut,[3] Philippe Rieu.[1] *[1]Nephrology, University Hospital, Reims, France; [2]UMR 6237, CNRS, Reims, France, Metropolitan; [3]Anatomo-Pathology, University Hospital, Reims, France.*

We observed two patients with persistent impaired renal function after post-ischemic acute renal failure. Renal biopsies of these 2 patients showed osmotic nephrosis-like lesions (ONLS). Hypovolemia and the administration of 3rd generation hydroxyethylstarch (HES 130/0,4) were the only factor we found to explain the ONLS. We therefore tried to identified by Raman microscopy the presence of HES in the kidneys of these two patients.

Methods: Raman spectrum was obtained for the pure commercial solution of HES 130/0,4. Raman spectral images acquired from renal biopsies of both patients and from control renal biopsies (normal kidney, amyloidosis , diabetes, chronic tubulo-interstitial renal disease) were examined to find out the HES spectral "fingerprints". Raman spectral images analysis were performed in a blinded fashion.

Results : Raman signal of HES 130/0.4 showed a characteristic spike at 480 cm-1 which was also found among the spectral images of tubular sections of the kidney with ONLS but not in control renal biopsies.

Discussion: While the renal consequence of the 1st and 2nd generation HES is well known, that of the 3rd generation HES remains controversial. A recent work performed in animal models finds ONLS after injection of 3rd generation HES. Our two observations show that renal impairment and ONLS may also occur in human after 3rd generation HES administration. HES is assumed to be present in empty-appearing intracytoplasmic vacuoles on light microscopy. However, HES deposition in kidney has never been demonstrated.

Conclusion: Our results obtained by Raman microscopy show for the first time that osmotic nephrosis like lesions are associated with the presence of HES in renal tubules.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1859**

**Association of Urinary Arsenic Excretion with Albuminuria in a Low Level Arsenic Exposure Population** Ernesto Sabath,[1] Itzel Aviles-Romo,[1] Diana Isabel Montero-Perea,[1] Hebert Hernandez-Montiel,[1] Ivan Perez-Maldonado,[2] Ma. Ludivina Robles-Osorio.[1] *[1]Department of Nephrology, Universidad Autonoma de Queretaro, Queretaro, Mexico; [2]Facultad de Medicina, Universidad Autonoma de San Luis Potosi, San Luis Potosi, Mexico.*

Chronic arsenic toxicity has become a human health threat and several epidemiologic studies had shown that chronic exposure is associated with type 2 diabetes mellitus, peripheral vascular disease and arterial hypertension; however its effects on kidney function have been relatively unexplored. Therefore we evaluate the association of urinary arsenic excretion with renal function measures and urinary protein excretion in a non-endemic arsenic exposure population.

We enrolled 180 adults with GFR >60 ml/min living in Central Mexico, 38% with type 2 diabetes. Age of patients at baseline ranged from 26 to 73 years and BMI was 28.5 ± 5.2. Spot urine samples for arsenic analysis were collected in arsenic free-containers and there was no difference in the urinary arsenic excretion between diabetic (18.94 µg/L) and non-diabetic (23.5 µg/L) persons. Micro or macroalbuminuria was found in 50% of the diabetic patients and mean HbA1c was 9.1% (4.8-14%). Univariate correlations analyzed with Pearson coefficient showed that urinary arsenic excretion was directly correlated with urinary albumin excretion in the diabetic ($r=0.28$ $p= <0.05$) but not in the non-diabetic ($r=0.05$) population. There was no correlation between urinary arsenic excretion and age, sex, educational status, uric acid, HbA1c and calculated GFR. Univariate analysis showed urinary arsenic excretion as independent risk factor for urinary albumin excretion in diabetic population ($p=<0.05$). Currently we are measuring α1-microglobulin levels as a marker of tubular injury to look for correlation with arsenic excretion in this population.

In conclusion this findings show no evidence of increased risk of diabetes in this population but our results suggests that even low urinary arsenic levels may be considered as a risk factor for urinary albumin excretion in diabetic persons with low level arsenic exposure.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1860**

**Prevalence and Magnitude of Nephrotoxicity by Iron Chelator Deferasirox; a Single Center Experience** Minako Shimazaki, Masahiko Yazawa, Yugo Shibagaki, Kenjiro Kimura. *Division of Nephrology and Hypertension, St. Marianna University Hospital, Kawasaki, Kanagawa, Japan.*

[Background] Deferasirox (DFX) is a novel oral iron chelator used to treat iron overload. Pre-marketing clinical trials revealed a few cases of kidney dysfunction and electrolyte disturbance but its prevalence in practice was not well studied. We recently experienced acute kidney injury with Fanconi syndrome in a patient receiving DFX, which resolved completely after drug withdrawal.

[Purpose and Method] To elucidate the prevalence and magnitude of nephrotoxicity induced by DFX, we reviewed charts of our prevalent patients who were receiving DFX and without significant kidney dysfunction [serum creatinine (SCr) above 2 mg/dL] before taking DFX in the outpatient clinic at the St. Marianna University Hospital. We retrospectively analyzed renal function, urine dipstick in these patients.

[Result] All the 10 prevalent patients who received DFX and without severe kidney dysfunction agreed to the consent. Average age was 64.9±20.4 year-old, 6 were men. Average period of taking DFX was 7.9 months (1.5-14), and 3 had to discontinue because of rising SCr during observation. Absolute elevation of SCr level after taking DFX until the last clinic visits during observation was not significant (0.16±0.23 mg/dL, P=0.08), but absolute elevation of SCr after taking DFX to maximal level of Scr was significant (0.26±0.15, P<0.01), indicating transient reduction of kidney function. Urine dipstick test was obtained in 4 out of 10 study patients. Urinary protein and/or urinary glucose turned positive in all the 4 patients after taking DFX. One patient was diagnosed as Fanconi syndrome after additional tests (presence of normal anion gap acidosis, hypophosphatemia and hypouricemia due to excess urinary excretion). In all the 3 patients who discontinued DFX during observation period, kidney function improved and urine protein and glucose disappeared immediately after discontinuation.

[Conclusion] Although mostly transient and mild, deferasirox-induced nephrotoxicity was prevalent. Careful monitoring of renal function, urinalysis, and electrolytes are necessary.

Disclosure of Financial Relationships: nothing to disclose

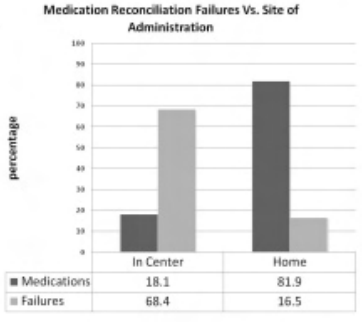
**F-PO1861**

**Medication Reconciliation in the End Stage Renal Disease Population (MrESRD)** Vijay Lapsia,[1] Ronald I. Shorr.[2] *[1]Medicine, University of Florida, Gainesville; [2]Geriatrics, Malcom Randall VAMC, Gainesville, FL.*

Medication reconciliation, comparing a patient's medication (RX) orders to RX a patient is taking, is recommended at every transition of care to reduce errors. Because they also receive in-center RX (ICRX), it is plausible that patients with End Stage Renal Disease (ESRD) on hemodialysis are at especially high risk for reconciliation failure, but this has not been well studied. **Methods**: Retrospective chart review of 75 patients with ESRD consecutively admitted at a University Hospital. Four sources of RX were ascertained: 1) home RX list, 2) hospital admission RX orders, 3) hospital discharge RX list, and 4) RX list maintained by the chronic dialysis unit. The RX list obtained from the chronic dialysis unit was presumed to be the "gold standard" for the purposes of reconciliation. If a RX was present *either* in the home RX list, hospital admission or discharge orders, *and* the chronic

dialysis unit RX list the RX was assumed to be reconciled. **Results:** The 75 patients (age 57 ± 13.8 yrs) were referred from 18 chronic dialysis units. Patient charactersitics are shown in figure. Patients were prescribed 11.3 ± 3.4 RX, of which 2 ± 1 were ICRX. Of these 75 patients, only 17 (22.7%) had perfect reconciliation. Reconciliation failure was most common in ICRX (Erythropoietin stimulating agents and vitamin D analogues) – see figure. Preliminary analysis revealed that neither patient demographics, dialysis unit, Medicare status, time of admission, elective admission, provider history, presence of dialysis records in the hospital chart, previous admission or type of dialysis were associated with reconciliation. **Conclusion:** Medication reconciliation among ESRD patients admitted to hospitals is poor, especially among medications administered in center. The health outcomes of poor medication reconciliation among hospitalized patients with ESRD are not yet known.

| Patient Characteristic | No | (%) |
|---|---|---|
| Female - Sex | 35 | (46.7) |
| Black - Race | 42 | (56) |
| Medicare Status | 47 | (62.3) |
| Dialysis records in hospital chart | 6 | (8) |
| Provider history | 18 | (24) |
| Elective Admission | 15 | (20) |
| Admission time (business hours) | 41 | (54.7) |
| Previous Adm | 53 | (70.7) |




Disclosure of Financial Relationships: nothing to disclose

**F-PO1862**

**Colestilan (MCI-196), a New Calcium-Free Phosphate Binder, Is Safe and Effective in Stage 5 CKD Dialysis Patients: Phase 3, Randomized, Double-Blind (DB), Placebo (PBO)-Controlled, Multicenter Study** Joachim E. Hertel,[1] Shigekazu Nakajima,[2] Hiroyuki Sano,[2] Forunato F. Senatore,[3] Dawn Buchanan.[3] *[1]Nephrology Associates, PC, Augusta, GA; [2]Mitsubishi Tanabe Pharma Corp., Tokyo, Japan; [3]Mitsubishi Tanabe Pharma Development America, Inc., Warren, NJ.*

**Objective**: Demonstrate superiority of colestilan (COL), a noncalcium-based, anion exchange resin phosphate (P) binder, over PBO in serum P control in stage 5 chronic kidney disease (CKD) pts on dialysis. Secondary measures: LDL-C, parathyroid hormone (PTH), calcium (Ca), Ca x P product, A1C, and safety/tolerability of COL.

**Methods:** After a 1-5 wk P-binder washout, pts received open-label (OL) COL (3-15 g/d; wkly flexible dose-titration to target P 3.5-5.5 mg/dL). After 12 wks, pts were randomized to COL or PBO for a 4-wk DB withdrawal period.

**Results:** Primary analysis demonstrated a statistically significant difference between COL (n=85) and PBO (n=83) in mean P of –1.01 mg/dL from wks 12 to 16 (or LOCF; *P*<.001). Statistically significant reductions were observed in P from baseline (wk 0) to wk 12 of –1.54 mg/dL with COL (*P*<.001). Pts with a baseline P ≥7.5 mg/dL (mean 8.76 mg/dL) showed a mean decrease of –2.41 mg/dL. During the OL phase, COL reduced mean LDL-C by 30.1% and mean A1C by –0.91% in pts with a baseline A1C ≥7% (mean 8.5%). Compared with PBO, COL significantly controlled LDL-C (mean difference, –51.9 mg/dL, *P*<.001), Ca x P (–7.91 mg/dL, *P*<.001), PTH (–66.6 pg/mL, *P*=.014), and A1C (mean difference, –0.28%; *P*=.002) with no difference in Ca (0.13 mg/dL, *P*=NS). Most frequently reported treatment-emergent AEs (%): during OL phase included nausea (9), diarrhea (9), vomiting (7.8), and constipation (6.1) and during DB phase (COL and PBO groups, respectively) were nausea (4.7 vs 0), diarrhea (3.5 vs 3.6), and vomiting (3.5 vs 0). Of 3 pts in the COL group who reported GI bleeds, 2 had a previous history.

**Conclusions:** COL was generally safe, well tolerated, and demonstrated superior efficacy versus PBO in P control in stage 5 CKD pts on dialysis with no impact on Ca levels. COL also demonstrated clinically relevant reductions from baseline in Ca x P and significantly reduced LDL-C and A1C.

Disclosure of Financial Relationships: Employer: Nephrology Associations, PCResearch Funding: Amgen, AMAG, Mitsubishi, Affymax, Luitpold, Rockwell, Roche; Honoraria: Amgen, AMAG.

**F-PO1863**

**Patient and Family Engagement in Informed Decision-Making about Renal Replacement Therapy (RRT) Initiation** Johanna Sheu,[1] Patti Ephraim,[1] Raquel Greer,[1] Tanjala S. Purnell,[1] Neil R. Powe,[2] Hamid Rabb,[1] Deidra C. Crews,[1] Bernard G. Jaar,[1] L. Ebony Boulware.[1] *[1]Johns Hopkins Medical Institutions; [2]University of California San Francisco.*

Background: Patient and family engagement in informed decision-making about initiating RRT is poorly described.

Methods: To develop a culturally-sensitive intervention to improve decision-making about RRT initiation, we performed 13 focus groups (optimal size=7-10) of patients with ESRD and their family members to elicit their prior experiences with RRT decisions. Patient and family groups (held separately) were stratified by race (African American (AA) or non-African American (non-AA)) and current RRT modality. We asked groups questions to assess: a) how sick patients were when they [or their family members] first learned about patients' initial RRT modality, b) if they had enough time to choose among modalities, c) if they learned about other modalities, and d) if they understood patients' initial modality prior to initiation.

Results: Patient and family groups (N=50 and 43) had experiences with initiating hemodialysis (HD)- (7 and 7 AA; 8 and 8 non-AA), peritoneal dialysis- (9 and 7 AA; 4 and 3 non-AA), and transplant (TP)- (11 and 9 AA; 11 and 11 non-AA, respectively). Over 1,400 statements were made during group sessions. Many patients in all groups initiated RRT on HD. Patients were often very sick at the time of RRT initiation. Both patients and families felt there was "not enough time to make a decision" about what form of RRT to initiate. They also often reported they "did not know about or weren't given another option or choice" about alternative treatment options prior to initiation or felt "rushed into" HD. Many patients who had received TP reported learning about TP while on HD, during which "there was more time to make decisions". Group discussions were similar among AA and non-AA groups.

Conclusions: Sickness, lack of time, and poor knowledge of various modalities at the initiation of RRT may limit patients' ability to make informed decisions regarding treatment options. Better patient education could improve patients' access to optimal therapies, such as early transplant.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1864**

**The Effects of Diet and Sodium Intake on Racial Differences in Plasma Renin Activity: Results from the Dietary Approaches To Stop Hypertension (DASH)-Sodium Trial** Sharon Turban,[1] Edgar R. Miller,[2] Mark Woodward,[3, 2] Lawrence J. Appel.[2] *[1]Nephrology, Johns Hopkins University, Baltimore, MD; [2]Internal Medicine and Epidemiology, Johns Hopkins University, Baltimore, MD; [3]Epidemiology and Biostatistics, University of Sydney, Australia.*

Blacks typically have lower plasma renin activity (PRA) than whites. The mechanisms for this racial difference are unknown. The lower potassium (K) intake of blacks may at least partially explain this racial difference in PRA. However, the effects of dietary K intake on PRA have been inconsistent, and data on whether changes in diet ameliorate black-white differences in PRA are sparse. We analyzed data from the DASH-Sodium (Na) randomized, controlled feeding trial (N=412) to determine if type of diet and/or sodium intake affects racial differences in PRA. Participants were randomized to either a control (1.7 g K/2100kcal/d) or DASH (4.1 g K/2100kcal/d) diet. Within each diet, participants received 3 levels of Na intake in random order for 30 days each.

Median plasma renin activity (PRA): ng/mL/hr in white and black participants on the control and DASH diets, by sodium intake

| | Intervention | | |
|---|---|---|---|
| **Control Diet** | **High Na** | **Medium Na** | **Low Na** |
| **White** | 0.58 | 0.66 | 0.98 |
| **Black** | 0.24 | 0.26 | 0.56 |
| ***p* -value*** | <0.0001 | 0.001 | 0.175 |
| | | | |
| **DASH Diet** | | | |
| **White** | 0.66 | 0.84 | 1.15 |
| **Black** | 0.36 | 0.47 | 0.60 |
| ***p* -value*** | 0.001 | 0.003 | 0.015 |

**p* -value for white-black difference, using log transformation, and adjusted for age, sex, caloric intake, baseline PRA, and clinical site

For both types of diet and at each Na level, blacks had lower PRA than whites (adjusted P value was significant in each diet-Na combination except the low Na, control diet). In both diets and in both blacks and whites, PRA increased progressively as sodium intake decreased and with the DASH diet compared to the control diet. In conclusion, racial differences in PRA are present even when individuals are on the same diets. Hence, differences in dietary intake are unlikely to be the sole cause for racial differences in PRA.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1865**

**How Long Should We Monitor the Patients for Bleeding after Percutaneous Renal Biopsy?** Jae-Yoon Park, Seong-Woo Lee, Hajeong Lee, Hayne C. Park, Ho Seok Koo, Dong Ki Kim, Kook-Hwan Oh, Kwon Wook Joo, Yon Su Kim, Curie Ahn, Jin Suk Han, Suhnggwon Kim. *Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.*

Percutaneous renal biopsy (PRB) may be complicated by serious bleeding. Overnight observation after renal biopsy is standard strategy for the safety. Although it was recently reported that outpatient observation is safe, appropriate observation time after the renal biopsy is still in debate. We evaluated prospectively the incidence, onset time and risk factors of hemorrhagic complications to determine the optimal duration of observation after PRB.

We enrolled 100 patients underwent renal biopsy for 6 months using a standard strategy. The biopsy was performed by two experienced nephrologists using biopsy gun under real-time ultrasound guidance. Serial color Doppler ultrasound was done immediately, 8 hours, 24 hours and 1 week after the PRB. Minor complication was defined as gross hematuria and/or sonographically detectable perinephric hematoma, and major complication as bleeding that needed transfusion or invasive interventions.

The 32 patients experienced hemorrhagic complications (32.0%, 10 with gross hematuria, 26 with hematoma, and 4 with both), and 1 major complication occurred 3 days after PRB. Baseline serum creatinine of the patient with major complication was 6.0 mg/dL. Gross hematuria was observed in 4 patients (40.0 %) within 8 hours after PRB, in 6 (60.0 %) between 8 and 24 hours. Hematoma was detected in 19 patients (73.1 %) within 8

hours, in 4 (15.4 %) between 8 and 24 hours, and in 3 (11.5 %) after the discharge. Serum creatinine and BMI were higher in complication group ($p < 0.05$). Number of needle passes, blood pressure, and degree of edema and proteinuria were not related to complication. In multivariate analysis, serum creatinine was the only significant risk factor of complication ($p = 0.007$). Hemorrhagic complications developed in 9 patients (28.1%) between 8 and 24 hours after PRB, all of which were minor.

The 8 hours' observation time after renal biopsy may be appropriate to the stable patients with normal creatinine. Larger scale safety study and analysis of cost-effectiveness should be made.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1866

**The UK Registry of Rare Renal Disease (RaDaR) Enables Research Studies on a National Scale** Hugh J. Mccarthy,[1] David Ansell,[2] Charles Tomson,[3] Moin Saleem.[1] *[1]Academic Renal Unit, University of Bristol, Bristol, United Kingdom; [2]UK Renal Registry, Bristol, United Kingdom; [3]Nephrology, Southmead Hospital, Bristol, United Kingdom.*

Objectives: In 2009 all member states of the European Union agreed to improve health care in rare disease.

Political backing was mirrored by financial support from the Medical Research Council's patient-cohort-initiatives, funding the development of this registry (RaDaR).

Methods: Administrative infrastructure has been provided by the UK Renal Registry - a well established registry of all patients in the UK with end stage renal disease.

RaDaR is governed by the Renal Association and backed by the UK renal community. It is operated by a RaDaR committee. Disease Specific Working Groups (DSWG), utilizing collaborations, provide research into multiple aspects of specific diseases including diagnostic, genetic, biomarker development and therapy.

Results: Two pilot projects into MembranoProliferative GlomeruloNephritis and Steroid Resistant Nephrotic Syndrome have commenced, with recruitment expanding rapidly. Clinical datasets for the website have been developed by the DSWGs in collaboration with RaDaR. Local teams in the collaborating centres populate those datasets, including histology which will be analysed by a central pathology steering group. The information collected is owned by RaDaR and given to the DSWG in link-anonymised form. Additional data that is generated by the DSWG following analysis will be returned to RaDaR at the end of the study.

Conclusions: This project illustrates a comprehensive model for study, management and dissemination of information for rare diseases on a National basis, overcoming governance, data protection and IT hurdles in a coordinated strategy.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1867

**Quality of Evidence Informing Patients' Choice of Renal Replacement Modality (RRM)** Priscilla Auguste,[1] Raquel Greer,[1] Patti Ephraim,[1] Johanna Sheu,[1] Deidra C. Crews,[1] Tanjala S. Purnell,[1] Temitope Olufade,[1] Julio Lamprea,[1] Neil R. Powe,[1] Hamid Rabb,[1] Bernard G. Jaar,[1] L. Ebony Boulware.[1] *[1]Johns Hopkins Medical Institutions; [2]University of California, San Francisco.*

Background: Patients and their physicians are encouraged to engage in informed decision-making regarding patients' RRM choice, but the quality of evidence available to inform patients' decisions is unknown.

Methods: To develop an intervention to improve decision-making about RRM choice, we identified data from national registries and systematically reviewed studies (English-language, published after 1987) to summarize evidence on clinical outcomes with different RRMs. Using modified Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group criteria, we assessed evidence quality ("very low" (e.g. case series), "low" (e.g. cross-sectional or pre-post observational studies), "medium" (e.g. longitudinal cohort studies or registry data), or "high" (e.g. randomized controlled trials (RCTs))) across 12 domains of outcomes previously identified by patients as important to RRM decisions.

Results: Registries provided evidence on 2 domains (8 outcomes). From 3,384 possibly relevant PubMed abstracts, 105 studies provided evidence on 10 domains (53 outcomes). (Table) There were a few (n=7) longitudinal cohort studies. Most (n=98) studies had qualitative, case-series, cross-sectional, or pre-post designs. There were no RCTs. Most (n=72) studies compared outcomes between hemodialysis (HD) versus peritoneal dialysis (PD) while fewer (n=21) compared HD versus transplant or PD versus transplant (n=3). The rigor of the design/methods/analysis was low across studies included in most domains.

Conclusion: There is little high-quality evidence to inform patients' RRM choice. Improved research is needed.

**Table.** Quality of evidence identified to inform decisions on RRM choice

| Domains of clinical outcomes relevant to decision-making | Studies (N) | Relevant Outcomes (N) | GRADE Quality Range |
|---|---|---|---|
| Mortality (e.g. 1-year, 5-year, 10-year) | Registry | 3 | Medium |
| Morbidity (e.g. infection, hospitalization) | Registry | 5 | Medium |
| Quality of Life | 15 | 4 | Very Low to Medium |
| Cognitive Function (e.g. attention and concentration) | 11 | 4 | Very Low to Low |
| Mental Health (e.g. depression and anxiety) | 20 | 6 | Low to Medium |
| Physical Function (e.g. limitations and activity level) | 20 | 6 | Very Low to Low |
| Fertility (e.g. conception, births) | 6 | 7 | Very Low |
| Sexual Function (e.g. libido, erectile dysfunction) | 17 | 13 | Very Low to Low |
| Work (e.g. employment) | 12 | 4 | Very Low to Low |
| Symptoms (e.g. pain, cramping) | 39 | 8 | Very Low to Low |
| Body Image | 9 | 2 | Very Low to Medium |
| Freedom | 6 | 1 | Very Low to Low |

Disclosure of Financial Relationships: nothing to disclose

### F-PO1868

**TNFα-Mediated Endocytosis of Nephrin** Magdalena Woznowski,[1] Dennis Sohn,[2] Ivo Quack,[1] Eva Koenigshausen,[1] Sebastian A. Potthoff,[1] Lars C. Rump,[1] Lorenz Sellin.[1] *[1]Department for Nephrology, University Hospital Duesseldorf, Duesseldorf, Germany; [2]Institute for Molecular Radiooncology, University Duesseldorf, Duesseldorf, Germany.*

**Introduction**

Podocytes are highly differentiated cells that form interdigitating foot processes. Podocyte damage leads to foot process retraction, disruption of the slit diaphragm and proteinuria. Human acquired glomerulopathies, such as IgA nephropathy, diabetic nephropathy, FSGS and other inflammatory glomerular diseases are associated with TNF-α elevation. Recent advances have revealed crucial roles of slit diaphragm-associated proteins, including nephrin, podocin and β-arrestin2. β-arrestin2 interaction was shown to induce nephrin endocytosis and is believed to cause thereby proteinuria. In addition, p38 MAPK is known to play a critical role in mediating podocyte injury. Therefore we investigated the influence of TNF-α on nephrin endocytosis and the involvement of p38.

**Methods**

Cells expressing nephrin and β-arrestin2 were stimulated with TNF-α. After cell lysis, co-immunoprecipitation with subsequent western blot analysis was performed. For the inhibitor studies, cells were treated with the p38 inhibitor SB202190. siRNA was used for knockdown of p38. For the endocytosis assay, cells expressing nephrin and β-arrestin2 were stimulated with TNF-α and incubated with biotin before cell lysis.

**Results**

The interaction of nephrin with β-arrestin2 is markedly enhanced through TNF–α stimulation. Incubation with SB 202190 attenuates the TNF-α mediated enhancement of the interaction between nephrin and β-arrestin2. Podocytes stimulated with TNF-α present an increased p38 phosphorylation. p38-knockdown by siRNA attenuates also the interaction of nephrin with β-arrestin2. Furthermore, p38 is shown to interact with nephrin and β-arrestin2. The most impressive finding is the TNF-α mediated enhancement of nephrin endocytosis through the interaction between nephrin and β-arrestin2.

**Conclusion**

TNF-α is shown to enhance nephrin endocytosis through increased interaction between nephrin and β-arrestin2. p38 phosphorylation is hereby of crucial importance. It is conceivable that this presents a molecular mechanism of TNF-α associated acquired proteinuria.

Disclosure of Financial Relationships: nothing to disclose

### F-PO1869

**Nephrin and Dynamin Share Functions in the Process of Glucose Stimulated Insulin Release** Jongmin Jeon,[1] Robier A. Aguillon Prada,[1,2] Christian Faul,[2] Jochen Reiser,[2] Camillo Ricordi,[1] Peter H. Mundel,[3] Alessia Fornoni.[1,2] *[1]Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL; [2]Medicine/Nephrology, University of Miami Miller School of Medicine, Miami, FL; [3]Medicine, Massachusetts General Hospital, Charlestown, MA.*

We have demonstrated an important function of nephrin in glucose stimulated insulin secretion. Nephrin undergoes endocytosis upon glucose stimulation and modulates actin cytoskeleton remodeling in pancreatic beta cells. Actin mobilization is dependent on guanosine triphosphatases (GTPase) such as dynamin, which is stimulated by glucose and is functionally coupled to insulin granule exocytosis. Therefore, we tested the hypothesis that nephrin and dynamin are functionally related.

Overexpression of wild type dynamin (WTD) in MIN6 cells caused increased glucose stimulated insulin release. This positive dynamin effect on insulin secretion was prevented by the down-regulation of nephrin expression via siRNA to an extent similar to the one observed when a dominant-negative dynamin mutant (K44A) was utilized. Furthermore, dynamin regulated glucose-induced nephrin trafficking from the plasma membrane to the cytoplasm. When GFP-nephrin transfected MIN6 cells were infected with K44A, nephrin remained localized at the cell surface despite glucose or protamine sulphate stimulation, suggesting that the GTPase activity of dynamin is essential to promote nephrin relocation from the plasma membrane into the cytoplasm. On the contrary, when MIN6 cells were

infected with WTD, nephrin trafficking from the cell surface into the cell was not impaired. Despite such a functional nephrin-dynamin interaction, coimmunoprecipitation experiments did not reveal a physical interaction between both proteins.

In summary, we demonstrate that nephrin is an important downstream effector of dynamin in the process of glucose stimulated insulin release. Our findings may shed new light on the pathway responsible for the regulated targeting of vesicles to the plasma membrane in pancreatic beta cells and may allow for the identification of new therapeutic targets for the augmentation of glucose stimulated insulin secretion.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1870

**GLEPP1 Interacts with src Family Kinases, Nephrin and Podocin** Eva Koenigshausen, Sarah Grabowski, Laura Gerbaulet, Ivo Quack, Magdalena Woznowski, Sebastian A. Potthoff, Lars C. Rump, Lorenz Sellin. *Department of Nephrology, Heinrich Heine University, University Hospital, Duesseldorf, Germany.*

**Introduction**

GLEPP1 (glomerular epithelial protein phosphatase 1) is a receptor tyrosine phosphatase expressed in the apical membrane of foot processes. GLEPP1 expression is reduced in proteinuric kidney diseases. Previously we could show that the interaction of nephrin with podocin and β-arrestin2 and endocytosis of nephrin is regulated by src family kinases. This observation led to the concept of the dynamic regulation of the glomerular slit diaphragm. Work by others implicated GLEPP1 to be a protective factor for the glomerular slit. We hypothesize a regulatory role for GLEPP1 in respect to nephrin endocytosis.

**Methods**

In our experiments, we used cells expressing GLEPP1 and nephrin, podocin, src, fyn, β-arrestin2 or paxillin. After cell lysis, co-immunoprecipitation with subsequent western blot analysis was performed. For immunofluorescence, cells expressing GLEPP1 and paxillin.GFP were fixed and permeabilized thereafter. GLEPP1 was visualized by immunofluorescence.

**Results**

GLEPP1 interacts with the slit diaphragm proteins nephrin and podocin as well as with the src family kinases src and fyn. Furthermore, GLEPP1 enhances the interaction of nephrin with podocin and reduces the interaction of nephrin with β-arrestin2. GLEPP1 expression induces cytoskeletal rearrangement and colocalizes with paxillin in focal complexes.

**Conclusion**

The integrity of the glomerular slit diaphragm is regulated by src kinase mediated tyrosine phosphorylation of the nephrin C-terminus. We assume that GLEPP1 activates src family kinases through interaction and dephosphorylation of src family kinases at their regulatory domain. Activated src family kinases consequently increase nephrin tyrosine phosphorylation at its C-terminus. Additionally, GLEPP1 seems to force cytoskeletal changes which might be of importance maintaining the delicate shape of healthy foot processes. Via this mechanism, GLEPP1 may support the integrity of the slit diaphragm and play a distinct role in protection against the development and course of glomerular proteinuric kidney disease.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1871

**The Cell Polarity Proteins, aPKC-Par Complex, Regulate the Turnover of Nephrin To Maintain Slit Diaphragm Integrity** Daisuke Satoh,[1] Yutaka Harita,[1] Chikara Daimon,[1] Tomonori Hirose,[1] Shigeo Ohno.[1,2] *[1]Department of Molecular Biology, Graduate School of Medical Science, Yokohama City University, Yokohama, Japan; [2]Advanced Medical Research Center, Yokohama City University, Yokohama, Japan.*

The appropriate localization of nephrin, a primary component of slit diaphragm (SD), is crucial for the selective glomerular filtration. Surface expression of Nephrin has been shown to be regulated by interaction with β-arrestin2, or CIN85, or by its tyrosine phosphorylation. However, the molecular mechanisms regulating physiological turnover of nephrin remain obscure.

Previously we have reported that selective depletion of aPKCλ, a component of cell polarity regulator aPKC-Par complex, in mouse podocytes results in SD disassembly and proteinuria. Further, aPKC-Par complex binds to nephrin, and the inhibition of kinase activity of aPKC alters the solubility of nephrin and podocin. These results raise the possibility that aPKC-Par complex may regulate the turnover of nephrin.

To this end, we used the biotinylation assay system to evaluate cell-surface localization and internalization of nephrin using HCT116 cells stably expressing nephrin (HCT116-nephrin cells) or isolated glomeruli from rat. The cells were incubate at 37°C to allow endocytosis after surface biotinylation followed by sequential stripping of the remaining cell surface biotin. In this system, in addition to total amount and cell surface localization, the amount of internalized nephrin can be quantitatively evaluated. Knockdown of Par3 in HCT116-nephrin cells led to decrease of cell surface localization of nephrin. Similar results were obtained by aPKC inhibitor treatment, overexpression of kinase negative form of aPKC, or knockdown of aPKC. Src kinase inhibitors did not abrogate the effect of aPKC inhibitor, indicating that these effects were independent of its phosphorylation. Furthermore, the amount of internalized nephrin was not affected by aPKC inhibitor treatment. These results suggest that nephrin turnover cycle is regulated by the balance between exocytosis and endocytosis, and aPKC-Par complex positively regulates the balance to maintain SD integrity possibly through the exocytosis of nephrin.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1872

**Crk1/2 Forms a Complex with Nephrin and p130Cas and Is Essential for Nephrin-Induced Cell Spreading** Britta George,[1] Rakesh Verma,[2] Abdul A. Soofi,[2] Lawrence B. Holzman.[1] *[1]Renal-Electrolyte and Hypertension Division, University of Pennsylvania, Philadelphia, PA; [2]Division of Nephrology, University of Michigan, Ann Arbor, MI.*

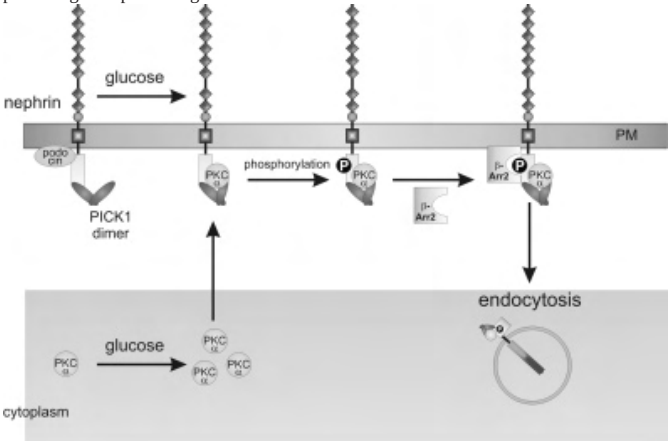
The slit diaphragm protein nephrin is essential for filter integrity of the kidney, transducing phosphorylation-mediated signals from the podocyte intercellular junction which regulate cytoskeletal dynamics. We previously reported that the adaptor protein Crk1/2 binds nephrin in a phosphorylation- and SH2-dependent fashion following nephrin activation. Crk1/2 is necessary for nephrin-induced lamellipodial dynamics in cultured podocytes since RNAi-mediated knockdown of Crk1/2 attenuates cell spreading. Glomeruli of mice deleted of Crk1/2 in a podocyte-specific fashion develop and age normally. However, Crk1/2 null podocytes in this model are protected from protamine sulfate injury-induced foot process spreading. Despite extensive mapping studies carried out in cell culture and *in vitro*, we were unable to identify a unique Crk binding site in nephrin that mediates nephrin-induced lamellipodial induction; this suggested that Crk1/2 interacts with nephrin both directly and indirectly. As such, we considered that Crk1/2 might alternatively function within the focal adhesion complex to mediate nephrin-induced lamellipodia formation. In support of this hypothesis, nephrin activation recruited tyrosine phosphorylated p130Cas to nephrin clusters; this required binding of the p85 subunit of pI3 kinase to nephrin and pI3 kinase activity. p130Cas recruitment occurred despite mutation of tyr residues necessary for Nephrin-Nck binding. However, Y to F mutation of these residues precluded nephrin-induced p130Cas phosphorylation suggesting that p130Cas recruitment and p130Cas tyrosine phosphorylation are mediated by independent signaling events. Consistent with these results, in developing mouse glomeruli we verified that phospho-p130Cas is present at the podocyte precursor adherens junction at all stages of development. We conclude that Crk interacts with nephrin directly through its SH2 domain as well as indirectly, through p130Cas in a pI3 kinase dependent manner, and plays an essential role in nephrin-induced lamellipodia formation.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1873

**PKCalpha and PICK1 Mediate beta-Arrestin2 Dependent Nephrin Endocytosis in Hyperglycemia** Ivo Quack,[1] Magdalena Woznowski,[1] Sebastian A. Potthoff,[1] Eva Koenigshausen,[1] Johannes Stegbauer,[1] Mario Schiffer,[2] Lars C. Rump,[1] Lorenz Sellin.[1] *[1]Department of Nephrology, Heinrich-Heine-University, Duesseldorf, Germany; [2]Department of Nephrology, Hannover Medical School, Hannover, Germany.*

Nephrin, the key molecule of the glomerular slit diaphragm, is expressed on the surface of podocytes and is critical in preventing albuminuria. In diabetes, hyperglycemia causes albuminuria and leads to loss of nephrin surface expression. Here, we report the underlying mechanism: hyperglycemia directly enhances nephrin endocytosis mediated by protein kinase C alpha. PKCalpha activity controls the rate of nephrin endocytosis via regulation of the beta-arrestin2-nephrin interaction. We identify PKCalpha and protein interacting with c kinase-1 (PICK1) as nephrin binding proteins. Hyperglycemia induces upregulation of PKCalpha and leads to the formation of a nephrin/PKCalpha/PICK1/beta-arrestin2 complex in vitro and in vivo. Beta-arrestin2 binding to the nephrin intracellular domain depends on nephrin threonine residues 1120 and 1125. PICK1 binds with its PDZ domain to nephrin amino acids 1160-1176 harboring a PDZ type III binding motif. Cellular knockdown of PKCalpha and/or PICK1 attenuates nephrin - beta-arrestin2 interaction and abrogates the amplifying effect of high glucose on nephrin endocytosis. In C57Bl/6 mice, hyperglycemia over 24 hours causes a significant increase in urinary albumin excretion supporting the concept of a rapid impact of hyperglycemia on glomerular permselectivity. In summary, the present study provides a molecular model of hyperglycemia-induced nephrin endocytosis and subsequent proteinuria and highlights PKCalpha and PICK1 as promising therapeutic targets.




Disclosure of Financial Relationships: nothing to disclose

**F-PO1874**

**NEPH1 Function Revisited: Role for Glomerular Development, Maintenance and Nephrin Trafficking** Tobias B. Huber. *Renal Division, University Hospital Freiburg, Freiburg, Germany.*

Podocytes form a complex 3D epithelial structure and depend on a temporally and spatially precise tuning of cellular interactions. Evolutionarily conserved Ig superfamily molecules between neighbouring podocytes interact to form the highly specialized SD that regulates interpodocyte positioning and dynamics.

To further investigate the precise function of NEPH1 within the SD we generated a conditional mouse where the transmembrane and intracellular domain was flanked by loxP sites. Sox2Cre*Neph1fl/fl mice recapitulated the described phenotype of Neph1 null mice with 95% lethality within 48h pp. EM revealed grossly disfigured secondary and aberrantly attaching primary processes correlating with a massive proteinuria on P1. In contrast, Nphs2Cre*Neph1fl/fl mice survived into adulthood. On the light and electron microscopic level the observed structural changes in the kidney were similar to the ones in the Neph1 null. These mice also showed an early onset massive proteinuria which persisted throughout their life. Histology progressed into classical FSGS which lead to uremia. The discrepancy between the Neph1 null and Nphs2Cre*Neph1fl/fl mouse might hint to an extrarenal function of NEPH1.

On the molecular level, loss of Neph1 resulted in a failure of correct SD assembly and in mis-directed Nephrin targeting as evidenced by confocal microscopy and Immuno EM.

To determine the function of neph1 in adult kidneys we generated a inducible Nphs2rtTACre line. After initiation of doxy treatment mice developed significant proteinuria and podocyte ultrastructural abnormalities indicating the critical importance of Neph1 for glomerular maintenance.

In summary we here demonstrate that NEPH1 is essential for SD assembly, foot process development and maintenance.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1875**

**Nephrin-Neph3 Bidirectional Gene Pair Is Synergistically Activated by Transcription Factors WT1 and NF-κB, and Silenced by DNA Methylation** Mervi Ristola,[1] Satu Arpiainen,[2] Moin Saleem,[3] Harry B. Holthofer,[1,2] Sanna H. Lehtonen.[1] *[1]Haartman Institute, University of Helsinki, Helsinki, Finland; [2]Dublin City University, Dublin, Ireland; [3]Academic and Children's Renal Unit, University of Bristol, Bristol, United Kingdom.*

Nephrin and Neph3 are homologous molecules expressed in the glomerular podocytes where they localize at the slit diaphragms. Nephrin, and apparently also Neph3, are crucial for podocyte function. NPHS1 and KIRREL2, encoding nephrin and Neph3, locate in human chromosome 19q13.12 in a head-to-head orientation and are separated by approximately 5-kb region constituting a bidirectional gene pair. Also mouse and rat have similar nephrin and Neph3 gene arrangement. Nephrin and Neph3 mRNAs are down-regulated and show positive correlation in human proteinuric diseases, which together with the genomic arrangement of their genes and similar protein structure and location proposes that nephrin and Neph3 genes may share key features in their regulation. In this study, we investigated if nephrin and Neph3 genes have similar mechanisms in their transcriptional regulation focusing on transcription factors WT1 and NF-κB, and DNA methylation. By overexpression studies and reporter gene assays, we show that both nephrin and Neph3 genes are activated by transcription factors WT1 and NF-κB in a synergistic manner. This cooperation was further supported by the physical interaction between WT1 and NF-κB shown by co-immunoprecipitation. Chromatin immunoprecipitation assay using cultured human podocytes demonstrated that WT1 and NF-κB bind to nephrin and Neph3 promoter. In cultured human podocytes, NF-κB activator TNF-α enhanced a time- and concentration-dependent induction of endogenous nephrin mRNA. Further, treatment of cultured podocytes and A293 cells with a demethylating agent showed that endogenous nephrin and Neph3 mRNAs are similary silenced by DNA methylation. In conclusion, these results show that nephrin and Neph3 share same mechanisms in their regulation. Both genes are synergistically activated by transcription factors WT1 and NF-κB in podocytes, and further, DNA methylation plays a role in silencing nephrin and Neph3 gene expression.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1876**

**Puromycin Aminonucleoside Induces Nuclear Translocation of IQGAP1 Protein While Decreasing Its Interactions with Slit Diaphragm Proteins in Podocytes** Claire Rigothier,[1-2] Moin Saleem,[1] Jean Ripoche,[2] Christian Combe,[2] Gavin Iain Welsh.[1] *[1]Academic Renal Unit, University of Bristol, Bristol, United Kingdom; [2]INSERM U577, Universite de Bordeaux, Bordeaux, France.*

Background

IQGAP1 is a scaffold protein that interacts with cytoskeletal proteins as well as proteins of the glomerular slit diaphragm (SD). IQGAP1 is implicated in actin cytoskeleton remodelling and in regulation of cell migration, cell adhesion, cell division and gene expression. We hypothesized that IQGAP1 is pivotal in maintaining glomerular barrier properties and podocyte cytoskeleton remodelling as observed in glomerulonephropathies (GN). We report changes in podocyte IQGAP1 subcellular localization and interaction with SD proteins upon exposure of podocytes to puromycin aminonucleoside (PAN), an inducer of experimental nephrotic syndrome.

Methods

In cultured human podocytes, a combination of Western blot (n=5) on cytoplasmic and nuclear extracts, quantitative PCR (n=3), immunoprecipitation (n=5) and immunocytolocalization (n=5) were performed to determine the subcellular localization, the transcription and interacting protein partners of IQGAP1, upon exposure to PAN.

Results

Upon exposure to PAN, IQGAP1 translocation from its usual localization on the plasma membrane to the nucleus was observed by immunocytolocalization (cytoplasm/nuclei ratio, ctl: 10.8±1.3 vs PAN: 6.6±0.5 intensity/mm2, p=0.02) and confirmed by Western blot after selective extraction (2.8±1.0 vs 1.4±0.4 intensity/mm2, p=0.03). qPCR excluded an increase of IQGAP1 transcription.

Interactions with proteins of the SD were also modified. We observed a reduction of IQGAP1 interactions with both nephrin and podocalyxin at each time of PAN exposure.

Conclusion

The alteration of IQGAP1 in PAN-induced GN may be important in the loss of the permeability of the glomerular barrier associated with this experimental model.

Also, the potential roles played by IQGAP1 in the nucleus in these conditions remain to be clarified, particularly on account of IQGAP1 implication in gene regulation. The PAN model provides a useful experimental tool to study the role of IQGAP1 in the alteration of podocyte cytoskeleton organization and remodelling as observed in GN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1877**

**WT1-Interacting Protein (WTIP) Is Necessary for Early Mouse Development and Glomerular Filtration Barrier Function in WTIP +/- Mice** Sethu M. Madhavan, Martha Konieczkowski, John O'Toole, Bingcheng Wang, Jane H. Kim, John R. Sedor. *Medicine, MetroHealth Campus, Case Western Reserve University, Cleveland, OH.*

WT1 mutations associate with familial glomerulosclerosis, and WT1 expression is reduced in acquired glomerulosclerosis. The podocyte adherens junction protein, WTIP, a member of the ajuba LIM-domain family, translocates to the nucleus after injury and represses WT1 activity. To define Wtip function in vivo, we generated a Wtip-deficient mouse line using β-galactosidase-neomycinR (β-geo) gene trap technology. The β-geo expression vector integrated into the second intron of Wtip and sequencing after RT-PCR confirmed that Wtip exon 2 spliced in frame to β-geo. Immunoblotting with an anti-β-gal antibody showed a Mr 170 protein, the size predicted for the Wtip-β-geo fusion protein. No Wtip $^{\beta\text{-geo}/\beta\text{-geo}}$ animals were identified among live-born mice or embryos as young as E8.5 (n=300 mice). The morphology and viability of Wtip $^{\beta\text{-geo}/+}$ and wild type mice was similar. Heterozygotes up to 1 year of age did not spontaneously develop proteinuria. After LPS injection, Wtip $^{\beta\text{-geo}/+}$ mice developed statistically greater and more prolonged proteinuria compared to the wild-type littermates. To map endogenous Wtip expression pattern, we assayed X-gal staining in Wtip $^{\beta\text{-geo}/+}$ mouse embryo and adult tissues. No X-gal staining was observed in the renal vesicle, coma shaped or S-shaped stages of glomerular development. However, X-gal was seen capillary loop stage and mature glomeruli, co-localizing with synaptopodin but not PECAM-1. Wtip is also expressed in developing myocardium as early as E9.5 but not in adult heart. X-gal staining of brain, the retina, ears and lungs was identified in E14.5 and E18.5 embryos. In summary, Wtip is essential for mouse embryogenesis and is expressed in podocytes from the capillary loop stage into adulthood. Resolution of abnormal proteinuria after LPS injection is delayed in Wtip$^{\beta\text{-geo}/+}$ mice, suggesting Wtip regulates glomerular filtration barrier function.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1878**

**WT1 Interacting Protein (WTIP) Regulates Podocyte Phenotype by Cell-Cell Contact Reorganization** Jane H. Kim,[1] Martha Konieczkowski,[2] John R. Sedor.[1,2,3] *[1]Physiology and Biophysics, Case Western Reserve University, Cleveland, OH; [2]Medicine, Case Western Reserve University, Cleveland, OH; [3]Division of Nephrology, Metro Health Medical Center, Cleveland, OH.*

Wtip translocation to the nucleus after injury causes actin cytoskeletal rearrangement and glomerular filtration barrier dysfunction. To define Wtip function in slit diaphragm assembly, we generated murine podocyte lines that stably express shRNAs directed against Wtip (shWtip) or an empty vector (shEmp). Wtip transcript and protein levels decrease by 90% in shWtip cells compared to shEmp and wild type cells. Although focal adhesion kinase, vinculin and paxillin expression is equivalent in shWtip and shEmp cells, shWtip cells adhere but fail to spread normally. shWtip podocytes do not assemble actin stress fibers and focal adhesions are reduced and fail to mature. Wtip expression in shWtip cells rescues the spreading defect. As shEmp podocytes establish cell-cell contacts, Wtip localizes to the adherens junction, colocalizing with β-catenin and cadherin. Epitope-tagged Wtip coprecipitates with both cadherin and β-catenin. In contrast, shWtip cells fail to establish stable cell-cell contacts but persistently elaborate lamellipodia and filopodia. Fluorescence microscopy demonstrated disordered F-actin structures and no synaptopodin. Although primarily intracellular, some cadherin and b-catenin cluster in brightly fluorescent but irregularly distributed spots in the plasma membrane that fail to laterally expand into mature adherens junctions. Cell surface biotinylation shows diminished plasma membrane expression of cadherin, β-catenin, and α-catenin in shWtip podocytes, although all are equivalently expressed in shWtip and shEmp podocytes by immunoblotting. In a model of calcium-regulated junction assembly, cadherin-based cell-cell contacts fail to reassemble in shWtip podocytes. In conclusion, stable assembly of podocyte adherens junctions requires Wtip, suggesting in vivo Wtip functions as a molecular switch regulating podocyte phenotype through cell-cell contact remodeling.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1879**

**Activation of RhoA in Podocytes Causes Foot Process Effacement and Proteinuria in Mice** Lei Zhu, Ruihua Jiang, Lamine Aoudjit, Tomoko Takano. *Medicine, McGill University, Montreal, QC, Canada.*

Podocytes are a major component of the glomerular filtration barrier and alterations to the morphology of podocyte foot processes are associated with proteinuria. RhoA is a small GTPase that regulates the actin cytoskeleton. The aim of the current study is to investigate the role of RhoA in the alternation of podocyte morphology and function. A mouse model of tetracycline-inducible, podocyte-specific expression of the constitutively active (CA)-RhoA was developed. When these mice were treated with doxycycline (Dox) for 4 wks, CA-RhoA was expressed in podocytes and significant proteinuria developed. In contrast, vehicle-treated mice did not express CA-RhoA, nor did develop proteinuria. Electron microscopy revealed focal and segmental foot process effacement in mice treated with Dox, but not in vehicle-treated mice. The degree of foot process effacement correlated with the degree of proteinuria. Dox-induced CA-RhoA expression and proteinuria were, in most cases, reversible within 2 wks after the Dox withdrawal. However, occasional mice showed extremely high proteinuria, which persisted even after the Dox withdrawal. In these mice, light microscopy showed focal and segmental glomerulosclesosis. Glomerular fibronectin mRNA was significantly increased in mice treated with Dox, supporting that RhoA activation leads to fibronectin upregulation. Expression of other genes such as nephrin, alpha-actinin-4, WT1, synaptopodin, and laminin 1 were unchanged. Differentiated cultured mouse podocytes transfected with GFP-CA-RhoA were significantly smaller and devoid of cellular processes, as compared with those transfected with GFP or GFP-dominant negative (DN)-RhoA. Thus, activation of RhoA in podocytes results in foot process effacement and proteinuria. Activation of RhoA causes reversible foot process effacement likely via modulating the actin cytoskeleton. In addition, when the degree/duration of the RhoA activation is severe/long, it may lead to upregulation of fibronectin and irreversible glomerulosclerosis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1880**

**Arhgap24 Controls Membrane Ruffling in Podocytes and Is a New Candidate Gene for Focal Segmental Glomerulosclerosis** Shreeram Akilesh,[1] Tobias B. Huber,[3] Michelle P. Winn,[4] Andrey S. Shaw.[2] *[1]Department of Pathology, Barnes-Jewish Hospital, St. Louis, MO; [2]Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO; [3]Division of Nephrology, University of Freiburg, Freiburg, Germany; [4]Division of Nephrology, Department of Medicine, Duke University, Durham, NC.*

The podocyte is the specialized epithelial cell of the kidney and is a key component of the filtration barrier. The podocyte has a complex membrane arborization that is effaced in proteinuric disease states such as focal segmental glomerulosclerosis (FSGS). We studied membrane dynamics *in vitro* and found that podocytes slow their membrane ruffling after differentiation. Since membrane dynamics are controlled by the small G-proteins Rac1 and RhoA, we examined the expression of regulators of these proteins in podocytes. We identified Arhgap24 as a GTPase activating protein (GAP) that inactivates Rac1 activity and arrests podocyte membrane ruffling. Sequencing of the *ARHGAP24* gene in patients with FSGS identified a mutation that tracks with disease in a pedigree. This mutation reduces the GAP activity of Arhgap24 *in vitro*. These findings imply that aberrant regulation of podocyte membrane dynamics, in part controlled by Arhgap24, contributes to FSGS.

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**F-PO1881**

**Drebrin Is a Novel Regulator of Podocyte Process Formation** Michelle N. Rheault,[1] Renee E. Koessler.[2] *[1]Pediatrics, University of MN, Minneapolis, MN; [2]Medicine, Northwestern University, Chicago, IL.*

Although they have different functions, both podocytes and neurons require the formation and maintenance of a complex arborized phenotype that is supported by the highly organized cytoskeleton. Drebrin, an actin binding protein concentrated in dendritic spines in neurons and involved in actin organization and spine elongation, is also present in podocytes. The purpose of this study was to examine the role of drebrin in the podocyte. Drebrin is present in podocytes and mesangial cells in human kidney. By confocal immunofluorescence (IF) microscopy, podocyte-specific drebrin expression was decreased in biopsies from 2 patients with minimal change nephrotic syndrome compared to controls. In cultured human podocytes, drebrin expression was induced in differentiated podocytes compared to undifferentiated podocytes and was localized to the periphery of the cell in a subcortial actin distribution. Treatment of differentiated human podocytes with 50μg/ml puromycin (PAN), a model of cytoskeletal reorganization and podocyte injury, led to a 50% reduction in drebrin protein levels after 48 hours. Drebrin localizes to adherens junctions in several cell types and podocyte slit diaphragms are known to be modified adherens junctions. Drebrin and the slit diaphragm protein, CD2AP, demonstrated co-localization in differentiated podocytes by confocal IF microscopy. They also demonstrated a specific interaction in co-immunoprecipitation studies in HEK cells suggesting that drebrin may be a novel slit diaphragm protein. To explore whether drebrin influences podocyte process formation, GFP-tagged drebrin was expressed in undifferentiated human podocytes. This caused numerous small actin and drebrin containing processes to form at the periphery of cells that did not express the classic filopodia marker fascin. Processes were not observed in cells expressing GFP alone. In conclusion, we found that podocyte drebrin protein levels are decreased in human minimal change nephrotic syndrome and a PAN culture model of cytoskeletal rearrangement in nephrotic syndrome. Drebrin may act as a novel linking protein between the slit diaphragm and actin cytoskeleton that regulates foot process structure.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1882**

**Def-6, a Novel Regulator of Small GTPases Acts Downstream of PAN and PKCλ/ι in Podocytes** Kirstin Worthmann,[1] Michael Leitges,[2] Oliver Dittrich-Breiholz,[3] Michael Kracht,[4] Hermann G. Haller,[1] Mario Schiffer.[1] *[1]Division of Nephrology, Medical School Hannover, Hannover, Germany; [2]Biotechnology Centre of Oslo, University of Oslo, Oslo, Norway; [3]Institute of Biochemistry, Medical School Hannover, Hannover, Germany; [4]Rudolf-Buchheim-Institute of Pharmakology, Justus Liebig University Giessen, Giessen, Germany.*

Background: Podocyte specific deletion of atypical PKCλ/ι in mice leads to a severe glomerular phenotype with gross proteinuria, glomerulosclerosis, atypical cell-cell junctions and mislocated slit diaphragms. This phenotype is similar to changes found in the puromycin aminonucleoside nephrosis (PAN) model in rats.

Methods and results: To test whether treatment of podocytes with PAN leads to inhibition of aPKCs we generated stable monoclonal PKCλ/ι knockout cell lines and compared these cells to podocytes stimulated for 24 hrs with PAN. We detected actin cytoskeletal rearrangements and an inbalance of the small GTPases RhoA and Rac1 in both conditions. When we compared gene expression profiles using one-color microarrays we detected several common target genes overrepresented in the PKCλ/ι KO cells and in podocytes stimulated with PAN. One of these genes is Def6, which is a known regulator of Rho-family GTPases as a GEF and cooperates with active Rac leading to morphological changes. Next, we investigated the Def-6 mRNA expression in PKCλ/ι WT and KO kidneys of different age. We could show that Def-6 mRNA expression decreases during development in the WT kidneys. In contrast, Def-6 expression levels increase in PKCλ/ι KO. During podocyte differentiation *in vitro* we detected a decrease of Def-6 expression in the WT and also in the PKCλ/ι KO cells, but interestingly the Def-6 level in the differentiated KO cells remains significantly higher similar to the Def-6 levels in the undifferentiated WT cells. Overexpression of Def-6 in podocytes resulted in an obvious phenotype with an increased number of lamellipodia and filopodia, similar to the phenotype in PKCλ/ι KO podocytes.

Conclusions: In summary, we provide evidence that Def-6 and PKCλ/ι are new downstream targets in PAN nephrosis. Dysregulation of Def-6 might be a key component of foot process effacement.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1883**

**The Role of SIRPα (SHPS-1) in Experimental Diabetic Nephropathy** Satoshi Takahashi,[#1] Keiju Hiromura,[#1] Hiroko Hamatani,[#1] Mai Kato-Tomioka,[#1] Akito Maeshima,[#1] Takashi Kuroiwa,[#1] Hiroshi Ohnishi,[#2] Takashi Matozaki,[#2] Aoki Takeo,[#3] Yoshihisa Nojima.[#1] *[1]Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan; [2]Laboratory of Biosignal Sciences, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Gunma, Japan; [3]Department of Anatomy and Cell Biology, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.*

SIRPα (SHPS-1) is a transmembrane protein that contains tyrosine phosphorylation sites in its cytoplasmic region, to which two tyrosine phosphatases, SHP1 and SHP2, are recruited in a phosphorylation-dependent manner. We previously reported that SIRPα was predominantly expressed in podocytes and that knock-in mice (C57BL/6 background) which express mutant SIRPα lacking the cytoplasmic region showed massive albuminuria by combination of uninephrectomy and adriamycin injection (ASN 2008). In this study, we sought to determine the role of SIRPα in diabetic nephropathy (DN). Type 1 DN was induced in SIRPα mutant mice (MU) and its wild type mice (WT) by streptozotocin (STZ) injection. The mean blood glucose levels between 2 to 32 weeks after STZ injection were similar in both groups (547.5±60.2 vs 528.7±63.6 mg/dL, MU vs WT). MU showed increased albuminuria compared to WT (178±33 vs 52±29 μg/day at 14 weeks; 600±168 vs 90±33 μg/day at 28 weeks; MU vs WT, $P<0.01$). Creatine clearance, measured just before sacrifice at 32 weeks, was elevated in MU compared to WT (0.78±0.11 vs 0.41±0.16 ml/min, MU vs WT, $P<0.05$), demonstrating that MU had increased glomerular hyperfiltration. Renal specimens revealed that MU had more thickened glomerular basement membrane (0.34±0.016 vs 0.27±0.034 μm, determined by electron microscopic examination, MU vs WT, $P<0.05$). Mesangial expansion was not different in both mice. These data suggested that loss of SIRPα signaling pathway enhance diabetic glomerular injury. In addition, taken together with the previous findings of adriamycin model, SIRPα signaling pathway is considered to play an important role in modulating glomerular barrier function upon podocyte injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1884**

**Down Regulation of SIRT1 Mediates Podocyte Apoptosis in Diabetes Mellitus** Peter Y. Chuang,[2] Yan Dai,[1] Ruijie Liu,[1] John C. He.[1,2] *[1]Medicine/Div. of Nephrology, James J. Peters VA Medical Center, Bronx, NY; [2]Medicine/Div. of Nephrology, Mount Sinai School of Medicine, New York, NY.*

Cellular events leading to podocyte loss in diabetic nephropathy are unclear. We previously found that advanced glycation endproducts (AGE) promoted podocyte apoptosis in a forkhead box O 4 (FOXO4)-dependent manner by increasing the expression of an apoptosis response gene Bim. The FOXO family of transcription factors (TF) is involved in the regulation of oxidant stress resistance, apoptosis, cell cycle inhibition, and cellular metabolism. Post-translation modification of FOXOs by phosphorylation and acetylation are essential for the regulation of their transcriptional activity. A mammalian sirtuin (SIRT1) targets FOXOs for deactylation and inhibits FOXO-induced transactivation of an apoptosis response gene, Bim, and enhances the expression of FOXO-target genes that are involved in oxidant stress resistance. In this study we tested the hypothesis that down regulation of sirtuin (SIRT1) in diabetes enhances the acetylation of FOXO4 to transactivate a pro-apoptotic, FOXO-induced gene BIM in the podocytes to promote apoptosis. We found that AGE enhanced FOXO4 binding to the Bim promoter in the podocytes by chromatin immunoprecipitation. AGE increased the acetylation of FOXO4 in cultured podocytes as determined by immunoprecipitation of endogenous FOXO4 and immunoblotting for acetylated lysine. The protein and mRNA levels of SIRT1 was reduced with AGE-treatment in podocytes. Over expression of SIRT1 using a lentiviral transduction of cultured podocytes abrogated AGE-induced apoptosis as assessed by annexin V labeling. Glomeruli isolated from diabetic db/db mice have a reduced expression of SIRT1 and increased acetylation of FOXO4. Treatment of db/db mice with a SIRT1 activator, resveratrol, attenuated glomerular Bim expression and podocyte apoptosis. This study suggest that reduced SIRT1 expression in the diabetic condition promotes FOXO4 acetylation, FOXO4-binding to the BIM promoter, BIM expression, and podocyte apoptosis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1885**

**Role of Non-Steroid Anti-Inflammatory Drugs in Regulation of TRPC Channels in Isolated Rat Glomerulus** Daria Ilatovskaya,[1,3] Robert P. Ryan,[1] Allen W. Cowley,[1] Alexander Staruschenko.[1,2] *[1]Physiology, Medical College of Wisconsin, Milwaukee, WI; [2]Kidney Disease Center, Medical College of Wisconsin, Milwaukee, WI; [3]Institute of Cytology RAS, Saint-Petersburg, Russian Federation.*

Non-steroid anti-inflammatory drugs (NSAIDs) are major drugs used in the treatment of inflammation in a wide variety of disorders. The best-known mechanism of action of NSAIDs is the inhibition of prostaglandin synthesis secondary to their action on cyclooxygenases (COXs). NSAIDs are generally used in treatment of acute and chronic inflammation-caused conditions, particularly in brain. However, an effect of NSAIDs in the kidney and particularly in the glomerulus is not clear. Transient receptor potential canonical channels (TRPC) are cation channels that are expressed along the nephron; furthermore, there has been proposed a role for TRPC channels in renal function. TRPC6 dysfunction has also been associated with the onset of focal segmental glomerosclerosis. We have addressed here a role of NSAIDs in regulation of TRPC channels in glomeruli. Western blotting confirmed that TRPC1, 3 and 6 are highly expressed in freshly isolated glomerulus of Sprague Dawley rat kidneys. Cell-attached patch clamp analysis revealed cation channel currents with distinct biophysical TRPC properties in podocytes in isolated decapsulated rat glomerulus. SKF-96365 (10 μM), a non-selective cation channels inhibitor, rapidly and markedly decreased these currents. Diclofenac (500 μM), one of well known NSAIDs, significantly decreased TRPC channel activity. Moreover, diclofenac inhibited TRPC6 when the channel was over-expressed in CHO cells. Thus this is the first report we are aware of describing single channel properties of endogenous TRPC channels in podocytes of freshly isolated rat glomerulus. In addition we provide a novel mechanism of NSAIDs regulation of TRPC channels which might be implicated in maintaining the glomerular filtration barrier.

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Disclosure of Financial Relationships: nothing to disclose

**F-PO1886**

**TRPC5 and TRPC6 Are Antagonistic Regulators of the Podocyte Actin Cytoskeleton through Differential Coupling to Rac1 and RhoA** Dequan Tian,[1] David L. Billing,[1] Anete Rozkalne,[1] Theodora Anagnostou,[1] Johannes S. Schlondorff,[2] Anna Greka.[1] *[1]Nephrology Division, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA; [2]Beth Israel Medical Center, Boston, MA.*

Dynamic regulation of the podocyte actin cytoskeleton is critical for the maintainance of the glomerular filtration barrier. Ca2+-dependent remodeling of the actin cytoskeleton is a dynamic process that drives cell migration through modulation of Rho GTPase activity. Previous work in podocytes has shown that RhoA mediates an adaptive, contractile phenotype, whereas Rac1 promotes proteinuria and progressive renal disease. Receptor-activated TRPC (Transient Receptor Potential Canonical) channels generate Ca2+ microdomains in many cell types, but antagonistic downstream pathways regulating cell migration have not been described. TRPC5 channels have been implicated in neuronal growth cone motility, and TRPC6 channel mutations have been linked to familial FSGS, but the specific mechanisms through which these channels act remain elusive.

Here we identify Angiotensin Type 1 receptor (AT1R) activated TRPC5 and TRPC6 channels as conserved antagonistic regulators of actin remodeling and cell motility in podocytes and fibroblasts. We show that TRPC5 is in a molecular complex with Rac1, whereas TRPC6 forms a molecular complex with RhoA. We further show that TRPC5-mediated Ca2+ influx induces Rac1 activation, thereby promoting cell migration. In contrast, TRPC6-mediated Ca2+ influx inhibits cell migration by increasing RhoA activity. These results suggest that under physiologic conditions, TRPC6/RhoA signaling confers a contractile phenotype, and that unopposed TRPC5 activity leads to a Rac1-mediated maladaptive migratory phenotype. In conclusion, increased TRPC5/Rac1 signaling may cause podocyte damage and proteinuria, and TRPC5 may therefore be a novel molecular target for anti-proteinuric therapies.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1887**

**Canonical Wnt/β-Catenin Signaling Mediates TGF-β1-Driven Podocyte Injury and Proteinuria** Dan Wang, Chun-Sun Dai, Youhua Liu. *Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

Podocyte dysfunction plays a causative role in the pathogenesis of proteinuria in a wide variety of primary glomerular diseases. TGF-β1, a fibrogenic cytokine that is upregulated in virtually all chronic kidney diseases, is known to induce podocyte injury, leading to defective glomerular filtration. However, how TGF-β1 causes podocyte dysfunction in vivo remains poorly understood. Here we show that canonical Wnt/β-catenin signaling plays an important role in mediating podocyte injury and proteinuria induced by TGF-β1. Incubation of mouse podocytes with TGF-β1 dramatically induced Wnt1 expression, which started as early as 1 h, reached the peak at 8 h, and sustained at least to 24 h. Several other Wnt genes including Wnt2, Wnt9a, Wnt10a, Wnt10b and Wnt11 were also induced, to a less extend, by TGF-β1. Induction of multiple Wnts by TGF-β1 resulted in marked activation of β-catenin in podocytes, as demonstrated by Western blot analysis and immunostaining. Ectopic expression of Wnt1 mimicked TGF-β1 and induced Snail expression in podocytes. In vivo, ectopic expression of constitutively active TGF-β1 via a hydrodynamic-based gene delivery approach induced Wnt1 and Snail expression in the glumeruli, which was associated with the onset of albuminuria in mice. Interestingly, simultaneous delivery of Dickkopf-1, an endogenous antagonist of Wnt/β-catenin signaling, significantly reduced TGF-β1-induced albuminuria in vivo. Taken together, these results indicate a critical role for the canonical Wnt/β-catenin signaling in mediating TGF-β1-driven podocyte injury and proteinuria.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1888**

**TGF-beta Reduces Wilms Tumor 1 Protein Expression in Podocytes In Vitro and In Vivo** Toru Sakairi, Yoshifusa Abe, Jeffrey B. Kopp. *KDS, KDB, NIDDK, Bethesda, MD.*

TGF-beta contributes to glomerulosclerosis (GS) and tubulointerstitial fibrosis. The Wilms tumor 1 protein (WT1) is a transcription factor required for normal podocyte differentiation; deletions and mutations in the WT1 gene cause syndromes associated with GS, including WAGR, Frasier and Denys-Drash. To address whether TGF-beta-induced podocyte injury might be mediated in part by altered WT1 expression, we evaluated the effect of TGF-beta1 on WT1 expression in podocytes using a human podocyte cell line (AB8/13) and Alb/TGF-beta transgenic (TG) mice, which manifest elevated plasma TGF-beta1 levels and undergo progressive GS. Following 24 hr exposure of human podocytes to TGF-beta1 (5 ng/mL), WT1 protein expression decreased as shown by Western blot and immunostaining. WT1 protein was consistently reduced with TGF-beta1, with a dose dependent effect from 0.2 ng/mL to 20 ng/mL. TGF-beta1 exposure (5 ngm/L) for 90 min reduced WT1 mRNA level, assessed by quantitative RT-PCR, 57 ± 8% ($p < 0.01$). In the presence of shRNA targeted to Smad4, WT1 mRNA was reduced with 5 ng/mL TGF-beta1 by 45±11% at eight hours compared with 80±4% in the presence of non-targeting control, suggesting a 44% abrogation of the inhibitory effect of TGF-beta1. The effect of Smad4 shRNA extended to WT1 protein as detected by Western blot. These data suggest that TGF-beta1 suppresses WT1 expression in part via a Smad dependent pathway. TG mice were studied at 1 week of age (prior to GS) and 3 weeks (early GS). By Western blot, WT1 protein expression in TG whole kidney was reduced by 23±10% ($p<0.05$) at 1 week and by 64±20% ($p<0.001$) at 3 weeks compared to wild-type mice. Immunostaining confirmed reduced WT1 expression in podocytes. WT1 mRNA expression in TG whole kidney was not altered, possibly indicating that regulation of WT1 expression in vivo differs from in vitro, or that there are species differences. In summary, TGF-beta reduced WT1 protein expression in podocytes in vitro and in vivo, at least in part via a Smad dependent pathway. These findings identify a novel pathway linking TGF-beta to podocyte injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1889**

**Transforming Growth Factor β1 Induced Podocyte Injury Is Partly Angiopoietin Mediated** Peter Margetts, Je Yen James Su, Anil Kapoor, Ayesha Ghayur. *Medicine, McMaster University, Hamilton, ON, Canada.*

Transforming growth factor β 1 (TGFβ1) has been demonstrated to cause injury to the glomerular filtration barrier. We used an adenovirus expressing either active TGFβ1 or green fluorescent protein (GPF), to transfect the kidneys of female Sprague Dawley rats. We

assayed for proteinuria along with tissue markers of fibrosis. We used immunohistochemistry and laser capture microdissection to study gene and protein expression in the glomeruli. In vitro, immortalized mouse podocytes were exposed to recombinant TGFβ1, angiopoietin-1 (ang1), or ang2. We blocked the TGFβ1 effects using antibodies against ang1, ang2 or the angiopoietin receptor Tie2. In the animal model, GPF co-localized with CD34 indicating that the glomerular endothelium was targeted by adenovirus. AdTGFβ1 induced significant proteinuria and podocyte effacement by EM. AdTGFβ1 treatment led to nephrinuria and downregulation of nephrin mRNA and synaptopodin protein in tissue samples. These changes were associated with in increase ang1 and ang2 expression in the glomeruli. In podocyte cell culture, TGFβ1 down regulated nephrin and synaptopodin in a dose dependent manner. Tie2 was not expressed on naïve podocytes, but its expression was induced by TGFβ1. Both ang1 and ang2 also downregulated these podocyte markers. A neutralizing anti-Tie2 antibody blocked TGFβ1 induced downregulation nephrin and synaptopodin. Antibodies against ang1 or ang2 also blocked TGFβ1 downregulation of nephrin.

We demonstrate that in vivo, TGFβ1 can lead to podocyte de-differentiation by suppressing key makers of podocyte integrity. In vitro, this TGFβ1 mediated process is suppressed by blocking angiopoietin signaling. This suggests that TGFβ1 mediated podocyte injury is partly angiopoietin mediated.

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**F-PO1890**

**Cathepsin L Is Transcriptionally Regulated by Dendrin and Drives Glomerular Disease Progression** Suma Yaddanapudi,[1] Mehmet M. Altintas,[2] Andreas D. Kistler,[2] Jochen Reiser.[2] *[1]Massachusetts General Hospital; [2]University of Miami Miller School of Medicine.*

Recently, a cytosolic variant of cathepsin L (CatL) has been implicated in the pathogenesis of human glomerular diseases and the LPS mouse model of proteinuria. Here, we show that cytosolic CatL is also involved in models of chronic progressive glomerular disease such as CD2AP deficient or TGF-β transgenic mice. Glomerular CatL expression in CD2AP-/- mice was normal by immunohistochemistry at the age of 1 week but increased at 3 weeks, i.e. the onset of glomerulosclerosis. Similar to the in vivo situation, conditionally immortalized podocytes from CD2AP-/- mice initially expressed low cytosolic CatL levels and normal actin cytsokeleton, but with increasing passage number, these cells undergo a phenotypic that is accompanied by the induction of high cytosolic CatL levels and cleavage of dynamin and synaptopodin along with rearrangement of the actin cytoskeleton. This phenotype could be rescued by treatment with CatL inhibitors or CatL shRNA. In both, in vivo and in vitro CD2AP-/- podocytes we found nuclear relocation of dendrin, a CD2AP-binding protein that promotes apoptosis upon nuclear import. Reporter assays in HEK cells demonstrated strong upregulation of CatL but not CatB promoter activity upon cotransfection with wild type dendrin but not with a dendrin construct devoid of the nuclear import signal. Knock down of dendrin rescued the actin phenotype of late passage CD2AP-/- podocytes and restored dynamin and synaptopodin levels. CD2AP-/- podocytes exhibit an increased susceptibility to TGF-β induced apoptosis, which was also rescued by dendrin shRNA, CatL shRNA or CatL inhibitor treatment. In line with the previous finding that TGF-β itself induces nuclear relocation of dendrin, CatL expression was also increased in TGF-β transgenic mice where it correlated with glomerular disease progression. In conclusion, we identified dendrin as a transcriptional activator of CatL. CatL not only induces acute podocyte FP effacement as shown previously, but enhances podocyte susceptibility to apoptosis and is thus involved in glomerular disease progression.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1891**

**PKCe Has Multiple Functions in Podocytes** Beina Teng, Irini Tossidou, Youying Mao, Joon-Keun Park, Hermann G. Haller, Mario Schiffer. *Nephrology, Medical School Hannover, Hannover, Germany.*

PKCε has multiple functions in podocytes

Background: Protein Kinase C (PKC) is a family of serine- and threonine protein kinases that is involved in diverse cellular functions. In recent studies it was demonstrated that the novel PKC isoform (PKCε) is a multifunctional kinase that influences a variety of cellular processes including proliferation, differentiation, apoptosis, motility, cell spreading, migration and adhesion in a wide range of cells. The role of PKCε in podocytes is still unexplored.

Methods and Results: *In vivo* in PKCε-/- mice we detected a progressive reduction of podocytes and a spontaneous glomerulosclerosis phenotype. We established immortalized cell lines for PKCε and performed migration and adhesion assays that revealed a diminished migration and adhesion of PKCε-/- podocytes. When we studied the composition of the fokal adhesion complex, we detected a strong downregulation of Integrin-β1, vinculin and Focal Adhesion Kinase (FAK) in PKCε-/- podocytes compared to PKCε+/+ podocytes. Stimulation of PKCε is required for the activation of focal adhesion components to regulate the assembly and disassembly of the focal adhesion, which influences the podocytes spreading, migration and adhesion. PKCε binds directly to actin or phosphorylates cytoskeletal proteins and plays a central role in the control of actin-cytoskeletal rearrangement by modulating multiple signalling pathways. Therefore PKCε is responsible for the extension of lamellopodia and filopodia during podocyte differentiation through turnover and actin-cytoskeletal reorganization. We performed the Actin Polymerization Assay with PKCε+/+ and deficient podocytes to see whether the deficiency of PKCε impacts the actin-polymerization in podocytes. PKCε plays also an important role in regulation of apoptosis. PKC deficiency leads to decreaded Akt and ERK1/2 activation and a prolonged apoptotic signalling after stimulation with growth factors. Moreover, PKCε is involved in Ras signalling cascade and PKCε deficiency causes arrest of cell proliferation.

Conclusion: We conclude that PKCε is an essential regulator of various cellular processes and thus is an indispensable component for normal podocyte function.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1892**

**Inhibition of MAPKAPK-2 (MK-2), a Downstream Target of p38 MAPK, Protects Podocytes from Injury** Ruma Pengal,[1] Adam Guess,[1] Shipra Agrawal,[1, 2] Rainer Benndorf,[1, 2] William E. Smoyer.[1, 2] *[1]Center for Clinical and Translational Research, The Research Institute at Nationwide Children's Hospital, Columbus, OH; [2]Department of Pediatrics, The Ohio State University, Columbus, OH.*

**Background:** The p38 mitogen activated protein kinase (MAPK) has been implicated in mediating podocyte injury in both clinical glomerulopathies and experimental models of nephrotic syndrome (NS). While targeted deletion of p38 results in prenatal death in mice, targeted deletion of one of its major downstream targets, MAPK-activated protein kinase-2 (MK-2), results in viable offspring. Based on this, we investigated the specific role of MK-2 in mediating NS-related podocyte injury.

**Methods:** We assessed the ability of MK-2 to regulate podocyte damage using C23, a novel specific inhibitor of MK-2, after exposure to puromycin aminonucleoside (PAN), a known toxic mediator of renal injury. PAN-induced injury was analyzed by measuring (i) cell viability using the MTT assay and (ii) actin cytoskeleton integrity by phalloidin staining. We also confirmed the efficacy of the MK-2 inhibitor using SDS- and isoelectric focusing followed by Western blotting to determine the degree of phosphorylation of p38 MAPK, MK-2, and the small heat shock protein Hsp25 which is a major substrate of MK-2. In addition, we measured the effect of MK-2 inhibition on the expression of Cox-2, another known mediator of podocyte injury, using qRT-PCR.

**Results:** Treatment of podocytes with C23 significantly reduced both PAN-induced cellular injury and actin filament disruption. In addition, C23 treatment also resulted in decreased MK-2 activity, as measured by phosphorylation of Hsp25. Further, qRT-PCR indicated that MK-2 inhibition led to reduced expression of Cox-2 mRNA.

**Conclusions:** These findings indicate that PAN-induced podocyte injury is mediated in part by MK-2 activation, and that MK-2 inhibition can effectively protect podocytes against injury, possibly via reduction in Cox-2 expression. We thus speculate that MK-2 inhibition may offer a more targeted therapeutic approach than p38 inhibition for podocyte protection in glomerular diseases.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1893**

**Cross-Talk between Nck1/2 and Synaptopodin Regulates Podocyte Actin Dynamics** Priyanka Rashmi,[1] Christian Faul,[2] Peter H. Mundel.[3] *[1]Graduate School, Mount Sinai School of Medicine, New York, NY; [2]Division of Nephrology, University of Miami, Miami, FL; [3]Division of Molecular Medicine, University of Miami, Miami, FL.*

Dynamic regulation of the podocyte actin cytoskeleton is paramount for proper kidney filter function and mutations affecting several podocyte proteins lead to the rearrangement of the actin cytoskeleton, disruption of the filtration barrier and renal disease. The ubiquitously expressed adapter proteins Nck1 and Nck2 modulate podocyte actin dynamics by linking phospho-nephrin to N-WASP:ARP2/3 mediated actin polymerization. Synaptopodin, another critical regulator of the podocyte actin cytoskeleton, induces stress fibers by blocking the Smurf-1 mediated ubiquitination of RhoA and suppresses Cdc42 signaling by blocking the binding of Cdc42 and Mena to IRSp53.

Here we show that in podocytes Nck1 but not Nck2 steady-state protein levels are regulated by Cbl mediated ubiquitination and subsequent proteasomal degradation. The inactivation of an evolutionarily conserved lysine residue reduces Cbl binding and proteasomal degradation of Nck1. Gene silencing of synaptopodin in podocytes reduces Nck1 but not Nck2 protein steady-state levels. Synaptopodin directly binds to Nck1 and competes with Cbl for Nck1 binding, thereby preventing its ubiquitination and subsequent proteasomal degradation. Of note, similar to synaptopodin, both Nck1 and Nck2 are targets of cathepsin L mediated proteolysis. Functionally, the overexpression of Nck1 or Nck2 in differentiated podocytes disrupts stress fibers and reduces synaptopodin levels, which can be rescued by the calcineurin inhibitor cyclosporine A or the cathepsin inhibitor E64.

Our data reveal a feedback loop whereby reciprocal control of synaptopodin and Nck1/2 steady state protein levels dynamically regulates the podocyte actin cytoskeleton. In summary, these results demonstrate a role for synaptopodin as inhibitor of Nck1 ubiquitination and proteasomal degradation. Together with the recently identified inhibition of Smurf1 mediated ubiquitination of RhoA, they reveal synaptopodin as a comprehensive inhibitor of E3 ubiquitin ligase signaling for the modulation of podocyte actin dynamics.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1894**

**Podocyte-Specific Deletion of Prohibitin-2 Leads to Progressive Proteinuria, Glomerular Sclerosis and Premature Death** Christina Ising, Sebastian Braehler, Bernhard Schermer, Thomas Benzing, Paul T. Brinkkoetter, Christine E. Kurschat. *Deparment of Nephrology, University of Cologne, Germany.*

Patients with mitochondrial disorders present with a variety of clinical symptoms including nephrotic syndrome and FSGS. The mechanisms how mitochondrial dysfunction leads to glomerular insufficiency are poorly understood. Prohibitin-1 (PHB1) and prohibitin-2 (PHB-2) are two closely related proteins that reside within the inner mitochondrial membrane. Both are essential for mitochondrial membrane stability and structure. They directly interact by forming multimeric ring complexes and stabilize each other. Here, we report a podocyte-specific prohibitin-2 knockout mouse model resembling the glomerular lesions observed in patients with mitochondrial dysfunction.

PHB-2-deficiency resulted in disorganized and swollen mitochondrial cristae in vitro. Mitochondrial membrane potential and enzymatic activities of respiratory complexes were unaffected. Depletion of PHB-2 led to decreased levels of PHB-1. The cells showed markedly reduced proliferation and an increased susceptibility to apoptotic stimuli.

PHB-2 knockout mice were born in normal mendelian ratios. At birth, glomerular development appeared to be normal. PHB-2 null mice rapidly developed significant proteinuria leading to growth retardation, massive loss of body weight and premature death. Kidney histology revealed collapsing glomerular capillaries, focal glomerulosclerosis and severe tubular atrophy with pseudostroma formation. Podocyte PHB-2 null mice did not survive longer than 5 weeks. In contrast, heterozygous floxed PHB-2;Cre positive mice showed normal kidney function.

In conclusion, the mitochondrial proteins PHB-1/-2 play an important role in glomerular function. Loss of these proteins leads to massively impaired glomerular filtration and limited survival. This knockout mouse model reflects the glomerular dysfunction observed in patients with mitochondrial disorders and provides a powerful model to further investigate the impact of mitochondrial function on glomerular biology.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1895**

**Role of CD2AP in Mitochondrial Fission and Fusion** Kumiko Moriwaki,[1] Mehmet M. Altintas,[1] Changli Wei,[1] Jing Li,[1] Hideyasu Kiyomoto,[2] Jochen Reiser.[1] *[1]Division of Nephrology and Hypertension, University of Miami, Miami, FL; [2]Division of Nephrology and Dialysis, Kagawa University, Kagawa, Japan.*

Mitochondrial dysfunction can be associated with focal segmental glomerulosclerosis (FSGS). Podocytes are target cells in FSGS and their mitochondrial regulation is not well investigated. We analyzed mitochondrial function in CD2AP knockout podocytes. Accordingly, mice lacking CD2AP or humans with CD2AP mutations develop FSGS. When we compared mitochondrial morphology of CD2AP-/- podocytes to wt podocytes, we found increased size and ballooned mitochondria that were often elongated in diseased cells similar to wt podocytes treated with puromycin, but clearly different from normal mitochondrial networks in untreated wt podocytes. We next studied mitochondrial fusion and fission, which are necessary to maintain functional integrity. In mammalian cells, fission requires dynamin-related protein 1 (DRP1) and fusion requires optic atorophy 1 (OPA1). DRP1 expression in CD2AP-/- podocytes was lower than in wt podocytes. In contrast OPA1 expression increased in CD2AP-/- podocytes. We monitored intracellular pH using 5-chloromethylfluorescein diacetate to investigate whether the alterations of mitochondrial morphology relate to its dysfunction known to affect the cytosol. Intracellular pH in CD2AP-/- podocytes was lower than in wt podocytes. To better understand the link of mitochondrial dysfunction and acidic cytosolic pH in CD2AP-/- podocytes, we analyzed expression of H+ transporters on mitochondrial membranes and found expression of SNAT3, a classical Glutamine-H+ antiporter by immnogold electron microscopy. SNAT3 expression was increased in podocytes during injury and might contribute to podocyte pH regulation. We are currently forcing expression of SNAT3 in podocytes using gene-transfer in normal and diseased mice to analyze effects on glomerular barrier function.

In summary, our data describes novel roles for CD2AP in mitochondrial morphogenesis via regulation of mitochondrial fission and fusion proteins. Mitochondrial function in podocytes is linked to podocyte cytosolic pH regulation via specific transporters such as SNAT3 to impact on the glomerular filter.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1896**

**Podocyte Specific crif1 KO Mice Show Severe Mitochondrial Deformity and Massive Albuminuria** Dae Eun Choi,[1] Jin Young Jeong,[1] Ji Yoon Jung,[1] Dong-Suk Chang,[1] Sarah Chung,[1] Yoon-Kyung Chang,[2] Ki Ryang Na,[1] Kang Wook Lee,[1] Young Tai Shin.[1] *[1]Internal Medicine, Chungnam National University Hospital, Daejeon, Korea; [2]Internal Medicine, Daejeon St Mary's Hospital, Daejeon, Korea.*

**Introduction**: Most of chronic renal diseases are associated with podocyte injury. Podocytes are important constituent of the glomerular filtration barrier. Although studies for podocyte injury using the models of diabetic glomerulonephritis (GN), drug induced GN, and hypertensive GN were done actively, there were few study of podocyte specific injury in vivo. Also, although some studies suggested mitochondrial injury of podocyte is important in progressive glomerular disease, there were few study of podocyte specific mitochondrial injury in glomerulonephritis. We made the model of podocyte specific mitochondrial injury using Cre-lox system. We studied the effects of mitochondrial injury to podocyte in vivo.

**Methods**: We made podocyte specific crif1 knockout (KO) mouse via mating C57Bl/6 background crif1-flox mouse with podocin Cre mouse. We compared control mice (crif1 (flox/flox)) to podocin specific crif1 KO mice (crif1 (Δ/Δ)). We observed metabolic finding including urine albumin, creatinine, and volume and body weight in these mice. We evaluated mouse kidney using H&E stain and electromicroscopy.

**Results**: Podocyte specific crif1 KO mice showed extensive mitochondrial swelling, broken cristae structure, and increase numbers of abnormal mitochondria in only podocyte at 5 weeks old. The levels of urine albumin/creatinine were increased markedly in crif1 (Δ/Δ) compared with crif1 (flox/flox) (45±14mg/mg vs. 1547±151mg/mg, $p<0.001$). There were no specific significant differences in body weight, urine volume, glomerular morphology in H&E stain, and the structure of foot process in electromicroscopy in both 5 weeks old mice.

**Conclusion**: podocyte specific crif1 KO mice showed severe podocyte specific mitochondrial deformity and massive albuminuria in spite of showing normal structure of foot process of podocyte.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1897**

**Angiotensin II Induces Reorganization of Actin Cytoskeleton and Myosin Light Chain in Podocytes through Rho/ROCK Signaling Pathway** Cheng Chen,[1] Wei Liang,[1] Zhilong Ren,[1] Guohua Ding,[1] Pravin C. Singhal.[2] *[1]Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [2]Department of Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.*

Accumulating evidence suggests that cytoskeleton reorganization is involved in podocyte injury, finally leading to glomerulosclerosis. Rho/ROCK(Rho-associated kinases) has been demonstrated to play a crucial role in cellular cytoskeleton remodeling through serine-theronine phorsphorylation of myosin light chain phosphatase (MLCP). In the present study, we evaluated the effect of Angiotensin II (Ang II) on actin cytoskeleton and myosin light chain (MLC) in podocyte to demonstrate whether Rho/ROCK pathway is involved in this process.

Ang II was infused in Sprague-Dawley rats via subcutaneous osmotic mini-pumps (400 ng/kg/min). Animals were sacrificed at week 4. Renal morphological changes were evaluated by light and electron microscopy. Immunohistochemistry and western blot were performed to detect the expression of p-MLC and ROCKα.

Cultured podocytes(MPC5) were incubated in media containing either buffer or Ang II($10^{-7}$mol/L) with or without the Rho-kinase (ROCK) inhibitor Y-27632 for variable time periods. F-actin was stained with FITC-conjugated phalloidin. P-MLC was evaluated by immunofluorescence and western blot. Activation of Rho/ROCK was evaluated by western blot.

P-MLC was mainly expressed in the podocytes of rat glomeruli. Compared with control group, MLC phosphorylation in Ang II-infused rat was significantly increased. ROCK expression has no significant change between two animal groups. In the cultured podocytes, Ang II stimulated the expression of Rho/ROCKα while the morphology of podocyte was simultaneously changed accompanied by increased phosphorylation of MLC and redistribution of actin. Addition of Y-27632 to podocytes treated with Ang II could ameliorate F-actin cytoskeleton remodeling and the increment of p-MLC.

These results suggest that Ang II induces remodeling of podocyte cytoskeleton via MLC phosphorylation and F-actin reorganization. Rho/ROCKα signaling transduction may play a pivotal role in this process.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1898**

**Abnormal Podocyte Structure in Nestin-Deficient and Nestin/Vimentin-Deficient Mice** Amanda Murphy,[1] Paria Mohseni,[2] Hoon-Ki Sung,[2] Richard K. Leung,[1] Susan E. Quaggin,[2,3,4] Paul S. Thorner.[1,3] *[1]Division of Pathology, DPLM, Hospital for Sick Children; [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital; [3]Faculty of Medicine, University of Toronto; [4]Department of Nephrology, St. Michael's Hospital, Toronto, Canada.*

Background: The intermediate filament nestin is expressed in podocytes at all stages of development. Most cells downregulate nestin upon differentiation, but human podocytes continue to express nestin after differentiation and in glomerular disease. Animal models of glomerular injury show increased podocyte nestin expression. Assembly of nestin requires other filaments, in particular vimentin, which co-localizes with nestin in podocytes in vivo and in vitro. We hypothesize that nestin plays important roles in podocyte development, function and/or glomerular disease.

Objective: Germline nestin knockout and nestin/vimentin double knockout mice were generated and assessed renal function, morphology and ultrastructure.

Results: Progeny are viable. Nestin-deficient mice show no increase in urinary protein excretion. Kidneys appear normal by light microscopy. Compared to wild-type controls, renal podocytes in nestin -/- mice show abnormally branched, dysmorphic primary and secondary processes. Foot processes have unusual profiles, but slit diaphragms appear normal. Nestin/vimentin double knockouts show a more severe ultrastructural phenotype, with collapsed 'limp' primary and secondary processes, stubby foot processes and no intermediate filaments in the cytoplasm.

Conclusion: Nestin deficiency has a unique phenotype not typical of any human disease nor transgenic model. This is exaggerated by concomitant deficiency of vimentin. We conclude that nestin plays an important role in process formation or maintenance. Vimentin

partially compensates for nestin deficiency in podocytes, indicating some redundancy of function. Despite the abnormal podocyte morphology, there is no obvious effect on glomerular function, suggesting the branching architecture is not critical for filtration function, as long as foot processes are intact, at least under normal conditions. Whether this holds true under conditions of stress remains to be determined.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1899**

**Podocyte-Specific Knockout of Myosin 1e Disrupts Glomerular Filtration** Mira Krendel,[1] Lawrence B. Holzman,[2] Sharon Chase.[1] *[1]Department of Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, NY; [2]Renal Electrolyte and Hypertension Division, University of Pennsylvania School of Medicine, Philadelphia, PA.*

We have previously demonstrated that knockout mice lacking an actin-dependent motor protein, myosin 1e (myo1e), develop proteinuria and effacement of podocyte foot processes (JASN, 2009, 20:86-94). In order to directly test the hypothesis that myo1e is necessary for normal podocyte functions, we have developed a new mouse strain, in which myo1e knockout is targeted to podocytes using Cre recombinase under the control of podocyte-specific NPHS2 promoter.

Immunofluorescence labeling of kidney cryosections from podocyte-specific myo1e knockout mice confirmed the loss of myo1e from podocytes. Mice lacking myo1e in podocytes developed moderate proteinuria by 8 weeks of age. The urinary albumin/creatinine ratio in podocyte-specific knockout mice was intermediate between that of control mice (normal kidney function) and myo1e-null mice (severe proteinuria). Since NPHS2 promoter becomes active only at the capillary loop stage of glomerular development, in the complete knockout myo1e may be absent from podocyte precursors at an earlier developmental stage than in the NPHS2-driven knockout and this may exacerbate glomerular abnormalities. Additionally, examination of myo1e expression pattern in podocyte-specific myo1e knockout mice revealed expression of myo1e in mesangial cells in some glomeruli, suggesting that myo1e may also be involved in mesangial cell development. In conclusion, podocyte-specific disruption of myo1e expression induces glomerular filtration defects, confirming that myo1e is required for normal podocyte activity. The timing and cell type specificity of myo1e depletion may determine the severity of kidney disease observed in myo1e-knockout mice.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1900**

**Distinct Contributions of Non-Muscle Myosin Heavy Chains IIA (Myh9) and IIB (Myh10) to Podocyte Traction and Adhesion** Joel M. Henderson,[1] Hui Chen,[1] Julie A. Tomolonis,[1] Mei Cao,[1] Martin R. Pollak.[2] *[1]Pathology, Boston University Medical Center, Boston, MA; [2]Medicine/Genetics, BIDMC/Harvard Medical School, Boston, MA.*

Purpose: Non-muscle myosin heavy chains are implicated in glomerular disease. These contractile proteins may help podocytes bolster glomerular capillary wall structure through tonic contraction. Changes which affect their function may compromise glomerular structural integrity, thereby leading to glomerular damage. The aim of this study is to characterize the functional role of non-muscle myosins in podocytes.

Methods: Myh9 and Myh10, the only myosin II heavy chain isoforms significantly expressed in cultured murine podocytes, were independently knocked down in these cells using lentiviral RNA interference. Knock down and control cells were plated on glass and polyacrylamide elastic substrates. Immunohistochemistry was performed to examine non-muscle myosin and filamentous actin distribution, and traction force microscopy was used to measure net contractile moment (an expression of inward pulling force). Blebbistatin (non-muscle myosin specific inhibitor) and lysophosphatidic acid (contractile stimulator) were applied in selected experiments.

Results: Highly efficient knockdown of Myh9 and Myh10 was achieved at the protein level using the lentiviral system. Both Myh9 and Myh10 knockdown were associated with disorganization and loss of actin stress fibers. Myh9 knock down resulted in a 52% decrease ($p=0.029$) in unstimulated traction, but only a 24% decrease in stimulated contraction (not statistically significant), relative to controls. Traction could not be measured in Myh10 knock down cells due to complete loss of adherent cells from elastic substrates. Blebbistatin almost completely abolished podocyte contractility in wild type cells.

Conclusion: Both non-muscle myosins IIA and IIB participate in cytoskeletal organization in murine podocytes, and together account for almost all traction force generation. Non-muscle myosin IIB may excede IIA in its relative contribution to stimulated traction force generation. Non-muscle myosin IIB also appears to play a unique role in podocyte adhesion.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1901**

**Zebrafish as a Model To Study MYH9-Associated Kidney Disease** Tobias Müller,[1] Elisabeth Rumpel,[1] Susanne Hradetzky,[1] Frank Bollig,[3] Christoph Englert,[3] Andreas Greinacher,[2] Karlhans Endlich,[1] Nicole Endlich.[1] *[1]Anatomy and Cell Biology, Ernst Moritz Arndt University, Germany; [2]Immunology and Transfusion Medicine, Ernst Moritz Arndt University, Greifswald, Germany; [3]Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, Germany.*

The simplicity and the rapid development of the zebrafish pronephros together with the similarity of the glomeruli of zebrafish and mammals make the zebrafish an ideal model system for studying kidney development and function. After 48-54 h post fertilization (hpf) the zebrafish larvae start with glomerular filtration. Furthermore, zebrafish larvae are translucent during the first 96-120 hpf, which makes it possible to study the filtration processes in vivo after microinjection of fluorescently labeled dextran. Using this model we studied the influence of the heavy chain of non-muscle myosin IIA (NMMHC-IIA) on pronephros development. Three days after microinjection of morpholinos against zMyh9 into fertilized eggs we observed a phenotype in 96% of the zebrafish larvae. Beside a defect in heart development, we observed strong pericardial edema. Similar effects were observed after incubation of the larvae for 24 h with 200 µM blebbistatin, an inhibitor of non-muscle myosin II. To study the effect of zMyh9 knockdown in detail we used transgenic zebrafish larvae expressing EGFP in podocytes and tubular epithelial cells (WT1b:EGFP). Immunohistological studies revealed that zMyh9 knockdown larvae exhibited morphological changes of the glomeruli. Electron microscopy showed impaired capillarization of the glomerulus, partly thickening of the glomerular basement membrane and filopodia-like foot processes in zMyh9 morpholino injected larvae compared to control morpholino injected ones. To study the influence of zMyh9 on glomerular filtration, we injected 10 kDa Alexa-467- and 500 kDa FITC-labeled dextrans in vivo into the caudal vein of zebrafish larvae around 96 hpf. With this method we found that glomerular filtration of zMyh9 morpholino injected larvae was significantly reduced in contrast to the controls. Taken together, our results demonstrate that zMyh9 plays an essential role in glomerular development in zebrafish.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1902**

***PAI-1*$^{-/-}$ Podocyte Resistance to Injury Is Not Overcome by Exogenous PAI-1 Suggesting Possible Intracrine PAI-1 Effects** Haichun Yang,[1] Jianyong Zhong,[2] Ji Ma,[2] Agnes B. Fogo.[1] *[1]Pathology, Vanderbilt University, Nashville, TN; [2]Pediatric Nephrology, Vanderbilt University, Nashville, TN.*

We investigated mechanisms of PAI-1 effects on podocyte injury by comparing podocytes with genetic deficiency of PAI-1 *vs.* exogenous restoration of PAI-1.

Primary podocytes were harvested from either *PAI-1*$^{-/-}$ (KO) or wild type (WT) mice, injured by puromycin aminonucleoside (PAN) and various human PAI-1 variants were added: control PAI-1, retaining native PAI-1 functions (CPAI); PAI-1 variant without VN binding but with normal proteolysis inhibition (Q123K); PAI-1 without proteolytic inhibition but with intact VN binding (T333R), and PAI-1 with reduced LRP-binding (R76E).

Endogenous PAI-1 entered cells resulting in increased PAI-1 in cell lysates. WT cells exposed to PAN had more apoptosis (by 34%) and cleaved caspase-3 (by 28%) *vs.* KO + PAN. In KO + PAN, exogenous PAI-1 with intact proteolytic inhibitory activity did not stimulate cleaved caspase-3 (CPAI 0.39±0.04, Q123K 0.26±0.04, R76E 0.46±0.10 *vs.* KO 0.57±0.08). In contrast, exogenous PAI-1 without proteolytic inhibitory activity (T333R) significantly restored caspase-3 to WT level (T333R 0.82±0.16, WT 0.79±0.07). In parallel, only T333R PAI-1 decreased Bcl-xl in PAN-injured KO cells (0.19±0.13), with no change in Bcl-xl in other groups (CPAI 0.48±0.03, Q123K 0.48±0.02, R76E 0.60±0.03). WT cells injured by PAN had decreased α-actinin 4 expression by 23.5% *vs.* KO, while none of the exogenous mutant PAI-1 variants changed the relative preservation of α-actinin 4 expression in KO podocytes injured with PAN.

Our data show that *PAI-1*$^{-/-}$ podocytes are protected from apoptosis and cytoskeletal disarray, and further, that addition of exogenous PAI-1 does not restore injury of cytoskeleton to WT levels, although apoptotic injury can be restored by adding proteolytically active PAI-1. Thus, these data suggest that endogenous and exogenous PAI-1 mediate podocyte cytoskeletal injury and apoptosis through different pathways. Endogenous PAI-1 may induce podocyte cytoskeletal injury as an intracrine peptide. Conversely, genetic deficiency of PAI-1 may protect cells against injury independent of classic PAI-1 pathways.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1903**

**Glomerular Destabilization Causing Autonomous Podocyte Loss and Progression. Restabilization by Angiotensin II Blockade, and Monitoring by Podocyte Urine Biomarkers** Akihiro Fukuda,[1] Yuji Sato,[2] Madhusudan M. Venkatareddy,[1] Larysa T. Wickman,[1] Mahboob A. Chowdhury,[1] Su Qing Wang,[1] Jocelyn E. Wiggins,[1] Roger C. Wiggins.[1] *[1]Nephrology Division, Departments of Internal Medicine, University of Michigan, Ann Arbor, MI; [2]Circulatoly and Body Fluid Regulation, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan.*

Podocyte depletion is the major mechanism driving glomerulosclerosis. Progression is the process by which progressive glomerulosclerosis leads to End Stage Kidney Disease (ESKD). The human diphtheria toxin receptor transgenic Fischer 344 rat model of controlled podocyte loss was used to dissect the progression process. After initial podocyte injury causing > 30% loss of podocytes the glomeruli became destabilized, resulting in continued

autonomous podocyte loss over time until global podocyte depletion (ESKD). Podocyte loss was measured by WT1 positive nuclear counting, the GLEPP1 positive podocyte tuft area, and by urine podocin mRNA. Autonomous podocyte loss from glomeruli eventually resulted in global depletion of podocytes with marked glomerular and interstitial fibrosis and ectatic tubules typical of ESKD. An identical process occurred in progression initiated by Puromycin aminonucleoside in the Sprague-Dawley rat, thereby demonstrating broad applicability of the observation. Angiotensin II blockade (using a combination of enalapril and losartan administered in the drinking water) prevented autonomous podocyte loss and progression (restabilization). Discontinuing angiotensin II blockade resulted in recurrent glomerular destabilization and podocyte loss. Furthermore, blood pressure control by non-angiotensin II blockade (using a combination of hydralazine, reserpine and hydrochrolothiazide administered in the drinking water) did not prevent autonomous podocyte loss. Therefore a critical degree of initial podocyte depletion destabilizes the glomerulus such that it autonomously loses podocytes in an angiotensin II-dependent manner until glomeruli become globally depleted of podocytes (ESKD). Glomerular destabilization can be monitored non-invasively by measuring podocyte mRNAs in urine.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1904**

**Podocyte-Specific Expression of Tamoxifen-Inducible Cre Recombinase in Mice** Hideki Yokoi,[1,2] Masato Kasahara,[1,2] Masashi Mukoyama,[1] Kiyoshi Mori,[1] Takashige Kuwabara,[1] Akira Sugawara,[1] Kazuwa Nakao.[1] *[1]Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Department of Nephrology and Blood Purification, Institute of Biomedical Research and Innovation, Kobe, Hyogo, Japan.*

Podocytes play an important role in maintaining normal glomerular function. A podocyte-specific conditional knockout technology has been established by the use of transgenic mice expressing podocyte-specific Cre recombinase to clarify the role of genes expressed in podocytes. Cre/loxP system has been used for conditional knockout mice. Although podocyte-specific conditional knockout mice are useful in investigating the role of the gene, several podocyte-specific knockout mice exhibit congenital renal abnormalities and perinatal death, which is caused by the disruption of the gene in the embryonic kidney stage. This strategy often has difficulty in elucidating the role of the gene in the diseased or postnatal kidney. Recently, efforts have been undertaken to create a temporal regulation for Cre recombination by tamoxifen or tetracycline. To introduce temporal control in genetic experiments targeting the podocyte, we constructed tamoxifen-inducible Cre-recombinase ($CreER^{T2}$) transgenic mice under the control of podocyte-specific promoter, 2.5-kb fragment of the human podocin (NPHS2) gene. Specificity and efficiency of Cre activity were examined by crossing NPHS2-$CreER^{T2}$ with the ROSA26 reporter (R26R) mouse in which a floxed-stop cassette has been placed upstream of the β-galactosidase gene. Four-week old double-mutant mice (NPHS2-$CreER^{T2}$/R26R) were intraperitoneally administered with 0.5 mg of 4-hydroxytamoxifen (4-OHT) for 3 consecutive days. NPHS2-$CreER^{T2}$/R26R treated with 4-OHT expressed β-galactosidase specifically in 85% of podocytes in glomeruli. Expression of Cre recombinase mRNA was mostly restricted in the kidney, especially in glomeruli. In conclusion, we have successfully generated podocyte-specific inducible Cre transgenic mice by tamoxifen administration. These mice allow us to disrupt genes specifically in podocytes after birth.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1905**

**Urinary Podocin: Nephrin mRNA Molar Ratio (PNMR) as a Non-Invasive Biomarker of Podocyte Stress** Larysa T. Wickman,[1] Akihiro Fukuda,[2] Madhusudan M. Venkatareddy,[2] Su Qing Wang,[2] Mahboob A. Chowdhury,[2] Jocelyn E. Wiggins,[2] David B. Kershaw,[1] Matthias Kretzler,[2] Roger C. Wiggins.[2] *[1]Pediatrics, University of Michigan, Ann Arbor, MI; [2]Internal Medicine, University of Michigan, Ann Arbor, MI.*

BACKGROUND: At the present time treatment decisions for patients with kidney disease are based on measurements of renal function which are inexact, or on repeat kidney biopsies which are invasive and carry risk. This means that therapeutic decisions are often delayed until substantial renal function has been lost. Progression to end stage kidney disease following glomerular injury is caused by persistent podocyte depletion. In rat model systems we have shown that podocyte loss can be monitored through podocyte specific urinary mRNAs (Sato et al. JASN, 2009). OBJECTIVE: To identify podocyte specific markers that can reflect changes in podocyte behavior related to progression using a combination of animal models and human urine samples from patients with glomerular diseases. METHOD: mRNAs from rat and human urine pellets and transgenic rat cortical renal tissues were extracted, reverse transcribed, and cDNAs quantitated using Q RT-PCR and cDNAs as standards. Because both Podocin and Nephrin are expressed in the same cell, the PNM ratio corrects for RNA stability, sample size, concentration, contamination from non-kidney sources, and variation in reverse transcription efficiency. RESULTS: The rates of podocyte mRNA decay for podocin and nephrin mRNAs in urine were paralleled, so that expressing data as a ratio gives reproducible values irrespective of urine collection conditions or amount of urine used. Animal data show a strong correlation between urinary and cortical PNMR, and also correlates with severity of the glomerular injury as determined histologically. Initial data from patients with various forms of glomerular diseases shows elevated levels of urinary PNMR. CONCLUSIONS: Combined animal and human data suggest that urinary PNMR reflects the progression process occurring in the glomerulus and can serve as a noninvasive biomarker of podocyte stress for use in the clinic.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1906**

**The Role of Circulating Proteases in FSGS** Jessica Harris, Gavin Iain Welsh, Moin Saleem. *Academic Renal Unit, University of Bristol, Bristol, United Kingdom.*

Focal Segmental Glomerulosclerosis (FSGS) is a steroid resistant form of nephrotic syndrome leading to end stage renal disease. The podocyte is known to be the target of injury for this disease, undergoing a dramatic change of shape and eventual loss from the glomerular basement membrane. However little is known about the external stimulus for these changes.

Here we have studied the effects of nephrotic and non-nephrotic plasma, obtained from patients with post transplant FSGS relapse, at commencement of plasma exchange and then when they are in remission, on conditionally immortalised cultured human podocytes (ciPods). In 4 consecutive patients we show that the actin associated protein VASP is phosphorylated in response to relapse plasma, with no response to remission plasma. Functionally, ciPods treated with relapse plasma show reduced adhesion compared to cells treated with remission plasma.

We investigated the role of circulating proteases in this signalling to VASP and show that inhibition of proteases in nephrotic plasma inhibits VASP phosphorylation. We show that the class of protease involved is likely to be a serine protease and, using siRNA technology, determine that plasma induced signalling is taking place via protease activated receptor 1 (PAR1).

Thus we hypothesise that excess activity of plasma proteases is involved in the pathogenesis of post transplant recurrence of FSGS, with VASP phosphorylation as a specific biomarker of disease activity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1907**

**MDR-1 Gene Polymorphisms in Steroid Resistant Nephrotic Syndrome** Narayan Prasad. *Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, UP, India.*

**INTRODUCTION AND AIMS:**

The putative genetic regulation of MDR-1gene expression and P-gp function has not yet been clearly delineated in Nephrotic syndrome(NS) patients. We undertook this study to see the distribution of 3 most frequent MDR-1 exonic polymorphisms G3435C, G2677T (A) and C1236T in our cohort of NS cases and age and sex matched healthy children to see their usefulness as marker of steroid responsiveness.

**METHODS:** 216 children of NS and 216 healthy age, sex and ethnically matched controls were genotyped for the three exonic MDR-1 polymorphisms (G3435C, G2677T (A) and C1236T) by the PCR-RFLP technique. Genotype/allele frequencies were compared in between steroid sensitive (SSNS) and steroid resistant (SRNS) patients and between patients and healthy controls.

**RESULTS:** Of the total 216 cases of NS (mean age 11 6.6 years, 165 males), 137 were responsive and 79 resistant to steroids. The genotype frequencies were in Hardy-Weinberg equilibrium. Homozygous mutants of MDR1-C3435T (TT versus CC, $\chi^2$= 4.51, p= 0.034) and MDR1-G2677T/A (TT+AA vs GG): $\chi^2$= 4.71, p= 0.030)] were significantly high in NS patients compared to control. On comparing SRNS versus SSNS, homozygous mutant TT+AA compared to wild genotype GG was significantly higher in SRNS than SSNS patients($\chi^2$= 6.53, p= 0.011) in SNPs MDR1-G2677T/A , while other markers C3435T and C1236T were not different. On analyzing synergistic effect of 2 or 3 SNPs in combination amongst SSNS, SRNS and controls , the combination bearing mutant genotype either of C3435T or G2677T/A exhibited significantly high frequency of mutant genotypes distribution in SRNS patients. MDR-1 haplotypes did not differ significantly between SSNS and SRNS patients. On linkage disequilibrium analysis, highest LD value ($r^2$ =0.08; D'=0.35) corresponds to SNP's C3435T-C1236T. Lower LD values was found between the SNP's G2677T/A-C1236T ($r^2$ =0.007; D'=0.16) and between C3435T-G2677T/A ($r^2$ =0.005; D'=0.11).

CONCLUSIONS:

Patients carrying homozygous mutant genotype (G2677T/A (GG + GT/GA v/s TT/AA)alone or in different combinations are more prone to develop steroid resistance in north Indian NS patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1908**

**Novel Podocyte Protein Pdlim2 Is Differently Expressed in Human Proteinuric Diseases** Fredrik S. Dunér,[1] Jaakko Patrakka,[3] Kjell R. Hultenby,[2] Annika Wernerson.[2] *[1]Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, Stockholm, Sweden; [2]Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; [3]Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.*

The cytoskeleton of podocyte foot processes is critical for their shape and function, and mutations in several cytoskeleton-associated proteins are associated with nephrotic syndrome. Pdlim2 is a novel member of an ALP subfamily of cytosolic proteins that associate with the cytoskeleton. Our aim was to study its expression in proteinuric disease.

Materials and methods:

Biopsies from nephrotic patients fulfilling the histological criteria for focal segmental glomerulosclerosis, perihilar type (FSGS, n=4), minimal change nephropathy (MCNS, n=5) and membranous nephropathy (MN, n=5) were studied. The expression of pdlim2 in

podocytes was assessed with immunofluorescence and semiquantitative immunoelectron microscopy (immunoEM) using gold-conjugated polyclonal anti-pdlim2 antibodies. The concentration of pdlim2 was expressed as the number of gold labels per $\mu m^2$, "Au/$\mu m^2$"

Results:

Immunofluorescence showed a strong linear staining for pdlim2 protein around glomerular capillaries in the normal kidney. No positive signal was detected outside glomeruli. By immunoEM, pdlim2 was localized centrally in the cytoplasm of the podocyte foot processes, corresponding to the actin microfilaments. The labeling was significantly lower in patients with MCNS (6.4 ± 3.6 Au/$\mu m^2$) and in MN (6.7 ± 2.6 Au/$\mu m^2$), whereas in FSGS patients, it was unchanged (19.7 ± 7.9 Au/$\mu m^2$) compared to controls (19.5 ± 8.7 Au/$\mu m^2$).

Conclusion:

In the normal human kidney, the novel protein pdlim2 is exclusively expressed in podocyte foot processes, associated with the actin cytoskeleton. Its expression was lower in MN and MCNS compared to FSGS, in which pdlim2 was unchanged compared to controls. This might be of pathogenetic and diagnostic importance.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1909**

**Steroid Resistance of Nephrotic Syndrome: Glucocorticoid Receptor-β and Receptor-γ Exert Dominant Negative Effect on Gene Repression but Not on Gene Induction** Yoshinori Taniguchi. *Endocrinology, Metabolism and Nephrology, Kochi Medical School, Nankoku, Kochi, Japan.*

**Background.** Glucocorticoid has diverse biological effects through induction or repression of its target genes via glucocorticoid receptor (GR). In addition to the wild-type GR (GR-α), a variety of GR variants has been reported, and these are thought to modify glucocorticoid action. Among others, GR-β is reported be responsible for the glucocorticoid resistance frequently observed in steroid-resistant nephrotic syndrome, rheumatoid arthritis, and hematologic tumors, although the precise molecular mechanism remains unclear.

**Methods.** In this study, we examined the function of GR-β and some GR variants (GR-γ and GR-Δ313-338) on (i) trans-activation, (ii) cis-repression and (iii) trans-repression using GR-deficient BE(2)C and T84 cells *in vitro*.

**Results.** We found that GR-β, when expressed alone, completely lost the capacity of both *trans*-activation and *trans*repression on GR target genes. Interestingly, however, GR-β showed a dominant-negative effect on GR-α only for its *trans*-repressive effects on cAMP-mediated and cAMP response elementdependent genes. Furthermore, both GR-β and GR-γ had dominant-negative effects on GR-α selectively for its *trans*-repressive effects on nuclear factor-κB-mediated and inflammation-related genes. **Conclusion.** These results suggest that 1) the GR-β variant by itself has no receptor function, but 2) GR-β and GR-γ have properties to exert dominant-negative effects on the GR-α-mediated *trans*-repression, which may be responsible for the steroid resistance frequently observed in chronic inflammatory diseases including nephritic syndrome and lupus under glucocorticoid therapy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1910**

**ACTH (Acthar Gel) Prevents Proteinuria and Renal Injury in the Remnant Kidney: Evidence for Direct Podocyte Protection** Rujun Gong, Lance D. Dworkin. *Brown Medical School, Providence, RI.*

Evidence suggests that corticotrophin (ACTH) reduces urine protein excretion in patients with a variety of glomerular diseases, however, the mechanism of its anti-proteinuric action remains elusive. We examined rats after 5/6 nephrectomy that received Acthar (ACTH) (10 IU/kg) or vehicle (CON) every other day for 5 weeks. Compared to CON, ACTH slowed body weight gain and diminished urine protein excretion (50.78 ± 13.37 *vs* 94.20 ± 13.69 mg/d, $P = 0.046$). Compared to CON, ACTH preserved kidney function as measured by increased renal plasma flow (0.77 ± 0.15 *vs* 0.41 ± 0.05 ml/min/100g *wt*, $P = 0.04$), glomerular filtration rate (0.24 ± 0.07 *vs* 0.14 ± 0.03 ml/mim/100g *wt*, $P = 0.07$) and lower serum creatinine levels (0.19 ± 0.03 *vs* 0.52 ± 0.13 mg/dl/100g *wt*, $P = 0.037$) . Glomerulosclerosis and tubulointerstitial fibrosis revealed by Masson's trichrome staining, renal inflammation characterized by infiltration of ED-1 positive macrophages, tubular atrophy, and tubular epithelial to mesenchymal transdifferentiation marked by expression of a-smooth muscle actin were all reduced by ACTH therapy. Of note, compared to CON, ACTH had no significant effects on mean arterial pressure (151.5 ± 9.8 *vs* 169.4 ± 8.1 mmHg, $P = 0.2$) or kidney hypertrophy assessed by kidney-to-body weight ratio (0.498 ± 0.033 *vs* 0.524 ± 0.034 g/100g *wt*, $P = 0.6$). Because glucocorticoid therapy exacerbates proteinuria and glomerulosclerosis in this model, the protective effect of ACTH is also unlikely to result from steroidogenesis. Instead, we found evidence for direct podocyte protection in remnant glomeruli. This included reduction in foot process effacement (by electron microscopy), diminished podocytic apoptosis (by TUNEL staining), and lesser reductions in glomerular expression of podocyte markers including vimentin, nephrin, podocin and WT-1 (by fluorescent and peroxidase immunohistochemistry and immune blot) in ACTH treated rats. Our data suggest that ACTH reduces proteinuria and injury in this model of progressive glomerulosclerosis *via* direct podocyte protection.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1911**

**Activation of Cell Cycle in Podocytes of Developing Glomeruli** Ji Ma, Nobuaki Takagi, Taiji Matsusaka, Iekuni Ichikawa. *Pediatric Nephrology, Vanderbilt University, Nashville, TN.*

Our preliminary studies in adult transgenic mice showed that induction of Simian Virus 40 T antigen (SV40T) gene expression in podocytes by doxycycline induced cell cycle re-entry, mitosis and cell division of podocytes. However, damage in podocytes was noted as albuminuria at 1 week after doxycycline treatment, followed by decreased expression of podocyte differentiation markers and increased expression of desmin at 2 weeks, then by glomerulosclerosis at 3 weeks. These data suggest that, in podocytes of adult glomeruli, forced re-entry of cell cycle alone does not lead to increased number of differentiated podocytes. In the present study we tested the possibility that, in developing glomeruli, SV40T-expressing podocytes may differentiate into normal podocytes and enrich the population of podocytes, which will decrease the susceptibility to later glomerular damage.

Starting at birth, double transgenic mice carrying both podocin-rtTA (R) and TRE-SV40T (S) transgenes and their littermate control mice carrying only podocin-rtTA were given doxycycline continuously through the nursing mother till 3 weeks of age, when urinary albumin excretion and kidney morphology were analyzed. While there was no significant difference in kidney weight or podocyte size, mice with SV40T expression in podocytes (RS) showed increased number of podocytes by 17.6±4.3% ($P<0.01$, N=5 pairs) with parallel increase in glomerular volume. There was slightly increased urinary albumin excretion in RS mice when compared with the control R mice (RS 90.4±22.6 *vs.* R 51.3±12.3 μg/mg creatinine, n=8 for RS and 7 for R, $P=0.2$ for group-wise comparison and $P<0.05$ for pair-wise comparison).

Thus, these data indicate that podocyte population can be increased in developing mice, which, by influencing glomerular development, may increase the resistance to glomerular injury after maturation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1912**

**Cytokine-Like Factor (CLF) Blocks the Effect of Cardiotrophin-Like Cytokine-1 (CLC-1) on Glomerular Permeability and May Point to Therapy for FSGS** Virginia J. Savin,[1] Mukut Sharma,[1] Ellen T. McCarthy,[2] Ram Sharma,[1] Jean-Francois Gauchat.[3] *[1]Medicine, MBRF, KC VA Medical Center, Kansas City, MO; [2]Kidney Institute, KU Medical Center, Kansas City, KS; [3]Pharmacology, University of Montreal, Montreal, Canada.*

A circulating substance causes recurrence of focal segmental glomerulosclerosis (FSGS) post-transplant and increases albumin permeability of isolated glomeruli ($P_{alb}$); plasma of normal humans blocks the permeability effect. We have identified cardiotrophin-like cytokine-1 (CLC1), a member of the IL6 family, as a candidate for this substance. CLC1 requires a tripartite receptor consisting of gp130, ciliary neurotrophic factor receptor (CNTFR) and leukemia inhibitory factor receptor-ß (LIFRß); podocytes express all 3 receptor components. CLC1 increases glomerular $P_{alb}$ in a dose-dependent fashion and decreases nephrin expression (RT-PCR); injection of CLC1 into rats increases urinary protein/creatinine by 3 fold. Strikingly, mAb to CLC1 prevents the increase in $P_{alb}$ by FSGS sera. CLC1 secretion requires a secretory partner, either CLF or soluble CNTFR (sCNTFR). The effect of secretory partners on activity of CLC1 is not known. To determine whether CLF alters the permeability effect of CLC1, we incubated normal rat glomeruli with CLC1 or with the complex cytokine CLC1/CLF and determined $P_{alb}$. Incubation with 0.1 ng/mL CLC1 increased $P_{alb}$ (0.45±0.04, N=3; mean ± SEM, N= # groups). Maximal effect of CLC1 occurred at concentrations of 5 ng/mL or higher ($P_{alb}$= 0.73±0.04, N=11, $p<0.001$). In contrast, CLC1/CLF complex, $10^{-3}$ to 10 ng/mL, had no effect on $P_{alb}$ (0.03±0.02, N=9). This result supports the concept that naturally occurring molecules related to CLC1 can block the its permeability effect. CLF may block CLC1 activity by binding to site III of CLC1 and interfering with interaction between CLC1 and the Ig-like domain of LIFRß. We propose that CLF may be the blocking substance of normal plasma and that CLF deficiency may increase CLC1 activity and cause glomerular injury in FSGS. In the future, CLF and similar molecules may be exploited in therapy of FSGS in humans.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1913**

**Lack of Heparan Sulphate in Zebrafish Results in Podocyte Foot Process Effacement and Ultrastructural Changes of the Glomerular Basement Membrane** Ramzi Khalil,[1] Danielle Cohen,[1] Malgorzata Wiweger,[1] Raimond B. G. Ravelli,[2] Cristina Avramut,[2] Wietske Van der Ent,[1] Emile De Heer,[1] Jan A. Bruijn,[1] Pancras C. W. Hogendoorn,[1] Hans J. Baelde.[1] *[1]Pathology, Leiden University Medical Center, Netherlands; [2]Molecular Cell Biology, Leiden University Medical Center, Netherlands.*

Heparan sulfate (HS) proteoglycans are thought to significantly contribute to the charge selectivity of the glomerular basement membrane. Their negative charge is conveyed by covalently attached HS. Key components in the HS chain elongation are the *ext1* and *ext2* gene products encoding subunits of HS co-polymerase. The aim of this project is to determine the contribution of HS to the function and morphology of podocytes in wild type and HS-deficient zebrafish embryos.

The zebrafish (*Danio rerio H*) *dackel* (*dak*) mutant has a premature stop codon in the *ext2* gene. As the result of this mutation the truncated transcript is degraded, resulting in Ext2-deficiency in these embryos. This leads to a complete lack of HS in homozygous mutants.

Wild type larvae and homozygous *dak* mutants at 5 days post fertilization (dpf) were anesthetized, chemically fixed and embedded in Epon resin. Ultrathin sections (100nm) were cut with Leica ultramicrotome EM UC6, post stained with 7% uranyl acetate and Reynolds's lead citrate, and examined at 120kV in a FEI electron microscope (Technai 12).

The homozygous *dak* mutant showed changes in podocyte morphology, such as foot process effacement and structural changes of the glomerular basement membrane. Similar changes were previously reported in mice with a podocyte-specific *Ext1* knockout, resulting in only a partial lack of HS, whereas the homozygous *dak* mutant has a complete lack of HS. We use this zebrafish model for the morphological validation of a potentially functional model to study the contribution of HS to the glomerular charge-selective barrier. Functional studies are underway to investigate whether the HS deficiency results in the loss of glomerular perm selectivity.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1914**

**α5 Integrin Expression and Signaling Is Downregulated in Podocytes Unable To Synthesize Heparan Sulfate Glycosaminoglycans** Kevin J. McCarthy,[1] Deborah J. McCarthy,[1] Kaitlin Marie McCarthy,[1] Shoujun Chen,[2] Yu Yamaguchi.[3] *[1]Pathology, LSU Health Sciences Center, Shreveport, LA; [2]Pathology, University of South Florida, Tampa, FL; [3]Developmental Neurobiology, Sanford-Burnham Medical Research Institute, La Jolla, CA.*

Podocytes from PEXTKO mice cannot synthesize heparan sulfate (HS) glycosaminoglycans thus their glomerular basement membranes lack significant amounts of HS. Despite the absence of HS, the animals do not develop significant proteinuria during the course of their lifetime, but their podocytes have effaced foot processes. A recent report from our laboratory showed that the HS on cell surface proteoglycans (PG) are critical determinants of podocyte adhesion and migration *in vitro* (Kidney International doi:10.1038/ki.2010.136), mediated via syndecan PGs. Microarray expression analysis of mRNAs isolated from HS+ and HS- podocytes was used to further investigate if other deficits occurred in the cell adhesion/migration pathway in HS-podocytes. The initial screen showed that compared to HS+ podocytes, the HS- cells showed a 5-fold decrease ($p<0.01$) in the expression of the α5 integrin subunit whereas α3 subunit expression was unchanged. Real time PCR assays further validated the microarray expression data. Western blot assays of cell extracts confirmed that α5 integrin subunit protein expression was downregulated to the same degree. Immunostaining of HS+ and HS- podocyte cell cultures with antibodies against α5 showed a significant decrease in the total area of the cell surface occupied by α5 integrin subunit in the HS- cells compared to HS+ cells. Immunostaining HS- cells for pFAK (phosphorylated focal adhesion kinase) showed lack of pFAK in focal adhesions compared to HS+ cells, indicating that signaling was compromised. Double label immunohistochemistry of renal tissue sections from wild-type and PEXTKO mice with antibodies against α5 integrin subunit showed a decrease in the staining intensity for α5 integrin subunit in the podocytes of the PEXTKO mice compared to control animals. These results show a previously unknown relationship between cell surface HS and the expression of specific integrin subunits in podocytes.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1915**

**Genetic Deletion of Dendrin Delays the Onset of Proteinuria and Improves Survival of CD2AP Null Mice** Kirk N. Campbell,[1] Ritu Gupta,[1] Jaakko Patrakka,[2] Andrey S. Shaw,[3] Karl Tryggvason,[2] Peter H. Mundel.[4] *[1]Nephrology and Hypertension, University of Miami Miller School of Medicine, Miami, FL; [2]Medical Biochemistry, Karolinska Institute, Stockholm, Sweden; [3]Immunobiology, Washington University, St Louis, MO; [4]Molecular Medicine, University of Miami Miller School of Medicine, Miami, FL.*

CD2 associated protein (CD2AP) is an important component of the glomerular slit diaphragm. CD2AP null mice develop albuminuria at 2-3 weeks of age and typically die from progressive renal failure at 6-7 weeks. We previously identified dendrin as a direct binding partner of CD2AP that relocates to the nucleus under the influence of transforming growth factor beta (TGF-beta) to promote podocyte apoptosis. We also demonstrated that nuclear dendrin enhances TGF-beta induced apoptosis. Dendrin null mice are viable with no identifiable disease phenotype. Here we demonstrate that dendrin accumulates in the nuclei of podocytes in CD2AP null mice. To test the role of nuclear dendrin in CD2AP null mice we generated CD2AP/Dendrin double knockout mice.

Our results show that the deletion of dendrin in CD2AP null mice (CD2AP-/-/Dendrin-/-) delays the onset of heavy albuminuria until 6-7 weeks of age compared to 3 weeks in CD2AP null mice expressing wild type dendrin (CD2AP-/-/Dendrin+/+). CD2AP null mice that were also dendrin heterozygotes (CD2AP-/-/Dendrin+/-) had an intermediate course with heavy albuminuria developing at 4-5 weeks. Most strikingly CD2AP-/-/Dendrin-/- mice had a mean survival of 13 weeks compared to 8-9 weeks for CD2AP-/-/Dendrin+/+ mice and 9-10 weeks for CD2AP-/-/Dendrin+/- mice. In summary, the deletion of dendrin delays the onset of proteinuria and improves survival in CD2AP null mice. These findings suggest a novel mechanism where the deletion of dendrin improves renal survival in progressive chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1916**

**Partioning Defective Par1 Polarity Protein Expression in Glomerular Development and Disease** Kimberly J. Reidy,[1] Zhong-Fang Du,[1] Jonathan M. Barasch,[6] James M. Pullman,[4] Anne Muesch,[3] Katalin Susztak.[2] *[1]Pediatrics/ Nephrology, Children's Hospital at Montefiore/ Albert Einstein College of Medicine, Bronx, NY; [2]Medicine/ Nephrology, Albert Einstein College of Medicine, Bronx, NY; [3]Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY; [4]Pathology, Montefiore Medical Center, Bronx, NY; [5]Pathology, Nephrocor, Uniondale, NY; [6]Medicine/ Nephrology, Columbia University, New York, NY.*

Partioning defective (Par) proteins establish cell polarity in columnar epithelial cells and neurons by localizing to distinct cell membrane domains. Par1a/b and Par3 are expressed in mature podocytes. Recent studies have suggested a role for Par proteins in establishing podocyte structure and glomerulosclerosis: Par3 (a scaffolding protein that forms a complex with Par6/aPKC) associates with nephrin, and conditional deletion of aPKC induced glomerulosclerosis. Par1 is a serine-threonine kinase that localizes asymmetrically from Par3/Par6/aPKC. We previously identified Par1a/1b in human, mouse and rat podocytes and blockade of Par1 signaling in cultured podocytes induced altered cell shape and decreased filopodia, suggesting a role for Par1 in modulating the podocyte architecture. Par1b expression was identified in embryonic day E13 and E15 kidneys (during glomerular differentiation). We examined Par1a/1b and Par3 expression in experimental proteinuric kidney disease and identified changes in expression pattern or decreased expression of Par1a/b and Par3 in adriamycin nephropathy and puromycin aminonucleoside nephrosis. To examine the relevance to human disease, we examined expression of Par1a in human kidney biopsies of focal glomerulosclerosis (FSGS, n=10), minimal change disease (MCD, n=5) and controls (normal tissue obtained from tumor nephrectomy, n=4). Similar to our findings in experimental models of kidney disease, we identified altered expression of Par1a in FSGS and MCD. Our data suggest that Par1a/1b signaling may play a role in defining podocyte polarity and in glomerulosclerosis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1917**

**Chronic Kidney Disease Stage 1-3 Increases Risk of Venous Thrombosis** Gurbey Ocak,[1] Marion Verduijn,[1] Carla Y. Vossen,[1] Willem Lijfering,[1] Friedo W. Dekker,[1] Frits R. Rosendaal,[1] Ron T. Gansevoort,[2] Bakhtawar Khan Mahmoodi.[3] *[1]Dep. Clinical Epidemiology, Leiden University Medical Center, Netherlands; [2]Dep. Nephrology; [3]Dep. Hematology, University Medical Center Groningen, Netherlands.*

**Background:** End-stage renal disease has been associated with venous thromboembolism (VTE). However, the risk for VTE in chronic kidney disease (CKD) stage 1-3 has not yet been investigated. The aim of this study was to investigate whether CKD patients with stage 1-3 are at increased risk for VTE.

**Methods:** This study was conducted on participants of a population based prospective cohort study, in which renal function and albuminuria were assessed, starting in 1997-1998, and were followed for the occurrence of VTE until June 1, 2007. CKD patients were staged according to the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines based on 24-hour urine albumin excretions and MDRD based estimated glomerular filtration rate (eGFR). Symptomatic and objectively verified VTE were considered as endpoint.

**Results:** Of the 8495 subjects, 243 had stage 1 CKD, 856 stage 2, and 491 stage 3. During a median follow-up period of 9.2 years, 128 individuals developed VTE. Age, sex, hypertension, diabetes, high sensitivity C reactive protein and body mass index adjusted hazard ratios (HRs) for CKD stage 1, 2, and 3 were respectively 2.1 (95% CI 0.9 -5.1), 1.9 (95% CI 1.1 -3.1), and 1.6 (95% CI 0.9 -2.8) relative to those without CKD. Subjects with CKD stage 3 and albuminuria (≥30 mg/day) had an adjusted HR of 3.0 (95% CI 1.4-6.4) and subjects with stage 3 without albuminuria had an adjusted HR of 1.0 (95% CI 0.4-2.4). The table shows HRs for eGFR adjusted for albuminuria and vice versa.

**Table.** Association between eGFR, albuminuria, and risk for venous thromboembolism

| | *Adjusted hazard ratios |
|---|---|
| eGFR | |
| > 90 ml/min | 1.0 (reference) |
| 60-90 ml/min | 1.2 (0.7-2.0) |
| 30-60 ml/min | 1.5 (0.7-3.1) |
| | †Adjusted hazard ratios |
| Albuminuria‡ | |
| No | 1.0 (reference) |
| Yes | 1.9 (1.3-2.8) |

eGFR indicates estimated glomerular filtration rate.
*Adjusted for age, sex, and albuminuria (continuous)
† Adjusted for age, sex, and eGFR (continuous)
‡ Albuminuria defined as urinary albumin excretion >=30 mg/d

**Conclusions:** CKD stage 1, 2, and CKD stage 3 in presence of albuminuria were risk factors for VTE. The risk for VTE seemed more related to albuminuria than to impaired eGFR.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1918**

**Association between Estimated Glomerular Filtration Rate, Proteinuria, and Adverse Cardiovascular Outcomes: A Longitudinal Population-Based Study** Aminu K. Bello, Brenda Hemmelgarn, Anita Lloyd, Scott Klarenbach, Matthew T. James, Braden J. Manns, Marcello Tonelli. *For Alberta Kidney Disease Network.*

Most studies of chronic kidney disease (CKD) and outcomes focus on mortality and endstage renal disease (ESRD), with limited data on other adverse outcomes. We examined the association between proteinuria, eGFR and cardiovascular (CV) events in a population-based cohort.

Of 1,526,437 subjects with at least 1 serum creatinine 920,985 (60.3%) and 102,701 (6.7%) had 1 dipstick proteinuria and 1 urine albumin-creatinine ratio (ACR) respectively. We considered time to hospitalization for 1 of 5 indications: (congestive heart failure [CHF], coronary bypass grafting [CABG] or percutaneous coronary intervention [PCI], peripheral vascular disease [PVD] and stroke/transient ischaemic attacks [CVA]).

During a median follow-up of 35 (range 0-59) months, 1,891 (0.2%) of those with dipstick proteinuria were hospitalized for PVD, 7,309 (0.8%) for PCI/CABG, 4,265 (0.5%) for CHF, and 4,692 (0.5%) for CVA. From the ACR cohort, 367 (0.4%), 2,218 (2.2%), 1,555 (1.5%), 1,157 (1.1%) had PVD, PCI/CABG, CHF and CVA respectively. In both cohorts, age-adjusted rates of CHF and CVA were increased ($p<0.001$) at lower levels of eGFR and with heavier proteinuria. While the risk of CABG/PCI and PVD increased ($p<0.001$) with heavier proteinuria, there were fewer events in subjects with lower eGFR. In fully adjusted Poisson models, compared to subjects with eGFR of 45-59 mL/min/1.73m$^2$ and no proteinuria, subjects with heavy proteinuria by dipstick and eGFR of 60mL/min/1.73m$^2$ had higher rates (events per 1000 patient years) of CABG/PCI and CVA; (1.4 [95% CI, 1.3-1.5]) vs (1.9 [95% CI, 1.6-2.3]) and 0.68 [95% CI, 0.62-0.74]) vs (1.43 [95% CI, 1.16-1.75]) respectively. Similar results were obtained in subjects with proteinuria measured by ACR.

Risks of major CV events at a given level of eGFR increased with higher levels of proteinuria. The findings extend current data on risk of mortality and ESRD, and suggest that proteinuria is of incremental prognostic value at every level of eGFR. These data support the use of proteinuria with eGFR for definition and risk stratification in CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1919**

**Association of eGFR and Albuminuria with All-Cause and Cardiovascular Mortality. A Collaborative Meta-Analysis of High-Risk Population Cohorts on Behalf of the CKD Prognosis Consortium** Marije Van de Velde,[1] Kunihiro Matsushita,[2] Josef Coresh,[2] Brad C. Astor,[2] Mark Woodward,[4] Andrew S. Levey,[3] Paul E. de Jong,[1] Ron T. Gansevoort.[1] *[1]Dept. Nephrology, University Medical Center, Groningen, Netherlands; [2]Dept. Epidemiology, John Hopkins Institute, Baltimore; [3]Div. Nephrology, Tufts Medical Center, Boston; [4]George Inst. Int. Health, University of Sydney, Australia.*

Screening for chronic kidney disease (CKD) in people at high risk for CKD is recommended, but data on the independent and combined associations of eGFR and albuminuria with all-cause mortality (ACM) and cardiovascular mortality (CVM) in these populations are limited.

We performed a collaborative meta-analysis of 10 cohorts with 266,975 participants selected because of increased CKD risk, including patients with hypertension, diabetes, or a history of CV disease. Albuminuria was ascertained by albumin-to-creatinine ratio (ACR) in 6 cohorts or dipstick in 4 cohorts.

Risk for ACM was not associated with eGFR (MDRD) between 60 and 105 ml/min/1.73m$^2$, but increased at lower eGFR. Hazard ratios (HRs) (95% Confidence Intervals) at eGFR 60, 45, and 15 (versus 95) ml/min/1.73m$^2$ were 1.03 (0.81-1.33), 1.38 (1.15-1.65) and 3.11 (2.26-4.27), respectively, after adjustment for ACR and CV risk factors. Albuminuria (ACR) was associated with risk for ACM linearly without thresholds. Adjusted HRs at ACR of 10, 30 and 300 (versus 5) mg/g were 1.08 (1.01-1.16), 1.38 (1.23-1.56), and 2.16 (1.99-2.35), respectively. eGFR and albuminuria were multiplicatively associated with ACM, without evidence for interaction. Similar associations were observed for CVM, and findings in cohorts with dipstick data were generally comparable to those in ACR cohorts. The trends for increased HRs were seen for subjects above and below age 65 years, although HRs for eGFR increased more steeply in younger subjects.

In conclusion, lower eGFR and higher albuminuria are risk factors for ACM and CVM in high risk populations, independent of each other and of CV risk factors. These associations are comparable to those observed in the general population, and are consistent with the current KDOQI thresholds defining CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1920**

**Development of a Risk Score for Chronic Kidney Disease in Population-Based Studies** Conall M. O'Seaghdha,[1,2] Asya Lyass,[1] Joseph Massaro,[1] Josef Coresh,[3] Brad C. Astor,[3] Caroline S. Fox.[1,2] *[1]NHLBI's Framingham Heart Study; [2]Harvard Medical School; [3]Welch Center, Johns Hopkins University.*

Background Stratification of individuals at risk for chronic kidney disease (CKD) may allow for optimization of preventive measures to reduce its incidence and complications. We aimed to develop a risk score that would estimate an individuals' absolute risk of developing CKD, and provide a tool for investigators to assess novel markers of renal risk.

Methods 2490 participants without baseline CKD from the Framingham Heart Study, who attended a baseline examination in 1995-1998 and a follow-up examination in 2005-2008, were included. CKD was defined as an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.732 by the Modification of Diet in Renal Disease Study equation. Participants were assessed for the development of incident CKD after 10 years of follow-up. Stepwise logistic regression was used to identify risk factors associated with the development of CKD. Risk factors meeting the inclusion threshold were used to construct a risk score for predicting the 10-year risk of CKD. Performance characteristics of the prediction algorithm were assessed using calibration and discrimination measures. The final risk score model was externally validated in the bi-ethnic ARIC Carotid MRI Study cohort (n=1777 participants).

Results At baseline, there were 1170 men and 1320 women, with a mean age of 53 years, who were free of pre-existing CKD; 9.2% (n=229) developed CKD at follow-up. Age, diabetes, hypertension, baseline eGFR and albuminuria were associated with CKD ($p<0.05$), and were incorporated into a risk function (c-statistic 0.813). In external validation in the ARIC study, the c-statistic was 0.79 in whites (n=1353) and 0.75 for blacks (n=424).

Conclusions Risk stratification for CKD is achievable using a risk score derived from clinical factors readily accessible in primary care. The utility of this score in identifying individuals in the community at high risk of CKD warrants further investigation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1921**

**Contrasting Incremental Value of Estimated Glomerular Filtration Rate and Albuminuria as Independent and Additive Predictors of Cardiovascular Versus Renal Outcomes in 27,000 High Risk People** Catherine M. Clase,[1] Peggy Gao,[1] Sheldon W. Tobe,[2] Anja Grosshennig,[3] Koon K. Teo,[1] Salim Yusuf,[1] Johannes F. Mann.[3] *[1]Medicine, McMaster University, Hamilton, ON, Canada; [2]Medicine, University of Toronto, Toronto, ON, Canada; [3]Nephrology, Schwabing General Hospital, Munich, Germany.*

**Background.** Glomerular filtration rate (eGFR) and albuminuria (uACR) are independent multivariable predictors of cardiovascular outcomes but their clinical utility is unclear. Further, few studies are available on the relationship between mild abmormalities of eGFR and uACR and major renal outcomes such as dialysis.

**Objective.** To examine the additional predictive impact of eGFR and uACR on cardiovascular and renal outcomes.

**Design.** Multivariable Cox regression; risk stratification tables.

**Setting.** ONTARGET and TRANSCEND randomized trials with 5 y follow up.

**Patients.** 27,620 patients; mean age 67 years, 30% female, 37% with diabetes, 76% with cardiovascular disease.

**Measurements.** Baseline eGFR, uACR, cardiovascular (CV) risk factors.

**Results.** In adjusted analysis, both eGFR and uACR predicted the primary CV composite outcome (eg, hazard ratio [HR] 2.53, 95% confidence intervals [CI] 1.61, 3.99 for eGFR < 30 mL/min/1.73m$^2$ and very high uACR). However, risk stratification tables showed that adding information of eGFR and uACR led to no improvement in calibration, and no decrease in the proportion of patients assigned to the intermediate risk category (31% without, and 32% with renal information). In contrast, eGFR and uACR were very strong predictors of risk of chronic dialysis (eg, adjusted HR 1338, 95% CI 402 to 4456 for eGFR < 30 mL/min/1.73m$^2$ and very high uACR), and when added to traditional CV risk factors greatly improved both model calibration and risk stratification capacity (65% assigned to intermediate risk categories without renal information, decreased to 18% with renal information)

**Limitations.** Creatinine not standardized.

**Conclusions.** In patients at high vascular risk, eGFR and uACR associate with increased cardiovascular risk but add modestly to classical risk factors. Conversely, both greatly improve the prediction of renal outcomes.

Disclosure of Financial Relationships: Research Funding: Astellas, Bayer, Bodystat (Quadscan); Scientific Advisor: Amgen.

**F-PO1922**

**Rate of Kidney Function Decline Is Associated with Increased Risk of Death** Ziyad Al-Aly, Tarek M. Elachkar, Michael I. Rauchman. *Division of Nephrology, Saint Louis Veterans Affairs Medical Center, Saint Louis, MO.*

The effect of rate of decline of kidney function on risk of death is not well understood. Using the Department of Veterans Affairs national databases, we built a cohort of 4,171 rheumatoid arthritis patients with early stage 3 CKD (estimated glomerular filtration rate [eGFR] 45-60 ml/min) and followed them longitudinally over time to characterize predictors of disease progression and the effect of rate of kidney function decline on risk of death. After a median follow up of 2.6 years, 1604 (38%) did not experience any kidney function decline, and 426 (10%), 1,147 (28%), 994 (24%) experienced mild, moderate and severe CKD progression (defined as eGFR loss of 0-1, 1-4, >4 ml/min/yr; respectively).

Peripheral artery disease was a significant predictor of moderate CKD progression. Black race, hypertension, diabetes, cardiovascular disease, and peripheral artery disease were predictors of severe CKD progression. After a median observation period of 5.7 years, compared to patients with mild progression, patients with moderate CKD progression exhibited a trend toward increased risk of death (HR=1.10, CI=0.975-1.304), and patients with severe CKD progression had significantly increased risk of death (HR= 1.539, CI=1.298-1.824). Our results show that among patients with early stage 3 CKD, some do not experience decline in eGFR and some experience mild, moderate and severe CKD progression. The results show that there is an independent and graded association between the rate of kidney function decline and the risk of death. Incorporating the rate of decline in the definition of CKD may transform a static definition into a dynamic one that more accurately describes the disease state in an individual patient.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1923

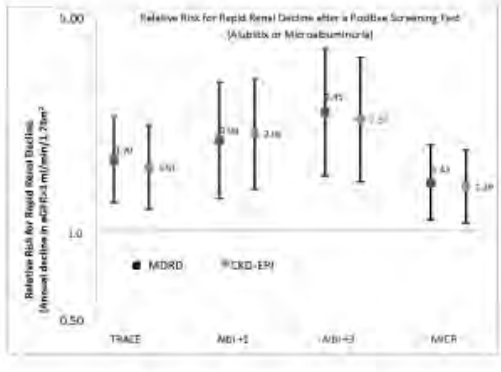
**Identifying Patients with Rapid Renal Decline in a Community-Based Population** William F. Clark, Jennifer J. Macnab, Jessica M. Sontrop, Louise M. Moist, Rita Suri, Marina I. Salvadori, Amit X. Garg. *University of Western Ontario.*

Background: To determine the value of simple measures of kidney impairment to detect patients with rapid renal decline (RRD) in a community-based population and in high-risk sub-populations.

Methods: Prospective cohort study of community-dwelling adults 18-87 years (2002-8, Canada). Change in eGFR was summarized using the ordinary least squares estimate of change for each participant. RRD was defined as average annual decline in eGFR >3ml/min/1.73m$^2$. The diagnostic accuracy of albustix (trace, >1g/L, >3g/L) vs. microalbuminuria (>17 mg/g if male or >25 mg/g if female) to identify a case with RRD was assessed.

Results: Of 3,371 participants, 2,560 had three eGFR measures and complete laboratory data. Median follow-up time was seven years with six serum creatinine measurements. The median change in eGFR was -0.76 ml/min/1.73m$^2$/yr; with 15.9% experiencing RRD. The incidence of RRD did not differ between males and females, and the highest rate occurred in those with baseline eGFR >90 or <30 ml/min/1.73m$^2$. The probability of identifying RRD from serial eGFR measurements was greatest following albustix >3g/L (0.41), with the lowest number needed to follow (2.4). The diagnostic accuracy of Albustix >trace was consistently better than microalbuminuria and was greatest with albustix >3g/L in those with multiple risk factors (age>60; diabetes or cardiovascular disease) with a post-test probability of 0.53 and number needed to follow of 1.9. Similar results were obtained when eGFR was calculated using the CKD-EPI equation.

Conclusions: Simple inexpensive screening tests in patients with or without risk factors will allow clinicians to follow a smaller number of patients with serial eGFR assessment in order to identify those with RRD who will potentially benefit from earlier therapeutic intervention.




Disclosure of Financial Relationships: nothing to disclose

## F-PO1924

**Lower Systolic BP Associated with Slower CKD Progression in the CKiD Study** Susan L. Furth,[1] Joseph T. Flynn,[2] Christopher B. Pierce,[2] Mark Mitsnefes,[2] Craig S. Wong,[2] Jeffrey M. Saland,[2] Marva M. Moxey-Mims,[2] Alison G. Abraham,[2] Bradley A. Warady.[2] *[1]Nephrology, Children's Hospital of Phila, Phila, PA; [2]Chronic Kidney Disease in Children Study, .*

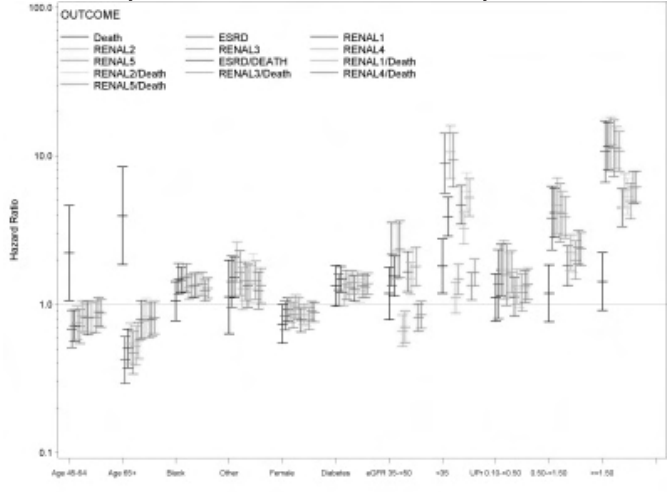
In the ESCAPE trial, subjects with mean ambulatory BP <50$^{th}$%ile had delayed CKD progression. We assessed if casual systolic (SBP) or diastolic BP (DBP)< 50%ile was associated with slower GFR decline than SBP or DBP 50- 90$^{th}$ or > 90%ile, after adjusting for age, follow-up yrs, CKD cause, baseline GFR, and presence of nephrotic proteinuria in the Chronic Kidney Disease in Children cohort study. Time-variable exposure analysis of the effect of SBP or DBP on the log-transformed annualized ratio of consecutive iohexol GFR (GFR) measures using multivariate generalized estimating equations with robust variance estimation and Cox proportional hazards models were used. 425 children, median age 11 yrs, median GFR 45 ml/min/1.73m$^2$, had BP measured at baseline, and at least one paired measure of GFR. 158 had SBP <50%ile, 175 had SBP 50 -90%ile and 92 had SBP > 90%ile. Median age (13 vs 10 vs 9 yrs) was higher in the <50%ile group (p<0.05), but median GFR (45,46,45 ml/min/1.73m$^2$), % with glomerular CKD diagnosis (23,15,24%) and nephrotic range proteinuria (11,10,12%) did not differ significantly between groups. In unadjusted analysis, after median 2.4 yrs follow-up, GFR in the SBP < 50%ile group declined at 2.3% per yr, compared to 3.5% in the 50-90%ile group, and 8.2 % in the SBP>90%ile group. In the adjusted time-variable exposure analysis, individuals with SBP<50%ile had annualized declines in GFR measurements on average 4% less than children with SBP 50th-90th%ile and similar demographic and clinical characteristics (p=0.047), while GFR decline in those with SBP>90%ile was 4% greater than in those with SBP 50-90%ile(p=ns). Point estimates for DBP were in the same direction, but not significant. In Cox proportional hazards analysis, children with SBP<50%ile had a lower risk of ESRD (initiation of dialysis or transplant) (HR 0.5, 95%CI 0.3-0.9) compared to those with SBP 50-90%ile (ref) or >90%ile (HR 1.0, 95%CI 0.6-1.7)). These data suggest that target SBP in children with CKD should be lower than the 50%ile for age, gender and height.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1925

**Failure Time Analysis of Renal Outcomes in the Chronic Renal Insufficiency Cohort (CRIC) Study** Dawei Xie, Wei Yang, Marshall M. Joffe, Valerie L. Teal, Amanda Hyre Anderson, Tom H. Greene, Harold I. Feldman. *CRIC.*

Studying the association between risk factors and renal outcomes in survival analysis is made complex by the multiple choices for renal outcome and the competing risk of death. We explored renal outcomes as ESRD alone, ESRD with 1) eGFR halving; 2) eGFR<15; 3) eGFR halving and <15; 4) eGFR drop 20, and 5) eGFR halving or drop 20. We considered 3 approaches for dealing with death; treat it as part of the outcome; a censoring event or a competing risk. We fit Cox proportional hazards models and obtained hazard ratios (HRs) for demographics and baseline characteristics adjusting for traditional risk factors. 3939 patients were included with an average of 3 years of follow-up. Rates of death, ESRD and eGFR halving were 6.9%, 10.7% and 7.0%, respectively. The associations between risk factors and renal outcomes were stable across varied definitions of renal outcomes, but were substantially different from their associations with mortality.




Risk factor associations were similar when treating death as a competing risk or censoring event.

HRs from failure time analyses

| Risk factors | | ESRD | | ESRD/eGFR halving | |
|---|---|---|---|---|---|
| | | M1[1, 2] | M2 | M1 | M2 |
| Age | 21-44 | 1 | | | |
| | 45-64 | 0.7 | 0.7 | 0.7 | 0.7 |
| | 65+ | 0.4 | 0.4 | 0.5 | 0.5 |
| Sex | Male | 1 | | | |
| | Female | 0.8 | 0.9 | 0.9 | 0.9 |
| Race | White | 1 | | | |
| | Black | 1.5 | 1.5 | 1.5 | 1.4 |
| | Other | 1.4 | 1.5 | 1.5 | 1.5 |
| Diabetes | No | 1 | | | |
| | Yes | 1.3 | 1.2 | 1.5 | 1.5 |
| Baseline eGFR | <35 | 8.9 | 8.3 | 3.9 | 3.7 |
| | 35-<50 | 2.2 | 2.1 | 1.6 | 1.5 |
| | 50+ | 1 | | | |
| Baseline 24 hour urine protein (g/day) | <0.1 | 1 | | | |
| | 0.1-<0.5 | 1.4 | 1.4 | 1.7 | 1.7 |
| | 0.5-<1.5 | 3.8 | 3.9 | 4.2 | 4.2 |
| | 1.5+ | 10.7 | 10.4 | 11.6 | 11.0 |

1. Adjusted for ABI, CVD, BMI, BP, hgb and smoking at baseline. 2. Death was treated as censoring in M1 and competing risk in M2.

Modeling ESRD or eGFR halving with death being censored is a robust approach to identify risk factors for renal progression in CRIC.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1926**

**Short-Term Prediction of Long-Term Renoprotective Treatment Effect: The Advantage of a Multiple Biomarker-Based Efficacy Score** Yan Miao,[1] Hiddo Jan Lambers Heerspink,[1] Hans-Henrik Parving,[2] Julia Lewis,[3] Dick De Zeeuw.[1] *[1]University Medical Center Groningen, Netherlands; [2]University Hospital Copenhagen, Denmark; [3]Vanderbilt University Medical Center.*

Efficacy on hard renal outcomes is needed for evaluation in late phase of drug development. This leads to expensive trials of long duration. Short term changes in biomarkers may be an alternative to predict the drug's renal efficacy and safety. We aimed to construct a multiple-biomarker based short-term efficacy score to predict the effect of a single drug on long-term hard renal outcomes.

Data from the merged RENAAL and IDNT trials were used (n=2661 type 2 diabetic and nephropathy). A multi-biomarker (blood pressure, uric acid, potassium, hemoglobin, and albuminuria) risk score was created using Multivariate Cox analysis in the placebo group. This score was subsequently applied to the baseline and month 6 measurements of the angiotensin receptor blocker (ARB) treatment arm to obtain the biomarker-based estimated long-term renal risk reduction. To test the validity of this estimate, the biomarker-based estimated risk reduction was compared with the actual measured long-term risk reduction on hard renal endpoints (doubling of serum creatinine or end stage renal disease).

The effect of ARB treatment on the multiple biomarker score (baseline, month 6) predicted a renal risk reduction of 20.7 %, which was remarkably equal to the actual observed risk reduction 21.0 (9.1 – 31.5) %. When single biomarker formulas were created (e.g. blood pressure) the score failed to predict the actual renal risk reduction (table).

We demonstrate the renal protective effect of an ARB (registered for blood pressure reduction) cannot be estimated from short-term BP changes alone. A multi-biomarker based efficacy score (based on 6 months changes in biomarkers) appears very accurate in predicting the long-term effect of an ARB on hard renal outcomes.

| | Predicted long-term renal risk reduction (%) using change in (single) biomarkers |
|---|---|
| ΔSystolic BP | -10.1 |
| ΔAlbuminuria | -19.8 |
| Δ Potassium | +6.7 |
| ΔHemoglobin | +5.8 |
| ΔUric Acid | -1.6 |
| | |
| ΔMultiple biomarker score | -20.7 |

Disclosure of Financial Relationships: nothing to disclose

**F-PO1927**

**Association between Changes in Serum Uric Acid and Renoprotection in the RENAAL Trial** Stefan Antonius Ottenbros,[1] Hiddo Jan Lambers Heerspink,[1] Gozewijn Dirk Laverman,[1] Stephan J. L. Bakker,[1] Shahnaz Shahinfar,[2] Hans-Henrik Parving,[3] Dick De Zeeuw.[1] *[1]Clinical Pharmacology, University Medical Center Groningen, Netherlands; [2]Philadelphia Children Hospital; [3]University Hospital Copenhagen, Denmark.*

Introduction: Increased serum uric acid concentrations have been shown to be a risk factor for progressive renal function loss. Treatment with the Angiotensin Receptor Blocker losartan lowers uric acid. Whether losartan induced reductions in uric acid levels are associated with renoprotection is unclear. Therefore, the aim of this study was to elucidate the association between changes in serum uric acid during losartan therapy and renal prognosis.

Methods: A post-hoc analysis in the RENAAL trial was conducted. Patients with diabetes and nephropathy were randomized to losartan or placebo for a median follow-up of 3.4 years. Multivariate cox regression analysis was used to determine the relationship between 6 month change in SUA and renal endpoints, defined as a doubling of serum creatinine or end stage renal disease.

Results: Baseline uric acid levels of the 1513 included patients were 6.71 and 6.70 mg/dL in placebo and losartan treated subjects respectively. During the first 6 months the placebo adjusted change in SUA was - 0.16 mg/dL (95% CI - 0.26 to - 0.05; p=0.032). A total of 489 renal events occurred during follow-up. Each 1 mg/dL change in SUA during the first 6 months of treatment was associated with a 17% risk change (Hazard ratio 0.83; 95% CI 0.77 – 0.90; p<0.001) for renal endpoints. This effect was independent of other risk markers or changes in risk markers, including age, eGFR, hemoglobin, log albuminuria. The magnitude of risk reduction was similar in male and female subjects. Similar associations were observed for the individual components of the renal endpoint.

Conclusion: Losartan lowers SUA levels compared to placebo treatment in patients with type 2 diabetes and nephropathy. This change in SUA is independently associated with a lower risk for renal endpoints. These data suggest that treatments specifically focused on lowering SUE may confer additional renoprotection.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1928**

**The Relationship between Blood Pressure and the Decline in Kidney Function in Community-Based Patients with Hypertension** Rebecca Hanratty,[1] John Powers,[2] Michael Ho,[4] Michel B. Chonchol.[3] *[1]Denver Health, Denver, CO; [2]Institute for Health Research, Kaiser Permanente of Colorado, Denver, CO; [3]Division of Renal Diseases and Hypertension, University of Colorado Denver, Denver, CO; [4]Denver Veterans Affairs Medical Center, Denver, CO.*

Background Hypertension is an important cause of chronic kidney disease (CKD). Identifying risk factors for progression to CKD in patients with normal kidney function and hypertension may help target therapies to slow or prevent decline of kidney function. Our objective was to identify risk factors for development of incident CKD and decline in estimated glomerular function (eGFR) in hypertensive patients.

Methods We performed a retrospective cohort study of adult patients with hypertension. Cox proportional hazards models were used to assess the relationship between incident CKD and potential risk factors for CKD, including systolic blood pressure. General linear mixed-effects models were used to estimate the relationship between potential risk factors and rate of decline in estimated glomerular filtration rate (eGFR).

Results Of 43,564 patients meeting the inclusion criteria, 12.2% (5292 patients) developed incident CKD. Diabetes was the strongest predictor of incident CKD (HR 1.98 95% CI 1.85 to 2.11) and was associated with the greatest rate of decline in eGFR (-2.17 mL/min/year). Time weighted systolic blood pressure was associated with incident CKD with odds increasing steadily above 120 mm Hg; each 10 mm Hg increase in time weighted systolic BP was associated with a 21% increase in the risk of developing incident CKD (HR 1.21 95% CI 1.18 to 1.23).

Conclusions We found that time-weighted systolic blood pressure was associated with incident CKD and more rapid decline of kidney function, with a steady increase in odds of incident CKD above a systolic blood pressure of 120 mm Hg.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1929**

**A Comparison of Absoulte and EGFR-Indexed PCR: Implications for Clinical Trials Interpretation of Proteinuria** Timothy Ellam, Pavez Hosain, Meguid El-Nahas. *Sheffield Kidney Institute, Northern General Hospital, Sheffield, Yorkshire, United Kingdom.*

Background

Declining GFR accompanies a reduction in the functioning nephron mass across which protein leak occurs, so the index of PCR:eGFR might reflect glomerular permeability/viability changes better than absolute PCR. To investigate how indexing PCR for eGFR affects patient stratification and measures of change in proteinuria, the relationship between changes in absolute PCR and PCR:eGFR was studied.

Methods

A cohort of N=807 CKD patients with at least two measures of PCR and corresponding eGFR were studied retrospectively. Patients were categorized on the basis of baseline PCR with a threshold of 100mg/mmol (1000mg/g) as per national management guidelines. Baseline PCR:eGFR was also calculated and patients recategorized according to thresholds derived from published trial populations (i.e. the quoted evidence) where both proteinuria and GFR were reported (e.g. proteinuria of 1g/24h in a population with mean GFR 40ml/min in the MDRD study.) Percentage changes in absolute and eGFR-indexed PCR (IPCR) were also compared.

Results

At baseline, 47% of patients had PCR>100. An IPCR threshold of 1 (as for eGFR 100 and PCR 100) labelled 64% proteinuric. IPCR threshold of 2.5 (corresponding to the MDRD study cohort) labelled 50% proteinuric. Among those with more advanced renal impairment there was naturally a greater increase in proteinuria classification on the basis of IPCR vs PCR (89% vs 59% at CKD 5 and 54% vs 47% at CKD 4). In patients with preserved excretory function (CKD 1+2) a reclassification based on IPCR of 2.5 reduced the proportion labelled as proteinuric from 54% to 45%. Among patients with ≥50% GFR decline over follow up, absolute PCR increased in 35%, but IPCR increased in 91%.

Conclusions

In this CKD population the distribution of PCR and eGFR was such that the effect on patient classification of indexing PCR for eGFR was modest overall, but marked in CKD5. In the setting of a substantial decline in GFR the protein leak per unit functioning nephron mass increases almost universally even when absolute PCR does not. Changes in sclerotic glomeruli, perhaps reflecting a 'remnant nephron' effect may underlie this phenomenon.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1930**

**Predicting Kidney Disease in the Korean Population** Keunsang Kwon,[1] Sik Lee,[1] Sung Kwang Park,[1] Ju-Hyung Lee,[1] Heejung Bang,[2] Abhijit V. Kshirsagar.[3] *[1]Chonbuk National University Medical School; [2]Cornell University; [3]University of North Carolina at Chapel Hill.*

Screening algorithms, used to identify early chronic kidney disease (CKD), have been developed and validated in US populations. Their utility and applicability in foreign populations has not been examined. In the current study, we developed a CKD prediction model based on SCORED (SCreening for Occult REnal Disease) in the Korean population, known to have a growing burden of CKD. We used a cross-sectional analysis of the Korean National Health and Nutrition Examination Survey version IV-1 (KNHANES IV-

1) n=6566, year 2008, for the model development. Model validation was performed in an independent dataset combining KNHANES IV-2 (n=2921, year 2007) and Korean Genomic Epidemiologic Study (n=8548). CKD was defined as glomerular filtration rate (GFR) < 60 ml/min/1.73 m² (abbreviated MDRD equation). Female gender, anemia, hypertension, diabetes mellitus, and proteinuria were again significantly predictive of CKD. Unlike SCORED, elevated waist circumference was significantly associated with CKD, and age retained a significant, yet slightly attenuated association, while cardiovascular disease was borderline significantly associated with CKD (p=0.08). Information on peripheral vascular disease was not available in this population. The Area Under Curve (AUC) for these variables was AUC=0.83. An integer value was assigned to variables based on the strength of the odds ratio: 2 for age 50-59 years, 3 for age 60-69 and 4 for age 70 or older, and 1 for female gender, anemia, hypertension, diabetes, proteinuria, cardiovascular disease and high waist circumference. Based on the Youden index, a value of 5 or greater was used to define a high risk population [Sensitivity 74% Specificity 67% Positive Predictive Value 10%, Negative Predictive Value 98%]. This prediction algorithm, weighted towards common variables associated with CKD, may be a useful tool to identify individuals with a high likelihood of kidney disease in the Korean population. Yet, further refinement of prediction models like this one is necessary prior to their application to populations of other countries or geographic regions.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1931

**Traditional Risk Factors Predict Renal Function Decline among Younger but Not Older CKD Patients** Sankar D. Navaneethan,[1] Susana Arrigain,[1] Welf Saupe,[3] Joseph V. Nally,[1] Jesse D. Schold.[2] *[1]Nephrology & Hypertension, Cleveland Clinic; [2]Quantitative Health Services, Cleveland Clinic; [3]eCleveland Clinic eResearch, Cleveland Clinic, Cleveland, OH.*

Purpose: Prior studies have shown African American race, diabetes, hypertension, and hyperlipidemia as risk factors for progression of chronic kidney disease (CKD). We examined whether these risk factors have differential impact on the progression of kidney disease in elderly CKD patients compared to younger CKD patients.

Methods: We examined factors associated with decline of renal function in stage 3 CKD patients (glomerular filtration rate [GFR] 30-59 ml/min/1.73m2) (n=33,756) from our institutional CKD registry. This registry included patients identified with CKD from January 2005- March 2010 based on outpatient GFR levels using CKD-EPI equation. We utilized multivariable mixed models to assess factors associated with progression of renal function over time following CKD. We utilized age >70 as a definition of older patients, which represented the median age of our study cohort.

Results: Median eGFR at baseline (time of CKD confirmation) was 51.5 ml/min/1.73 kg/m². Factors explaining progressive decline of renal function over time included older age, male gender and African American race. The effect of demographic characteristics and pre-existing comorbidities had a statistically significantly greater association with renal function decline among patients aged <70 years as compared to patients ≥70 years: males (p<0.001), African Americans (p<0.001), patients with pre-existing diabetes (p=0.003) and obesity (p=0.001). Pre-existing hyperlipidemia was less predictive of decline among older patients (p<0.001) and pre-existing hypertension did not have differential effect between age groups (p=0.39).

Conclusion: These results suggest that the impact of traditional risk factors of kidney disease progression are important among stage 3 CKD patients aged <70 years but are not clear discriminators of risk among stage 3 CKD patients ≥70 years of age. These results may have important implications for management and interventions of various risk factors of kidney disease.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1932

**EGFR Change and Mortality Risk among Patients with Non-Dialysis Dependent CKD** Robert M. Perkins,[1] Ion D. Bucaloiu,[2] H. Lester Kirchner,[1] Nasrin Ashouian,[2] James E. Hartle,[2] Taher M. Yahya.[2] *[1]Center for Health Research, Danville, PA; [2]Nephrology/Geisinger, Danville, PA.*

**Background:** Prior estimates of the impact of rate of eGFR decline on mortality have focused on populations with normal kidney function, or have been limited by lack of information on characteristics known to influence mortality risk. **Methods:** We analyzed all adults with non-dialysis dependent CKD (NDD-CKD, eGFR 15-59 ml/min/1.73m²), stratified by tertile of rate of annualized eGFR change, at a tertiary care facility between 2004 and 2009. Independent predictors of mortality were assessed using Cox proportional hazards models incorporating demographics, comorbidities, medication use, and laboratory results. **Results:** 16,481 patients with NDD-CKD were followed for a median (IQR) of 33.6 (21.0—55.5) months. Rapid decliners across all CKD stages were more likely to be male, older, suffer from diabetes and other comorbid conditions, have proteinuria and lower serum albumin and HDL levels than those in the highest tertile of eGFR change. Death occurred more frequently in rapid decliners than in those in the highest tertile of eGFR change (116.3 vs 83.1, 176.4 vs 140.3, and 309.4 vs 241.6 deaths/1000 p-y for mild stage 3, moderate stage 3, and stage 4 CKD, respectively (p < 0.05 for all comparisons)).

Cox proportional hazard model of time to death associated with eGFR change among patients with NDD-CKD*

| | Stage 3 CKD | | Stage 4 CKD |
|---|---|---|---|
| | **Mild (baseline eGFR 45-59), n=11,222** | **Moderate (baseline eGFR 30-44), n=3,972** | **Baseline eGFR 15-29, n=830** |
| HR (95% CI), p value for death associated with eGFR decline of 1 ml/min/1.73m²/yr | 1.030 (1.026—1.034), p<0.0001 | 1.015 (1.008—1.022), p<0.0001 | 1.007 (0.994—1.020), p=0.29 |

*Model adjustments: mild and moderate stage 3 CKD = age, gender, HTN, hyperlipidemia, charlson comorbidity index, proteinuria, hemoglobin A1C, serum albumin, serum HDL; stage 4 CKD = age, smoking status, CAD, HTN, hyperlipidemia, proteinuria

**Conclusions:** Among patients with stage 3 CKD, rate of eGFR decline adds incrementally and independently to mortality risk.

Disclosure of Financial Relationships: Consultancy: American RegentResearch Funding: Amgen; Honoraria: American Regent.

## F-PO1933

**Analysis of Repeated Measures of Renal Functions in the Chronic Renal Insufficiency Cohort (CRIC) Study** Wei Yang, Marshall M. Joffe, Dawei Xie, Xin Wang, Amanda Hyre Anderson, Tom H. Greene, Harold I. Feldman. *CRIC.*

To study risk factors associated with renal progression measured as eGFR change over time, methods are needed to deal with missing eGFR due to informative dropout from ESRD or death. We fit 3 models: 1) a linear mixed effects model using only observable eGFR values; 2) a censored regression model, in which eGFR was assumed less than 10.8 ml/min/1.73m2(the average eGFR value before ESRD in USRDS) when participants developed ESRD and 3) a shared parameter model for modeling eGFR and dropout events simultaneously. There were 3939 patients with an average of 3 years of follow-up. The estimated overall eGFR slope was -1.8 ml/min/1.73m2/year. Female sex, black race, diabetes, high baseline eGFR and proteinuria were associated with faster renal progression. These relationships were stable across modeling strategies.

Estimated differences of mean change (SE) of eGFR over time (ml/min/1.73m2/year)

| Risk factors | | Linear mixed effects model[1] | Censored regression model | Shared parameter model[2] |
|---|---|---|---|---|
| Age | 21-44 | Reference | | |
| | 45-64 | 0.3 (0.2) | 0.3 (0.2) | 0.5 (0.2) |
| | 65+ | 0.2 (0.2) | 0.0 (0.3) | 0.4 (0.2) |
| Sex | Male | Reference | | |
| | Female | -0.3 (0.1) | -0.3 (0.1) | -0.2 (0.1) |
| Race | White | Reference | | |
| | Black | -0.2 (0.1) | -0.3 (0.2) | -0.3 3 (0.1) |
| | Other | -0.6 (0.2) | -0.5 (0.3) | -0.5 (0.2) |
| Diabetes | No | Reference | | |
| | Yes | -0.2 (0.1) | -0.5 (0.2) | -0.3 (0.1) |
| Baseline eGFR | <35 | 1.5 (0.2) | 1.7 (0.2) | 1.3 (0.2) |
| | 35-<50 | 0.8 (0.1) | 1.1 (0.2) | 0.9 (0.1) |
| | 50+ | Reference | | |
| Baseline 24 hr urine protein (g/day) | <0.1 | Reference | | |
| | 0.1-<0.5 | -0.6 (0.1) | -0.7 (0.2) | -0.6 (0.1) |
| | 0.5-<1.5 | -2.0 (0.2) | -1.9 (0.2) | -2.0 (0.2) |
| | 1.5+ | -4.4 (0.2) | -3.8 (0.3) | -4.3 (0.2) |

1. All models adjusted for ABI, CVD, BMI, BP, hemoglobin and smoking status at baseline. 2. Survival time assumed to follow piecewise exponential distribution.

Linear mixed effects model is appropriate for identification of risk factors for renal progression.

Disclosure of Financial Relationships: nothing to disclose

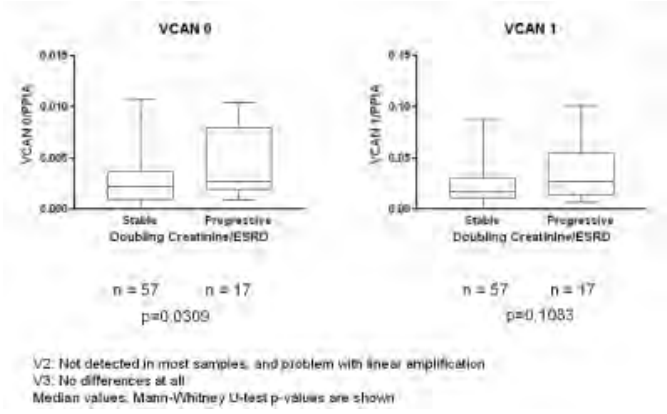
## F-PO1934

**Versican as a Novel Biomarker for Progression of Chronic Kidney Disease** Michael Rudnicki. *Nephrology and Hypertensiology, Medical University Innsbruck, Innsbruck, Austria.*

Patients with progressive chronic kidney disease are at high risk for ESRD and cardiovascular events. Prediction of progression using standard markers shows weak correlation with the clinical course and high intraindividual variation.

Using data from five published gene expression datasets we identified the proteoglycan versican (VCAN) as a marker of renal damage. Increased expression of VCAN was associated with (i) age, (ii) impaired renal function in diabetic nephropathy, (iii) progressive proteinuric nephropathies, (iv) increased histological damage scores in transplant biopsies and (v) renal graft loss.

In an independent cohort of 74 subjects with biopsy proven glomerulopathies RNA levels of VCAN isoforms V0, V1, V2 and V3 were evaluated. VCAN isoform V0, but not V1, V2 and V3 correlated significantly with doubling of serum creatinine or ESRD during a median follow up of 50 months (4-94).




V2: Not detected in most samples, and problem with linear amplification
V3: No differences at all
Median values. Mann-Whitney U-test p-values are shown

V0 and V1 correlated significantly with creatinine and proteinuria at biopsy, creatinine at follow-up, and interstitial inflammation, but not with histological diagnosis, tubular atrophy and interstitial fibrosis. V0 and V1 RNA levels predicted the course of disease significantly better than creatinine, proteinuria and the degree of histological damage. Immunohistochemical staining revealed VCAN expression mainly in the tubulointerstitial compartment. In a glomerulonephritis mouse model versican was significantly increased in nephritic mice as compared to control animals.

In summary, these data support the potential use of VCAN isoforms V0 and potentially V1 as biomarkers to predict a progressive clinical course of CKD.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1935

**Can the Examination of the Ocular Fundus Be a Predictor of Chronic Kidney Disease?** Yoshinari Yasuda,[1] Kiyoshi Shibata,[2] Sadao Suzuki,[3] Shoichi Maruyama,[1] Enyu Imai,[1] Seiichi Matsuo.[1] *[1]Department of Nephrology/CKD Initiatives, Nagoya University Graduate School of Medicine, Nagiya, Aichi, Japan; [2]Kasugai City Health Care Center, Kasugai, Aichi, Japan; [3]Nagoya City University, Nagoya, Aichi, Japan.*

*Background:* In recent years, chronic kidney disease (CKD) has been increasingly highlighted as a risk factor for dialysis treatment and cardiovascular diseases. CKD is reported to have an association with ageing, smoking, hypertension, diabetes mellitus or hyperlipidemia. Arteriosclerosis is believed to plays an important role for CKD onset and progression, its precise mechanisms has not yet been clarified.

*Objective:* In this report, we examined the relationship between the findings in the ocular fundus (EOF), a marker for arteriosclerosis, and CKD.

*Methods:* The subjects were 3,464 men and 3,251 women, who underwent health check including the EOF in Kasugai City Medical Care Center, from 2006 to 2007. Estimated GFR (eGFR) was calculated by the Japanese eGFR equation, and cases with eGFR less than 60 mL/min/1.73m$^2$ and/or with proteinuria were diagnosed as CKD. EOF was evaluated by Keith-Wagner classification (KW) and by Scheie classification (SC). Multivariate odds ratios for CKD for abnormality of the EOF in KW and SC were calculated using logistic regression adjusted for age and sex, BMI, diabetes, hyperlipidemia and hypertension.

*Results:* By EOF examination, KW and SC abnormalities were observed in 601 (8.95%) and in 214 cases (3.19%), respectively. eGFR among patients with EOF abnormalities was significantly lower than those without. Multivariate analysis revealed that age, male, obese, hyperlipidemia and KW abnormality was significant factors for CKD.

*Multiple regression analysis of the risk factor for CKD*

| Risk factors | OR | 95%CI | p-value |
|---|---|---|---|
| Age | 1.07 | 1.06-1.08 | <0.01 |
| Male | 1.58 | 1.40-1.78 | <0.01 |
| Obese | 1.37 | 1.18-1.59 | <0.01 |
| Hypertention | 1.08 | 0.95-1.23 | 0.22 |
| Diabetes | 1.15 | 0.94-1.39 | 0.17 |
| Hyperlipidemia | 1.32 | 1.15-1.53 | <0.01 |
| KW | 1.34 | 1.07-1.67 | <0.05 |
| SC | 1.02 | 0.71-1.45 | 0.92 |

*Conclusion:* It is suggested that KW abnormality from the EOF would predict the CKD.

Disclosure of Financial Relationships: Honoraria: Astellas, Banyu, Takeda, Novartis, Daiichi-Sankyo, Dainippon-Sumitomo, Kowa, Kirin, Mochida, Ohtsuka, Chugai, Phizer, Eisai, Tokyo-Tanabe-Mitsubishi,Fuji; Scientific Advisor: Astellas; Other Relationship: Astellas, Banyu, Chugai, Dainippon-Sumitomo, Phizer, Novartis.

## F-PO1936

**Should We Redefine Classification of Chronic Kidney Disease in Elderly Population?** Arash Rashidi,[1] Ashok K. Ananthasayanan,[1] Mirela A. Dobre.[2] *[1]Internal Medicine, Fairview Hospital, A Cleveland Clinic Hospital, Cleveland, OH; [2]Internal Medicine, Huron Hospital, A Cleveland Clinic Hospital, Cleveland, OH.*

Background: According to the National Kidney Foundation, people with an estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73m2 have CKD. It is not clear if the same cut off for eGFR for CKD should be considered in all age groups. Our objective is to find whether people older than 65 years with an eGFR of 45 to 59 should be classified as CKD.

Methods. The Cardiovascular Health Study limited data base, was used to identify a cohort of patients with a baseline history of CKD (eGFR<60). Patients were categorized in three groups with eGFR more or equal to 60, between 45 to 59, and 30 to 44. Estimated GFR was derived from the calibrated creatinine level and using MDRD equation. The studied population was followed for a mean duration of 10.3 years. Total and cardiovascular mortality and cardiovascular events were compared between the different groups, using the Cox regression survival analysis. The data was adjusted based on age, race, gender, body mass index, diabetes, hypertension, coronary artery disease at baseline, lipid profile, smoking status and medications.

Results: There were 4504 subjects with an eGFR more or equal to 60 and 1212 subjects with eGFR less than 60 at baseline in which 940 people had an eGFR between 45 to 59. During the follow up period 1170 people died because of cardiovascular causes. In adjusted Cox model, there was no difference between the group with eGFR of 45 to 59 and the group with eGFR more than 60 in regards to cardiovascular events (HR=1.09; 95%CI: 0.96-1.24). The result was similar in regards to the cardiovascular mortality (HR=1.12; 95%CI: 0.97-1.30). The subjects with eGFR between 30 to 44 had significantly higher cardiovascular events and mortality (HR=1.40; 95%CI: 1.11-1.76 and HR=1.36; 95%CI: 1.06-1.74).

Conclusion: There is no significant difference in cardiovascular mortality between the elderly subjects with eGFR between 45 and 59 and eGFR more or equal to 60. Probably the cutoff point of eGFR for definition of CKD in elderly population should be different than younger population.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1937

**Chronic Kidney Disease in Octogenarians** Shani Shastri, Hocine Tighiouart, Ronit Katz, Dena E. Rifkin, Linda F. Fried, Michael Shlipak, Anne B. Newman, Mark J. Sarnak. *Tufts Medical Center.*

**Introduction:** Chronic kidney disease (CKD) is a major public health problem in older adults. However, there are limited data on the prevalence of CKD and its clinical importance in those older than 80 years. We examined the prevalence of CKD in octogenarians, and its association with cardiovascular disease (CVD) in Cardiovascular Health Study (CHS) All Stars participants.

**Methods:** Serum creatinine and cystatin C were measured in 1028 CHS All Stars participants. Kidney function was estimated using CKD-Epi creatinine and cystatin C equations that incorporated coefficients for age, gender, and race ($eGFR_{EPI}$, $eGFR_{CYS3var}$) and one variable cystatin C equation ($eGFR_{CYS1var}$). CKD was defined as estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73m$^2$. Prevalent CVD was defined as a composite of coronary heart disease, heart failure or stroke. Logistic regression was used to examine the association of CKD, defined by each equation, with CVD in unadjusted and adjusted analyses.

**Results:** Mean age was 86 years, 64% were females, 86% were Caucasians, 66% had hypertension, 14% had diabetes, and 39% had prevalent CVD. Mean $eGFR_{EPI}$, $eGFR_{CYS3var}$ and $eGFR_{CYS1var}$ were 59, 62, and 70 ml/min/1.73m$^2$, respectively and 51%, 46%, and 33% of participants had CKD using the three equations. In adjusted analyses associations of CKD with CVD varied by equation (Table).

**Conclusion:** CKD is highly prevalent in octogenarians though its prevalence varied upon the equation used. The $CKD_{CYS1var}$ equation yielded the lowest prevalence but the strongest association with CVD. As there are no validated estimating equations in the elderly, estimation of kidney function based on any one equation should be interpreted with caution.

Association of CKD with CVD Based on eGFR Equations Using Serum Creatinine and Cystatin C

| | **Adjusted* Odds Ratio (95% CI)** | | |
|---|---|---|---|
| **eGFR (ml/min/1.73 m2)** | **CKD-Epi** | **CKD-CYS 3var** | **CKD-CYS 1var** |
| ≥60 | Reference | Reference | Reference |
| <60 | 1.53 (1.15, 2.03) | 1.67 (1.25, 2.23) | 2.09 (1.55, 2.83) |

* Adjusted for age, gender, race, body mass index, smoking, diabetes, hypertension, systolic blood pressure, low and high density lipoprotein cholesterol, vitamin D, and log C-reactive protein.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1938

**Risk Factors for ESRD among African American Adults in the Community: A Genetic Epidemiology Network of Arteriopathy (GENOA) Study** LaTonya J. Hickson,[1] Andrew D. Rule,[1] Kenneth R. Butler,[2] Gary L. Schwartz,[1] Tom Mosley,[2] Stephen T. Turner.[1] *[1]Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [2]Geriatrics, University of Mississippi, Jackson, MS.*

Background: African Americans have an increased risk for the development of end stage renal disease (ESRD).

Aim: To determine the incidence of ESRD and its primary risk factors in predominately hypertensive African American adults in the community

Methods: The GENOA cohort includes African American (Af-Am) sibships from Jackson MS with two or more adult members having hypertension. The cohort was assessed from 1996 to 1999 with a medical questionnaire, physical examination, and a blood sample. The risk of subsequent ESRD was assessed using the USRDS database with censoring at death.

Results: Of the 1846 Af-Am adults, 69% were females, 72% had hypertension, 23% had diabetes, mean (±SD) age was 58±10 years, and estimated glomerular filtration rate 85±21 mL/min/1.73 m$^2$. After 9.7±1.3 years of follow up, 48 (2.6%) developed ESRD. The listed cause of ESRD was diabetes in 34 (71%), unspecified/missing in 13 (27%), and multiple myeloma in 1 (2%). MYH9, non-muscle myosin IIA heavy chain, genotype (rs5756130) was available for 1525 participants. Allele frequency was 88%. Baseline characteristics adjusted for age and sex that predicted ESRD were **MYH9** genotype (HR 3.5, CI 1.0-11.8, p=0.02), **hypertension** (HR 12.9, CI 1.8-95.5, p=0.01), and **diabetes** (HR 24.2, CI 9.3-62.7, p<.0001). In a multivariate model, diabetes remained the strongest risk factor (HR 19.4, CI 7.5-50.5, p<.0001), see Table.

Multivariable-adjusted hazard ratios for incident ESRD

| Predictors | HR | 95% CI | P value |
|---|---|---|---|
| Age | 1.0 | 0.7-1.5 | 0.92 |
| Male | 2.0 | 1.0-3.9 | 0.05 |
| Diabetes | 19.4 | 7.5-50.5 | <.0001 |
| Hypertension | 5.6 | 0.7-43 | 0.10 |
| MYH9 genotype | 3.5 | 1.1-11.1 | 0.04 |

HR: Hazard Ratio, CI Confidence Interval

Conclusion: After a decade of follow-up, 2.6% of these predominately hypertensive African American adults in the community developed ESRD. While those with diabetes were at highest risk of ESRD, MYH9 genotype made an additional contribution to the prediction of ESRD.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1939

**Apolipoprotein E and Kidney Function Decline in Older Adults** Rebecca Kurnik Seshasai,[1] Ronit Katz,[2] Ian H. de Boer,[2] David Siscovick,[2] Michael Shlipak,[3] Dena E. Rifkin,[4] Mark J. Sarnak.[1] *[1]Tufts Medical Center, Boston, MA; [2]University of Washington, Seattle, WA; [3]San Francisco VA Medical Center, San Francisco, CA; [4]University of California San Diego.*

Chronic kidney disease is a public health problem that mostly affects older adults. Although studies in middle-aged adults have suggested that the apo ε4 is associated with slower progression of kidney disease and the apo ε2 allele with more rapid progression of kidney disease, there are limited data in older adults.

We evaluated 4,015 participants from the Cardiovascular Health Study (CHS), a cohort of adults aged >65. An established genotype score (JAMA 2005) was determined for each individual: ε2 /ε2 (+2), ε2/ ε3 (+1) ε2/ ε4 (0), ε3/ ε3 (0), ε3/ ε4 (-1), ε4/ ε4 (-2). In cross sectional analysis, we evaluated the association of CKD (eGFR cystatin <60 ml/min/1.73m2) with genotype score in unadjusted and adjusted logistic regression. In longitudinal analysis we evaluated the association of the genotype score with rapid kidney function decline, defined by eGFR cystatin C decline >3ml/min/1.73m$^2$/year.

Mean age was 72 years, 40% were men, 13% black and 14% had diabetes. Mean baseline eGFRcys was 80 ml/min/1.73 m$^2$. The predominant genotypes were ε3/ ε3 (60.6%), ε3/ ε4 (20.6%) and ε2/ ε3 (13.6%). 12.4% had CKD at baseline and 24.9% had rapid progression over a median follow up of 6.8 years. There was no association between apoE genotype score and CKD in cross-sectional analysis or longitudinal analysis (Table).

Conclusions: Apo E allelic frequency does not appear to be a major determinant of prevalent CKD or declining kidney function in older adults.

Association of Genotype Score with Prevalent CKD and with Rapid Kidney Function Decline

| | Cross Sectional (OR per unit increase in genotype score) | | Longitudinal (OR per unit increase in genotype score) | |
|---|---|---|---|---|
| Unadjusted | 1.05 (0.93, 1.18) | 0.44 | 0.99 (0.88, 1.10) | 0.83 |
| Adjusted* | 0.98 (0.85, 1.12) | 0.73 | 0.96 (0.86, 1.08) | 0.52 |

* Adjusted for age, gender, race, DM, SBP, DBP, antihypertensive medications, BMI, prevalent CHD, LDL, HDL, triglycerides and lipid lowering medications.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1940

**Chronic Kidney Disease Japan Cohort (CKD-JAC) Study** Enyu Imai,[1] Seiichi Matsuo,[1] Hirofumi Makino,[2] Tsuyoshi Watanabe,[3] Kosaku Nitta,[4] Tadao Akizawa,[5] Satoshi Iimuro,[6] Yasuo Ohashi,[6] Akira Hishida.[7] *[1]Nagoya University; [2]Okayama University; [3]Fukushima Medical University; [4]Tokyo Women's Medical University; [5]Showa University; [6]University of Tokyo; [7]Fujinomiya Munichipal Hospital.*

Prevalence of chronic kidney disease (CKD) is estimated to 13.3 million in Japan, but the patient characteristics during the predialysis period (CKD stage 3-5) are not well studied. We established the Chronic Kidney Disease Japan Cohort (CKD-JAC) to study the incidence of CVD, end stage renal disease (ESRD), and all-cause mortality in pre-dialysis patients treated by nephrologists for 4 years. The inclusion criteria were 1) Japanese and Asian patients living in Japan; 2) age 20-75 years; and 3) estimated GFR (eGFR) of 10-59 ml/min/1.73 m$^2$. We analyzed 2977 participants for baseline characteristics. Mean eGFR was 28.6±11.8ml/min/1.73m$^2$ and mean albuminuria was 976±1340 mg/g Cr. In our study, 91.9% of the participants had hypertension, but it was well-controlled (131/76 mmHg). ACE inhibitors and ARBs were used by most of the participants. The number of participants with eGFR >45ml/min/1.73m$^2$, 45 to 30 ml/min/1.73m$^2$, 30 to 15 ml/min/1.73m$^2$, and <15 ml/min/1.73m$^2$ were 304, 1037, 1160, and 476, respectively. Less than 15% of participants had a history of ischemic heart disease, and 11.5% had a history of stroke. Heart failure and arteriosclerosis obliterance were present in 3.9% and 2.9% of patients, respectively. Comorbidity of cardiovascular diseases was more prevalent in proportion to declining eGFR. Indicators of arteriosclerosis, higher pulse wave velocity (PWV), and high pulse pressure were associated with declinig eGFR, diabetes and particularly with diabetic nephropathy. Patients due to glomerulonephritis seemed to be at low risk for atherosclerosis and also to show lower levels of hypertension. In conclusion, the difference between causative diseases is associated with different comorbidity and level of arteriosclerosis. Future analysis of the cohort will clarify whether the incidence of ESRD and CVD will differ among causative diseases.

Disclosure of Financial Relationships: Consultancy: Daiichi-Sankyo, Kyowa-Hakkou-Kirin, KakenResearch Funding: Daiichi-Sankyo, Kyowa-Hakko-Kirin, Chugai, Banyu; Honoraria: Daiichi-Sankyo, Kyowa-Hakko-Kirin, Chugai, Takeda, Banyu, Novartis,; Scientific Advisor: Japanese Society of Nephrology, KDIGO, JASN, KI, NDT, Clincal Nephrology, Nature Review Nephrol,.

## F-PO1941

**Development and Validation of an Electronic Health Record-Based Chronic Kidney Disease Registry** Sankar D. Navaneethan,[1] Stacey Jolly,[2] Jesse D. Schold,[3] Susana Arrigain,[3] Welf Saupe,[4] John W. Sharp,[3] James F. Simon,[1] Martin J. Schreiber,[1] Anil K. Jain,[2,4] Joseph V. Nally.[1] *[1]Nephrology and Hypertension, Cleveland Clinic; [2]Medicine Institute, Cleveland Clinic; [3]Quantitative Health Sciences, Cleveland Clinic; [4]eCleveland Clinic eResearch, Cleveland Clinic.*

**Objectives:** Chronic Kidney Disease (CKD) is increasing and outcomes related research from diverse health care settings are needed to target appropriate efforts and interventions. We developed an Electronic Health Record (EHR) based CKD registry at the Cleveland Clinic and validated its contents.

**Methods:** Patients who had at least one face-to-face outpatient encounter with a Cleveland Clinic health care provider and a) had two estimated glomerular filtration rate (eGFR) values <60 ml/min/1.73m$^2$ more than 90 days apart as of January 1, 2005 and/or b) patients with International Classification of Diseases (ICD-9) codes for kidney disease were included.

**Results:** Our registry includes 57,276 patients (53,399 patients with two eGFR <60 ml/min/1.73m$^2$ and an additional 3,877 patients who met the ICD-9 code criteria) as of March 2010. Mean age was 69.5 ±13.4 years with 55% females and 12% African Americans. Medicare is the primary insurer for over half of the study cohort. The kappa statistics to assess the extent of agreement between the administrative dataset derived from the EHR and actual EHR chart review showed substantial agreement (≥ 0.80) for all conditions except coronary artery disease and hypertension which had moderate agreement (<0.60).

| Condition | Kappa statistic | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|---|
| Diabetic nephropathy | 0.75 | 85% | 90% | 90% | 85% |
| Glomerulonephritis | 0.85 | 95% | 90% | 90% | 85% |
| Polycystic kidney disease | 0.90 | 100% | 90% | 90% | 100% |
| Diabetes | 0.90 | 91% | 100% | 100% | 90% |
| Hypertension | 0.45 | 65% | 90% | 95% | 50% |
| Hyperlipidemia | 0.85 | 95% | 90% | 90% | 95% |
| Coronary artery disease | 0.60 | 80% | 80% | 80% | 80% |
| Congestive heart failure | 0.85 | 87% | 100% | 100% | 85% |
| Cerebrovascular disease | 0.90 | 95% | 95% | 95% | 95% |

**Conclusions:** Development of EHR based CKD registry is feasible in a large health system and the comorbid conditions included in the registry are reliable. Apart from research purposes, such a registry would help to improve the quality of care delivered to CKD patients and complement the ongoing nationwide efforts to develop a CKD surveillance project.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1942

**Multiple Admissions for Suspicion of Acute Coronary Syndrome: Effect of Kidney Function** Claudine T. Jurkovitz,[1] Xin Xu,[1] James R. Bowen,[1] Angela J. Disabatino,[2] Michael Stillabower,[2] William S. Weintraub.[1,2] *[1]Center for Outcomes Research, Christiana Care Health System, Newark, DE; [2]Division of Cardiology, Christiana Care Health System, Newark, DE.*

***Background.*** Chronic kidney disease (CKD) is a well known risk factor for cardiovascular disease. Whether patients with CKD are more likely to be admitted multiple times for suspicion of acute coronary syndrome (ACS) is unclear. ***Methods.*** We studied the likelihood of emergency department readmission in all patients for whom Troponin T (cTn) was measured between 2004 and 2010 at a large regional health care institution. A total of 83959 patients had complete records for cTn, serum creatinine, and demographic variables. Estimated glomerular filtration rate (GFR) was stratified as follows: GFR≥60, 30-59, 15-29, <15 mL/min/1.73m$^2$. CKD was defined as GFR<60. The maximum cTn achieved was stratified into cTn≥0.03, 0.04-0.3, > 0.3 ηg/mL. Logistic regression was used to estimate the association between one or more readmissions (dependent variable) and CKD stages after adjusting for age, race, sex, and cTn. ***Results.*** Readmissions involved 34.5% of the patients. Among those, 16.8% had only 1 readmission and 17.7% had 2 or more. CKD was present

in 24.7% (n=20754) of the study population. The population characteristics are described in the table. Compared to patients with normal kidney function (GFR≥60), patients with CKD were more likely to be readmitted (Odds ratio (OR), 95% Confidence Interval), OR = 1.32 (1.27-1.38) for those with GFR 30-59, OR=1.40 (1.30-1.51) for those with GFR 15-29 and OR=1.64 (1.48-1.81) for those with GFR <15. ***Conclusion.*** The decision-making process regarding the evaluation and treatment of CKD patients suspected of ACS should take into account the high likelihood of readmissions for these patients.

Characteristics of the population

| | All | GFR<60 | GFR≥60 | p |
|---|---|---|---|---|
| >1 readmission (%) | 34.5 | 45.9 | 30.8 | <0.0001 |
| Age≥ 65 (%) | 40.8 | 72.5 | 30.4 | <0.0001 |
| Male (%) | 47.3 | 42.4 | 48.9 | <0.0001 |
| White (%) | 71.4 | 79.0 | 68.8 | <0.0001 |
| Black (%) | 23.9 | 18.4 | 25.7 | |
| cTn>0.3 (%) | 4.0 | 6.7 | 3.2 | <0.0001 |

Disclosure of Financial Relationships: nothing to disclose

## F-PO1943

**Incidence of End-Stage Renal Disease among Type 2 Diabetes Patients – A Nationwide Register-Based Cohort Study** Patrik Finne,[1] Marjo Helena Kervinen,[2] Carola Gronhagen-Riska,[3] Reijo Sund.[4] *[1]Finnish Registry for Kidney Diseases, Helsinki, Finland; [2]Department of Internal Medicine, Kuopio University Hospital, Kuopio, Finland; [3]Division of Nephrology, Helsinki University Central Hospital, Helsinki, Finland; [4]National Institute for Health and Welfare, Helsinki, Finland.*

Background

Incidence of type 2 diabetes has increased rapidly due to increase in sedentary life-style and improved detection of the disease. Type 2 diabetes is the most common cause of end-stage renal disease (ESRD) in most industrialized countries. Type 2 diabetes patients' risk of developing ESRD has not previously been estimated in nationwide cohorts.

Material and methods

Finland has 5.3 million inhabitants. Through linkage of several national healthcare registers (hospital diagnoses from registers of National Institute for Health and Welfare, drug prescriptions and reimbursements from the registers of The Social Insurance Institution of Finland) we identified 332,340 patients aged 40 years or older with a first registration of type 2 diabetes in 1988 to 2007. Statistics Finland provided information on patients' deaths. Time of initiation of renal replacement therapy (RRT) was obtained from the Finnish Registry of Kidney Diseases which contains data on all RRT patients in Finland. ESRD was defined as initiation of RRT.

Results

By the end of 2007, 2,048,474 patient-years were accrued, during which 941 type 2 diabetes patients developed ESRD and 105,625 died. Overall, the incidence rate of ESRD was 46 per 100,000 person-years and all-cause mortality rate was 5156 per 100,000 person-years. Incidence rate of ESRD was 51–55 per 100,000 person-years in those aged 40–69 years at time of diagnosis of type 2 diabetes. In those diagnosed at age 70–79 years the rate was 32 and in older patients it was 8 per 100,000 person-years. Mortality rate was 1531 deaths per 100,000 person-years in 40–49-year-olds and 8377 in 70–79-year-olds. Men had higher risk of ESRD than women (incidence rate ratio 2.2, 95% CI 1.9–2.5) and this did not change with adjustment for age.

Conclusions

Although type 2 diabetes is a common cause of ESRD, type 2 diabetes patients' risk of ESRD is minimal and much smaller than their risk of death.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1944

**Predictors of Decline in Renal Function in Older Adults: Results from the Einstein Aging Study (EAS)** Jennifer Yi-Chun Lai,[1] Mindy Katz,[2] Andrzej Galecki,[3] Richard B. Lipton,[2] Markus Bitzer.[1] *[1]Internal Medicine, University of Michigan, Ann Arbor, MI; [2]Neurology, Einstein, Bronx, NY; [3]Institute of Gerontology, University of Michigan, Ann Arbor, MI.*

Background: Decreased kidney function (DKF) in older adults harbors increased morbidity and mortality, but risk factors for rapid decline of renal function are not established. Therefore, we examined rates and predictors of estimated glomerular filtration rate (eGFR) decline in a sub-study of the EAS, a systematically-recruited prospective cohort of community-dwelling adults. Method: eGFR decline rate was determined in 161 subjects based on early serum creatinine measurements and MDRD formula using mixed effect modeling. Average eGFR (AeGFR, ml/min/1.73m2) was calculated for each subject across the observation period. Multivariate linear and logistic regression analyses were used to identify factors associated with more rapid eGFR decline. For latter the sample was dichotomized into those with high versus lower GFR decline. Albuminuria was determined by dipstick analysis. Results: Mean age of all subjects was 81 years (70-96 years; 96 female, 65 male). The AeGFR was 64 in males and 66 in females; the difference was statistically not significant. The average loss of renal function was -2.2% and -3.3% per year in male and female subjects, respectively. In multivariate linear analysis with GFR % decline as outcome, subjects of female gender (β= -0.93, P value <0.0001) and higher AeGFR (β= -0.02, P value <0.0001) were more likely to have more rapid GFR decline. Similar findings were obtained using multivariate logistic analysis predicting high eGFR decline for female subjects and subjects with higher AeGFR (OR>999, P value <0.0001; OR=1.1, P value=0.006, respectively). No association of eGFR decline with albuminuria was detected. Conclusions: Female gender and higher AeGFR are independent risk factors for more rapid eGFR decline in subjects in the EAS, but albuminuria is not associated with eGFR decline. This suggests that other risk factors for rapid loss of eGFR exist in older adults compared with younger adults. We are expanding the sample size to further test our findings.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1945

**Association of Estimated Glomerular Filtration Rate and Albuminuria with Mortality and End-Stage Renal Disease: A Collaborative Meta-Analysis of Kidney Disease Cohorts** Brad C. Astor,[1] Kunihiro Matsushita,[1] Ron T. Gansevoort,[2] M. van der Velde,[2] Mark Woodward,[4] Andrew S. Levey,[3] Paul E. de Jong,[2] Josef Coresh.[1] *[1]Department of Epidemiology, Johns Hopkins University, Baltimore; [2]Department of Nephrology, University Medical Center, Groningen, Netherlands; [3]Division of Nephrology, Tufts Medical Center, Boston; [4]George Inst. Int. Health, University of Sydney, Australia.*

Limited data are available on the independent associations of estimated glomerular filtration rate (eGFR) and albuminuria with mortality and end stage renal disease (ESRD) among individuals with chronic kidney disease (CKD). We conducted a collaborative meta-analysis of 21,688 participants selected for CKD from 13 cohorts.

A total of 4,374 deaths occurred in the 10 studies from which information on mortality was captured. A total of 4,128 ESRD events occurred in the 12 studies from which such information was captured. After adjustment for potential confounders and albuminuria, a 15 mL/min/1.73m$^2$ lower eGFR below 45 mL/min/1.73 m2 was significantly associated with mortality (pooled hazard ratio [HR] 1.47 [95% CI: 1.22-1.79]), and ESRD (pooled HR 6.24 [95% CI: 4.84-8.05]). There was significant heterogeneity between studies for both HR estimates. After adjustment for risk factors and eGFR, an eight-fold higher albumin:creatinine ratio (ACR) or protein:creatinine ratio (PCR) was significantly associated with mortality (pooled HR 1.40 [95% CI: 1.27-1.55]), without evidence of significant heterogeneity. An eight-fold higher ACR or PCR was also strongly associated with ESRD (pooled HR 3.04 [95% CI: 2.27-4.08]), with significant heterogeneity between HR estimates.

Lower eGFR and more severe albuminuria independently predict mortality and ESRD among individuals selected for CKD. The associations are stronger for ESRD than for mortality. The observed associations are consistent with CKD classification based on eGFR stages, and suggest that albuminuria provides additional prognostic information among individuals with CKD.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1946

**Pregnancy as the First Occasion for CKD Diagnosis** Rossella Attini,[1] Arianna Pagano,[1] Valentina Consiglio,[2] Pietro Gaglioti,[1] Tullia Todros,[1] Giorgina B. Piccoli.[2] *[1]Materno-Fetal Unit, Department of Obstetrics, ASO Universitaria OIRM-S.Anna, University of Torino, Italy; [2]Struttura Semplice Nefrologia Department of Clinical and Biological Sciences, ASO Universitaria San Luigi Gonzaga, University of Torino, Italy.*

Background

CKD and pregnancy share a complex relationship. Outside of pregnancy, CKD is frequently asymptomatic, and often diagnosed only if specifically searched for. Pregnancy may be the first occasion for diagnosis of CKD, as blood and urine tests are rarely performed in asymptomatic patients.

Aim of the study was to evaluate the prevalence and the main clinical features of CKD diagnosed during pregnancy in a Materno-Foetal unit and Outpatient facility dedicated to pregnant CKD patients.

Methods

Prospective analysis of CKD cases referred to the Unit, January 2000– June 2010. Start of observation: referral to the unit. End of observation: 1 month after delivery. All diagnosed were posed and reviewed by the same nephrology gynaecology team.

Results

Over 189 pregnancies, 68 patients had a diagnosis of kidney disease during pregnancy: in 38 the clinical history was silent, while in 30 a previous diagnosis was not considered as relevant (Single kidney, acute pyelonephritis with kidney scars). Excluding 8 cases with acute pyelonephritis, CKD was diagnosed in 60 new patients.

The diagnosed were glomerular diseases in 12/60 patients (2 dropped from follow-up; 7 underwent a renal biopsy: IgA nephropathy in 4, membranous nephropathy in 3); in 6 cases CKD stage 4-5 was diagnosed. The main diagnostic clue was either a small for gestational age baby or alteration in biochemical tests during pregnancy. Interstitial nephropathy or urological anomalies or previous acute pyelonephritis were diagnosed in 34/60 cases, and persistent urinary anomalies in 9/60 cases; the remaining 5/60 patients had other diagnoses.

At referral median age was 30 years; 57 were Caucasians; referral occurred late (52% in the second and 17% in the third trimester), median creatinine 0.79 mg/dl (0.3-4.9 mg/dl) , median proteinuria 0.66 g/24 hours (0-7.8).

Conclusions

Pregnancy may be an occasion for early diagnosis of CKD. The awareness of this condition may be precious tool for ensuring woman's health.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1947**

**The Impact of Moderate Chronic Kidney Disease (CKD) on All Cause Mortality, Cardiovascular (CV) Mortality, and Defibrillation in Patients with Implantable Cardioverter Defibrillators (ICD) for Sudden Cardiac Arrest (SCA) Prevention** Sejan B. Patel, Eric W. Raasch, Patrick H. Pun, John Paul Middleton. *Division of Nephrology, Duke University Medical Center, Durham, NC.*

Patients with end stage renal disease (ESRD) have an inordinate risk for SCA, but compared to those without ESRD, they have reduced survival despite presence of an ICD for SCA prevention. Less is known about patients with CKD. We evaluated the impact of CKD on all cause mortality, CV mortality, and ICD shocks in patients with an ICD for SCA prevention.

We performed a retrospective study of subjects who received a de novo ICD between 2002 and 2004 in our institution. We defined CKD as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m2. The primary outcome was time to all cause and cardiovascular (CV) death. The secondary outcome was time to ICD shock.

Of 102 subjects, 46 subjects had CKD (45%). Subjects with CKD were older (age 70±10 vs. 60±15 years, p<0.001) and had lower left ventricular (LV) ejection fraction (27±13% vs. 35±18%, p<0.05) than those without CKD. There was no difference in comorbidities. Subjects with CKD had 12 all cause and 6 CV deaths and those without CKD had 3 all cause and 3 CV deaths over a mean period of 14.5±6.5 months associated with a 12-month survival of 84% with CKD vs. 98% without CKD (p<0.01) and 12-month CV survival of 88% with CKD vs. 98% without CKD (p=0.20). Subjects with CKD had 12 ICD shocks and those without CKD had 14 ICD shocks over a mean period of 5.9±4.5 months associated with a 6-month survival from ICD shock of 78% with CKD vs. 74% without CKD (p=0.60). Cox regression adjusting for demographics, comorbidities, and LV ejection fraction showed that an increase in eGFR by 1 mL/min was associated with a 3.4% hazard reduction for all cause death (HR 0.966, 95%CI 0.938-0.995, p<0.05).

Patients with CKD have reduced survival despite presence of an ICD for SCA prevention. No difference in CV death and ICD shock suggests that mechanisms of death other than SCA may limit ICD effectiveness in this population. Clinicians should consider competing non-cardiovascular causes of death in assessing benefit and risks of an ICD in this population.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1948**

**Nationwide Outcomes of Patients with Chronic Kidney Disease Undergoing Coronary Artery Bypass Surgery** Ankit Sakhuja, Abhishek Deshmukh, Nilay Kumar, Rahul S. Nanchal, Aaron T. Dall, Gagan Kumar. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

**Background:**

Chronic kidney disease (CKD) has been associated with adverse outcomes after coronary artery bypass grafting (CABG). However data from a national perspective are limited. We sought to investigate outcomes of patients with CKD undergoing CABG with a focus on development of acute kidney injury (AKI) and the need for dialysis.

**Methods**

Retrospective analysis was performed using National Inpatient Sample from the year 2007. All adult patients (age 18 years or more) with discharge diagnosis of CKD and the procedure CABG were identified using appropriate ICD-9-CM codes. Patients with end stage renal disease were excluded. The primary outcomes measured were frequency of AKI, need for dialysis, all cause in hospital mortality, length-of-stay, total charges and disposition of the patients. Pearson correlation and Chi square were used to compare the variables for unadjusted analysis and logistic regression was used to obtain adjusted odds ratios. $\alpha$ was set at 0.05.

**Results**

There were an estimated 212,237 patients undergoing CABG in 2007of which, 8.3% had CKD. After multivariate logistic regression and controlling for demographic factors and co-morbid conditions, the CKD patients were more likely (OR 8.5; 95%CI 7.7-9.4) to develop AKI and require dialysis. Patients with CKD developing AKI and requiring dialysis had significantly higher in-hospital mortality (OR 3.92; 95%CI 2.21-6.94) when compared to those who did not require dialysis.

Length of stay was 3.1 days longer (95%CI 2.8-3.4) and charges were $27,440 higher (95%CI $23,502-$31,337) in CKD patients when compared to those with normal kidney function.

The CKD patients were discharged to long term care, significantly more than those with normal renal function (31% vs. 16%)

**Conclusion**

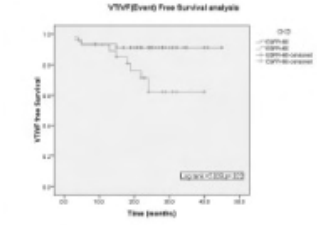
In patients undergoing CABG, CKD predicts higher in-hospital mortality and increased frequency of AKI, including need for dialysis. CKD predicts longer length of stay, higher incurred charges and discharges to long term care facilities.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1949**

**Moderate Chronic Kidney Disease Is an Independent Risk Factor for Malignant Ventricular Arrhythmias in Chronic Heart Failure Patients with Primary Prevention Cardiac Resynchronization Therapy-Defibrillator (CRT-D) Devices** Sanjeev Kumar,[1] Girish Babu,[2] A. Gopalamurugan,[2] Anthony Chow,[2] Pierre Lambiase,[2] Oliver Segal.[2] *[1]Department of Nephrology, Royal Free Hospital, United Kingdom; [2]The Heart Hospital, University College London Hospitals, United Kingdom.*

Chronic kidney disease (CKD) in the context of chronic heart failure (CHF) occurs frequently and is associated with increased mortality. We sought to determine whether baseline CKD is associated with increased incidence of malignant ventricular arrhythmias (sustained ventricular tachycardia/fibrillation;VT/VF) in CHF patients. A retrospective analysis of prospectively collected data for all CHF patients with no previous history of VT/VF implanted with primary prevention CRT-D devices between Jan'04 and Nov'08. VT/VF incidence was analyzed by device follow-up records from stored electrograms. The cohort was divided into 2 groups, G1 and G2 with MDRD eGFR < 60 and eGFR > 60 mls/min per 1.73m2 respectively. 117 patients met the inclusion criteria and there were 54(46%) in G1 and 63(54%) in G2. The mean eGFR in CKD group (G1) was 44±9 mls/min. The two groups were similar with respect to age, follow-up duration (mean 2 years) , baseline left ventricular ejection fraction (LVEF), NYHA class and anti-arrhythmic drugs. Significantly greater proportion of VT/VF occurred in G1 when compared to G2 (31% vs 12%, G1vs.G2; p< 0.05). Kaplan Meier survival analysis revealed significantly lower event-free survival in G1 (p<0.01). Multivariate analysis revealed eGFR<60 ml/min per 1.73m2 (HR 3.7, p<0.05) and LVEF (HR 0.9, p<0.05) as independent risk factor for sustained VT/VF. CONCLUSION: Even moderate CKD is a strong independent risk factor for malignant ventricular arrhythmia in HF patients with no previous history of VT or VF.



Disclosure of Financial Relationships: nothing to disclose

**F-PO1950**

**The Impact of Renal Dysfunction on Overall Mortality in Cancer Patients** Sun Young Na, Jiyoon Sung, Wookyung Chung, Hyun Hee Lee, Sejoong Kim, Jae Hyun Chang, Ji Yong Jung. *Department of Internal Medicine, Gachon University of Medicine and Science, Incheon, Republic of Korea.*

**Background**

Patients with renal dysfunction are at increased risk for cardiovascular and all-cause mortality. The effects of renal dysfunction on the risk of death with malignant disease are uncertain. The aim of this study is to determine the association between estimated glomerular filtration rate (eGFR) and overall mortality in cancer patients.

**Methods**

In this retrospective study, we identified 8226 cancer patients with one or more in and outpatient serum creatinine measurements from January 1, 2000 to December 31, 2004. Patients were followed up to December 31, 2008. We estimated glomerular filtration rate (eGFR) using the abbreviated Modification of Diet in Renal Disease (MDRD) equation. Renal dysfunction was defined as an eGFR of less than 60ml/min/1.73m$^2$. Cox's proportional hazards models adjusted for age, gender, diabetes mellitus, hypertension, proteinuria, serum hemoglobin and albumin level.

**Results**

The mean age of the patients was 56.3 years, and the proportion of male patients was 50.7%. The cancer types were included kidney and urinary tract cancer in 312 (3.8%), other solid organ cancer in 7621 (92.6%), and hematologic malignancy in 293 (3.6%) patients. A total of 1054 (12.8%) patients had renal dysfunction (baseline eGFR less than 60ml/min/1.73m$^2$). Lower eGFR was associated with increased risk of death in cancer patients. The adjusted hazard ratios were 1.17 with an eGFR of 30 to 59 ml/min/1.73m$^2$ (95% confidence interval 1.04 to 1.31, $p$=0.01), 2.22 with an eGFR of less than 30ml/min/1.73m$^2$ (95% confidence interval 1.76 to 2.80, $p$<0.001), compared to patients who had eGFR more than 60ml/min/1.73m$^2$.

**Conclusions**

The prevalence rate of renal dysfunction was 12.8% in cancer patients and higher than 4.7% of general population. The risk of death with cancer was greater at lower eGFRs. This association may contribute to excess mortality in cancer patients with renal dysfunction. Therefore, nephrologists should be aware of renal dysfunction as a predictor of mortality and should make efforts to preserve renal function in cancer patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1951**

**Use of Renin-Angiotensin System Blocking Agents and Retarded Progression of End-Stage Renal Failure: A Retrospective Study** Akira Fujiwara,[1] Nobuhito Hirawa,[2] Gen Yasuda,[2] Satoshi Umemura.[1] *[1]Department of Medical Science and Cardiorenal Medicine, Yokohama City University School of Medicine, Yokohama, Japan; [2]Division of Nephrology and Hypertension, Yokohama City University Medical Center, Yokohama, Japan.*

Since angiotensin II receptor antagonists have been available around 2000, many studies have revealed their effects in retarding the deterioration of renal function. However, before these trials, physicians were reluctant to use renin-angiotensin system blocking agents (RASBA) in end-stage renal failure, because of the risk of renal function deterioration and hyperkalemia. In the present retrospective cohort study, we reviewed the records of uremic patients before initiation of dialysis therapy to evaluate whether RASBA changed the natural course of renal function deterioration.

**Methods.** Between 1990 and 2010, 274 patients were followed from 3 to 5 years before dialysis therapy. The patients were categorized into 4 groups: diabetic and non-diabetic, and 1990s and 2000s. The slope of the reciprocal of the serum creatinine levels versus time (1/Cr), an index of the rate of renal failure progression, was evaluated.

**Results**. Ninety-one percents of patients in the 2000s diabetic group (n=52, men/women 33/19, mean age 74±5 years) received RASBA, compared with 4% in the 1990s diabetic group (n=55, men/women 40/15, mean age 71±5 years). The 1/Cr was 0.11±0.05 dL/min/month in the 2000s diabetic group, which was lower than 0.15±0.08 dL/min/month in the 1990s diabetic group, showing retardation of progression of renal impairment. Meanwhile, there was no difference in 1/Cr (0.07±0.04 vs. 0.08±0.04 dL/min/month) between the non-diabetic 1990s (n=100, men/women 71/29, mean age 73±6 years) and 2000s groups (n=67, men/women 33/34, mean age 75±15 years), although the proportion of patients receiving RASBA was higher in the 2000s non-diabetic group (92%) than that in the 1990s non-diabetic group (4%).

**Conclusion**. Our study suggests that increasing use of RASBA is associated with retarded deterioration of renal failure caused by diabetes, whereas the use of these agents is not associated with a clear retardation in renal failure progression in non-diabetic uremic patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1952**

**Improvement in GFR in Patients with Chronic Kidney Disease** Lise Weis,[1,3] Marie Metzger,[2] Benedicte Stengel,[2] Emmanuel Letavernier,[1,3] Marc C. Froissart,[4] Jean-Philippe Haymann,[1,3] Jean-Jacques Boffa.[1,3] *[1]U 702, INSERM, Paris, France; [2]U 780, INSERM, Paris, France; [3]Hopital Tenon, APHP, Paris, France; [4]Hopital Georges Pompidou, APHP, Paris, France.*

Renal function decline in chronic kidney disease (CKD) patients is considered ineluctable. We report patients with CKD whose GFR improved.

We studied 406 patients from the Nephrotest cohort who underwent at least 3 mGFR measures by $^{51}$Cr-EDTA clearance, over at least 2 year-follow up. Patients were assigned as having improved *vs* declined mGFR, on the basis of visual examination of mGFR slopes by 4 independent nephrologists who further reached consensus about discrepancies. We compared patients with improved mGFR first with those with moderate mGFR decline of 0 to -2ml/min/1.73m$^2$/yr, then with those with declined mGFR, on number of achieved nephroprotection targets: systolic and diastolic blood pressure,<130 and <80 mm Hg, U-albumin/creatinin ratio<30 mg/mmol, diabetes control and RAS blocker use. We identified 63 patients (15.5%) with improved mGFR over time. Their mean mGFR slope was 2.8 ± 2.2 ml/min/1.73m$^2$/yr *vs* -3.0±3.0 for the 332 patients with mGFR decline and -0.6± 1.1 for the 144 with moderate decline. They had various nephropathies except diabetic and polycystic kidney disease. As compared to the 332 with declining mGFR, those who improved did not differ in age, sex and cardiovascular history, but their baseline mGFR was higher, 42.3 vs 39.7±13.2 ml/mn/1.73 m$^2$, they had less often 2 or more metabolic complications, 30% vs 48%, and higher number of achieved targets, 3.7±0.9 vs 3.3±0.8. After adjusting for baseline mGFR, the OR (95%CI) for improved mGFR significantly increased 1.7 (1.2-2.3) with each increase of achieved target unit. However, when compared to patients with moderate mGFR decline, the adjusted OR was no longer significant, 1.3 (0.9-1.9).

This is the first description of a rather large cohort of CKD patients whose mGFR improved over time, including a fourth with severe CKD. This study revealed that nephroprotection alone may not explain this evolution, suggesting the existence of other factors. Our data suggest the reversal of renal fibrosis in human pathology, as previously described in experimental models.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1953**

**The Impact of Moderate Chronic Kidney Disease (CKD) on Survival in Patients with Implantable Cardioverter Defibrillators (ICD) for Primary and Secondary Sudden Cardiac Arrest (SCA) Prevention** Sejan B. Patel, Eric W. Raasch, Patrick H. Pun, John Paul Middleton. *Division of Nephrology, Duke University Medical Center, Durham, NC.*

Patients with CKD have an inordinate risk for SCA. While an ICD attenuates the risk of SCA in the general population, it is not evident whether patients with CKD have similar benefit. We evaluated the impact of CKD on survival in patients with an ICD for SCA prevention.

We performed a retrospective study of subjects who received a de novo ICD between 1997 and 2009 in our institution. We defined CKD as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m2. The primary outcome was time to all cause death.

Of 400 subjects, 201 subjects had CKD (50%). Subjects with CKD were older and had more comorbidities than those without CKD (age 68±11 vs. 58±13 years, coronary artery disease 80% vs. 67%, diabetes mellitus 36% vs. 27%, hypertension 94% vs. 83%, left ventricular (LV) ejection 27±10% vs. 32±13%, p<0.01). Subjects with CKD had a eGFR 43±13 mL/min and those without CKD had a eGFR 80±19 mL/min. There was no difference in primary and secondary prevention indications for an ICD among subjects with and without CKD (primary 81% vs. 73%, secondary 19% vs. 27%, NS). There were 135 deaths during a mean period of 4.6±2.7 years. The primary prevention cohort was associated with a 5-year survival of 61% with CKD vs. 88% without CKD (p<0.001). The secondary prevention cohort was associated with a 5-year survival of 61% with CKD vs. 77% without CKD (p<0.05). Cox regression adjusting for demographics, comorbidities, LV ejection fraction, and ICD indication showed that an increase in eGFR by 1 mL/min was associated with 1.1% hazard reduction in death (HR 0.989, 95%CI 0.979-0.999, p<0.05).

Patients with CKD have reduced survival despite presence of an ICD for primary and secondary SCA prevention. Effectiveness of an ICD may be limited by mechanisms of death other than SCA that are significant in this population.

Disclosure of Financial Relationships: nothing to disclose

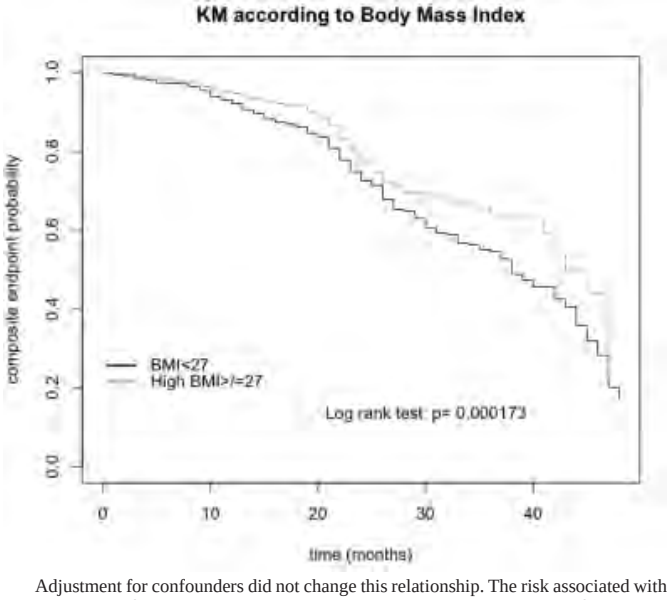
**F-PO1954**

**Chronic Kidney Disease Progression and Mortality in Moderate Kidney Dysfunction According to Body Mass Index** Antonio Bellasi, Marcora Mandreoli, Antonio Santoro. *On Behalf of the PIRP Study Group, Policlinico S.Orsola-Malpighi, Italy.*

In the general population the excess of weight represents a strong risk factor for End Stage Renal Disease (ESRD) and all-cause mortality. Conversely, in dialysis patients a high Body Mass Index (BMI) is inversely related to overall mortality. This study sought to investigate the impact of BMI on the risk of the composite endpoint of dialysis inception or all-cause mortality in a large cohort of Chronic Kidney Disease (CKD) patients not on dialysis.

We utilized the patient's records from the "Prevenzione Insufficienza Renale Progressiva" (PIRP) database, a large project sponsored by the Emilia-Romagna Institute of Health, aimed at optimizing CKD patients care. Survival analyses estimated the relationship between BMI at baseline and outcomes.

A total of 4174 patients with CKD 3-5 (male 65,7%; mean age 73,3 (1.2) yrs; diabetes 30,5%) patients registered in the PIRP database between year 2004 and 2007 were identified. The median eGFR (MDRD4) and BMI were 29 ml/min/1.73m$^2$ [IQ: 21-37] and 26 Kg/m$^2$ [IQ: 24-29], respectively. The mean follow-up was 14.5 (10.7) months. An inverse relationship between BMI and the risk of commencing dialysis or dying was noted. Indeed, a moderate overweight was linked with a better survival (long-rank test: p<0.001).




Adjustment for confounders did not change this relationship. The risk associated with a BMI ≥27 Kg/m$^2$ was 38% lower than that associated with a BMI <27 Kg/m$^2$ (HR 0.62, 95% IC: 0.40-0.95; p=0.03).

In summary, as documented in dialysis patients the presence of CKD modify the prognostic significance of traditional CV risk factors. Indeed, a moderate overweight probably due to a better nutritional status, is associated with a better prognosis. Finally, this results further underlie the need of a dedicated nutritional approach in elderly with moderate chronic kidney dysfunction

Disclosure of Financial Relationships: nothing to disclose

**F-PO1955**

**Epidemiology and Risk Fcators of Chronic Kidney Disease in Sichuan Province** Li Wang. *Department of Nephrology, Sichuan Provincial Hospital, Chengdu, Sichuan, China.*

Objective: To investigate the epidemiology and its risk factors of chronic kidney diseaes.Method:3024 people older than 18 years in chengdu (represent urban area and Guanghan (represent rural area)were enrolled by cluster random sampling.This reseach included a questionnaire , physical exminations and laboratory examinations CKD was diagnosed by decreased eGFR(<60ml/min/1.73m$^2$) or presentation of microalbuminuria, macroalbuminuria, proteinuria or hematuria. .**Results:***1.Prevalence of CKD in Sichuan province*: The overall prevalence and age-and-sex-standardized prevalence of CKD in Sichuan was 19.1% and 16.48% respectively. The prevalence of hematuria, albuminuria, proteinuria and decreased eGFR was 7.2%, 11.4%, 1.4% and 2.9%; and the standardized prevalence was 6.74%, 9.49%, 1.11% and 2.34%, respectively. Prevalence of CKD, hematuria, microalbuminuria and macroalbuminuria were 15.1%, 11.2%, 12.4% and 1.1% in female , and everyone was lower in male population with the corresponding prevalence of 6.2%, 3.3%, 8.4% and 1.0%(p<0.001). Prevalence of CKD in Chengdu was lower than in Guanghan(10.4% vs 19.1%, p<0.001). And prevalence of hematuria, proteinuria, albuminuria and decreased eGFR was also lower in Chengdu than in Guanghan(p<0.001). Prevalence of CKD and microalbuminuria increased with age. In different age groups(18~39, 40~49, 50~59, 60~69, 70~95), the prevalence of CKD was 11.6%, 16.8%, 19.9%, 27.4% and 41.3% respectively. The awareness rate of CKD was 7.9%. The logistic regression analysis showed that hypertension(OR =1.933, 95.0%CI: 1.506-2.482), diabetes(OR=4.528, 95.0%CI: 3.239-6.329), hyperuricemia(OR=1.644, 95.0%CI: 1.233-2.192) and history of reral disease(OR=1.682, 95.0%CI: 1.137-2.487) was risk factors of CKD. And male(OR=0.496, 95.0%CI: 0.379-0.648), high degree of education(OR=0.839, 95.0%CI: 0.738-0.955), and high income(OR=0. 804, 95.0%CI: 0.727-0.889) was negative associated with CKD.Conclusion:The prevalence of CKD in Sichuan province is relatively high in China with a higher prevalence in rural population than in urban population. Female gender, hypertension, hyperuricemia, diabetes, past history of renal disease, low degree of education and low income are risk factors of CKD.

Disclosure of Financial Relationships: nothing to disclose

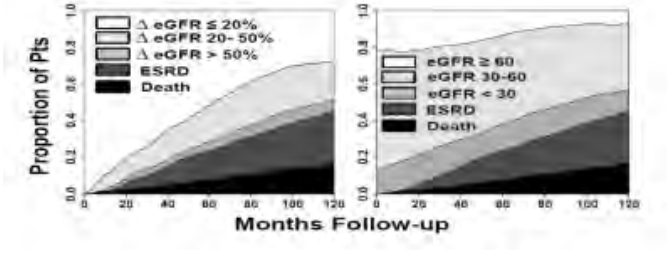
**F-PO1956**

**Multistate Representations of the Evolution of Cohorts with Chronic Kidney Disease (CKD)** Bo Hu,[1] Liang Li,[1] Lawrence J. Appel,[2] Brad C. Astor,[2] Julia Lewis,[3] Michael S. Lipkowitz,[4] Robert D. Toto,[5] Xuelei Wang,[6] Jackson T. Wright,[6] Tom H. Greene.[7] *[1]Cleveland Clinic; [2]Johns Hopkins; [3]Vanderbilt; [4]MSSM; [5]UTSW; [6]CWRU; [7]Utah.*

**Introduction** Longitudinal analyses of CKD progression often use as outcome the mean slope of estimated GFR (eGFR) or time to a designated event (eGFR level or ESRD). These approaches use limited portions of available data, restricting statistical power and clinical insight, and are susceptible to informative censoring bias. The time-to-event approach emphasizes patients at greatest risk for adverse events and is not well suited to assessment of favorable outcomes. We show alternative representations of evolution of CKD cohorts that avoid these problems by jointly incorporating information from clinical events **and** eGFR.

**Methods** We propose a hybrid multistate method which applies competing risk methods to estimate cumulative incidence of ESRD and death in conjunction with non-parametric regression analysis for change in eGFR.

**Results** Multistate representations of the evolution of the cohort of 1094 African American Study of Kidney Disease (AASK) pts over 10 yrs are in 2 panels showing proportion of pts with ESRD and death with additional states defined by change in eGFR from baseline (left) or absolute level (right). After 10 yrs, an estimated 17% of patients died, 29% had ESRD, 6% had 50% eGFR decline w/o ESRD, 21% had 20% - 50% eGFR decline. The remaining 28% had stable function (10-yr eGFR ≥ 80% of initial eGFR).




**Conclusion** The proposed multistate approach avoids bias from informative censoring by death and/or ESRD and accounts for the full spectrum of negative and positive outcomes ranging from death to stable function. The method extends to states jointly defined by eGFR and other markers such as protein excretion and can be adapted to increase power for treatment comparisons.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1957**

**Impaired Fasting Glucose Is Associated with Renal Hyperfiltration in the General Population – Results from the Renal Iohexol-Clearance Survey in Tromsø 6 (RENIS-T6)** Toralf Melsom,[1.2] Ulla Dorte Mathisen,[1.2] Trond G. Jenssen,[2] Marit D. Solbu,[1.2] Bjorn Odvar Eriksen.[1.2] *[1]University Hospital of North Norway; [2]University of Tromsø, Norway.*

**Background**: Approximately 30% of the adult U.S. population have impaired fasting glucose (IFG), which is associated with increased prevalence of chronic kidney disease. Elevated glomerular filtration rate (GFR), or hyperfiltration (HF), has been proposed as an underlying mechanism for renal injury in diabetes and obesity. The purpose of this study was to assess whether impaired fasting glucose (IFG) or insulin resistance were associated with HF in the general middle-aged population.

**Method**: From the 6$^{th}$ Tromsø study, we investigated 1627 individuals from the general population between 50 to 62 years without self-reported diabetes, cardiovascular disease or kidney disease. GFR was measured by single sample iohexol-clearance. 34 persons with GFR< 60 ml/min/1,73m$^2$ and 20 with fasting glucose ≥ 126 mg/d were excluded. Insulin resistance was estimated by using the Homeostasis Model Assessment (HOMA-IR). We defined HF as GFR above the 90 percentile, adjusted for sex, age, weight and height. Multiple logistic regression was performed with HF (yes/no) as the dependent variable and fasting glucose, fasting insulin, HOMA-IR and IFG (yes/no) as independent variables in different models.

**Results**: IFG was present in 33 % of males and 15% of females.

Table 1. Adjusted odds ratio (OR) for hyperfiltration

| | OR | 95% CI |
|---|---|---|
| Model 1: | | |
| Glucose, per mmol/L | 1.87 | 1.31 to 2.69 |
| | | |
| Model 2: | | |
| IFG (yes/no) | 1.77 | 1.21 to 2.58 |
| | | |
| Model 3: | | |
| Insulin, per µU/ml | 1.03 | 0.99 to 1.06 |
| | | |
| Model 4: | | |
| HOMA-IR, per mU x mmol/L² | 1.13 | 1.01 to 1.27 |
| | | |
| Model 5: | | |
| HOMA-IR, per mU x mmol/L² | 1.05 | 0.93 to 1.20 |
| Glucose, per mmol/L | 1.77 | 1.20 to 2.61 |

All models were adjusted for age, gender, weight, height, smoking status, diastolic blood pressure and the use of inhibitors of the renin-angiotensin system.

**Conclusion**: Fasting serum glucose and IFG but not fasting insulin levels, were associated with HF. This indicates a similar mechanism for HF in prediabetes and diabetes. IFG could be one of the causes of renal injury in the general population.

Disclosure of Financial Relationships: nothing to disclose

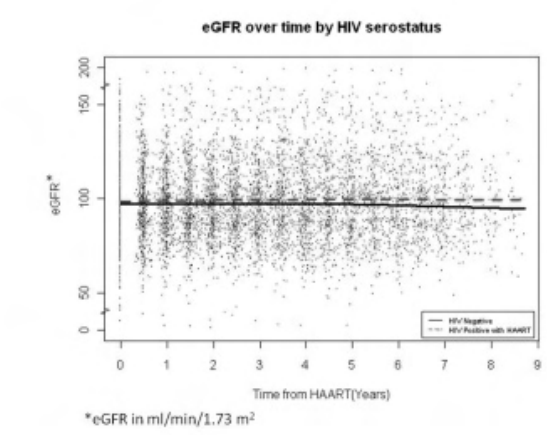
**F-PO1958**

**Antiretroviral-Treated HIV-Infected Women Have Kidney Function Trajectories Similar to HIV-Uninfected Women** Michelle M. Estrella,[1] Alison G. Abraham,[1] Yuezhou Jing,[1] Rulan S. Parekh,[2] Phyllis Tien,[3] Kathryn Anastos,[4] Mardge H. Cohen,[5] Jack A. Dehovitz,[6] Lynda A. Szczech,[7] Stephen J. Gange.[1] *[1]Johns Hopkins University; [2]University of Toronto; [3]University of California, San Francisco; [4]Montefiore Medical Center; [5]Stroger Hospital; [6]SUNY Downstate Medical Center; [7]Duke University.*

To determine whether HIV infection in the context of highly active antiretroviral therapy (HAART) was associated with faster kidney function decline, we compared estimated glomerular filtration rates (eGFR) between HIV-infected and uninfected women comparable at baseline on known risk factors for kidney disease. Using data from 3766 women in the Women's Interagency HIV Study, we identified 127 HIV-infected women who were matched at HAART initiation to 493 HIV-uninfected women on calendar time, age, and kidney disease risk factors (systolic blood pressure ±20 mm Hg, total cholesterol ±20 mg/dl, hepatitis C status, and diabetes; see Table). We used linear mixed models to examine the difference in annual eGFR decline by HIV status. The effect of HIV disease severity was assessed by stratifying eGFR slope by time-varying CD4$^+$ cell count in HIV-infected women. Baseline mean eGFRs were similar between HIV-infected and uninfected women (HIV$^+$=98 vs HIV$^-$=97 ml/min/1.73 m$^2$). We found no significant difference in eGFR slope by HIV status ($ß_{HIV+}$=-0.08 vs $ß_{HIV-}$=0.18, P=0.13), though the mean difference by 7 years was marginally significant (P=0.06). No trend was noted in eGFR decline by CD4$^+$ cell count. Our results suggest that HAART-treated HIV-infected women with a mean age of 39 years at baseline experience a similar eGFR trajectory as those of HIV-uninfected women.

Table. Baseline characteristics

| Characteristic | Mean | |
|---|---|---|
| | HIV+ | HIV- |
| Age, y | 39 | 39 |
| Systolic BP, mm Hg | 115 | 116 |
| Total cholesterol, mg/dl | 182 | 182 |
| Hepatitis C infected, % | 29 | 29 |
| Diabetic, % | 16 | 16 |




Disclosure of Financial Relationships: nothing to disclose

**F-PO1960**

**Serum Uric Acid Predicts End Stage Renal Disease and Mortality in Chronic Kidney Disease Patients in Southern Taiwan** Chi-Chih Hung, Shang-Jyh Hwang. *Division of Nephrology, Department of Internal Medicine, Kaohsiung, Taiwan.*

Background: Serum uric acid (UA) is a risk factor for cardiovascular disease in general population. However, whether UA is a risk factor for end stage renal disease (ESRD) is controvertial. Taiwan has high incidence of both hyperuricemia and ESRD. We would thus hypothesize that UA is a risk factor for ESRD in chronic kidney disease (CKD) patients.

Method: From 2002.11.11 to 2008.12.31, 3272 patients joined the integrated CKD care cohort study in Kaohsiung Medical University Affiliated Hospitals. All biochemical data within 3 months of the enrollment were averaged. Renal outcome and survival were followed till 2009.12.31. Chi-square test, t-test, Kaplan-Meier survival analysis and multivariate cox regression analysis were used and p value less than 0.05 was considered as significant.

Results: UA level increased significantly from CKD stage 1 to 5 ($p<0.05$, compared with CKD stage 1). In the multivariate linear regression, UA correlated with male gender, use of anti-hyperuricemic drug, phosphate, proteinuria and GFR ($p<0.01$). The patients were thus divided by UA level quartiles as UA1 to UA4 (from low to high UA level). The UA4 group had higher percentage of DM, hypertension, cardiovascular disease, and use of anti-hyperuricemic drug than the other groups (all $p<0.05$). Also the UA4 group had lower glomerular filtration rate (GFR), lower albumin, lower hematocrit, higher body mass index and higher phosphate than the other groups (all $p<0.05$). Kaplan-Meier survival analysis showed lower renal survival rate in UA4 group compared with all other three groups ($p<0.05$). The 5 year renal survival is 78% and 55% in UA1 and UA4 group, respectively. The mortality was different only between UA1 and UA4 groups with 93% and 82% 5 year survival rate, respectively. In the multivariate cox regression analysis, albumin, UA groups, male, proteinuria, and GFR were risk factors for ESRD. In the multivariate cox regression analysis, higher C-reactive protein, lower GFR, UA groups and lower albumin were risk factors for all-cause mortality.

Conclusion: Serum UA level at the time of enrollment predicts ESRD and mortality in CKD patients in Southern Taiwan.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1961**

**Illicit Drug Use and Chronic Kidney Disease: Findings from the National Health and Nutrition Examination Survey** Arjun Das,[1] Lydia Bazzano,[2] James P. Lash,[1] Sanjeev Akkina.[1] *[1]Nephrology, University of Illinois at Chicago, Chicago, IL; [2]Epidemiology, Tulane University, New Orleans, LA.*

Studies have reported a possible link between illicit drug use and chronic kidney disease (CKD). The purpose of this study was to examine this relationship in a nationally representative sample.

**Methods:** We performed a cross-sectional analysis of data from the National Health and Nutrition Examination Survey 1999 – 2004. The sample included 8270 participants between the ages of 20 - 59 years who completed a survey regarding use of illicit drugs. CKD was defined as an MDRD estimated glomerular filtration rate (eGFR) ≤60 mL/min/1.73m$^2$ or the presence of microalbuminuria (>20mg/g). We compared non-drug users to those who used any illicit drugs (apart from marijuana) and to those who used IV drugs. Using logistic regression models, we estimated odds ratios for CKD by type of illicit drug use adjusted for age, gender, race (white vs. non-white) and diabetes (DM).

**Results:** We found that drug users had similar mean eGFR (106.0 ± 0.8 vs. 106.6 ± 0.7 ml/min/1.73m$^2$, p = 0.51) and albumin/creatinine ratio (23.5 ± 4.6 vs. 21.7 ± 2.5 mg/gm, p = 0.73) as compared to non-drug users. The unadjusted risk for CKD was lower in the drug user group but this difference became insignificant after adjustment (see table). Similarly, IV drug users did not differ from non-drug users in terms of eGFR (105.7 ± 2.9 vs. 106.6 ± 0.7 ml/min/1.73m$^2$, p = 0.74) and albumin/creatinine ratio (45.9 ± 23.6 vs. 21.7 ± 2.5 mg/gm, p = 0.32). The risk of CKD was also not significantly different among the two groups (see table).

**Table: Risk of CKD in Drug users and IV Drug users versus Non-Drug users**

| | Model Attributes | OR (95% CI) | p - value |
|---|---|---|---|
| **Illicit drug use** | *Unadjusted* | 0.739 (0.590-0.926) | 0.0086 |
| | Multivariate Adjusted* | 0.829 (0.657-1.046) | 0.1133 |
| **IV drug use** | *Unadjusted* | 0.644 (0.317-1.308) | 0.2235 |
| | Multivariate Adjusted* | 0.655 (0.309-1.389) | 0.2699 |

* Age, gender, race and DM

**Conclusion:** In a representative sample of the U.S. population, we did not find an association between illicit drug use and CKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1962**

**Oral Paricalcitol Effects from Hyperparathiroidism Treatment to Renoprotection Role in Patients with Chronic Kidney Disease (CKD) Stages 3-5nd** Secundino Cigarran,[1] Francisco Coronel,[2] Montserrat Pousa,[1] Ignacio Docal.[3] *[1]Nephrology, Hospital Da Costa, Burela, Lugo, Spain; [2]Nephrology, Hospital Clinico Universitario San Carlos, Madrid, Spain; [3]Radiology, Hospital Da Costa, Burela, Lugo, Spain.*

The new understanding of vitamin D endocrine/intracrine system alters the view of renal disease and its treatment.Vit D analogs are expanding because of its role on inflammation and fibrosis related to renin-angiotensin-aldosterone axis (RAAS) in which is important as renoprotective effect. The aim of this study is to assess the effect of oral paricalcitol on PTH and on Proteinuria in CKD stage 3-5ND patients with 25D and 1,25 D normal levels on 6 months of follow up. 42 patients, were followed during 6 months, mean age 70.34± 12.4 yr, 34.2% female, 34% diabetic, estimated glomerular filtration rate (GFR-MDRD): 33.93±13.0 ml/min. Inclusion criteria were Vitamin 25D &1,25D in normal range and PTHi >150 pg/ml. Paracalcitol 1µ/daily p.o. was added to treatment. The primary end point was to compare change in PTHi levels and secondary, the effect in mean spot urinary albumin / creatinine ratio between baseline measurement and the last of the study evaluation. Parameters analyzed at start and every 3 months there were: PTHi, serum calcium, serum phosphorous, phosphorous fractional excretion, GFR, and urine spot albumin/creatinine ratio.

t-paired analysis

| Variable | Baseline | 6 months | P |
|---|---|---|---|
| GFR (ml/min) | 33.9±13.2 | 24.9±13.8 | .003 |
| Serum Albumin (gr/dl) | 4.25±.24 | 4.23±.35 | NS |
| Serum Phosphorous(mg/dl) | 3.7±.52 | 3.96±1.01 | .032 |
| Serum Calcium (mg/dl) | 9.17±.52 | 9.19±.73 | .005 |
| CaxP (mg2/dl2) | 34.12±9.09 | 36.40±11.1 | .006 |
| PTHi (pg/ml) | 259.93±134.92 | 124±15 | .000 |
| 25D Vitamin (ng/ml) | 46±9.3 | 49±4.2 | NS |
| 1,25D Vitamin (pg/ml) | 33.0±10 | 36.86±13.4 | NS |
| Urinary spot Albumin/creatinine (mg/gr crea) | 1044.51±1786.7 | 408.57±793.26 | .030 |

NS Not significance

There were not serious adverse events.

Paricalcitol resulted in significant reduction of PTHi, significant increase of calcium and phosphorous and significant reduction in urinary albumin/creatinine ratio showing a renoprotective action.GFR reduction could be RAAS effect.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1963**

**Impacts of rHuEPO Treatment on Progression of CKD and on Mortality and Hospitalization during 1 Year after HD Onset in Japan** Tadao Akizawa.[1,2] *[1]Department of Nephrology, Showa University School of Medicine, Tokyo, Japan; [2]On Behalf of JET Study Group.*

Background and Objective: There are a few evidences with respect to the clinical significance of anemia treatment in CKD patients with recombinant human erythropoietin (EPO). We retrospectively evaluated the effects of EPO treatment on progression of CKD and prognosis after onset of HD in the sub-cohort (3,286 patients) of the ongoing multicenter, prospective JET study in Japan.

Method: The effect on time to renal failure of EPO treatment during the non-dialysis period was estimated using the inverse probability weighted (IPW) analysis to adjust for time-dependent confounding. The weights used in IPW analysis were calculated using the logistic model, which included baseline confounders and time-dependent variables : hemoglobin (Hb) and serum creatinine (sCre). The cumulative proportion (Kaplan-Meier method) without death, evaluated in 3,280 patients, excluded 6 patients with no data on first EPO treatment after HD.

Results: During the non-dialysis periods, 69.3% of CKD patients were treated with EPO at the mean Hb level of 8.73 g/dL. The effect of covariates on the motivation to start EPO treatment were significant in some parameters of Hb, sCre, age, diabetes, cardiac insufficiency, hyperlipidemia and hypertension. The adjusted Hazard Ratio of HD introduction by EPO treatment was 0.65 (0.54-0.78; $p<0.0001$). Compared to non-EPO treatment group, in EPO treatment group, the cumulative proportion without death was significantly higher, with 98.59%, 97.10% and 95.36% at 3, 6 and 12 months after onset of HD, respectively, and the risk of death during 12 months was also lower (HR 0.598; 0.419-0.853; $p=0.005$). Likewise, patients treated with EPO had lower risk of hospitalization/death (HR 0.887; 0.790-0.995; $p=0.041$).

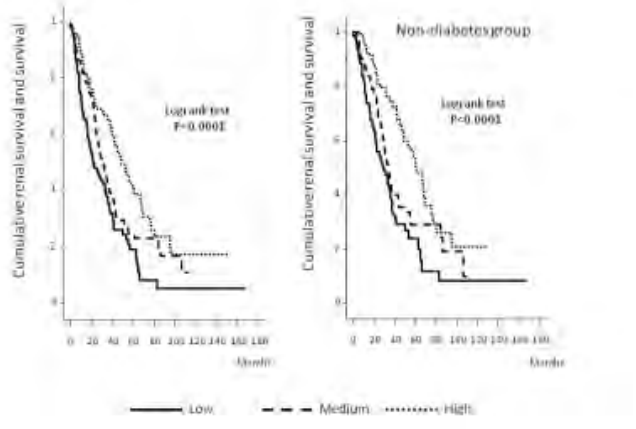
Conclusion: This retrospective research strongly suggests that EPO treatment during non-dialysis in CKD patients has preventive effects not only on the progression of CKD but also on mortality and hospitalization after onset of HD.

Disclosure of Financial Relationships: Consultancy: Chugai, Kirin,AbbottResearch Funding: Chugai, Kirin.

**F-PO1964**

**Association between Body Mass Index (BMI) and Renal Outcome in Predialysis Chronic Kidney Disease** Daijo Inaguma. *Nephrology, Nagoya Daini Red Cross Hospital, Nagoya, Aichi, Japan.*

[Introduction] It is claimed that the higher the BMI values of maintenance dialysis patients are, the more likely their life expectancy is to be favorable, but there have been hardly any reports in regard to the predialysis period. [Purpose] The purpose of this study was to investigate the association between BMI and both renal outcome and life expectancy in predialysis chronic kidney disease. [Subjects] The subjects were 455 patients with chronic kidney disease who presented to the nephrology department during the period between January 1995 and December 2008 and whose BMI was recorded. There were 143 women, and the subjects' mean age was 65.9 ± 12.8 years. Their estimated GFR was 17.4 ± 8.6 ml/min/1.73 m². [Methods] This was a retrospective observational study. The patients were divided into 3 groups according to their BMI values at the first presentation: low-BMI group (14.3-21.2), medium-BMI group (21.3-24.2), and high-BMI group (24.3-35.1), and the endpoint was renal death or death. [Results] The observation period (median) was 20.0 months, and during the observation period 233 patients were started on dialysis and 15 patients died. According to the Kaplan-Meier survival curves the low-BMI group had the poorest outcome (Log-rank test, $P < 0.0001$), especially limited to non diabetes group.




The results of a multivariate analysis using the Cox proportional hazard model showed that BMI was a factor associated with outcome even after adjusting for age, sex, presence or absence of diabetes, albumin values, hemoglobin concentrations, and whether the patient was treated with a renin-angiotensin inhibitor (95% CI 0.890-0.974, HR = 0.931, $P = 0.021$). [Discussion] The results suggested that nutritional status deteriorates as chronic kidney disease progresses, probably influencing the rate of progression of renal dysfunction and life expectancy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1965**

**Genetic Investigation of JunD Mediated Activation Networks in Primary Rat Macrophages** Richard P. Hull,[1] Santosh S. Atanur,[1] Jacques Behmoaras,[2,3] Zelpha D'Souza,[1] Jennifer Smith,[3] Charles D. Pusey,[3] H. Terence Cook,[2,3] Timothy J. Aitman.[1] *[1]MRC CSC, Imperial College; [2]CCIR, Imperial College; [3]Renal Medicine, Imperial College, London.*

Activated macrophages are major cellular mediators of injury in proliferative and crescentic glomerulonephritis (Crgn). Our previous work on a rat model of Crgn identified a genetically determined macrophage activation phenotype associated with Crgn susceptibility. Using congenic, linkage and microarray studies we found that the AP-1 transcription factor JunD was a primary determinant of macrophage activation in the Crgn-susceptible WKY rat. *Jund* is markedly overexpressed in WKY compared to Crgn-resistant Lewis bone marrow derived macrophages (BMDMs) and, using *Jund* congenic strains, we demonstrated *Jund* dependant control of IL10 expression. *Jund* knockdown in WKY BMDMs led to a decrease in Fc receptor-mediated macrophage activation. Our current work aims to identify genome-wide JunD mediated macrophage activation networks in the WKY rat and to understand the genetic determinants of JunD-mediated overactivity in WKY BMDMs.

We have performed chromatin immunoprecipitation combined with high throughput sequencing (ChIP-Seq) in BMDMs from WKY and *Jund* congenic strains to give a genome wide profile of JunD binding. Our preliminary analysis has identified differential binding between basal and LPS stimulated WKY BMDMs with 65-67% of peaks in each condition located in intergenic regions and 25% located within introns. 52% of intragenic peaks were found within 20 kilobases (kb) of a transcriptional start site (TSS) suggesting preferential JunD binding to enhancer regions. Gene ontology analysis of peaks located ±20kb from a TSS is consistent with a role for JunD as a mediator of macrophage activity enriching, for example, for processes such as intracellular signaling cascades ($P=2.5x10^{-9}$) in basal BMDMs. We have now performed comparative microarray experiments in WKY, Lewis and *Jund* congenic BMDMs and by integrating these gene expression data sets with the ChIP-Seq data sets, we aim to identify the key genes in networks through which JunD mediates macrophage overactivity and novel targets through which to modulate macrophage activation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1966**

**Developmental Tissue-Specific Expression of the Prorenin Receptor** Renfang Song, Graeme James Preston, Ihor V. Yosypiv. *Pediatrics, Tulane School of Medicine, New Orleans, LA.*

The prorenin receptor [(P)RR] has two major roles: 1) Mediate specific intracellular effects of prorenin and renin and 2) Enhance their enzymatic activity on the plasma membrane. In this study, we examined (P)RR gene and protein expression during mouse organogenesis. (P)RR mRNA and protein levels were determined in the brain, kidney, lung and heart of CD1 mice on embryonic (E) days E12.5-18.5 and postnatal (P) days P1, P10 and P60 (adult) by quantitative RT-PCR and Western blot analysis, respectively. Cellular distribution of the (P)RR protein and mRNA in the metanephros was mapped by immunohistochemistry and *in situ* hybridization (ISH), respectively. RT-PCR demonstrated that brain, kidney and lung (P)RR mRNA levels increase progressively during gestation, peak on P10 and decline in adulthood. In contrast, (P)RR protein levels were high during early gestation and decreased with maturation. In the heart, (P)RR mRNA and protein contents remain stable during gestation to subsequently decline on P10 and P60. The (P)RR protein is weakly expressed in the developing metanephros in inner tubular structures as early as on E14.5. On E16.5 and E18.5, (P)RR is present in the ureteric bud (UB) epithelia followed by glomerular mesangium. On P1 and P10, (P)RR is most abundant on the luminal aspect of collecting ducts followed by proximal tubules and mesangium. This expression pattern persists into adulthood. ISH revealed weak diffuse presence of (P)RR mRNA in the inner mesenchyme and UBs on E13.5. A significant increase in the intensity of (P)RR mRNA expression throughout the kidney was observed on E14.5. These results demonstrate that (P)RR gene and protein expression is developmentally regulated in a tissue-specific manner. During early metanephric development, (P)RR mRNA is broadly distributed in the mesenchyme and UB epithelia. (P)RR protein expression in the kidney is spatially restricted to UBs/collecting ducts followed by glomerular mesangium and tubules. The enrichment of (P)RR in UB-derived collecting ducts and glomeruli suggests a novel function for the (P)RR in the regulation of nephron and renal collecting system development.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1967**

**Establishment of a Rapid and Simple Protein Enrichment Method in Clinical Proteomics Study: Enrichment of Proteins by Nitrocellulose Membrane** Xuejiao Liu,[1] Lulu Jia,[2] Mingxi Li,[1] Bixia Gao,[1] Xiaohong Fan,[1] Xuemei Li,[1] Youhe Gao,[2] Xue-Wang Li.[1] *[1]Nephrology, Dept Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences,Peking Union Medical College, Beijing, China; [2]Perking Union Medical College, Beijing, China.*

**Objective:** To establish a rapid and simple protein enrichment method by nitrocellulose (NC) membrane and compare it with acetone precipitation (AP). **Methods:** The morning urine samples of fourteen healthy donors (7M/7F, 20-30 year old) were collected. Twelve samples (6M/6F) were pooled together. The urinary proteins of pooled, single male and female samples were enriched by AP overnight, NC membrane for 30, 60 and 90 minutes respectively. The concentration of protein was measured by Bradford method, and the differences of protein bands between AP, NC membrane enrichment (30, 60 and 90 min) was evaluated using 1D-SDS-PAGE electrophoresis. The proteins of pooled sample enriched by AP (Group A) and NC membrane for 90 minutes (Group B) were identified by LC-MS/MS (Thermo,LTQ Velos). **Results**: The proteins were enriched by NC membrane rapidly. (figure1) The amounts of proteins enriched by AP were higher than that by NC membrane in pooled, male and female samples. The amount of proteins enriched by NC membrane was increased with the enriching time in pooled, male and female samples. The NC membrane method selectively reduced the amount of albumins.(figure1)The number of proteins identified by LC-MS/MS in group B was more than that in group A.**Conclusion:** A rapid and simple protein enrichment method was established. This new method enriched less urinary albumin which diminishes the suppression of albumin on low-abundant proteins in the urine and may be used in biomarkers discovery studies. Further research is needed to evaluate the profile of proteins and the significance of this new method in clinical proteomics study.

Figure 1

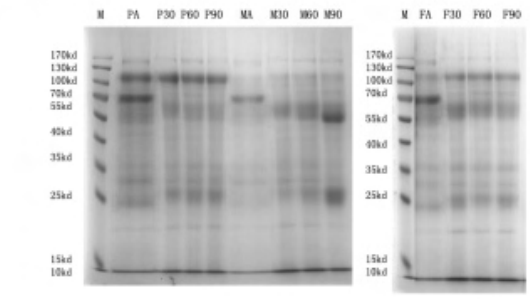



Figure 1: Proteins enriched by AP and NC membrane. (M):marker, (PA): the pooled protein precipitated by acetone, (P30-P90):the pooled protein enriched by NC membrane for 30-90 minutes. (MA): male proteins precipitated by acetone, (M30-90): male proteins enriched by NC membrane for 30-90 minutes. (FA):Female proteins precipitated by acetone. (F30-90): Female proteins enriched by NC membrane for 30-90 minutes.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1968**

**Isolation of Whole Glomeruli from a Needle Biopsy Sample of a Patient with Glomerulonephritis** Kenichiro Koitabashi,[1,2] Takashi Yasuda,[2] Kenjiro Kimura.[2] *[1]Clinical Proteomics and Molecular Medicine, St. Marianna University Graduate School of Medicine, Kawasaki, Japan; [2]Division of Nephrology and Hypertension, Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Japan.*

*Background:* Renal biopsy samples are important not only for the diagnosis of glomerulonephritis, but also for the investigation of its pathogenesis. However, it remains difficult to biochemically analyze proteins extracted solely from the glomeruli of the needle biopsy samples. To overcome this difficulty, we established a simple but reliable method, named as "micro-sieving," to isolate whole glomeruli from a single needle biopsy sample of a patient with glomerulonephritis.

*Method:* We isolated glomeruli from each of four renal needle biopsy samples by the micro-sieving and from each of the two surgically resected kidneys by a conventional sieving method. In the micro-sieving, the biopsy sample was sieved the 180 μm mesh, then the sieved fraction was sieved again on the 75 μm mesh. The glomeruli were recovered on the 75 μm mesh. The number of the isolated glomeruli was counted by microscopy. This procedure took about one hour. The glomerular proteins (2.5 μg) extracted by the micro-sieving and the conventional sieving were compared by 2-dimensional electrophoresis (2-DE).

*Result:* The glomeruli isolated from the biopsy samples showed no obvious destruction. On average, 55 glomeruli were isolated from a single renal biopsy and 23 μg proteins were extracted from the glomeruli. Further, we successfully obtained clear 2-DE images of the glomerular proteins from a single biopsy sample. The 2-DE images were very similar to that by the conventional sieving method.Conclusion: The "micro-sieving" can be used widely as a fundamental technique to analyze glomeruli in renal needle biopsy samples.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1969**

**Alternative Splicing of a Comprehensive ESRP-Regulated Network Contributes an Extensive Additional Layer of Complexity to the Global Changes in Gene Expression That Occur during the Epithelial Mesenchymal Transition (EMT)** Russ P. Carstens. *University of Pennsylvania School of Medicine.*

The Epithelial Mesenchymal Transition (EMT) is a critical process during the development and has also been implicated in tissue fibrosis and tumor metastasis. Furthermore, the reverse process of Mesenchymal Epithelial Transition (MET) is also crucial for development, as shown during renal organogenesis. Changes in the transcriptional profile of epithelial cells that undergo the EMT have been well studied, including the downregulation of E-cadherin and upregulation of vimentin. However, the extent to which changes in splicing contribute an additional layer of gene regulation that impacts the EMT has not been systematically investigated. Alternative splicing achieves coordinated changes in gene expression and generates protein isoforms with differential and often opposing activities. In a genome-wide cell-based screen we recently discovered Epithelial Splicing Regulatory Proteins 1 and 2 (ESRP1 and ESRP2) as essential regulators of transcripts that switch splicing during the Epithelial to Mesenchymal Transition (EMT), including genes with important roles in the EMT. The ESRPs are transcriptionally inactivated during the EMT and direct knockdown of ESRP expression promotes the EMT indicating that they are crucial components of an epithelial-specific gene signature. Using splicing sensitive microarrays and massively parallel high throughput sequencing (RNA-seq) we uncovered a global ESRP-regulated splicing network defined by hundreds of transcripts that switch splicing during the EMT. These ESRP-regulated transcripts encode numerous proteins associated with the EMT, regulation of the actin cytoskeleton, cell-cell adhesion, cell polarity, and cell migration. Our results strongly suggest that the definition of this ESRP-regulated splicing regulatory network defines protein interaction networks and isoform-dependent functional properties of numerous proteins that underlie the EMT. These studies thus highlight the importance of coordinated changes in alternative splicing, in addition to changes in transcription, to promote dynamic changes in proteomic complexity during the EMT.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1970**

**RNA-Seq Analysis of TGFβ-Induced Gene Expression in Tubular Epithelial Cells Identifies Novel Low-Copy Number Transcripts and Correlation with Differentially Expressed Genes in Renal Biopsy Material** Eoin P. Brennan,[1] Melissa Morine,[2] David Walsh,[1] Derek P. Brazil,[1] Catherine Godson,[1] Finian Martin,[1] The Genie Consortium.[1] *[1]UCD Diabetes Research Centre, Conway Institute, University College Dublin, Dublin, Ireland; [2]Nutrigenomics Research Group, Conway Institute, University College Dublin, Dublin, Ireland.*

TGF-β1 is implicated in disease progression in diabetic nephropathy (DN). We have assessed the global transcriptional profile in cultured proximal tubule epithelial cells (HK-2) stimulated with TGF-β1 (10 ng/ml, 48h) using 'next generation' sequencing (RNA-seq). We identified 624 differentially expressed genes (304 upregulated, 320 downregulated; p≤0.05), and network analysis indicated upregulation of key pathways including TGF-β1 signalling, extracellular matrix (ECM)-receptor interaction and focal adhesion. Differential expression of six representative genes was validated by qRT-PCR. Of particular note, was the induction by TGF-β1 of expression of transcripts of all 7 chorionic gonadotropin/LH beta chain genes in a locus on chromosome 19q13.3. We noted a shared motif which contained a HNF1α/Tcf transcription factor binding element in the proximal promoters of all 7 genes. This may identify the path by which TGF-β1 is acting in this context. Comparison of the RNA-Seq analysis with microarray [Affymetrix HGU133A] analysis of an identical TGF-β1 cell treatment showed 103 genes were detected as differentially expressed in both analyses and an additional 109 genes ('low expressors'; <100 transcript reads) were detected as differentially expressed by RNA-Seq only. Preliminary analysis of the differentially expressed gene cohort detected by RNA-seq and microarray analysis with genes selectively expressed in biopsies from patients with DN [Affymetrix HGU133A microarray analysis; Schmid H et al.,2006] identified a shared subset of 65 transcripts, thereby detecting a TGF-β1-driven pro-fibrotic 'finger-print' in the DN-biopsy transcriptome. RNA-seq therefore achieves a more sensitive and comprehensive transcriptomic analysis that will facilitate our improved understanding of the molecular events that underpin DN progression including integration with GWAS-derived data.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1971**

**Genome-Wide Microarray Coupled with ChIP-Seq Identify the Wnt Signaling Pathway as a Major p53 Target in the Developing Kidney** Zubaida R. Saifudeen, Meera Harshad Velankar, Nathaniel J. D. Mclaughlin, Jiao Liu, Samir S. El-Dahr. *Pediatrics, Tulane University, New Orleans, LA.*

p53 regulates expression of cell cycle, apoptosis, differentiation and senescence pathway genes. We recently described a requirement for p53 function in kidney development. Interestingly, $p53^{-/-}$ kidneys have fewer nephrons than wild-type littermates. Early in development, $p53^{-/-}$ metanephroi exhibit an atypical large cap mesenchyme suggesting a defect in conversion of mesenchyme to epithelial progenitors. To identify pathways directly regulated by p53, genome-wide microarray coupled with ChIP-Seq was done on E15.5 metanephroi of $p53^{+/+}$ and $p53^{-/-}$ littermates (n=3/group). 3627/44000 (~8%) mapped genes showed significant differential expression ($p<0.05$). 40% (1422/3627) of these are potential direct targets of p53 as they have at least one p53 binding site, discovered by p53 ChIP-seq. In addition to known p53-target genes (cancer, cell cycle, DNA damage, metabolism), pathway analysis identified many renal developmental regulators (Osr1, HNF1b, Pax2, Pax8, Fgf8) and signaling pathways (Wnt, Bmp, FGF, Notch) as p53-targets. Importantly, p53 is enriched *in vivo* at genes of several members of the Wnt signaling pathway that are differentially expressed in $p53^{-/-}$ kidneys, including Fzd4, Fzd7, LRP1/6, Dvl2, Gsk3β, Csnk1g1 and cyclin D1 suggesting these Wnt pathway members as primary targets for p53 regulation in the developing kidney. Using a TCF-reporter, we show p53 potently represses β-catenin transcriptional activity in metanephric mesenchyme cells, associated with reduced β-catenin levels. P53 repression of TCF-reporter activity was maintained in the presence of LiCl, a GSK3β inhibitor, implying p53 acts downstream of the β-catenin destruction complex. siRNA-mediated knockdown of p53 up-regulates Axin2, a Wnt responsive gene. This study demonstrates that p53 regulates nephrogenesis via multiple mechanisms, including direct transcriptional effects on the Wnt signaling pathway. Our data suggest aberrant Wnt signaling in the mesenchymal cap cells in the absence of p53 as a possible mechanism for the defect in differentiation of the cap mesenchmye to nephron progenitors.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1972**

**Uncovering Genes and Regulatory Pathways Related to Urinary Albumin Excretion Using Linkage Analysis and Genetical Genomics in Mice** Rachael S. Hageman, Magalie S. Leduc, Christina R. Caputo, Shirng-Wern Tsaih, Gary Churchill, Ron Korstanje. *The Jackson Laboratory, Bar Harbor, ME.*

Identifying the genes underlying quantitative trait loci (QTL) for disease has proven difficult, mainly due to the low resolution of the approach and the complex genetics involved. However, recent advances in bioinformatics and the availability of genetic resources now make it possible to narrow the genetic intervals and test candidate genes. In addition to

identifying the causative genes, defining the pathways that are affected by these QTL is of major importance as it can give us insight into the disease process and provide evidence to support candidate genes. In this study we mapped three significant and one suggestive QTL on Chromosomes (Chrs) 1, 4, 15, and 17, respectively, for increased albumin excretion (measured as albumin-to-creatinine ratio) in a cross between the MRL/MpJ and SM/J mouse inbred strains. By combining data from several sources and by utilizing gene expression data, we identified Tlr12 as a likely candidate for the Chr 4 QTL. Through the mapping of 33,881 transcripts measured by microarray on kidney RNA from each of the 173 male F2 animals, we identified several downstream pathways associated with these QTL. Among these were the glycan degradation, leukocyte migration, and antigen presenting pathways. We demonstrate that by combining data from multiple sources, we can identify not only genes that are likely to be causal candidates for QTL, but also the pathways through which these genes act to alter phenotypes. This combined approach provides valuable insights into the causes and consequences of renal disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1973**

**Systems Approach Identify HIPK2 as a Critical Regulator of Kidney Tubulointerstitial Fibrosis** Yuanmeng Jin,[1] Yifei Zhong,[1] John C. He.[1,2] *[1]Medicine/Nephrology, Mount Sinai School of Medicine, New York, NY; [2]Medicine/Nephrology, James J. Peters VA Medical Center, Bronx, NY.*

Tubulointerstitial fibrosis is a common pathway leading to the progression of kidney disease for which there are limited treatment options. We describe an integrated computational/experimental approach to identify upstream protein kinases that regulate gene expression changes in kidneys of HIV-1 transgenic mice (Tg26), a model for HIV-associated nephropathy (HIVAN) where significant tubulointerstitial injury is present. With this approach we identified that the homeo-domain interacting protein kinase 2 (HIPK2), a previously unrecognized kinase for kidney disease, is a highly expressed kinase in the renal tubulointerstitium of Tg26 as well as in patients with HIVAN or with other kidney diseases. HIPK2 mediates transforming growth factor-β-induced Smad3 phosphorylation and expression of epithelial-mesenchymal transdifferentiation markers in renal tubular epithelial cells. Knockout of HIPK2 attenuated tubulointerstitial fibrosis in kidneys of mice with unilateral ureteral obstruction, a well-established model for renal fibrosis. We conclude that HIPK2 is a critical regulator of kidney fibrosis and a potential target for anti-fibrosis therapy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1974**

**Application of Laser Capture Microscopy (LCM) and Proteomic Tissue Analysis To Characterize Fibronectin Glomerulopathy (FNG)** Anjali A. Satoskar,[1] John P. Shapiro,[2] Cherri N. Bott,[1] Gyongyi Nadasdy,[1] Michael A. Freitas,[2] Lee A. Hebert,[3] Tibor Nadasdy,[1] Brad H. Rovin.[3] *[1]Pathology, Ohio State University, Columbus, OH; [2]Molecular Virology Immunology Medical Genetics, Ohio State University, Columbus, OH; [3]Nephrology, Ohio State University, Columbus, OH.*

FNG is a rare familial glomerulopathy characterized by proteinuria, progressive renal dysfunction, and pathologically by lobular glomerular enlargement, obliteration of capillary loops and mesangial deposition of acellular finely granular/fibrillary material. Although these deposits were shown to stain for fibronectin, staining can be weak and inconsistent. It was therefore postulated that other proteins may contribute to these deposits. To address this question and to demonstrate the utility of LCM and proteomic analysis for studying glomerular diseases, we examined renal biopsy material from two affected members of a known FGN family. Snap frozen renal biopsy tissue was sectioned, fixed in ethanol and stained. LCM was used to collect glomeruli by dissecting around the Bowman's capsules. For comparison, glomeruli were collected from baseline allograft biopsies (n=3), diabetic nephropathy (DN) (n=2), and lupus nephritis (LN) (n=5) biopsies. Glomeruli were digested with trypsin and a proteomic profile obtained using liquid chromatography-tandem mass spectrometry. Compared to normal, DN, and LN glomeruli, FNG glomeruli showed a significantly greater content of fibronectin isoforms 1, 3, 4, 5, 8, fibulin-1 and 5, and transthyretin. Several proteins were down-regulated in FNG glomeruli including vimentin, laminin, HSP70, podocin and synaptopodin (also decreased in DM and LN glomeruli). The interstitium of FNG biopsies uniquely showed an increased content of apolipoprotein-A1, A4, and serpin. This study demonstrates that LCM followed by tissue proteomics is a promising technique to identify unique proteins in kidney compartments. This will be useful in dissecting the pathogenesis of glomerular diseases. The data suggest that fibulin-1 and 5 may play a role in FNG. Several basement membrane and podocyte proteins were under-represented in FNG. This may account for the proteinuria seen in FNG.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1975**

**Using Gene Knock-Outs and Transposon Based Transgenics in Sensititized Rat Strains To Study Genes Nominated by Human GWAS for Renal Disease** Howard J. Jacob,[1,2] Rebecca R. Schilling,[1] Jozef Lazar,[1,3] Carol Patricia Moreno Quinn.[1] *[1]Physiology & Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI; [2]Pediatrics, Medical College of Wisconsin, Milwaukee, WI; [3]Dermatology & Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI.*

Genome Wide Association Studies (GWAS) and other types of genetic studies in humans have nominated genes contributing to hypertension and associated renal disease. Functional studies must now be undertaken for these genes. The rat is the dominant model for the physiological assessment of the cardiovascular system, but has lacked the ability to target genes. Using zinc finger nucleases (ZFN), which takes a fraction of the time of ES cell technology, we have knocked out (KOed) 45 genes in 9 months. An additional 55 genes will be KOed over the next 15 months. As there is interplay between hypertension and renal disease, we are knocking the genes out on the hypertensive backgrounds of SS and FHH rats. For select genes we also use transgenic rats generated using the *Sleeping Beauty* transposon system to over expressing a gene of interest.

Twelve renal genes are currently under evaluation. As an example, we have studied *Rab38* a gene responsible for a quantitative trait locus for hypertension-associated renal failure and a host of other traits associated with protein trafficking using both transgenic rescue and site directed gene KO. In the FHH rat, *Rab38* is responsible for the coat color, eye color, platelet storage pool deficit and proteinuria. We completely rescued all 5 classes of phenotypes with addition of a transposon carrying the wild-type *Rab38*. To validate the utility of the ZFN for functional studies, we also knocked out the *Rab38* gene in a consomic strain (FHH.BN1), which all the wild type phenotypes. The KO completely replicated the disease phenotypes proving both the gene and methodology. Data from the phenotyping of other renal strains will come online before the meeting and will also be presented.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1976**

**RNA-Seq Defined Glomerular Cell-Lineage Enriched Transcipts in Human Glomerular Disease** Jeffrey B. Hodgin,[1] Felix H. Eichinger,[2] Courtenay M. Vining,[2] Ann Randolph,[2] Preethi Sankaranarayanan,[2] Simon C. Satchell,[3] Moin Saleem,[3] Matthias Kretzler.[2] *[1]Pathology, University of Michigan; [2]Nephrology, University of Michigan, Ann Arbor; [3]University of Bristol, United Kingdom.*

To perform their specialized functions, glomerular endothelial, epithelial and mesangial cells have developed unique structural and molecular components. For a comprehensive survey of cell lineage-enriched transcripts, deep-sequencing (RNA-Seq) was employed on conditionally immortalized human endothelial and podocyte cell lines and microdissected glomeruli. Transcript abundance was determined using Affymetrix U133plus and RNA-Seq with 30 million reads per experiment(Illumina Genome Analyzer), yielding an average expression correlation of 0.80 for transcripts detected on both platforms. On NGS, a total of 17441 transcripts were detected above noise for podocytes, 17305 for endothelial cells, and filtered against 18244 transcripts in glomeruli to confirm in vivo expression. Cell lineage enrichment was defined by the absolute difference in normalized expression exceeded by 2 standard deviations across all transcripts and yielded 449 podocyte and 283 endothelial transcripts. The podocyte-enriched transcripts were interrogated for protein-protein interactions and the resulting networks analyzed for shared transcriptional responses using promoter models (Genomatix ModelInspector). Comparative promoter analysis defined a model containing transcription factor matrices V$ZBPF and V$EGRF (including WT1) in THRB, CAMK2B, COL4A4, MATN2. Among the 656 human genes containing the promoter model, 264 were present on Affymetrix HGU133A microarrays and investigated for their ability to classify expression profiles from 34 patients with glomerular diseases. These could also segregate glomerular transcriptional profiles of MGN from FSGS. Functional analysis of this gene set revealed significant overrepresentation of the Wnt canonical pathway and PI3K signaling events. Identifying novel cell-lineage enriched transcripts in the glomerular context will allow better definition of the unique molecular pathways essential for the glomerular ultrafiltration barrier and it's failure in disease.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1977**

**Common Peptide Motifs Binding HLA-DQ in Caucasian and Japanese Idiopathic Membranous Nephropathy Patients** Satoru Ogahara, Yasuhiro Abe, Kenji Ito, Maho Watanabe, Takao Saito. *DIvision of Nephrology and Rheumatology, Department of Internal Medicine, Fukuoka University, Fukuoka, Japan.*

Thirty Japanese adult patients with idiopathic membranous nephropathy (IMN) and 238 Japanese normal controls were analysed for HLA-DRB, DQA1 and DQB1 gene polymorphism using second exon amplification and sequence-specific oligonucleotides. A highly significant increased frequency of the DRB1*15:01 (0.80 vs 0.15, OR 23.2, $p = 2.8 \times 10^{-15}$), DRB5*01:01 (0.80 vs 0.15, 23.2, $2.8 \times 10^{-15}$), DQA1*01:02 (0.87 vs 0.26, 18.6, $1.1 \times 10^{-10}$) and DQB1*06:02 (0.80 vs 0.13, 25.8, $2.2 \times 10^{-16}$) alleles were found in Japanese IMN patients. Caucasoid patients with IMN have been reported to be associated with a HLA-DRB1*03:01, DQA1*05:01 and DQB1*02:01(Vaughan et al., 1995). It was interesting to note that a HLA-DR15 subtype in Japanese and that of HLA-DR3 in Caucasoid were associated with susceptibility to IMN. HLA-DR3 was very rare in Japanese. Thus, no disease associated with these antigens has been reported in Japanese. We thought that

HLA molecule primarily contribute to autoimmune disease by binding peptides that were presented to antigen-HLA molecule specific T-cell receptors in a context that results in the loss of tolerance to self-antigens. We evaluated the presentation of peptide motifs on HLA-DR and DQ associated with both ethnic IMN in comparison to other HLA-DR or DQ using SYFPEITHI algorithm (www.syfpeithi.de) to predict the binding peptide to specific HLA molecules. Common amino acid residues were found within pocket 1 (A, F, W, Y, I, L or V), pocket 4 (L, V or I), pocket 6 (P or A), pocket 7 (D or E) pocket 9 (I, L or V) between HLA-DQ2 (DQA1*05:01-DQB1*0201) associated with Caucasoid IMN and HLA-DQ6 (DQA*01:02-DQB1*06:02) with Japanese IMN. There was no common peptide motif in HLA-DR associated with IMN. M-type phospholipase A2 receptor (PLA2R) have been proposed to be target antigen in IMN (Beck LH et al., 2009). We predicted that PLA2R(24-32, AAALTPERL) was one of the antigenic peptide binding HLA-DQ molecules using protein-BLAST. To seek a putative antigenic peptide might be important for achieving a understanding of disease pathogenesis and designing antigen-specific therapy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1978**

**Homozygosity Mapping of Recessive Disease Genes for Steroid-Sensitive Nephrotic Syndrome** Xuewen Song,[1] Pingzhao Hu,[2] Ning He,[1] Andrew D. Paterson,[2] York P. Pei.[1] *[1]Divisions of Nephrology, University Health Network and University of Toronto, Toronto, ON, Canada; [2]Genetics and Genome Biology, Hospital for Sick Children, Toronto, ON, Canada.*

Recent studies of familial steroid-sensitive (SS-) nephrotic syndrome (NS) due to minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) suggest that these disorders may be underpinned by mutations of rare recessive genes (JASN 12:374-8, 2001; 14:1897-900, 2003). Homozygosity mapping is a powerful approach for mapping rare recessive disease genes in inbred families and has been recently extended to outbred populations. We completed a genome-wide homozygosity scan in 30 patients (Caucasian, n=21; Chinese, n=3; Native Indian, n=3; South Asian, n=2; Japanese, n=1) with SS-MCD/FSGS by using the Illumina Human CNV 370-Quad arrays. All patients except three Native Indians were unrelated. Using the software PLINK, we calculated the inbreeding coefficient F and found cryptic relatedness in the parents of 8 patients (F > 0.03). We catalogued all runs of homozygosity (ROHs) > 1Mb in individual patients and found a total of 284 tracts in 21 Caucasian patients and 42 tracts, in 3 Chinese patients. Using the ethnic-specific database from the HapMap reference populations for comparison, we identified 21 unique ROH tracts shared by > two Caucasian patients and 4 unique ROH tracts shared by > two Chinese patients. Among the Caucasian study patients, six shared a ROH on Chr. 5, five shared a ROH on Chr. 7 and two unique ROH tracts on Chr. 11, and 4 shared a ROH on Chr. 22, and none of these tracts were seen in 60 Caucasian control subjects. Of interest we found only two ROH tracts on Chr. 1 and 12 shared by all three affected cases from a Native Indian family. Genome-wide ROH scan with an expanded number of patients with familial and sporadic SS-MCD/FSGS and ethnicity-specific control subjects from HapMap3 database is currently in progress. Our preliminary study suggests homozygosity mapping is a promising approach for discovering recessive disease genes for SS-MCD/FSGS.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1979**

**Genome-Wide Analysis of DNA Methylation in Monocytes and Naïve T Helper Cells in Minimal Change Nephrotic Syndrome Patients between Relapse and Remission** Yasuko Kobayashi. *Pediatrics, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.*

DNA methylation in the promoter region of a gene is associated with transcriptional inactivation, while demethylation contributes to the activation. Changes in the DNA methylation lead to differences in gene expression and influence disease development. Minimal change nephrotic syndrome (MCNS) is a major cause of nephrotic syndrome in children and has been shown to involve immunological disturbance. Since one of the most characteristic features of the disease is relapse, the pathogenesis might be in part due to epigenetic changes such as DNA methylation. However little is known about epigenetic changes in MCNS. To know if the epigenetics is underlying MCNS, we investigate genome-wide DNA methylation in monocytes and naïve T helper cells between pediatric MCNS patients in relapse and remission.

We performed Microarray-based Integrated Analysis of Methylation by Isoschizomers; HpaII/MspI (MIAMI) method which provides high throughput global analysis of DNA methylation with 18,014 sites in the vicinity of genes in monocytes (n = 6) and naïve T cells (n = 4) isolated from the peripheral blood of the MCNS patients during both relapse and remission, and from healthy controls (n = 5). The results were confirmed by bisulfite-pyrosequencing analysis. We found three loci (GATA2, PBX4 and NYX ) less methylated in relapse compared with remission in naïve T cells from the same MCNS patients, when the data were analyzed with standard threshold. The loci were less methylated in relapse compared with controls either. We found no significant difference in monocytes between relapse and remission.

Consequently, this study demonstrates that DNA methylation patterns change from remission to relapse of MCNS and that the change is cell-type specific. We are currently studying what brings this difference in DNA methylation and how it could be linked to pathogenesis of relapse of MCNS.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1980**

**Genome-Wide Analysis of the Change of DNA Methylation in Naïve T Helper Cells in Minimal Change Nephrotic Syndrome** Yasuko Kobayashi. *Pediatrics, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.*

An increasing body of evidence indicates that epigenetic modifications including DNA methylation play critical roles in development of diseases. Minimal change nephrotic syndrome (MCNS) has been associated with immunological disturbances such as T cell dysfunction, but its pathogenesis is not fully understood. To know if DNA methylation states are involved in pathogenesis of MCNS, we have analyzed genome-wide DNA methylation in naïve T cells.

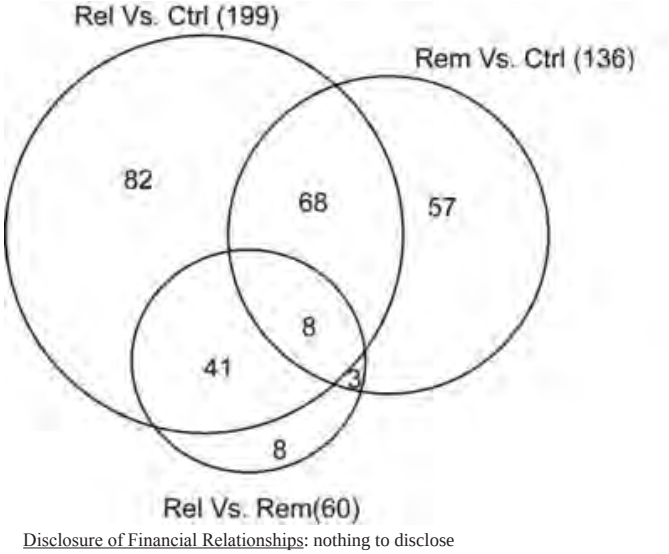
We performed a genome-wide profiling assay called Microarray-based Integrated Analysis of Methylation by Isoschizomers; HpaII/MspI (MIAMI) at 18,328 sites in the vicinity of genes in naïve T cells using three sets of comparison between: 1) relapse vs. controls (CompA), 2) remission vs. controls (CompB) and 3) relapse vs. remission of MCNS (CompC). Naïve T cells were extracted from peripheral blood of the patients (n = 4) both during relapse and remission, and from healthy controls (n = 5).

The number of detected genes are shown in Table1. We found a larger number of genes changing in methylation in CompA and B than CompC (analyzed with lower threshold), suggesting DNA methylation in T cells play roles in MCNS. There were many co-occurrent genes between each comparison (Figure1). Intriguingly, 93.5% genes in CompA except co-occurrent ones with CompB were less methylated. Gene ontology(GO) analysis in CompA revealed that most of the GO categories of which false discovery rate were under 0.1 shows the close interaction between regulation of biological processes leading to control of gene expression and protein modifications.

The genome wide approach we have performed here for DNA methylation in T cells will provide further insights into our understanding of pathogenesis of MCNS.

Number of the genes detected more or less methylated

| | More Methylated Gene | Less Methylated Gene |
|---|---|---|
| CompA | 60 | 139 |
| CompB | 94 | 42 |
| CompC | 4 | 56 |




Disclosure of Financial Relationships: nothing to disclose

**F-PO1981**

**Urine Peptide Profiling by MALDI-TOF Mass Spectrometry Can Distinguish FSGS from Four Common Glomerulopathies** Michael G. Janech,[1,2] Elizabeth G. Favre,[2] Benjamin Neely,[2] Jonas S. Almeida,[3] John M. Arthur.[1,2] *[1]Research Service, Dept. Veterans' Affairs, Charleston, SC; [2]Medicine, Medical University of South Carolina, Charleston, SC; [3]Bioinformatics and Computational Biology, Univ. of Texas, MD Anderson Cancer Center, Houston, TX.*

Clinical presentation of patients with focal segmental glomerulosclerosis (FSGS) is similar to patients with other glomerulopathies (GN). Renal biopsy provides a definitive diagnosis, but is contraindicated for some patients and carries risk for bleeding. Previous reports suggest low molecular weight polypeptides have diagnostic value as non-invasive markers for the classification of glomerular disease. We investigated the potential for urine peptides as diagnostic classifiers of FSGS relative to four common GNs (membranous, MPGN, minimal change, IgA). Urine was collected from 41 patients [biopsy-proven FSGS=18; biopsy proven other GN=23]. Peptides were isolated from equivalent volumes using two different resins [strata-x or graphite]. Spectra were collected using MALDI-TOF, aligned and background subtracted. Peak areas were normalized to total ion current(TIC) or an internal standard(IS). Few differences between FSGS and other GNs were detected by parametric statistics. Peaks at 3459, 3387, 3283, and 2569 were 3-4 fold higher in the FSGS group compared to minimal change using graphite; whereas peak 2360 was 4 fold higher in the minimal change group compared to FSGS using strata-x resin. A non-

parametric support vector machine was trained to classify patients. Receiver operator characteristic(ROC) curves were used to estimate model performance to classify FSGS patients. For each model, between 16 and 28 peptides were utilized as classifiers. Area under the ROC curve(AUC) to predict FSGS with strata-x resin was 0.99 for TIC and 0.92 for IS normalized data. AUC for peptides isolated by graphite was 0.99 for TIC and 0.97 for IS normalized data. Regardless of isolation or normalization method, modeling MALDI-TOF data by support vector machine classified FSGS from other GNs with high specificity and sensitivity. If validated, analysis of urine peptides is a promising alternative to renal biopsy to diagnose FSGS.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1982

**Expression of TSPAN8, a Susceptible Gene for IgA Nephropathy in Human Renal Tissues** Takashi Hirukawa,[1] Qiong Wu,[1] Sanae Saka,[2] Nobuhito Hirawa,[2] Masayuki Endoh,[1] Taiji Matsusaka,[1] Takatoshi Kakuta,[1] Masafumi Fukagawa,[1] Satoshi Umemura,[2] Hidetoshi Inoko,[3] Iekuni Ichikawa.[3] *[1]Division of Nephrology, Endocrinology and Metabolism, Department of Internal Medicine, Tokai University School of Medicine, Isehara, Japan; [2]Department of Medical Science and Cardiorenal Medicine, Yokohama City University, Japan; [3]Department of Molecular Life Science and Molecular Medicine, Tokai University School of Medicine.*

Through a large-scale genome-wide association study using 23,000 microsatellite markers, we have found that TSPAN8, encoding tetraspanin-8, is a candidate susceptible gene for the onset of IgA nephropathy (IgAN), the most common form of primary glomerulonephritis. Tetraspanin-8 is a cell surface glycoprotein of unknown function and suggested to be involved in angiogenesis of colon cancer. In the present study, we analyzed the expression of tetraspanin-8 in human renal tissues with various kidney diseases.

We performed immunohistochemical examination using polyclonal rabbit anti-human tetraspanin-8 in renal biopsy samples [20 IgAN, 5 ANCA associated nephropathy (ANCA), 5 interstitial nephritis (IN), and 5 normal].

Tetraspanin-8 was occasionally expressed on renal tubular epithelial cells. Analysis in serial sections revealed that tetraspanin-8 was co-expressed with cytokeratin 7 (84.6%), aquaporin 2 (49.0%), and aquaporin 1 (14.0%), thus indicating that it was predominantly expressed in distal and collecting tubules. In IgAN, on average, 2.38±1.21% of tubule cross sections contained tetraspanin-8 positive cells. This percentage was not significantly different from those in ANCA (3.80±1.19) or IN (2.84±1.42), but tended to be higher than that in normal (0.50±0.48). Analysis of clinical data at biopsy revealed that tubule tetraspnin-8 positivity was negatively correlated with eGFR (correlation coefficient -.659, $p < 0.001$). In addition to tubular cells, approximately 70% of vascular smooth muscle cells of the interlobular artery intensely expressed tetraspanin-8, uniformly in all samples.

These findings indicate that renal injury causing decline in eGFR, irrespective of nature of disease, enhances expression of tetraspanin-8 in tubule cells.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1983

**TRPC6 Polymorphisms in Membranous Nephropathy** Julia M. Hofstra,[1] Marieke Coenen,[2] Tom Nijenhuis,[1,3] Jeroen Schoots,[2] Mascha M. V. A. P. Schijvenaars,[2] Jo H. M. Berden,[1] Joost G. Hoenderop,[3] Johan Van der Vlag,[1] Rene J. Bindels,[3] Nine V. Knoers,[2] Jack F. Wetzels.[1] *[1]Nephrology; [2]Antropogenetics; [3]Physiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.*

BACKGROUND: Mutations in the transient receptor potential channel TRPC6 cause autosomal dominant focal segmental glomerular sclerosis (FSGS). TRPC6 expression is up-regulated in renal biopsies of patients with idiopathic membranous glomerulopathy (iMN). Hence, we hypothesized that genetic variants (single nucleotide polymorphisms (SNPs)) in TRPC6 might confer susceptibility to development or progression of iMN.

METHODS AND RESULTS: Genomic DNA was isolated from blood samples of 101 iMN patients and 292 controls. In the patient cohort 13 SNPs were identified, using direct sequencing of the TRPC6 gene. Clinical outcome in patients was determined (remission n=26, renal failure n = 47, persistent proteinuria n= 28, follow-up median 80 months [range 51-166]). The 13 identified SNPs showed no correlation with remission nor renal failure.

Five SNPs were selected, based on frequency and possible functionality, for genotyping in controls. No statistically significant differences in genotypes nor allele frequencies between patients and controls were observed for the 4 SNPs genotyped hitherto.

Genotype of 5 selected SNP's in iMN patients and controls

| | Patients (n=101) | Controls (n=292) | p-value* |
|---|---|---|---|
| **C1-10C>A** | | | 0.31 |
| CC | 95.0% | 97.5% | |
| CA | 5.0% | 2.5% | |
| **rs3802829** | | | 0.65 |
| CC | 82.2% | 85.7% | |
| CT | 16.8% | 13.4% | |
| TT | 1.0% | 0.8% | |
| **rs36111323** | | | 0.07 |
| GG | 67.3% | 77.9% | |
| GA | 32.7% | 21.3% | |
| AA | 0% | 0.7% | |
| **rs12366144** | genotyping in progress | | |
| **rs12805398** | | | 0.11 |
| CC | 68.0% | 77.7% | |
| CT | 31.0% | 21.6% | |
| TT | 1.0% | 0.7% | |

* Fischer exact test

CONCLUSION: TRPC6 polymorphisms do not seem to play a role in clinical outcome in our iMN cohort. One SNP (rs36111323) showed a tendency to higher minor allele frequency in patients compared to controls. However, this suggestive finding needs to be replicated in a larger, independent patient cohort.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1984

**An Integrative Genomics Approach to HIV Associated Nephropathy** Roel Sterken,[1] Murim Choi,[2] Natalia Papeta,[1] Paul E. Klotman,[3] Vivette D. D'Agati,[1] Richard P. Lifton,[2] Ali G. Gharavi.[1] *[1]Columbia University Medical Center, New York, NY; [2]Yale University School of Medicine, New Haven, CT; [3]Mount Sinai School of Medicine, New York, NY.*

Introduction: The goal of this project is to identify a transcriptional network of risk attributable genes to HIV associated nephropathy (HIVAN). HIVAN is a complication of HIV infection characterized by dedifferentiation and depletion of glomerular podocytes and collapsing glomerulopathy.

Method: The HIV transgenic mouse model TgFVB develops significant HIVAN in 80% of mice. We integrated phenotype, genotype and gene expression data in a TgFVB×B6 mouse cross segregating for HIVAN, and performed genome-wide quantitative trait locus analysis of both disease and gene expression (called "eQTL mapping") to identify mediators of HIVAN.

Results: 141 (TgFVB×B6) F2 mice were phenotyped, genotyped for ~1,400 SNPs and whole genome expression data for 33,881 transcripts was collected. Linkage mapping of HIVAN identified two major HIVAN QTL on Chr. 13 (HIVAN2, LOD = 3.77) and Chr. 4 (HIVAN3, LOD = 3.65), accounting resp. for 10% and 7% of the variance in HIVAN. Linkage analysis identified 9,818 expression QTL (eQTL) with LOD score above 3.0 ($P < 0.0001$). 2,438 eQTL had a LOD peak within 20Mb of their genome location (cis-QTL), while 8,062 genes had a LOD peak beyond 20Mb (trans-eQTL). 513 eQTL linked to HIVAN2 and 335 eQTL linked to HIVAN3, several of which are known podocyte expressed genes, such as Nphs2, Podxl, Vegfa, Stat3 and Pkd2. To identify eQTLs causal to HIVAN, we fitted HIVAN regulated eQTLs to a conditional causality model. We found 88 and 48 trans-eQTLs for HIVAN2 and HIVAN3 resp., that fitted a causal model. Pathway analysis indicated overrepresentation of interferon pathways ($P < 1.36$ e-5) and Tcf21 regulated cell differentiation pathways ($P < 3.06$ e 7). In addition we found 12 cis-eQTLs located on the HIVAN2 locus and 4 cis-eQTLs on HIVAN3, making these eQTLs prime candidate genes to be the HIVAN QTL itself.

Conclusion: We identified candidate mediators to HIVAN using an integrated eQTL approach. Identifying regulatory networks underlying HIVAN can help provide insight into the pathogenesis of HIVAN and kidney disease in general.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1985

**Urinary Biomarkers To Distinguish Class IV from Class V Lupus Nephritis** Michael R. Bennett, Michiko Suzuki, Shannen Nelson, Joshua David Pendl, Hermine Brunner, Prasad Devarajan. *Cincinnati Children's Hospital.*

Up to 80% of children with systemic lupus erythematosus (cSLE) have lupus nephritis (LN). The ISN/RPS Morphologic Classification of LN reports on histological features that differentiate forms of LN, such as Diffuse Proliferative Class IV and Membranous Class V lesions. Kidney biopsies are the choice for diagnosing LN, but are impractical to accurately assess the course of LN in clinical practice.

We set out to discover non-invasive urinary biomarkers that can discriminate LN subtypes using 4 independent proteomic techniques.

We used 2 dimensional gel electrophoresis (2-DGE), NMR based metabonomics, surface-enhanced laser desorption/ionization time of flight MS (SELDI), and liquid chromatography tandem ms (LC MS/MS2) to investigate novel biomarkers that could distinguish Class IV vs. Class V LN. Urine samples from children with Class IV (n=6) and (pure) Class V LN (n=7) collected within 60 days of kidney biopsy and those of controls with focal segmental glomerulosclerosis (n=4) were tested. Samples were normalized for total protein (2-DGE and LC-MS/MS2) or urine creatinine (NMR and SELDI). Using 2-DGE and MALDI-TOF-MS/MS, we found albumin fragments (25kDa) and α1-B glycoprotein (A1BG, 60kDa) significantly over-expressed in class IV vs. class V LN. Using SELDI, we identified Alpha-1 Antitrypsin (A1AT). This protein was significantly ($p < 0.01$; AUC 0.90) over-expressed in Class V vs Class IV LN and FSGS controls. Principal component analysis of NMR metabonomics spectra suggests decreased levels of citrate and increased levels of taurine in Class V when compared to Class IV patients, while LC-MS/MS2 uncovered the most differences between the groups. Among proteins upregulated in Class V LN were apolipoprotein D, lipocalin-like prostaglandin D synthetase, ITIH4, Caspase 10, uromodulin and CD14. Those most upregulated in Class IV LN were vitamin D binding protein, ceruloplasmin, hemopexin, A1BG and orosomucoid. A1AT has been linked to SLE flares and hemopexin is associated with glomerular disease.

The validation of these novel non-invasive biomarkers that can distinguish LN subtypes would greatly aid in diagnosing and monitoring treatment in LN.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1986**

**Identification of Shared Transcriptional Networks of Diabetic Glomerulopathy in Man and Mouse** Jeffrey B. Hodgin,[1] Viji Nair,[2] Hongyu Zhang,[2] Ann Randolph,[2] Raymond C. Harris,[3] Robert G. Nelson,[4] Frank C. Brosius,[2] Matthias Kretzler.[2] *[1]Pathology, University of Michigan; [2]Nephrology, University of Michigan, Ann Arbor, MI; [3]Medicine, Vanderbilt University, Nashville, TN; [4]Epidemiology and Clinical Research Branch, NIDDK, Phoenix, AZ.*

Though mouse models of diabetic nephropathy have been quite useful, none reliably mimics human disease. This has made it challenging to identify specific factors that cause or predict diabetic glomerulopathy (DG). Therefore, we wished to define where mouse models faithfully recapitulate human DG on a functional level using a cross-species comparison to identify shared transcriptional networks. Transcriptional networks for diabetic humans and three AMDCC mouse models were generated using glomerular mRNA, Affymetrix microarrays, transcriptional pathway mapping, and promoter modeling tools. The human transcriptional network was derived from albuminuric (>30 mg/g Alb/Cr) versus nonalbuminuric (<30 mg/g Alb/Cr) Pima Indians, a cohort with early DG. Mouse transcriptional networks were derived from streptozotocin treated DBA/2 mice, db/db C57BLKS mice, and eNOS-deficient db/db C57BLKS mice, each versus control. Integrating the gene expression alterations with biological knowledge resulted in complex networks of 1000s of genes linked by multiple co-citations and promoter binding sites (Genomatix Bibliosphere). TALE (Tool for Approximate Large Graph Matching, University of Michigan) was used to align the human and mouse transcriptional networks to derive three conserved network structures for each human-mouse comparison, comprised of approximately 100 nodes representing key hubs of conserved regulatory events. Shared gene nodes were found in all three networks, many of them reflecting established pathogenetic mechanisms of diabetic complications including JAK-STAT and VEGFR signaling pathways. Shared top biological processes included endothelial cell differentiation, angiogenesis, and phospolipase C activity. This approach can guide the selection of disease pathways in mouse models that are the most relevant to the human disease process and identify new pathways that are excellent targets for future study.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1987**

**Prediction of Diabetic Nephropathy by Urine Metabolomic Profiling** Robert G. Nelson,[1] Robert C. Williams,[1] Robert L. Hanson,[1] William Knowler,[1] Frank J. Gonzalez,[2] Andrew Patterson.[2] *[1]NIDDK, Phoenix, AZ; [2]NCI, Bethesda, MD.*

We performed a global, unbiased urine metabolomic screen to identify candidate biomarkers of diabetic kidney disease in 68 Pima Indians with type 2 diabetes. In a nested case-control study, 34 cases were matched for age, sex, and duration of diabetes with 34 controls. All participants had normal urinary albumin excretion (albumin/creatinine ratio (ACR) <30 mg/g) at baseline; cases progressed to diabetic nephropathy, defined by macroalbuminuria (ACR≥300 mg/g), during 10 years of follow-up, whereas controls remained normoalbuminuric during the same period. Baseline urine samples stored at -20°C were profiled using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry operating in either electrospray ionization (ESI) positive or negative mode. The urine samples contained 21,041 features (13,809 ESI+, 7,232 ESI-) in which at least 17 cases had different relative concentrations than their matched controls. Fisher's permutation test was used to test the statistical significance of the difference in relative concentration of these candidate biomarkers between cases and controls; the false discovery rate procedure was used to adjust for multiple comparisons. The analysis identified 66 biomarkers with p-values $<10^{-3}$, corresponding to a false discovery rate of 56.2%. These biomarkers are now being structurally identified by co-chromatography and tandem mass spectrometry comparison with authentic standards; the first to be identified, ranked 32 on the list of top putative biomarkers, was indoxyl sulfate ($p=5.6x10^{-4}$) , a protein-bound endogenous uremic toxin excreted in the proximal tubule that increases tubulointerstitial fibrosis and glomerulosclerosis by increasing the expression of transforming growth factor-β1. The relative concentration of this toxin was higher in cases than in controls (3.1 vs. 1.8). Structural identification of the other metabolites continues and may provide new insights into mechanisms of diabetic kidney disease and a means of identifying those at risk before they can be identified by conventional clinical tests.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1988**

**Global MicroRNA Gene Profiling in Autosomal Dominant Polycystic Kidney Disease (ADPKD)** Xuewen Song, Ning He, Kairong Wang, York P. Pei. *Divisions of Nephrology, University Health Network and University of Toronto, Toronto, ON, Canada.*

MicroRNAs (miRNAs) play an important role in regulating gene expression in health and disease. Previously, we have shown that aberrant activation of gene pathways and transcriptional networks were associated with human PKD1 renal cysts (Hum Mol Genet 18: 2328-2343, 2009). In the current study, we examined the global expression of miRNAs and related their changes to the expression of genes/gene pathway in human PKD1 renal cysts of different size, minimally cystic tissue from six PKD1 human polycystic kidneys, as well as normal renal cortical samples. We identified 158 differentially regulated miRNAs (Up: 59; Down: 99) using a false discovery rate (FDR) of 0.1%. Among these miRNAs, 76 were located in 23 genomic clusters. Within each of 19 genomic clusters, all differentially expressed miRNAs were changed in one direction, suggesting co-expression of these miRNAs. Pathway analysis of miRNA-predicted target genes suggested some miRNA families may regulate gene pathways associated with renal cyst growth in ADPKD. For example, down-regulation of miR-424/503 were associated with increased expression of pathway components for G1-S cell cycle progression (e.g. CDC25A, CDK6, CCND1, CCDN2, CCND3, CCNE1 and E2F3), mTOR signalling (IGF1, IGF1R, PIK3R1, AKT3, RICTOR, EIF4E, EIF4B, RAF1 and VEGFA); and miR-301a may promote EMT by targeting transcriptional factors ZEB1 and ZEB2 as well as TGF β pathway components (e.g. TGFBR2, BMPR2, ACVR1, SMAD5). By integrating dysregulated miRNAs and our previously reported dysregulated mRNAs, we also found a inverse correlation between expression of these miRNAs and most of their predicted target genes. Taken together, our results indicate that a unique panel of miRNAs is associated with renal cyst growth in ADPKD. Targeting these specific miRNAs may provide a novel approach in the treatment of ADPKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1989**

**Computational Prediction of MicroRNA Targets in ADPKD** Priyanka Pandey, Shan Qin, Jacqueline Ho, Jordan A. Kreidberg. *Division of Nephrology, Children's Hospital Boston, Harvard Medical School, Boston, MA.*

microRNAs (miRNAs) have emerged as a new area of interest in renal development and pathology. They are a class of several hundred short non-coding RNAs that have been implicated in a wide spectrum of biological processes and their expression levels have been found to be increased or decreased in several disease processes including kidney cancer, diabetic nephropathy and polycystic kidney disease. Autosomal dominant polycystic kidney disease (ADPKD) is a relatively common genetic kidney disease characterized by the formation of cysts throughout the kidney parenchyma. In the vast majority of cases, this disease is caused by mutations in PKD1 or PKD2. Although the involvement of several genes and transcriptional networks, as well as miRNAs has been previously shown in PKD, specific miRNA:mRNA pairings that affect the initiation and progression of cyst formation remain to be identified. Kidneys of Pkd1-/- embryos develop a rapidly progressive cystic disease during embryogenesis. We first performed gene expression profiling using embryonic kidneys of wild type and Pkd1-/- embryos, to identify genes whose expression were highly mis-regulated in Pkd1-/- embryonic kidneys. By using computational approaches (TargetScan, miRanda, microT and miRDB), we then predicted miRNAs that were suggested to target these mRNAs, and selected a set of candidate miRNAs for further study suggested by at least two of four prediction tools. Differential expression of 9 candidate miRNAs and 17 potential target mRNAs was then confirmed by qRT-PCR. These studies predicted 14 candidate interactions that will be subject to experimental validation. Gene Ontology and gene set enrichment analysis identified overrepresented signaling pathways between Pkd1 mutant and wild-type kidneys, including calcium, Wnt, Notch, TGF-β, MAPK and VEGF signaling. Our results suggest a possible role of miRNAs in specific signal transduction pathways mis-regulated in ADPKD.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1990**

**Endocytosis of α-Galactosidase A in Human Podocytes in Fabry Disease Is Mediated by Different Receptors** Thaneas Prabakaran,[1] Rikke Nielsen,[1] Jakob V. Larsen,[1] Moin Saleem,[3] Claus Munck Petersen,[1] Pierre J. Verroust,[4] Erik I. Christensen.[1] *[1]Department of Anatomy and Department of Medical Biochemistry, Aarhus University, Aarhus, Denmark; [2]Department P and Department of Medical Endocrinology, Rigshospitalet, Copenhagen, Denmark; [3]Children's Renal Unit and Academic Renal Unit, University of Bristol, Southmead Hospital, Bristol, United Kingdom; [4]UMRS 592, Institut de la Vision, Paris, France.*

Background: Injury and loss of glomerular podocytes are leading factors of glomerular disease and renal failure. Podocyte repair is therefore an important therapeutic target. In Fabry disease, podocyte injury is caused by the progressive intracellular accumulation of glycosphingolipid in form of globotriaosylceramide (GL-3). Aim: To determine how recombinant α-galactosidase A (α-Gal A) (agalsidase beta; Fabrazyme®) designed for enzyme replacement therapy in Fabry disease is taken up by human podocytes in the renal glomeruli. Methods: The uptake of α-Gal A in podocytes was investigated using affinity chromatography, immunochemistry, laser capture microdissection, RT-PCR and Western blotting analyses. Results: This study identifies three endocytic receptors, mannose 6-phosphate receptor (M6PR), megalin, and sortilin, with drug delivery capabilities in human podocytes. All receptors are localized on the surface membrane of podocytes indicating their endocytic capabilities. The receptors mediate the uptake and lysosomal targeting of recombinant α-Gal A. Conclusions: These findings highlight the importance of receptor-mediated endocytosis as a key mechanism to maintain podocyte integrity by delivering α-Gal A to lysosomes for degradation of GL-3 deposits. The present study provides the rationale for the renal effect of treatment with α-Gal A and identifies potential pathways for future non-carbohydrate based drug delivery to the kidney podocyte and other tissues.

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**F-PO1991**

**Analysis of the Foetal Urinary Proteome Predicts Postnatal Renal Outcome of Posterior Urethral Valves (PUV)** Julie Klein,[1] Chrystelle Lacroix,[2] Françoise Muller,[3] Benjamin Breuil,[1] Justyna Siwy,[4] Harald Mischak,[4] Flavio Bandin,[1,5] Bernard Monsarrat,[2] Jean-Loup Bascands,[1] Stéphane Decramer,[1,5] Joost Schanstra.[1] *[1]U858, Inserm, Toulouse, France; [2]IPBS, CNRS, Toulouse, France; [3]Nephrologie Pediatrique, Hôpital Robert Debré AP-HP, Paris, France; [4]Mosaiques Diagnostics & Therapeutics AG, Hannover, Germany; [5]Paediatric Nephrology, Hôpital des Enfants, Toulouse, France.*

Posterior urethral valves (PUV) are rare diseases associated with a wide spectrum of outcomes ranging from prenatal death with terminal renal failure to survival with normal renal function. *In utero* the most severe cases can be easily identified based on severe oligohydramnios or sonographic evidence of bilateral renal dysplasia with microcystic hyperechogenic kidneys and absence of corticomedullary differentiation. For the less severely affected patients, the long term outcome is nearly impossible to predict. We hypothesise that foetal urine (FU) contains biomarkers that allow assessing the severity of renal lesions *in utero* and subsequent prediction of postnatal renal outcome.

The FU proteome was analysed by capillary electrophoresis coupled to mass-spectrometry (CE-MS). In addition, parallel LC-MS/MS analysis of a number of FU samples yielded peptide sequence data allowing calibration of the CE-MS data and therefore patient comparison.

We have compared the FU proteome of 20 foetuses with PUV leading to normal-acceptable renal function up to at least the age of two years (*controls*) with that of 18 foetuses with PUV leading to termination of pregnancy due to non-functional kidneys (*cases*). This lead to the identification of 30 FU biomarkers that classified with 100% sensitivity and specificity this training set.

These 30 biomarkers were validated in an independent blinded cohort of 22 PUV patients (7 terminations of pregnancy + 7 neonatal deaths (*cases*) and 8 patients with good postnatal function (*controls*)), predicting the post-natal outcome with 93% sensitivity, 100% specificity and an AUC=0,973. Three of the 30 biomarkers were identified as collagen fragments.

These data strongly suggest that the analysis of the FU proteome allows predicting post-natal renal outcome of posterior urethral valves.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1992**

**Identification and Functions of microRNAs in the Developing and Adult Mouse Kidney** Vishal Patel, Sachin S. Hajarnis, Donovan Huynh, Ryan Hunter, Darren Williams, Rajiv Parmar, Peter Igarashi. *Internal Medicine-Nephrology, UT Southwestern Medical Center, Dallas, TX.*

MicroRNAs (miRNAs) are short, non-coding RNAs that inhibit mRNA translation and promote mRNA degradation. miRNAs play critical roles in embryonic development and tissue homeostasis in the heart, lung, and blood vessels, but their roles in the kidney are poorly understood. We performed microarray analysis of 707 known mature miRNAs in the adult and immature mouse kidney. Analysis of kidneys at different ages from postnatal day (P) 2 ,P7, P14, P21, and P35 revealed stage-dependent expression of 114 miRNAs. 60 miRNAs were highly expressed in the immature kidney and down-regulated in the adult, whereas 54 miRNAs showed a reciprocal pattern of expression. To examine the functions of miRNAs in the kidney, we generated mutant mice that lack Dicer, an enzyme required for miRNA biogenesis, specifically in renal epithelial cells. Approximately 70% of neonatal *Dicer* mutant mice developed hydroureter and hydronephrosis. A subset of neonatal *Dicer* mutant mice also developed kidney cysts. *Dicer* mutant mice that survived to adulthood developed interstitial fibrosis and de-differentiation of tubular and parietal epithelial cells. Tamoxifen-inducible, kidney-specific inactivation of *Dicer* in adult mice resulted in increased interstitial fibrosis and tubular epithelial de-differentiation. Target prediction analysis revealed that the 3'UTRs of mRNAs encoding tumor suppressors contained binding sites for miRNAs that were enriched in the developing kidney. Conversely, miRNAs that were enriched in the adult kidney targeted oncogenes and genes involved in the prevention of epithelial-to-mesenchymal transition and fibrosis. Taken together, these studies show that distinct subpopulations of miRNAs are expressed in the developing and adult kidney. Expression of miRNAs in renal epithelial cells is essential for both kidney development and the prevention of renal fibrosis. miRNAs represent novel therapeutic targets for the treatment of kidney diseases, such as PKD and renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1993**

**Renal Gene Expression Profile in Radiation Nephropathy** Marek Lenarczyk,[2] John E. Moulder,[2] Brian L. Fish,[2] Martin Hessner,[3] Eric P. Cohen.[1] *[1]Medicine, Medical College of Wisconsin, Milwaukee, WI; [2]Radiation Oncology, Medical College of Wisconsin, Milwaukee, WI; [3]Pediatrics, Medical College of Wisconsin, Milwaukee, WI.*

Oxidative stress may play a significant role in kidney disease. But neither protein nor lipid oxidative stress (OS) were found in radiation nephropathy (Rad Res 171:164, 2009), in which chronic OS is said to occur. We therefore tested for OS using a genome-wide expression array, with attention to the latent period before physiologically-significant renal damage. WAG/RijCmcr male rats were divided into experimental and age-matched control groups (6 per group). At age 2 months rats underwent 10 Gy total body irradiation (TBI) , followed by a syngeneic bone marrow transplant. Controls were sham-treated. Half of the irradiated and control animals were placed on captopril 500 mg/L in their drinking water at 2 days after TBI and thereafter.

Animal groups according to day post-TBI and CAP treatment

| | 1 day | 21 days |
|---|---|---|
| 0 Gy, no drug | x | x |
| 0 Gy, captopril | | x |
| 10 Gy, no drug | x | x |
| 10 Gy, captopril | | x |

x denotes gene array testing

Differentially expressed genes were defined as those having greater than a 2-fold change and a false discovery rate<0.01. We identified 41, 50, and 6, differentially expressed genes for group comparison between 1d 10 Gy vs. 1d 0 Gy, 21d 10Gy vs. 21d 0 Gy, and 21d 10Gy with CAP vs. 21d 10 Gy no CAP, respectively.Comparison between 21d 10Gy+CAP vs. 21d 10Gy identified 4 down-regulated (Ucp1 uncoupling protein 1, Slco4a1-solute carrier organic anion transporter family, Grem2 -gremlin 2, Eif4g2-eukaryotic translation initiation factor 4) and 2 up-regulated (Gadd45g-growth arrest and DNA-damage-inducible, Ren-renin) transcripts. Up-regulation of renin is expected with use of captopril and confirms drug delivery to the rats. Gadd45g enables DNA repair. The down-regulation of eIF4G2 could affect the translation of laminin, which would be a mechanism for the benefit of captopril in this model. None of the 97 annotated genes are associated with oxidative stress, which suggests that persistent oxidative stress does not occur in radiation nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1994**

**Proteomics Reveals Potential Biomarkers of Chronic Kidney Disease in Patients after Hematopoietic Cell Transplantation** Sangeeta R. Hingorani,[1,2] Qing Zhang,[2] Jacob Kennedy,[2] Hong Wang,[2] Samir Hanash.[2] *[1]Pediatrics, University of Washington/Seattle Children's Hospital, Seattle, WA; [2]Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA.*

Chronic kidney disease (CKD) is a cause of morbidity and mortality within the first year after hematopoietic cell transplant (HCT). We studied 18 patients with CKD (defined as a GFR <60 ml/min/1.73m$^2$ at 1 year post HCT) and 18 age and gender-matched control patients without CKD. Urine samples were collected between 0800-1000 hours on day 100 after transplant (prior to development of CKD). Pooled samples were created using equal amounts of protein. The pooled urinary proteins were separated by SDS-PAGE, and digested with trypsin. Acquired mass spec data from LTQ-Orbitrap was automatically processed by the CPAS pipeline. Identified peptides were validated through PeptideProphet and inferred into proteins via ProteinProphet. Tandem mass spectra were searched against the human IPI database. For each protein identified, the number of MS/MS events was normalized and the G-test was performed. The calculated G-value was used to assess whether the protein was differentially expressed.

We identified 609 unique proteins in the urine at day 100 post-HCT; 435 in cases and 516 in controls. Fifty-nine proteins were expressed only in cases at day 100 and undetectable in controls; candidate biomarkers are listed in Table 1. These proteins were chosen based on a p-value of <0.05, their presence in 2 or 3 runs and biologic plausibility. These proteins are involved in inflammatory responses, dermatologic diseases, and cell adhesion, signaling and migration.

Candidate biomarkers of CKD post-HCT

| Gene | Description |
|---|---|
| ANXA2 | Annexin a2 |
| ANXA3 | Annexin a3 |
| ANXA5 | Annexin a5 |
| C3 | Complement C3 precursor |
| FLG | Filaggrin |
| IL-10RB | Interleukin-10 receptor beta chain precursor |
| IVL | Involucrin |
| PLAU | Urokinase-type plasminogen activator |
| RDX | Radixin |
| S100A8 | Calgranulin a |
| TAGLN2 | Transgelin-2 |

Further work is needed to replicate our findings in a larger number of patients and to determine if these proteins are expressed in the kidney using EIA to measure proteins in the urine and immunohistochemistry of autopsy or biopsy specimens.

Disclosure of Financial Relationships: nothing to disclose

**F-PO1995**

**Upregulation of Inflammatory Mediators in End-Stage Renal Disease as Measured Via Biochip Array Technology** Vinod K. Bansal,[1] Rachael Davis,[2] Evangelos Litinas,[2] Debra Hoppensteadt,[2] Evangelos Litinas.[2] *[1]Department of Nephrology, Loyola University Medical Center, Maywood, IL; [2]Department of Pathology, Loyola University Medical Center, Maywood, IL.*

End-Stage Renal Disease (ESRD) is a complex syndrome in which systemic vascular pathophysiologic changes contribute to adverse cardiovascular and cerebrovascular manifestations. Given that inflammatory and hemostatic aberrations contribute to the overall pathogenesis of the syndrome, the purpose of this study is to profile several inflammatory mediators in order to better understand their role in the underlying mechanism of vascular changes in ESRD. Plasma samples from 49 patients with ESRD were collected prior to maintenance hemodialysis sessions. A group of 56 normal individuals, both male and female, was included as control. Cerebral Array II chips were used in the Randox® system to simultaneously measure Neuron Specific Enolase (NSE), Neutrophil Gelatinase-associated Lipocalin (NGAL), Soluble Tumor Necrosis Factor Receptor I (TNFRI), D-Dimer (DD), Thrombomodulin (TM), and C-reactive protein (CRP). The Randox® technology utilizes

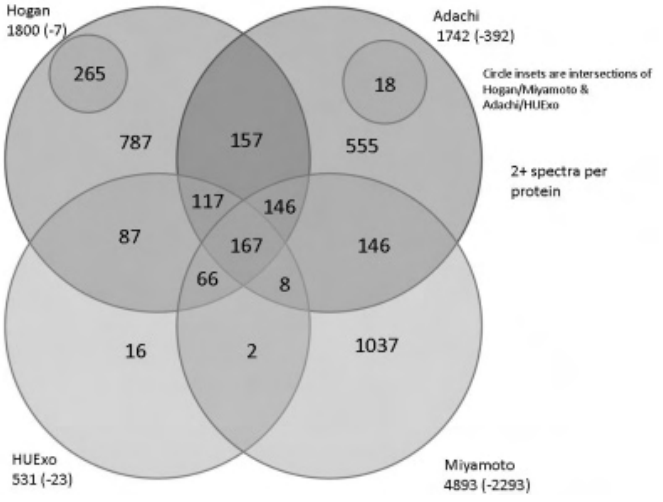
multiple discrete test regions of immobilized antibody to simultaneously quantify multiple markers from a single patient plasma sample. As compared to the normals, all of the markers studied showed an upregulation in patients with ESRD. Most notably, TNFRI showed a 19.8 fold increase in patients with ESRD (mean 7.8 ± 2.8 ng/ml, range 0.8 to 13.7) compared to the control (mean 0.4 ± 0.2, range 0.1 to 1.0). TM was increased 5.2 fold (mean 6.5 ± 2.6, range 0.7 to 14.1) compared to control (mean 1.2 ± 0.4, range 0.6 to 2.3). Also, NGAL showed a 4.6 fold increase (mean 1390 ± 257, range 406 to 1729), compared to control (mean 299 ± 99, range 115 to 603), and CRP a 4.2 fold increase (mean 5.7 ± 4.2 ug/ml, range 0.6 to 13.2) compared to control (mean 1.4 ± 1.7, range 0.2 to 11.4). DD and NSE were also increased 3.0 and 1.8 fold respectively. These studies show that inflammatory markers such as TNFRI, NGAL and NSE are upregulated in ESRD. The marked increase in TM and TNFRI is highly suggestive of endothelial damage in this syndrome.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1996

**In-Depth Proteomic Analysis of Podocin-Rich Exosomes in Human Urine** Marie C. Hogan,[1] Roman Zenka,[2] Kenneth L. Johnson,[2] Cristine Charlesworth,[2] Peter C. Harris,[1] H. Robert Bergen,[2] Christopher James Ward.[1] *[1]Nephrology, Mayo; [2]Proteomics Center, Mayo Clinic, Rochester, MN.*

Ready isolation of glomerular proteins would be of great significance for identifying diagnostic & prognostic markers of glomerular disease. To isolate such proteins we performed a comprehensive proteomic study of pooled urines containing the podocin exosome subfraction using modifications to prior protocols (Hogan et al JASN 2009). **Methods:** Fresh urine from 14 subjects (seven with normal renal function & no microalbuminuria (age 28±3.3yrs) & nine ADPKD individuals (27.7± 6.2yrs)) were used for exosome isolation by centrifugation on 5-30% sucrose ($D_2O$). 30ug protein (pooled podocin rich fraction) of each individual was separated with 1D PAGE (to isolate 10 slice sections from high to low Mw). Slices were trypsinized, eluted & analyzed by quantitative MS/ MS. Elucidator® compared relative peptide M/Z intensities. ID mapping to Uniprot facilitated comparison of our proteome with urine, glomerular & existing exosome proteomes. **Results:** Of a confident set of 1792 proteins identified (≥2 peptides), 594 are shared with the glomerular proteome but 1476 were not. 1039 are involved in cellular process.




Ribonucleotide & purine nucleotide binding proteins were overrepresented. Podocyte disease related proteins, podocin, podocalixyn, podocalixyn-like protein, ACTN4, CD2AP & MYH9 were present. **Conclusions:** Our data substantially expands the number of urine exosome proteins identified to date. Exosome of podocyte origin are shed in urine in individuals without proteinuria & can be isolated for label free MS. As these vesicles are enriched in membrane & intracellular proteins of glomerular origin, this offers the prospect of their use for biomarkers in glomerular diseases. Our future studies will concentrate on simplifying extraction methods & studies in albuminuric patients with glomerulopathies.

Disclosure of Financial Relationships: Consultancy: Hoffmann-La RocheResearch Funding: Novartis USA.

## F-PO1997

**Outcomes as a Function of Donor Type from a Phase III Study of Belatacept vs Cyclosporine in Kidney Transplantation (BENEFIT-EXT)** Sander Florman,[1] Antoine Durrbach,[2] Christian Larsen,[3] Jose Medina-Pestana,[4] Yves Vanrenterghem,[5] Flavio Vincenti,[6] Alan Block,[7] Pushkal Garg,[7] J. Brian Copley,[7] Tao Duan,[7] Josep Grinyo.[8] *[1]Mount Sinai Med Cntr; [2]Bicetre Hosp; [3]Emory Univ; [4]Hosp do Rim; [5]Univ Hosp Leuven; [6]UCSF; [7]BMS; [8]Univ Hosp Bellvitge.*

**Purpose:** To assess 2-yr outcomes of belatacept vs cyclosporine (CsA) in adults receiving extended-criteria donor (ECD) kidney transplants as a function of donor type.

**Methods:** BENEFT-EXT is a 3-yr, randomized, Phase III study of belatacept in adults receiving ECD kidney transplants. Pts were randomized 1:1:1 to receive a more intensive (MI) or less intensive (LI) regimen of belatacept or CsA; all pts received basiliximab induction, MMF, and corticosteroids. Randomized and treated patients met protocol-specified ECD criteria of donor age>60, UNOS EC, CIT≥24 hrs and/or DCD. Yr 2 outcomes included composite patient/graft survival, measured GFR (mGFR), calculated GFR (cGFR) and protocol-defined acute rejection (AR).

**Results:** 543 pts composed the ITT population; 384 and 55 pts received UNOS ECD and DCD kidneys, respectively. Similar AR rates were observed in pts regardless of donor type. Patient/graft survival with belatacept was comparable to CsA across the ITT, UNOS ECD, and DCD populations. Outcomes in recipients of protocol-defined ECD kidneys are consistent with recipients of UNOS ECD kidneys. Belatacept pts demonstrated better GFR compared with CsA in ITT and UNOS ECD.

Outcomes as a Function of Donor Type at Yr 2

| | Patient/Graft Survival, % | mean, mGFR, mL/min | mean, cGFR, mL/min | AR, % |
|---|---|---|---|---|
| ITT: MI (n=184) | 83 | 52 | 44 | 17 |
| ITT: LI (n=175) | 84 | 50 | 43 | 18 |
| ITT: CsA (n=184) | 83 | 45 | 35 | 15 |
| UNOS ECD: MI (n=129) | 81 | 48 | 40 | 19 |
| UNOS ECD: LI (n=122) | 82 | 46 | 40 | 21 |
| UNOS ECD: CsA (n=133) | 81 | 41 | 31 | 15 |
| DCD: MI (n=18) | 83 | 56 | 50 | 11 |
| DCD: LI (n=19) | 100 | 42 | 56 | 21 |
| DCD: CsA (n=18) | 72 | 46 | 36 | 17 |

**Conclusions:** Belatacept demonstrated better renal function, comparable patient/graft survival and comparable AR compared to CsA in ECD kidney recipients irrespective of donor type at Yr 2. The outcomes among donor types were consistent with the overall ITT population.

Disclosure of Financial Relationships: nothing to disclose

## F-PO1998

**Genetic Differences in Native Americans Affects Tacrolimus Dosing after Kidney Transplant** Harini A. Chakkera,[1] Anita Grover,[2] Jason K. Bodner,[1] Senaida Behmen,[1] Raymond L. Heilman,[1] Kunam Sudhakar Reddy,[1] Adyr A. Moss,[1] Khaled Hamawi,[1] Lynda A. Frassetto.[2] *[1]Mayo Clinic AZ; [2]UCSF.*

**Background:** Tacrolimus (FK) pharmacokinetics (PK) is influenced by enzymes cytochrome P450 (CYP) 3A4 and 5, and the transporters P-glycoprotein -MDR1, MRP1 and BCRP. Racial differences are seen in SNPs encoding these transporters and enzymes. Genetic variabilities among Native Americans (**NA**) have not been studied but clinically NA require lower FK to achieve therapeutic levels.

**Methods:** 12-hour PK studies and SNPs for CYP3A4 (*1, *1B) & 5 (*1, *3), MDR1 (C1236T, G2677T, C3435T), MRP1 (G1299T) and BCRP (G34A, C376T) performed on 24 NAs with kidney tx on stable dose FK. PK estimated using NONMEM and fit to a two compartment model using an empirical Bayesian approach. Genotyping using TaqMan. Genetic, PK and demographic correlations assessed.

**Results:** Mean age: 51±13 yr, 70% male, 15 were Navajo. Average NA FK dose was 0.03±0.02 mg/kg/day. Literature averages 0.2-0.25mg/kg/day for AA and 0.05-0.1 mg/kg/day for Chinese. Genotype frequencies (table 1).

| Gene | NA % (WT/HT/HZ) | AA % (WT/HT/HZ)* | As % (WT/HT/HZ)* |
|---|---|---|---|
| CYP3A5*1/*1, *1/*3, *3/*3 | 0.00/0.12/0.88 | 0.4-0.7/0.05-0.15/0.3-0.45 | 0-0.1/0.45-0.5/0.455-0.5 |
| MDR1 C1236T | 0.25/0.58/0.17 | 0.87/0.13/0 | 0.1/0.45/0.45 |
| MDR1 G2677T | 0.57/0.19/0.24 | 0.85/0.15/0 | 0.13/0.29/0.47 |
| MDR1 C3435T | 0.25/0.65/0.10 | 0.86/0.14/0 | 0.3/0.4/0.3 |

WT=wild type; HT=heterozygote; HZ=homozygous; AA = African Americans; As = Asians. *population frequency - http://www.ncbi.nlm.nih.gov/snp;

Significant interactions in the entire cohort include: *G2677T* and absorption rate (p=0.01), CYP3A5 and intercompartmental clearance (p=0.01) and steady-state distribution volume (p=0.03), and among Navajo only, between *G2677T* and absorption rate (p=0.01).

**Conclusion:** Native Americans have lower drug clearance and often have CYP3A5*3 genotype and MDR variants. Compared to WT, variants have lower FK absorption and metabolism, thus NA require less FK to achieve trough levels.

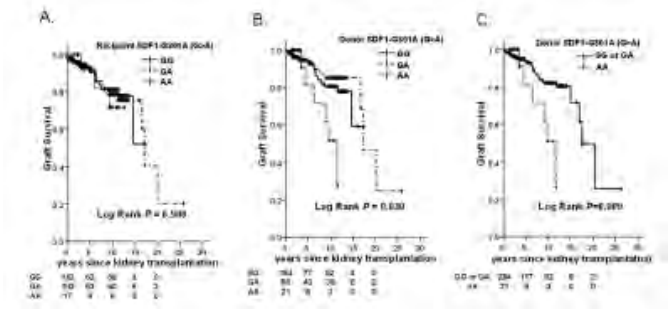
Disclosure of Financial Relationships: Research Funding: Astellas.

## F-PO1999

**Genetic Predisposition of Donors Affects the Allograft Outcome in Kidney Transplantation; Stromal-Derived Factor-1 Polymorphism** Jung Pyo Lee,[1] Seung Hee Yang,[1] Shin-Young Ahn,[2] Jae-Yoon Park,[2] Kwon Wook Joo,[2] Dong Ki Kim,[2] Yon Su Kim.[2] *[1]Kidney Research Institute, Seoul National University, Seoul, Republic of Korea; [2]Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.*

Background and method; Genetic predisposition may be an important aspect to discern impending responses after transplantation. In this study, we evaluated the role of the genetic predisposition of SDF1 at the 3' untranslated region (G801A) on renal allograft outcomes. A total of 263 pairs of recipients and donors were enrolled.

Results; SDF1 was differentially expressed in renal tissues with acute rejection according to genetic variations of donors showing higher expressions in the grafts from GG donors. Biopsy-proven acute rejection (BPAR) within 1 year and long-term graft survival were traced. Despite similar allele frequencies between donors and recipients, A allele from donors, not from recipients, has a protective effect on the development of BPAR (p=0.037). Adjustment for multiple covariates did not affect this result (OR 0.49, 95% C.I 0.26–0.91, $P$ = 0.023). However, patients who received AA homozygote grafts showed poor graft survival compared to recipients from GG or GA donors ($P$ =0.009).



This association was significant after adjusting for several risk factors (hazard ratio 3.14; 95% C.I = 1.19-8.29; *P* = 0.021). The allelic variation of recipients, however, was not associated with BPAR and graft loss.

Conclusion; A donor-derived genetic polymorphism of SDF1 has influenced the graft outcome. Thus, the genetic predisposition of donor should be carefully considered in transplantation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2000**

**Access to the Waiting List and Renal Transplantation within Europe** Vianda S. Stel,[1] Reinhard Kramar,[2] Torbjorn Leivestad,[3] Keith Simpson,[4] Jacqueline Smits,[5] Kitty J. Jager.[1] *[1]ERA-EDTA Registry, Netherlands; [2]Austrian Dialysis and Transplant Registry, Austria; [3]Norwegian Renal Registry, Norway; [4]Scottish Renal Registry, United Kingdom; [5]Eurotransplant International Foundation, Netherlands.*

The aim of the study is to examine 1) access to the kidney transplant waiting list (WL) and 2) access to renal transplantation for those who are waitlisted. We focus on time trends, international differences and patient subgroups.

Of the incident RRT patients from 1995 to 2007 in the ERA-EDTA Registry database, the date on the WL from 1995 to 2005 was available for Austria (AT) and The Netherlands (NL) (from Eurotransplant), Norway (NO) (Scandiatransplant) and Scotland (UKs) (Scottish Renal Registry). Patients receiving a transplant without waitlisting were put on the WL for 1 day.

From 1995 to 2007 the incidence of RRT per million age related population (pmarp) was highest in AT, and increased slightly in patients <65 years (AT:77-80; NO:53-61; NL:58-61, UKs:64-68 pmarp) and importantly in patients ≥65 years (AT:287-520; NO:216-399; NL:277-415, UKs:223-298 pmarp). The number of transplants performed pmp increased over time (AT:38-48, NO:43-55, NL:31-51, UKs:27-36 pmp), with the highest number of deceased donors in AT (41 pmp in 2007), and of living donors in NO until 2006 (17 pmp) and in NL in 2007 (23 pmp).

Of the patients starting RRT from 1995 to 2003 at age <65 years, the proportion of patients waitlisted within 2 years was 52, 78, 57 and 52% in AT, NO, NL and UKs respectively. In patients ≥65 years it was 24% in NO and 6% in the other countries. In NO, 92% of waitlisted RRT patients <65 years were transplanted within 4 years after the start of RRT whereas this was less in AT (73%), NL and UKs (both 65%). In patients ≥65 years it was 71% (AT), 77% (NO), 52% (NL) and 36% (UKs). There was no gender difference, and no clear trend in access to WL and transplantation for those who were waitlisted.

In conclusion, in contrast to the other countries, Norway transplanted virtually all waitlisted patients <65 years and most patients ≥65 years within 4 years. This may be due to their relatively high number of (live donor) kidney transplants and a lower RRT incidence.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2001**

**Depressed Kidney Function in Kidney Donors Causes Significant Endothelial Dysfunction without Cardiovascular Risks** Masahiko Yazawa,[1] Ryo Kido,[2] Takashi Yasuda,[1] Yugo Shibagaki,[1] Kenjiro Kimura.[1] *[1]Center for Kidney Diseases, St. Marianna University Hospital, Kawasaki, Kanagawa, Japan; [2]Department of Epidemiology and Healthcare Research, Kyoto University, Kyoto, Japan.*

[Purpose] Living kidney donors (LKDs) who were proved to be free from CVD risks by pretransplant evaluation are theretically good models of depressed kidney function without CVD risk factors. In order to elucidate the role of kidney function per se in the pathogenesis of CVD in CKD, we evaluated vascular function in LKDs compared to general CKD patients (CKDs).

[Methods] Twenty-eight prevalent LKDs were compared to the same number of CKDs who were matched with age, sex and estimated GFR. Same number of healthy control volunteers were also studied as reference. Flow mediated dilatation (FMD), as a maker of endothelial function, ankle brachial index (ABI) and pulse wave velocity (PWV) as markers for arteriosclerosis were measured and compared between LKDs and CKDs. Urine nitric oxide (NOx) excretion was measured as another maker of endothelial function. We also tried to clarify factors that affect results of FMD using multiple regression analysis.

[Results] The median age were 62.5 and 53.5 years old, median eGFR were 49.4 and 50.0 ml/min/1.73m$^2$, in LKDs and CKDs, respectively. LKDs had lower prevalence of hypertension, use of the RAS inhibitors and smoking. Their ABI and PWV were better than those of the CKDs (1.18 vs. 1.13 and 1383 vs. 1571, respectively, P<0.05 for both markers). On the other hand, FMD was equally low in the LKDs and CKDs (median 3.3% vs. 3.4% P=0.56). The LKDs had higher prevalence of severe endothelial dysfunction expressed as FMD less than 2.0% (35.7% vs. 17.6%, P=0.13). The median Urine NOx excretion in LKDs was 109.2 μmol/ mmolcr, which was much less than that of healthy controls. The multiple regression analysis showed that the presence of hypertension (p<0.001), LKDs (p=0.03) and age (p<0.001) were significantly correlated with low FMD.

[Conclusion] Endothelial function in living kidney donors was at most as low as CKD patients, which suggests depressed kidney function per se causes endothelial dysfunction without CVD risk factors.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2002**

**What Happens to Patients Returning to Dialysis after Renal Transplant Failure? An Analysis of UK Renal Registry Data** Lynsey Helen Webb,[1] Anna Casula,[1] Charles Tomson,[2] Chris Maggs,[1,3] David Ansell,[1] Yoav Ben-Shlomo.[4] *[1]UK Renal Registry, Bristol, United Kingdom; [2]Richard Bright Renal Unit, Southmead Hospital, Bristol, United Kingdom; [3]University of Hull, Hull, Yorkshire, United Kingdom; [4]Department of Social Medicine, University of Bristol, Bristol, United Kingdom.*

**Background:** Around 22,000 patients in the UK have functioning renal transplants, 3% of which fail each year. Patients with established renal failure who undergo successful transplantation have better quality of life and longer survival compared to those patients remaining on dialysis. This study is the first large UK study examining survival following transplant failure.

**Methods:** Using the UK Renal Registry database, two cohorts were identified. The control cohort comprised patients commencing haemodialysis (HD) or peritoneal dialysis (PD) as an initial form of renal replacement therapy (RRT) between 01/01/2000-30/09/2008, who were wait-listed for transplantation within 2 years of starting RRT. The case cohort were patients commencing HD or PD after failure of a first transplant between 01/01/2000-30/09/2008. Patients were followed until death, loss to follow up or the 31/12/2008, and were censored from the relevant cohort if transplanted. Hazard ratios (HR) and Kaplan-Meier survival were calculated. Cox regression modelling was performed, adjusting for age, gender, ethnicity and diabetic status.

**Results:** The study cohort consisted of 11,280 controls and 3,417 cases. The adjusted HR for death within 90 days of starting dialysis post-graft failure was 5.4 (95%CI 3.5-8.4) compared to the control group. The adjusted HR for death in the 1st year was 4.5 (95%CI 3.3-6.0) which steadily fell to a HR 1.2 (95%CI 0.3-1.6) at >5 years post-graft failure.

**Conclusions:** In keeping with previous North American work, return to dialysis following graft loss is associated with significant mortality, most pronounced in the 1st year after transplant failure. Patients commencing dialysis after graft failure do significantly worse than "fit" (i.e. suitable for transplantation) patients starting dialysis as initial RRT. Further investigation of the patient- and centre-level causes of this increased mortality risk is required.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2003**

**Impact of State Living Donor Policies on Rates of Living Kidney Donation** Caroline E. Jennette,[1] James Bradley Layton.[1,2] *[1]UNC Kidney Center, NC; [2]Gillings School of Global Public Health, NC.*

Kidney transplantation is limited by the number of organs available. Since 2004, efforts have been made on a state level to increase opportunities for transplantation through a tax deduction/credit to reimburse living donors for non-medical expenses related to donation. It is unclear whether policy enactment has influenced living kidney (LK) donation.

**Methods**: States with enacted bills (N=15) were compared to states without (N= 30). States without LK donor information were excluded (n=5), as was the District of Columbia. Average changes per year in the number of LK donations, LK donation rate per 10,000 population, and proportion of all kidney donations made by living donors were considered from 2001 to 2009 using mixed models for repeated measures. States with enacted laws promoting LK donation were considered for the years on and after the law's passing and compared to states without enacted laws. Models were adjusted for the proportion of the state population on dialysis. Donation and dialysis numbers were obtained from the United States Renal Data System and the Organ Procurement & Transplantation Network. Population sizes were obtained from census estimates.

**Results:** Bill enactment did not have a significant effect on the percentage of LK donors nor on the increase of total LK donors. Rates of LK donation increased in all states, but enacted states had a significantly lower rate of increase. Enacted states tended to have higher LK donor rates in the years before policy enactment, but this was not statistically significant.

LK Donation in Enacted vs. Non-Enacted States

| Average per year changes | Enacted States | Non-Enacted States | P Value |
|---|---|---|---|
| Proportion of kidneys from living donors | -1.21% | -1.11% | 0.8 |
| Number of LK donors | 19.13 | 17.11 | 0.9 |
| LK donor rate per year per 10,000 pop | 0.015 | 0.030 | <0.0001 |

**Conclusion:** State enactment of policies to reimburse LK donors for expenses has not significantly increased LK donation. Further research is needed to understand and improve upon methods of policy implementation and educational outreach related to financial reimbursement for living organ donation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2004**

**Gender-Matching in Renal Transplant Allograft Allocation – Does Mismatch Matter?** G. Junge, Alexander Karpov, H. Schwende, C. Cornu-Artis. *Novartis Pharma AG.*

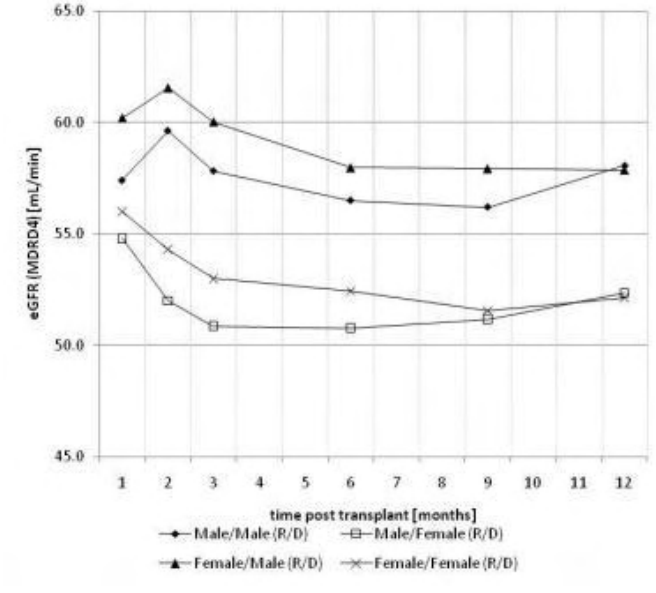
Long-term renal function (RFct) is dependent on a multitude of recipient-related and a few definable donor-related factors that represent the quality of the renal parenchyma being transplanted. However, the effect of donor/recipient gender mismatch on evolution of RFct is rather poorly delineated.

**Method**

RFct at M12 was assessed in 807 KTx recipients from Study A2309, a randomized, open-label, non-inferiority trial comparing TDM-everolimus with reduced CsA vs MPA with standard dose CsA. A2309 demonstrated non-inferiority at M12 which allowed looking at pooled data, neglecting the impact of the immunosuppressive regimen (non significant in univariate RFA). Recipient age, gender, race, BMI, graft type, PRA, donor age, race and gender were tested in univariate/multivariate risk factor analysis (RFA) of RFct(SCR) at M12.

**Results**

Male(M) donor organs showed 5.7 mL/min higher eGFR at M12 compared to female(F) allografts (Fig 1). Lowest change in eGFR (-0.7 mL/min) from M1-12 was observed in the M/M recipient/donor combination and highest in F/F (-4.1 mL/min). SCR-change from M1-3, M6 and M12 will be presented. The univariate RFA of SCR at M12 revealed statistical significance for younger recipient age (p=0.017), F-gender (p<0.001), Afro-American (AA) race (p=0.022), higher BMI (p=0.025), deceased donor allograft (p=0.023), and older donor age (p<0.001) which all remained significant in the multivariate RFA.




**Conclusion**

Male allografts showed higher eGFR at M12 compared to female grafts with highest eGFR measured in the M/M constellation (58.1 mL/min). Highest eGFR decline from M1-12 was observed in F/F recipient/donor situation with max change in SCR (+11.1 µmol/L). Significant RF of SCR were older donor- and younger recipient age, female gender, AA-race, higher BMI and allografts of deceased donors. The two different immunosuppressive regimens did not appear to influence RFct differently.

Disclosure of Financial Relationships: Other Relationship: Medical Scientific Expert, Novartis Pharma AG Basel/CH

Research association, Charite Berlin/GER.

**F-PO2005**

**Medical Tourism: Organ Trafficking and Kidney Transplantation** Snezana H. Mijovic-Das. *Nephrology Division, Albany Medical College, Albany, NY.*

Despite great success of kidney transplantation there is a fair amount of frustration because of inability to provide enough kidneys to address the need for a rapidly growing population of patients with ESRD. In the USA 65 000 patients are on the waiting list and 3000 of them die yearly while being on such a list.

Because of this sad reality organ sale from commercial living donors (CLDs) or vendors has now become evident. According to WHO's estimates, 5-10% of nearly 70, 000 kidneys transplanted annually are obtained by organ trafficking. Patients with enough resources may travel from one country to another to purchase a kidney mainly from a poor person for an amount between $1000 and $5000, while brokers take from wealthy recipients between $100, 000 and $200, 000. China, India, Pakistan, Egypt, Brazil, The Philippines, Moldavia, Romania and Colombia are known to be "hot spots" for organ trafficking, well known to encourage the sale of organs to the tourist recipients from the client countries.

Selling and buying of organs should be prohibited. Organs should be donated only freely and without monetary reward. The entire international community should support the global fight against organ trafficking and illicit medical tourism. The alternative programs must be developed to make more organs available and to provide a good care of donors, their needs and social benefits. Engaging governments to make laws on transplantation and Ministry of Health to control the transplant practices is essential to improve the global situation of Organ Trafficking/Transplant Tourism. The alliance with international professional organizations such as International Society of Nephrology and other societies, all working with WHO to influence health authorities at World Health Assembly to fight the organ Trafficking and Medical Tourism, is a must.

However, the prohibition of selling and buying organs should not affect reimbursement to donors for documented costs including loss of income or expenses related to cost of recovering and medical expenses incurred for post- discharge care.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2006**

**Association of Race and Insurance Type with Discussion of Transplant Options at Dialysis Initiation** Kirsten L. Johansen,[1,3] Rebecca H. Zhang,[2,3] Yijian Huang,[2,3] Nancy G. Kutner.[2,3] *[1]UCSF; [2]Emory; [3]USRDS Rehab/QOL SSC.*

Black pts are wait listed for kidney transplant (txp) within the first year of dialysis less often than White pts. We hypothesized that there would be differences in rates of early txp discussion based on race and insurance. Adults starting dialysis in 2005-2008 who were not on the wait list were studied (n=317,434). Demographic, clinical, and insurance information was taken from USRDS files, along with whether txp options had been discussed and reasons for not discussing. Logistic regression was performed within age strata with txp discussion as the outcome.

Logistic regression of being informed by age

| | | OR (95% CI) by age | | | |
|---|---|---|---|---|---|
| Variable | 18-34 | 35-49 | 50-64 | 65-79 | 80+ |
| Black race | 0.86 (0.76,0.96) | 1.05 (0.99,1.11) | 1.15(1.11,1.20) | 1.15 (1.11,1.19) | 0.99 (0.94,1.04) |
| Pvt ins (ref) | | | | | |
| Medicare | 0.74 (0.60,0.90) | 0.74 (0.68,0.79) | 0.80 (0.77,0.84) | 0.89 (0.86,0.92) | 0.94 (0.90,0.99) |
| Medicaid | 0.80 (0.69,0.93) | 0.73 (0.68,0.79) | 0.76 (0.73,0.80) | 0.87 (0.79,0.95) | 1.02 (0.85,1.22) |
| Other | 0.62 (0.51,0.76) | 0.73 (0.65,0.81) | 0.81 (0.76,0.85) | 0.90 (0.84,0.96) | 1.15 (1.04,1.26) |
| None | 0.65 (0.56,0.67) | 0.67 (0.51,0.72) | 0.74 (0.70,0.78) | 0.90 (0.79,1.02) | 0.96 (0.75,1.23) |

Txp was less likely to be discussed with Black pts age 18-34 but more likely to be discussed with those 50-79. Non-private insurance was associated with lower odds of discussion in all but the 80+ age group. When we examined reasons, Black pts were more likely not to have been assessed in the youngest age group, and the same was true for every category of non-private insurance in all age groups except 80+. Pts with private insurance are more likely to have txp discussed with them at the time of dialysis initiation. Even after adjustment for insurance, younger Black pts - but not older Black pts - have lower odds of being informed about txp options.

Disclosure of Financial Relationships: Research Funding: Amgen, Abbott Laboratories; Scientific Advisor: Amgen Nephrology Advisory Board.

**F-PO2007**

**The Impact of Living Versus Deceased Kidney Donation on Renal Allograft Loss Due to Recurrence of Common Glomerular Diseases** Phuong-Chi T. Pham,[1] Phuong-Thu T. Pham.[2] *[1]Nephrology Division, UCLA-Olive View Medical Center, Sylmar, CA; [2]Kidney and Pancreas Transplantation, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Recurrence of the primary kidney disease in renal allografts may reflect a persistent environmental factor or systemic condition associated with the recipient, an intrinsic inheritable kidney abnormality, or a combination of these factors. We propose that allograft loss due to recurrence of the primary kidney disease may be highest in recipients of kidneys from closely related relatives where intrinsic kidney abnormalities play a key role in recurrence and lowest in recipients of deceased donors. In conditions where environmental or recipient systemic factors play a key role in disease recurrence, minimal, if any, difference in the rates of allograft loss due to disease recurrence should be observed between deceased and living related renal transplantation. In the current study, we review the OPTN/UNOS database for the difference in Kaplan Meier rates of renal allograft loss due to disease recurrence between deceased donors and living donors, the latter further stratified by haplotype match. Primary kidney transplants performed during 1988-2003 (as of February 5, 2010) with the native renal diagnoses of diabetes mellitus (DM), IgA nephropathy (IgAN), membranous glomerulonephropathy (MGN), membranoproliferative glomerulonephropathy (MPGN), lupus nephritis (LN), and focal segmental glomerulonephropathy (FSGS) were included.

There were negligible differences in the rates of allograft loss due to disease recurrence between deceased and living renal transplantation for all aforementioned glomerulonephropathies over a 10 year follow-up. There was a trend for worse allograft loss with higher number of haplotype match in DM, IgAN, and FSGS. Most interestingly, increasing haplotype match was associated with significantly lower rates of allograft loss due to reccurence for LN (4.3 vs 2.7 vs 0.5% for zero (n=187), 1 (n=602), and 2 (n=192) haplotype match, respectively). Further studies and analyses to elucidate contributing factors for these differences are needed.

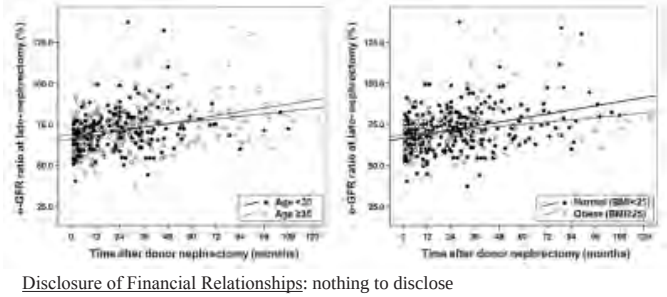
Disclosure of Financial Relationships: nothing to disclose

**F-PO2008**

**Long-Term Compensation of Renal Function after Donor Nephrectomy Is Not Affected by Donor Factors** Dong Jin Joo, Kyu Ha Huh, Beom Seok Kim, Yu Seun Kim. *Yonsei University, Transplantation Center, Seoul, Korea.*

***Background:*** We retrospectively evaluated the factors that influence the compensatory ability of the remaining kidney after nephrectomy in donors for kidney transplantation.

***Patient and Methods:*** Follow-up renal function data of 396 donors (46.2%) were available from 857 living kidney donors from January 1999 to December 2007 at Yonsei University, Severance Hospital, Korea. The renal function was expressed as estimated glomerular filtration rate (e-GFR) was calculated by Modification of Diet in Renal Disease (MDRD) formula. Post-nephrectomy renal function was expressed as relative renal function (post-nephrectomy e-GFR ratio versus pre-nephrectomy e-GFR,%). ***Results:*** Two cases (0.05%) of renal failure resulted during 26.0±24.2 months of follow-up. The mean e-GFR decreased to 62.0±13.1 ml/min immediately after nephrectomy from 93.2±18.5 ml/min at pre-nephrectomy. Donors with high body mass index (BMI) and male sex showed a greater decrement of renal function at the immediate post-nephrectomy period (within 7 days). The long-term follow-up relative renal function significantly increased by post-nephrectomy duration. The slope of relationship between the relative e-GFR and post-nephrectomy duration was 0.176 (y=67.022+0.176*x, $R^2$=0.103, $p$<0.0001). It indicates that the estimated e-GFR increased 2.11± 3.12% of its initial e-GFR per post-nephrectomy year. Unlike the immediate post-nephrectomy change of renal function, the long-term renal function was not affected by donor factors such as age, gender and BMI. The compensation of renal function persisted after nephrectomy regardless of donor factors in univariate and multivariate linear regression analysis.




Disclosure of Financial Relationships: nothing to disclose

**F-PO2009**

**Utility of Estimated Glomerular Filtration Rate in Live Kidney Donation** Soo Kun Lim, Kok Peng Ng, Li Ping Tan, Chew Ming Wong, Tee Chau Keng, Yip-Boon Chong, Wai Yew Kong. *Renal Unit, Department of Medicine, University Malaya Medical Centre, Kuala Lumpur, Malaysia.*

**Introduction:** Creatinine-based prediction equations have been widely used to estimate glomerular filtration rate (GFR). However, these formulas have not been validated to be used in certain group of patients, including previous kidney donors. **Objectives:** To compare the utility of GFR estimating formulae based upon creatinine and cystatin-C in assessing residual renal function after living donor nephrectomy. **Methods:** A cross-sectional study that included live kidney donors who had their nephrectomy at least 6 months before. Demographic and transplant data were collected and laboratory investigations including renal profile, cystatin-C were done. Residual kidney function was assessed using Cr-51 EDTA method. The estimated GFR (eGFR) was calculated using Cockroft-Gault formula, modified 4-variable Modification of Diet in renal Disease (MDRD) formula and cystatin C-based formula (Dade Behring Calibration). **Results:** There were a total of 38 kidney donors in this study. The median age was 52.0±10.5 years and 66% were females. Most of the donors were Chinese (76%) followed by Indians (13%) and Malay (11%). The duration of kidney donation ranged from 8 to 227 months with mean of 81.7 months. Mean serum creatinine was 96µmol/L and mean GFR was 82ml/min/1.73m$^2$. Thirty five donors (92%) have GFR of 60ml/min/1.73m$^2$ and above. Estimated GFR (eGFR) of 60ml/min/1.73m$^2$ and above was only present in 61% and 66% of the donors using MDRD and Cockroft-Gault formula respectively. Using cystatin C-based formula, the mean eGFR was 94ml/min/1.73m$^2$ and 92% have GFR of 60ml/min/1.73m$^2$ and above. Cystatin C-based formula overestimated GFR by at least 20% in almost half of the donors (47.4%). **Conclusion:** Our preliminary results showed that eGFR using creatinine-based formula is not validated in live kidney donors. Estimated GFR using MDRD and Cockroft-Gault formulas tend to underestimate the GFR. Cystatin C-based formula correlates better with GFR but can significantly overestimate the GFR in almost half of the donors. Further study is needed to validate the use of cystatin C in estimating GFR in this patient population.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2010**

**Accepting Kidneys from Older Living Donors: Impact on Transplant Recipient Outcomes** Ann Young,[1,2] Joseph Kim,[3] Mark Speechley,[2] Anjie Huang,[4] G. V. Ramesh Prasad,[3] Greg A. Knoll,[5] Darin Treleaven,[6] Michael Diamant,[1,2] Amit X. Garg.[1,2] *[1]Division of Nephrology, University of Western Ontario, Canada; [2]Department of Epidemiology and Biostatistics, University of Western Ontario, Canada; [3]Division of Nephrology, University of Toronto, Canada; [4]Institute for Clinical Evaluative Sciences, Toronto, ON, Canada; [5]Division of Nephrology, University of Ottawa, Canada; [6]Division of Nephrology, McMaster University, Canada.*

Background: Due to increasing demand for kidney transplant, selection criteria for living kidney donors have been relaxed. Older individuals are now accepted. Age-associated changes in renal morphology and function may affect transplant survival.

Methods: This retrospective cohort study examined outcomes of kidney recipients whose donors were: Older-living (≥60 years), younger-living (<60 years), and deceased standard criteria (SCD). Transplants occurred from 01/2000 to 03/2008 in Ontario, Canada. The study used Canadian health administrative data, supplemented by original medical records. The primary outcome was time to death or graft loss.

Results: Recipients received kidneys from: 73 older-living donors, 1187 younger-living donors, and 1400 deceased SCDs. Older-living kidney recipients were often older, but had similar co-morbidity scores. Baseline GFR of older-living kidneys was 13 ml/min per 1.73 m2 lower than younger-living kidneys. Time after transplant was 4 (IQR: 2-5) years. There was no significant difference in recipient death or graft loss between recipients of older or younger living donor kidneys (23 vs. 15%, adjusted HR (95%CI): 1.56 (0.98-2.49)). However, this hazard may increase with time (time-dependent interaction: p=0.01). Recipient death was higher for older-living kidney recipients (15 vs. 6%, HR: 2.73 (1.39-5.35)). Associations were not modified by recipient age or donor GFR (all interactions tested: p<0.05). Survival of older-living kidneys was similar to kidneys from deceased SCDs (HR: 0.98 (0.95-1.02)). This hazard may increase with time (p=0.01).

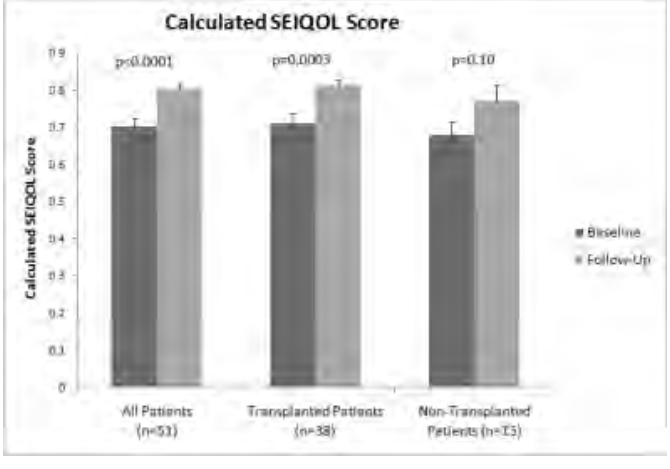
Conclusions: Accepting older-living kidney donors results in acceptable outcomes for the recipient. Continued and expanded use may help with the demand for transplantation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2011**

**A Novel Measure of Individualized Quality of Life in Patients Being Evaluated for Kidney Transplant** Jay A. Shah,[1] Khaled Abdel-Kader,[1] Irina Karpov,[3] Larissa Myaskovsky,[2] Rachel Hess,[2] Mark L. Unruh.[1] *[1]Renal Division, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Department of Medicine, University of Pittsburgh School of Medcine, Pittsburgh, PA; [3]Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Health-related quality of life (HRQOL) is important in kidney transplant. The Schedule for the Evaluation of Individual Quality of Life (SEIQOL) is a novel tool to evaluate individualized quality of life (IQOL). It allows patients to nominate domains they view as valuable to their IQOL. The SEIQOL has previously been studied in various patient populations, including a cohort of patients with chronic kidney disease (CKD). We followed the change in SEIQOL score in patients being evaluated for kidney transplant. A cohort of 51 patients with CKD who were undergoing evaluation for kidney transplant completed the SEIQOL at baseline and a single 6-12 month follow up. We considered two separate groups: 38 patients who received a kidney transplant between assessments and 13 who did not. The average age of the cohort was 49 years, 66% were men, 27% were black, 31% had diabetes, 39% were on dialysis, baseline glomerular filtration rate among non dialysis patients and hemoglobin were 17.9 and 11.8, respectively. The baseline characteristics were similar in both groups. Domains of importance on the SEIQOL included family, health, leisure, and relationships. On follow up, finance became a prominently listed domain, while leisure became less frequently listed among transplanted patients. The SEIQOL score improved significantly in the transplanted group.



Future work should examine if the SEIQOL better correlates with health outcomes in this population. We conclude the SEIQOL is an effective tool for assessing change in IQOL in kidney transplant patients.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2012**

**Reaching the Original GFR of a Single Kidney after Living Donor Transplantation: Impact of Donor and Recipient Characteristics** Bertram Hartmann,[1] Christiane Manderscheid,[2] Ulf Schoenermarck,[2] Markus Guba,[3] Michael Fischereder,[2] Holger Schmid.[2] *[1]Medical Department I, Section of Nephrology, Ulm University, Ulm, Germany; [2]Medical Department I, Section of Nephrology, Munich University Hospital, Campus Grosshadern, Munich, Germany; [3]Department of Surgery, Munich University Hospital, Campus Grosshadern, Munich, Germany.*

Background

The aim of the study was to analyze living donor (LD) and recipient's characteristics after kidney transplantation (KT) and their impact on reaching the original donated GFR of the kidney.

Methods

242 consecutive living donor kidney recipient/donor pairs from 1997 – 2007 were included. Pairs were comparable concerning HLA match, ischemia time, operative time, and induction immunosuppressive treatment. GFR of the transplanted kidney was evaluated by using creatinine-based estimation equations (MDRD2 formula) at 3, 10 and 180 days after KT.

Results

A total of 45.9% of recipients reached the original donated GFR already 3 days after LD KT and 64.9% 10 days after LD KT. Significant predictors for not reaching the transplanted GFR in the early posttransplant period were older age of the donor (mean 52±9 vs. 49±10 years, $p<0.05$), a higher weight of the recipient (mean 74±10.7 vs. 67±12 kg, $p<0.001$) and a higher recipient´s BMI (24.3±3 vs 23±2.5 kg/m², $p<0.01$), as well as the gender constellation of a male donation to a female recipient ($p<0.01$). Adaptive hyperfiltration (GFR >60 ml/min) of the recipient after transplantation was a frequent finding.

Conclusions

The findings suggest that donor's age, recipient's weight, and the gender disparity of the donor-recipient pair should be considered as a criterion in the choice of donor and recipient pairs for successful short-term outcome in living donor renal transplantation. The problem of hyperfiltration in the early posttransplant period that may have a pivotal long-term prognostic impact deserves further exploration.

Disclosure of Financial Relationships: Research Funding: financial support by Wyeth for one research project.

**F-PO2013**

**Older Dialysis Patients Are Unlikely To Receive a Deceased Donor Transplant, Even If Listed for Transplantation** Kathryn K. Stevens,[1] John Douglas Mcclure,[2] Marc J. Clancy,[1] Jonathan Fox,[1] Colin C. Geddes.[1] *[1]Western Infirmary, Glasgow, United Kingdom; [2]University of Glasgow, Glasgow, United Kingdom.*

**Introduction:** Most transplant centres have no upper age limit for transplantation, unless there is a clear contra-indication. The probability of older patients actually receiving a deceased donor (DD) kidney transplant is unclear preventing informed choice about the option of transplantation.

**Aim:** To determine the probability of receiving a DD kidney transplant in patients commencing RRT categorised by age.

**Methods:** Patients commencing dialysis in our centre between 1992 and 2009 were identified. Time to listing on the DD transplant waiting list (WL) and to first DD transplant were determined by Kaplan Meier analysis for patients, categorised by age, with censoring at the date of first living donor kidney transplant, death or last dialysis.

**Results:** 1513 patients were categorised into groups by age in years (1:<35 (n=134), 2:35-49.9 (n=207), 3:50-64.9 (n=415), 4:>65-74.9 (n=438) and 5:≥75 (n=319)). The probability of being listed for DD transplant was 75%, 54%, 27%, 4%, and 0.8% in groups 1-5 respectively. If listed, the probability of receiving a DD transplant within 5 years of starting RRT was 81%, 48%, 26%, 8% and 0% in groups 1-5. 93% (n=63), 87% (n=65), 76% (n=45) and 100% (n=7) of patients in groups 1-4 respectively who received a DD transplant were alive and off dialysis 1 year after transplant. The reason patients who were listed did not receive a transplant was usually death on the WL.

**Conclusion:** The likelihood of being listed for DD transplant falls with increasing age at the time of starting RRT. Even for patients listed for transplant, the probability of older patients actually receiving a transplant is lower than for younger patients. Death on the WL offers some explanation for this and organ allocation policies (e.g. matching by age of donor and recipient) may also contribute. Assessment for possible DD transplantation involves a considerable investment for both patient and healthcare resources. A patient's decision to proceed with assessment should be informed by similar local data.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2014**

**HLA Frequency Differences in Mexican-American and Caucasian** Steve Ogechi Egwuonwu,[1] Kantibhai M. Patel,[2] Zuber D. Mulla,[3] Ramin Tolouian.[1] *[1]Nephrology and Hypertension, Texas Tech University Health Sciences Center, El Paso, TX; [2]HLA Lab, Las Palmas Medical Center, El Paso, TX; [3]Obstetric and Gynecology, Texas Tech University Health Sciences Center, El Paso, TX.*

**Introduction**: We have learned during the years that Hispanic patients with renal transplant needs more immunosuppressants compared to Caucasian patients. Although the exact mechanism of this difference is not clear, we hypothesize that the differences in the HLA of these populations could have some role. As HLA frequencies differ from one ethnic group to another, there is a paucity of literature regarding HLA frequencies in the Mexican American (MA) population residing on the US-Mexico border.

**Methods**: We determined the distribution of HLA Class I and Class II antigen frequencies in Mexican American (MA) and compared it with Caucasian subjects from the kidney transplant program in El Paso, Texas, from 1987 to 2007.

Our analysis included 1455 donors and recipients: 1106 MA and 349 Caucasians. The HLA Class I (HLA-A, HLA-B, and HLA-C locus) and Class II (HLA-DR and DQ locus) typing were performed using Dynalbead HLA Cell Prep I and HLA Cell Prep II serology technique and Terasaki HLA Tissue Typing Class I and Class II Trays. Comparison of the HLA frequencies between these two racial/ethnic groups was done using the chi square test.

**Results**: The B7 antigen was significantly most frequent among Caucasians than among MA: ($p<0.0001$). In the Mexican American group HLA B35 appeared to be most significant ($p<0.0001$). Other antigens that were common among the MA compared to the Caucasians include A24 ($p<0.0001$), A31 ($p<0.0001$), and A68 ($p<0.0001$). Neither B4005 nor B48 were found in Caucasians but were detected in MA. In HLA class II locus, HLA DR4 was significantly most common in MA.

**Conclusion**: In our knowledge this is the largest study about the frequency of HLA in MA in US-Mexico border region. This study reveals clear differences in the distribution of HLA frequencies between Mexican Americans and Caucasians. Further studies should be conducted to determine if HLA differences have a role in different immune response in these two populations.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2015**

**Clinical Impact of Kidney Living Donors with Thin Basement Membrane Compared to Normal Donors** Monica X. Inofuentes, Francisco E. Rodríguez Castellanos, Patricia C. Ruiz Palacios, Bernardo Moguel, Maria Carmen Avila-Casado. *Nephropathology, Instituto Nacional de Cardiologia Ignacio Chavez, Mexico City, Mexico.*

We have previously presented studies of Asymptomatic Mexican patients with Thin basement membranes (TBM) diagnosed at time-0 biopsy.

**AIMS.**

The aim of the present study was to assess the outcome at one-year follow up of patients with thin basement membrane (TBM) diagnosed at time-zero biopsies (TZB) compared to normal basement membrane (NBM) donors.

**METHODS.**

We analyzed the clinical and histopathological characteristics of the TZB from January 2007 to January 2008; 30 subjects in total divided in two groups- Group 1(n=15) TBM and Group 2 (n=15) NBM donors. The biopsies were processed for light microscope and electron microscopy.

**RESULTS.**

The basal serum Cr were different in both groups ($p=0.24$), at the end of the follow up period the comparison of the different parameters with significance in both groups were Creatinine clearance (CrCl) ( $p=0.19$), proteinuria ($p=0.10$) and systolic blood pressure ($p=0.19$). Serum Cr (SCr) and diastolic blood pressure showed a tendency for significance.

Group 2 and Group 1 in basal (prenephrectomy) SCr and CrCl and at the end of the year of follow up had differences ($p=0.001$) in both parameters. But for the development of Proteinuria postnephrectomy only de group Group 1 showed differences ($p=0.33$).

Interstitial inflammatory cells, tubulointerstitial Fibrosis and glomerular sclerosis weren't different in both groups.

**CONCLUSIONS.**

We find differences for CrCl and SCr in both groups, with worst outcome for TBM.

The most important finding in this trial was the association of TBM and the development of Proteinuria. This could be relevant in naturally occurring renal injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2016**

**Information Needs of Living Kidney Donors To Improve the Informed Consent Process: A Qualitative Study** Elisa J. Gordon,[1,3] Elizabeth A. Reddy,[1] Jeffrey Beaubien,[2] Jane L. Holl,[1] John J. Friedewald.[3] *[1]Institute for Healthcare Studies, Northwestern University; [2]Aptima, Inc; [3]Surgery, Northwestern University.*

Informed consent is ethically and legally required for living donation. Transplant clinicians must disclose sufficient information for living kidney donors (LKDs) to make an informed decision and feel adequately prepared to donate. We investigated LKDs' information needs. A random sample of LKDs who donated in the past year was interviewed

via telephone about their information needs, perceptions of the informed consent process and about the independent donor advocate (IDA). Interviews lasted 20 minutes. Responses were recorded by hand-written notes and analyzed by content for repetitive themes and patterns. The response rate was 75% (15/20 LKDs). LKDs desired more information about: recovery duration and experience; impact of donation on long-term health (i.e., side effects, medications, diet, having children); surgical logistics; recipient's health; scar size; and donor testing process. Most LKDs (n=12) viewed information about short- and long-term recovery as most crucial. LKDs suggested improving the informed consent process by: providing more information about recovery, long-term health effects of donation, the donor evaluation process, and showing the donation informational video earlier. Most LKDs felt the IDA helped protect their interests (n=14) and was a good source of information (n=6), but two did not appreciate the IDA's help.

Table 1. Illustrative Quotations of LKDs' Information Needs

| |
|---|
| "Things you have to do after surgery. I underestimated how long it would take my body to readjust. I was kind of tired. Even recently, I'm just getting back." |
| "I needed to know if there would be issues getting insurance later in life and if there might be health problems early on like diabetes or kidney failure." |

Greater efforts are needed to inform LKDs about all steps involved in donation early in the evaluation process and post-operative donor health and recovery via video and IDA to ensure realistic expectations and optimize informed consent. As this was a single center study, further research is needed to more generally assess LKDs' information needs.

Disclosure of Financial Relationships: nothing to disclose

### F-PO2017

**Long-Term Outcomes in Highly Sensitized and ABO Incompatible Patients Undergoing Kidney Transplants** Kumar Gaurav, Rasheed A. Balogun, Douglas Scott Keith, Scott Leonard Sanoff, Peter I. Lobo. *Division of Nephrology, University of Virginia, Charlottesville, VA.*

**Introduction**: There is no consensus on how low the anti-HLA antibodies need to be reduced prior to transplant so as to avoid post-transplant rejection in highly sensitized and ABO incompatible patients. Similarly there is no data regarding the level of maintenance immunosuppression required to prevent chronic graft loss.

**Methods**: Since May 1st 2006, we performed 20 kidney transplants in highly sensitized patients who were crossmatch positive against their living donor and in 8 patients who were ABO incompatible with their living donors. All these patients underwent a desensitization protocol treatment which included the use of Cellcept, Rituximab, as well as Plasmapheresis and IVIG to decrease antibody levels such that they may only be detectable by either the flow crossmatch or by the single antigen Luminex beads but not detectable by the cytotoxic assay. Post transplant, patients were maintained on higher doses of prednisone, CellCept and Prograf.

**Results**: Out of the 20 patients who underwent the treatment protocol and subsequently the transplant, 18 still have a functioning graft at an average follow-up of 27.7 months with a mean serum creatnine of 1.3 mg/dL and no albuminuria. 7 of 9 patients with a positive flowcrossmatch had humoral rejection episodes resulting in loss of 1 allograft. None of the 11 patients that had antibodies detectable only by Luminex had a rejection episode. The difference in rejection episodes between the flow crossmatch positive patients and flow crossmatch negative patients is statistically significant ($p<0.0003$). 2 of these 20 patients died after one year of follow-up, 1 with severe heart failure and other with sepsis. All 8 ABO incompatible patients have a functioning graft after a mean follow-up of 27 months and a mean serum creatnine of 1.1 mg/dL. Only 1 of these 8 patients had a rejection episode.

**Conclusion**: Our data indicates that for successful long-term outcome in highly sensitized and ABO incompatible patients, it would be beneficial to significantly reduce antibodies pre-transplant as well as maintain these patients on more immunosuppression.

Disclosure of Financial Relationships: nothing to disclose

### F-PO2018

**Impact of the Model for End-Stage Liver Disease Allocation Policy on Patient Survival after Liver Transplantation** Aastha Sethi,[1] Michelle M. Estrella,[2] Mohamed G. Atta.[2] *[1]Renal Division, Johns Hopkins Bayview Medical Center, Baltimore, MD; [2]Renal Division, Johns Hopkins Hospital, Baltimore, MD.*

To determine the effect of implementing the model of end-stage liver disease (MELD) among liver transplant individuals on all-cause mortality, we performed a survival analysis from the time of transplant to death in individuals who underwent orthotopic liver transplantation (OLT) at a tertiary referral hospital from 05/16/1995-04/22/2009. Individuals who were <18 years old, received multiple organs, underwent status 1A transplantation, or had no follow-up 3 months after the transplant were excluded (n=199). For the remaining 419 individuals (163 in pre-MELD era, 256 in MELD era) included in the analysis, hazard ratios (HR) and 95% confidence intervals (CI) were estimated using Cox proportional hazard models. Participants were censored at the time of last follow-up or 12/31/09. Socio-demographic characteristics were similar between the two groups. Mean follow-up times post transplant in the pre-MELD and MELD patients were 8.4 and 3.1 years, respectively, with 120 deaths observed. Adjusting for age, gender, race, pre-transplant renal replacement therapy (RRT), hepatitis C status, hypertension, diabetes, and time-varying binary estimated glomerular filtration rate [eGFR < 60 vs. ≥ 60 ml/min/1.73 $m^2$], there was no survival difference between patients transplanted in the pre-MELD versus the MELD era. However, need for pre-transplant RRT (HR=2.90, CI 1.24–6.80) and low eGFR post-transplant (HR= 1.53, CI 1.10–2.13) were adversely associated with worse survival in the adjusted analyses. These estimates were unchanged even when observation time was truncated to allow similar follow-up times between the pre-MELD and MELD era groups. In conclusion, patient mortality remains high after OLT. However, there is no difference in patients' survival between the pre-MELD and MELD era. The need for RRT prior to liver transplantation and impaired kidney function after transplantation are associated with increased mortality risk after liver transplantation.

Disclosure of Financial Relationships: nothing to disclose

### F-PO2019

**Similar 10-Year Graft Survival of Living Related and Unrelated Donor Kidney Transplantation, in Spite of Differences in HLA-Matching** Igor Marques, Gustavo Ferreira, Clarice Park, Lilian P. F. Carmo, Elias David-Neto. *Nephrology, University of Sao Paulo School of Medicine, Sao Paulo, Brazil.*

Aim of the study: Living unrelated donors (LUD) constitute an incremental source of kidneys for transplantation at a global level. This study examines the outcome of allograft and patient survival in LUD transplantation compared with living related donor kidney transplantation (LRD) and analyzes influencing factors. Methods: We retrospectively analyzed 389 first living donor kidney transplantations performed between 1998 and 2007 in a single center, 281 recipients from LRD and 108 from LUD. Results: In the LRD group 50 patients (17.8%) were HLA-identical siblings, 181 (64.4%) had parent donor with haploidentical HLA and the other 50 patients (17.8%) had a distinct HLA-matching. The LUD group differed from the LRD group in age, gender and HLA-matching. The patients who received a kidney from a relative were younger at the time of transplantation. The female gender represented more than half of the patients in the LRD group, while in the LUD group represented close to a third part. As expected, the LUD group had a poor HLA-matching. All the other variables had a similar distribution in both groups, including: cause of end-stage renal disease, preemptive transplantation, donor age and gender, panel reactive antibodies and immunosuppression. The incidence of acute rejection was similar in both groups (LRD 25% and LUD 26%, p=0.89). There was no significant difference in patient survival when analyzed from the time of transplantation. The 1-year patient survival in the LUD and LRD groups was 96% and 95.3%, 5-year 91.6% and 92.4% and 10-year 89.1% and 84.7%, respectively. The death-censored graft survival rates for LUD transplants were 94.8% at 3 years, 91.7% at 5 years and 68.9% at 10 years, comparable to those for LRD transplants (excluding HLA-identical siblings), which were 92.5% at 3 years, 88.9% at 5 years and 81.1% at 10 years (p=0.77). Conclusion: In spite of poor HLA-matching, long-term graft survival of LUD kidney transplantation is similar to haploidentical and distinct LRD transplantation.

Disclosure of Financial Relationships: nothing to disclose

### F-PO2020

**Designation of Long Term Outcome Program for Iranian Living Unrelated Kidney Donors (LURD): Update Report** Ali Nobakhthaghighi, Ezzatollah Abdi, Tahereh Malakoutian, Ehsan Nobakht, Mohammad Kamgar, Niloofar Nobakht, Varshasb Broumand, Laila Nobakhthaghighi, Monir Sadat Hakemi, Behrooz Broumand, Iraj Fazel. *Academy of Medical Sciences of Iran.*

Introduction:The government-regulated compensation for LURD transplantation has decreased the waiting time and the number of patients in Iran national waiting list. However, the long term outcome of living donors is not well understood. A 10 year controlled cohort study has been started by Academy of Medical Sciences in cooperation with Charity Foundation for Especial Diseases since June 2006 to evaluate long term medical complications of kidney donors.

Methods: LURDs who had donated their kidneys after1995 were asked to participate in the study. Age, gender, BMI, systolic blood pressure(SBP), diastolic blood pressure(DBP), blood sugar(BS), hemoglobin, BUN, creatinine,lipid profile, urinalysis, urine albumin/creatinine and GFR are recorded.

Results: 1616 donors have been registered so far. 963 underwent nephrectomy between 2006 -2007. 97 donors underwent nephrectomy between1995-2003. The average time between nephrectomy and the study enrolment was 316.7 days. Mean age at the time of first visit was 30.4± 6.1 with 1333 (82.5%) male and 283(17.5%) female with mean BMI 23.5 ± 4.4 mean SBP 107 ±12.2 and mean DBP 75.3± 7.7. Mean GFR was 87.6±24 at the time of enrolment. Mean LDL was 98.12±32.2. Mean urine albumin/creatinine ratio was 6.1± 9.1mg/gram.67 of the kidney donors who underwent nephrectomy at least 5 years before the first visit and had at least 2 annual visits were selected. No statistically significant differences in BMI, SBP,DBP, BS, hemoglobin, LDL, urine albumin/creatinine ratio and GFR were observed in their follow up visits.

Conclusion: Most of the study participants were young with normal BMI and SBP. No worsening of SBP,GFR and proteinuria was observed in donors who had at least two annual follow up, however those are only small percentage of our study population.Most of the donors underwent nephrectomy within 1 year of study enrollment and long term follow up is needed to assess their prognosis. Further monitoring and comparison to normal subjects will be reported in future.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2021**

**Characteristics of an Online Renal Data-Sharing Initiative – The PatientsLikeMe Transplants Community** C. Brownstein,[1] P. Wicks,[1] T. Vaughan,[1] M. Massagli,[1] G. Junge.[2] *[1]Research and Development, PatientsLikeMe, Cambridge, MA; [2]Global Program, Novartis Pharma AG, Basel, Switzerland.*

PURPOSE: Renal transplant patients face medical and personal challenges such as monitoring their health, adhering to medication, and coping with emotions. While well-supported during the immediate post transplant period by healthcare professionals, patients may benefit from other means of support in the long term, such as peer to peer networking.

METHODS: The PatientsLikeMe Transplants Community allows patients to share detailed information about medical history, diagnosis leading to end stage renal disease, health-related quality of life, lab values (including blood pressure, serum creatinine, and GFR-4D), symptoms, and treatments with other patients.

RESULTS: Since its launch in Spring 2010, PatientsLikeMe hosted data from 681 kidney transplant recipients (792 transplants). Mean current age is 42±11 years, with 66% females. Mean age at transplant is 36±12 years; patients report spending a median of 11 months on the waiting list (range 0–11.8 years). Of those reporting a causative condition (N=566, 83%), the most common are hypertension (50%), diabetes (21%), glomerulonephritis (18%), and PKD (13%). 714 organs are of known origin (90%). 53% of organs were donated by living donors (81% related), 47% were from cadaveric donors. HLA mismatch data is available for 393 kidneys (50%) [Table 1]; there are significant differences in HLA mismatch between deceased (1.5±1), living (2.3±2), and living-related donors (1.9±1, respectively, F=6.5, df=2, p=0.002). Sibling-donated kidneys have the fewest HLA mismatches (1.4±1, N=79), followed by parents (2.0±1, N=36), children (2.1±1, N=19), and any other relation (2.5±2, N=48) (F=5.4, df=3, p=0.001).

CONCLUSION: Many patients who experience a life-changing event such as a renal transplant are willing to share detailed health data. This growing Community may advance scientific and medical research efforts as well as provide educational and support opportunities for patients willing to participate.

Table 1. HLA-A, B, DR mismatches

| Mismatches | N | % |
|---|---|---|
| 0 | 113 | 29 |
| 1 | 66 | 17 |
| 2 | 87 | 22 |
| 3 | 80 | 20 |
| 4 | 23 | 6 |
| 5 | 24 | 6 |
| 6 | 0 | 0 |
| Total | 393 | 100% |

Disclosure of Financial Relationships: Employer: PatientsLikeMe; Ownership: PatientsLikeMe.

**F-PO2022**

**Serum Angiotensin Converting Enzyme-2 Activity as a Marker of Cardiovascular Risk in Kidney Transplant Patients** Maria Jose Soler, Marta Riera, Marta Crespo, Judit Rigol, Marisa Mir, Eva Rodriguez, Josep M. Puig, Julio Pascual. *Nephrology, Hospital del Mar. Parc de Salut Mar. Fundació IMIM, Barcelona, Spain.*

Angiotensin converting enzyme(ACE)-2 is an enzyme that counterbalances ACE activity. The role of ACE2 in kidney transplant(KT) patients is unknown. The aim of this study is to investigate whether ACE2 activity is altered in KT patients as compared with controls. In addition, we studied the correlation between serum ACE2 activity and age, gender, graft function and analytical cardiovascular risk markers in KT patients.

ACE2 activity was assessed using a fluorescent assay in 113 KT patients(age 55±12yr, GFR-MDRD 44.8±11.3mL/min). Chronic kidney disease(CKD)-Stage 3 patients(n=27, age 57±10yr, GFR-MDRD 41.4±8.6)age, gender and MDRD-matched served as controls.

Serum ACE2 activity was decreased in KT patients as compared to CKD patients(35.4±2.8 vs 67.6 ±13 RFU/mL/h, p<0.05). In the univariate analysis, ACE2 activity was increased in KT patients with ischemic heart disease as compared with KT without ischemic heart disease (45.1±7.6 vs 34.3±2.9, p<0.05). In addition, ACE2 activity was increased in male as compared with female KT patients (42.7±3.7 vs 21.9±2.8, p<0.05). Renin angiotensin system blockade did not influence serum ACE2 activity. In multiple regression analysis, creatinine, LDL-cholesterol, gamma glutamyl transferase, gender, and hemoglobin were independent predictors of serum ACE2 activity.

Multiple linear regression analysis of independent predictors of serum ACE2 activity in KT patients.

| Risk factor | Standardized coefficient (Beta) | p-value |
|---|---|---|
| Gender | -0.284 | 0.002 |
| Serum creatinine (mg/dL) | 0.223 | 0.014 |
| Gamma glutamyl transferase (UI/L) | 0.222 | 0.008 |
| LDL-cholesterol (mg/dL) | 0.182 | 0.029 |
| Hemoglobin (g/dL) | 0.209 | 0.020 |

Data are expressed as regression coefficients and p-values. Dependent variable: serum ACE2 activity RFU/µL/h expressed in lnACE2; multiple r2 = 0.34, p < 0.001.

In conclusion, serum ACE2 activity in KT patients is increased when graft function is decreased and it is directly correlated with clinical and biochemical cardiovascular risk markers.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2023**

**Gonadal Dysfunction Associated with mTOR Inhibitor Treatment Is Reversible** Jordi Rovira, Barbara Vodenik, Maria J. Ramirez-Bajo, Elisenda Banon-Maneus, Daniel Moya-Rull, Marta Arias, Luis F. Quintana, Fritz Diekmann, Josep M. Campistol. *Nephrology and Renal Transplantation, Laboratori Experimental de Nefrologia I Trasplantament (LENIT), Hospital Clinic de Barcelona, Fundacio Clinic per la Recerca Biomedica, Barcelona, Spain.*

Recent studies observed an association of SRL treatment with gonadal dysfunction in transplant recipients.

The aim of this study is to characterize the effect of mTOR inhibition on spermatogenesis, showing the morphology changes on seminiferous tubules and sexual hormone profile, as well as to analyze the withdrawal of mTOR inhibition.

**Material and methods:**

Adult male *Wistar* rats were distributed in two groups according to intraperitoneal administration of vehicle (VEH; n=8), SRL (n=24) 1mg/kg three times a week. VEH group was treated for 12 weeks. Rats treated with SRL were sacrificed at 4, 8 and 12 weeks. A group of rats was treated with SRL for 4 weeks and then continued with vehicle injection during 8 weeks. This group was used to analyze the possible reversibility of the effect of mTOR inhibition. Body and testicular weight, testosterone, FSH and LH levels were measured. The testes were collected to perform histological measurements; seminiferous tubules area and inner diameter. The proliferation was performed by immunohistochemistry using Ki67 and PHH3 stain.

**Results:**

Testosterone levels were decreased after sirolimus treatment and recovered after withdrawal. The testes weight was significantly lower in SRL groups compared with VEH group. 8 weeks after a 4 cycle of SRL treatment permitted testicular weight had partially recoverd. The spermatogenesis was totally blocked on the spermatogonial level by SRL treatment. Withdrawal of treatment led to complete recovery spermatogenesis.

**Conclusions:**

mTOR inhibition in healthy animals produces sexual hormone dysfunction, a seminiferous tubule dystrophy and the blockade of spermatogenesis. This blockade is reversible.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2024**

**Effect of Blood Transfusions on Mortality and Long Term Renal Graft Outcome in Renal Transplant Patients** Frank J. O Brien, James Lineen, Claire Kennedy, Peter J. Conlon. *Department of Nephrology, Beaumont Hospital, Dublin, Ireland.*

Background

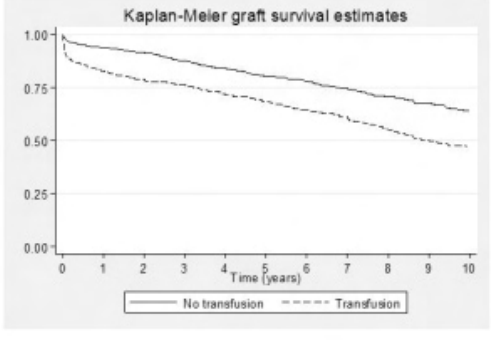
It has been established that patients who receive blood transfusions pre transplant spend a longer time on the transplant pool, and have higher rates of rejection. Little evidence exists on the long term graft outcomes in patients who receive transfusions at the time of transplant. This study aims to establish if there is a relationship between the receipt of blood transfusions at time of transplant and long term graft survival.

Methods

This was a single center, national, retrospective cohort study. Data was collected on patients who received kidney transplants over a 14 year period(n=2013). The primary outcomes were graft survival and mortality in patients who received blood transfusions compared to those that did not. Subsequent multi variate analysis using Cox's proportional hazard model was used to examine the effect of hemoglobin, donor and recipient age, CMV status, acute rejection, PRA and HLA. Log rank test was used to compare the two groups and Kaplan Meier survival curves were subsequently created.

Results

In the first year post transplant there was a lower rate of graft survival in those that received a blood transfusion compared to those that did not, p<0.001.




There was a strong association between number of units transfused and graft survival. Patients who received one blood transfusion had a ten year graft survival of 52%, while those that received four had a graft survival rate of 41% at ten years.

Using a Cox's proportional hazard model, perioperative blood transfusions had an independent negative effect on graft survival when adjusted for donor and recipient age, acute rejection and PRA group. Hemoglobin levels prior to transfusion did not have an influence on graft outcome.

Conclusion

Perioperative blood transfusion is associated with adverse effects on long term graft outcome.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2025**

**Endothelin Receptor Plays Role in BK Polyoma Virus Infection of Renal Epithelial Cells** Andrey Sorokin,[1] Bradley S. Miller,[1] Takahito Moriyama.[2] *[1]Department of Medicine, Medical College of Wisconsin, Milwaukee, WI; [2]Department of Medicine, Tokyo Women's Medical University, Tokyo, Japan.*

Although potent immunosuppressive therapies are useful for acute and chronic rejection, they become a risk factor for the progression of latent BK virus (BKV) to BKV nephritis after renal transplantation. Little is known about the mechanisms for BKV entry into human tubular epithelial cells (natural target of BKV), but it is likely that virus initially attaches to the α(2,3)-linked sialic acid of an N-linked glycoprotein of unknown nature. Expression of endothelin receptors is markedly higher in proximal tubular epithelial cells in transplanted kidney. We have established that antagonist of Endothelin Receptor B (ETRB) BQ788 prevented BKV infection of cultured human renal proximal tubular epithelial cells (HRPTEC). The percentage of infected cells and the cellular levels of BKV large T antigen expression were significantly decreased in HRPTEC treated with BQ788, but not in HRPTEC treated with BQ123 (antagonist of Endothelin Receptor A). In order to confirm that BQ788 decreases BKV entry into HRPTEC we have analyzed distribution of Alexa Fluor 488 labeled BKV particles in HRPTEC after 4 hours incubation. We observed that in control HRPTEC labeled BKV particles were located mostly in the cytoplasm, whereas in HRPTEC treated with BQ788 a significant portion of BKV particles was located on the cell surface indicating that BQ788 interferes with entry pathway of BKV. We also tested ability of PD145065, an unselective antagonist which blocks both ETRB and ETRA, for its ability to prevent infection by BKV. It appears that PD145065 was unable to mitigate infection, suggesting that inhibition of endothelin signaling is not sufficient to prevent BKV infection. Thus, BQ788 acts not through inhibition of signaling from ETRB, but because it possesses the unique ability to interfere with BKV infection likely by preventing viral particles from attachment to cellular BKV receptor. Further studies are necessary to test whether blocking ETRB could represent a simple feasible alternative strategy to treat BKV infection without any long-term negative impact to renal allograft.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2026**

**Everolimus May Reduce Need for Cytomegalovirus Infection Prophylaxis Versus Mycophenolate in *De Novo* Renal Transplant Recipients: Results from a Pooled Analysis of Three Prospective Studies** Fuad S. Shihab,[1] K. Mange.[2] *[1]University of Utah School of Medicine; [2]Novartis Pharmaceuticals Corporation.*

**Purpose**: Organ transplant recipients at risk for cytomegalovirus **(**CMV) infections typically require prolonged antiviral prophylaxis therapies that increase the economic burden in post-transplant care. Use of everolimus (EVR) with cyclosporine (CsA) in renal transplant (RTx) patients decreases the incidence of CMV, which may translate as an economic benefit of EVR vs. mycophenolate (MPA).

**Methods:** CMV data from 2004 *de novo* RTx recipients from three EVR studies A2309 (N = 833), B201 (N = 588) and B251 (N = 583) were analyzed to identify differences between EVR dosing groups and MPA control groups. In all studies, EVR groups received either 1.5 mg/day, or 3 mg/day with either standard or reduced dose cyclosporine based on individual study designs. All control groups received MPA with standard dose CsA. Steroids were given as per center practice. CMV events were reported as per local center evaluations.

**Results**: CMV prophylaxis was used in approximately 30% of all RTx recipients, primarily in those with D+R- serology. Time to first CMV event (infection/syndrome, viremia, or disease) was analyzed between two treatment groups: EVR group (EVR 1.5 and 3.0 mg combined, N=1335) vs. MPA group (EC-MPA or MMF, N=669). Mean time to first CMV event for EVR 1.5 mg, EVR 3.0 mg and MPA groups were 194, 190 and 124 days after transplant. Hazard ratio (HR) adjusted for study effect and donor/recipient serology was 2.214 for MPA vs. EVR ($p<0.0001$). In the populations who did not receive prophylaxis, adjusted HR was 2.769 for MPA vs. EVR ($p<0.0001$). An analysis of the D+R- serology subgroup who did not receive prophylaxis identified a HR of 2.167 for the MPA vs. EVR ($p<0.0179$).

**Conclusion**:This pooled analysis documents significant reduction in risk (measured as HR) for CMV events in EVR-treated *de novo* RTx recipients compared with the MPA control group. In addition, time to first CMV event is prolonged for EVR vs. MPA, and although this additional analysis is not rigorous, this delay in onset of CMV may positively impact the economic burden of post-transplant management.

Disclosure of Financial Relationships: Employer: University of UtahResearch Funding: Novartis; Honoraria: Novartis.

**F-PO2027**

**Diagnosis and Monitoring of UTIs in Renal Transplant Recipients: A Higher Than Expected Incidence of Shed Urothelial Cell Colonization by Bacteria** Holly R. Courtneidge,[1] Alberto Contreras-Sanz,[1] Rita La Rovere,[1] Rebecca Birch,[1] Chloe J. Goonewardene,[2] Chris Mark Ambrose,[2] Harry Horsley,[2] James Malone-Lee,[2] Scott S. P. Wildman,[1] Mark A. Harber.[2] *[1]Royal Veterinary College, London, United Kingdom; [2]UCL Medical School, London, United Kingdom.*

Renal transplant recipients (RTRs) are susceptible to recurrent UTIs. It is acknowledged that there is considerable insensitivity of routine urinalysis when screening for UTIs. A large number of patients with genuine urine infections go undiagnosed and develop chronic recalcitrant infections; which can be associated with morbidity and graft loss. We investigated alternative UTI detection techniques and used these to investigate the natural history of UTIs in a cohort of RTRs.

MSUs were collected from 113 RTRs and 31 controls. Microscopic pyuria counts (in 1μl) and ATP measurements (normalised to creatinine levels), were performed on fresh unspun samples and compared to conventional nitrite and leukocyte dipsticks and bacterial culture results. Cytospun urine samples were stained with acridine orange (and crystal violet) to identify intracellular bacteria (IB) in shed urothelial cells.

Of the RTRs, 22.6% were deemed to have a UTI by 'gold standard' conventional culture, whereas 7.5% and 69.8% had UTIs according to nitrite and leukocyte dispsticks, respectively. Significant pyuria (≥10 wbc in 1μl urine [1]) revealed 84.9% of RTRs had a UTI. ATP levels were found to be 6.7±3.6 nM and 4.5±0.5 nM in RTRs deemed to have a UTI (by conventional culture) and those that did not, respectively. In controls 3.2% had significant pyuria,and ATP level was 3.8±2.1 nM. IB were visualised in shed urothelial cells in 45.3% of RTRs, and 0% of controls. Only 1.8% of RTRs with IB were deemed to have a UTI by conventional culture.

It is apparent that standard bedside tests for UTIs give variable results and that quiescent bacteria in urothelial cells is more common in RTRs than previously thought. Our results suggest that measuring urinary ATP is not an alternative test for UTI detection, but significant pyuria (≥10 wbc in 1μl fresh unspun urine) may be a more sensitive test to determine UTIs and quiescent IB.

[1] Stamm WE Am J Med 75:53–58 1983

Disclosure of Financial Relationships: nothing to disclose

**F-PO2028**

**Risk Factors for Post Renal Transplant BKV Infection** Ankit Sakhuja, Kumar Sujeet, Y Ran Zhu, Brahm S. Vasudev, Ehab Saad, Barbara Bresnahan, Sundaram Hariharan. *Division of Nephrology, Medical College of Wisconsin, Milwaukee, WI.*

Introduction: BKV infection can progress to BKV nephritis leading to graft failure. This study evaluated our ability to predict BKV infection.

Methods: This prospective study was undertaken at our center after a formal IRB approval. A total of 189 renal transplant recipients from July 2007 – Sept 2009, followed till May 2010. All subjects were screened for quantitative BKV DNA (blood and urine) at 1, 3, 6,and 12 months post-tx. Detection of BKV in plasma and or urine was defined as BKV infection. Immunosuppressive therapy consisted of MMF, Tac and Pred. Recipient, Donor demographics, transplant and post transplant variables were correlated to post-transplant BKV infection. ANOVA and Chi Square tests were used for statistical analysis.

Results: A total of 65/189 (34%) had BKV infection, 48 (25%) BK viremia and viruria; and 17 (9%) BK viruria alone. The following table shows the results of univariate analysis for various risk variables to post-transplant BKV infection.

Table 1

| | BK viremia and viruria (n =48) | BK viruria alone, (n = 17) | No BKV infection, (n = 124) | P |
|---|---|---|---|---|
| Recipient: Mean Age (yrs) | 51.6 ±10.7 | 46.3 ± 11.0 | 50.7 ± 11.9 | 0.222 |
| Sex (M/F) | 34/14 | 11/6 | 73/51 | 0.252 |
| Race (W/B/O) | 31/6/10 | 14/2/1 | 70/44/10 | **0.003** |
| PRA Class I (Mean, %) | 16.8 ± 31.6 | 22.7 ±381. | 10.8 ± 24 | 0.686 |
| PRA Class I (Mean, %) | 7.3 ± 20.9 | 13.7 ± 30.9 | 8.2 ±22.8 | 0.814 |
| CIT - minutes | 930 ± 399 | 840 ± 506 | 815 ±396 | 0.401 |
| HLA AB mismatch | 2.7 ±1.2 | 1.9 ±1.4 | 2.8 ± 1.2 | 0.05 |
| HLA DR mismatch | 1.3 ±0.59 | 1.1 ± 0.66 | 1.3 ± 0.65 | 0.372 |
| Induction Ab (Thymo vs IL2 R blocker vs none) | 19/26/3 | 5/11/1 | 54/63/7 | 0.514 |

In addition, donor demographics, donor source (LD vs DD), Kidney vs Kidney/Pancreas Txs, post-transplant variables (DGF, acute rejection) did not correlate with BKV infection (data not shown).

Conclusion: The current study shows a lower prevelance of BKV infection in African Americans as opposed to Caucasians. However, there was no other overt risk factors identified to the occurrence of BKV infection.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2029**

**Impact of Steroid Avoidance on BK Virus Infection in Renal Transplantation**
Maria Marin, Saurabh K. Goel, Robert A. Sessions, Mohamed A. El-Ghoroury. *Dept of Nephrology, St John Hospital and Medical Center, Detroit, MI.*

Polyomavirus-associated nephropathy (PVAN), caused by BK virus, is an increasingly recognized cause of renal allograft dysfunction and graft loss. While the only widely accepted risk factor for PVAN is the degree of overall immunosuppression, the specific role for different immunosuppressants remains poorly characterized. We sought to determine if steroid use impacts the incidence of BK virus infection.

We retrospectively reviewed the charts of 101 consecutive renal transplant recipients screened for BK virus by plasma/urine PCR. Screening was performed at 1, 3, 6, 12 months after transplantation. BK virus infection was defined as plasma viral load of ≥ 10,000 DNA copies/ml and/or urine viral load of ≥ 10,000,000 DNA copies/ml. All patients received induction with rabbit ATG. Immunosuppression at hospital discharge included tacrolimus, mycophenolic acid ± prednisone, per institution protocol. Mean follow-up was 18.5 months. Total of 19 patients (18.8%) had evidence of BK virus infection including 12 (11.9%) with significant viremia. Renal biopsy performed (for increasing creatinine by >25%) in 7 patients with viremia showed PVAN in 6 cases.

BK viremia, viremia and/or viruria and PVAN depending on the steroid treatment (ITT population)

| | Patients on steroids (n=57) | Patients not on steroids (n=44) | p-value |
|---|---|---|---|
| Viremia n (%) | 6 (10.5%) | 6 (13.6%) | 0.632 |
| Viremia+/viruria n (%) | 12 (21.1%) | 7 (15.9%) | 0.512 |
| PVAN n (%) | 3 (5.3%) | 3 (6.8%) | 0.743 |

Viremia was defined as > 10,000 DNA copies /ml, viruria as >10,000,000 DNA copies/ml, PVAN was defined by renal allograft biopsy positive immunohistochemical staining for BK virus

eGFR was significantly lower at 18 months in patients with BK virus infection than in those without infection (41.3 vs. 57.5 ml/min; p=0.05). Two patients with PVAN progressed to ESRD. One patient needed ureteral stent placement for ureteral stenosis.

In conclusion, treatment with steroids does not appear to increase the incidence of BK virus infection in renal transplant patients. Larger, prospective studies are needed to address the specific effect of steroids on the incidence and outcome of BV virus infection.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2030**

**Circulating Endothelial Cells Are Elevated in Cytomegalovirus Infection in Transplantation**
Uta Erdbruegger,[1] Emily Dey Hazra,[2] Barbara Hertel,[2] Alexander Woywodt,[3] Torsten Kirsch,[2] Hermann G. Haller,[2] Marion Haubitz.[2] *[1]Dep. of Medicine, Div. of Nephrology, University of Virginia Health System, Charlottesville, VA; [2]Dep. of Medicine, Div. of Nephrology, Hannover Medical School, Hannover, Germany; [3]Lancashire Teaching Hospitals NHS Foundation Trust, Nephrology, Lancashire, United Kingdom.*

Cytomegalovirus (CMV) infection is a frequent complication in the early post-transplant period. CMV pp65 antigenemia testing and CMV PCR are used to diagnose and monitor disease activity. However, additional markers to better predict the clinical course are needed. Since CMV infection is associated with endothelial damage we hypothesize that circulating endothelial cells (CEC) are elevated in CMV infection and correlate with disease activity.

The study cohort consisted of 11 transplant recipients with CMV (2 bone marrow transplant recipients, 6 renal -, one liver-, one heart- and one lung transplant), 11 disease controls (DC) (renal transplant recipients without CMV, matched per age and transplant age) and 11 healthy controls (HC).

CEC were isolated with anti-CD146 driven immunomagnetic isolation and counted after staining with Ulex Europaerus lectin 1. CEC were measured once a patient was found to have active CMV disease reflected by positive CMV PCR and CMV antigenemia testing and repeated every 4 to 5 days until a negative result for CMV antigenemia and CMV PCR testing was obtained.

CEC were significantly elevated in patients with CMV (155.6/ml ±158 STD) compared to DC (33.6/ ml ±14.9, p=0.0324) and HC (10.9/ml ±6.0, p=0.0112). CEC levels between DC and HC also differed significantly (p=0.0026). 32 serial measurements of CEC were obtained in patients with active CMV. CEC correlated significantly with CMVpp65 (R 0.46, p=0.008), but not with CMV PCR (R 0.249, p=0.264) whereas CMVpp655 and CMV PCR correlated significantly (R 0.993, p=0.0001).

CEC are elevated in CMV infection after transplantation and correlate with CMV antigenemia. This might reflect endothelial damage caused by CMV. Routine and serial testing of CEC needs to be done to establish CEC as a sensitive clinical marker compared to CMV pp65 and CMV PCR which reflect only virus replication.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2031**

**Correction of Metabolic Acidosis with Potassium Citrate in Renal Transplant Recipients**
Alf Corsenca,[1] Astrid Starke,[2] Rudolf P. Wuthrich,[1] Patrice M. Ambühl.[2] *[1]Division of Nephrology, University Hospital Zurich, Zurich, Switzerland; [2]Renal Division, Stadtspital Waid Zurich, Zurich, Switzerland.*

Persisting disturbances in acid/base homeostasis may negatively impact on several metabolic pathways in renal transplant patients (RTP), specifically in muscle and bone mineral metabolism. The aim of this study was to prospectively examine the efficacy and safety of potassium citrate (K-Cit) versus potassium chloride (K-Cl) with regard to normalization of acid/base derangements in RTP with chronic metabolic acidosis.

30 RTP with chronic metabolic acidosis (serum bicarbonate < 24 mmol/L) and stable renal graft function were randomized to receive an individualised dose of either K-Cit (N=19) or K-Cl (N=11) to achieve a bicarbonate level of ≥ 24 mmol/L over 12 months of treatment.

| | Baseline | | Month 1 | | Month 6 | | Month 12 | |
|---|---|---|---|---|---|---|---|---|
| | K-Cit | K-Cl | K-Cit | K-Cl | K-Cit | K-Cl | K-Cit | K-Cl |
| Bicarbonate, mM/L | 21.3±2 | 20.4±2 | 24.9±2* | 21.8±2 | 24.1±3* | 21.6±2 | 24.3±3* | 21.4±2 |
| Potassium, mM/L | 4.2 | 4.1 | 4.7 | 4.4 | 4.7* | 4.2 | 4.4 | 4.3 |
| eGFR, ml/min | 51±13 | 60±19 | -- | -- | -- | -- | 50±11 | 56±14 |
| Citrate dosage, gr | 0 | 0 | 3.3±0.4 | 0 | 4.0±2.3 | 0 | 4.7±2.1 | 0 |
| Potassium dosage, mmol | 0 | 0 | 58±7* | 25.4±9 | 71±41* | 27.3±10 | 83±37* | 28.2±10 |

*) P < 0.05 vs. K-Cl

Serum potassium did not reach critical concentrations at any time point in either treatment group. Adverse treatment effects consisted mainly of mild gastrointestinal symptoms in both groups.

This is the first study demonstrating that metabolic acidosis can effectively and safely be normalized in patients with a renal graft. K-Cit was associated with a trend to slightly better preservation of renal graft function. In analogy to demonstrated benefits in patients with native CKD improving acid/base homeostasis may be advantageous in preserving GFR of grafted kidneys too. Further analysis of our data will focus on the impact of K-Cit on bone structure, mineral metabolism, physical performance and quality of life in RTP.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2032**

**Factors Relating Renal Resisitive Index in Renal Transplant Recepients**
Siren Sezer, Sebnem Karakan, Nurhan Ozdemir. *Nephrology, Baskent University Hospital, Ankara, Turkey.*

**Background:** An increased renal allograft renal resistive index (RRI), which represents the percentage reduction of the end-diastolic flow as compared with the peak systolic flow, is associated with long-term greft survival. We aimed to find the clinical and laboratory parameters correlated with RRI measurements.

**Method:** We included 82 patients at the first year of transplantation and followed for 5 years prospectively. We recorded parameters as demographic and clinical features, laboratory values including rutine parameters with fetuin-A and HOMA index. Patients RRI was measured with Doppler ultrasonography.

**Results:** Baseline GFR was estimated as 82.4±11.3 mL/min/1.73 m². Fifty-nine (71,9%) had low RRI and 23 patients (29.1%) had high RRI according to the accepted RRI cut off 0.70. There were positive correlations between Fetuin A (r=+0.22, p=0.04), HOMA index (r=+0.24, p=0.02), systolic blood pressure (r=+0.53, p=0.00), diastolic blood pressure (r=+0,04, p=0,00) and proteinuria (r=+0.01, p=0.03), phosphorous (r=+0.19, p=0.04), C-reactive protein (r=+0.13, p=0.02). Comparison of variable factors in patients with low and high RRI is shown in table [table]. In linear regression analysis, independent factors affecting RRI were systolic BP (β=+0,36, p=0,00), serum CRP(β=+0,21, p=0,02), albumin(β=+0,29, p=0,00) and phosphorus (β=+0,21, p=0,00). At the end of five years follow up, increased RRI was associated with poor renal outcome (19% vs 8%, p=0,00) and had lower survival (p<0.05, log-rank test).

Comparison between patient groups

| | Low RRI(<0,70) | High RRI(≥0,70) | p |
|---|---|---|---|
| Fetuin A(g/L) | 43,1±21,1 | 53,6±20,4 | 0.02 |
| HOMA index | 2,2 ±1,0 | 2,8±1,1 | 0.00 |
| Systolic blood pressure(mm/Hg) | 113,3±8,1 | 126,6±13,7 | 0.00 |
| Diastolic blood pressure(mm/Hg) | 77,6±6,6 | 86,2±9,5 | 0.00 |
| Proteinuria(mg/d) | 282,2±167,7 | 668,7±364,7 | 0.01 |
| Albumin (g/d) | 3,7±0,3 | 3,9±0,2 | 0.02 |
| C-reactive protein (mg/l) | 2,1 ±0,7 | 2,6±1,2 | 0.01 |

none

**Conclusion:** RRI is a valuable tool for evaluating intrarenal damage, target organ damage and subclinical atherosclerosis. RRI should be monitored and it should be included in the routine investigations of renal allograft recipients during follow-up period.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2033**

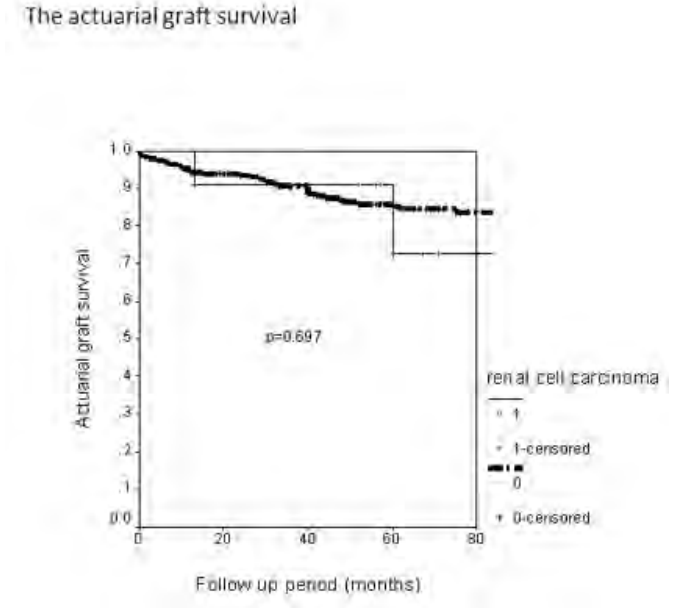
**Accidental Discovery of Renal Cell Carcinoma in Patients with ESRD at Time of Renal Transplantation: Impact on Transplant Outcome**
Hussein M. A. Sheashaa, Helmut G. Rennke, Helen Mah, Edgar L. Milford, Anil Chandraker, Abdelaziz A. Elsanjak. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Background: Renal tumors are common in the pre-transplant ESRD population. Their impact on transplant outcome has not been well addressed.

Methods: Our study is a retrospective follow-up observational study conducted in 258 renal transplant recipients. All of them had an ipsilateral native nephrectomy at the time of transplantation. We reviewed the histopathology of their native nephrectomies to measure

the prevalence of renal cell carcinoma (RCC) and to address the impact of accidental discovery of RCC on graft and patient outcome.

Results: RCC was found in 12 cases (4.7%); clear type in 9 cases, chromphobe and combined clear and papillary type in 1 and 2 cases respectively. There was no significant difference in HLA mismatch, primary immunosuppression, occurrence of rejection, graft function and patient and graft survival between patients with or without RCC (Figure 1). RCC presented in the other native kidney in 3 cases (25%) post-transplantation and one of them developed metastasis 4 years after renal transplantation in RCC group in comparison to 8 cases in the control group (3.3%) one of which developed metastasis 7 years after renal transplantation ($p<0.001$). The median follow up was period was 56 months for RCC group and 65 months for the control group.




Conclusions: We found that renal transplant outcome is not adversely affected by the presence of accidently discovered RCC at the time of transplantation. Patients with an accidentally discovered RCC at time of renal transplantation appear to be at significantly higher risk of the occurrence of RCC in the remaining native kidney. Further studies are warranted to confirm our results.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2034**

**Urinary Free Light Chains in Patients with Kidney Transplant Rejection** Min Li, Kanwaljit K. Chouhan, Kristine E. Gullo, Altaf-M. Khan, Nazih L. Nakhoul, Eric E. Simon, Rubin Zhang, Vecihi Batuman. *Section of Nephrology and Hypertension, Department of Medicine, Tulane University School of Medicine, New Orleans, LA.*

Urinary levels of polyclonal *kappa* and *lambda* free light chains (FLC) were evaluated as a potential novel biomarker for kidney transplant rejection. FLC were measured with a nephelometric immunoassay and correlations with kidney function were sought in 103 patients. Urinary FLC- to -creatinine ratios were compared with other low-molecular weight urinary biomarker proteins, including retinol-binding protein (RBP), β2-microglobulin (β2M), cystatin C (CSC), and immunoassayed urinary microalbumin to creatinine ratio (UACR). As controls, urinary FLC levels were obtained from healthy individuals. In kidney transplant rejection, patients with a biopsy diagnosis of acute tubular necrosis (ATN), chronic allograft nephropathy (CAN) and acute rejection (AR) exhibited highly increased urinary κFLC and λFLC and as well as urinary total (κ+λ) FLC/creatinine and FLC/CSC ratios. UACR, β2M and RBP fluctuated widely in subjects with ATN, CAN and AR. β2M and RBP showed no significant difference among groups compared to normal individuals. Significant correlations were noted with urinary FLC/creatinine and kidney function (serum creatinine and estimated glomerular filtration rate) in the ATN, CAN and AR groups. However, the urinary FLC/CSC ratio did not correlate with kidney function. In acute kidney rejection, urinary total (κ+λ) FLC/creatinine was markedly increased and correlated highly with UACR. Among all potential biomarkers examined for AR, FLC yielded the best predictive value at 87.8%, with 61% sensitivity and 78.3% specificity, RBP was the second best predictor (78.1% positive predictive value, 58.2% sensitivity and 66.7% specificity) and UACR was the third, while β2M and CSC were not significantly correlated with AR. These results suggest that monitoring the levels of urinary FLC would be useful for predicting early allograft rejection.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2035**

**Influence of Different HAART Regimen on Interstitial Fibrosis and Tubular Atrophy in HIV Kidney Transplant Recipients** Lissa B. Levin, Gregory Malat, Blair Weikert, Alden Michael Doyle, Karthik M. Ranganna. *Medicine, Drexel University College of Medicine, Philadelphia, PA.*

Transplantation in HIV+ recipients is safe and effective, but a high degree of awareness of complex pharmacology is required. Cyclosporine, sirolimus and prednisone is our maintenence immunosuppression regimen in HIV recipients. There is known to be interaction between the protease inhibitors (PI) and the differential metabolism of immunosuppressants, which may potentiate the calcineurin-inhibitor nephrotoxic effect. Protease inhibitors are known to be potent inhibitors of CYP3A4. Cyclosporine and sirolimus are CYP3A4 substrates, administration of these immunosuppressants with an inhibitor like any of the aforementioned protease-inhibitors decreases the dosage requirement of the immunosuppressants and increases the potency and possible toxicity of a comparable immunosuppressant dose.

We hypothesized that the the biopsies of the patients on PI therapy would show more severe IFTA (interstitial fibrosis and tubular atrophy) scores than patients on non-nucleoside reverse transcriptase inhibitor based regimen (NNRTI). At our institution, we have transplanted 92 HIV-positive patients since 2001. Of those patients, 50 were on PI-based regimens and 29 patients were on NNRTI-based regimens. We excluded those patients on combination of PI and NNRTI, or on neither a PI or NNRTI. Surveillance and for-cause allograft biopsies were graded using the Banff classification schema for interstitial fibrosis (ci) and tubular atrophy (ct), with scores ranging from 0-3 for both parameters. The sum of ci and ct scores provided the basis for analysis between the two groups.Analysis of the biopsy data from the two groups showed no difference between the average scores of subjects on PI vs. and those on NNRTI-based regimens. One year IFTA scores within the PI-based group averaged 1.8 vs. average scores within the NNRTI group of 2.1 ($p=0.592$). Three year IFTA scores within the PI-based group averaged 2.3 vs. average scores within the NNRTI group of 2.6 ($p=0.39$). Our analysis indicates that NNRTI-based and PI-based regimens are similar with respect to chronic allograft injury.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2036**

**ImmuKnow™ (Cylex) Assay Results in the First Year Post-Transplant and Relationship to BK Virus Infection and Acute Rejection** Jane Gralla, Janna L. Huskey, Alexander C. Wiseman. *University of Colorado.*

**Background:** The ImmuKnow Assay is a functional T cell assay (TCA) that may quantify cellular immune responsiveness following renal transplantation. Using a standard protocol of TCA sampling in the first year post-transplant (tx), we hypothesized an association between TCA and BK virus infection (BKV) and acute rejection (AR) events.

**Methods:** We performed a single-center retrospective analysis of 897 TCA results in 414 renal tx recipients obtained at 0 (N=122), 1 (N=316), 6 (N=258), and 12 (N=201) months post-tx from 2005-2009 with concurrent urine and blood BKV PCR measurements.

**Results:** Mean TCA values were similar 0 to 1 month (419 ng/ml vs. 441 ng/ml, $p=0.32$), decreased 1 to 6 months (466 ng/ml vs. 356 ng/ml, $p<0.0001$), and remained stable 6 to 12 months post-tx (357 ng/ml vs. 370 ng/ml, $p=0.33$) "Low" TCA values (≤225 ng/ml) at 12 months were associated with a diagnosis of BKV reactivation (urine BKV PCR $>10^8$ copies/ml or detectable BKV viremia by blood PCR)($p=0.006$), but not at 1 or 6 months. Reductions in TCA of 100, 150 or 200 ng/ml over time were not predictive of BKV. "High" values of TCA (≥525 ng/ml) were not predictive of AR.

Table 1: BKV diagnosed at time of protocol test and corresponding TCA value

| TCA value | N | BKV+ | BKV- | p-value |
|---|---|---|---|---|
| 1 month TCA≤ 225 ng/ml | 29 | 1 (3%) | 28 (97%) | 0.99 |
| 1 month TCA >225 ng/ml | 287 | 13 (5%) | 274 (95%) | |
| 6 month TCA ≤ 225 ng/ml | 55 | 2 (4%) | 53 (96%) | 0.26 |
| 6 month TCA >225 ng/ml | 190 | 18 (9%) | 172 (91%) | |
| 12 month TCA ≤ 225 ng/ml | 37 | 6 (16%) | 31 (84%) | 0.006 |
| 12 month TCA >225 ng/ml | 142 | 4 (3%) | 138 (97%) | |

**Conclusions:** In this large single-center series, 1) TCA values were shown to significantly decrease from 1-6 months but not thereafter, 2) changes in TCA were not predictive of BKV reactivation, 3) low absolute TCA values (≤ 225 ng/ml) at 12 months were more commonly noted in patients with BKV reactivation. These findings suggest that TCA monitoring is not warranted prior to 6 months post-transplant, but patients with low TCA values at 6 months may benefit from potential tailoring of immunosuppression or more aggressive monitoring for infections between 6 and 12 months post-transplant.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2037**

**Intense C4D Staining and Presence of Donor Specific Antibody Is Associated with Higher Proteinuria in HIV Positive and HIV Negative Renal Transplant Recipients** Aniruddha V. Palya, Parmish Lalit Kohli, Gregory Malat, Simi Shahabdeen, William Yang, Blair Weikert, Alden Michael Doyle, Karthik M. Ranganna. *Medicine, Drexel University College of Medicine, Philadlphia, PA.*

HIV positive kidney transplant recipients have lower long term renal allograft survival compared with HIV- recipients of allograft. C4D positivity on biopsy has been found to be a known prognostic indicator for long term allograft outcome.

Our study analyzed the degree of proteinuria in relation to C4d deposition in renal biopsy specimens and donor specific antibodies in plasma (DSA) in HIV+ and HIV- recipients. 27 HIVpositive and 167 HIV negative renal transplant recipients transplanted between March 2005 and September 2008 were included.

C4d was positive in 100 biopsy specimens. Proteinuria data was obtained on all patients at the time of renal biopsy. HIV positive recipients had a higher incidence of C4D positivity. Increasing proteinuria was noted in both HIV positive as well as HIV negative renal transplant recipients (p-trend 0.01) with increasing intensity of C4D staining. HIV positive renal transplant recipients had a higher proteinuria when compared with HIV negative recipients across different C4D intensities (p value 0.01).

Presence of DSA was associated with significantly higher proteinuria (p value <0.001). Higher proteinuria was noted in HIV positive renal transplant recipients with DSA. Both C4D positivity and DSA remained significant in a multivariate analysis after adjusting for known confounders.

From our study we found that increasing intensity of C4D staining and donor specific antibodies appear to be associated with increasing proteinuria in both HIV positive and negative renal transplant recipients.

TABLE: Mean urine protein to creatinine ratio across HIV Positive and HIV Negative renal Transplant recipients

| | HIV positive(N=27) | HIV negative(N=167) | Unadjusted p-value | Adjusted p-value* |
|---|---|---|---|---|
| Recipients with C4D1 | 1.2 | 0.8 | 0.07 | 0.01 |
| Recipients with C4D2 | 1.9 | 1.7 | | |
| Recipients with C4D3 | 2.4 | 2.1 | | |
| DSA positive | 1.7 | 1.3 | <0.01 | <0.001 |

* Included in the final model: Hepatitis C, Graft or patient survival, Immunosuppression

Disclosure of Financial Relationships: nothing to disclose

## F-PO2038

**An Increase in Body Mass Index Is a Risk Factor for Persistent New Onset Diabetes after Transplantation in Renal Transplant Recipients** Yoonjung Kim, Junam Shin, Hye Ryoun Jang, Jung Eun Lee, Yoon-Goo Kim, Dae Joong Kim, Ha Young Oh, Wooseong Huh. *Nephrology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.*

The association of an increase in BMI with the development of type 2 DM is well known , but it has not been clear in renal transplant recipients. This study investigated the relationship between an increase in BMI after kidney transplantation(KT) and the development of persistent new onset diabetes after transplantation (P-NODAT) in recipients treated with tacrolimus. Study design was retrospective case-matched control(1:3) study. Thirty-four patients developing P-NODAT were identified among 186 adult renal transplant recipients with no evidence of pre-transplant DM, who underwent KT from September 1997 to March 2008 and were treated with a triple regimen including tacrolimus. The controls were selected by pre-transplant BMI, age at transplant (±5 years) and date of transplantation(±12 months). Finally, 21 P-NODAT patients and 63 controls were enrolled. The pre- and post-transplant BMI data were collected every 16 weeks until 80 weeks. Between the two groups, the proportion of deceased donor was higher in P-NODAT patients (38.1%, vs.15.9%, $p$=0.02). There were no significant differences in sex, family history of DM, pre-transplant BMI, HCV infection, donor age, acute rejection rate, CMV infection, and tacrolimus levels. Pre-transplant BMI was 23.7±3.0 Kg/m$^2$ in P-NODAT group and 23.2±2.2 Kg/m$^2$ in the control group. BMI was decreased to 22.5±3.3 Kg/m$^2$ in P-NODAT group and to 22.0±2.2 Kg/m$^2$ in control group 16 weeks after KT. And then, in P-NODAT group, BMI was rapidly recovered to 23.4±3.3 Kg/m$^2$ at 32 weeks. However, in control group, BMI was recovered to 22.9±2.1Kg/m$^2$ at 80 weeks (ANOVA for repeated measures $p$=0.03 for between groups analysis). Logistic regression analysis revealed a significant association between weight gain from 16 weeks to 32 weeks and development of P-NODAT. The odds ratio was adjusted for donor type. Adjusted odds ratio was 1.37 (95% CI, 1.10-1.72) for every 1 kg increase in body weight from 16 weeks to 32 weeks (P=0.006). The increase in BMI after KT was related to a higher incidence of P-NODAT.

Disclosure of Financial Relationships: nothing to disclose

## F-PO2039

**Severe Vascular Lesions and Poor Functional Outcome in Kidney Transplant Recipients with Lupus Anticoagulant Antibodies** Guillaume Canaud,[1] Bienaime Frank,[3] Laure-Helene Noel,[2] Dany Anglicheau,[1] Eric Thervet,[1] Christophe M. Legendre,[1] Julien Zuber.[1] *[1]Service de Transplantation, Hôpital Necker, Paris, France; [2]Laboratoire d'Anatomopathologie, Hôpital Necker, Paris, France; [3]Service d'Explorations Fonctionelles, Hôpital Necker, Paris, France.*

Antiphospholipid syndrome (APS), the leading cause of acquired thrombophilia, has been associated with severe vascular changes in native kidney. However, the impact of anti-phospholipid antibodies (APA) on clinical outcome and graft histology following renal transplantation remains poorly known and controversial. Therefore, we retrospectively explored, using a nested case-control study, the functional and histological significance of APA, primarily lupus anticoagulant, in kidney transplant recipients, systematically evaluated with 3- and 12-month post-transplant screening biopsies and measured glomerular filtration rate (mGFR).

Among 1359 kidney transplant recipients during the study period, 37 had APA (2.7%), primarily lupus anticoagulant, and 12 fulfilled APS diagnostic criteria (0.8%) at the time of transplantation. During the early post-transplant course, 4 of the 12 APS patients died. Early thrombosis of graft vessels and deep venous thrombosis occurred more frequently in APA+ patients than in controls (27 vs 7%, p<0.05 and 35 vs 14%, p<0.05, respectively). The patient survival rate was significantly lower in patient with APS than in the controls. Strikingly, hallmark lesions of APS-associated nephropathy (APSN) were found in most of screening graft biopsies in APA+ patients but not in the controls. Accordingly, APA+ patients had a dramatic increase of chronic vascular cv and ah scores and a faster decline in mGFR at one year. In conclusion, renal transplantation may be life-threatening in APS patients, and the presence of lupus anticoagulant at the time of transplantation is associated with a high rate of allograft APSN and a poor transplantation outcome.

Disclosure of Financial Relationships: nothing to disclose

## F-PO2040

**Malignancies Confined to Disused Brachiocephalic Arteriovenous Fistulae in Renal Transplant Patients: A Rare but Important Differential** Philip Webster, Lareina Wujanto, David Taube, Neill D. Duncan. *Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom.*

**Background**

Swelling in an arteriovenous fistula (AVF) is commonly due to thrombosis, aneurysm and infection. However due to the significantly increased risk of malignancy after transplantation, this should also be considered. We present 4 patients, all of whom had renal transplants at our centre, who developed malignant change confined to their AVF.

| Patient | Diagnosis | Immunosuppression | Treatment | Outcome |
|---|---|---|---|---|
| 1. Male, 59yrs, Caucasian | Angiosarcoma | Mycophenolate mofetil (MMF)/steroids for underlying glomerulonephritis. Rituximab/plasma exchange/ daclizumab/steroids/ tacrolimus for ABO incompatible transplant. | Arm amputation | Alive 2 years after presentation, no metastasis |
| 2. Female, 41yrs, Afro-Carribean | Angiosarcoma | Alemtuzumab/steroids/ tacrolimus for transplant | Nil | Died 4 months after presentation with lung metastases |
| 3. Male, 44yrs, Caucasian | T-cell Lymphoma | Steroids/tacrolimus for transplant. MMF/steroids for rejection | Reduction of immunosuppression | Alive 3 months after presentation, under close monitoring |
| 4. Male, 56yrs, Caucasian | Diffuse Large B-cell Lymphoma with Epstein-Barr virus | Steroids/tacrolimus/MMF for transplant | Reduction of immunosuppression. Rituximab | Alive 3 months after presentation undergoing treatment |

**Discussion**

Predilection for malignancy at the site of an AVF is not understood. Turbulent blood flow within a fistula leads to endothelial shear stress causing up-regulation of growth peptides, heightening proliferative responses. Angiosarcoma behaves aggressively with a poor outcome. There are 6 similar cases previously reported. All were initially thought to have thrombosed, aneurysmal fistulae. 5 died within 16 months of diagnosis. The other had lung metastases at time of publication. Surgical resection of a non-metastasized lesion offers the only chance of cure at present. There is no previous documentation of post-transplant lymphoproliferative disease confined to AVFs.

**Conclusion**

Whilst rare, malignancy should be considered when a new swelling occurs at a haemodialysis fistula in immunosuppressed patients. Further research into pathogenesis is required.

Disclosure of Financial Relationships: nothing to disclose

## F-PO2041

**Hypercalcemia after Kidney Transplantation in Patients with ou without Cinacalcet before Transplantation** Amélie Belloi,[1] Emmanuel Villar,[1] Marie-Christine Carlier,[2] Anne Charrie,[3] Rémi Cahen,[1] Claire Pouteil-Noble.[1] *[1]Néphrologie, CHLS, Pierre-Bénite, France; [2]Biochimie, CHLS, Pierre-Bénite, France; [3]Radioanalyse, CHLS, Pierre-Bénite, France.*

Use of phosphate binders or calcimimetics in dialysis have modified phosphate and calcium balance after transplantation. The aim of this prospective study was to compare the evolution of phosphate and calcium balance after transplantation and parathyroidectomy incidence between D0 and M1, M3, M6, M12 in patients with or without cinacalcet on the day of transplantation. All the patients transplanted between 01.01.00 and 12.31.08 with a functioning graft more than 1 month were included(n = 338). Serum phosphate and calcium balance (PTH, Ca, P and 25OHD), graft function, calcium and vitamin treatment, parathyroidectomy history were collected until M12 and compared between 2 groups : without (group 1, n = 310) and with (group 2, n = 28) cinacalcet. After transplantation, mean calcemia in group 2 (between 2.53± 0.21mmol/L and 2.57±0.12mmol/L) was significantly increased at M1, M3, M6, M12 in comparison with group 1(between 2.37±0.20mmol/L and 2.45±0.17mmol/L) (p<0.0005) while on D0, calcemia was no different. Mean PTH was significantly higher in group 2 than in group 1 at M3, M6 and M12 (p<0.05). Graft function was not different in the 2 groups. The rate of hypercalcemia was significantly more common in group 2 than in group 1 at M1(36% vs9%), M3(39% vs15%), M6(32% vs15%), M12(32% vs10%) (p<0.05). Cinacalcet treatment was required in 25% of patients of group 2 and only 0.65% of patients of group 1. Parathyroidectomy was performed in 7% of patients of group 2 vs 0.65% of patients of group 1. During the first year of transplantation, hypercalcemia is more common in patients with cinacalcet treatment before transplantation and may require cinacalcet treatment or parathyroidectomy. Cinacalcet represents a suspensive treatment of hyperparathyroidism in dialyzed patients. Side effects of cinacalcet (hypercalciuria,

nephrocalcinosis, bone mineralization) are not well known on the long term kidney graft function. The cost-benefit of cinacalcet treatment after transplantation must be evaluated in comparison with parathyroidectomy.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2042**

**Impact of Polymorphisms of the Renin-Angiotensin-Aldosterone System on Renal Transplantation** Magdalena Siekierka-Harreis, Christos Bantis, Christina Schwandt, Lars C. Rump, Katrin Ivens. *Department of Nephrology, Heinrich-Heine University, Düsseldorf, Germany.*

In the present study we evaluated the impact of the three major polymorphisms of the renin-angiotensin-system on long-term outcome after renal transplantation.

We studied n=205 consecutive patients, who underwent renal transplantation in our center from 1998 to 2001 (cadaveric: n=161, living related: n=44), followed up for 5.0±2.0 years. One hundred healthy volunteers were analyzed as controls. Aldosterone synthase gene C-344T, angiotensinogen gene M235T and angiotensin converting enzyme gene I/D polymorphisms were determined by PCR.

The genotype distribution was similar in patients and control subjects (ns). During follow up n=103 (50.2%) patients had at least one episode of acute rejection and n=28 (13.7%) experienced graft failure. No association between the polymorphisms and the incidence of acute rejection was detected (ns). Patients carrying the aldosterone synthase CT/TT genotypes experienced graft failure more frequently (16.1%) compared to the CC genotype (4.5%, $x^2$: 4.0, OR: 1.22, 95%CI: 1.07-1.39, $p<0.05$). An increased frequency of graft loss was also observed among the patients carrying the angiotensinogen TT genotype (27.8% vs. 10.7% in MM/TT, $x^2$: 7.4, OR: 2.43, 95%CI: 1.32-4.48, p=0.007). Combined analysis of the polymorphisms allowed a more precise identification of patients predisposed to graft loss: patients with the genotype combination of aldosterone synthase CT/TT and angiotensinogen TT had an almost three times higher risk for graft loss (31.3% vs. 10.4% in the other genotype constellations, $x^2$=10.0, OR=2.87, 95% CI: 1.53-5.41, p=0.002). The Kaplan Meier analysis confirmed the impact of C-344T ($p<0.05$) and M235T (p=0.005) polymorphisms as well as their combined analysis ($p<0.001$) on graft survival. This effect was prominent after the first two years after renal transplantation. No association between angiotensin converting enzyme gene I/D polymorphism and any of the parameters studied was observed.

Our results suggest that aldosterone synthase gene C-344T and angiotensinogen gene M235T polymorphisms influence the long-term graft survival in renal transplantation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2043**

**Copeptin in Heart Transplant Recipients and Hemodialyzed Patients Is Dependent on Kidney Function and Heart Structure, but Not in Kidney Transplant Recipients** Jolanta Malyszko,[1] Jacek S. Malyszko,[1] Piotr Przybylowski,[2] Ewa Koc-Zorawska,[1] Michal Mysliwiec.[1] *[1]Nephrology Department, Medical University, Bialystok, Poland; [2]Cardiac Surgery and Transplantation, Jagiellonian University, Cracow, Poland.*

Introduction: Copeptin is cosynthesized with vasopressin, thereby directly mirroring vasopressin levels – but copeptin is more stable in plasma and serum. Chronic heart failure-CHF is present in more than one-third of incident dialysis patients as well as in kidney allograft recipients. The aim of the study was to assess copeptin in kidney allograft recipients kidney, orthotopic heart recipients and hemodialyzed in relation to NYHA class and kidney function.

The studies were performed on 136 prevalent patients after orthotopic heart transplantation- OHT, 100 prevalent kidney allograft recipients and 100 HD patients. Plasma copeptin was measured using a commercially available kit.

Results: Copeptin correlated with parameters of kidney function: creatinine, eGFR by MDRD, eGFR by CKD-EPI, cystatin C (r=0.46, $p<0.001$), and HDL, NT-proBNP (r=0.25, $p<0.01$), IVS (intraventricular septal diameter) (r=-0.30, $p<0.01$), EF, ferritin. In multiple regression analysis predictors of copeptin were cystatin C and IVS explaining 51% of copeptin variations. In kidney allograft recipients copeptin was significantly lower than in orthotopic heart transplants. Lower NYHA classes were more prevalent in kidney allograft recipients. Copeptin was higher in CKD stage 4 when compared to stage 2, similarly it was higher in NYHA class III than I. However, all the correlation did not reach statistical significance. Copeptin correlated with age, residual renal function, HD dose, heparin dose, Kt/V, pH, TSAT, ferritin, presence of CAD, hsCRP, IL-6, NYHA. In multiple regression analysis NYHA class, Kt/V and IL-6 predicted copeptin levels in HD.

Copeptin in heart transplant population is independently associated with kidney function and heart structure but not in kidney allograft recipients. Copeptin in HD population is independently associated cardiac function, but additionally may be associated with dialysis adequacy and chronic inflammation.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2044**

**Association of Aldosterone Synthase Gene Polymorphisms and Histopathology of Calcineurin-Inhibitor Toxicity in Renal Transplant Patients** Patricia C. Ruiz Palacios,[1] Monica X. Inofuentes,[1] Rafael G. Toledo,[1] Francisco E. Rodríguez Castellanos,[1] Eduardo Mancilla Urrea,[3] Maria Carmen Avila-Casado.[2] *[1]Nephrology, Instituto Nacional de Cardiologia Ignacio Chavez, Mexico, DF, Mexico; [2]Nephropathology, Instituto Nacional de Cardiologia Ignacio Chavez, Mexico, DF, Mexico; [3]Renal Transplant, Instituto Nacional de Cardiologia Ignacio Chavez, Mexico, DF, Mexico.*

INTRODUCTION. Recent observations suggest that aldosterone plays a central role in the pathogenesis of Calcineurin Inhibitors (CI) nephrotoxicity. The aim of this work was to test the association of polymorphisms in the aldosterone synthase (AS) gene with the histopathology of CI toxicity in renal transplant patients.

METHODS. A renal biopsy and blood sample was taken from 50 male and 33 female patients treated with CI, to determine histophatological findings secondary to CI nephrotoxicity and their polymorphisms in the AS gene. The -344T/C, Int 2W/C polymorphisms were screened by modification of standard PCR-RFLP. Renal biopsies were analyzed by light microscopy.

RESULTS. The patients with nephrotoxicity (group A) had higher systolic and diastolic blood pressure than patients without nephrotoxicity (group B): 140±26.1 mmHg vs 124±18.6 (p= 0.011), and 87±13.7 vs 77±12.6 (p=0.003), respectively. The uric acid tended to be higher in group A than group B (8.2±2.3 vs 6.7±1.9 mg/dL, p=0.008). CI levels were higher in the group A than group B (114 vs 54 ng/ml, p = 0.02). Development of arterial hypertension occurred in 71% of patients in group A and 53% of patients in group B (p=NS). There was a trend of higher levels of aldosterone in patients with genotype CC of the polymorphism Int 2W/C and the genotype TC of the polymorphism -344 T/C. There was no significant difference in the genotypes between group A and group B. Also, there was no difference in the grade of interstitial fibrosis between groups.

CONCLUSION. This study proves that there is no association between polymorphisms of AS gene and histopathological findings of CI toxicity in patients with renal transplant. The potential involvement of the aldosterone in the development of nephrotocixity due to CI may not be dependent of the specific polymorphisms of the AS gene.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2045**

**New Manifestation of Henoch-Schönlein Purpura Following Renal Transplantation Due to IgA Nephropathy** Ulf Schoenermarck, Simon Rau, Michael Fischereder. *Medical Clinic I, Nephrology Div., University Hospital Munich-Grosshadern, Munich, Germany.*

Henoch–Schönlein purpura (HSP) is a rare systemic small vessel vasculitis, typically presenting with palpable purpura, arthralgia, abdominal pain and renal disease. Renal histology shows a similar picture as in IgA nephropathy, the most common glomerulonephritis in adults. Recurrence of IgA nephropathy or HSP after renal transplantation is frequent. Here, we report on a patient with first manifestation of HSP following a renal transplantation due to IgA nephropathy.

We report the case of a 69-year-old male who underwent cadaveric renal transplantation 21 months prior to admission due to a bioptically proven IgA nephropathy. The immunosuppressive regimen consisted of tacrolimus, mycophenolate mofetile and steroids. Steroids were tapered and finally stopped completely a few months after transplantation. Baseline creatinine was ~1.6 mg/dl.

The patient was admitted with palpable purpura of all limbs, skin ulcerations and diffuse arthralgias. Serum creatinine was 2.1 mg/dl with mild proteinuria (0.3 g/l) and hematuria (50/µl). The skin biopsy showed a classical leukocytoclastic vasculitis with deposition of complement C3, IgA and fibrinogen which is pathognomonic of HSP. Steroid therapy was reintroduced (starting with 100 mg/d prednisolone p.o.). Skin lesions and kidney function rapidly improved. The patient was discharged from hospital after 13 days with a serum creatinine of 1.4 mg/dl. On a follow-up examination 4 weeks after discharge, serum creatinine was 1.5 mg/dl and the skin lesions were hardly visible. Hematuria or proteinuria was no longer detectable.

Recurrence of preexisting IgA nephropathy or HSP is frequent after renal transplantation. In contrast, HSP with cutaneous leukocytoclastic vasculitis following a renal transplantation due to IgA nephropathy has not been described yet. In this case remission of HSP could be rapidly achieved by steroid therapy. The withdrawl of steroids after renal transplantation due to isolated IgA nephropathy might have favoured the occurence of HSP in this patient.

Disclosure of Financial Relationships: Other Relationship: Travel grant from Astellas.

**F-PO2046**

**Predictors of Serum Hepcidin Levels in Renal Transplant Patients** Stephan Brincat,[1] Chris Jones,[1] Jolanta Malyszko,[2] Iain C. Macdougall.[1] *[1]Department of Renal Medicine, King's College Hospital, London, United Kingdom; [2]Department of Nephrology and Transplantology, Medical University, Bialystok, Poland.*

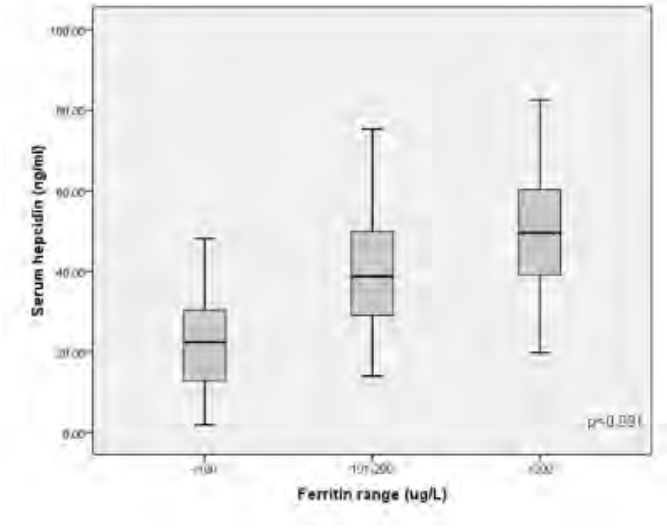
Introduction: Hepcidin, a 25-amino acid peptide, is a key regulator of iron metabolism. It is modulated in response to anaemia, inflammation, and hypoxia. Post-transplant anaemia is common with a multifactorial aetiology.

Aim: To identify predictors of serum hepcidin levels in renal transplant patients.

Methods: Using a commercially available ELISA, serum levels of hepcidin were measured in a cohort of stable renal transplant patients and compared to markers of anaemia, inflammation and iron status. Variables were log transformed to satisfy normality assumptions.

Results: In 96 transplant patients (aged 23-86 years, 63 male, 62 Caucasian, 14 diabetic, mean eGFR=53ml/min/1.73m$^2$), serum hepcidin levels were higher than healthy controls (36.8+/-19.4 v 20.6+/-10.0ng/ml; p<0.001). 49% of patients were anaemic (WHO classification). Higher hepcidin levels were observed in anaemic patients, compared to non-anaemic patients (43.9+/-16.9 v 29.1+/-19.2ng/ml; p<0.001). Hepcidin levels were positively correlated with ferritin (r=0.62, p<0.01) and interleukin-6 (r=0.265, p=0.02) and negatively correlated to haemoglobin (r=-0.44, p<0.01) and eGFR (r=-0.27, p<0.01). Mean hepcidin levels showed a stepwise increase with 3 different ferritin ranges (p=<0.001) [Figure 1]. Using multiple linear regression ($R^2$=0.5), the only independent predictors for hepcidin were ferritin (p<0.001), haemoglobin (p=<0.005) and raised C-reactive protein (p<0.02).

Conclusions: Serum hepcidin levels are independently predicted by ferritin, haemoglobin, and raised C-reactive protein, but not kidney function in renal transplant patients. Further studies are required to characterise the role of serum hepcidin in post-transplant anaemia.



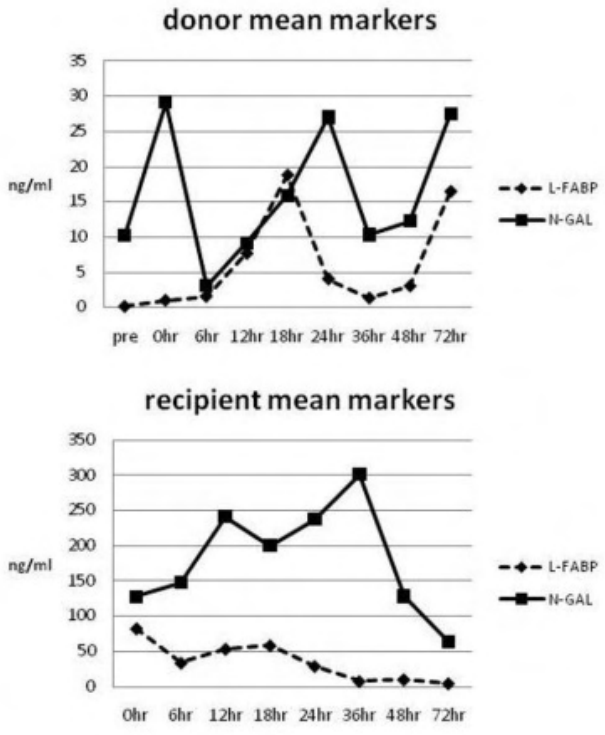

Disclosure of Financial Relationships: nothing to disclose

**F-PO2047**

**Trend Differences between Two Urinary Biomarkers in Living Donated Kidney Transplant Donors and Recipients during the Perioperative Period**

Junko Kohei, Kosaku Nitta, Ken Tsuchiya, Takumi Yoshida, Shunji Shiohira, Hidekazu Sugiura. *Nephrology, Tokyo Womens Medical University, Shinjuku, Tokyo, Japan.*

Urinary liver-type fatty acid binding protein (L-FABP) and neutrophil gelatinase-associated lipocalin (N-GAL) variations have previously been associated with acute kidney injury in perioperative period, however the trend of these variations post renal surgery has not been studied in depth. This study's objective is to examine whether there are differences in the variations between the two markers during the perioperative period in both donor's and recipient's urine pre, post and during living-donor transplantation. We randomly selected 10 donors and their recipients undergoing kidney transplantation to use as test samples for a prospective investigation. Urinary samples were collected immediately before surgery and 0, 6, 12, 18, 24, 36, 48, and 72 hours post operation. L-FABP and N-GAL from these test samples were measured with a standard commercial ELISA kit. In the results that followed from recipients, within a three day post operative period, there were no cases demonstrating delayed graft function and in every case serum creatinine levels fell below 2.0mg/dl. Mean donor L-FABP and N-GAL were different between the peak time. Furthermore 72hour after surgery both markers had risen. On the other hand, mean recipient markers were also different between the peak time, their score was gradually decreased form 36hours after surgery.




N-GAL drastically changed after donor and recipient surgery and elevated early postoperative period with some peak; it tended to reflect the surgical kidney injury rather L-FABP. N-GAL will not be a reliable marker alone to help identifying acute kidney injury or delayed graft function.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2048**

**Influence of Different HAART Regimen on the Incidence of C4D Deposition and Donor Specific Antibodies in HIV Positive Renal Transplant Recipients**

Aniruddha V. Palya, Blair Weikert, Gregory Malat, Suganthi Soundararajan, Nauman Shahid, Alden Michael Doyle, Karthik M. Ranganna. *Medicine, Drexel University College of Medicine, Philadelphia, PA.*

It is increasingly being noted that HIV+ renal transplant recipients tend to have more rejection. We have been seeing increased antibody mediated rejection in them. C4D deposition in the biopsy and donor specific antibodies are features of antibody mediated rejection. HIV recipients have previously been reported to have a significantly higher incidence of C4D positivity and DSA than non-HIV recipients. We decided to look whether there is any difference in their incidence when the recipients are on different HAART regimen. Induction agent we use is basiliximab and the mainetenence immunosuppression include cyclosporine, sirolimus and prednisone. There was no difference between the two groups as far as demographics were concerned.

We studied 71 biopsies which included surveillance and indication biopsies and panel reactive antibody titers performed between March 2005 and September 2008 on 27 HIV positive renal transplant recipients. 21 out of 71 biopsies (29%) were indicated biopsies.

Degree of C4D positivity in biopsies and incidence of DSA

| | PI based (n=9) | NNRTI based (n= 14) | Combination (N = 4) | P value |
|---|---|---|---|---|
| Recipients with C4D negative | 8 | 2 | 2 | |
| Recipients with C4D 1 | 3 | 1 | 2 | 0.17 |
| Recipients with C4D 2 | 0 | 1 | 0 | |
| Recipients with C4D 3 | 3 | 5 | 0 | |
| DSA positive | 4 | 7 | 0 | 0.013 |
| DSA negative | 10 | 2 | 4 | |
| Serum Creatinine | 3.1±2.2 | 2.2±1.3 | 2.7 ± 1.6 | 0.49 |
| Urine Protein/Creatinine ratio | 1.34 | 1.07 | 0.88 | 0.82 |
| Days since transplant | 482 | 717 | | |
| Functioning graft | 6 | 12 | 3 | 0.64 |

non-nucleoside reverse transcriptase inhibitor based regimen (NNRTI); protease inhibitor based regimen (PI).Donor specific antibody (DSA)

We found that there was no significant difference in the incidence of C4D deposition across the different HAART regimen. We also found no significant difference with respect to serum creatinine and graft survival. However, NNRTI based HAART regimen appeared to be associated with higher incidence of DSA.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2049**

**Incidence of BK Viremia and BK Nephropathy in Renal Transplant Patients Induced with Alemtuzumab or Antithymocyte Globulin: Single Center Experience** Farnaz Mohammadi, Abdul Moiz, Nadia W. Iqbal, Krista L. Lentine, Bahar Bastani. *Division of Nephrology, Saint Louis University School of Medicine, Saint Louis, MO.*

**OBJECTIVES:** To evaluate the incidence of BK viremia (BKV), BK nephropathy (BKVN), renal transplant outcomes and predictors for developing BKVN.

**METHODS:** We retrospectively analyzed 306 consecutive renal transplant patients (pts) from 2006 to 2010. Pts had received either alemtuzumab (30mg, one dose) or antithymocyte globulin (ATG; 1.5mg/kg, 5 doses) or basiliximab as induction therapy. Maintenance regimen comprised of tacrolimus and mycophenolate-mofetil (MMF). Prednisone was used only in ATG and basiliximab groups. Serum BK PCR was serially monitored post-transplant. Reduction in immunosuppression was carried upon diagnosis of BKV. Leflunomide replaced MMF in cases with concomitant rejection and BK infection.

**RESULTS:** Mean (±SD) age of pts at BKV was 52±12 yrs, of whom 78% were male. For induction 236(77.1%) pts had received alemtuzumab, 68(22.2%) pts ATG and 2 basiliximab. Total number of BKV pts was 40 (13.1%). Time from transplant to serum BK PCR positivity was 9±9 months. BKV lasted for a mean of 5.3±5 months. Out of 40 pts with BKV, 27(67.5%) had received alemtuzumab and 12(30%) ATG. BKVN was noted in 5 pts; concomitant acute rejection was present in 2 pts. Mean tacrolimus trough level was 9.2±3.1ng/ml at the time of BKV. Tacrolimus dose was reduced in 19(41.3%) pts. MMF dose was decreased in 38(96%) pts and it was discontinued in remaining 4%. Biopsy proven acute rejection during follow-up was seen in 17(42.5%) pts; leflunomide replaced MMF in 14 of them. Four pts lost allograft (10% of those with BKV). In 5(12.5%) pts BKV lasted for more than 6 months. There was no statistically significant difference in the incidence of BKV in alemtuzumab vs ATG (11.4% vs 17.6%, P=0.14).

**CONCLUSION:** Early diagnosis of BKV based on serial monitoring of serum BK PCR and pre-emptive reduction of immunosuppression resulted in resolution of BKV and reduced progression to BKVN. Substitution of MMF with leflunomide in patients with concomitant BKV and rejection was associated with progressive decrease in viral load and stabilization of allograft function.

Disclosure of Financial Relationships: nothing to disclose

**F-PO2050**

**An Adjuvanted Influenza A H1N1 Vaccine Does Not Provide a Protective Immune Response in the Majority of Renal Transplant Recipients** Susanne Brakemeier, Petra Glander, Fritz Diekmann, Hans-Hellmut Neumayer, Klemens Budde. *Nephrology, Charite, Berlin, Germany.*

In the course of the Influenza A H1N1 pandemic especially immunocompromised patients were recommended to receive vaccination. In healthy controls, between 80% and 95% of adults develop a sufficient immune response after a single vaccination, however no data are available for immunosuppressed patients so far, therefore we evaluated the immune response to an adjuvanted Influenza A H1N1 vaccine (Pandemrix®) in renal transplant patients.

A total of 60 patients (12 female/48 male) aged 52 ± 14 years who received a transplant at least six months ago agreed to participate in the study. 22 healthy subjects served as controls.

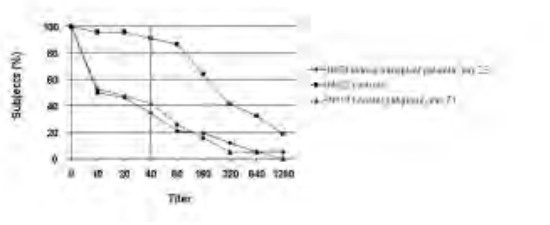
A single dose of Pandemrix® (3,75µg per dose, adjuvanted) was administered at day 0. In a subgroup of 19 patients, booster vaccination was performed after a median of 21 days and booster titers were analysed at a median of 71 days after the second vaccination.

Two patients had an elevated titer before vaccination, although being completely asymptomatic.

Of the remaining 58 patients, after a median of 25,8 days only 34,5% developed a titer of 1:40 or more, which is considered as a sufficient immune response. The other 38 patients (65,5%) showed no or only a weak response. In contrast to this 91% of the control group developed a protective titer of 1:40 or more.

In the booster subgroup, a titer of 1:40 or more was present in 42% after the second vaccination.

Figure 1




Immunosuppressive regimens consisted of mycophenolate in combination with either tacrolimus, cyclosporine, everolimus, or sirolimus, with a median through level of 5.5 ng/ml, 92.4 ng/ml, 6.9 ng/ml, and 7,4 ng/ml, respectively. 50% of patients were off steroids.

These data suggest that in renal transplant patients a single dose of Pandemrix® as well as booster vaccination is not sufficient to induce a protective immune response.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2051**

**Fool's Gold: Creatinine as the Diagnostic Standard for AKI Biomarker Studies** Sushrut S. Waikar,[1] Rebecca A. Betensky,[2] Joseph V. Bonventre.[1] *[1]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Biostatistics, Harvard School of Public Health, Boston, MA.*

Serum creatinine concentration (SCr) has been used as the diagnostic test for acute kidney injury (AKI) for decades, despite being an imperfect gold standard due to limited sensitivity and specificity. Novel tubular injury biomarkers may revolutionize the diagnosis of AKI and eventually replace SCr in the evaluation of patients at risk for AKI. However, if novel biomarkers are compared against SCr as the gold standard, then errors in SCr's diagnostic performance may adversely affect the apparent sensitivity and specificity of the biomarkers. The apparent sensitivity and specificity of a biomarker depend not only on the biomarker's test characteristics but also on disease prevalence, sensitivity and specificity of the gold standard, and the conditional dependence between the tests. Assuming conditional independence (i.e., the gold standard and the biomarker are independent given the disease status), the apparent sensitivity and specificity of a biomarker can be calculated for different estimates of disease prevalence and gold standard performance characteristics. The following table shows how varying estimates of disease prevalence and SCr's sensitivity and specificity affect the apparent sensitivity and specificity of a novel biomarker that is assumed to be perfectly accurate for the diagnosis of AKI.

Apparent performance of a perfect biomarker compared to an imperfect gold standard

| Gold standard | Prevalence | Apparent Sn | Apparent Sp |
|---|---|---|---|
| | 0.1 | 31% | 97% |
| SCr rise of 0.3 mg/dL (Sn 80%, Sp 80%) | 0.2 | 50% | 94% |
| | 0.3 | 63% | 90% |
| | | | |
| | 0.1 | 100% | 91% |
| Need for dialysis (Sn 10%, Sp 100%) | 0.2 | 100% | 82% |
| | 0.3 | 100% | 72% |

Sn, sensitivity; Sp, specificity

Misclassification of AKI may lead to substantial misinterpretations of the diagnostic ability of novel biomarkers. Biomarkers that perform best relative to SCr may in fact be conditionally dependent and recapitulate the shortcomings of SCr. Our findings suggest that small changes in SCr should not be used to define AKI in biomarker or interventional AKI studies.

Disclosure of Financial Relationships: Consultancy: Data safety monitoring board for Takeda; expert witness testimony in product liability litigation for GE Healthcare and Salix PharmaceuticalsResearch Funding: NxStage, Pfizer, Satellite Healthcare; Honoraria: Otsuka.

**SA-PO2052**

**Pattern of Change in Serum Creatinine Predicts Need for Dialysis in Acute Kidney Injury (AKI)** Yang Luo,[1] Etienne Macedo,[1] Josee Bouchard,[1] Sharon Soroko,[1] Glenn M. Chertow,[2] Jonathan Himmelfarb,[3] T. Alp Ikizler,[4] Emil P. Paganini,[5] Ravindra L. Mehta.[1] *[1]University of California San Diego; [2]Stanford University School of Medicine; [3]University of Washington; [4]Vanderbilt University School of Medicine; [5]Cleveland Clinic Foundation.*

*Background:* The course of AKI is characterized by the rate, magnitude and duration of serum creatinine (Scr) change. These changes are affected by comorbidities, and nature and timing of acute insults. We hypothesized that pattern of Scr increase is associated with need for RRT. *Methods:* Using data from 351 out of 618 PICARD study (KI, 2004, 66: 1613-1621) patients whose Scr (corrected for fluid balance) increased consecutively for 3/+ days, we determined the pattern of AKI during the development phase before dialysis. The slope, Scr delta and area under curve (AUC) of Scr were calculated for the 1st 3 days after ICU admission and at peak value (Max). The predictive ability of these variables was assessed with a ROC analysis and cut-off points established. Patients were assigned to categories based on the cut points to compute odds ratios. *Results:* Daily slope and delta Scr of the 1st 3 days were similar in the 2 groups. Dialyzed patients had higher peak Scr (delta max Scr p=0.04). Increases in AUC in the 1st 3 days and at peak were predictive of need for RRT.

Table

| | Dialyzed(n=141) | Non-dialyzed(n=210) | ROC curve | Cut-Off value | OR (95%CI) |
|---|---|---|---|---|---|
| AUCmedian (IQR) (mg*day/dl) | | | | | |
| 1st day | 3.9(2.7-5.7) | 3.5(2.7-4.9) | 0.84 | 5.9 | 2.0(1.1-3.7) |
| 2nd day | 7.1(5.0-9.7) * | 5.9(4.7-8.1) | 0.81 | 5.9 | 2.1(1.4-3.3) |
| 3rd day | 11.0(8.1-14.3) * | 9.0(7.2-11.6) | 0.79 | 11.3 | 2.3(1.5-3.6) |
| to Max | 19.6 (13.0-37.4) * | 15.4 (11.5-25.9) | 0.81 | 27.8 | 1.9(1.2-2.9) |
| delta Max median (IQR )(mg/dl) | 2.7 (1.6-4.2) * | 2.1 (1.2-3.2) | 0.56 | 2.8 | 1.7(1.1-2.6) |

*P<0.05 compared with non-dialyzed group

*Conclusion.* Patterns of serum Scr change can identify patients who will need RRT. AUC Scr relative to other parameters may facilitate optimal timing of dialysis in AKI patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2053**

**Glomerular Filtration Rate, Proteinuria, and the Incidence and Outcomes of Acute Kidney Injury** Matthew T. James,[1,2] Brenda Hemmelgarn,[1,2] Natasha Wiebe,[3] Neesh I. Pannu,[3] Braden J. Manns,[1,2] Scott Klarenbach,[3] Marcello Tonelli.[3] *[1]Medicine, University of Calgary, Canada; [2]Community Health Sciences, University of Calgary, Canada; [3]Medicine, University of Alberta, Canada.*

Lower levels of estimated glomerular filtration rate (eGFR) predispose to acute kidney injury (AKI), and modify the risks of death and kidney failure following AKI. Proteinuria is also a marker of kidney disease but whether it is associated with the risk and subsequent outcomes of AKI is unknown. We performed a cohort study of 920,985 adults residing in Alberta, Canada between 2002 and 2007 to examine how proteinuria modified the associations between eGFR and AKI, as well as the risks of adverse clinical outcomes following AKI. Participants not requiring chronic dialysis at baseline and with at least one outpatient measurement of both serum creatinine and proteinuria (using urine dipstick or albumin-creatinine ratio) were included. We assessed hospitalization with AKI using validated administrative codes. Over median follow-up of 35 months (range, 0-59 months), 6,520 (0.7%) participants were hospitalized with AKI. Among those with eGFR ≥ 60 mL/min/1.73m$^2$, the adjusted rate of hospitalization with AKI was 4.4-fold higher among those with heavy proteinuria by dipstick (rate ratio 4.4 vs no proteinuria, 95% CI 3.7-5.2). The adjusted rates of hospitalization with AKI remained up to 3.7 fold higher in participants with heavier proteinuria across lower levels of baseline eGFR. Similar findings were observed when urine albumin-creatinine ratio was used to assess proteinuria, or when AKI was defined by receipt of dialysis. Further, compared to participants without proteinuria, the adjusted rates of death and end-stage renal disease or doubling of serum creatinine following AKI were up to 1.6-fold and over 3-fold higher, respectively, in participants with heavy proteinuria but similar baseline eGFR. At all levels of eGFR, people with proteinuria are at markedly higher risk of developing AKI and of death or advanced kidney disease after experiencing AKI. These findings suggest that proteinuria, in addition to baseline eGFR, should be incorporated into systems for assessing the risk or outcomes of AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2054**

**Accuracy of Morning Urine Protein-to-Creatinine Ratio in Acute Kidney Injury** Jonathan J. Taliercio, Susana Arrigain, Jesse D. Schold, James F. Simon. *Department of Nephrology and Hypertension, Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, OH.*

The purpose of the study was to assess the accuracy of proteinuria quantification using a morning urine protein-to-creatinine (PC) ratio compared to 24-hour urine collection in hospitalized patients with non-oliguric acute kidney injury (AKI).

The 24-hour urine collection is the gold standard for quantifying protein excretion. The first morning urine PC ratio has been validated as an alternative to the 24-hour collection in stable kidney function. While the PC ratio has not been validated in AKI, practitioners are using it in this setting.

This was a prospective cohort study of adult subjects who had non-oliguric AKI (≥2-fold rise in serum creatinine and urine output ≥400 cc/day) and ≥ +1 proteinuria admitted to the Cleveland Clinic main hospital. Exclusion criteria included CKD stage ≥ 4, initiation of hemodialysis, and AKI recovery before consent. Morning urine samples were obtained before noon the day of enrollment or the following morning. Lin's concordance coefficient was used to analyze the linear association and deviation from the 45 degree line between 24-hour protein and the PC ratio.

175 subjects were screened; 35 subjects met inclusion criteria and were enrolled in the study. The median and interquartile range for the 24-hr protein and the PC ratio were 0.47 (0.26, 1.33) and 0.89 (0.33, 2.07) respectively. Only 4 patients had >2 grams of proteinuria. Lin's concordance coefficient was 0.85 (95% CI 0.74-0.91) using all raw data, and a more conservative 0.72 (0.52-0.85) using log transformed values and excluding 3 influential data points. The PC ratio tended to overestimate the 24-hour collection, with the difference increasing with greater proteinuria.

The morning urine protein-to-creatinine ratio is strongly associated with 24-hour urine protein collections in patients with non-oliguric AKI and <2 gram proteinuria. The accuracy is poor with a bias toward over-estimation of proteinuria, potentially limiting its utility in patients with heavier proteinuria. The 24-hour urine collection should remain the standard practice for proteinuria quantification in AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2055**

**Pre-Operative Proteinuria and Adverse Renal Outcomes in Patients Receiving Coronary Artery Bypass Grafting Surgery** Tao-Min Huang, Vincent Wu, Guang-Huar Young, Fan-Chi Chang, Pi-Ru Tsai, Wen-Je Ko, Kwan-Dun Wu. *Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan.*

Background: Proteinuria has been recognized as an independent risk for adverse outcomes in patients with chronic kidney disease (CKD), However, the effect of preoperative proteinuria on renal outcome after cardiac surgery has never been evaluated. We hypothesized that preoperative proteinuria (detected by dipstick test) is predictive of cardiac surgery associated acute kidney injury (CSA-AKI).

Methods:Data was obtained from a teaching hospital and its two affiliate hospitals. From 2003 to 2007, all adult patients undergoing coronary artery bypass grafting surgery

(CABG) were included. Patients with stage 5 CKD or previous dialysis were excluded. Proteinuria was defined as mild (trace to 1+) or heavy (2+ to 4+) according to dipstick tests. Multiple stepwise logistic regressions were used to assess the factors associated with CSA-AKI (defined by Acute Kidney Injury Network criteria) and postoperative renal replacement therapy (RRT).

Results:A total of 1052 patients were enrolled with a mean age of 65.7±11.0 years; 75.7% were male. CSA-AKI developed in 183 (17.4%) patients, of which 50 (4.8%) underwent RRT. Mild proteinuria (OR = 1.576, p = 0.043) and heavy proteinuria (OR = 2.041, p = 0.0110) were associated with CSA-AKI, independent of CKD stages and the presence of diabetic mellitus.

Factors associated with post-operative acute kidney injury

| Variables | OR (95%CI) | p |
|---|---|---|
| Age | 1.04 (1.02-1.06) | <0.001 |
| DM | 2.22 (1.49-3.31) | <0.001 |
| Recent MI | 1.84 (1.19-2.83) | 0.006 |
| IABP | 6.17 (3.49-10.90) | <0.001 |
| Proteinuria | | |
| No proteinuria | 1 | - |
| Mild | 1.58 (1.02-2.45) | 0.043 |
| Heavey | 2.04 (1.18-3.54) | 0.011 |
| CKD stage | | |
| <3 | 1 | - |
| 3 | 1.65 (1.08 - 2.52) | 0.021 |
| 4 | 2.61 (1.31 - 5.19) | <0.001 |

Heavy proteinuria was also associated with postoperative RRT (OR = 3.440, p = 0.0036), independent of serum creatinine.

Conclusion:This study demonstrates that proteinuria, detected with urine dipstick, is an important predictive factor of mild and severe CSA-AKI in patients undergoing cardiac surgery.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2056

**Plasma NGAL for Diagnosis of Established AKI** Dinna N. Cruz,[1] Sergio Gaiao,[1,2] Francesco Garzotto,[1] Massimo De Cal,[1] Claudio Ronco.[1] *[1]S Bortolo Hosp, Vicenza, Italy; [2]Sao Joao Hosp, Porto, Portugal.*

The conceptual model of AKI is a rapid worsening of kidney function from pre-morbid levels. Therefore ideally a baseline serum creatinine (sCr) should be known; unfortunately this is not always possible. Diagnosis of AKI present on ICU admission is frequently done retrospectively, when old records are obtained or when a significant decrease in sCr from the admission value is seen in subsequent days. In some studies renal SOFA (rSOFA) score has been used to define AKI using a single sCr value. NGAL has been shown to be an early marker of AKI; however, our aim was to use it for discrimination of established AKI on ICU admission.

**METHODS**

We selected 90 ICU patients who had AKI on ICU admission and 168 ICU patients who never had AKI. Diagnosis was based on RIFLE, using a baseline sCr retrospectively obtained from hospital records. Plasma NGAL and sCr were measured on the first ICU day; rSOFA was calculated based on admission sCr. Patients were considered rSOFA(+) if rSOFA>1 and NGAL(+) if NGAL>150ng/ml. We then constructed a "panel" based on sequential approach using both rSOFA and NGAL. Patients were considered panel (+) if (rSOFA>1) or (rSOFA≤1 but NGAL>150ng/ml); and panel (-) if both rSOFA(-) and NGAL(-). We compared the diagnostic test accuracy of the 3 methods for AKI diagnosis.

**RESULTS**

Median sCr on admission were 1.7 (IQR 1.2,2.6) mg/dl and 0.9 (IQR 0.8,1.1) mg/dl in AKI and nonAKI groups, respectively. The diagnostic test accuracy of each method is reported in Table 1.

Table 1: Diagnostic test accuracy (with 95%CI)

| | rSOFA | NGAL | rSOFA-NGAL panel |
|---|---|---|---|
| SENSITIVITY (%) | 38.9 (29.0-49.8) | 68.9 (58.1-78.0) | 71.1 (60.5-79.9) |
| SPECIFICITY (%) | 96.4 (92.0-98.5) | 80.4 (73.4-85.9) | 80.4 (73.4-85.9) |
| PPV (%) | 85.4 (70.1-93.9) | 65.3 (54.7-74.5) | 65.9 (55.6-75.1) |
| NPV (%) | 74.7 (68.2-80.2) | 82.8 (76.8-88.1) | 83.9 (77.0-89.0) |

**CONCLUSION**

rSOFA alone has high specificity but low sensitivity for AKI on ICU admission. When NGAL is used together with it, the panel has acceptable specificity but higher sensitivity. When the baseline sCr is not readily available, NGAL may provide additional information to the physician and help in recognizing established AKI.

Disclosure of Financial Relationships: Honoraria: Speaker Honoraria for Biosite/ Inverness Medical.

## SA-PO2057

**Neutrophil Gelatinase Associated Lipocalin (NGAL) as a Biomarker of Acute Kidney Injury (AKI) after Cardiac Surgery in Adults** Rafael Carlos Miranda, Thayza Lopes, Emmanuel A. Burdmann, Emerson Quintino Lima. *Nephrology Division, Hospital de Base - Sao Jose do Rio Preto Medical School, Sao Jose do Rio Preto, Sao Paulo, Brazil.*

**Introduction:** Acute kidney injury following cardiac surgery is associated with high morbidity and mortality. The aim of this study was to evaluate the utlity of urinary NGAL excretion as an early biomarker for AKI after cardiopulmonary bypass (CPB) in adults. **Methods:** Patients submitted to elective coronary artery bypass or valve surgery requiring CPB were enrolled. Patients with ongoing AKI, baseline GFR < 30 ml/min (MDRD equation) or renal transplant were excluded. AKI was defined as an increase of 50% or at least 0.3mg/dL in baseline serum creatinine level within 4 days after surgery (AKIN criteria). Urinary NGAL was measured by an ELISA kit (BioPorto, Denmark) before surgery and 2, 4, 6, 12, 24, 48, 72 and 96 hours after CPB. Preoperative, intraoperative and postoperative data were evaluated by unpaired t-test, Mann Whitney and Fisher's exact tests. The performance of urinary NGAL as a biomarker for AKI was evaluated by ROC curve. P<0.05 was considered as significant. **Results:** 103 patients, 58 males, 55 ± 14 years, were submitted to 75 coronary artery bypass and 28 valve surgeries. AKI developed in 28 (27%) patients (19 males, 58 ± 12 years, preoperative serum creatinine 1.2 ± 0.3 mg/dL). There was no difference regarding age, gender, preoperative serum creatinine, EuroSCORE, surgical, anesthesia and CPB times and hospital length of stay between AKI and non AKI patients. Mortality (32% vs 2.7%, P<0.001) was higher in AKI patients. The median NGAL after 4 h (73 ng/ml [8-398] vs 11 ng/ml [(2-63]; P=0.008), 6 h (39 ng/ml (3-253) vs 9 ng/ml [3-27]; P= 0.009) and 12h (19 ng/ml [6-50] vs 6 ng/ml [3-30]; P=0.02) after CPB were higher in AKI group and preceded the rise in serum creatinine in at least 12h. The ROC curve area for urinary NGAL 4 h, 6 h and 12 h after CPB were 0.66 (95% CI 0.54-0.79; P=0.008), 0.66 (95% CI 0.53-0.79; P=0.009) and 0.64 (95% CI 0.52-0.76;P=0.02). **Conclusions:** These results suggest that NGAL might be a useful tool for the early identification of cardiac surgery patients who will develop AKI.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2058

**Performance of Plasma Neutrophil Gelatinase-Associated Lipocalin (pNGAL) as Marker of Acute Kidney Injury. A Prospective Study in Emergency Department** Karina Soto,[1] Silvia Coelho,[1] Ana Luisa Papoila,[2] Qing Ma,[3] Bruno Rodrigues,[1] Pedro Fidalgo,[1] Michael R. Bennett,[3] Prasad Devarajan.[3] *[1]Nephrology, Hospital Fernando Fonseca, Lisbon, Portugal; [2]Biostatistic, Faculdade de Ciências Médicas, Lisbon, Portugal; [3]Nephrology, Cincinnati Children's Hospital, Cincinnati, OH.*

**Background**: The search for early and accurate biomarkers of acute kidney injury (AKI) is currently a top priority, particularly in the emergency department (ED), where an immediate approach is crucial. In a prospective cohort study we evaluated the discriminative and predictive ability of pNGAL for AKI diagnosis.

**Methods**: Patients (>18y - <80y and CKD<stage IV) admitted to the ED of Fernando Fonseca Hospital, were classified according to clinical criteria. pNGAL was measured at 0, 6, 12, 24 and 48 hours from admission (Triage® NGAL Device; Biosite). Diagnosis of AKI was based on RIFLE and AKIN criteria. To determine the predictive and discriminative power of pNGAL, the area under the receiver operating characteristic curve (AUC) and predictiveness curves were used. Cut-off values were determined through Generalized Additive Models.

**Results**: Patients (n=616) mean age of 59.1y, were adjudicated to have AKI (21.1%), pre-renal azotemia (preR- 25.8%), stable chronic kidney disease (CKD-2.4%) and normal function (NF- 50.7%). The highest levels of pNGAL were shown in the AKI group, differentiating AKI patients from preR and NF (p<0.001). The discriminative ability of pNGAL was good, reaching AUCs from 0.77 to 0.80 (CI 0.77 to 0.84). Increasing levels of pNGAL were associated with increasing risk of AKI and with higher grades of AKIN and RIFLE classifications, showing better performances with AUCs 0.88; 0.86; 0.89; 0.87; and 0.85 at different study times (AKIN>2 grade). At a cut-off of 100 ng/mL, pNGAL showed specificity and sensitivity values of 70.2 and 78.3%, respectively at 12h of admission.

**Conclusions**: pNGAL is a useful biomarker for diagnosis of AKI in a heterogeneous ED population, with good discriminative ability, reliably differentiating AKI from preR azotemia. pNGAL is also an excellent marker of AKI severity.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2059

**Plasma Neutrophil-Gelatinase-Associated Lipocalin (pNGAL) for the Prediction Risk of Acute Kidney Injury, in the Presence of Comorbidities. *A Prospective Study*** Silvia Coelho,[1] Qing Ma,[2] Pedro Fidalgo,[1] Bruno Rodrigues,[1] Michael R. Bennett,[2] Ana Luisa Papoila,[3] Prasad Devarajan,[2] Karina Soto.[1] *[1]Nephrology, Hospital Fernando Fonseca, Lisbon, Portugal; [2]Nephrology, Cincinnati Children's Hospital, Cincinnati, OH, Portugal; [3]Biostatistics, Faculdade de Ciências Médicas, Lisbon, Portugal.*

**Background:** In the emergency department (ED) a prompt diagnosis and appropriate treatment for acute kidney injury (AKI) could improve patient's outcome. A multivariable (clinical and biomarkers) approach could tremendously improve diagnosis accuracy.

The purpose of this study was to evaluate the pNGAL performance associated with clinical risk factors of AKI in a heterogeneous ED population.

**Methods**: Patients (>18y - <80y; CKD stage <IV) admitted to the ED were included. AKI was defined according to the RIFLE and AKIN criteria. pNGAL was measured (by Triage® NGAL Device; Biosite) at 0, 6, 12, 24 and 48h from admission. Significant AKI risk factors were selected and a multifactorial analysis was developed associating with pNGAL levels.

**Results**: 616 patients (mean age 59.1 y) were classified by clinical criteria as AKI (21.1%), pre-renal azotemia (PreR-25.8%), stable CKD (2.4%) or normal kidney function (NF- 50.7%), and were assigned as I to IV stages of susceptibility (on the basis of baseline kidney function): 66.9%, 21.5%, 4.9%, and 6.6%, respectively. Most stages III-IV corresponded to AKI patients and median values of pNGAL increased with grade of susceptibility (p<0.001).

The risk of developing AKI was associated with increasing stages of susceptibility, cardiovascular disease (CVD), and older age (≥75 y) and pNGAL discriminatory performance was better in presence of these variables. Multiple logistic regression model

at T12 showed that patients with pNGAL>100 ng/mL had 7-fold higher risk of AKI (OR 7.4), with an OR=2.7 for CVD; an OR=4.1 for >III susceptibility; and OR=1.8 for older age. (Hosmer-Lemeshow p-value=0.616; AUC 0.83).

**Conclusions**: The presence of elevated pNGAL levels in ED is associated with heightened risk of developing AKI, even more so in patients with risk factors such as CVD, previous renal dysfunction and older age.

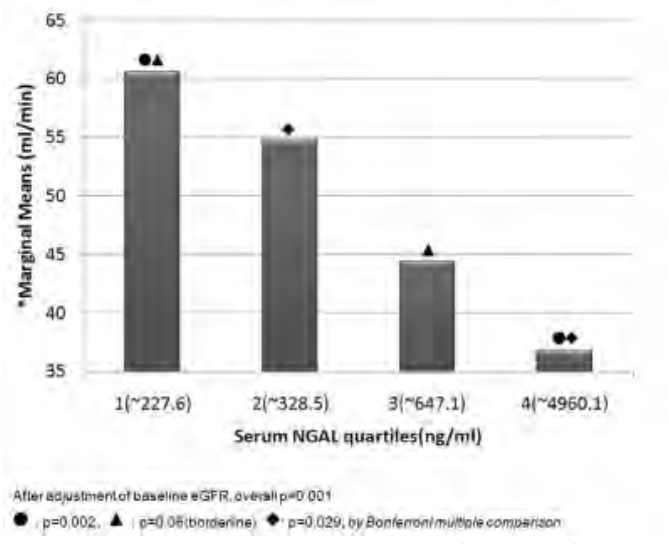
Disclosure of Financial Relationships: nothing to disclose

### SA-PO2060

**Serum Neutrophil Gelatinase-Associated Lipocalin Predicts Long-Term Renal Outcomes in Patients with Acute Kidney Injury** Kichul Yoon, Eunjung Cho, Sang-Kyung Jo, Won-Yong Cho, Hyoung-Kyu Kim. *Division of Nephrology, Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea.*

**Background :** There has been insufficient research on long-term renal prognosis of AKI patients. Objectives of this study were to determine long-term renal outcome in patients with AKI and to find factors that can predict long-term renal function. **Methods:** Out of 100 hospitalized AKI patients who were enrolled in this prospective, observational, cohort study, 55 patients who had a mean follow-up of 506 days were included in the analysis. The serum and urine neutrophil gelatinase-associated lipocalin (NGAL) levels were measured at the time of AKI diagnosis. Patients were divided into 'worsened' or 'preserved' renal function group at 6 month by criteria of 25% reduction in eGFR from baseline. Variables associated with the groups and the factors that might predict eGFR at 6 months and 1 year were also determined. **Results:** Thirty two percent of the patients fell into the 'worsened' group at 6 months. Baseline eGFR, initial serum & urine NGAL and initial presence of proteinuria were associated with 'worsened' renal function. Baseline eGFR was the strongest predictor in predicting eGFR at 6 months, followed by initial serum NGAL. Serum NGAL was found to be a useful predictor of 6 month eGFR.

Figure 1. Estimated marginal means of 6 month eGFR outcome(ml/min/1.73m$^2$) for serum NGAL in quartiles




After adjustment of baseline eGFR, overall p=0.001

● : p=0.002, ▲ : p=0.06(borderline), ◆ : p=0.029, by Bonferroni multiple comparison

The serum NGAL level was also a good diagnostic marker for prediction of the 6-month eGFR <30ml/min/1.73m$^2$, based on area under the receiver operating characteristic curve analysis. **Conclusion:** Significant proportion of this cohort who suffered AKI episode had worsened long-term renal function after AKI. Initial serum NGAL level might be a useful independent biomarker for predicting long-term renal function after AKI.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2061

**Serum Neutrophil Gelastinase-Associated Lipocalin (NGAL) and Cystatin C (CyC) Are Early Biomarkers of Contrast Induced Nephropathy (CIN) in CKD Patients after Coronary Catheterization** Norella C. T. Kong. *Medicine, Hospital Universiti Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia.*

Purpose: We investigated whether serum NGAL and CyC are early predictive biomarkers of CIN in CKD patients undergoing coronary catheterization.

Methods: CIN was defined as a rise in serum creatinine ≥25% above baseline levels in the absence of other aetiology. Serum creatinine levels were measured (and eGFR, MDRD, calculated) at baseline, 24 hrs and 48 hrs post-procedure. Blood for serum CyC were collected at baseline and 24hrs and for serum NGAL pre-, at 4 hrs post- and at 24 hrs post-procedure.

Results: CIN was diagnosed in 11/100 (11%) patients and one patient required dialysis. We found a significant rise in serum NGAL ≥25% from baseline at 24 hrs in 7 of these CIN patients(Z=-1.97, p = 0.04*) but NGAL did not change in the other four. Serum CyC at 24hrs was increased ≥25% from baseline in 4 of CIN patients (4/11) (Z=-2.76, p=0.008) - these patients also had a rise in serum NGAL. Serum CyC did not change in the other 7 of the 11 CIN patients. Serum NGAL also rose ≥25% in 12 of the 89 non-CIN patients and one subsequently developed CIN. This patient also had a rise in serum CyC level. The remaining patients were discharged at 48 hrs. This subgroup could well have had 'incipient CIN'. Receiver operating characteristic curve (ROC) showed that both NGAL and CyC could diagnose CIN 24hrs earlier than serum creatinine. The areas under the ROC curves for serum CyC and for serum NGAL between 0-24 hrs were 80.7% (p =0.001**; 95% CI= 0.68– 0.93) and 85.1% (p <0.001**; 95% CI=0.75– 0.952) respectively. An increase in serum NGAL ≥ 25% from baseline at 24 hrs post-procedure and advanced age were independent predictors for the development of CIN (OR=1.012, p=0.005**), (OR =1.188, p=0.019*) respectively.

Conclusions: Both serum NGAL and serum CyC were earlier predictive biomarkers for CIN than serum creatinine and the use of either would enhance CIN diagnosis for this at-risk population.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2062

**Serum Neutrophil Gelastinase-Associated Lipocalin (NGAL) as an Early Marker of Contrast Induced Nephropathy (CIN) in CKD Patients after Coronary Catheterization** Norella C. T. Kong.[Hospital Unive] *[1]Medicine, Hospital Universiti Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia; [2]Inst#1; [3]Inst#1; [4]Inst#1; [5]Inst#1.*

Purpose: To investigate whether serum NGAL is an early predictive biomarker of CIN compared with serum creatinine in CKD patients who undergo coronary catheterization.

Methods:

This prospective study involved patients with CKD Stages 2-4 who underwent coronary catheterization. CIN was defined as an increase of ≥25% in the baseline serum creatinine within 48 hours (hrs) of exposure to contrast medium in the absence of an alternative aetiology. All patients received IV N saline and oral N acetylcysteine prophylaxis. Serum creatinine levels were measured at baseline, 24 hrs and 48 hrs post- procedure. Blood samples for serum NGAL were collected pre-, at 4 hrs and at 24 hrs post- procedure.

Results:

100 patients completed the study. The frequency of CIN was 11% and one patient required dialysis. In patients with CIN, serum creatinine started to rise at 24 hrs but achieved significance only at 48 hrs (Z=0.155, p =0.006). Serum NGAL increased ≥25% from baseline at 24 hrs in 7 of the CIN patients (7/11) (Z = -1.97, p = 0.04) but did not change in the other 4 (4/11). However, serum NGAL also rose ≥25% in 12 of the 89 non-CIN patients, one of whom subsequently developed CIN but the rest were discharged at 48 hrs. This subgroup may have had 'incipient CIN'. At both baseline and at 24 hrs, serum NGAL correlated with serum creatinine (r1=0.42, p1 <0.005; r2 =0.423, p2 <0.001) and eGFR (r1 = -0.356, p1 <0.005; r2 = -0.399, p2 <0.001 respectively). It was also highly associated with CKD stages at both time points (p=0.01 and <0.001 respectively).Using ROC curves, the area under the curve was 85.1% (p <0.001; 95% CI = 0.75– 0.952)giving a sensitivity and specificity for serum NGAL of 81.0% and 75.3% respectively. Risk factors for CIN in CKD patients included age, female gender, Indian ethnicity, diabetes mellitus and anaemia. Age alone was the independent risk predictor (p=0.026).

Conclusions: Serum NGAL was an early predictive biomarker for CIN in the said patient population.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2063

**Increased IL-6 and NGAL with Transaminitis after Laparoscopic Donor Nephrectomy** Steven C. Yap, Sang Won Park, H. Thomas Lee. *Anesthesiology, Columbia University College of Physicians and Surgeons, New York, NY.*

Patients with AKI frequently sustain extra-renal organ dysfunction. We showed previously that murine models of AKI led to increased IL-6 and TNF-α that directly contributed to hepatic dysfunction. Despite the large number of nephrectomies performed in the United States, the impact of these findings in humans is unclear. In this study, we examined whether patients undergoing laparoscopic donor nephrectomy show increased postoperative IL-6 and TNF-α levels with injury to the liver and whether the remaining kidney sustains injury. Serial serum and urine samples were collected from 25 laparoscopic donor nephrectomy patients and 13 control patients subjected to non-renal laparoscopic surgery. IL-6, TNF-α and NGAL were quantified by ELISA. Serum was also analyzed for creatinine (Cr), AST and ALT. Our results show that patients after nephrectomy not only show increased Cr, but also have increased serum IL-6, AST (Figure 1) and ALT (not shown) compared to patients after control laparoscopic surgery. Increases in serum TNF-α did not reach significance most likely due to small sample size. Elevated serum cytokines correlated with transaminitis (IL-6 vs. ALT, Pearson r=0.42, P=0.02). Donor nephrectomy patients had increased urine NGAL normalized to urine Cr at 24 hours (0.22±0.05, N=19 for donor nephrectomy vs. 0.10±2.2, N=7 for control laparoscopy, p<0.005). Increases in urine NGAL directly correlated with age (Figure 1D) suggesting that decreased renal reserve resulting in tubular stress may occur in the remaining kidney in older nephrectomy patients. Taken together, these findings suggest that donor nephrectomy results in increased production of cytokines, possibly explaining the relationship between AKI and hepatic dysfunction. In addition, patients with advanced age may sustain additional injury to the remaining kidney after nephrectomy.

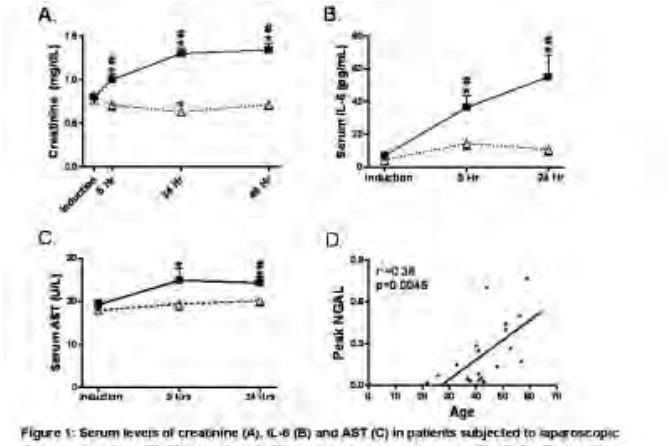



Figure 1: Serum levels of creatinine (A), IL-6 (B) and AST (C) in patients subjected to laparoscopic donor nephrectomy (N=25, ●) and control laparoscopic non-renal surgery (N=13, △). Peak urine NGAL corrected for urine creatinine after laparoscopic donor nephrectomy (N=19, ●) correlated with patient age (D). *P<0.05 vs. Control, #P<0.05 vs. Induction. Error bars represent SEM.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2064**

**Test Characteristics of Urinary Neutrophil Gelatinase-Associated Lipocalin (uNGAL) for Differential Diagnosis and Risk Stratification in Patients with Established Acute Kidney Injury** Eugenia Singer,[1,2] Antje Elger,[1] Saban Elitok,[2] Ralph Kettritz,[1,2] Thomas L. Nickolas,[3] Jonathan M. Barasch,[3] Friedrich C. Luft,[1,2,4] Kai M. Schmidt-Ott.[1,2,4] *[1]Charité – Universitaetsmedizin, Berlin, Germany; [2]Helios Clinics Berlin, Germany; [3]Columbia University College of Physicians and Surgeons; [4]Max-Delbrueck Center for Molecular Medicine, Germany.*

Background: Serum creatinine dynamics are used for the diagnosis of acute kidney injury (AKI) in current classification schemes (e. g. RIFLE), but are temporally and diagnostically poor. Urinary NGAL, a biomarker of nephron damage, may help to differentiate intrinsic kidney damage from prerenal AKI. Methods: The relationship between uNGAL levels at the time of diagnosis of AKI and outcomes was evaluated prospectively. uNGAL levels were determined on the Abbott ARCHITECT(R) standardized clinical platform. The primary outcome was worsening of AKI (progression to higher RIFLE category, dialysis initiation, or death) during hospitalization. In those patients with a likely or definitive diagnostic attribution based on clinical criteria, we tested uNGAL in distinguishing intrinsic from prerenal AKI. Results: We studied 162 hospitalized AKI patients. We excluded 17 with postrenal obstruction or insufficient clinical information. From the remaining 145 patients, 75 patients had a clinical diagnosis of intrinsic AKI, 32 patients had prerenal AKI, and 38 patients had an uncertain or ambiguous diagnosis. We found that uNGAL levels effectively discriminated intrinsic AKI from prerenal AKI (ROC 0.87, CI 0.81-0.94). NGAL levels at a cutoff > 100 ng/ml had a sensitivity of 0.75, a specificity of 0.88, a positive predictive value of 0.93, and a diagnostic accuracy of 0.78 in separating intrinsic AKI versus prerenal AKI. Patients with the primary endpoint had significantly higher uNGAL levels ($p<0.001$). In logistic regression analysis, uNGAL levels independently predicted worsening AKI, when corrected for demographics, comorbidities, serum creatinine, and RIFLE class. Conclusion: uNGAL is useful in the differential diagnosis and prognostic stratification of patients with established AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2065**

**Urinary L-FABP Predicts Recovery from Severe Acute Kidney Injury in Critically Ill Patients** Kent Doi,[1] Hiroko Yamamoto,[1] Kousuke Negishi,[1] Tomoko Ishii,[1] Toshiro Fujita,[1] Takehiro Matsubara,[1] Naoki Yahagi,[1] Takeshi Sugaya,[2] Eisei Noiri.[1] *[1]University of Tokyo; [2]CMIC, Co. Ltd.*

Acute kidney injury (AKI) is one of the serious complications for critically ill patients in intensive care unit (ICU). New AKI biomarkers including urinary L-type fatty acid-binding protein (L-FABP) have been developed mostly for early detection and severity prediction. However, their prognostic utility for severe and established AKI remains unclear. This study is aimed to evaluate whether urinary L-FABP can predict renal recovery from AKI that requires continuous renal replacement therapy (CRRT). Urinary L-FABP was measured at the initiation of CRRT in 34 critically ill patients treated at a mixed ICU of our university hospital. In 22 patients (64.7%), CRRT was discontinued because of renal recovery, whereas 12 patients needed CRRT until they died. There were significant differences between the recovery group and the non-recovery group in urinary L-FABP [75.0 (IQR 10.5–260.8) ng/ml versus 251.9 (52.0–5785.9) ng/ml, $p<0.05$] and blood lactate level (2.4±0.9 mmol/l versus 6.6±1.3 mmol/l, $p<0.05$). The non-renal SOFA score in the recovery group was significantly lower than the non-recovery group (7.9±0.6 versus 11.3±0.8, $p<0.05$). However, distributions of RIFLE criteria classes based on serum creatinine and urine output were variable and failed to predict renal recovery. Receiver operating characteristic (ROC) analysis revealed urinary L-FABP had a good performance for predicting renal recovery [area under the ROC curve 0.71 (95%CI 0.51–0.86)], which was improved by being combined with non-renal SOFA score [area under the ROC curve 0.85 (0.61–0.96)]. Six patients in the recovery group with the higher urinary L-FABP values than the cut-off value (103.2 ng/ml) showed significant reductions of urinary L-FABP at one day prior to CRRT termination [263.5 (IQR 259.9–2109.2) ng/ml → 54.0 (10.0–133.4), $p<0.05$]. In conclusion, urinary L-FABP measured at CRRT initiation and sequentially can predict renal recovery.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2066**

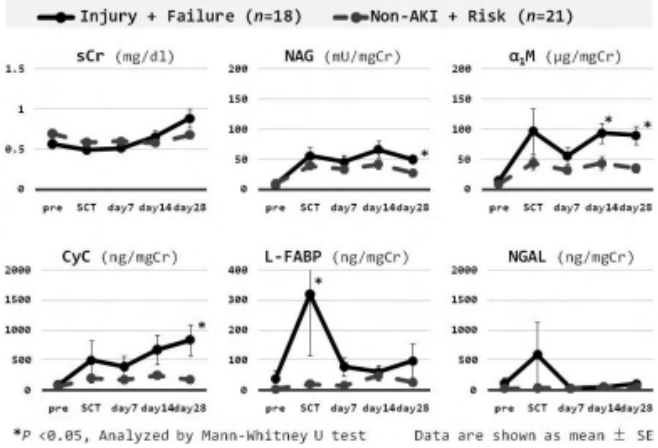
**Highly Elevated Levels of Urinary Liver-Type Fatty Acid Binding Protein Is Likely To Be an Early Warning Sign of Acute Kidney Injury Following Hematopoietic Stem Cell Transplantation** Taku Morito,[1,2] Minoru Ando,[2] Ken Tsuchiya,[2] Kosaku Nitta.[1] *[1]Department IV of Internal Medicine, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan; [2]Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Bunkyo-ku, Tokyo, Japan.*

INTRODUCTION: Acute kidney injury (AKI) is a common complication after myeloablative allogeneic hematopoietic stem cell transplantation (SCT). The use of urine as a diagnostic medium may allow the non-invasive detection of incipient nephropathy.

METHODS: A prospective cohort study was conducted in 39 patients (48.1±13.1 years) receiving SCT. To detect subclinical tubular damage, urinary biomarkers such as N-acetyl-β-glucosaminidase (NAG), $\alpha_1$ microglobulin ($\alpha_1$M), Neutrophil gelatinase associated lipocalin (NGAL), Liver-type fatty acid binding protein (L-FABP) and cystatin C (CyC) were consecutively measured before and 4 points after SCT. AKI was classified by the RIFLE criteria.

RESULTS: Incidence of any AKI was 76.9% after SCT (10.3% within 14 days and 15.4% within 28 days), and high-grade AKI (≥ Injury) was 46.2%. Changes of 5 biomarkers were compared between patients with and without the emergence of high-grade AKI (Figure1). While serum Cr remained statistically constant until day 28, in high-grade AKI group, any biomarker manifested the first peak at SCT, which was followed by a transient decrease at day 7 and a subsequent increase up to day 28. Only the peak value at SCT of L-FABP was statistically significant. These results suggested that: (1) subclinical tubular damage is induced likely by pre-conditioning regimens, the degree of which could be involved in the emergence of high-grade AKI, and (2) L-FABP may be most sensitive for early detection of tubular damage in the setting of SCT.

CONCLUSIONS: L-FABP has shown promise as a marker of identification of patients at risk for high-grade AKI following myeloablative allogeneic SCT.




Disclosure of Financial Relationships: nothing to disclose

**SA-PO2067**

**Urinary IL-18 and L-FABP as Early Predictive Biomarkers in Contrast-Induced AKI (Acute Kidney Injury) on Chronic Kidney Disease (CKD) Stage 3 Patients** Kosuke Inoue. *Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Nankoku, Japan.*

BACKGROUND/AIMS: Contrast-induced nephropathy (CIN) is the important cause of hospital-acquired AKI on CKD patients. However, some urinary biomarkers for AKI were reported to be high in CKD patients. Thus, sensitivity and specificity of these urinary biomarkers are not determined for the diagnosis of AKI in CKD patients. This study was designed to investigate whether human urinary IL-18, neutrophil gelatinase-associated lipocalin (NGAL), and liver-type fatty acid binding protein (L-FABP) are early predictive markers for CIN after coronary angiography (CAG) in CKD patients. METHODS: 24 patients of CKD Stage 3 undergoing CAG were enrolled. Urine samples were collected before, 3h, 6h, 24h after CAG and IL-18, NGAL, and L-FABP levels measured by using an ELISA kit. Urinary creatinine values were measured and the values of urinary biomarkers were corrected by the creatinine concentration because of oligohydruria. This study was approved by Kochi Medical School review boards. All patients provided written informed consent. RESULTS: eGFR (Estimated glomerular filtration rate) decreased more than 5ml/min in 12 patients (decreased eGFR group) and did not decrease in remaining 12 patients (non-decreased group) after CAG. At 3h, 6h, and 24h after the procedure, the ratio with the previous value of the urinary IL-18 and L-FABP were significantly increased in the decreased eGFR group, but not in the non-decreased group. In contrast, NGAL are rapidly

increased in both group, however, no statistically significant difference of NGAL was observed between two groups. When we used uncorrected biomarker values by creatinine, the specificity and sensitivity were significantly decreased.

CONCLUSIONS: We conclude that urinary IL-18 and L-FABP could be early biomarkers of CIN in CKD Stage3 patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2068**

**IL-18 Is the Best Predictor of Renal Replacement Therapy after Cardiac Surgery from among a Panel of Markers** John M. Arthur,[1,5] Evelyn C. Lewis,[1] Timothy P. Taylor,[1] Michael G. Janech,[1,5] James A. Tumlin,[3] Lakhmir S. Chawla,[2] Andrew Shaw.[4] *[1]MUSC, Charleston, SC; [2]George Washington University, Washington, DC; [3]UT Chattanooga, Chattanooga, TN; [4]Duke University, Durham, NC; [5]Ralph H Johnson VA Medical Center, Charleston, SC.*

Acute kidney injury (AKI) has a high mortality but treatments to prevent it have not been identified. Markers that can predict which patients will require renal replacement therapy (RRT) are needed to help develop new therapies. Urine samples from 117 patients who developed at least stage I AKI after cardiac surgery were collected at 4 centers. Mean bypass time (2:47±0:07 vs 2:36±0:22), preoperative creatinine (1.2±0.05 vs 1.3±0.11 mg/dl) and time after surgery of urine collection (1.4±0.07 vs 1.8±0.23 days) were not different between the groups. The increase in serum creatinine was greater in the RRT group (0.7±0.06 vs 1.4±0.28 mg/dl, p<0.05). We measured 27 analytes in urine obtained after the patients met criteria for AKI. Receiver Operator Characteristic curves were created to determine the ability of the analytes to predict RRT. Adjusting the concentration of the analyte by dividing by urine creatinine concentration improved the ability of the analytes to predict RRT. Urinary IL-18 was the best predictor of RRT with an AUC value of 0.86. Using a cutoff value of 249 pg/mg Cr, the RRT outcome of 101 of the 117 patients was correctly predicted. The sensitivity was 73% and the specificity was 87%. The AUC values for the remaining markers was VCAM-1 (0.82), NAG (0.82), IL-6 (0.82), NGAL (0.80), L-FABP (0.80), Cystatin C (0.79), HGF (0.79), Clusterin (0.78), IL-1ra (0.77), albumin ((0.77), MCP-1 (0.76), IL-8 (0.75), Fetuin A (0.74), Urine protein (0.72), VEGF (0.69), LIF (0.69), GROα (0.67), RBP (0.67), β2 microglobulin (0.66), IL-10 (0.65), Eotaxin (0.65), Netrin 1 (0.63), MIP1α (0.58), TRAIL (0.55), RANTES (0.54) and TNFα (0.43). We conclude that IL-18 is the best predictor of the need for RRT after cardiac surgery from among a panel of commonly used markers. The predictive value in this population of post surgery patients is good enough to help select patients for clinical trials in AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2069**

**Utility of Urine Biomarkers for Differential Diagnosis of Acute Kidney Injury in Cirrhotic Patients** Won K. Han, Svastijaya Daviratanasilpa, Bonita E. Falkner. *Medicine/Division of Nephrology, Thomas Jefferson University, Philadelphia, PA.*

The purpose of this study was to evaluate the diagnostic utility of urinary hepatocyte growth factor (HGF), kidney injury molecule-1 (KIM-1), and N-acetyl-β-D-glucosaminidase (NAG) for detection of established acute kidney injury (AKI) in a cross-sectional study and then to differentiate the cause of AKI among patients with cirrhosis in a case-control study. In the cross-sectional study, spot urine samples were collected from 79 patients with established AKI and from 233 non-AKI subjects. The case-control study included urine samples from 48 cirrhotic patients (31 AKI and 17 non-AKI). AKI was defined as ≥ 50% increase in serum creatinine from baseline. Biomarkers were measured at 3 time points during hospitalization and normalized to the urine creatinine concentration. Receiver-operating characteristic curves were generated and the areas under the curve (AUC) compared for performance of biomarkers. In the cross-sectional study, urinary HGF, KIM-1, and NAG had an AUC of >0.90 to detect the established AKI. In the prospective study of patients with cirrhosis, the AUCs to differentiate acute tubular injury (n=10) from prerenal azotemia (n=10) at 24 and 48 hours after enrollment were 0.90 and 0.98 for HGF; 0.82 and 0.89 for KIM-1; 0.76 and 0.84 for NAG, respectively. The AUCs to differentiate acute tubular injury (n=10) from hepatorenal syndrome (n=11) at 24 and 48 hours after enrollment were 0.65 and 0.83 for HGF; 0.56 and 0.77 for KIM-1; 0.66 and 0.89 for NAG, respectively. Our results demonstrate that urinary biomarkers can aid to differentiate the cause of AKI in patients with cirrhosis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2070**

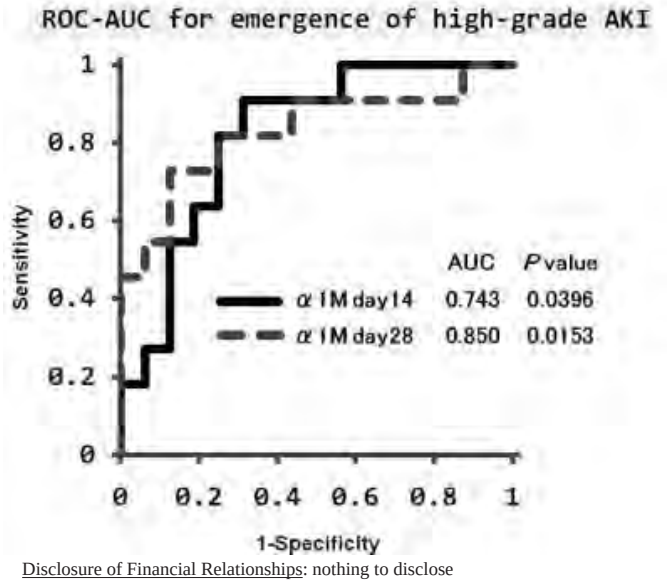
**Early Elevation of Urinary α1 Microglobulin Following Myeloablative Allogeneic Hematopoietic Stem Cell Transplantation Patients Predicts Incidence of High-Grade Acute Kidney Injury** Taku Morito,[1,2] Minoru Ando,[2] Ken Tsuchiya,[1] Kosaku Nitta.[1] *[1]Department IV of Internal Medicine, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan; [2]Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Bunkyo-ku, Tokyo, Japan.*

INTRODUCTION: Allogeneic myeloablative (m-allo) hematopoietic stem cell transplantation (SCT) involves a great risk of acute kidney injury (AKI). Early detection and treatment of AKI is necessary to improve the outcome.

METHODS: A prospective cohort study was conducted in 27 patients (46.3±13.4 years) receiving m-allo SCT between Dec 2008 and Oct 2009. We measured urinary biomarkers for renal tubular injury such as N-acetyl-β-glucosaminidase (NAG), $\alpha_1$ microglobulin ($\alpha_1$M), Neutrophil gelatinase associated lipocalin (NGAL), Liver-type fatty acid binding protein (L-FABP) and cystatin C (CyC) levels before and 4 points after SCT. AKI was classified by the RIFLE criteria. Discriminative power of biomarkers for emergence of AKI was evaluated using area under the ROC curve. Variables relevant to emergence of AKI were analyzed using multivariate logistic regression analysis.

RESULTS: Any AKI occurred at day 51 (median) following m-allo SCT. Incidence of any AKI and high-grade AKI (≥ Injury), which is associated with 1-year mortality for post-SCT patients, was 66.7% and 40.7%, respectively. Among biomarkers we measured, $\alpha_1$M at day 14 and 28 had moderate power to discriminate subsequent high-grade AKI (figure 1). Multivariate logistic regression analysis showed that only $\alpha_1$M level at day 14 and 28 were associated with the emergence of high-grade AKI. ($\alpha_1$M at day 14: OR; 1.039, 95%CI; 1.005-1.113, $P$ = 0.0177 and $\alpha_1$M at day 28: OR; 1.122, 95%CI; 1.023-1.442, $P$ = 0.0007, respectively).

CONCLUSIONS: Among currently-available biomarkers, urinary $\alpha_1$M could be useful for identification of patients at risk for subsequent high-grade AKI following m-allo SCT.




Disclosure of Financial Relationships: nothing to disclose

**SA-PO2071**

**Soluble Thrombomodulin Levels in Acute Kidney Injury** Josee Bouchard,[1] Rakesh Malhotra,[1] Sharon Soroko,[1] Akanksha Gupta,[2] David T. Berg,[2] Ashita J. Tolwani,[3] Ravindra L. Mehta.[1] *[1]University of California San Diego; [2]Eli Lilly Research Laboratories; [3]University of Alabama at Birmingham, .*

*Background.*

Markers of endothelial dysfunction have been implicated in the pathophysiology of acute kidney injury (AKI) in septic animal models. There are limited data on markers of endothelial dysfunction in clinical AKI. We hypothesized that soluble thrombomodulin (sTM) is involved in the development of AKI in critically ill patients and that sTM levels can predict AKI.

*Methods.*

We conducted a prospective, multicenter observational study to evaluate the role of sTM levels as an early biomarker for AKI in critically ill patients. AKI was defined according to the AKIN criteria. Samples were collected every 12 hours after ICU admission for ≥48 hours and up to 10 days.

*Results.*

From the 80 patients enrolled, 18 developed AKI on average 1.5 ± 1.7 days after ICU admission. Mean sTM levels were higher in patients who developed AKI compared to patients without AKI over the study (6.5 ± 7.5 ng/ml vs. 1.7 ± 2.0 ng/ml; p < 0.001). The area under the curve (AUC) to predict AKI using sTM was 0.729 (95% CI, 0.630 to 0.828). STM levels were also significantly higher in patients with sepsis than without sepsis (3.6 ± 5.9 ng/ml vs. 2.3 ± 3.2 ng/ml; p= 0.005). In septic patients, sTM levels were higher in patients who developed AKI than in those who did not over the study (7.4 ± 9.2 ng/ml vs. 2.1 ± 2.6 ng/ml; p < 0.001) and the AUC to predict AKI in this group was 0.709 (95% CI 0.578-0.839).

*Conclusion.*

Soluble thrombomodulin is a biomarker of endothelial dysfunction potentially involved in the pathophysiology of AKI and which can help to predict AKI. Further studies are required to evaluate its role in endothelial dysfunction, sepsis and AKI.

Disclosure of Financial Relationships: Research Funding: Astute Medical Inc. $95 000 per year for one year.

**SA-PO2072**

**Serum Nutritional Markers Are Predictors of Early Mortality in Hospital-Acquired Acute Kidney Injury** Chuan-Ming Hao. *Huashan Hospital, Fudan University.*

**Background:** Preexisting malnutrition is common in patients with acute kidney injury (AKI), but few studies have shown the relationship between the changes of nutritional status and the outcomes of these patients. **Methods:** 194 patients with hospital-acquired AKI, as determined by RIFLE staging criteria were included. Nutritional evaluations, including Subjective Global Assessment (SGA), anthropometric and laboratory evaluations were conducted. The primary outcome was early mortality (<7 days) and late mortality (>7 days, <28 days) after inclusion into the study. **Results:** AKI patients at enrollment were characterized by a high prevalence of malnutrition. Univariate analysis showed that the SGA, serum prealbumin, cholesterol and total lymphatic cells, and the Maastricht index were significantly different among early mortality, late mortality and survival groups. The serum prealbumin and cholesterol levels in the early death group were significantly lower than those in the survival and late death groups ($p < 0.05$). Multivariate analysis revealed that SGA, albumin, prealbumin and cholesterol remained independently and significantly associated with early mortality after adjusting for age, sex, dialysis, ventilation, hemoglobin, platelets, bilirubin, and Glasgow coma score. The ROC area value to predict early mortality for albumin, prealbumin and cholesterol were 0.591, 0.736 and 0.603, respectively, with that of prealbumin significantly higher than others ($p < 0.05$). **Conclusions:** Lower levels of serum prealbumin, albumin and cholesterol at enrollment were independently associated with increased early mortality in hospital-acquired AKI patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2073**

**Serum Uric Acid Level as a Marker for Mortality and Acute Kidney Injury in Patients with Acute Paraquat Intoxication** Sujin Seok, Junghoon Kim, Su-Ji Kim, Hyo-Wook Gil, Jong-Oh Yang, Eun-Young Lee, Sae-Yong Hong. *Internal Medicine, Soonchunhyang University, Cheonan, Korea.*

Paraquat (PQ) is a non-selective herbicide that generates reactive oxygen species (ROS) *in vivo*. Uric acid emerged as a marker of oxidative stress, and may enhance ROS-mediated injury. Therefore, we investigated the association between uric acid levels and mortality and acute kidney injury (AKI) in patients with acute PQ intoxication.

**Methods:** From January 2007 to December 2008, 513 patients arrived at our hospital with acute PQ intoxication. We excluded the patients of initial serum creatinine >1.2 mg/dL, arrival at hospital more than 24 hours after PQ ingestion, death on the day of admission, and those who left the hospital against medical advice. Subsequently, a total of 247 patients were included in the study. Patients were divided into two groups (hyperuricemia *vs.* non-hyperuricemia) based on uric acid levels. Mortality and AKI were analyzed in reference to uric acid level.

**Results:** The mean serum uric acid level was 5.6±1.6 mg/dL in men, 4.1±1.3 mg/dL in women and the mean serum creatinine level was 0.7±0.2 mg/dL. Patient mortality was higher in hyperuricemia group than in non-hyperuricemia group (68.4% *vs.* 38.3%, $p<0.05$). Hyperuricemia significantly increased the risk of mortality and the adjusted odds ratio for mortality was 3.67 (1.35-9.98) with hyperuricemia. The frequency of AKI was higher in the hyperuricemia group (78.9% *vs.* 61.2%, $p<0.05$). The mortality in the kidney failure group was 75.7%, while that of the counterpart was 17.9%. After adjustments for age, gender, and the amount of PQ ingestion, hyperuricemia was still significantly associated with an increased risk of kidney failure (OR, 3.30; 95% CI, 1.33-8.20). There was an increased prevalence of respiratory failure associated with hyperuricemia. The incidence of liver injury and pancreatic injury were not statistically significant. The mean uric acid level increased in association with an increase in the number of organ injuries.

**Conclusions:** Baseline serum uric acid level might be a good clinical marker for patients at risk of mortality and AKI after acute PQ intoxication.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2074**

**Urinary Kallikrein Activity and Tissue Kallikrein Promoter DNA Methylation Analysis in Acute Kidney Injury** Sunwoo Kang,[1] Pei-An (Betty) Shih,[2] Roy Mathew,[3] Fangwen Rao,[2] Rakesh Malhotra,[2] Ravindra L. Mehta,[2] Daniel T. O'Connor.[2] *[1]Nephrology, College of Medicine, Inje University, Busan, Korea; [2]Medicine, University of California San Diego, Lajolla, CA; [3]Veteran's Affair Medical Center, Albany, NY.*

Transplanted subjects with acute kindy injury (AKI) excreted less kallikrein. Kallikrein gene transfer protected against AKI by inhibiting apoptosis and inflammation without affecting blood pressure. Therefore, we hypothesized that changes in urine epigenetics for kallikrein could be a biomarker approach during the AKI.

We examined the DNA for aberrant methylation of the promoter KLK1 gene and LINE-1 elements by pyrosequencing from 20 AKI patients and 38 healthy controls. Results were compared with clinical data and the urine activity of the kallikrein.

The AKI patients had lower systolic blood pressure than controls (119.8 ± 4.4 vs. 131.7 ± 1.7 mmHg; p = 0.02) and higher heart rate (89.3 ± 3.6 vs. 68.0 ± 1.6 bpm; p = 1.73E-05). Unexpectedly it turned out that our AKI subjects have high kallikrein excretion, about 10 times higher than controls ( 6.74 ± 1.92 vs. 0.63 ± 0.08 mU/mg creatinine; p < 0.001). Our AKI subjects had high epinephrine excretion, about 3 times higher ( 20.1 ± 2.4 vs. 7.5 ± 1.1 ng/mg creatinine; p < 0.001). Urinary kallikrein excretion was controlled by urinary epinephrine excretion in these samples. Promotor KLK1 specific methylation was high in blood. But, it was significantly lower in urine (66.38 ± 1.00 vs. 33.43 ± 4.67%; p < 0.001). KLK1 methylation in blood DNA was significantly higher in the AKI patients than controls (70.32 ± 2.27 vs. 65.36 ± 1.05%; p = 0.011). However, KLK1 methylation in urine DNA trended higher in the AKI patients (40.95 ± 7.06 vs. 30.35 ± 5.88%; p = 0.22).

In conclusion, increased epinephrine production under hypotensive condition induced significant increase in urinary kallikrein excretion in AKI. KLK-1 promoter DNA methylation study confirmed that this gene is the kidney specific expressed one because it was lower in urine. Since KLK1 promotor methylation was higher in the AKI patients than controls, KLK1 promoter CpG methylation may have potentially pathogenic role on AKI susceptibility.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2075**

**Urine Sediment from Patients with Acute Kidney Injury Contains Potential Biomarker Proteins** Robert Arthur,[1] Michael G. Janech,[1,2] Benjamin Neely,[1] John M. Arthur,[1,2] Juan Carlos Q. Velez.[1] *[1]MUSC, Charleston, SC; [2]Ralph H Johnson VA, Charleston, SC.*

Acute kidney injury (AKI) is an important clinical problem. Many potential biomarkers have been proposed in the urine but the urine sediment has not been investigated as a potential source of biomarkers. The sediment is used to help diagnose acute tubular necrosis and is a likely source of informative biomarkers. We compared the proteins in the urine supernatant to those in the urine sediment using 2D gel electrophoresis. Patterns of proteins were strikingly different between the sediment and supernatant. Uromodulin (Tamm Horsfall Protein) was far more abundant than any other protein on the sediment gels. Carbonic anhydrase, keratin, alpha and beta actin were also identified. Albumin, zinc α2 glycoprotein, α-1-microglobulin and prostaglandin H2 isomerase were most abundant in the urine supernatant. To further characterize the proteins present in the sediment, we performed two dimensional chromatography tandem mass spectrometry on tryptic peptides from sediment proteins. 578 proteins were identified with high confidence. Protein abundance was determined using Scaffold by dividing number of assigned spectra by number of total spectra to give a percent total spectra. Uromodulin, several histone proteins, keratin, hemoglobin subunits and protein S100-A8/9 were the most abundant proteins. Gene Ontology (GO) terms were assigned to the proteins. 21% of the proteins were assigned to cellular processes. Protein phosphorylation and proteolysis were the most common cellular processes with 10 proteins each. Proteins involved in signal transduction, DNA processes, cell adhesion, nucleosome assembly, signal transduction and transcription were also abundant. 16% of the proteins were assigned to GO terms associated with biological regulation. Regulation of cell growth and transcription were the most common terms in this group. For the cellular component GO terms, 72% of proteins were unassigned, 15% were in intracellular organelles, 11% were in membrane and 9% each were in cytoplasm and nucleus. These proteins in the urine sediment of patients with AKI are candidates to be used as diagnostic and prognostic markers.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2076**

**Diagnostic Marker Pattern of Sequenced Urinary Peptides for Early and Accurate Detection of Acute Kidney Injury** Jochen Metzger,[1] Torsten Kirsch,[2] Marion Haubitz,[2] Harald Mischak,[1,3] Peter Rossing,[4] Stefan Herget-Rosenthal.[5] *[1]Mosaiques Diagnostics GmbH, Germany; [2]Hannover Medical School, Germany; [3]University of Glasgow, United Kingdom; [4]Steno Diabetes Centre, Denmark; [5]Rotes Kreuz Krankenhaus, Germany.*

Acute kidney injury (AKI) is a common condition in intensive care unit (ICU) patients and associated with high mortality. In order to prevent its progression and to improve its outcome, early and accurate detection of AKI is crucial. In pilot studies kidney injury molecule 1 (KIM-1) was proposed as a promising early marker for AKI. However, latter studies could not confirm these findings making it necessary to search for other, more predictive markers.

We used capillary electrophoresis mass spectrometry to identify urinary peptide markers predictive for AKI in urine samples obtained from indwelling bladder catheters of ICU patients who later developed AKI (maximum 5 days prior AKI) defined by a serum creatinine increase ≥ 50% in ≤ 48 hours or maintained normal kidney function. The statistically most significant markers were combined and validated on a blinded set of ICU patient samples. Classification accuracy of the proteomic model in this patient collective was compared to that based on urinary KIM-1 levels.

A combination of 20 peptides allowed classification of a blinded test set of ICU patient samples (n=20) with 89% sensitivity and 82% specificity. In order to evaluate general applicability, the urinary proteomic model was further applied to the classification of single-void urine samples from hematopoietic stem cell transplanted patients of which 13 developed AKI after transplantation and 18 did not. AUC in this validation set was 0.90 with sensitivity and specificity values of 94 and 82 %, respectively. Compared to the proteomic model, ROC curve analysis revealed poorer classification accuracy of KIM-1 with the respective AUC values being outside the statistical significant range (0.67 for the ICU and 0.57 for the HSCT validation set).

In contrast to KIM-1, the proteomic marker pattern allowed accurate detection of AKI as early as 5 days in advance of serum creatinine irrespective of the patient population in whom AKI occurred.

Disclosure of Financial Relationships: Employer: Empolyee of mosaiques diagnostics.

**SA-PO2077**

**Clinical Significance of Tubular and Podocyte Biomarkers in Acute Kidney Injury** Katsuomi Matsui,[1] Atsuko Kamijo,[1] Masanori Hara,[2] Takashi Yasuda,[1] Kenjiro Kimura,[1] Takeshi Sugaya.[1] *[1]Department of Nephrology and Hypertension, Internal Medicine, St. Marianna University School of Medicine, Kanagawa, Japan; [2]Department of Pediatrics, Yoshida Hospital, Niigata, Japan.*

*Background*: Acute kidney injury (AKI) is a common complication in critically ill patients. Urinary excretion of liver-type fatty acid-binding protein (L-FABP), which is expressed in the proximal tubules, reflects the presence of tubular injury. Urinary excretion of podocalyxin (PCX), a glycoprotein prominently expressed on podocytes, is associated with podocyte injury. Our aims were to evaluate the utility of urinary L-FABP for the early detection of AKI and to examine whether podocyte injury is present in AKI patients using the biomarker of urinary PCX.

*Methods*: Patients admitted to the intensive care unit (ICU) were divided into the AKI group (n=14) and non-AKI group (n=11), according to the occurrence of AKI during hospitalization in the ICU. Changes in various biomarkers were evaluated.

*Results*: In the AKI group, elevation of urinary L-FABP level [maximum value of L-FABP, 199.0 (92.5–433.6) μg/g creatinine, median (25%–75% interquartile range)], which reflects tubular injury (area under the curve 0.95, cut-off value 44.1 μg/gCr), occurred between -30 and 0 hour before the occurrence of AKI (i.e., the time at which serum creatinine peaked), and elevation of urinary PCX level [maximum value of PCX, 389.5 (267.0–501.0) μg/g creatinine; upper limit of reference value, 160 μg/g creatinine] occurred during the time of recovery from AKI when serum creatinine levels were decreasing between 34.0 and 72.0 hours after the occurrence of AKI.

*Discussion and Conclusions*: Our study suggests that L-FABP is a useful biomarker for early detection of AKI and that podocyte injury was induced during the recovery phase of AKI. Moreover, podocyte injury observed by elevation of urinary PCX was induced even in patients with mild or moderate AKI. It is speculated that the more severe the episode of AKI, the more severe is the podocyte injury. Because a recent study revealed that AKI is associated with increased risk for CKD, podocyte injury may be closely related to renal impairment after AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2078**

**Time-Dependent Changes of Urinary Biomarkers in Lung Cancer Patients after Cisplatin-Based Chemotherapy** Tomoko Sato,[1] Satohiro Masuda,[1] Aiko Ozawa,[1] Yosuke Togashi,[2] Young Hak Kim,[2] Michiaki Mishima,[2] Takaharu Ichimura,[3] Joseph V. Bonventre,[3] Ken-Ichi Inui.[1] *[1]Department of Pharmacy, Kyoto University Hospital, Kyoto, Japan; [2]Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [3]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

**[Backgrounds]** Acute kidney injury occurs after cisplatin-based chemotherapy with about 20% of patients with lung cancer. Therefore, serial monitoring of kidney function is crucial in patients with lung cancer treated with cisplatin. Because the classical biomarkers for renal function such as serum creatinine (Scr) and blood urea nitrogen (BUN) are insensitive and nonspecific, some algorithm(s) should be established in combination with some urinary biomarkers specific for cisplatin-induced nephrotoxicity. **[Aim]** The aim of the present study is to identify useful urinary biomarkers for the monitoring of the kidney damage in the lung cancer patients treated with cisplatin. **[Methods]** The urine samples were collected at the day before the administration of cisplatin (60mg/m$^2$) and at days 3, 7, 14 and 21. The urinary neutrophil gelatinase-associated lipocalin (NGAL), Liver-type fatty acid-binding protein (L-FABP) and osteopontin were measured by commercially available ELISA kits. The urinary Kidney Injury Molecule-1 (KIM-1) and forty cytokines/chemokines were also measured by microbead-based sandwich ELISA with the Bio-Plex® system. Eight patients were enrolled in this study with written informed consent. **[Results]** In the case with severe nephrotoxicity after the cisplatin-based chemotherapy with elevated Scr, most of the urinary biomarkers examined were markedly increased at day 3. However, the urinary KIM-1 and NGAL, but not L-FABP or osteopontin, were increased up to 3-fold in four patients without change in Scr. Because the pre-treatment urinary KIM-1 and NGAL was increased at the second course of chemotherapy, cumulative nephrotoxicity was suggested in the patients receiving repeated cisplatin-based chemotherapy. **[Conclusion]** Urinary KIM-1 and NGAL can be sensitive and specific biomarkers reflecting the cisplatin-induced kidney injury in lung cancer patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2079**

**Changes in Urinary Cytokines during 3 Days after Ischemia-Reperfusion Predict Renal Functional Outcome on Day 7 and 1, 2, and 3 Later** Osun Kwon, Binzhi Zhang. *Medicine/Nephrology, Penn State College of Medicine, Hershey, PA.*

Acute kidney injury (AKI) is a common disorder associated with high morbidity and mortality. Ischemia is the leading cause of AKI. To date, however, no specific treatment modalities are available to treat sustained AKI. Noninvasive parameters which reliably reflect the ongoing phenomenon in the kidney and predict renal functional outcome are required to facilitate improved management and decrease the morbidity and mortality of the patients. We recently reported that urinary IP-10, IL-8, VEGF, TGF-alpha and EGF may predict renal functional outcome during the first week of diagnosis of ischemic AKI. In the current study, we questioned whether changes in multiple urinary cytokine levels early after an ischemic insult predict long-term as well as short-term renal functional outcome. Fifteen recipients of a renal allograft were studied since freshly transplanted kidneys are known to be an optimal model for pure postischemic AKI in humans. Urine samples were obtained about 4 hrs after reperfusion of the renal allograft and 3 days later. Markers of inflammation and regeneration were assessed in the urine samples. During 3 days after ischemia-reperfusion, high proinflammatory cytokine levels tended to decrease and low EGF levels tended to increase, especially in subjects with recovering renal function compared to the subjects destined to have sustained AKI. The sum of the changes (% increase or decrease) in 8 urinary proinflammatory cytokine levels; IL-1beta, IL-6, IL-8, MCP-1, IP-10, RANTES, TGF-alpha, VEGF, and the change in urinary EGF between day 0 and 3 were analyzed for renal outcome on postoperative day 7 and year 1, 2, and 3. More than 53.3 % and -48.3 % increase in urinary EGF between day 0 and 3 predicted renal recovery (s-Cr ≤ 1.3 mg/dl) 1 and 2 years later, and 3 years later, respectively. Less than 709.3 % decrease in the sum of 8 urinary cytokine changes tends to predict no renal recovery on day 7 and 1 and 2 years later. We suggest that increase in urinary EGF and decrease in multiple urinary proinflammatory cytokines over 3 days after an ischemic insult may predict renal functional outcome on day 7 and 1, 2, and 3 years later.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2080**

**Association of Urinary Alpha-GST and Pi-GST with Dialysis Requirement in Acute Kidney Injury** Victor F. Seabra,[1,3,4] Mary Celine R. Perianayagam,[1,3] Hocine Tighiouart,[2,3] Orfeas Liangos,[3] Oscar Pavao Dos Santos,[4] Bertrand L. Jaber.[1,3] *[1]Kidney and Dialysis Research Laboratory, St. Elizabeth's Medical Center, Boston, MA; [2]Biostatistics Research Center, Tufts Medical Center, Boston, MA; [3]Department of Medicine, Tufts University School of Medicine, Boston, MA; [4]Division of Nephrology, Federal University of Sao Paulo, Sao Paulo, SP, Brazil.*

**Background.** There is lack of information on the potential usefulness of single measurements of urinary alpha glutathione S-transferase (GST), a proximal tubular injury marker, and pi-GST, a distal tubular injury marker, for the prediction of dialysis requirement in acute kidney injury (AKI).

**Methods.** We examined the association of urinary alpha-GST and pi-GST levels with dialysis requirement or in-hospital death in 246 hospitalized adults with AKI using logistic regression analyses. A single urine sample obtained at the time of nephrology consultation was assayed by ELISA, and the results were normalized to urinary creatinine.

**Results.** Mean age was 66 years, 54% were men, mean APACHE II score 20, 44% had sepsis, 39% required dialysis, and in-hospital mortality was 22%. In unadjusted analyses, increases in pi-GST were associated with higher odds for dialysis requirement or the composite of dialysis requirement or in-hospital death. These associations were attenuated after adjustment for APACHE II score. Urinary alpha-GST was not associated with the outcomes of interest.

| | **Dialysis requirement** | | **Dialysis requirement or in-hospital death** | |
|---|---|---|---|---|
| | OR per doubling (95%CI) | | OR per doubling (95%CI) | |
| | alpha-GST | pi-GST | alpha-GST | pi-GST |
| **Unadjusted** | 1.10 (0.94,1.29) P=0.22 | 1.14 (1.01,1.29) P=0.03 | 1.11 (0.96,1.30) P=0.16 | 1.15 (1.02,1.29) P=0.02 |
| **Adjusted for APACHE II score** | 1.05 (0.89,1.24) P=0.57 | 1.11 (0.98,1.26) P=0.09 | 1.04 (0.88,1.23) P=0.64 | 1.12 (0.98,1.27) P=0.10 |

**Conclusion.** A single measurement of the distal urinary tubular marker pi-GST obtained at the time of nephrology consultation might provide prognostic discrimination for predicting dialysis requirement in AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2081**

**Evaluation of Endotoxin Activity Assay in Acute Kidney Injury and Continuous Renal Replacement Therapy** Kent Doi, Takehiro Matsubara, Eisei Noiri, Toshiro Fujita, Naoki Yahagi. *University of Tokyo.*

Blood endotoxin level is one of the most important predictive factors for the outcomes of critically ill patients in ICU. The EAA™ Endotoxin Activity Assay is the only FDA approved rapid whole blood assay for detection of human endotoxemia. This assay is based on the ability of antigen-antibody complexes to prime neutrophils for an augmented respiratory burst response. Although neutrophil activation has been observed in acute kidney injury (AKI) and patients treated by continuous renal replacement therapy (CRRT), it is unclear whether the EAA assay will be influenced by these conditions. Blood endotoxin levels of 14 AKI patients who were treated by CRRT were measured sequentially (0, 24, 48 hr) by the EAA assay. All the patients were diagnosed as AKI by the AKIN criteria (BUN 66.9 ± 25.2 mg/dl, Cre 5.1 ± 2.5 mg/dl, plasma NGAL 533.1 ± 100.3 ng/ml). There was no significant correlation between the endotoxin activity (EA) values and BUN ($R^2$ = 0.1981), serum creatinine ($R^2$ = 0.088), or plasma NGAL ($R^2$ = 0.046). Four patients had severe gram-negative rod infections that eventually caused septic shock. In 10 AKI patients without septic shock, the EA values did not show any significant changes during the three different time-points. The highest EA values of the septic shock patients were significantly higher than the non-septic shock patients (0.81 ± 0.10 vs 0.37 ± 0.05, p<0.05). Plasma NGAL of the septic shock patients was also significantly higher than the non-septic patients (847.8 ± 163.1 ng/ml vs 407.2 ± 103.2 ng/ml, p<0.05). Combination of EA value (cut-off >0.40) and plasma NGAL (cut-off >500 ng/ml) could perfectly distinguish the septic shock patients. These data indicate that the EAA assay was not influenced by AKI/CRRT and that the EAA assay and plasma NGAL can detect severe sepsis in AKI/CRRT patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2082**

**Stroke Volume Variation (SVV) Monitoring and Acute Kidney Injury (AKI) in Abdominal Aortic Aneurysm (AAA) Surgery** Paolo Lentini,[1-2] Vincenzo Catena,[1] Rudi Stramanà,[3] Valentina Pellanda,[1] Massimo De Cal,[2-4] Alexandra Chronopoulos,[4] Claudio Ronco,[4] Marco Baiocchi,[1] Diego Cognolato,[3] Roberto Dell'Aquila.[1] *[1]Nephrology-Intensive Care Unit, S. Bassiano Hospital, Bassano Del Grappa (VI), Vi, Italy; [2]University of Padua, Padua, Italy; [3]Vascular Surgery, St. Bassiano, Bassano Del Grappa (VI), Italy; [4]Nephrology, St. Bortolo, Vicenza, Italy.*

AAA surgery patients are at high risk for AKI, which may be due to hypovolemia, haemorrhage, decreased cardiac output, vascular disruption, or inflammation. Large variations in SVV can predict volume responsiveness in mechanically ventilated patients. AIM: We conducted a pilot study to assess if patients with wide variations of SVV before and after clamping of the aorta are at higher risk of AKI development than those with lesser variations. MATERIALS AND METHODS: We enrolled 8 consecutive patients undergoing elective AAA surgery with supra-renal clamping. Patients were all on volume-control mechanical ventilation (8 ml/kg) and positive end expiratory pressure of 4 cmH20. Patients with sustained arrhythmias, spontaneous ventilations, and those extubated before 12 h post-operative were excluded. SVV was measured with the FlowTrack/Vigileo (Edwards®) device every 3 minutes during the procedure and for 24 h after surgery. A detailed log of ins and outs was kept. Patients received 70ml/kg/h of cristalloids, and fluid boluses and transfusions were given if needed according to medical team preference. Data was compared with non-parametric tests. RESULTS: Out of 8 patients, 3 developed AKI, defined as RIFLE Risk category. As compared to patients without AKI, AKI patients had a significantly larger SVV after aortic clamping (13.25% vs. 24.5%, p=0.01). The increase in SVV at aortic declamping time, as compared to SSV at clamping time, was also significantly higher in AKI patients (-3.75% vs. 12.5%, p=0.04). CONCLUSION: SVV monitoring during and after suprarenal AAA surgery is a feasible non-invasive tool that can help identify patients at risk for AKI. Particularly, an increase in SVV at aortic declamping time, as compared to SSV at clamping time, may be a marker for risk of AKI development that should be further studied.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2083**

**Urine Biomarkers Predict Acute Kidney Injury in Very Low Birth Weight Infants** David J. Askenazi,[1] Stuart Goldstein,[2] Prasad Devarajan,[2] Chirag R. Parikh,[3] Ravindra L. Mehta,[4] Anupam Agarwal.[5] *[1]Pediatrics, Univeristy of Alabama, Birmingham, AL; [2]Pediatrics, Cincinatti Children's Medical Center, Cincinnati, OH; [3]Medicine, Yale, New Haven, CT; [4]Medicine, University of California, San Diego, CA; [5]Medicine, University of Alabama, Birmingham, AL.*

Objective: Acute kidney injury (AKI) is a strong predictor of mortality in premature infants. Our objective is to determine the utility of 6 potential urine biomarkers to predict AKI in very low birth weight (VLBW, birth weight between 500-1500 g) infants.

Method: 78 VLBW infants were enrolled from a regional quaternary care NICU. Urine samples were collected on postnatal days 1 through 6 and the maximum concentration for each biomarker was determined. Candidate biomarkers evaluated include neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule -1 (KIM-1), interleukin-18 (IL-18), Osteopontin (OPN), β2 Microglobulin (β2 M), and cystatin C. As data was non-normally distributed, the Mann-Whitney U test was used to determine differences between groups.

Results: Urinary concentrations of NGAL, KIM-1, IL-18, OPN were increased in infants with AKI compared to those without AKI. No difference was seen with β2 M and Cystatin C. Similar findings were seen if values were corrected for urine creatinine (data not shown).

Urine Biomarkers Predict AKI in VLBW infants

| | No AKI ( n = 60 ) | AKI (n =18 ) | p | ROC AUC |
|---|---|---|---|---|
| NGAL (ng/mL) | 349 ( 168,651) | 562 (358,847) | <0.05 | 0.65 |
| KIM-1 (pg/mL) | 313 ( 129,592) | 711 ( 307,1190) | <0.01 | 0.73 |
| IL-18 (pg/mL) | 75 (26,385) | 192 ( 87,810) | <0.05 | 0.67 |
| Osteopontin (ng/mL) | 214 (136,329) | 379 ( 231,536) | <0.05 | 0.72 |
| Cystatin C (ng/mL) | 1776 (335,4560) | 2884 ( 1373,4545) | 0.39 | 0.58 |
| β2 Microglobulin (ug/mL) | 1.6 (0.9,2.4) | 1.7 ( 1.1,3.3 ) | 0.33 | 0.59 |

Median (25%,75%) AUC for AKI

Conclusion: Urine concentration of 4/6 candidate biomarkers are elevated in VLBW infants with AKI. Single or panel biomarkers may improve our ability to detect AKI, predict outcomes, and design interventions in this population.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2084**

**Urine Biomarkers of Acute Kidney Injury (AKI) Predict Mortality in Very Low Birthweight (VLBW) Infants** David J. Askenazi,[1] Stuart Goldstein,[2] Chirag R. Parikh,[3] Prasad Devarajan,[2] Ravindra L. Mehta,[4] Anupam Agarwal.[5] *[1]Pediatrics, University of Alabama, Birmingham, AL; [2]Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [3]Medicine, Yale, New Haven, CT; [4]Medicine, University of California, San Diego, CA; [5]Medicine, University of Alabama, Birmingham, AL.*

Background: AKI is a strong predictor of mortality in VLBW infants. Novel urine biomarkers of AKI predict mortality in other populations. We evaluate the utility of 6 biomakers to predict mortality in VLBW infants.

Method: Urine samples were collected on postnatal days 1 through 6 for 105 VLBW infants in whom 13 died at (age 26 +/- 6 days). The maximum concentration for each biomarker was compared between survivors and non-survivors.

Results: Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), interleukin 18 (IL-18) and osteopontin (OPN) levels were higher in non-survivors vs. survivors, while cystatin C and B2 M were not statistically different.

| | survived (n=94) | died (n=13) | p | ROC AUC |
|---|---|---|---|---|
| NGAL (ng/mL) | 376 ( 181,569) | 555 (300,1176) | <0.05 | .68 |
| KIM-1 (pg/mL) | 315 ( 146,611) | 384 ( 252,1215) | <0.01 | .66 |
| IL-18 (pg/mL) | 93 (54,353) | 114 (40,213) | <0.05 | .50 |
| Osteopontin (ng/mL) | 229 (169,354) | 480 (280,631) | <0.05 | .78 |
| Cystatin C (ng/mL) | 1938 (570,4469) | 1884 ( 400,4588) | 0.39 | .47 |
| β2 Microglobulin (ug/mL) | 1.7 (1.1,2.5) | 1.7 ( 0.9 ,2.9 ) | 0.33 | .51 |
| GA ( wks ) | 27 +/- 3 | 25 +/- 2 | < 0.01 | |
| BW (gm) | 1000 +/- 304 | 681 +/- 237 | < 0.01 | |

Median (25-75%) , AUC for Mortality

Conclusion: Urine AKI biomarkers predict survival in VLBW infants. Single or panel biomarkers may improve our ability to detect predict outcomes, and design intervention studies in this population.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2085**

**Urine Biomarkers for Gentamicin-Induced Acute Kidney Injury in the NICU** Suzanne Heemskerk,[1,2] Diana Jansen,[1] Linda Koster-Kamphuis,[3] Martijn P. W. J. M. Bouw,[1] Arno F. J. van Heijst,[4] Peter Pickkers.[1] *[1]Intensive Care Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Pediatric Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [4]Neonatal Intensive Care Unit, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

*Background:* Gentamicin (GM) is an aminoglycoside frequently used in the neonatal intensive care unit (NICU) to treat infections. Despite low resistance and costs, GM is also nephrotoxic and may cause acute kidney injury (AKI). Serum creatinine appears to be an insensitive and unreliable marker in this setting.

*Objective:* To determine whether urine biomarkers are useful for early detection of gentamicin-induced AKI in neonates in the NICU.

*Subjects:* Twenty-five neonates (18M/7F, gestational age 35.4 weeks) with a bladder catheter without pre-existent kidney disease were divided in a GM group (n=13) and a reference group (n=12).

*Study design and procedures:* A prospective, clinical observational trial with non-invasive procedures. Demographics, vital signs and clinical conditions were recorded. Every two hours, during the period of bladder catheter, urine samples were collected and renal injury biomarkers glutathione S-transferase A1-1 (GSTA1-1), GSTP1-1, Kidney Injury Marker-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) were determined. Residual blood samples were used to measure serum creatinine (sCr).

*Results:* Demographics were similar between both groups expect for gender. No significant differences were found in baseline kidney function, hemodynamics, ventilation support and reason for admission.Treatment with GM resulted in higher levels of sCr compared to the reference group (80[64-87] vs 60[55-76] µmol/L;P<0.05). The average time until the highest peak was shorter for all biomarkers compared with sCr (P<0.05). Furthermore, higher levels of sCr corresponded with higher levels of KIM-1, GSTA1-1 and GSTP1-1.

*Conclusion:* Treatment with GM results in higher levels of sCr. In addition, the urinary biomarkers KIM-1, GSTA1-1 and GSTP1-1 might be useful for early detection of AKI in the NICU.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2086**

**Predicting Acute Kidney Injury Development after Cardiac Surgery in Children Using Neutrophil Gelatinase Associated Lipocalin (NGAL)** Ana Carolina Kozak, Thayza Lopes, Emmanuel A. Burdmann, Emerson Quintino Lima. *Nephrology Division, Sao Jose do Rio Preto Medical School, Sao Jose Rio Preto, Sao Paulo, Brazil.*

**Introduction:** Serum creatinine is currently the most used biomarker for diagnosis of acute kidney injury (AKI). The aim of this study was to evaluate the role of urinary NGAL excretion as an AKI biomarker after cardiac surgery in children. **Methods:** Children (<18

y) submitted to elective cardiac surgery were enrolled. AKI was defined as a reduction in baseline estimated GFR (Schwartz formula) ≥25% (pediatric RIFLE) in the first 4 days after surgery. Urinary NGAL was measured by an ELISA kit (BioPorto, Denmark) before and 2, 4, 6, 12, 24, 48, 72 and 96 hours after surgery or cardiopulmonary bypass (CPB) beginning. Pre, intra and postoperative data were evaluated by unpaired t-test, Mann Whitney and Fisher's exact tests, as appropriated. The performance of urinary NGAL as an AKI biomarker was evaluated by ROC curve. P<0.05 was considered significant. **Results:** 96 children submitted to cardiac surgery were assessed. AKI developed in 19 (20%) patients. There was no difference regarding weight, preoperative estimated GFR and previous cardiac surgery between AKI and non AKI groups. Children who developed AKI were younger (29.8 ± 43.3 months vs 52.8 ± 51.7 months in non-AKI, P=0.01). The CPB duration (111 ± 51 min vs 67 ± 49 min in non AKI; P<0.001), surgery time (243 ± 78 min vs 170 ± 71 min in non AKI; P<0.001) and anesthesia time (332 ± 93 min vs 253 ± 88 min in non AKI; P=0.001) were higher in the AKI group. The median urinary NGAL was higher in AKI patients after 2 h (43 ng/ml [ 10-100] vs 4 ng/ml [0.9-22]; P=0.001), 4 h (27 ng/ml [3-300] vs 3.7 ng/ml [1.6-15.8]; P=0.005) and 6 h (11.7 ng/ml [2.4-33] vs 2.5 ng/ml [0.9-10.8]; P=0.03) following CPB or surgery beginning and preceded the rise in serum creatinine for at least 12h. The ROC curve areas for urinary NGAL 2 h, 4 h and 6 h after CPB were 0.74 (95% CI 0.62-0.87; P=0.008), 0.72 (95% CI 0.59-0.85; P=0.002) and 0.71 (95% CI 0.58-0.84; P=0.004), respectively. **Conclusions:** Urinary NGAL was an efficient and early biomarker for diagnosis of AKI development after heart surgery in children.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2087

**Renal Outcomes Following Acute Kidney Injury in the Pediatric Intensive Care Unit: A Prospective Cohort Study** Cherry Mammen,[1] Douglas G. Matsell,[1] Daniel S. Levine,[3] Helen Ruth Nadel,[3] Jean Paul Collet.[2] *[1]Division of Nephrology, UBC/BC Children's Hospital, Vancouver, BC, Canada; [2]Dept. of Pediatrics, UBC/BC Children's Hospital, Vancouver, BC, Canada; [3]Dept. of Radiology, UBC/BC Children's Hospital, Vancouver, BC, Canada.*

**Background**: The development of standardized AKI definitions has allowed for a better understanding of AKI epidemiology, but the long term renal outcomes of AKI in the pediatric critical-care setting have not been well-established. **Objectives**: 1) To determine the incidence of chronic kidney disease (CKD) in children who sustained AKI at a tertiary care PICU from 2006-2008 and 2) To explore the relationship between AKI severity and the probability of developing CKD 1-3 years following AKI. **Methods:** All AKI patients admitted to BC Children's Hospital's PICU from 2006-2008 were identified by their maximal AKI stage: 1 (mild), 2 (moderate) or 3 (severe), according to the Acute Kidney Injury Network (AKIN) definition. Patients were contacted for a renal assessment at either 1, 2, or 3 years following their AKI. The outcome of CKD was defined as the presence of either proteinuria, microalbuminuria, hypertension (HTN), or abnormal GFR through estimated or nuclear (DTPA) methods. The proportion of CKD patients from each AKI stage was compared by chi-square test. Multivariate logistic regression was used to model the association between several ICU admission characteristics (age, weight, diagnosis, PRISM score, use of acute dialysis, nephrotoxin use, lowest Hb and paO2) and the likelihood of developing CKD. **Results:** 31/108 (28.7%) patients revealed at least one sign of CKD (18: microalbuminuria or proteinuria, 16: abnormal GFR, 2: HTN). Incidence rate of CKD was 12 cases/100 person-years of follow-up. No significant differences were seen in the proportion of patients with CKD between the 3 AKI stages (p=0.332). None of the selected ICU admission factors predicted CKD. **Conclusions:** The severity of AKI, as defined by the AKIN classification system, does not predict long term renal injury in tertiary-care PICU patients. However, the high incidence of CKD in this population signifies that all critically-ill children should be followed-up regularly after an episode of AKI.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2088

**Degree and Outcome of AKI in Children and Neonates in a Single Pediatric Nephrology Center** Isabel Roberti, Shefali Vyas. *Pediatric Nephrology and Transplantation, Saint Barnabas Medical Center, Livingston, NJ.*

Data is sparse regarding the **degree and outcome of AKI in pediatrics** despite an increase in incidence of AKI in hospitalized children.

We reviewed consults between January 2008 and March 2010 with the diagnosis of AKI - 125 children (27 s/p kidney txp, 7 with known CKD and 91 with new onset AKI). Of these 91, 4 were lost to f/u. Demographic data, etiology of AKI, co-morbidities, clinical findings, need for dialysis and outcome were analyzed by location (ICU, PICU, Floor) for 87 children.

Seven of the 87 children had 2 or more episodes of AKI. Demographics: M/F = 47/40; Ethnicity: C/AA/H/O = 26/40/17/4; Age: 1 day - 19yrs (median NICU = 10d, PICU and Floor = 11yrs).

**Characteristics of AKI in 87 Children**

| Variable | NICU N= 34 | PICU N=17 | Floor N= 36 |
|---|---|---|---|
| AKI grade: R/I/F/L# | 0/3/27/4 * | 0/3/9/5* | 4/17/14/1 |
| Etiology: PR/R/PostR## | 18/11/5 | 7/9/1 | 24/9/3 |
| HTN | 2 (6%) | 7(41%)* | 5(14%) |
| Dialysis | 3(10%)* | 6(35%)* | 1(0.02%) |
| Outcome: D/R/CKD/ERSD### | 13*/17/4/0 | 2/10/3/2 | 1/31*/4/0 |

#=pRIFLE; ##PR=pre-renal, R=Renal, PostR= Post renal; ### D=death, R=complete recovery; *p<0.05

**Pre-Renal AKI (N=31):** NSAIDs induced AKI was seen in 1/7 (14%)(PICU) and 5/24 (21%)(Floor).

**Renal AKI (N=18)(Floor and PICU):** AGN (variable diagnosis) was the most common etiology -Floor (5/9 = 55%), PICU (4/9 = 44%) - Postinfectious GN - 2/18 (11%), HUS 4/18(22%).

As expected, **AKI grade was significantly higher in the PICU and NICU** compared to the Floor. However, 5/15 (29%) children with AKI on the Floor progressed to CKD or ESRD. Overall 13/87 (15%) had CKD or ESRD on f/u. **The grade of AKI did not correlate with outcome**. Among **children who required dialysis** (N=7) (PICU+Floor), 4 **(57%) developed CKD or ESRD - p<0.01**.

**AKI in the NICU cohort had multiple co-morbidities and significantly higher mortality** (38%); 6 had severe IUGR, 6 were twins or triplets, 19 were extreme premies with Bwt<1000g.

We conclude that **the grade of AKI presentation is high** in all the units and the prognosis is particularly poor in neonates due to multiple co-morbidities. The need of long-term f/u of AKI survivors is imperative in view of the high rate of CKD and ESRD.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2089

**Acute Kidney Injury in Congenital Diaphragmatic Hernia Requiring Extracorporeal Life Support: An Insidious Problem** David T. Selewski, Samir K. Gadepalli, Robert Drongowski, David B. Kershaw, George Mychaliska. *Mott Children's Hospital, University of Michigan, Ann Arbor, MI.*

**Purpose:** Patients with congenital diaphragmatic hernia (CDH) requiring extracorporeal life support (ECLS) are at increased risk for acute kidney injury (AKI.) We hypothesized that AKI would be associated with increased mortality. We also hypotesized that vasopressor requirement and nephrotoxic medication exposure would be associated with AKI.

**Methods:** This is a single center retrospective chart review in all patients with CDH requiring ECLS from 1999-2009 (n=68). Patient variables that could potentiate renal failure were collected. We used a rise in creatinine from baseline by the RIFLE (risk: 1.5x, injury: 2x, failure: 3x, loss, and end-stage renal disease) criteria to define AKI. Statistical analysis was performed via SPSS using student's t-test and Chi-square analysis with p < 0.05 considered significant.

**Results:** Survival to hospital discharge was 37/68 (54.4%). AKI was identified in 48/68 (71%) patients with 15 (22% of all patients) qualifying as injury and 33 (49% of all patients) qualifying as failure by the RIFLE criteria. Patients that qualified as failure by the RIFLE criteria had a significant decrease in survival (27.3% with failure vs. 80% without failure, $p$ =0.001.) This represents an absolute increase in mortality of 52.7% when patients qualified as failure. Patients who qualified as failure also had increased length of ECLS (314 ±145 vs. 197 ±115 hours, $p$=0.001) and decreased ventilator free days in the first 60 days (3.83±8.5 days vs. 25.21±17.8, $p$=0.001). There was no significant difference in survival when patients qualified as risk or injury. AKI was not associated with vasopressor requirements, diuretic exposure or antibiotic days.

**Conclusions:** This is the first report using a systematic definition of AKI in patients with CDH on ECLS. There is a high incidence of AKI in these patients and when it progresses to failure is associated with higher mortality, increased ECLS duration and increased ventilator days. This highlights the importance of recognizing AKI in CDH patients requiring ECLS and the potential benefit of preventing progression of AKI or early intervention.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2090

**Weight-Based Determination of CRRT Fluid Overload Status Predicts Pediatric ICU Mortality** David T. Selewski, Theresa Mottes, Rebecca M. Lombel, Mallika Kommareddi, Neal B. Blatt, Yong Y. Han, Michael Heung. *University of Michigan, Ann Arbor, MI.*

**Purpose:** In pediatric patients fluid overload (FO) at continuous renal replacement therapy (CRRT) initiation is an independent risk factor for mortality. Previous studies have calculated FO based on daily fluid balance during ICU admission, which is labor intensive and error-prone. We hypothesized that a simpler weight-based assessment of FO at CRRT initiation would be effective in predicting mortality.

**Methods:** This is a retrospective single-center review of all PICU patients requiring CRRT from 07/2006-02/2010 (n=119). Percent FO was determined by a published standard [Method 1: (fluid in-fluid out from ICU admission to CRRT start)/ICU admission weight] and by a weight-based method [Method 2: (CRRT initiation weight-ICU admission weight)/ICU admission weight]. We calculated %FO at CRRT discontinuation: (CRRT discontinuation weight-ICU admission weight)/ICU admission weight. Median [IQR] %FO was compared between survivors and non-survivors using Mann-Whitney U-test. Mortality odds ratios (OR) were determined by logistic regression modeling adjusted for age and PRISM score.

**Results:** ICU mortality for this cohort was 58%. Percent FO at CRRT initiation was significantly different between survivors and non-survivors (Method 1: 8.0% [2.0-14.0] vs 23.0% [12.0-37.0], $p$<0.001; Method 2: 3.0% [0-15.0] vs 16.0% [3.0-38.0], $p$=0.001). FO scores by each method were significantly correlated ($r$=0.77, $p$<0.001). The adjusted mortality OR for a 1% increase in FO was 1.05 (95%CI 1.01-1.09) by Method 1 and 1.04 (95%CI 1.01-1.07) by Method 2. At CRRT discontinuation survivors had lower %FO than non-survivors (2.7% [-4.7-9.7] vs 11.1% [-1.0-23.5], $p$=0.001).

**Conclusion:** Our results confirm that FO is associated with greater mortality in PICU patients requiring CRRT. We have extended the literature by demonstrating that weight-based assessments of both initial and discontinuation %FO have prognostic significance. We propose weight-based FO determination as a simple and effective way to routinely assess FO in the ICU setting.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2091**

**Impact of AKI on Development and Progression of CKD** Neville R. Dossabhoy,[1,2] Cherinet Adgeh,[1,2] Mukesh Sharma,[1,2] Sunanda J. Ram.[2] *[1]Medicine, VA Medical Center, Shreveport, LA; [2]Medicine, LSUHSC, Shreveport, LA.*

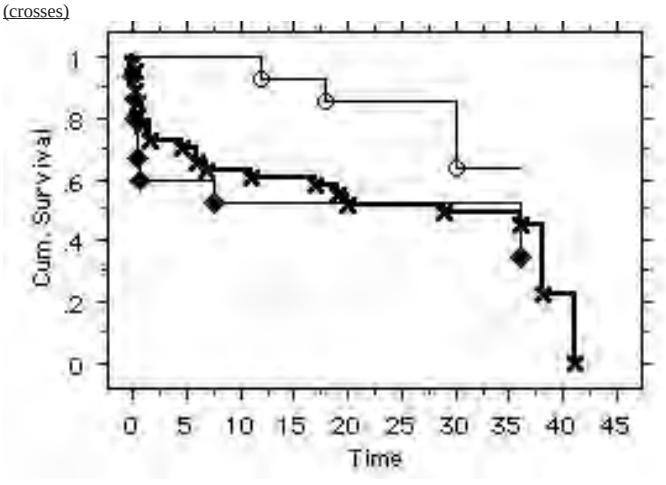
Background: Emerging evidence indicates that the incidences of Acute Kidney Injury (AKI) and CKD are rising. However, much of the growth of End Stage Renal Disease (ESRD) seems independent of the growth in CKD. Relatively little is currently known regarding the potential for evolution from AKI to CKD, or the impact of AKI on CKD progression.

Purpose: To study the impact of AKI episodes on development of CKD and on progression of preexisting CKD.

Methods: We conducted a retrospective analysis of clinical data spanning a 3 year period from 2006-09. Patients with CKD (stages 3 and 4) and AKI were identified using ICD codes. Patient demographics and comorbid conditions were noted. Lab values were recorded including baseline creatinine and GFR, and serial GFR's after AKI. Primary end points of this study were rate of progression of CKD after AKI insult; mortality; and incidence of ESRD in patient with AKI, CKD, and AKI on CKD. Kaplan-Meier survival curves were plotted for outcomes of ESRD and death.

Results: 80 patients with complete data were analyzed. Those with >1 episode of AKI were excluded. Mean age 67±1 years; all males; follow-up upto 38 months. 16 had AKI only, 16 CKD only, and 48 had AKI on CKD. AKI did not significantly impact on time to ESRD (P=NS). There was a trend towards AKI increasing patient mortality, regardless of presence of baseline CKD.

Survival curves for CKD only (open circles), AKI only (diamonds) and AKI on CKD (crosses)




Conclusion: AKI may worsen risk of death but did not significantly affect attainment of ESRD compared to patients with preexisting CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2092**

**Long-Term Clinical Outcomes in Patients with Chronic Kidney Disease Complicated by Dialysis-Requiring Acute Kidney Injury** Po-Hung Lee, Yung-Ming Chen, Tun-Jun Tsai, Kwan-Dun Wu. *Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan.*

**Background**: Information is limited regarding the clinical outcome in patients with preexisting chronic kidney disease (CKD) who developed dialysis-requiring acute kidney injury (AKI). This study investigated the long-term outcome of these patients, with emphasize on patient overall survival and end-stage renal disease (ESRD).

**Patients and Methods**: Between January and June in 2002, we consecutively recruited patients with CKD stage 3 or worse who received emergent hemodialysis via a temporary catheter due to fluid overload or uremic emergency. These patients were monitored over a period of 5 years for all-cause mortality and ESRD till the end of 2007. All parameters and biochemical data were obtained by reviewing medical charts by a nephrologist. Factors predicting clinical outcomes were analyzed with appropriate statistics.

**Results**: Among the 131 patients investigated, 21 (16%) were successfully withdrawn from acute hemodialysis for at least 3 months after an average of 8 sessions of dialysis therapy (ranging from 1 to 44, group A). In contrast, 110 (84%) patients were unable to be withdrawn from acute hemodialysis (group B). Multivariate logistic regression revealed that larger kidney size, lower predialysis Cr levels, and not having diabetes were predictors for withdrawl from dialysis. During the subsequent 5 years, all patients in group B became ESRD requiring chronic renal replacement therapy (RRT), whereas only 8/21 (38%) patients in group A developed ESRD. Being diabetes and hyperkalemia at basal were predictors for ESRD in the latter group. Further analysis with Cox's proportional hazards model showed that older age, prerenal azotemia, and propensity score for assigning to dialysis withdrawl were independent risk factors for death from any cause. There was no statistically significant difference in all-cause mortality between groups A and B patients.

**Conclusion**: 10% (13/131) of our CKD patients with dialysis-requiring AKI could be removed from acute RRT and remain dialysis-free after 5 years. These patients were mainly nondiabetics, having larger kidney size, and displaying lower Cr and potassium levels at baseline.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2093**

**Is the Severity of Acute Kidney Injury (AKI) Associated with Long-Term Mortality?** Mariana B. Pereira,[1] Dirce M. T. Zanetta,[2] Regina C. R. M. Abdulkader.[1] *[1]Discipline of Nephrology, School of Medicine. University of S. Paulo, Brazil; [2]School of Public Health University of S. Paulo, Brazil.*

AKI increases long-term mortality. However, the causes and factors associated with long-term mortality have not been studied. We studied 530 patients 1 and 2 years after AKI, defined by AKIN criteria (creatinine). Exclusion criteria: age<18 years, presumed etiology other than acute tubular necrosis, baseline creatinine > 3.5 mg/dL, renal transplant and unknown outcome. Analyzed variables: age; gender; type of admission (medical or surgical); patient location (ward, emergency service or ICU); AKI etiology (ischemia, nephrotoxicity, sepsis); baseline and discharge GFR (evaluated by MDRD equation); AKIN classification(1, 2, 3); need for dialysis or mechanical ventilation; comorbidities (hypertension, diabetes, heart failure, cancer, chronic liver or pulmonary diseases). Causes of death were identified by death certificate and classified by ICD-10. Survivors and nonsurvivors were compared 1 and 2 years after hospital discharge. Multivariable logistic regression was used to analyze the independent variables associated with mortality, by backward stepwise method. Goodness of fit was evaluated by the Hosmer-Lemeshow test. One- and two-year mortality were respectively: 27% and 37%. Adjusted odds ratios (OR) and 95% confidence intervals (CI) are presented in the table.

| | OR (CI) | P |
|---|---|---|
| At 1 year | | |
| Clinical admission | 2.78 (1.68-4.60) | <0.01 |
| Chronic liver disease | 2.65 (1.30-5.42) | <0.01 |
| Heart failure | 1.69 (1.03-2.77) | 0.03 |
| Cancer | 3.44 (2.02-5.85) | <0.01 |
| At 2 years | | |
| Age >65 years | 1.54 (1.03-2.32) | 0.03 |
| Clinical admission | 2.23 (1.38-3.61) | <0.01 |
| Heart failure | 2.37(1.47-3.81) | <0.01 |
| Chronic liver disease | 3.05 (1.48-6.29) | <0.01 |
| Cancer | 3.40 (1.99-5.82) | <0.01 |

Causes of death were similar (P<0.05) at 1 and 2 years after discharge, respectively: diseases of circulatory (26 and 39%), pulmonary (15 and 17%), and digestive systems (12 and 5%), or malignant neoplasms (22 and 12%). In conclusion, long-term mortality after AKI is associated with previous patient´s condition, not with the AKI severity.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2094**

**Chronic Renal Replacement Therapy (C-RRT) after Acute Renal Failure (ARF) Treated with Acute Dialytic Support in a Big Population** Maria Teresa Tenorio,[1] Fernando Liano.[2] *[1]Nephrology, Hospital Universitario Ramon y Cajal; [2]On Behalf of the Madrid Acute Renal Failure Study Group (GEFRAM).*

The new ARF classification systems, the introduction of the use of AKI and the analysis of huge administrative data bases suggest that a great number of AKI patients could be on chronic dialysis in the future.

The aim of this work is to test if this hypothesis is true in our community.

Methods: Observational, multicenter, retrospective study analyzing how many of the patients, who started chronic hemodialysis (C-HD) or peritoneal dialysis (CPD) in the Madrid region (6.328.106 inhabitants) along 2009, had an AKI episode that required acute RRT, either in 2008 or 2009. Only AKI patients having a free-dialysis period after hospital discharge or those who were on dialysis for more than 3 months were included. Twenty hospitals and 15 satellite units (SU) with c-RRT facilities covered these treatments in Madrid. The population served by each hospital was provided by local authorities. A simple questionnaire was mailed. Minimal disagreements were later resolved.

Results: Adequate information, corresponding to 91% of the Madrid population, was received from 19 hospitals and all the SU. On 2009 December 31st, 2040 patients were on CHD (339.9 pmp/y) and 341 on CPD (56.8 pmp/y). Along 2009, 790 patients started c-RRT (incidence:131 pmp/y) due to: diabetes, 24.3; glomerulonephritis (GN), 17.4; vascular dis, 17.1; interstitial dis, 8.8; PKD, 8.1; other congenital 1.1; others 9.6 and unknown 13.5 % respectively. Among the incident patients, 54 (7%) had needed acute RRT for an ARF episode in 2008 or 2009. This figure, representing an incidence of chronic RRT due to severe ARF of 9 cases pmp/y, has the following etiology: 13 acute tubular necrosis, 10 GN, 8 vasculitis, 7 other causes and unknown origin in 16. Of these patients,53 were placed on C-HD.

Conclusions: The contribution of the ARF needing dialytic treatment during the acute episode over the total incidence of c-RRT in Madrid is important, but that of the paradigmatic form of the syndrome, ATN is small (incidence 2 pmp/y). These data should be borne in mind when the new denomination, AKI, is used as its utilization is not uniform.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2095**

**Survival and Quality of Life in Patients with Acute Kidney Injury (AKI)** Denise H. M. P. Diniz,[1, 3] Marcelo Andery Naves,[1, 3] Nestor Schor,[1] Sergio L. Blay.[2] *[1]Division of Nephrology, Department of Internal Medicine, Federal University of São Paulo, São Paulo, SP, Brazil; [2]Departament of Psychiatric, Federal University of São Paulo, São Paulo, SP, Brazil; [3]Division of Stress and Quality of Life, Federal University of São Paulo, São Paulo, SP, Brazil.*

Background: Survival and Quality of life (QoL) has not been a priority goal for patients with AKI. Aim: To evaluate survival rate and QoL domains in patients with AKI in two public hospitals in Sao Paulo, Brazil. Methods: Study design: Case-control study. Setting: Hospital São Paulo-UNIFESP and Hospital dos Servidores do Estado –HSPE in São Paulo,Brazil. Study participants: Cases and controls were selected among patients admitted at ICU during 2008 and 2009. Patients were randomly selected after, inclusion and exclusion criteria were verified at an initial interview. Cases were patients at ICU with a confirmed diagnosis of AKI, with no chronic comorbities, that needed renal therapeutic support. The control group consisted of patients at ICU with acute diseases. Out of 579 patients initially identified, 372 (156 cases and 216 controls) survived up to the first application of SF-36, but 284 patients (142 cases and 142 controls) were matched according to age and gender. Main outcome measures: QoL was assessed through a Portuguese version of the SF-36 Health Survey. Results: Significant differences in mortality rate were observed between cases and controls. Out of 311 cases total number of identified cases), 155 died ( 49.84%) and 52 controls out of 268 (total of controls) died (19.40%). There was a significant correlation between AKI and death rate. Beside, there was also significant correlation between dialysis and death rate, in both hospitals. However, in the UNIFESP there was also association between death rate and health insurance (social security and private security). Other significant differences were observed according to QoL domains, like general health condition( 0.007), vitality ( 0.004), mental health (0.016).. Conclusion: Our data demonstrated that mortality rates are higher and QoL domains are significantly impaired among cases when compared to controls.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2096**

**AKI Accelerates Progression of CKD: Prospective Cohort Study** Ha Na Yang, Hye Won Kim, Eunjung Cho, Inhye Cha, Won-Yong Cho, Hyoung-Kyu Kim, Sang-Kyung Jo. *Department of Internal Medicine, Korea University Anam Hospital, Seoul, Korea.*

Although recent studies showed the important role played by acute kidney injury(AKI) in the progression of chronic kidney disease(CKD), lack of prospective controlled studies makes it difficult to reach a meaningful conclusion. The purposes of this study was to examine whether the presence of AKI accelerates progression of CKD and also to examine whether severity of AKI or new biomarkers could be served as useful predictor of CKD progression. We constructed a cohort of CKD patients who were hospitalized under diagnosis of AKI on CKD(n=93). We classified severity of AKI by RIFLE criteria and measured initial serum, urine NGAL and urine KIM-1. We also constructed an age-sex matched cohort for control of CKD patients without AKI episodes(n=89). We followed-up their clinical course every three months, and observed change of eGFR for primary outcome. The secondary outcome was defined as doubling of serum creatinine, initiation of maintenance renal replacement therapy or all-cause mortality. Kaplan-Meire curve was used to obtain probability to reach to the outcomes and we also assessed the prognostic value of RIFLE criteria and biomarkers of AKI by logistic regression. Mean age in AKI on CKD cohort were 65.14 ± 14.95 yrs(male:44.1%). The prevalence of diabetes and hypertension was 56.5 and 73.9% and the causes of AKI were ischemic 39.8%, septic 35.2% and toxic 13.6%. Severity of AKI were risk(33%), injury(26.1%), and failure(40.9%). Mean follow-up period were 116.24 ± 98.27days. Change of eGFR 6 months later were -3.54 in AKI on CKD cohort, and -0.17 mL/min/1.73 $m^2$ in stable CKD cohort ($p<0.05$). Kaplan-Meier curve showed significantly higher probability to reach to secondary outcome in AKI on CKD cohort compared to stable CKD cohort(p=0.03). Although failure in RIFLE criteria also tended to show higher probability of progression (p=0.07), initial NGAL or KIM-1 could not predict the progression. In conclusion, this prospective study shows that episodes of AKI definitely accelerate the progression of CKD and might also suggest the usefulness of RIFLE criteria in prediction of CKD progression.

Disclosure of Financial Relationships: nothing to disclose

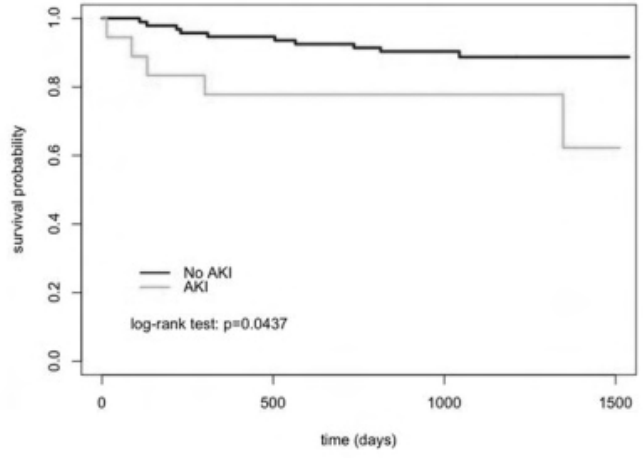
**SA-PO2097**

**The Long Term Impact on Survival of Acute Kidney Injury after Liver Transplantation** Elena Mancini,[1] Antonio Bellasi,[1] Alessia Mordenti,[2] Sandra Giannone,[2] Andrea Zanoni,[2] Antonio Santoro.[1] *[1]Nephrology Unit, Policlinico S.Orsola-Malpighi, Bologna, Italy; [2]Intensive Care Unit, Policlinico S.Orsola-Malpighi, Bologna, Italy.*

Data on long-term impact on survival of Acute Kidney Injury (AKI) after surgery are still scanty. In particular, only a few papers reported on a follow up longer than 1 year. Thus, we tested the association between AKI and the 4-year all-cause mortality in a cohort of patients undergoing Orthotopic Liver Transplantation (OLT), a procedure frequently complicated by AKI.

Demographic and clinical variables of all consecutive patients undergoing OLT between January 2006 and December 2007 at our institution were collected at study entry and during the first week after surgery. AKI was defined according to the AKIN criteria as a 50% o greater increase of serum creatinine during the first week after surgery. Patients were followed until study end or death.

We recruited 144 middle-age [53.4 (11.7)] male (74.7%) and female (25.3%) patients. The most common cause of liver failure was a hepatotropic viral infection (75.7%). The median MELD score was 21 [Interquartile Range (IQ): 12-27]. The median follow-up time was 37.2 [IQ: 28.7-44.4]. The overall incidence of AKI within 7 days from surgery was 16.2%. As illustrated in figure 1, patients who developed AKI were at increased risk of death (log-rank test $p<0.0437$).




Even after adjustment for age, sex, liver failure etiology and graft function, patients with AKI had a significant 268% increase in the risk of all-cause mortality (Hazard ratio 3.68; 95% confidence interval 1.15-11.71; p=0.02).

Early AKI complicating OLT has a strong impact on patient long-term survival. Concerted efforts should be undertaken to limit the occurrence of such devastating complication in the peri-operative period after OLT.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2098**

**Long Term Effect on Renal Function of Chronic Kidney Disease Patients by Radiocontrast Media** Il Young Kim, Soo Bong Lee, Jungmin Son, Harin Rhee, Jung Sub Kim, Sang Heon Song, Eun Young Seong, Ihm Soo Kwak. *Internal Medicine, Pusan National University School of Medicine, Busan, Republic of Korea.*

**Background**: The risk of acute kidney injury(AKI) by intravascular radiocontrast in patients with chronic kidney disease(CKD) has been well known, but long term effect on renal function in patients with CKD has been less evaluated.

**Methods**: We retrospectively reviewed medical records of 176 CKD patients with estimated glomerular filtration rates (eGFR) <60 ml/min/1.73$m^2$ who underwent computed tomography (CT) with intravenous radiocontrast at Pusan National University Hospital. Patients were divided into 3 groups(CKD stage 3, N=104; CKD stage 4, N=52; Peritoneal dialysis(PD), N=20). We used reciprocal of serum creatinine(1/sCr) as a parameter of renal function. Baseline 1/sCr and follow-up 1/sCr after CT was tracked in each patients. Follow-up 1/sCr values were assessed in monthly based manner(up to 8 months). Statistical analysis was performed by paired t-test using values before and after CT.

**Results**: In baseline characteristics, there are no significant differences among the 3 groups(CKD stage 3, stage4, PD) in variables of sex(Male(%); 47.2% vs 59.6% vs 60%, p=0.256), diabetes(40.3% vs 53.8% vs 55%, p=0.094), and age(68.29±10.21 vs 67.15±11.44 vs 65.00±9.62, p=0.448). There are no significant differences between values of 1/sCr before and after CT in each of 3 groups. (CKD stage 3: 0.6847 ± 0.1287 vs 0.6832 ± 0.1596, p=0.897, CKD stage 4: 0.4475 ± 0.1225 vs 0.4389 ± 0.0732, p= 0.474, Peritoneal dialysis: 0.1133 ± 0.0451 vs 0.1102 ± 0.0348, p=0.171). In each group, data analysis according to the presence of diabetes mellitus (DM) did not show significant differences of 1/sCr before and after CT

**Conclusion**: Overall, these results illustrate that intravenous contrast using in CT scan has no significant long term effect on renal function in CKD patients irrespective of DM. Large prospective study could give more solid evidence.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2099**

**Post-Operative Acute Kidney Injury in Patients with Renal Cell Carcinoma Is a Potent Risk Factor for New-Onset Chronic Kidney Disease after Radical Nephrectomy** Ajin Cho, Junseok Jeon, Hye Ryoun Jang, Wooseong Huh, Yoon-Goo Kim, Dae Joong Kim, Ha Young Oh, Jung Eun Lee. *Internal Medicine, Samsung Medical Centre, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.*

Radical nephrectomy is a significant risk factor for chronic kidney disease (CKD). The aim of this study was to determine the incidence of AKI and whether post-operative AKI is associated with new-onset CKD after radical nephrectomy for renal cell cancer (RCC).

We conducted a retrospective study of 519 adult patients (>40 years old) with normal renal function who underwent unilateral radical nephrectomy for a solitary renal cortical tumor and were pathologically diagnosed with RCC between January 2000 and February 2007. Postoperative AKI was classed using risk, injury, failure, loss, and end-stage kidney disease (RIFLE) criteria. To evaluate the renal outcome of post-operative AKI, serum creatinine levels at 3 months, 12 ±3 months, and 36 ±3 months after the nephrectomy were recorded for all patients. CKD was defined as a decrease in estimated glomerular filtration rate (GFR) to less than 60 mL/min per 1.73 $m^2$. One hundred and seventy-five (33.7%) patients experienced post-operative AKI. Most of them (94%) fell into the AKI risk category. Older age [odds ratio (OR) 1.02, 95% confidence interval (C.I.) 1.00–1.05], male gender (OR 3.13, 95% C.I. 1.91–5.12), high body mass index (OR 1.08, 95% C.I. 1.01–1.15), small RCC size (OR 0.87, 95% C.I. 0.81–0.93), and high preoperative GFR (OR 1.04, 95% C.I. 1.03–1.06) were independent risk factors for postoperative AKI. CKD was more prevalent in the AKI group than in patients without AKI 3 years after surgery (50% vs. 32%, respectively; *P*=0.003). Patients who experienced postoperative AKI had a 4.24-fold higher risk of new-onset CKD after multiple adjustments were made to the data (95% C.I. 2.28–7.89, *P*<0.001). Postoperative AKI was a potent risk factor for new-onset CKD after radical nephrectomy in patients with RCC. Prevention of postoperative AKI is essential for reducing the incidence of CKD after nephrectomy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2100

**Incidence, Etiology, and Outcome of Acute Renal Failure in Patients with Influenza A during 2009-2010 Season** Wiroon Sangsiraprapha,[1] Vikyath Prakash,[1] George M. Dolson.[1,2] *[1]Internal Medicine, Baylor College of Medicine, Houston, TX; [2]Nephrology, Houston Veteran Affairs Medical Center, Houston, TX.*

Influenza A uncommonly causes acute kidney injury (AKI). We described a patient with influenza A and AKI due to rhabdomyolysis (index case, Clin Micro Infect 16, 330, 2010), then retrospectively reviewed influenza A cases at 2 urban hospitals to determine the incidence, etiology, and outcome of AKI.

Positive influenza A was identified in 87 patients. Thirteen (14.3%) developed AKI (creatinine 0.9±0.1 mg/dL, increased to 4.9±1.0, p<0.02, mean±SE). AKI patients were older (50.2±3.7 years vs 32.4±2.1, p<0.001), had shortness of breath (92.3±7.7% vs 30.9±5.5 , p< 0.001), and pneumonia (76.9±12.1% vs 9.4±3.4, p< 0.001). HIV infection increased risk of AKI (30.7±13.3% vs 4.1±2.3, p<0.05). AKI patients more likely received antiviral medications (92.3±7.7% vs 52.8±5.9, P, <0.001). AKI increased risk of death (46.114.4% vs 4.12.3, p<0.01). Diabetes, obesity, coronary disease, chronic lung disease, malignancy, or immunosuppressiant did not increase risk of AKI.

Etiology and outcome of AKI varied among the 13 patients. Eight (61.5%) had acute tubular necrosis (ATN). Seven ATN patients had shock and ARDS with SOFA scores greater than 13. Rhabdomyolysis was present in 3 ATN patients. Six ATN patients died (46%). Five patients (38% of AKI, all with ATN) required renal replacement therapy (RRT). Among patients who received RRT, 3 died, 1 continued RRT, and 1 recovered renal function. AKI in 5 patients was prerenal (38.5%). All patients with prerenal AKI recovered.

In summary; 15% patients with influenza A developed AKI. These patients were older, had severe respiratory symptoms, and had a higher mortality than patients without AKI. Antiviral medications did not appear to decrease risk of AKI. Underlying HIV increased probability of AKI. ATN was the most common etiology of AKI associated with influenza A. Rhabdomyolysis occurred in 23% of the cases of AKI.

In conclusion, AKI developing during influenza A infection predicts a worse outcome with increased risk of death. Etiology of AKI appears multifactorial and associated with pulmonary and cardiovascular failure.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2101

**Acute Kidney Injury (AKI) at the Time of Nephrology Consultation (NC) in H1N1-Infected Patients** Márcia Fernanda Arantes Oliveira,[1] Regina C. R. M. Abdulkader,[1] Yeh Li Ho,[2] Sigrid S. Santos,[2] Renato Antunes Caires,[1] Lucia Andrade.[1] *[1]Nephrology Department, University of São Paulo School of Medicine; [2]Infectious Diseases Department, University of São Paulo School of Medicine, São Paulo, SP, Brazil.*

Early NC has been associated with a better prognosis in AKI. The characteristics of AKI that lead the intensivist to request a NC have not been studied. The H1N1 epidemic provided this opportunity. From July to August, 52 adult patients with H1N1 infection were treated in the intensive care unit (ICU). AKI was identified in 25 H1N1-infected patients. We defined AKI using the RIFLE criteria and based on an increase in serum creatinine ($S_{creat}$) level from baseline to 72 hours after ICU admission. We compared the patients to whom a NC was requested (NC+, n=16) with those who were never followed by the nephrologist (NC-, n=9). Continuous variables were compared by t-test or Mann-Whitney test, and presented as mean±SD or median (interquartile range). Categorical variables were compared by Fisher's exact test or qui-square test. NC+ patients were older (45 ±15 and 35±16 years, p=0.04) and 56% needed dialysis. NC+ patients had a trend to higher mortality (50% and 11%, P=0.08). Although the frequency of baseline GFR<75 mL/min/1.73$m^2$ was similar in both groups: 33% and 23%, NC+ patients had higher values of peak $S_{creat}$ up to 72 hs after ICU admission [2.89 (1.75-4.39) and 1.65 (1.57-2.84), p<0.01]. Other comorbidities had similar distribution in both groups.

| | NC+ | NC- |
|---|---|---|
| Mechanical ventilation (yes/no) | 13/3 | 6/3[a] |
| Vasopressor support (yes/no) | 10/6 | 9/0[b] |
| APACHE II | 18±6 | 15±3 |
| pH | 7.23±0.14 | 7.35±0.06[c] |
| Bicarbonate(mEq/L) | 20.4±6.6 | 21.7±4.2 |
| Creatine kinase (U/L) | 305 (139-788) | 160 (72-349) |
| LDH (U/L) | 959 (511-1951) | 1042 (559-2101) |
| C-reactive protein (mg/dL) | 177 (111-316) | 113 (90-156) |
| Lactate (mg/dL) | 25 (19-39) | 21 (14-34) |
| Bilirubin (mg/dL) | 0.8 (0.4-1.0) | 0.3 (0.2-0.7)[c] |

[a] p=0.02 vs. NC+; [b] p=0.05 vs. NC+; [c] p=0.04 vs. NC+

AKI was identified by 72 hours after ICU admission in 25 patients. However, only 64% of those were treated by a nephrologist. Those patients had higher baseline creat levels and were more severely ill.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2102

**Incidence and Clinical Characteristics of Acute Hepatitis A Complicated with Acute Renal Failure** So Yeon Choi,[2] Dong Hyun Sinn,[4] Eun Hee Jang,[3] Hyun-Jung Kim.[1] *[1]Medicine, Gyeongsang National University, Jin-Ju, Republic of Korea; [2]Medicine, Seoul Adventist Hospital, Seoul, Republic of Korea; [3]Medicine, Cheju National University, Cheju, Republic of Korea; [4]Medicine, Armed Forces Capital Hospital, Sung nam, Republic of Korea.*

Background/Aims: Acute renal failure (ARF) was regarded to be a rare complication of acute hepatitis A. However along with increase in the incidence of acute hepatitis A in Korea, more cases of ARF among acute hepatitis A patients are encountered nowadays. We aim to see the recent aspects of incidence and clinical characteristics of ARF in acute hepatitis A patients.

Methods: In overall, 363 patients (age; 27.6 ± 6.5, male; 263 (73%)) who had been diagnosed as acute hepatitis A at two local hospitals located in Korea (Seoul Adventist Hospital and Armed Forces Capital Hospital) during January 2002 to December 2009 were enrolled. Diagnosis of acute hepatitis A was made by confirmation of anti HAV-IgM antibody and corresponding symptoms and sign. ARF was defined by serum creatinine level above 1.5 mg/dL.

Results: ARF was noticed in the 14 of 363 (3.9%) patients. Five out of 14 patients (36%) with ARF required dialysis therapy, and all of the 14 patients with ARF recovered from ARF. Patients with ARF tend to be older (30.7 ± 6.7 vs. 27.5 ± 6.5, p = 0.074), and showed more prolonged prothrombin time (59.8% ± 28.5 vs. 77.8 ± 26.1, p = 0.011), higher peak of total bilirubin (13.2 ± 8.8 vs. 7.5 ± 4.9, p = 0.001), higher peak of alanine aminotransferase (4,447 ± 1,777 vs. 2,746 ± 1,939, p = 0.001), higher peak of aspartate aminotransferase (4,688 ± 3,146 vs. 2,746 ± 1,939, p = 0.006), and lower nadir level of platelet (136,857 ± 51,992 vs. 203,805 ± 139,478 p = 0.009) compared to patients without ARF. If ARF developed, days in hospital were significantly increased (32.1 ± 28.6 vs. 18.4 ± 15.0, p = 0.029). Proteinuria more than grade 1 was noticed in 239 of 340 (66%) patients who underwent urine analysis. But the degree of protienuria was not different between patients with ARF and without ARF.

Conclusions: We report the recent aspects of incidence and clinical characteristics of acute renal failure in acute hepatitis A patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2103

**Acute Kidney Injury (AKI) by Cortinarius Orellanus Intoxication – The Largest German Case Series** Stefan Herget-Rosenthal,[1] Duaa Aresmouk,[2] Martin Langenbeck,[1] Siegmar F. Berndt,[3] Andreas Schaper,[4] Scott O. Grebe.[2] *[1]Div. of Internal Medicine, Rotes Kreuz Krankenhaus, Bremen, Germany; [2]Dept. of Nephrology, Helios Klinikum, University of Witten/Herdecke, Wuppertal, Germany; [3]German Mycological Society, University, Kassel, Germany; [4]Poison Information Centre North, Georg-August-University, Goettingen, Germany.*

The fungus Cortinarius orellanus contains the tubulotoxin orellanine, which may cause AKI by interstitial nephritis and oxygen-free radicals. Besides case reports only 2 larger series have been reported in 1957 and 1990. We report current data on AKI, therapeutic options and outcome of orellanine intoxication.

We analyzed the largest German case series of AKI due to orellanine intoxication after mushroom ingestion confused with chanterelles. Renal biopsy was performed in 1 patient. Orellanine intoxication was confirmed by an expert of the German Mycological Society. Data are presented as mean ± SD. The semi-quantitative amount of ingested mushrooms, and the therapy with N-acetylcysteine (NAC) and steroids were correlated with chronic kidney disease (CKD) stages 12 months after intoxication.

All patients (n = 8, age 52±10 years, 50% male) presented with typical symptoms of orellanine intoxication (nausea, epigastric pain, headache) with a latency of 7±1 days after ingestion. None had preexisting CKD. At admission all patients showed severe AKI (S-Crea 10.0±6.5 mg/dl, Urea 177±68 mg/dl) and tubular proteinuria of 405±320 mg/d. In 5 patients (63%) AKI was oligo-anuric and immediate renal replacement therapy (RRT) was required. The biopsy showed severe acute tubular necrosis and interstitial nephritis. After 12 months, 3 patients (38%) still required RRT, 4 patients developed CKD stage 3 and 1 stage 4. CKD severity was correlated with the amount of ingested mushrooms (r = 0.8, p = 0.02) but not with the therapy with NAC and steroids (r = -0.1, p = 0.87).

Orellanine intoxication is a rare cause of severe AKI. Our limited data suggest, that orellanine intoxication is associated with poor renal outcome reflected by a high rate of advanced CKD. CKD severity correlates with the amount of ingested mushrooms and is not influenced by antioxidant or antiinflammatory therapy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2104**

**Cortinarius Speciosissimus Mushroom Poisoning Causing Irreversible Renal Failure** Carol Brunton,[1] Colin G. Millar,[1] Christopher O. C. Bellamy.[2] *[1]Aberdeen Royal Infirmary, Aberdeen, United Kingdom; [2]Edinburgh Royal Infirmary, Edinburgh, United Kingdom.*

4 fit and healthy adults (2 male, 2 female) aged between 46-58yrs, presented to our unit 36 hrs after ingesting "wild mushrooms",subsequently identified as belonging to the Cortinarius species (Cortinarius Speciossimus)

Initial symptoms were vomiting and diarrhoea, "burning throat", headache and increasing oliguria. 3 were completely anuric on transfer. All were dialysed by day 6. There is no specific treatment although previous case reports suggest improved outcome with the use of N-acetyl cysteine [NAC], Selenium, and steroids. All patients received NAC infusion followed by oral therapy for 3 weeks, both iv and oral Selenium and Prednisolone 60mg daily with tapering doses. All were biopsied by day 17 post poisoning, and exhibited severe diffuse toxic tubular injury with associated interstitial inflammation and areas of fibrosis. Blood and urine samples were analysed for Orellanine but were all negative.

The Cortinarius genus has over 300 species, but 2 are highly poisonous. The toxic compound is Orellanine, a bipyridine similar in structure to paraquat. The charged nitrogen atoms confound important redox reactions. In vitro studies demonstrate increased reactive oxygen species and depletion of glutathione, hence the use of anti-oxidants as potential treatments. Orellanine appears to have a predilection for the proximal tubular epithelium and the exact mode of action is uncertain. It is not destroyed by heat, freezing, drying or cooking and once ingested cannot be removed. Acute renal failure has been reported, and often presents after a delay of 7-21days. It is irreversible in up to 70% cases depending on dose and individual sensitivity.

This is a rare cause of renal failure; the last reported cases in the UK were in 1980. The changing climate and encouragement of foraging may make this species more common. The cases we present had a much more rapid presentation, with very early requirement for renal replacement therapy. 2 years after ingestion, 3 of our patients remain dialysis dependant. One of the female patients recovered renal function after 4 months and has a current creatinine of 124µmol/l (1.4mg/dl).

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2105**

**Response to Vasoconstrictor Therapy in Hepatorenal Syndrome Parallels Increases in Mean Arterial Pressure: A Pooled Analysis of Clinical Trials** Juan Carlos Q. Velez, Paul J. Nietert. *Department of Medicine, Medical University of South Carolina, Charleston, SC.*

Hepatorenal syndrome (HRS) is an ominous complication of end-stage liver disease. Vasoconstrictor therapy has been advocated as a modality to revert HRS with mixed results. We hypothesize that, regardless of the pharmacological agent used, achievement of a substantial rise in arterial blood pressure is critical for the recovery of kidney function in HRS. Using PubMed search engine, we identified 28 studies on pharmacotherapy of HRS, including retrospective and prospective clinical trials. After the exclusion of 9 studies due to unavailable relevant data, we conducted an analysis of data pooled across 489 subjects from 17 eligible studies (12 tested terlipressin, 3 norepinephrine, 3 octreotide, 2 midodrine and 1 dopamine), 6 of which were randomized controlled trials. Means and standard errors were identified for the populations' mean arterial pressure (MAP), serum creatinine (sCr), and urine output (UOP) measurements at baseline and at varying subsequent time points during treatment (range = 3 to 14 days). When possible, values specific to study subgroups (e.g. responders vs. non-responders, active treatment vs. placebo or comparator) were abstracted. Inverse variance weighted regressions were conducted to estimate the mean change per day in MAP, sCr, and UOP for each of the study subgroups (n=32), since some measurements were conducted on 3 or more occasions. The associations between changes in MAP and changes in sCr and UOP were then assessed using Spearman rank correlations. Increases in MAP were strongly associated with declines in sCr (rho = -0.80, $p < 0.0001$) and moderately associated with increase in UOP (rho = 0.37, $p = 0.087$). The magnitudes of these associations were stronger when the correlations were restricted to randomized clinical trials (MAP vs. sCr: rho = -0.90, $p < 0.0001$; MAP vs. UOP: rho = 0.60, $p = 0.12$), and the findings were similar among populations whose mean baseline MAP was ≤75 mmHg or >75 mmHg. These results support consideration for a goal-directed approach to the treatment of HRS. A substantial rise in MAP may improve kidney function in HRS irrespective of baseline MAP.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2106**

**Clinical Characteristics and Renal Prognosis from 111 Patients with Acute Interstitial Nephritis** Tao Su, Liqiang Meng, Li Yang, Xiaomei Li. *Renal Division, Department of Medicine, Peking Universtity First Hospital, Institution of Nephrology, Beijing, China.*

Acute interstitial nephritis (AIN)is a common cause of acute renal dysfunction. However the true outcome may be underestimated by several reasons involving basal condition and factors during disease progress. In this study an attempt was made to find prognostic factors in AIN. **Methods:** Patients pathologically diagnosed as AIN 2002.1-2009.12 were analyzed. Assessment of renal function was defined as full-, partial- or no-recovery when Scr returned to normal, higher than normal but had a more than 50% decrease of highest level or had no change. Risk factors including etiology of AIN, offending drugs, history of essential hypertension(EHT), diabetes(DM) and chronic kidney disease(CKD) were enrolled into logistic regression analysis for long-term renal outcome prediction. **Results:** 111 patients were enrolled (41 male and 70 female), averaged 45.3 years old. 63 cases were followed up for average 29.0 months. Aetiology of AIN involved in drug (80.2%), TINU syndrome (11.7%), idiopathic (4.5%). Severe renal failure was the major clinical pattern. Hyperglobulinemia was observed in 27.9% of all, 53.9% of TINU. 42% of TINU showed uveitis 6 months later after occurrence of AIN. There were 22.5%, 27.9% and 14.4% of patients suffered from basal CKD, EHT and DM. Six patients had autoimmune thyroid disease (AITD), while three were TINU. Among 111 patients, cases with full-, partial- or no-recovery in a short-term (1-2 month) renal assessment was 36.0%, 18.9% and 45.0% respectively. From the followed-up 63 cases, 54(85.7%) patients got a final full renal recovery, including 21 cases recovered shortly and 33 cases gradually within 6 months. Only nine (14.3%) remained renal insufficiency, four of them with CKD history despite of normal Scr level before the onset of AIN. In risk factor analysis, CKD was the only factor associated with outcome (OR =4.53). **Conclusion:** From this analysis, AIN patients with hyperglobulinemia or history of AITD should be closely followed-up to rule out TINU. Most AIN patients show good prognosis within 6 months, but a few may progress into renal chronicity, especially in those with history of CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2107**

**Improving Renal Outcome in Multiple Myeloma and Acute Kidney Injury: Extracorporeal Elimination of Free Light Chains Using High Cut-Off (HCO) Hemodialysis** Nils Heyne,[1] Martina Guthoff,[1] Hans-Ulrich Haering,[1] Katja C. Weisel.[2] *[1]Department of Endocrinology and Diabetology, Angiology, Nephrology and Clinical Chemistry, University of Tübingen, Tübingen, Germany; [2]Department of Hematology, Oncology and Immunology, University of Tübingen, Tübingen, Germany.*

About 50% of multiple myeloma patients present with impaired renal function upon diagnosis, up to 10% are dialysis-dependent. Cast nephropathy is the most common form of light-chain associated acute kidney injury (AKI). Novel therapeutics have substantially improved outcome in multiple myeloma disease, nonetheless, renal function determines patient prognosis. Timely initiation of chemotherapy and reduction of serum free light-chain (sFLC) concentrations are paramount for renal functional recovery.

We and others shown extended high cut-off (HCO) hemodialysis an effective means for extracorporeal sFLC removal. Here we report outcome data from 19 patients with multiple myeloma and dialysis-dependent AKI, treated with chemotherapy and concomitant sFLC removal. 10 patients were newly diagnosed and 9 had relapsed or refractory disease. Median eGFR was 7.4 (range 3.3 - 10.9) ml/min/1.73m², median sFLC concentration was 9.680 (range 1.590 - 66.100) mg/l. Chemotherapy mainly included new therapeutic drugs. Follow-up > 3 months was obtained from 17 patients.

In parallel to chemotherapy, a median of 8 (range 3 - 23) HCO dialyses were required to reduce sFLC concentrations below 500 mg/l. Sustained renal functional recovery was achieved in 14 out of 17 patients (83.2 %) with a median time of 17 days until off hemodialysis. Patients who did not recover renal function suffered refractory myeloma disease. Response to chemotherapy and duration of AKI prior to initiation of therapy were independent predictors of renal functional outcome.

In combination with effective chemotherapy, extracorporeal elimination of sFLC by HCO hemodialysis enables renal functional recovery of dialysis-dependent AKI in a high proportion of myeloma patients. A prospective, randomized European multicenter trial (EuLITE), adressing the influence of extracorporal sFLC elimination on renal and patient outcome in multiple myeloma and cast nephropathy is ongoing.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2108**

**Diagnostic Uncertainty of Kidney Biopsy for Evaluation of Renal Sarcoidosis in Patients with Acute Renal Failure and Sarcoidosis – A Case Report and Series** Ravish Shah,[1] Alia S. Albawardi,[2] Anil K. Agarwal,[1] Tibor Nadasdy,[2] Ganesh B. Shidham.[1] *[1]Internal Medicine, Ohio State University Medical Center, Columbus, OH; [2]Pathology, Ohio State University Medical Center, Columbus, OH.*

**Purpose of study**: Sarcoidosis is an idiopathic multisystem disease characterized by noncaseating granulomatous inflammation. The incidence of kidney involvement ranges from 3-23%. We hereby report a case of acute renal failure (ARF) in a patient with sarcoidosis, and review 21 kidney biopsies in patients with history of sarcoidosis. **Method**: A 63-year-old Caucasian male was treated for pulmonary sarcoidosis with a 6-month course

of prednisone. Two months after finishing treatment, he presented with creatinine of 4 mg/dl (baseline 1.5 mg/dl) for which no obvious etiology could be identified. A kidney biopsy was performed. This showed nonspecific changes without evidence of epitheloid granulomata. Even though the biopsy was negative for renal sarcoidosis, he was empirically started on high-dose prednisone for a clinical diagnosis of renal sarcoidosis. Serum creatinine improved to 2 mg/dl within a month of treatment. Patient continues to be stable on low-dose prednisone. This case shows the importance of prednisone trial for clinical diagnosis of renal sarcoidosis in patients with an inconclusive renal biopsy.

To further investigate the incidence of inconclusive renal biopsies and absence of epitheloid granuloma (hallmark of renal sarcoidosis) we performed a retrospective review of 21 renal biopsies in patients with ARF and sarcoidosis.

| Kidney biopsy results | N = 21 |
|---|---|
| Granulomatous interstitial nephritis | 4/21 (19%) |
| Membranous GN | 4/21 (19%) |
| Diabetic nephropathy | 4/21 (19%) |
| Nonspecific changes of chronic renal injury | 9/21 (43%) |

**Conclusion**: These data underscore the diagnostic uncertainty of renal biopsy for ARF in the setting of sarcoidosis, as classical granulomatous changes are seen in only a minority of cases. It is possible that biopsy sampling error is higher in sarcoidosis as granulomatous lesions are focal. Empiric treatment with steroids may be initiated in cases in which there is a strong clinical suspicion of renal sarcoidosis even with a non-diagnostic biopsy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2109**

**Clinical Features and Outcome of Anti-Glomerular Basement Membrane Disease in Older Patients** Zhao Cui,[123] Juan Zhao,[123] Xiao-Yu Jia,[123] Ming Hui Zhao.[123] *[1]Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China; [2]Institute of Nephrology, Peking University, Beijing, China; [3]Key Laboratory of Renal Disease, Ministry of Health of China, Beijing, China.*

**Background** Anti-glomerular basement membrane (GBM) disease is increasingly recognized in older patients. The difference in disease presentation and outcome between older and younger patients remains controversial. It makes treatment difficult to the older patients. We conducted the current study to analyze the characteristics of patients over 65 years with anti-GBM disease and to compare the younger and older cohorts.

**Methods** 221 consecutive Chinese patients with anti-GBM disease diagnosed from 1998 to 2008 in our referral center were recruited. Clinical and pathologic characteristics as well as outcomes were analyzed retrospectively.

**Results** The 50 (22.6%) older patients had a male predominance (male/female = 1.9:1). In comparison with the 171 younger patients, the older patients had higher proportion of positive ANCA ($p<0.001$), lower prevalence of hemoptysis ($p=0.011$), lower urine protein excretion ($p=0.001$) and lower initial serum creatinine ($p=0.001$). During follow-up, patient survival was worse in older patients ($p=0.007$) and renal survival was similar to the younger patients ($p>0.05$). Multiple regression analysis showed that the serum level of anti-GBM antibodies was the independent predictor for patient death ($p=0.004$) and the initial serum creatinine was the independent predictor for renal failure ($p<0.001$). Plasma exchange combining with corticosteroids and cyclophosphamide was the independent factor to improve both patient survival ($p=0.001$) and renal survival ($p=0.016$).

**Conclusions** Older patients with anti-GBM disease had milder renal damage and less pulmonary involvement, but with similar renal outcome and worse patient survival. It implied that early diagnosis and intensive therapy were crucial to improve the outcome of older patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2110**

**Outcome of Chinese Patients with Anti-Glomerular Basement Membrane Disease Receiving Different Therapeutic Regimens: A Large Cohort Study from a Single Center** Zhao Cui,[123] Juan Zhao,[123] Xiao-Yu Jia,[123] Qi-Zhuang Jin,[123] Xu-Yang Cheng,[123] Ming Hui Zhao.[123] *[1]Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China; [2]Institute of Nephrology, Peking University, Beijing, China; [3]Key Laboratory of Renal Disease, Ministry of Health of China, Beijing, China.*

Anti-glomerular basement membrane (GBM) disease usually presents with rapidly progressive glomerulonephritis, accompanied by pulmonary hemorrhage. The low incidence and fulminant course of disease prevent any large randomized control study defining the benefits of any given therapy. We conducted a retrospective survey of 221 consecutive patients from 1998 to 2008 in our hospital, with an aim to elucidate the effect of different therapeutic regimens on the outcome. Disease presentation and outcomes were compared among patients received different treatments. We found that the initial serum creatinine and positive ANCA were independent predictors for patient death. The initial serum creatinine and percentage of crescents in glomeruli were independent predictors for renal death. The combination therapy, plasmapheresis plus corticosteroids and cyclophosphamide, had an overall benefit effect on both the patient survival ($p=0.016$) and renal survival ($p=0.008$), particularly to the patient survival of Goodpasture disease ($p=0.015$) and the renal survival of anti-GBM nephritis with initial serum creatinine over 600μmol/L ($p=0.014$). Treatment using corticosteroids alone was a predictor for patient and renal death ($p<0.001$). Corticosteroids plus cyclophosphamide had no effect on patient or renal outcome ($p>0.05$). In conclusion, the combination therapy was preferred, especially in patients with pulmonary hemorrhage or severe renal damage. Early diagnosis was crucial to improve the outcome.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2111**

**Phase 1 Evaluation of the Novel C5aR Antagonist CCX168** Daniel Dairaghi, Daniel A. Johnson, Kara Deshayes, Shichang Miao, Lisa C. Seitz, Yu Wang, Manmohan Reddy Leleti, Jay P. Powers, Pirow Bekker, Thomas J. Schall, Juan C. Jaen. *ChemoCentryx, Inc.*

Purpose

CCX168 is an orally active C5aR antagonist. A Phase 1 study has been conducted in healthy volunteers (HVs) to establish the safety, tolerability, pharmacokinetic (PK) and pharmacodynamic (PD) profile of oral CCX168. This work has paved the way for clinical evaluation of CCX168 in patients with ANCA-associated vasculitis/glomerulonephritis (GN).

Methods

In the Phase 1 program, 40 male or female HVs received either placebo or 1 – 100 mg CCX168 orally, in a single-dose or a multiple-dose (7 days) regimen. Blood was collected at pre-specified time points for PK and PD analyses. Ex vivo analysis of C5aR receptor coverage on blood neutrophils was performed based on C5a-induced CD11b expression. A similar PD assay was performed on blood samples from mice dosed orally with CCX168. PK/PD requirements were defined for complete inhibition of C5aR-mediated neutrophil activation and anti-MPO-induced GN by CCX168 in mice.

Results

CCX168 potently blocks C5a-mediated chemotaxis of human neutrophils in human blood (IC50 2 nM) and CD11b upregulation (IC50 4 nM). CCX168 was well tolerated, with excellent oral bioavailability and dose-proportional increases in exposure in both periods of the Phase 1 study. There were no serious adverse events (AE) or withdrawals due to AE. Plasma levels of CCX168 of 197 ng/mL (~400 nM) were reached after a 100-mg dose, 12 hrs after which there was a 94% reduction in CD11b upregulation (ex vivo PD assay). Similar plasma levels of CCX168 were required in the anti-MPO mouse model for near-maximal prevention of GN (see related abstract). Plasma distribution half-life was 7.8 hours and terminal half-life was 29 hours.

Conclusions

CCX168 showed an excellent safety and tolerability profile in HVs. PK and PD data indicate that 30-50 mg CCX168 bid in humans should result in greater than 90% C5aR coverage in blood at all times, considered optimal for CCX168 evaluation in Phase 2 trials in vasculitis.

Disclosure of Financial Relationships: Employer: ChemoCentryx.

**SA-PO2112**

**AQP2 Affects Renal Epithelial Cell Adhesion, Migration and Tubule Formation by Interacting with Integrin β1 Via an External RGD Motif** Ying Chen,[1] William Rice,[1] Wei Li,[1] Yawei Kong,[1] Robert A. Fenton,[2] Jian Li,[3] Victor Hsu,[3] Dennis Brown,[1] Hua Ann Jenny Lu.[1] *[1]Program in Membrane Biology/Divison of Nephrology, Massachusetts General Hospital and Harvard Medical School, Boston, MA; [2]The Water and Salt Reaserch Center, Department of Anatomy, Aarhus University, Aarhus, Denmark; [3]Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.*

Aquaporin 2 (AQP2) is a classic water channel that mediates water reabsorption by principal cells of collecting duct (CD) of mammalian kidney in response to vasopressin. However, we and others have observed an unexpected phenotype in AQP2 knockout animals, with severe tubular defects, renal failure and neonatal mortality. Here, we report the discovery of a conserved Arg-Gly-Asp (RGD) sequence, an integrin-binding motif, in the second external loop of AQP2. AQP2 was able to interact with integrin β1 via this RGD motif in co-immunoprecipitation assays. AQP2 colocalized with integrin β1 on the basal and lateral membranes in CD of wild type mouse kidneys. A synthetic peptide consisting of the entire 2nd external loop of AQP2 bound to integrin β1 on overlay assays, and blocked migration and tubule formation by cultured AQP2 expressing cells. Mutation of this AQP2 RGD motif to RGA in AQP2 expressing cells resulted in significant defects in cell adhesion, migration and in vitro tubule formation. RGA expressing cells formed cyst-like structures in 3D cultures. The functional significance of the interaction of AQP2 and integrin β1 was further investigated. Alteration of the AQP2-RGD motif led to a significant increase in integrin β1 accumulation at the plasma membrane in cell cultures. To summarize, we have shown that AQP2 interacts with integrin β1 via its RGD motif and modulates the trafficking of integrin β1. We propose that AQP2 may affect important cellular functions related to epithelial cell/matrix interactions that are mediated by integrin β1.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2113**

**Vasopressin-Induced Phosphorylation of S261 in AQP2-P262L, a Mutant in Recessive Nephrogenic Diabetes Insipidus, May Underlie Recessive Nephrogenic Diabetes Insipidus** Peter M. T. Deen, Christiane Trimpert, Lise M. Verhoef. *Physiology, Radboud Univ. Nijmegen Med. Centre, Nijmegen, Netherlands.*

Vasopressin regulates water homeostasis through insertion of aquaporin-2 (AQP2) water channels in the apical plasma membrane of renal cells. AQP2 mutations cause nephrogenic diabetes insipidus (NDI), a disease in which the antidiuretic response is lacking, resulting in polyuria and polydipsia. While most AQP2 mutants in recessive NDI are misfolded, retained in the endoplasmic reticulum and unable to interact with wild-type (wt)-AQP2, cell culture studies suggest that AQP2-P262L in recessive NDI folds properly and interacts with wt-AQP2 in healthy parents, but is impaired in its vasopressin-induced translocation from

vesicles to the apical membrane in NDI patients. The underlying mechanism, however, is unknown. As it was recently shown that, besides S256, vasopressin-induced translocation of AQP2 also coincides with AQP2 phosphorylation at S264 and S269 (pS269), and de-phosphorylation at S261, and P262 lies adjacent to S261, we tested vasopressin-induced phosphorylation of AQP2-P262L. In MDCK cells, wt-AQP2 only shows an unglycosylated 29 kDa band, while AQP2-P262L is expressed as a 29 and 30 kDa band. Using AQP2 antibodies insensitive to phosphorylation, dDAVP increased the presence of the 30 kDa band. This band felt back to 29 kDa upon treatment with phosphatases, indicating that phosphorylation of AQP2-P262L underlies the appearance of the 30 kDa band. Using phosphor-specific antibodies, it appeared that, similar to wt-AQP2, dDAVP increased pS256 and pT269 in human AQP2-P262L. however, in contrast to wt-AQP2, pS261 was hardly detectable with control AQP2-P262L, and was extensively increased with dDAVP. Moreover, compared to the 29 kDa band, 30 kDa AQP2-P262L was strongly phosphorylated at S256 and S261. Our data reveal that vasopressin induces pS261 in AQP2-P262L and that this difference from wt-AQP2 may underlie its inability to sort to the plasma membrane and to cause NDI in the AQP2-P262L NDI patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2114

**Transient Receptor Potential Classic Channel 6 Activation Participates in the Regulation of Aquaporin-2 Trafficking** Vedrana Tabor,[1] Doerte Faust,[2] Jelena Milic,[2] Aline Kirschner,[2] Beate Eisermann,[1] Anita Neumann,[1] Andrea Geehlhaar,[1] Janin Junker,[1] Walter Rosenthal,[2,3] Enno Klussmann.[1] *[1]Signal Transduction, Leibnitz Institute for Molecular Pharmacology, Berlin, Germany; [2]Max Delbrueck Center for Molecular Medicine, Berlin, Germany; [3]Charite University Medicine, Berlin, Germany.*

Transient receptor potential classic 6 (TRPC6) channels belong to a superfamily of transient receptor potential (TRP) channels. Proteins from this family form cation channels permeable for Ca2+ and Na+. TRPC 6 can be specifically and selectively activated by hyperforin, one of two main constituents of St. Johns wart. TRPC6 is co-expressed with aquaporin-2 (AQP2) in renal collecting duct principal cells. It has been suggested that the arginine vasopressin (AVP)-induced redistribution of AQP2 acts not only through elevation of cAMP levels, but also through the increase of cytosolic Ca2+. These Ca2+ influxes might be controlled by TRPC6 channels.

We observed that by activating TRPC6 channels AQP2 changes it localization from cytoplasmic to predominant plasma membrane in rat primary inner medullary collecting duct (IMCD) cells. Further, we have shown that V2R stimulation and consequent rise in the cAMP levels induce the TRPC6 redistribution from intracellular domains to the plasma membrane. We have tested the potential co-localization of AQP2 and TRPC6 on AQP2 bearing vesicles as one of the possible reasons for the coordinated trafficking. However, our results strongly suggest that TRPC6 does not reside on the AQP2 bearing vesicles.

Specific TRPC6 activation changed the phosphorylation patterns of AQP2.

In addition, we have identified two drug-like small molecules inhibiting TRPC6, and tested their impact on AQP2 and TRPC6 trafficking in rat primary IMCD cells as well as in the mouse model of syndrome of inappropriate secretion of antidiuretic hormone (SIADH). The compounds inhibited TRPC6 activity, this was associated with the inhibition of the AVP-induced redistribution of AQP2, and in addition normalization of Na+ levels in vivo. Thus inhibition of TRPC6 with the small molecules may pave the way to a new concept for the treatment of diseases associated with excessive water retention.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2115

**Dislocation of Mouse Collecting Duct Aquaporin-2 by Metabolic Acidosis: Role of Vasopressin V1a Receptor** Yukiko Yasuoka,[1] Mizuka Kobayashi,[2] Yuichi Sato,[3] Hiroshi Nonoguchi,[4] Akito Tanoue,[5] Katsumasa Kawahara.[1] *[1]Physiology, Kitasato U. Sch. of Med, Sagamihara, Kanagawa, Japan; [2]Anesthesiology, Kitasato U. Sch. of Med, Sagamihara, Kanagawa, Japan; [3]Mol. Diagnostics, Kitasato U. Sch. of Allied Health Sci., Sagamihara, Kanagawa, Japan; [4]Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [5]Pharmacology, National Research Institute for Child Health and Development, Okura, Tokyo, Japan.*

Metabolic acidosis decreases urine concentrating ability by interfering with aquaporin-2 (AQP2) translocation in the luminal membrane of the principal cell (PC) through the kidney collecting duct (CD). However, the cellular mechanisms involved in urinary AQP2 excretion are still controversial. We determined whether vasopressin V1a receptor (V1aR), localized in the CDs and stimulated by acidosis, played key roles in the luminal AQP2 translocation. Wild (WT) and V1aR knockout (KO) mice were placed in metabolic cages and subjected to normal diet (control) and dietary $NH_4Cl$ loading (acidosis) with free access of water for 6 d. Some of them were treated without water for additional 1 d (dehydration). Urinary excretion of AQP2, estimated by Western blotting analysis, was 2.2% and 25% of control, respectively, in WT and KO mice (n=8, each) after 6-d metabolic acidosis, although it was markedly (160%) increased in WT, but remained unchanged in KO mice after dehydration (1 d) (n=3, each). Immunocytochemistry of the kidney cortex (WT) showed the diffuse presence of AQP2 in the cytoplasm of the PC (control, acidosis) and the dense staining in the apical membrane (dehydration). On the other hand, in the KO mice CDs, it showed weak staining of AQP2, with mainly staining in the apical membrane in the above three conditions. By using highly sensitive *in situ hybridization* technique, a level of the V2R mRNA expression remained unchanged during acidosis. We conclude that luminal AQP2 translocation in the mice kidney CDs is inhibited by metabolic acidosis even in the absence of V1aR signaling, and that V1aR may be essential for expression of AQP2, especially its upregulation during dehydration.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2116

**Crucial Role of p38MAPK in Control of Aquaporin-2 Abundance in Renal Principal Cells** Vedrana Tabor,[1] Pavel I. Nedvetsky,[1] Grazia Tamma,[2] Philipp Skroblin,[1] Aline Kirschner,[3] Beate Eisermann,[1] Andrea Geehlhaar,[1] Burkhard Wiesner,[1] Walter Rosenthal,[3,4] Enno Klussmann.[1] *[1]Signal Transduction, Leibniz Institute for Molecular Pharmacology, Berlin, Germany; [2]General and Environmental Physiology, University of Bari, Bari, Italy; [3]Max Delbrück Center for Molecular Medicine, Berlin, Germany; [4]Molecular Pharmacology and Cell Biology, Charité University Medicine, Berlin, Germany.*

The water channel aquaporin-2 (AQP2) is mediating arginine-vasopressin (AVP)-dependent increases in water reabsorption in renal collecting duct principal cells by binding to vasopressin V2 receptors (V2R) expressed on the basolateral cell surface. This leads to activation of adenylyl cyclase and elevation of cAMP levels. cAMP activates protein kinase A (PKA) which phosphorylates AQP2 at serine 256 (AQP2-Ser256). Short term stimulation with forskolin (FSK), another cAMP elevating agent induces increases in AQP2 abundance in primary IMCD cells. Recently, it was discovered that Ser261 within the C-terminus of AQP2, is phosphorylated under the resting conditions. This phosphorylation decreases in response to cAMP elevation induced by AVP or FSK. We observed that p38MAPK is phosphorylating Ser261 in vitro. Phosphorylation by p38MAPK represents a hallmark for ubiquitination and proteasomal degradation of its targets, indicating that it might regulate AQP2 protein levels in renal principal cells.

According to our in vivo studies in cells and mice, within 30 minutes of exposure to AVP and FSK the abundance of AQP2 increased by decreasing the extent of AQP2-pUb, thus decreasing its proteasomal degradation. This event is associated with a decrease of p38MAPK-induced Ser261 phosphorylation in a PKA-dependent manner. Physiologically, hitherto this unrecognized regulatory mechanism of AQP2 expression might play a key role in rapidly increasing water reabsorption.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2117

**Abnormal Function of the Vasopressin-Cyclic-AMP-Aquaporin2 Axis during Urine Concentrating and Diluting in Patients with Reduced Renal Function** Ingrid Moeller Thomsen,[1] Thomas G. Lauridsen,[1] Erling B. Pedersen.[1,2] *[1]Departments of Medicine and Medical Research, Holstebro Hospital, Holstebro, Denmark; [2]University of Aarhus, Aarhus, Denmark.*

*Background*

The kidneys ability to concentrate and dilute urine is deteriorated during progressive renal insufficiency. We wanted to test the hypothesis that these phenomena could be attributed to an abnormal function of the principal cells in the distal part of the nephron.

*Methods*

Healthy control subjects and patients with chronic kidney diseases were studied. Group 1 comprised healthy subjects, n=10. Groups 2-4 comprised patients with chronic kidney disease (Group 2, n=14, e-GFR ≥ 90 ml/min; Group 3, n=11, 60 ml/min ≤ e-GFR < 90 ml/min; and Group 4, n= 16, 15 ml/min < e-GFR ≤ 60 ml/min). The subjects collected urine during 24 hours. A urine concentrating test was done by thirsting during the following 12 hours. Thereafter, a urine diluting test was performed with a water load of 20 ml / kg body weight. The effect variables were urinary excretions of aquaporin2 (u-AQP2), cyclic-AMP (u-c-AMP), urine volume (UV), free water clearance ($C_{H2O}$), urine osmolarity (u-Osm), and plasma arginine vasopressin (p-AVP).

*Results*

After fluid deprivation, u-Osm increased. In all groups, UV and $C_{H2O}$ decreased and u-AQP2 and u-c-AMP increased in Groups 1and 2, but were unchanged in Group 3 and 4. P-AVP was significantly higher in Group 4 than in the other groups. During urine diluting, UV and $C_{H2O}$ reached significantly higher levels in Groups 1-3 than Group 4. Both before and after water loading, u-AQP2 and p-AVP were significantly higher and u-c-AMP was significantly lower in Group 4 than the other groups. Estimated-GFR was correlated negatively to p-AVP and positively to u-c-AMP during the diluting test.

*Conclusion*

Patients with moderately severe chronic kidney disease have a reduced renal concentrating and diluting capacity compared to both patients with milder chronic kidney disease and healthy control subjects. These phenomena can be attributed, at least partly, to an abnormally decreased response in the AVP-c-AMP-AQP2 axis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2118**

**Renal Aquaporin Expression and Osmotic Water Permeability Is Altered in Mice Fed a High Fat Diet** Grazia Tamma, Patrizia Gena, Domenica Lasorsa, Lisa Mastrofrancesco, Anna Rosito, Maria Svelto, Giuseppe Calamita, Giovanna Valenti. *Department of General and Environmental Physiology, University of Bari.*

The prevalence of obesity, one of the metabolic syndrome forms, has increased dramatically during the past decade with an estimated incidence of over 300 million adults worldwide. Obesity is an established independent risk factor for the development of chronic kidney disease. On the other hand, activation of the sympathetic nervous system and the renin-angiotensin system, leading to renal sodium and water retention, links obesity with hypertension. Considering these particular clinical features, in this study we focused our attention on the potential dysregulation of renal aquaporins (AQPs) in the kidney of a mouse model of obesity and type 2 diabetes mellitus (T2DM).

Mice were fed a high fat (HFD) or normal (ND) diet for 20 weeks. The expressions of AQP1, 2, 3 and 4 were analyzed by Western blotting and the subcellular distribution was investigated by immunocytochemistry. The osmotic water permeability (Pf) of the whole renal plasma membranes was evaluated by stopped flow light scattering.

Western blotting of total lysates from HFD mice revealed higher immunoreactivity for AQP1, AQP2 and AQP4 compared to mice fed a normal diet; no change was detected for AQP3. However, AQP1, AQP2, AQP3 and AQP4 abundance decreased in a crude kidney membrane preparation suggesting a reduced cell surface expression of these AQPs. Immunolocalization experiments confirmed an altered subcellular distribution of renal AQPs in HFD mice kidney.

Stopped flow light scattering showed a reduced Pf from 254.8±30.5 μm/s to 169.8±16.2 (P<0,0001) in membrane from ND versus HFD mice, respectively, consistent with the observed decrease in AQPs cell surface expression.

Altogether, these data reveal altered AQPs expression and osmotic water permeability of HFD mice kidney. These observations suggest a patho-physiological relevance for aquaporins in renal complications associated with metabolic syndrome.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2119**

**Urinary Exosomal (Microvesicular) Aquaporin-2 Is a Possible Early Biomarker for Cisplatin-Induced Acute Kidney Injury** Hiroko Sonoda,[1] Hiroaki Kondo,[1] Naoko Yokota-Ikeda,[2] Masahiro Ikeda.[1] *[1]Veterinary Pharmacology, University of Miyazaki, Miyazaki, Japan; [2]Nephrology, Miyazaki Prefectural Miyazaki Hospital, Miyazaki, Japan.*

Cisplatin, an antineoplastic agent, is known to cause acute kidney injury (AKI). The lack of appropriate biomarkers has hampered initiation of potential therapeutics in cisplatin-induced AKI in a timely manner. Of the known aquaporins (AQPs), water channel proteins, AQP1 and AQP2 have been found in the urinary exosomes (microvesicles) that are secreted from tubular epithelial cells. It was reported that cisplatin-injured rats showed urinary concentrating defect associated with down-regulation of AQP1 and AQP2 proteins levels in the kidney. In order to evaluate urinary exosomal AQP2 as an early noninvasive biomarker for cisplatin-induced AKI, this study examined the effect of the treatment of rats with cispaltin on the excretion of exosomal AQP2. Cisplatin (7.5 mg/kg) or saline was administered intraperitoneally in rats. Urine and blood samples were collected at various time points after the injection. Urinary exosomes were isolated from the collected urine by differential centrifugation. Plasma creatinine concentration of cisplatin-injected rats significantly increased 72 to 168 hours after the injection, compared to that of control rats. Urinary exosomal AQP2 abundance, judged by immunoblotting, was significantly lower in cisplatin-injected rats than in control rats 24 to 168 hours after the injection. Renal AQP2 protein level tended to be increased 24 hours, and was significantly decreased 168 hours after the injection. RT-PCR analyses showed that the renal AQP2 mRNA level was not changed at 24 to 72 hours and was significantly decreased 168 hours after the injection. Based on these data, we conclude that urinary AQP2 may represent an early to later noninvasive biomarker for cisplatin-induced AKI. Furthermore, we speculate that the reduction of urinary exosomal AQP2 level by cisplatin was due to retention of renal AQP2 abundance in early phase of cisplatin-induced kidney injury and due to lowered renal AQP2 level in later phase.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2120**

**Angiotensin (1-7) Induces Downregulation of AQP1 in Rat Proximal Cell Via Release of Bradykinin and Prostaglandin** Zaira Palomino Jara,[1] Richard Bouley,[2] Dulce Elena Casarini,[1] Flavia F. Jung.[3] *[1]Department of Medicine, Federal Univerisity of São Paulo, São Paulo, Brazil; [2]Nephrology Division, Massachussetts General Hospital and Harvard Medical School, Boston, MA; [3]Division of Pediatric Nephrology, Georgetown University, Washington, DC.*

Aquaporin 1 (AQP1) is the major constitutively expressed water channel in the renal proximal tubule and thin descending limb where nearly 80% of water is reabsorbed. However, the factors regulating its expression are still elusive.

We previously showed that Angiotensin II (AngII) $10^{-8}$M upregulated the AQP-1 expression in immortalized rat proximal tubule cells (IRPTC). We now show that Angiotensin-(1-7) (Ang(1-7) downregulated AQP1 in IRPTC. Ang(1-7) $10^{-8}$M downregulated AQP-1 mRNA and protein expression ( p<0.01,n=4) and these inhibitory effects were completely blocked by $10^{-5}$M D-[Ala$^{7}$]- Ang-(1-7) (p<0.001,n=4). Therefore in this study, we investigated Bradykinin (Bk), Prostaglandin E2 (PGE2), Angiotensin Converting Enzyme (ACE) and ACE2 as possible mediators of Ang (1-7) effect on AQP1 expression. IRPTC were treated with $10^{-8}$M Ang(1-7) for 0, 5, 15, 30, 60 and 120 min. After each time point, the release of Bk and PGE2 induced by Ang(1-7) stimulation were measured by both HPLC and by ELISA while the enzymatic activity of ACE and ACE2 were measured by fluorometric assay. At 15 min there was a significant elevation of Bk release compare to in absence of Ang(1-7) (time 0) (1.53 vs. 0.43 nM/mg; p<0.01, n=5). PGE2 release was significantly higher after 30 min of stimulation (169,10 vs. control 23,30 pg/mL, p<0.01, n=3). Interestingly, the presence of Ang(1-7) inhibited the enzymatic activity of ACE (ZPhe-HL – control: 0,46 ± 0,02 vs 5min: 0,04 ± 0,001 nM/min/mg, p<0,01, n=4;) while it stimulated ACE2 activity (control: 180 ± 33,88 vs. 120 min: 1041,9 ± 77,2 nM/min/mg, p<0,01, n=4). This study suggest that Ang(1-7) effect on AQP1 downregulation is mediated by release of BK and PGE2, as well as, inhibition of ACE and stimulation of ACE2. This mechanism may play a important role in the homeostasis of water reabsortion in proximal tubule.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2121**

**AQP5: Renal Aquaporins Family Got a New Member** Giuseppe Procino,[1] Lisa Mastrofrancesco,[1] Fabio Sallustio,[2] Vincenzo Costantino,[2] Francesco Paolo Schena,[2] Maria Svelto,[1] Giovanna Valenti.[1] *[1]Department of General and Environmental Physiology, University of Bari, Bari, Italy; [2]Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy.*

In an attempt to investigate the regenerative potential of adult multipotent renal progenitor/stem cells (ARPCs) isolated from human kidneys (Sallustio et al., 2009) we characterized them for the expression of aquaporins. ARPCs expressed measurable levels of the proximal tubule-specific AQP1, both at mRNA and protein levels. When ARPCs were differentiated in vitro into epithelial cells, the expression of the collecting duct-specific AQP2 was also induced.

Surprisingly, ARPCs also expressed measurable levels of AQP5, an aquaporin known to be selectively expressed in lung, salivary and lachrymal glands in mammals. This evidence prompted us to investigate the presence and the localization of AQP5 in the mammalian kidney.

Total RNA was isolated from adult human, rat and mouse kidneys and subjected to RT-PCR. Interestingly, AQP5 transcripts were found in all the species tested.

Western blotting analysis, revealed an AQP5 band of 27 kDa as well as a glycosylated form. Consistent with that, neither the transcript nor the protein was found in AQP5 null mice. AQP5 abundance was higher in the renal cortex than in the medulla.

Immunolocalization indicated that AQP5 was expressed at the apical membrane of the cortical collecting ducts (CCDs) epithelial cells with negligible staining in the inner medulla. Triple immunostaining indicated that, in rat CCDs, AQP5 did not colocalize either with AQP2 or with the intercalated cells marker V-ATPase, suggesting a cell specific expression of AQP5 in cells not expressing AQP2 but likely involved in water reabsorption. The ratio between AQP2- and AQP5-expressing cells was approximately 3:1.

In conclusion, the expression of AQP5 in the ARPCs, might suggest a role in the differentiation/regeneration processes of the collecting duct epithelial cells. Moreover, its constitutive expression at the apical membrane in the CCD, renders AQP5 a possible target for improving water reabsorption in the collecting duct when AQP2 apical expression is unpaired as in nephrogenic diabetes insipidus.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2122**

**Systemic Distribution of AQP11 and Phenotypic Analysis of AQP11-Null Mice** Kenichi Ishibashi. *Pathophysiology, Meiji Pharmaceutical University, Kiyose, Tokyo, Japan.*

AQP11 is a member of a new aquaporin subfamily with unusual NPA boxes, but has a high water permeability. AQP11 is expressed widely most abundantly in testis, thymus and moderately in liver, kidney, intestine and brain. However, its cellular localizations other than kideny are poorly characterized. Little is known about the phenotypes of AQP11 knockout mice other than polycystic kidney disease which is fatal due to uremia within a month. In this study, we examined AQP11 distribution and extrarenal phenotypes of AQP11-null mice. In situ hybridization analyses revealed that AQP11 was expressed at epithelial cells in the small intestine, hepatocytes, lymphocytes in the thymus and spermatocytes in the testis. Immunohistochemical analyses revealed that AQP11 was expressed intracellularly in the epithelial cell of the choroid plexus, vascular endothelial cells in the brain and the spinal cord, epithelial cells in the small intestine, and lymphocytes in the thymus. In AQP11-null mice, we observed intracellular vacuoles in hepatocytes around portal area, spermatocytes, and the epithelial cells of the choroid plexus and the small intestine. However, other cells expressing AQP11 appeared unchanged in AQP11-null mice.The vacuoles of hepatocytes (postnatal day 6) and proximal tubular cells (postnatal day 3) were compared by staining with an early endosome marker (EEA1), a lysosome marker (M6PR), and an ER marker (KEDL) and microarray analyses. Both vacuoles were strongly stained with M6PR and KEDL but weakly with EEA1, suggesting a common defect in endocytosis in these organs. The microarray analyses in both organs showed the absence of the enhanced expression of ER-stress related genes which has been observed at postnatal day 7 in the kidney. In conclusion, AQP11 disruption will initially lead to early endosome dysfunction and the intracellular vacuole develops in organs where active solute transports are conducted.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2123**

**Importance of Cysteine 227 on Water Permeability and Multimerization of AQP11 in Transfected Mammalian Cells** Kanako Muta, Sayaka Oshikawa, Masahiro Ikeda. *Veterinary Pharmacology, University of Miyazaki, Miyazaki, Japan.*

The aquaporins (AQPs) are a family of membrane channel protein and thirteen members (AQP0 - AQP12) have been identified in mammals. The AQPs exist as a multi-subunit oligomer and this structure is suggested to be important for their function. Among the AQPs, it has been reported that AQP11 is expressed at the proximal tubules in the kidney and the knockdown of the protein causes polycystic kidney disease in mice. Recently, an amino acid substitution (Cys227Ser) of AQP11 was reported to be responsible for renal cystic phenotype in mice, suggesting the importance of Cys227 in AQP11 function. However, roles of Cys227 in AQP11 water permeability and multimerization have yet to be elucidated. Although AQP11 is originally thought to be an intracellular AQP, a significant amount of AQP11 has been shown to be expressed at the plasma membrane, allowing us to measure the water permeability through AQP11 by a conventional cell swelling assay. In this study, we investigated the effect of Cys to Ser substitution at position 227 in AQP11 on its water permeability and multimerization in transfected CHO cells. When we measured the osmotic water permeability of the cell using swelling assay, the cells expressing AQP11-Cys227Ser had significantly increased osmotic water permeability (Pf = 9.9 +/- 0.8 cm/s x $10^{-4}$, n = 15, P < 0.05) compared with the cells expressing wild-type AQP11 (Pf = 7.1 +/- 0.6 cm/s x $10^{-4}$, n = 15). We also performed biotinylation assay and the results showed that the plasma membrane expression level of AQP11-Cys227Ser was lower than that of the wild-type protein. A chemical cross-linking experiment with paraformaldehyde revealed less multimer formation by AQP11-Cys227Ser, compared with the wild-type form. Collectively, these results indicate that Cys227 had an important role in 3D- structure of AQP11 molecule in association with its normal water transport function. Furthermore, this study suggests that both loss- and gain-of-function mutations of AQP11 appear to cause polycystic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2124**

**Chronic Use of Chloroquine Disrupts the Urine Concentration Mechanism by Lowering cAMP Levels in the Inner Medulla** Mitsi A. Blount, Tobias N. Von Bergen, Janet D. Klein, Jeff M. Sands. *Department of Medicine - Renal Division, Emory University School of Medicine, Atlanta, GA.*

Chloroquine, a widely used anti-malaria drug, has gained popularity for the treatment of rheumatoid arthritis, SLE, and HIV. Unfortunately, chloroquine may also negatively impact renal function for patients whose fluid and electrolyte homeostasis is already compromised by diseases such as chronic kidney disease (CKD). Chronic administration of chloroquine also results in polyuria which may be explained by suppression of the antidiuretic response of vasopressin. Several of the transporters responsible for concentrating urine are vasopressin-sensitive including the urea transporters UT-A1 and UT-A3, the water channel aquaporin-2 (AQP2), and the Na+-K+-2Cl- cotransporter (NKCC2). To examine the effect of chloroquine on these transporters Sprague-Dawley rats received daily subcutaneous injections of 80 mg/kg/day of chloroquine for 4 days. Twenty-four hour urine output was 2-fold higher and urine osmolality was decreased by 2-fold in chloroquine-treated rats compared to controls. Urine analysis of treated rats detected the presence chloroquine as well as decreased urine urea and cAMP levels compared to control rats. Western blot analysis showed a down regulation of AQP2 and NKCC2 transporters, however UT-A1 and UT-A3 abundances were unaffected by chloroquine treatment. Immunohistochemistry showed a marked reduction of phosphorylated UT-A1 and total AQP2 in the apical membrane in IMCDs of chloroquine-treated rats. Furthermore, activated CREB, a transcription factor for AQP2 and NKCC2 but not for the urea transporters, is decreased. RT-PCR analysis confirms a decrease of AQP2 and NKCC2 mRNA, while UT-A1 and UT-A3 mRNA levels remained unchanged. In conclusion, chloroquine-induced polyuria likely occurs as a result of lowered cAMP production. Acutely, low cAMP levels cause improper membrane association of UT-A1 and AQP2. Chronically, AQP2 and NKCC2 expression is downregulated on a transcriptional level. These findings suggest that chronic chloroquine treatment would exacerbate the already compromised fluid homeostasis observed in diseases like CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2125**

**Renal Phenotype of Transgenic Mice Over-Expressing hCD39/NTPDase1** Yue Zhang,[1] Keiichi Enjyoji,[2] Kaiya L. Morris,[1] Shannon K. Sparrow,[1] Karen M. Dwyer,[3] Simon C. Robson,[2] Bellamkonda K. Kishore.[1] *[1]Medicine, VA Med Ctr & Univ. of Utah, Salt Lake City, UT; [2]Medicine, BIDMC, Harvard Med School, Boston, MA; [3]Nephrology, St. Vincent's Hospital & Univ. of Melbourne, Melbourne, Victoria, Australia.*

Extracellular nucleotides regulate urinary concentration in a type-2 purinergic receptor (P2-R)-dependent manner. Ectonucleoside triphosphate diphosphohydrolase-1 (NTPDase1/CD39) hydrolyzes ATP and ADP to AMP, and thus regulates the availability of ligands for P2-Rs. CD39 and related ectonucleotidases are expressed by the vasculature and other structures in kidney (Kishore et al, *AJP Renal* 288:F1032, 2005). Here we evaluated the urinary concentration/dilution capability of transgenic mice (TG) globally over-expressing hCD39 (Dwyer et al, *JCI* 113:1440, 2004). When allowed *ad libitum* access to solid chow and drinking water, the TG mice tend to drink more water and void quantitatively more and dilute urine as compared to wild type (WT) control mice, without significant differences in urinary excretion of vasopressin (AVP). Under stable and basal hydrated conditions (feeding gelled diet for 7 days), TG mice concentrated urine to a significantly lesser degree, despite lack of significant alterations in water intake, urine output and AVP, and protein abundance of AVP-regulated AQP2 in the kidney relative to WT mice. TG mice could dispose of an acute water load more rapidly, albeit total amounts excreted were not significantly different from the WT mice. When subjected to water restriction for 3 days, the TG mice could not concentrate urine to the level noted in WT mice, and had significantly lesser urinary AVP levels (TG 229 vs. WT 555 pg/mg creatinine, N = 5, $P < 0.02$). However, the increases in urinary osmolalities in response to sub-acute or chronic dDAVP treatment were similar or somewhat higher, respectively, in TG mice. Our observations suggest that although hCD39 over-expressing TG mice fail to concentrate urine as efficiently as the WT mice in response to water restriction, collecting ducts are responsive to dDAVP. Heightened nucleotide scavenging by the over expression of CD39 likely alter the release of endogenous AVP in response to water restriction.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2126**

**Mice with Disrupted COX-2 Expression Display Impaired Urine Concentration Ability** Rikke Norregaard,[1] Kirsten Madsen,[2] Pernille B. Hansen,[2] Peter Bie,[2] Jorgen Frokiaer,[1] Boye Jensen.[2] *[1]Institute of Clinical Medicine, University of Aarhus, Aarhus, Denmark; [2]Cardiovascular and Renal Research, University of Southern Denmark, Odense, Denmark.*

Recent data showed that hypothalamic $PGE_2$-EP1 receptor interaction promotes urine concentrating ability through stimulation of vasopressin (AVP) release. We hypothesized that hypothalamic COX-2 activity promotes urine concentrating ability through stimulation of AVP release. To address this we measured water turnover, AVP expression, AVP plasma concentration and renal transport proteins in normohydrated and water deprived (WD) COX-2 deficient ($^{-/-}$) and wildtype (WT) littermate controls. Basal urine output and water intake was enhanced and urine osmolality was lower in male COX-$2^{-/-}$ mice on a pure C57/bl6 background. Water deprivation (WD) resulted in lower urine concentration ability with a higher plasma osmolality and $Na^+$ concentration in COX-$2^{-/-}$ mice with no gender difference. Hypothalamic COX-2 expression was not altered by WD. Cerebral tissue $PGE_2$ concentration was enhanced in COX-$2^{-/-}$. Hypothalamic AVP mRNA was enhanced by WD in both genotypes. Total AVP peptide content was higher in COX-$2^{-/-}$ compared to WT at baseline and not changed by WD. Plasma AVP concentration in conscious catheterized WD mice did not differ between genotypes. WD increased inner medulla COX-2 protein abundance and $PGE_2$ urine excretion in WT. This was abolished in COX-$2^{-/-}$. Baseline medullary interstitial osmolality was higher in COX-$2^{-/-}$ and increased similar to WT in response to WD. The protein abundance of AVP V2 receptor in inner medulla (IM) and cAMP urine excretion was lower in COX-$2^{-/-}$ after WD. Baseline abundance and apical targeting of total aquaporin-2 (AQP2) was not different after WD in IM whereas pAQP2 was stimulated similarly in WT and COX-$2^{-/-}$. In summary, COX-$2^{-/-}$ mice exhibit chronically increased AVP stores but insufficient plasma levels to attain urine concentrating ability similar to WT despite elevated interstitial medullary osmolality and enhanced AQP levels. We speculate that increased flow in fewer nephrons and/or impaired local, COX-2 mediated, cAMP-dependent effects in the IM cause the observed attenuation in urine concentration.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2127**

**Mice with Disrupted Acyl-CoA Binding Protein Gene Display Impaired Urine Concentrating Ability** Stine Langaa,[1] Maria Bloksgaard,[2] Signe Bek,[2] Ditte Neess Pedersen,[2] Pernille B. Hansen,[1] Susanne Mandrup,[2] Boye Jensen.[1] *[1]Cardiovascular and Renal Research, University of Southern Denmark, Odense, Denmark; [2]Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark.*

Acyl-CoA binding protein (ACBP) is a lipid-binding protein expressed in most eukaryotic cells. To elucidate the role of ACBP in the intact organism, the ACBP gene was disrupted in mice. ACBP knock-out mice (ACBP$^{-/-}$) were grossly normal but displayed a skin/fur phenotype and increased water turnover.

It was hypothesized that transepithelial transport of water in the kidney is supported by ACBP.

Mice were placed in metabolic cages for quantitative studies of NaCl balance and urine concentrating ability. Blood was sampled by orbital- and cardiac puncture. Organs were rapidly removed and frozen for molecular analyses.

Under baseline conditions, adult ACBP$^{-/-}$ did not differ from wild type mice (WT) with respect to food intake. Water intake and diuresis were significantly higher in ACBP$^{-/-}$ whereas urine osmolality was lower. Renal excretion of $Na^+$ and $K^+$ did not differ between genotypes. After water deprivation for 20 hours, ACBP$^{-/-}$ exhibited higher diuresis, lower urine osmolality, elevated hematocrit and higher relative weight loss compared to WT. Kidney and adrenal weight compared to body weight did not differ between genotypes. $Na^+$ and $K^+$-excretion in urine were similar in the two groups. Renal papillary interstitial fluid osmolality, $Na^+$, $K^+$ and urea concentrations did not differ significantly between genotypes. ACBP$^{-/-}$ did not differ from WT with respect to kidney mRNA levels of aquaporin 1 (AQP1) and the $Na^+$-$K^+$-2 $Cl^-$ co-transporter. AQP2 mRNA and protein abundances and the intracellular localization of AQP2 were not different between the two groups. Adrenal mRNA level of cholesterol side-chain cleavage enzyme and plasma corticosterone concentration did not differ between genotypes. Adrenal aldosterone synthase mRNA was significantly elevated in ACBP$^{-/-}$ compared to WT whereas plasma renin and aldosterone levels were similar in the two groups.

We conclude that ACBP is necessary for an intact urine concentrating ability most likely through effects on collecting duct water transport.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2128**

**Transporters and Urine Concentration in Low-Protein Fed Mice with P2Y2 Receptor Knock-Out** Janet D. Klein,[1] Jeff M. Sands,[1] Christopher F. Martin,[1] Yue Zhang,[2] Bellamkonda K. Kishore.[2] *[1]Dept. of Medicine, Renal Div., Emory University, Atlanta, GA; [2]Dept. of Medicine, VA Medical Center and Univ. of Utah, Salt Lake City, UT.*

Transport of urea and movement of water to produce concentrated urine must respond to changes in protein load. Previous work in rats showed that low protein diet (LPD) causes UT-A1 to increase in inner medullary base and decrease in inner medullary tip (Kim, AJP Renal 296: F66, 2009). The contribution of UT-A in the LPD-induced polyuria is, therefore, unclear. AQP2 in LPD-fed rats is markedly reduced (Sands, JCI 97:2807, 1996). We examined the possible role of the P2Y2 receptor in the response of transporters involved in urine concentration by comparing P2Y2 knock-out (KO) mice with wild type (WT) controls fed normal protein (23%) vs low protein (8%) diet for 2 weeks. Water intake increased 63% in WT but not KO mice (n=5/grp). Urine output increased in both WT (133%) and KO (81%) mice after LPD, with a commensurate decrease in urine osmolalities of both WT (2115 pre vs 1583 mosmol/kg $H_2O$ post LPD) and KO (2493 pre vs 1966 mOsmol/kg $H_2O$ post LPD) mice. UT-A1 protein was increased 65% in IM of LPD-fed P2Y2 KO mice (n=9, $p<0.05$) but was unchanged in the WT mice. AQP2 tended toward decrease in the LPD WT mice, but the change was not statistically different. The P2Y2 KO mice showed no decrease in AQP2 upon LPD feeding. Similarly, AQP1 was decreased by 36% ($p<0.05$, n=5) in the LPD-fed WT mice but showed no decrease in the LPD-fed P2Y2 KO mice (n=5, p=n.s.). These results suggest that the relatively milder polyuria in the LPD-fed P2Y2 KO mice may reflect a combination of lower AQP2 impairment and increased compensatory action from the upregulated urea transporters. Conversely, this may suggest that the P2Y2 receptor could exacerbate the effect of LPD on the normal kidney's ability to concentrate urine.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2129**

**Disrupting Interactions of Protein Kinase A (PKA) and A-Kinase Anchoring Proteins (AKAP) with Small Molecules as a Novel Strategy for the Treatment of SIADH** Vedrana Tabor,[1] Kerstin Zuehlke,[1] Sabine Friedl,[1] Solveig Grossmann,[1] Jelena Milic,[2] Nico Kotzur,[1] Aline Kirschner,[2] Anita Neumann,[1] Beate Eisermann,[1] Andrea Geehlhaar,[1] Walter Rosenthal,[2,3] Enno Klussmann.[1] *[1]Signal Transduction, Leibniz Institute for Molecular Pharmacology, Berlin, Germany; [2]Max Delbrueck Center for Molecular Medicine, Berlin, Germany; [3]Molecular Pharmacology and Cell Biology, Charité University Medicine Berlin, Berlin, Germany.*

Increased secretion of arginine vasopressin (AVP or ADH) leads to the development of the syndrome of inappropriate ADH secretion (SIADH), which is the most frequent cause of hyponatremia in humans. This syndrome is associated with a three-fold increased mortality. Elevated AVP levels are also common in patients suffering from chronic heart failure. AVP binds to vasopressin V2 receptors (V2R) and increases water reabsorption in renal collecting duct principal cells via activation of adenylyl cyclase (AC) and the consequent increase in cAMP levels. cAMP activates PKA and induces PKA-driven AQP2 phosphorylation of serine 256 (AQP2 Ser256). This elicits AQP2s insertion into the plasma membrane thereby facilitating water reabsorption from primary urine. This redistribution of AQP2 depends on the interaction of PKA with A-kinase anchoring proteins (AKAPs), which position PKA at defined sites within cells and thereby coordinate PKA signalling temporally and spatially. The objective of our study was to develop new strategies for targeting the excessive water retention caused by inappropriate AVP secretion.

According to our in vitro, as well as in vivo studies in mice, a novel drug-like small molecule, FMP-API-3, inhibits AKAP-PKA interactions. The compound blocked the AVP-induced AQP2 translocation in primary renal principal cells of rats and mice in vivo. This inhibition is a consequence of reduced PKA-dependant phosphorylation of AQP2-Ser256. In addition, Na+ blood serum levels, water uptake and urine excretion in the SIADH model normalised upon the long-term FMP-API-3 treatment. Thus disruption of AKAP-PKA interaction might lead to a new concept for the treatment of SIADH and diseases associated with AVP-dependent water retention (such as chronic heart failure).

Disclosure of Financial Relationships: nothing to disclose

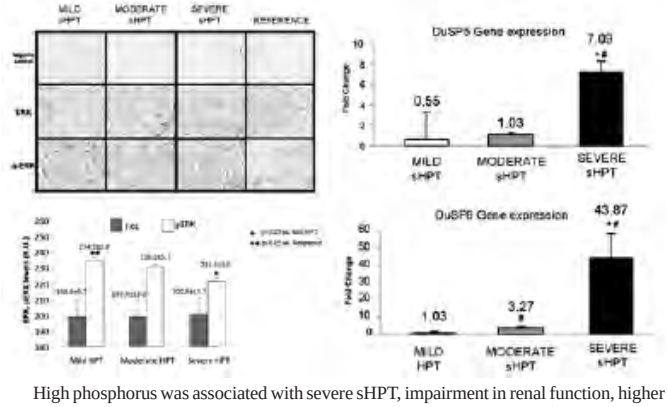
**SA-PO2130**

**Phosphorus Load and Severe Secondary Hyperparathyroidism Are Associated with Widespread Gene Expression Downregulation but Upregulation of Antiproliferative Pathway** Pablo Roman-Garcia, Natalia Carrillo-Lopez, Ana Rodriguez-Rebollar, Jose L. Fernandez-Martin, Manuel Naves-Diaz, Jorge B. Cannata-Andia. *Servicio de Metabolismo Óseo y Mineral, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain.*

Secondary hyperparathyroidism (sHPT) is a common complication of CKD that can be worsened by phosphate load. FGF23 is a phosphaturic hormone that also inhibits PTH secretion through MAPK pathway activation. The aim of this study was to study the molecular mechanisms involved in severe FGF23-resistant sHPT in CKD.

Rats were nephrectomized (7/8) and subsequently divided in two groups: normal P diet (0.6% P) and high P diet (0.9% P). In addition, a group of animals with a normal P diet without nephrectomy was used as reference group. Rats were sacrificed at 4, 8, 12, 16 and 20 weeks. Blood samples were collected, and parathyroid glands extracted and pooled according to PTH levels into three groups: no sHPT, mild sHPT and severe sHPT (Mean PTH: 60±63, 583±545 and 2238±851 pg/mL, respectively). Biochemical parameters, differential PTGs gene expression (microarrays and qPCR) and ERK and pERK levels were assessed.

Severe sHPT was associated with PTGs, higher serum PTH, FGF23, P levels and higher mortality. It was also associated with low serum Ca levels, severe impairment of renal function, together with a marked widespread gene expression down-regulation. Interestingly, Dual Specificity Phosphatases (Dusps) 5 and 6, involved in the de-phosphorilation of pERK (inhibition of PTGs growth pathway) were highly and significantly over-expressed.




High phosphorus was associated with severe sHPT, impairment in renal function, higher mortality, severe gene expression downregulation. And overexpression of Dusp 5 and 6. The latter, together with the likely inactivation of MAPK pathway reflects the mechanism triggered to counteract the progression of sHPT .

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2131**

**Acute Decrease of Serum Parathyroid Hormone after Iron Dextran Administration in Experimental Rat Model of Chronic Renal Failure with Secondary Hyperparathyroidism** Sylvie Sulkova,[1] Jitka Kuncova,[2] Renata Slavickova,[1] Jitka Sviglerova,[2] Ondrej Viklicky,[1] Milan Stengl,[2] Martin Matejovic.[3] *[1]Dept Nephrol, IKEM, Prague, Czech Republic; [2]Dept Physiol, Charles Univ Med Faculty, Pilsen, Czech Republic; [3]Dept Medicine, Charles Univ Med Faculty, Pilsen, Czech Republic.*

**Background**: Calcium-sensing receptor in parathyroid tissue (PT) is sensitive also to other methals, including iron (Fe). We tested the hypothesis that Fe administration may decrease parathyroid hormone secretion.

**Methods:** Secondary hyperparathyroidism was induced by high-phosphate (1,29%) diet in 5/6 nephrectomy rat model of chronic renal failure (CRF). Basal and time-course (30, 60, 90 and 120 min) serum PTH after bolus administration of Fe-dextran (100 ug/kg) was measured in 4 experimental groups /sham-operated rats with normal diet (SH-N) and with high-P diet (SH-P); CRF with normal diet (CRF-N) and with high-P diet (CRF-P)/. PT tissue was weighted at the end of experiment. At least 5 animals in each group completed experiment. Statistics was tested by ANOVA followed by post-hoc Sheffe´s test or by repeated measures ANOVA (STATISTICA Cz, version 7, StatSoft, CZ).

**Results:** Controls animals (SH-N) had significantly lower PT weight as well as basal serum PTH. The highest PT weight was found in CRF-P. Iron administration induced marked acute decrease in serum PTH in all subgroups with basal high PTH (CRF-P, CRF-N, SH-P). This decrease of PTH started as early as in 1st hour after Fe administration.

Weight of PT nodule and PTH levels in subgroups

| | Weight of PT nodule (mg) | PTH (pg/nodule) | Baseline serum PTH (pg/ml) | Serum PTH 60 min after Fe administration (pg/ml) |
|---|---|---|---|---|
| SH-N | 0.6 ± 0.08 | 32.7 ± 7.2 | 319 ± 38 | 364 ± 73 |
| SH-P | 2.4 ± 0.48 | 88.4 ± 19.2 | 1157 ± 110 | 691 ± 96 |
| CRF-N | 1.9 ± 0.28 | 82.5 ± 16.6 | 397 ± 27 | 309 ± 19 |
| CRF-P | 3.7 ± 0.26 | 146.15 ± 30.5 | 501 ± 66 | 373 ± 53 |

a: $p<0.05$ vs SH-N; b: $p<0.05$ vs SH-H; c: $p<0.05$ vs CRF-N; d: $p<0.05$ vs CRF-P; e: $p<0.05$ vs respective baseline value

**Conclusion:** Parenteral iron administration induced acute decrease of serum PTH. These findings should be further explored in clinical practice.

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Disclosure of Financial Relationships: nothing to disclose

**SA-PO2132**

**Comparative Gene Expression in Parathyroid Oxyphil and Chief Cells** Cynthia S. Ritter,[1] Bruce H. Haughey,[2] Brent W. Miller,[1] Alex J. Brown.[1] *[1]Renal Division, Washington University School of Medicine, St. Louis, MO; [2]Department of Otolaryngology, Barnes Jewish Hospital, St. Louis, MO.*

The parathyroid glands (PTGs) of young adults are composed almost entirely of chief cells, but with age and stress another cell type appears, the oxyphil cell. These cells are thought to be derived by transdifferentiation of chief cells, but how and why they form, as well as their function, is totally unknown. Oxyphil cell number increases in PTGs of patients with chronic kidney disease (CKD) and increases further after treatment of hyperparathyroidism with calcitriol and cinacalcet (Lomonte, CJASN 2008). Here, we compared the gene expression in oxyphil and chief cells of PTGs of CKD patients by immunostaining for PTH, PTHrP, CaR, VDR and 25-hydroxyvitamin D-1a-hydroxylase (1aOHase or CYP27B1). Oxyphil cells, found in defined nodules or as diffuse, undefined "islands," were identified by H&E staining. The most impressive differential expression was seen with the 1aOHase, which was very highly expressed in oxyphil islands, to a lesser degree in oxyphil nodules, with virtually none in chief cells. PTHrP was also highly expressed in oxyphil islands, compared to trace amounts in oxyphil nodules and chief cells. Both oxyphil islands and nodules expressed PTH at a level equal to or greater than chief cells. Both the CaR and VDR were expressed in oxyphil cells, but like the chief cells, expression was variable in diffuse and nodular areas. The hypothesis that CaR activation by cinacalcet or calcitriol (indirect via Ca) leads to an increase in oxyphil cells is supported by our observation that incubation of normal rat parathyroid glands in high Ca medium led to the formation of oxyphilic cells. Studies are underway to further characterize the gene expression profile and secretome of parathyroid oxyphil cells, and to determine the role of these cells (positive or negative) in the morbidity and mortality of CKD patients.

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Genzyme Corporation
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**SA-PO2133**

**The Effects of Cyclooxygenase 2 Downstream mPGES1-PGE2-EP2 on the Hyperplasia of Parathyroid Gland in Uremic Patients** Chuan-Ming Hao. *Huashan Hospital, Fudan University.*

**Objective**

To explore the potential role of COX2 downstream pathway mPGES1-PGE2-EP2 in hyperplasia of uremic parathyroid gland.

**Methods**

mPGES1 and EP2 were detected by immunohistochemistry and immunoblot. Freshly excised PTG tissues were incubated for 24 hours: normal phosphate 1mM(NP), high phosphate 4mM (HP), HP with COX2 inhibitor NS398, HP with mPGES1 inhibitor MK886, and HP with EP2 antagonist AH6809. The dose-dependent effects of PGE2 and EP2 agonist butaprost ($10^{-7}$M-$10^{-5}$M) were also observed. The PTH levels were determined by DPC IMMULITE. preproPTH and PCNA of cultured tissues were assessed by realtime PCR and immunoblot.

**Results**

mPGES1 and EP2 were located in cytoplasmic, especially in the perinuclear region of PTG cells. They were expressed much more in nodular hyperplasia than those in diffuse hyperplasia glands. Immunoblot analysis supported the expression of mPGES1 and EP2 in the hyperplastic PTGs. High-phosphate treatment further increased the production of PTH compared to normal phosphate treated group (NP1.00±0.49, HP4.54±2.11, $p<0.05$). COX2 inhibitor NS398, mPGES1 inhibitor MK886 and EP2 antagonist AH6809 significantly inhibited this effect, respectively (2.00±1.24 1.88±0.58 0.80±0.76, $p<0.05$). The changes of preproPTH mRNA levels of PTGs showed the similar trend. HP treatment also induced an elevated expression of PCNA in PTGs (1.68±0.13) compared to NP treatment (1.06±0.02 $p<0.05$). Such stimulation was significantly blocked by either NS398(0.72±0.42), MK886(1.13±0.12) or AH6809(0.89±0.30). PGE2 directly induced a dose-dependent over-production of PTH and high expression of PCNA in cultured PTG tissues. Butaprost ($10^{-6}$M) produced similar changes of high PTH synthesis and PCNA expression in PTGs.

**Conclusion**

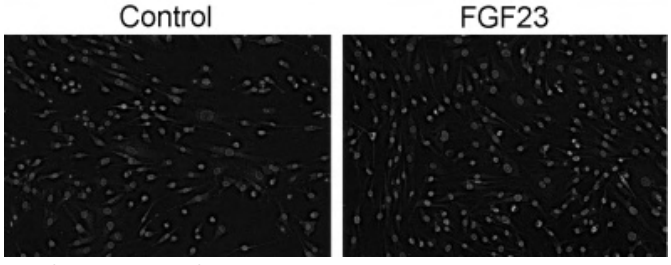
Increased expression of mPGES1-EP2 was detected in PTGs of uremic patients. Inhibition this pathway reduced the secretion of PTH and PTG cell proliferation, while PGE2 and EP2 agonist directly stimulated such reactions. These data suggest that this pathway plays an important role in the pathogenesis of secondary hyperparathyrodism.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2134**

**FGF23 Modulate Osteocytes Life Span** R. C. Pereira, Harald Jueppner, Isidro B. Salusky, Katherine Wesseling-Perry. *Pediatrics Nephrology, David Geffen School of Medicine, Los Angeles, CA.*

Impaired osteoblast/osteocyte viability contributes to bone loss in pts. with osteoporosis and in kidney transplant recipients. ROD contributes to increased fracture rates, and osteocyte function is altered in CKD pts, as evidenced by increased FGF-23 and DMP1 expression in osteocytes. However, rates of osteocyte apoptosis and factors that regulate this process in CKD are unknown. We exame apoptosis in BBX from 14 pediatric pts. with CKD stages 3-5. Biochemical values were: Ca 9.1±0.2 mg/dl, P 5.1±0.4 mg/dl, Alk P'tase 364±70 IU/l, PTH (1st IMA, Immutopics$^{R}$) 357±100pg/ml, C-term FGF-23 (2nd generation Immutopics$^{R}$) 1106±295 RU/ml. Bone histology was OF (n=1), mixed disease (n=4), adynamic (n=4) and osteomalacia (n=2) and normal (n=3). Apoptosis in bone sections was assessed via *in situ* nick-end labeling (TUNEL) Klenow T-deoxynucleotidyl transferase per manufacturer's instructions (Oncogene Products). Sections incubated with vehicle alone served as negative controls; control bone samples were obtained from individuals with normal kidney function. Apoptotic osteoblasts and osteocytes were not detected in bone from CKD pts. whereas 10-20% of osteocytes were apoptotic in normal controls. To assess potential mechanisms underlying this decreased apoptotic rate in CKD bone, the mouse osteocyte cell line MLOY-4 was cultured in serum-free medium in the presence of FGF-23 (10, 50 and 100 ng/ml) or PTH (10 nM) for 24 and 48 hours. Cell death was assessed by acridine orange/ethidium bromide staining (orange death /green alive cells).




After 24 and 48 hrs of FGF23 treatment, osteocyte viability was increased by 50% at all concentrations. PTH failed to affect osteocyte viability. Thus FGF-23, which is increased in CDK pts, may prevent osteocyte cell death. Further studies are warranted to determine the mechanism by which FGF-23 mediates this affect in the context of CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2135**

**PTH-Dependent Regulation of Npt2a and Npt2c Expression Appears To Involve Predominantly cAMP/PKA-Dependent Mechanisms** Hiroko Segawa,[1, 2] Akira Maeda,[1] Thomas J. Gardella,[1] Ken-Ichi Miyamoto,[2] Harald Jueppner.[1] *[1]Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA; [2]Department of Molecular Nutrition, Institution of Health Bioscience, the University of Tokushima Graduate School, Tokushima, Japan.*

Parathyroid hormone (PTH), a major regulator of renal ionic phosphate (Pi) reabsorption, regulates brush border membrane (BBM) expression of three distinct Na/Pi transporters, Npt2a, 2c, and PiT-2. It activates several signaling pathways down-stream of the PTH/PTHrP receptor (PTHR), including phospholipase C (PLC)/protein kinase C (PKC), which has been suggested to be prominently involved in the control of renal Pi transport. However, patients with pseudohypoparathyroidism (PHP), a disorder caused by maternally inherited GNAS mutations that inactivate the stimulatory G protein (Gαs), present with hyperphosphatemia due to impaired PTH-stimulated Pi excretion, thus implicating cAMP/PKA-dependent mechanisms in renal regulation of Pi homeostasis. To examine which signaling pathways down-stream of PTHR are involved in regulating Npt2a and Npt2c expression, we compared the phosphaturic actions of M-PTH(1-34) (M: Ala 1,12,Aib3,Gln10,homoArg11,Trp14,Arg19), a long-acting analog which stimulates both cAMP and IP3 formation, to those of Trp1-M-PTH(1-34), a PLC-defective variant. When injected into WT or Npt2c-null mice, each analog induced similar reductions in plasma Pi levels, as well as similar reductions in Npt2a expression. When injected into Npt2a-null mice, each analog again induced similar decreases in plasma Pi and Npt2c expression levels. Effects of each analog on Npt2a expression were rapid, whereas those on Npt2c expression were gradual. The two analogs also showed similar reductions in plasma Pi levels in mice expressing a mutant PTHR (DSEL) that signals via cAMP/PKA but not PLC/PKC. The data thus suggest that the cAMP/PKA pathway is importantly involved in the acute PTHR-dependent regulation of Npt2a and Npt2c expression, which is consistent with findings in PHP patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2136**

**FGF Receptors 3 and 4 Mediate Regulation of 1,25 Vitamin D Metabolism by FGF23** Jyothsna Gattineni,[1] Regina Goetz,[2] Moosa Mohammadi,[2] Michel G. Baum.[1] *[1]Pediatric Nephrology, UT Southwestern Medical Center, Dallas, TX; [2]Pharmacology, NYU Medical Center, New York, NY.*

Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone implicated in many hypophosphatemic disorders. FGF23 causes phosphaturia and decreased serum 1,25 Vitamin D levels. We have shown that FGF receptor 1 (FGFR1) is the predominant receptor for the hypophosphatemic actions of FGF23 by decreasing renal NaPi-2a and 2c expression. Studies in individual FGFR null mice have not identified the FGFR(s) mediating the regulation of vitamin D metabolism by FGF23. We have studied FGFR3FGFR4 double mutant mice (DKO) as these mice have shortened life span and are growth retarded similar to *fgf*23 null mice. The effect of FGF23 (12 mcg/mouse/BID for 4 days intraperitoneally) was examined on serum levels of phosphate, PTH, 1,25 Vitamin D, renal BBMV NaPi-2a expression and phosphate transport:

Effect of Pharmocological doses of FGF23 on Control and DKO mice

| | Control Vehicle | Control FGF23 | DKO Vehicle | DKO FGF23 |
|---|---|---|---|---|
| Serum Phosphate (mg/dl) | 8.3±0.4 (n=12) | 5.6±0.2* | 6.9±0.3 (n=9)† | 11.6±0.5* |
| Serum 1,25 Vitamin D (pmol/L) | 77.4±13.1 (n=11) | 19.2±3.7* | 173.4±32.7 (n=11)† | 216.9±47.6 |
| Serum PTH (pg/ml) | 86.8±16.2 (n=9) | 88.3±9.5 | 126.8±25.6 (n=9) | 21.3±5.1* |
| BBMV phosphate transport pmol/mg/10 secs | 176.7±10.0 (n=7) | 129.2±8.4* | 167±6.5 (n=9) | 192.7±4.2* |
| Renal BBMV NaPi-2a-ßactin expression | 0.9±0.04 | 0.7±0.06* | 0.5±0.04 | 0.8±0.05* |

* Vehicle vs FGF23 <0.05; †control vehicle vs DKO vehicle <0.05

FGF23 administration to DKO mice increased serum phosphate levels, increased renal BBMV NaPi-2a expression and renal BBMV phosphate transport, unlike control mice. Basal 1,25 Vitamin D levels were higher in DKO mice relative to control mice and did not decrease in response to FGF23. PTH levels decreased in response to FGF23 in the DKO mice while they were unchanged in the control mice. We conclude that hyperphosphatemia in DKO mice in response to FGF23 was due to decreased PTH levels and unchanged 1,25 Vitamin D levels. These data indicate that FGFR3&4 are the receptors that mediate the regulation of 1,25 Vitamin D metabolism by FGF23.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2137

**Fibroblast Growth Factor 23 (FGF23) and Left Ventricular Hypertrophy (LVH) in Children on Dialysis** Wacharee Seeherunvong,[1] Carolyn L. Abitbol,[1] Jayanthi Chandar,[1] Paolo Rusconi,[2] Gaston E. Zilleruelo,[1] Michael Freundlich.[1] *[1]Pediatric Nephrology, University of Miami, Miami, FL; [2]Pediatric Cardiology, University of Miami, Miami, FL.*

Cardiac death in children on dialysis is 700-fold higher than normal children and accounts for 40% of pediatric dialysis deaths. Vitamin D (D) abnormalities and ↑FGF23 levels associate with ↑ mortality risk and LVH in CKD adults. Comparable studies in children are lacking. Children and adolescents (n=36), age 15.7 ± 5.0 yr on maintenance dialysis (7 peritoneal; 29 hemo) underwent echocardiography with LV mass index (LVMI= mass/height in m $^{2.7}$) calculation (LVH defined as height-age adjusted LVMI >95%tile). All required phosphate (P) binders and 31 (86%) calcitriol or paricalcitol treatment. D insufficiency (25-OHD < 30 ng/ml) was detected in 58% (< 20 ng/ml in 29%). Serum Ca was normal (9.42 ± 0.7 mg/dl) and most had ↑serum P (6.6 ±1.2 mg/dl). Parathyroid hormone (PTH) was adequately controlled in most (384 ± 418 pg/ml). Median FGF23 concentration (n=17) was 21,450 RU/ml (normal <230 for age < 17 yr), mean log FGF23 was 4.29 ± 0.46. Plasma Pro brain-natriuretic peptide (BNP) was ↑ in all (7179 ± 14952 pg/ml, normal 0-125). Average LVMI was 49.6 ± 27.4 g/m $^{2.7}$ with LVH in 49% of patients. In univariate analyses, age, time on dialysis, and serum levels of P, PTH, 25(OH)D or $1,25(OH)_2D_3$ did not associate significantly with LVMI. However, systolic and diastolic BP centiles (p=0.03), Pro BNP (<0.001) and logFGF23 (p=0.03) correlated with LVMI. Multiple regression analysis for cardiac risk variables significantly (p<0.05) associated with LVMI in univariate analysis revealed that the log FGF23 provided the highest contribution to LVMI (p=0.07). Each standard deviation ↑ in log FGF23 resulted in a 7.4 g/m $^{2.7}$ LVMI increase. Thus, ↑LVMI and LVH in children and adolescents on maintenance dialysis correlate strongly with the biomarker Pro BNP. Our preliminary observations indicate that ↑FGF23 levels are strongly associated with LVMI in young dialysis patients. Larger studies are needed to detect more robust associations between FGF23 and LVMI and its use as a predictive marker of cardiovascular outcomes.[/bold]

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2138

**Elevation of Phosphate but Not Circulating FGF23 Increases Mortality in the Jck Model of CKD-MBD** Shiguang Liu, Joseph H. Boulanger, Wenping Song, Yves Sabbagh, Susan Schiavi. *Endocrine and Renal Sciences, Genzyme Corp, Framingham, MA.*

A biphasic increase in serum FGF23 is observed during chronic kidney disease (CKD) progression. Small increases (2-3X) are associated with stage 2-3 suggesting that FGF23 induction may be an adaptation to initial renal damage that promotes increased fractional phosphate (Pi) excretion. In late stages of CKD, FGF23 levels become overwhelming elevated by tens of thousand fold and significantly correlate with mortality. This association is independent of serum Pi levels despite the known role of Pi on mortality. To first confirm the influence of Pi on mortality of jck mice, a genetic model of polycystic kidney disease that mimics the progression of CKD-Mineral and Bone Disorder, animals were maintained on diets containing either normal (0.4%) or high (1.0%) Pi. Although increased dietary Pi content had no apparent influence on renal function, it significantly increased serum Pi, PTH, $1,25(OH)_2D_3$ and FGF23. Importantly, the life span of Jck mice was significantly decreased from an average of 25 to 20 weeks ($p < 0.05$). As a means of further assessing the role of FGF23 and Pi on mortality, we utilized high pressure tail vein (HPTV) injection to induce a more rapid and severe induction of supra-physiological FGF23 levels in the Jck mouse fed either normal or high Pi diets. HPTV injection of FGF23 plasmid increased serum FGF23 levels from ~100 to 100,000 pg/ml at 8 weeks of age that was sustained for at least 8 weeks post HPTV injection. FGF23 over-expression slightly lowered serum Pi levels in the early stages of CKD (from 8 to 13 weeks of ages) with both diets, however, the serum Pi levels of the FGF23 group on high-Pi diet was not lower than the vector group on normal Pi diet. In addition, overexpressing FGF23 did not prevent the hyperphosphatemia accompanying declining kidney function. Importantly, the supra-physiological FGF23 induction by HPTV early in CKD did not significantly increase renal dysfunction or mortality regardless of Pi levels. These results suggest that Pi rather than FGF23 contributes largely to mortality in the setting of CKD and underscores the importance of managing Pi early in CKD.

Disclosure of Financial Relationships: Employer: Genzyme Corp.; Ownership: Genzyme stock.

## SA-PO2139

**Effects of 1, 25 Dihydroxyvitamin D3 on Calcium and Phosphorus Homeostasis in the Mouse** David J. Berlove, Alexander Brezzani, Kelly Keefe, Robert Sacchiero, Zhonglin Zhao. *Preclinical Pharmacology, Genzyme, Waltham, MA.*

Maintenance of calcium and phosphorus balance is critical for a wide range of physiologic functions. In the presence of renal disease, dysregulation of these essential minerals is common and a variety of pathological sequelae ensue, including vascular calcification, bone disorders and acceleration of kidney damage.

One consequence of renal damage is a loss of 1, 25 dihydroxyvitamin D3 (D3) which is essential for mineral balance and other functions. Renal failure patients are typically supplemented with D3 which may have unintended consequences in hyperphosphatemic patients. We have chosen to explore some of the effects of D3 administration on phosphate and calcium-related parameters in adult C57/Bl6 mouse.

Our initial study surveyed the effects of a single injection of D3 (IP, dose range 0-5000ng/kg) in mouse. Ileum, jejunum and kidney were collected at 6 hours. Treatment had no effect on mRNA expression of the renal phosphorus transporter, NaPi-2a. The renal phosphorus transporter, NaPi-2c, which was expressed at lower basal levels, increased around 50% at all doses from 300ng/kg. The intestinal phosphorus transporter NaPi-2b was up-regulated in jejunum 4-5X at doses from 600-5000ng/kg. There was a pronounced dose-dependent increase in the D3 metabolizing enzyme, cyp24a1, in both kidney and jejunum. D3 receptor expression was not affected in any tissue. Urinary phosphorus excretion was elevated for the 6 hours after D3 administration with a strong trend towards increased Ca excretion.

A follow-up study exploring the time-course of changes (0-24 hours) demonstrated that the massive increase in Cyp24a1 mRNA peaked at 3-6 hours and then rapidly subsided. Expression of NaPi-2b mRNA was more complex with significant elevations at 3 and 6 hours followed by a subsequent decrease, below basal expression at 24 hours. Serum phosphorus was increased while PTH levels were reduced. We conclude that pharmacologic treatment with D3 significantly alters expression of phosphate transporters in the intestine and urinary excretion of phosphate. However the effects over longer periods of treatment may be different than those observed very acutely.

Disclosure of Financial Relationships: Employer: Genzyme Corporation; Ownership: Genzyme CorporationResearch Funding: Genzyme Corporation.

## SA-PO2140

**Analysis of the Parathyroid Glands in Hemodialysis Patients with Secondary Hyperparathyroidism Refractory to Cinacalcet Hydrochloride** Keiichi Sumida, Yoshifumi Ubara, Junichi Hoshino, Rikako Hiramatsu. *Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

Cinacalcet hydrochloride (cinacalcet) increases sensitivity of calcium sensing receptors (CaSR) to activation by extracellular calcium, and thus suppresses parathyroid hormone (PTH) release, while controlling calcium-phosphate balance. There have been few studies of histopathological alterations in hyperplastic parathyroid glands in secondary hyperparathyroidism (SHPT) after cinacalcet administration.

Hemodialysis patients with severe SHPT were divided into those treated with and without cinacalcet (cinacalcet group and conventional group, respectively; both *n*=12). Total maximal parathyroid gland weight and maximal to minimal parathyroid gland weight ratio were significantly higher in the cinacalcet group compared with the conventional group (1798.7±1658.3 mg *vs.* 764.2±471.1 mg, *P*=0.018, 15.8±13.9 *vs.* 6.6±4.2, *P*=0.047, respectively).

Significant increases were observed in oxyphil cell area (61.7%±17.1% *vs.* 36.7%±15.6%, *P*=0.001), hemosiderosis score (1.50±1.24 *vs.* 0.42±0.51, *P*=0.029), and CaSR expression (1.67±0.75 *vs.* 0.75±0.75, *P*=0.025) in the former than the latter group. These findings indicated that cinacalcet induced specific qualitative alterations of hyperplastic parathyroid glands in patients with severe SHPT.

Our results suggest new effects of cinacalcet on hyperplastic parathyroid glands, although further analyses are needed to confirm the physiopathological mechanisms responsible for these alterations.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2141**

**Combination Therapy with Cinacalcet for Patients with Secondary Hyperparathyroidism (SHPT) during Intravenous Vitamin D Treatment – A Randomized Controlled Trial Comparing Dose Escalation of Vitamin D Pharmaceutical Infusion and Dose Escalation of Calcium Carbonate** Shigeo Negi,[1] Takashi Shigematsu.[2] *[1]Division of Nephrology and Blood Purification Medicine, Wakayama Medical University, Wakayama City, Kimiidera, Japan; [2]Division of Nephrology and Blood Purification Medicine, Wakayama Medical University, Wakayama City, Kimiidera, Japan.*

[Aims] (1) To investigate (1) the efficacy of combined administration of Cinacalcet for patients with SHPT during Maxacalcitol (OCT) treatment. (2) To compare dose escalation of OCT and dose escalation of calcium (Ca) preparation for hypocalcemia associated with Cinacalcet administration in a prospective, multicenter, randomized controlled trial.

[Methods] Cinacalcet was concurrently administered to patients undergoing treatment with OCT and having an intact-PTH ≥ 180pg/ml, adjusted Ca ≥ 9.0mg/dl, and serum phosphorus concentration ≥ 3.5mg/dl. The patients were randomly divided into either the OCT dose-escalation group or Ca preparation dose-escalation group, followed for 48 weeks, and analyzed for achievement rate according to the Clinical Practice Guideline for the Management of Secondary Hyperparathyroidism in Chronic Dialysis Patients from the Japanese Society for Dialysis Therapy (JSDT).

[Results] (1) The combination therapy with Cinacalcet was effective in all 94 patients with SHPT. (2) Of 52 patients, 25 were divided into the OCT dose-escalation group, and of 42 patients, 19 were divided into the Ca preparation dose-escalation group. There was no significant difference in the achievement rate for each factor of Ca, P, and PTH between the two groups. The achievement rate for the two factors of Ca and P was significantly higher in the OCT dose-escalation group ($p < 0.05$) and that for the three factors of Ca, P, and PTH tended to be higher in the OCT dose-escalation group ($p = 0.058$).

[Conclusions]

(1) The combination therapy of OCT and Cinacalcet was effective for patients with SHPT showing resistance to OCT. (2) Dose escalation of OCT was more effective than that of Ca preparation for hypocalcemia induced by Cinacalcet.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2142**

**Cinacalcet for Secondary Hyperparathyroidism Lowers FGF23 Levels in Patients Receiving Hemodialysis** Masahiro Koizumi,[1] Hirotaka Komaba,[1] Hisae Tanaka,[1] Shohei Nakanishi,[2] Akira Fujimori,[3] Masafumi Fukagawa.[1] *[1]Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine, Isehara, Japan; [2]Chibune Kidney and Dialysis Clinic, Osaka, Japan; [3]Division of Blood Purification and Kidney Center, Konan Hospital, Kobe, Japan.*

**Background.** Fibroblast growth factor 23 (FGF23) is a bone-derived hormone that plays an important role in the regulation of phosphate and vitamin D homeostasis. Serum levels of FGF23 are markedly elevated in patients with end-stage renal disease and associated with resistance to active vitamin D therapy for secondary hyperparathyroidism (SHPT). However, little is known whether cinacalcet therapy modulates FGF23 secretion and whether FGF23 levels predict response to this therapy.

**Methods.** This was a post hoc analysis of data from a 52-week, multicenter, open-label, single-arm trial that examined the effect of cinacalcet on SHPT in patients undergoing hemodialysis. Alteration of vitamin D dosage was permitted only on the grounds of patient safety. Full-length intact FGF23 was measured at baseline and study weeks 12, 24, and 52, using serum samples of subjects who completed the study (N = 55).

**Results.** Treatment with cinacalcet resulted in a significant decline in log-transformed FGF23 levels by 9% and this response was sustained over 52 weeks. Changes in log FGF23 levels were significantly and positively associated with changes in serum phosphate ($r = 0.332$, $P = 0.013$) and calcium ($r = 0.590$, $P < 0.001$) independently of changes in parathyroid hormone levels and active vitamin D dose. Logistic-regression analysis showed that baseline FGF23 levels did not predict the likelihood of achieving an intact PTH <180 pg/ml at the study end (odds ratio, 0.134; 95% CI, 0.901 to 2.177).

**Conclusions.** Cinacalcet lowers serum FGF23 levels in patients with SHPT, even in the setting of sustained active vitamin D therapy which should increase FGF23 secretion from the bone. Further studies are needed to elucidate the precise mechanism of FGF23 reduction by cinacalcet and whether this results in improved patient survival.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2143**

**Cinacalcet HCl Influences Frequency of Parathyroidectomy for Advanced Secondary Hyperparathyroidism** Yoshihiro Tominaga. *Transplant & Endocrine Surgery, Nagoya Second Red Cross Hospital, Nagoya, Aichi, Japan.*

Requirement of parathyroidectomy (PTx) for secondary hyperparathyroidism (SHPT) due to chronic kidney disease (CKD) may be influenced by the medical treatment, guideline of the treatment and medical insurance system ect in each country. Recent induction of Cinacalcet HCL (cinacalcet) may significantly reduce the frequency of PTx for SHPT. However, the influence by cinacalcet has not been clarified.

We established the Japanese Association of Parathyroid Surgeons. The aims of the association are (1) to establish registration system to evaluate the annual number of PTx, the surgical indications, procedure and outcomes (2) to propose surgical indications for advanced SHPT to physicians on surgeons view point (3) to distribute adequate surgical technique and network system (4) to perform clinical studies concerning advanced SHPT and PTx in multi-centers.

We picked up the institutes in which PTx for SHPT was routinely performed base on questionnaires to 4036 hemodialysis (HD) centers in Japan.Out of 340 institutes 109 agreed to participate in the association. Based on DOPPS and Japanese Society of Dialysis Therapy (JSDT) studies, more than 90% of PTx for SHPT could be recruited in our association.

At first we evaluated the annual number of initial PTx and re-operation for SHPT in each institute by questionnaires. Total annual number of initial PTx for SHPT from 2004 to 2009 were 1137, 1154,1375,1749,1042,490 operations respectively. In Japan the number of PTx increased from 2004 to 2007. In 2006 JSDT established the guideline of treatment for SHPT in HD patients and the guideline proposed that the PTx should be performed at relatively early stage of SHPT to acquire good patient's survival. And the number of PTx significantly decreased from 2008 to 2009. Cinacalcet has been available from the beginning of 2008 in Japan.

In conclusion in Japan the frequency of PTx for SHPT significantly decreased after the induction of cinacalcet. Surgical indications and outcomes in era cinacalcet should be evaluated and the adequate treatment including PTx for advanced SHPT should be established.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2144**

**Residual Renal Function Is an Independent Determinant of Serum FGF-23 Levels in Prevalent Hemodialysis Patients** Liesbeth Viaene,[1] Bjorn K. I. Meijers,[1] Bert Bammens,[1] Vanrenterghem Yves,[1] Dirk Vanderschueren,[2] Pieter Evenepoel.[1] *[1]Nephrology, University Hospital, Leuven, Belgium; [2]Endocrinology, University Hospital, Leuven, Belgium.*

Introduction: Poor residual renal function (RRF) and high FGF-23 levels are associated with arterial stiffness, left ventricular hypertrophy and increased (cardiovascular) mortality. Therapy with active vitamin D, dietary phosphate loading and high serum phosphate and calcium levels have been associated with high serum FGF-23 levels. The present study aimed to investigate the impact of RRF on FGF-23 levels in hemodialysis (HD) patients. Methods: We performed a cross-sectional observational study in 68 prevalent HD patients (36 male, age 71 ± 12 yrs, dialysis vintage 31 ± 33 months). Mid-week predialysis blood samples were collected and analyzed for parameters of mineral metabolism including calcidiol,calcitriol, biointact PTH, and FGF-23 (Kainos). Weekly total phosphate clearances as well as mass removals were calculated from urine and dialysate collections. Residual glomerular filtration rate (rGFR), determined as the arithmetic mean of renal urea nitrogen and creatinine clearance, below 1 ml/min/1.73m² and/or a 24-h urine output < 100 ml was defined as anuria. Results: In multivariate regression analysis, rGFR was found to be inversely associated with serum FGF-23 levels, independent of dialysis vintage, total phosphate clearance, estimates of dietary phosphate intake (i.e. nPNA and total phosphate mass removal), active vitamin D therapy and serum phosphate and calcium levels. rGFR, serum phosphate and calcium levels and active vitamin D therapy explain 66 % of the variation in FGF-23 ($p<0.0001$). The 38 anuric patients had borderline higher FGF-23 levels (2219 vs. 1129 ng/L, median, $p = 0.09$) but similar serum phosphate (4.6 vs. 4.4 mg/dL, mean) and calcium levels (9.4 vs. 9.5 mg/dL, mean). Conclusion: Our data indicate that RRF is an important determinant of FGF-23 and favour the hypothesis that the ailing kidney directly contributes to raising FGF-23 levels, e.g. by a decreased expression of an inhibitor or by less clearance. Whether FGF-23 is associated with poor outcomes independent of RRF, or vice versa, remains to be clarified.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2145**

**Hepcidin and Disordered Mineral Metabolism in CKD** Cristiane Carvalho,[1] Tamara Isakova,[1] Gordana Olbina,[2] Myles S. Wolf,[1] Mark E. Westerman,[2] Orlando M. Gutierrez.[1] *[1]University of Miami; [2]Intrinsic Life Sciences.*

**Intro:** Hepcidin regulates iron homeostasis. Elevated hepcidin levels block iron absorption in the gut and iron release from macrophages and hepatocytes, restricting iron availability for erythropoiesis. Because hepcidin secretion is induced by inflammation, increased hepcidin levels are thought to play a key role in anemia of chronic inflammatory conditions, such as chronic kidney disease (CKD). Disorders of mineral metabolism are common in CKD and have been implicated in the pathogenesis of inflammation. Few studies examined the association between hepcidin and markers of mineral metabolism in CKD.

**Methods**: We examined the associations between hepcidin, iron, and markers of mineral metabolism in 125 patients with pre-dialysis CKD. Hepcidin was measured by competitive ELISA.

**Results:** Hepcidin levels were inversely correlated with eGFR ($r=-0.3$, $P<0.001$), and linearly correlated with TSAT ($r=0.2$, $P=0.01$) and ferritin ($r=0.7$, $P<0.001$). In univariate analyses, higher phosphate and parathyroid hormone (PTH) levels and lower calcitriol levels were associated with higher hepcidin levels ($P<0.001$ for all). In addition, there was a trend towards an inverse association between fibroblast growth factor 23 (FGF23) and hepcidin ($P=0.05$). In multivariable-adjusted analyses, log FGF23, log calcitriol, phosphate, and log PTH levels were independently associated with log hepcidin levels, whereas the association between eGFR and hepcidin was no longer significant (Table).

**Conclusion:** Abnormalities in phosphate and vitamin D metabolism were associated with increased hepcidin levels in patients with pre-dialysis CKD, and attenuated an inverse association between eGFR and hepcidin. These results suggest that disorders of mineral metabolism contribute to increased hepcidin secretion in CKD. Whether inflammation mediates this link requires further study.

| Variable | ß | t-value | P |
|---|---|---|---|
| Log FGF23 | -0.41 | -5.0 | <0.001 |
| eGFR | -0.004 | -1.0 | 0.3 |
| Phosphate | 0.43 | 4.6 | <0.001 |
| Log PTH | 0.24 | 2.9 | 0.01 |
| Log calcitriol | -0.18 | -2.1 | 0.04 |
| TSAT | 0.5 | 0.9 | 0.4 |
| Log Ferritin | 0.69 | 7.9 | <0.001 |

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2146

**Stanniocalcin-1 (STC1) Is Upregulated in the Tunica Media of Calcifying Aortas of Uremic Rats** Luis F. Michea, Peter Murphy, Magdalena Gonzalez. *ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile.*

Medial vascular calcification (VC) of large arteries is frequent in end-stage renal disease and correlates with hyperphosphatemia. Is now recognized that VC involves phenotypic trans-differentiation of vascular smooth muscle/mesenchymal cells into osteogene-expressing, sharing many features with embryonic bone formation. STC1 is a homodimeric glycoprotein, acting as an autocrine/paracrine factor that promotes osteochondrogenic differentiation in developing bone of mammals. We hypothesized that the expression of STC1 would be increased in VC observed in chronic renal failure (CRF). Male SD rats were nephrectomized (5/6 kidney ablation) and separated into the following groups: standard diet, high phosphate diet (HP), vitamin D plus HP. These groups were compared to sham operated rats under standard or HP diet. Physiological parameters were determined. After 5 weeks animals were killed to obtain blood, urine and aorta samples for biochemical and histological studies. We observed an increase in blood pressure and creatinine in all NPX groups (n=6,P<0,05). HP diet caused increased plasma phosphate levels in all NPX groups (n=6,P<0,05). NPX increased the expression of STC1 in rat aorta (1,6 fold,P<0.05). HP diet increased STC1 expression in aorta of NPX rats (2,3 fold compared to normal,P<0.05) and in aorta from NPXVD+HP. STC1 protein abundance increased 3.0±0.4 fold in NPX-HP and 4.5±0.9 fold in NPXHP+VD, as compared to SHAM aorta (P<0.05). Aortic sections from NPX rats showed the presence of STC1 in tunica media. HP and HP+VD caused a further increase in STC1 immunodetection, consistent with western blot studies. To evaluate the potential role of phosphate and VD as upregulators of STC1 expression in aorta, aortic explants were incubated in medium supplemented with HP (2.5 mM), VD (100nM), VD+HP or vehicle during 48 hours. As compared to vehicle treated control explants, HP, VD and HP+VD increased STC1 mRNA abundance (25,8±10,3;15,3±9,9;48,2±6,1 fold;n=4;P<0.05). Our data demonstrate increased expression of STC1 in calcifying aorta of NPX, which can be induced by HP and VD. Supported by FONDECYT 1090223, NMII P07/088F, Fondecyt-FONDAP 15010006.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2147

**Regulation of Calcitriol and Urinary Phosphate Excretion in the Immediate Post Transplantation Period: A Role for FGF-23** Katherine Wesseling-Perry,[1] Justine Bacchetta,[3] Eileen W. Tsai,[1] Robert B. Ettenger,[1] Harald Jueppner,[2] Isidro B. Salusky.[1] *[1]Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Endocrine Unit, Mass General Hospital, Boston, MA; [3]Hospices Civils de Leon, Leon, France.*

Levels of FGF-23 have been implicated in hypophosphatemia and suppressed calcitriol levels in the first year post transplantation. FGF-23 levels are elevated in dialysis pts. and profound changes in mineral metabolism occur immediately post-transplantation; however, the role of pre-transplant FGF-23 levels in these alterations is unknown. Biochemical values were obtained in 44 pediatric renal transplant pts. age 16.3 ± 2.9 yrs at baseline, daily for the first 5 days post-transplant, and at 3 and 6 mos. FGF-23 correlated with serum creatinine (r=0.37, p<0.05) and 1,25$(OH)_2$D (r= -0.32,p<0.05), and PTH (r= -0.31,p<0.05) at baseline. Subjects were divided into two subgroups: high (above the median) v. low (below the median) baseline FGF-23. Data are expressed as median (IQ range).

| | Baseline FGF-23<759 RU/ml ("low FGF") | | Baseline FGF-23>759 RU/ml ("high FGF") | |
|---|---|---|---|---|
| | Baseline | Day 5 | Baseline | Day 5 |
| S-Cr (mg/dl) | 5.9 (2.7, 8.6) | 1.7 (1, 2.6)* | 8 (5.5, 10) | 1.2 (0.8, 2.1)* |
| S-P (mg/dl) | 4.6 (4.1, 5.2) | 5.4 (2.6, 3.9)* | 4.8 (4.3, 6.1) | 5.5 (2.1, 4.5)* |
| 1$^{st}$ PTH (pg/ml) | 288 (193, 410) | 95 (58, 158)* | 152 (82, 290) | 68 (52, 126)* |
| 1,25$(OH)_2$ vitamin D (pg/ml) | 23.8 (10.7, 57.2) | 44.4 (28.4, 62.9)* | 15.1 (2, 21.4) † | 15.4 (5.8, 35.5) † |
| C-term FGF-23 (RU/ml) | 406 (221, 534) | 77 (61, 265)* | 2085 (1069, 7400) † | 362 (102, 760)* † |
| $FE_{PO4}$ (%) | 49 (42, 73) | 24 (12, 37)* | 50 (39, 64) | 36 (18, 59)* † |
| U-FGF-23/Cr (RU/mg) | 1.68 (0.48, 5.96) | 0.36 (0.22, 0.90)* | 12.4 (1.4, 183) † | 0.35 (0.18, 1.07)* |

*p≤0.05 from baseline
†p≤0.05 between groups

The effect of baseline FGF-23 level on 1,25$(OH)_2$D, phosphate, and $FE_{PO4}$ after kidney transplant was evaluated using a mixed model. Baseline 1,25$(OH)_2$D levels did not differ between groups. 1,25$(OH)_2$D were higher in pts with low baseline FGF-23 levels than in pts. with high FGF-23 during the first five days; no differences were observed by 3 and 6 mos. Baseline FGF-23 was not associated with S-P following kidney transplant; however, $FE_{PO4}$ was significantly higher in pts with high baseline FGF-23 in the first 5 days. Thus, pre-transplant FGF-23 values have significant impact on calcitriol levels and phosphate excretion in the early transplant period; the effect of pre-transplant FGF-23 levels are attenuated by 3 months post-op.

Disclosure of Financial Relationships: Honoraria: Genzyme.

### SA-PO2148

**Serum Phosphate and FGF-23 Levels Increase in Incident Peritoneal Dialysis Patients along the Decline of Residual Renal Function** Liesbeth Viaene,[1] Bert Bammens,[1] Vanrenterghem Yves,[1] Dirk Vanderschueren,[2] Pieter Evenepoel.[1] *[1]Nephrology, University Hospital, Leuven, Belgium; [2]Endocrinology, University Hospital, Leuven, Belgium.*

Introduction: Poor residual renal function (RRF) and a disordered mineral metabolism are associated with increased mortality in peritoneal dialysis (PD) patients. Information about the impact of RRF on mineral metabolism is scanty. Methods: A prospective observational cohort study with data collection 2, 6, 12 and 24 months after start of PD was performed in 35 patients (19 male, age 55 ± 17 year). Mid-day blood samples were collected and analyzed for parameters of mineral metabolism including calcidiol, calcitriol, biointact PTH and FGF-23 (Kainos). Residual glomerular filtration rate (rGFR) was determined as the arithmetic mean of renal urea nitrogen and creatinine clearance. Differences between time points were analyzed by Wilcoxon signed-rank test (month 2 vs 24) and linear mixed models (LMM). Results: rGFR significantly declined over time (LMM p<0.0001). Peritoneal clearances tended to increase but did not compensate for the declining renal clearances. Dietary protein intake, as estimated by nPNA, and phosphate binder therapy did not change, however serum phosphate and FGF-23 levels significantly increased over time (LMM). Similar results were obtained in 18 patients free of active vitamin D therapy during the study period. Conclusion: Serum phosphate and FGF-23 levels increase in incident PD patients along the loss of RRF. Whether the increasing FGF-23 levels are a direct consequence of the ailing kidney (e.g. less expression of an inhibitor, less clearance) and/or secondary to the increased phosphate levels remains to be investigated.

Evolution of biochemistry

| | Month 2 | Month 6 | Month 12 | Month 24 | p month 2 vs month 24 | p LMM |
|---|---|---|---|---|---|---|
| Phosphate (mg/dl) | 3.8 | 4.7 | 4.3 | 4.6 | 0.01 | 0.0007 |
| Calcitriol (ng/L) | 23 | 18 | 18 | 24 | 0.51 | 0.15 |
| Biointact PTH (ng/L) | 95 | 99 | 133 | 118 | 0.30 | 0.21 |
| FGF-23 (ng/L) | 718 | | | 1516 | 0.002 | |
| rGFR (ml/min/1.73m²) | 5.3 | 4.5 | 3.8 | 2.4 | <0.0001 | <0.0001 |
| nPNA (g/kg/d) | 0.9 | 0.8 | 0.8 | 0.7 | 0.02 | 0.92 |

Median values are shown.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2149

**Effect of Different Ergocalciferol Dosing on Mineral Metabolism and Anemia Management in End Stage Kidney Disease** Neenoo Khosla, Junine Darby Degraf, Hongyan Du, Jaime N. Sua, Derek Larson, L. Tammy Ho, Stuart M. Sprague. *Nephrology, Northshore University Healthsystem, Evanston, IL.*

There is increasing interest in the impact of 25-hydroxyvitamin D (25D) repletion in patients with end stage kidney disease (ESKD). It is not known what dose of 25D should be given or what clinical effect repletion of 25D will achieve. To explore these questions Ergocalciferol (Ergo) was administered to hemodialysis (HD) patients at two dosing strategies. In 2 HD units (Group 1), patients with 25D levels <30 ng/ml were prescribed 50,000 units of Ergo weekly, while patients with levels>30 ng/ml were given 50,000 units monthly. In the third unit, all patients were given 50,000 units weekly (Group 2) for 25D <50 ng/mL. Patients were followed prospectively with baseline and quarterly labs for 6 months. Change from month 0 to 6 was calculated from difference of values with assigned rank test to assess significance. Spearman's correlation coefficient and a Wilcoxon two sample test was used to assess the difference between the two groups.

Baseline 25D median values for Group 1 was 19.5 versus Group 2 at 22.7 ng/mL (p=.135). Additional baseline lab values were similar except Group 1 had a higher baseline alkaline phosphatase value (82 vs 62.5 I.U.).

Mean 25D levels increased to 33.4 and 44 ng/ml for Groups 1 and 2 respectively, with a significantly greater increase in those with the more aggressive treatment. There was no effect on parameters of mineral metabolism in either group. There was a significant decline in Epo dosing with no change in Hgb for both Group 1 and 2. Although there was no change in iron dosing or % iron saturation in either group, those who received the higher dose of Ergo had a significant decrease in ferritin.

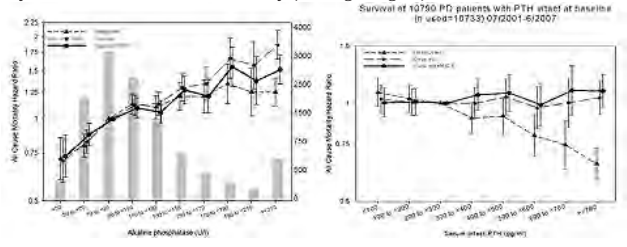
In conclusion, weekly dosing of Ergo for 25D <50 raises 25D levels to a greater extent compared to dosing based on 25D<30 and this was associated with a lower ferritin and decreased requirement for Epo. These data suggest that increasing the 25 D level may result in a decreased inflammatory state. Further studies are needed to explore these findings.

Disclosure of Financial Relationships: Research Funding: Shire.

**SA-PO2150**

**Association of Mortality with Serum Alkaline Phosphatase (AlkPhos) and Parathyroid Hormone (PTH) in Chronic Peritoneal Dialysis (CPD) Patients** Kamyar Kalantar-Zadeh,[1] Uyen Duong,[1] Csaba P. Kovesdy,[3] Allen R. Nissenson,[4] Keith C. Norris,[5] Rajnish Mehrotra.[2] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Salem VA Medical Center, Salem, VA; [4]Davita, Lakewood, CO; [5]King-Drew School of Medicine, Lynwood, CA.*

**Background:** Serum AlkPhos may be a better mortality predictor than PTH in dialysis patients (pts). We examined this hypothesis in CPD pts, since the spectrum of bone disease may vary by dialysis modality.**Methods:** We examined cohort of all CPD pts from July 2001 through June 2007. **Results:** We identified 12,422 CPD pts who had baseline AlkPhos and PTH measures. They were 54±16 years old, 47% women and 23% African Americans. Serum AlkPhos was divided into 10 preselected groups by increments of 20 U/L. Higher AlkPhos was associated with increased death risk including adjustment for case-mix and malnutrition-inflammation complex syndrome. Death hazard ratios (HR) and 95% CIs for AlkPhos groups >130 U/L were 1.3(1.1-1.4), 1.2(1.1-1.4), 1.6(1.3-1.8), 1.4(1.1-1.7), and 1.5(1.3-1.7) respectively. We also found that lower AlkPhos levels were independently associated with better survival: Death HRs (95% CI) for AlkPhos in 50-<70 and ≤50 U/L were 0.7(0.6-0.9), 0.8(0.7-0.9), respectively (see Left Figure). By contrast, eight a preselected groups of PTH <100 pg/ml to ≥ 700pg/ml and 6 groups of 100 pg/ml increments in-between did not show any notable association with mortality (see Right Figure).




**Conclusions:** In this CPD cohort, increase in AlkPhos (≥130U/L) was associated with increase in all-cause mortality and decrease in Alkphos (≤70U/L) with greater survival, whereas PTH did not exhibit death association. Additional studies to examine utility of AlkPhos for clinical decision making in CPD patients are indicated.

Disclosure of Financial Relationships: Employer: Harbor-UCLA/MFI & LABioMed; Consultancy: Abbott, Amag, Amgen, Affymas,DaVita, Fresenius, Genzyme, Otsuka, Shire, WatsonResearch Funding: Abbott, Shire, NIH, NKF; Honoraria: Abbott, Amag, Amgen, Affymas, DaVita, Fresenius, Genzyme, Shire, Watson, Otsuka; Scientific Advisor: Editorial Board: AJKD, CJASN, AJN, ACKD, JREN, Renal & Urology News.

**SA-PO2151**

**Evaluation of 16 Months of Clinical Use of Cinacalcet in Haemodialysis and Peritoneal Dialysis Patients: An Observational Study in Belgium (ECHO-B)** Frederic D. Debelle,[1] Gert Meeus,[2] Michel Dhaene,[1] Georges F. M. Cornet,[3] Xavier Warling,[4] Sofie Jamar,[5] Evemie Schutyser,[6] Michel Y. Jadoul.[7] *[1]RHMS, Baudour; [2]AZ Groeninge, Kortrijk; [3]CH Peltzer-La Tourelle, Verviers; [4]CHR de la Citadelle, Liège; [5]Imelda, Bonheiden; [6]Amgen Belgium; [7]UCL St-Luc, Brussels.*

In this Belgian observational study we investigated the effectiveness and treatment patterns of cinacalcet in dialysis patients with secondary hyperparathyroidism (SHPT). Data from 81 SHPT patients from 20 centers were collected from 6 months before to 16 months after cinacalcet initiation. Results are presented as means±SD, unless indicated otherwise. Follow-up time after cinacalcet initiation was 15.4±2.7 months. Patients were 48% male, aged 58±16 (SD) years and on dialysis for 5.2±5.7 years. They had markedly elevated intact parathyroid hormone (iPTH) levels (860±526 pg/ml) and elevated phosphorus (P) levels (5.7±1.5 mg/dL) whereas albumin-corrected calcium (Ca) concentration was 9.4±0.9 mg/dL at baseline (cinacalcet initiation).

At baseline, the proportion achieving the NKF-K/DOQI™ targets was: PTH (1%), P (40%), Ca (54%), and CaxP (48%). These values were 25%, 47%, 48% & 81% at 6 months, and 42%, 52%, 34% & 80% at 16 months after cinacalcet initiation, respectively. The proportion of patients reaching both the iPTH and CaxP targets was 21% at 6 months and 33% at 16 months *versus* 2% at baseline. Maximum reductions of Ca (-7.5%), P (-11.9%) and CaxP (-17.6%) were seen at 4 months, whereas iPTH levels were even further reduced by 36% at 4 months and 51% at 16 months. The primary endpoint (achievement of an iPTH level between 150 and 300 pg/ml and/or a reduction of 30% or more within 4 months of cinacalcet start) was achieved in 80% of patients.

Mean and median Cinacalcet doses were 48 and 30 mg in the 6th month, and 52 and 51 mg in the 16th month, respectively. Sevelamer use decreased upon cinacalcet initiation, while doses of Ca-containing P binders slightly increased. The data from this observational study are in line with published randomized controlled trials and larger observational studies, and further support the use of cinacalcet for the treatment of SHPT in dialysis patients.

Disclosure of Financial Relationships: Consultancy: Amgen; Honoraria: Amgen.

**SA-PO2152**

**Effect of Intravenous Iron Therapy on Serum FGF23 Levels and Mineral Metabolism in ESRD Patient Undergoing Hemodialysis** Yoko Takeda,[1] Hirotaka Komaba,[2] Shunsuke Goto,[1] Keiji Kono,[1] Kentaro Nakai,[1] Hideki Fujii,[1] Shinichi Nishi,[1] Masafumi Fukagawa.[2] *[1]Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan; [2]Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine, Isehara, Kanagawa, Japan.*

**INTRODUCTION AND AIMS:** Fibroblast growth factor 23 (FGF23) induces urinary phosphate excretion, suppresses 1,25-dihydroxyvitamin D synthesis, and inhibits parathyroid hormone (PTH) secretion. Recent data suggest that FGF23 plays a role in the development of iron-induced hypophosphatemia in subjects with normal kidney function. The aim of this study was to examine the effect of intravenous iron therapy on serum FGF23 levels and mineral metabolism in patients undergoing hemodialysis.

**METHODS:** This prospective study enrolled 27 patients who were receiving hemodialysis for more than three months and had iron-deficiency anemia defined by a hemoglobin concentration less than 10.5 g/dl and serum ferritin less than 100 ng/ml. Intravenous saccharated ferric oxide(SFO) at a dose of 40 mg was administered thrice weekly over three weeks. Serum FGF23, intact PTH and other parameters were prospectively monitored for five weeks.

**RESULTS:** Intravenous iron therapy resulted in a significant increase in hemoglobin and ferritin levels at wk 3 (10.0 ± 0.5 g/dl to 10.6 ± 0.5 g/dl; P < 0.001, 30.1 ± 22.7 to 153.83 ± 83 ng/ml; P <0.001, respectively). Serum FGF23 increased from 4906 ± 6201 pg/ml at baseline to 8824 ± 11041 pg/ml at wk 3 (P = 0.031), whereas intact PTH decreased from 146 ± 109 pg/ml to 109 ± 77 pg/ml (P <0.001). TRACP-5b decreased from 477 ± 295 mU/dL to 412 ± 254 mU/dL (P <0.001) at wk 3. These levels gradually returned to baseline by wk 5. There were no significant changes in serum calcium and phosphorus during the study period.

**CONCLUSIONS:** Intravenous iron therapy results in further increase in FGF23 levels in hemodialysis patients. This increase does not induce hypophosphatemia in the absence of functioning kidney, but may result in transient PTH suppression and its related decreased bone resorption.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2153**

**Racial Differences in Vitamin D, Parathyroid Hormone and Fibroblast Growth Factor-23 Levels in Patients with Severe Chronic Kidney Disease** Anna Jeanette Jovanovich,[1] Michel B. Chonchol,[1] Alfred K. Cheung,[2,4] James S. Kaufman,[3] Tom H. Greene,[4] William L. Roberts,[4] Gerard John Smits,[1] Jessica B. Kendrick.[1] *[1]University of Colorado Denver Health Sciences Center, Denver, CO; [2]VASLCHCS, Salt Lake City, UT; [3]VA Boston Healthcare System, Boston, MA; [4]University of Utah, Salt Lake City, UT.*

Purpose: Abnormalities of mineral metabolism have not been described across races in patients with severe CKD.

Methods: Study was conducted among patients with severe CKD, but not on dialysis, who participated in the Homocysteine in Kidney and End Stage Renal Disease study. 25-hydroxyvitamin D (25(OH)D), calcitriol (1,25(OH)2D), intact parathyroid hormone (iPTH), and fibroblast growth factor (FGF-23) levels were measured in plasma samples. Multivariable regression analyses were performed to examine the association between race and vitamin D, iPTH, and FGF-23 levels.

Results: There were a total of 1099 patients with an eGFR of 18 ± 6.5 mL/min/1.73m2. 57% of the cohort was non-Hispanic white (NHW), 26% was non-Hispanic black (NHB) and 17% were categorized as other races. NHB had the lowest 25(OH) D levels when compared to NHW and others (15.8±9.0 vs. 22±10 vs. 24±10 ng/mL respectively; p<0.0001). However, there was no significant difference in 1,25(OH)2D levels among races. NHB had higher iPTH levels than NHW and others (248±208 vs. 167±131 vs. 210±156 pg/mL respectively; p<0.0001). The median [IQR] FGF-23 levels among NHB, NHW and others were 324 [181-655], 431 [232-1026], and 364 [219-895] RU/mL, respectively (p=0.0001). After adjustment for demographics, cardiovascular risk factors and eGFR, NHB was independently associated with lower 25(OH)D (β= -0.25; p<0.0001) and higher iPTH (β=0.22; p<0.0001) levels than non-Blacks. NHB was independently associated with increased iPTH when further adjusted for calcium, phosphorus, 25(OH)D, 1,25(OH)2D and FGF-23 suggesting that other mechanism play a role in elevated iPTH levels in NHB. There were no racial differences in multivariable-adjusted 1,25(OH)2D and FGF-23 levels.

Conclusions: Low 25(OH)D and elevated iPTH levels are more severe in NHB when compared to non-blacks with severe CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2154**

**Preliminary Validation of Three Commercially Available Assays for Measurement of Human Plasma FGF23** Sandra M. Malakauskas,[1] Jun Ling Lu,[2] Ali Iranmanesh,[1,3] Csaba P. Kovesdy.[1,3] *[1]Salem VA Medical Center, Salem, VA; [2]Salem Research Institute, Salem, VA; [3]University of Virginia, Charlottesville, VA.*

FGF23 is a bone-derived peptide hormone that is emerging as an integral regulator of phosphorus balance and vitamin D hydroxylation, yet its clinical significance in CKD and ESRD is poorly understood. The purpose of this study is to perform preliminary validation of 3 commercially available assays for measurement of FGF23 in human plasma

and to examine the influence of sample handling on assay performance. 30 patients were enrolled from a single center and included those with normal GFR, CKD (eGFR < 60 mL/min/1.73m$^2$), and ESRD on HD. Blood was collected, and plasma FGF23 levels were determined according to the manufacturers' instructions using assays from Millipore (intact, MiFGF) and Immutopics (C-terminal, IcFGF; intact, IiFGF).

Intra-assay coefficients of variation (CV) were calculated. To examine matrix influence, plasma was spiked with known concentrations of standard FGF23 at the low and high end of the detectable range and % recovery was calculated. To assess for FGF23 degradation with delayed processing, measured FGF23 concentrations were compared between samples immediately processed ($t_0$) with those incubated at room temperature (RT) for 30-465 minutes (RT- $t_0$/ $t_0$*100).

The CV for intra-assay sample replication ranged from 3.1-36% for MiFGF and was greatest at the lower FGF23 levels observed in controls. For the IcFGF and IiFGF assays, the CV ranged 3.7-10% and 2.3-7.5% respectively. % recovery ranged 87-140% (median 110%) and 91-245% (108%) for IcFGF and IiFGF, respectively (MiFGF pending). Within each patient group, the overestimation of recoverable FGF23 was greatest at the higher concentration. RT incubation trended toward overestimation for all assays and all patient groups: -4.3-91% (median 13%) for MiFGF, -8.2-36% (15%) for IcFGF, and -5.0-11% (3.3%) for IiFGF.

Our preliminary validation confirms the previously published adequacy of IcFGF, suggests inferiority of IiFGF based on % recovery analysis, supports near-equivalence of MiFGF, and suggests that FGF23 is sufficiently stable to allow routine sample processing by clinical labs.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2155

**The Association of Steroid Use and 25(OH)D Deficiency: Results from the National Health and Nutrition Examination Survey (NHANES) 2001-2006**

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**Background**: In many kidney disorders requiring glucocorticoid therapy, there is a substantial decrease in bone mineral density. The etiology of glucocorticoid-induced osteoporosis is multifactorial and may involve 25(OH)D deficiency. The association between steroid use and 25(OH)D deficiency is theoretical and has not been confirmed in large population based studies. Recommendations for vitamin D supplementation are 400 IU/day. There are no specific recommendations for steroid users.

**Methods**: Cross-sectional analysis using children, adolescents and adults from NHANES 2001-2006 (n= 24,235), representative of 251 million US residents. We evaluated the association of serum 25(OH)D deficiency (defined as 25(OH)D <10ng/mL) with oral or IV steroid use within the past 30 days.

**Results**: 1% of the population used steroids within the past 30 days. These participants were more likely to be older, black, obese, and have eGFR <60. 5% of the population had 25(OH)D levels <10ng/ml. Among those with 25(OH)D deficiency, 10% reported steroid use. The odds of having 25(OH)D deficiency were two-fold higher in those who reported steroid use as compared to those without steroid use (OR 2.1, 95% CI 1.1, 4.0). This remained after multivariate adjustment (OR 2.1, 95% CI 1.0, 4.5). Consistent with recent studies, females (OR 1.7, 95% CI 1.5, 2.0), those >21y (OR 1.5, 95% CI 1.3, 1.7), black (OR 11.4, 95% CI 9.2, 14.2), Hispanic (OR 2.7, 95% CI 1.9, 3.8), obese (OR 1.9, 95% CI 1.6, 2.2), and those who drank milk less than once per week (OR 3.2, 95% CI 2.6, 4.1) were more likely to be 25(OH)D deficient. Those who used either 200 IU or 400 IU vitamin D supplementation per day were less likely to be 25(OH)D deficient (OR 0.2, 95% CI 0.1, 0.4, and OR 0.3, 95% CI 0.2, 0.5, respectively). When defining 25(OH)D deficiency as levels <15ng/mL, the OR for steroid users was 1.4 (95% CI 0.99, 1.94).

**Conclusion**: Steroid use is independently associated with 25(OH)D deficiency suggesting the need for screening and repletion in patients with kidney disease on chronic steroids.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2156

**Repletion of Vitamin D Deficiency by Cholecalciferol Is Blunted in Chronic Kidney Disease (CKD)**

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**BACKGROUND:** Previous studies have shown that ergocalciferol in pts with CKD stage 3+4 is not effective with less than 33% of pts achieving a 25-Vitamin D (VitD) target of >30 ng/ml and only pts with CKD 3 demonstrating a partial reduction in PTH. The aim of this study was to test the response to cholecalciferol (presumably more potent than ergocalciferol) in CKD. We attempted to replete VitD to a target level of 40-60 ng/ml using PTH suppression as an outcome measure.

**METHODS:** Demographics, creatinine and VitD were extracted from the Columbia CKD Program database. The MDRD eGFR was calculated. Pts with initial VitD ≥40 were labeled REPLETE. Pts were treated with cholecalciferol 10,000 IU weekly. Doses were titrated to a maximum of 50,000 IU weekly for levels <40. Pts achieving a VitD of ≥40 were labeled RESPONDER. Suboptimal response was labeled INTERMEDIATE. No change or a decrease in VitD was labeled NON-RESPONDER.

**RESULTS:** 389 pts (345 CKD, 44 transplant) were included with a mean follow up of 2.4 years. 48 (11.2%) were REPLETE, 100 (23%) were RESPONDER, 205 (47.8%) were INTERMEDIATE, 76 (17.7%) were NON-RESPONDER. The initial VitD in the NON-RESPONDER (27) and RESPONDER (24) were similar (p=NS). The initial eGFR in the RESPONDER (44) was significantly higher than the NON-RESPONDER (29)(p<0.001). The final eGFR in the REPLETE and RESPONDER were significantly higher than the INTERMEDIATE and NON-RESPONDER (46 and 41 vs. 32 and 25 respectively, p<0.001). This finding was similar in the transplant population (p<0.02). The post-treatment PTH in the RESPONDER decreased (121 to 87 pg/ml, p<0.05); in the NON-RESPONDER, PTH increased (148 to 216 pg/ml, p=0.002). Initial VitD and final eGFR predicted response to treatment by multivariate logistic regression (p=0.03 and p=0.005).

**CONCLUSION:** Despite aggressive repletion, only 23% of pts achieved a clinically significant increase in VitD levels. Lower initial VitD and lower final eGFR were associated with a blunted response to treatment. Treatment resistance to cholecalciferol is similar to ergocalciferol; the mechanisms for both remain unclear.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2157

**Association of Markers of Mineral Metabolism with Arterial Stiffness in Community-Living Elders: The Health ABC Study**

Magdalena Madero,[1] Christina Wassel,[2] Carmen A. Peralta,[3] Samer Najjar,[8] Kim Sutton-Tyrrell,[4] Linda F. Fried,[4] Ian H. de Boer,[5] Michael Shlipak,[3] Anne B. Newman,[4] Dorothy B. Hausman,[7] Mark J. Sarnak,[6] Stephen B. Kritchevsky,[9] Joachim H. Ix.[2] *[1]Instituto Nacional de Cardiologia; [2]U San Diego; [3]U San Francisco; [4]U Pittsburgh; [5]U Washington; [6]Tufts; [7]U Georgia; [8]Wash Hosp Ctr; [9]Wake Forest.*

Disorders of mineral metabolism are associated with greater risk for cardiovascular disease (CVD) events in the general population, even in persons without kidney disease. This risk may be mediated through arterial stiffness.The aim of the study was to evaluate the association of mineral metabolism markers with arterial pulse wave velocity (aPWV), in 2,229 older adults from the Health ABC study.Mean age was 72 years, 52% were woman, 39% were black, and 17% had chronic kidney disease (CKD). In unadjusted analyses, lower 25-hydroxyvitamin D, lower phosphorus and higher iPTH were associated with greater aPWV.

Association of Serum Markers of Mineral Metabolism with Pulse Wave Velocity

| | 25-OH-Vitamin D * | PTH * | Phosphorus * | Calcium * |
|---|---|---|---|---|
| | Δ aPWV (m/sec) (95% CI) | | | |
| Model 1 (unadjusted) | -2.86 (-4.38, -1.31) | 3.04, (1.42, 4.68) | -2.37(-3.90, -0.81) | 0.52 (-1.01, 2.08) |
| Model 2 ** | -0.59 (-2.20, 1.05) | 1.01(-0.60, 2.65) | -1.67 (-3.25, -0.07) | -0.83 (-2.34, 0.71) |

** Adjusted for age, sex, race, field, season, eGFR, BMI, smoking, physical act, DM, LDL, HDL, tryg, lipid med, SBP. DBP, BP meds

Except for phosphorus, these associations were attenuated and no longer significant after adjustment for demographic variables, site, season, kidney function and CVD risk factors.Contrary to our hypothesis, higher phosphorus was associated with lower aPWV. There was no association between serum calcium and aPWV in unadjusted or adjusted models. Results were similar by sex and the presence of CKD.We found no evidence that abnormal mineral metabolism is associated with greater aPWV in community-living elders. This study suggests that pathways other than vascular stiffness may mediate the relationship between disordered mineral metabolism and CVD events in older adults.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2158

**1, 25 Dihydroxyvitamin D3 Promotes Medial Vascular Calcification in Uremic Rat**

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1, 25 dihydroxyvitamin D3 (D3) is a hormone with multiple essential functions including regulation of mineral balance. Levels fall sharply in advanced renal disease and replacement therapy is widely practiced. Our study was designed to study the effects of D3 treatment on vascular calcification in a severe rat uremia model.

We first conducted a dose-ranging study in rats with uremia, testing 5 different doses of D3 (0-100ng/kg IP, twice weekly) for 8 days. Serum calcium and phosphorus began trending higher at 60mg/kg and were significantly higher at 100ng/kg. We selected the highest non-calcemic dose, 40ng/kg, for our treatment study.

We then began a four week study using our previously reported uremia induction (1% adenine for 1 week followed by 0.35% for 3 weeks). This model recapitulates many changes associated with human disease including changes in serum creatinine, BUN, phosphorus, and calcium and inflammatory state.

Following a week of uremia induction, half of the animals began 3 weeks of treatment with D3, 40ng/kg IP, 3 times weekly. Uremic groups had mean serum creatinine of 0.85mg/dL. At study termination, aorta was collected for mineral quantitation and histopathological analysis.

Serum - Week 4

| Group | Calcium | Pi | BUN | Creatinine | PTH | FGF-23 |
|---|---|---|---|---|---|---|
| Non-uremic | 12.6 | 12.0 | 19.6 | 0.4 | 1052 | 771 |
| Non-uremic/D | 12.4 | 10.9 | 20.4 | 0.4 | 1125 | 4563 |
| Uremic | 10.8 | 14.7 | 99.6 | 2.0 | 3626 | 5761 |
| Uremic/D | 12.0 | 15.6 | 99.9 | 1.9 | 2383 | 305416 |

There was no significant change in aortic mineral content except for uremic rats treated with D3 which had a 66X elevation in calcium, with 8-9X increases in Pi and Mg.

Aortic medial calcification was observed by Von Kossa staining only in uremic rats supplemented with D3. This was true even though serum calcium levels were normal (compared to non-uremic) and PTH was not suppressed below normal. This suggests that D3 can be a potent stimulator for calcification even at doses with no apparent hypercalcemia. The massive increase in FGF-23 in D3-treated uremic rat may play a role in this process.

Disclosure of Financial Relationships: Employer: Genzyme; Ownership: GenzymeResearch Funding: Genzyme.

**SA-PO2159**

**Comparison of Fetuin A, 25-Hydroxyvitamin D, Erythrocyte Fatty Acid Contents and Vascular Calcification on Plain Radiographs According to Dialysis Modality** Won Suk An, Young Ki Son, Seong Eun Kim, Ki Hyun Kim. *Internal Medicine, Dong-A University, Busan, Republic of Korea.*

Low fetuin-A, vitamin D insufficiency and vascular calcification (VC) on plain radiographs are related to mortality in hemodialysis (HD) patients. However there are few reports about differences of fetuin-A level, vitamin D status, erythrocyte fatty acid (FA) contents and VC on plain radiographic films according to dialysis modality. The present study was designed to demonstrate associations and any difference of fetuin-A level, vitamin D status, erythrocyte FA contents and VC on plain radiographic films according to dialysis modality. We recruited 31 HD patients and 30 peritoneal dialysis (PD) patients from a single dialysis center. We checked the plain radiographic films of the feet, hands, pelvis and lateral lumbar spine and defined significant VC as any one finding among the abdominal aortic calcifications scores >5, VC scores of the pelvis and hands >3 or arterial media calcifications of the feet on plain radiographs. Erythrocyte membrane FA contents were measured by gas chromatography. The mean age, dialysis duration, the prevalence of VC on plain radiographs (60.0 *vs* 61.3 %) were not significantly different in PD patients compared to HD patients. But Fetuin-A levels (408.1±79.0 *vs* 297.8±71.1), body mass index, total cholesterol, LDL cholesterol, monounsaturated FA, oleic acid were significantly higher in PD patients compared to HD paitients. Serum albumin and 25-hydroxyvitamin D (8.6±5.7 *vs* 21.7.±6.0) was significantly lower in PD patients compared to HD paitients. Patients who showed significant VC on plain radiographs had longer dialysis vintage, higher prevalence of coronary artery disease and higher monounsaturated FA than patients without significant VC in HD patients. Patients who showed significant VC on plain radiographs had lower Fetuin-A and higher CRP than patients without significant VC in PD patients. Fetuin-A was independent risk factor related with VC on plain radiographs in PD patients. Fetuin A, 25-hydroxyvitamin D and monounsaturated FA were significantly different, although the prevalence of VC on plain radiographs was not different according to dialysis modality.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2160**

**Detection of Vascular Calcification Patients with Stage 3, 4, and 5 Chronic Kidney Disease Who Are Not on Dialysis. OSERCE II Study** Jose L. Gorriz,[1] Javier Nieto,[2] Alberto M. Martinez-Castelao,[3] Celestino Pinera,[5] Guillermina Barril.[4] *[1]Servicio de Nefrologia, Hospital Universitario Dr Peset, Valencia, Spain; [2]Servicio de Nefrologia, Hospital General de Ciudad Real, Ciudad Real, Spain; [3]Servicio de Nefrologia, Hospital de Bellvitge, Barcelona, Spain; [4]Servicio de Nefrologia, Hospital de la Princesa, Madrid, Spain; [5]Servicio de Nefrologia, Hospital M. Valdecilla, Santander, Spain; [6]Servicio de Nefrologia, Fundació Puigvert, Barcelona.*

**OBJECTIVE:** To analyze the presence of vascular calcifications through plain X-rays of hands, hip, and abdomen in patients included in the OSERCE II study, and to assess the factors associated with the calcifications.

**METHODS:** OSERCE II is an observational study conducted in 39 sites in 3, 4, and 5 CKD patients not on dialysis. K/DOQI stage (39.6%, 46.3%, and 14.1% in 3, 4, and 5 not on dialysis stages). 37% were diabetic.

Vascular calcifications were detected using the Adragao index (plain pelvis and hand X-rays) and the Kauppila index (spinal X-ray). The films were forwarded to a hospital for centralized reading by two radiologists. Complete data were available for 572 and 568 patients (Adragao and Kauppila indices, respectively).

**RESULTS:** Significant calcifications (Adragao ≥ 3 or Kauppila > 6) were present in 30.3% and 31% respectively.

Multivariate analysis: Adragao index ≥3 was associated with male gender (OR:0.67-[0.41-1.08]), diabetes mellitus (OR:3.27-[1.86-5.77]), cerebrovascular disease (OR:2.47-[0.94-6.52]), pathological ankle-brachial index (OR:1.81-[1.11-1.2.93]), higher levels of phosphorus (OR:1.55-[1.15-2.08]), and age (OR:1.10-[1.08-1.12]).

Multivariate analysis: Kauppila index >6 was associated with peripheral vascular disease (OR:2.07-[1.22-3.51]), elevated phosphorus levels (OR:1.90-[1.44-2.50]), diabetes mellitus (OR:1.49-[0.95-2.30]), pathological ankle/brachial index (OR:1.48-[0.95-2.30]), and age (OR:1.07-[1.05-1.10]).

**CONCLUSIONS:** Stage 3, 4, and 5 CKD patients not on dialysis have a high prevalence of vascular calcifications detected with the Adragao and Kauppila indices. The prognostic significance for cardiovascular morbidity and mortality of these patients is yet to be established.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2161**

**Lack of Association of Klotho Gene Variants with Valvular and Vascular Calcification in Caucasians: A Candidate Gene Study of the Framingham Offspring Cohort** Navdeep Tangri, Ahsan Alam, Eric C. Wooten, Gordon S. Huggins. *Division of Nephrology, Tufts Medical Center, Boston, MA.*

Background: Valvular and vascular calcification are important early aging phenotypes and represent risk factors for cardiovascular morbidity and mortality. Klotho is a gene primarily expressed in the kidney that has an important role in calcium phosphate homeostasis. The functional KL-VS variant of Klotho has been associated with aging and cardiovascular disease in human studies, but its role in valvular and vascular calcification remains unknown. We performed a candidate gene study in the Framingham Offspring cohort to evaluate the effect of KL-VS variant of the Klotho gene on valvular calcification.

Methods: We analyzed the Klotho KL-VS genotype (rs9536314) from the Affymetrix 550K genome-wide dataset, distributed by dbGAP, on 1389 cases and 2139 controls from the Framingham Heart Study Offspring Cohort. Allele and genotype frequencies were compared between cases and controls. Valvular calcification was defined as presence of calcification on the mitral annulus or the aortic valve as determined by echocardiography. A sensitivity analysis of coronary artery calcification by EBCT was performed on 1363 patients.

Results: The frequency of the TT vs the TG allele was not different between the cases and the controls (39 % vs 41 %). The KL-VS variant of Klotho was not associated with valvular or vascular calcification, despite adequate power to detect association (86% for OR 1.2 or greater). In sensitivity analyses, no association (p>0.001) between other common variants of Klotho, beta klotho or FGF23 and the endpoints of valvular or vascular calcification was observed.

Conclusions: In our adequately powered candidate gene study, we did not observe an association with the functional KL-VS variant of Klotho and presence of valvular or vascular calcification. Future studies aimed at combining cohorts with echocardiographic phenotypes need to be conducted to identify genetic variants associated with valvular calcification.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2162**

**Value of Plain Bone X-Ray and Blood Chemistry for Detection of Vascular Calcification (VC) of Patients on Different Dialysis Modalities** Moon-Jae Kim, Seoung Woo Lee, Woo Chul Joo, Joon Ho Song. *Kidney Center, Inha University Hospital, Incheon City, Korea.*

**Purpose:** To evaluate detection for vascular calcification (VC) of patients between hemodialysis(HD) and peritoneal dialysis(PD) with plain radilologic bone series and blood chemistry.

**Methods**: VC was assessed using plain X-ray on L-spine, pelvis and hand in 51 HD and 74 PD patients who used calcium-based phsophate binder more than 5 years. Calcification scoring proposed by Kauppila and Adragao were used to evaluate the presence of VC in abdominal aorta, iliac and femoral, radial and digital arteries. We analyzed the difference in VC according to dialysis modality.

**Results:** One hundred and twenty five patients were included. Mean age were 57±13, 54±13 years in HD and PD groups respectively. Dialysis duration was 6.4±3.4 years in HD, 5.6±2.3 years in PD. Diabetics (DM) were 29(56.9%) in HD and 25(33.3%) in PD. Calcification scores of lumbar was correlated with scores of pelvis(r=0.561, p<0.001) and of hand(r=0.236, p=0.008). In HD patients, lumber score was correlated with age (r=0.378, p=0.006) and albumin(r= -0.284, p=0.046). In PD patients, lumbar score was correlated with age. However, HD showed more VC of digital bone(37.3 vs. 17.6%, p=0.013) and had higher scores than PD ( 0.9+1.3 vs. 0.5+1.0, p=0.054). Diabetics and male were also high scores of VC on abdominal arteries in both groups. Daily calcium load of phasphate binder and presence of residual renal function were similar between two modailties. But serum creatinine, HDL-cholesterol and iPTH levels were significantly lower in patients with low scores of VC in radial and digital arteries in both groups.

**Conclusion:** There seems to be significant difference in the location and degree of VC according to dialysis modality. HD showed more VC of digital bone and higher scores than PD on long-term use of calcium-based phosphate binders. Routine bone X-ray and blood chemistry had some value for detection for VC of patients on dialysis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2163**

**Intact PTH Overestimates Parathyroid Function in Patients under Cinacalcet Hydrochloride Treatment** Ryo Koda, Junichiro J. Kazama, Minako Wakasugi, Ichiei Narita. *Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.*

The stimulation of calcium sensing receptor in parathyroid cells increases the relative amount of 7-84PTH secretion to 1-84PTH secretion besides it decreases absolute amount of PTH secretion. Intact PTH detects both 1-84PTH and 7-84PTH, while whole PTH does only 1-84PTH. Therefore the ratio of whole PTH level / intact PTH level (w/i) was expected to decrease in patients under cinacalcet hydrochloride (CH) treatment. However, this expectation was not fully confirmed by clinical observations, because CH therapy also decreases extracellular Ca level, which is the major regulator of 1-84PTH/7-84PTH in parathyroid cells. Forty CKD5D patients who were going to receive CH treatment due to secondary hyperparathyroidism (M27:F13, 63.3±14.2yo, hemodialysis vintage 11.8±8.2 year, intact PTH 480.2±267.6 pg/ml, Ca10.0±0.7 mg/dl) were included for the analysis as the treatment group. CH was administrated to them for 24 weeks. Eighty seven of those without need of CH therapy (M56:F31, 64.5±13.1yo, hemodialysis vintage 12.0±7.7 year, intact PTH 203.3±128.7 pg/ml (p<.001 vs the treatment group), Ca9.1±0.9 mg/dl (p<.001

vs the treatment group)) were registered as control group. The serum Ca and intact PTH levels in the treatment group decreased along with CH treatment, and the levels became comparable with those of the control group at the 8, 12, 16 and 24 weeks. The w/i ratio was significantly greater in the control group than that of the treatment group at those time points (.532±.112 vs .456±.067, .448±.081, .447±.077, .453±.066, p<.0001 respectively). No significant difference was found in w/i levels in the treatment group before and during the CH therapy. Since CH therapy decreases Ca levels, it is difficult to detect the direct effect of CH on w/i in the data obtained from same patients before and during the therapy. We could successfully demonstrate it by comparing the treatment group and control group that share comparable serum Ca levels. Thus, intact PTH assay overestimates parathyroid function in patients under CH therapy, which possibly leads to the risk of the over treatment.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2164

**Discrete Effect of Cinacalcet with Maxacalcitol on the Parathyroid Growth and PTH Secretion in Patients with Secondary Hyperparathyroidism** Tatsuo Tsukamoto. *Division of Nephrology and Dialysis, The Tazuke Kofukai Medical Research Institute, Kitano Hospital, Osaka, Japan.*

Cinacalcet has decreased PTH secretion through the activation of calcium-sensing receptor in the hyperplastic parathyroid grand of dialysis patients. In addition, cinacalcet has a suppressive effect on the parathyroid growth not only in experimental animals but also patients with severe hyperparathyroidism. Thus, we established a prospective clinical study to prove this regressive effect of cinacalcet combined with intravenous maxacalcitol, a vitamin D derivative, on hyperplastic parathyroid gland in moderate to severe SHPT (iPTH 300-1000pg/ml) (UMIN 000001793), of which interim report was demonstrated in the last meeting [JASN 20, F-PO1894, 2009]. The starting dose of cinacalcet was 25mg daily, which was increased by 25mg every 2-4 weeks, until intolerance. Ca and phosphorus were adequately controlled by increase of maxacalcitol and calcium carbonate along the Japanese guideline. 53 patients with 157 of enlarged parathyroid glands were enrolled, and followed for 1 year. The gland size was measured every 6 months by a high-resolution color Doppler sonography. The average dose of cinacalcet and maxacalcitol were 39mg daily and 14μg per week at the end of the study, respectively. PTH decreased significantly from 565.5±32.0pg/ml to 164.3±20.1pg/ml after the protocol treatment. Although the averaged annual volume expansion rate was 7.5% increase in all of the parathyroid glands, 54% of the glands showed significant volume reduction after the one-year treatment. In addition, 10.2% of the glands displayed cystic degeneration during the treatment, which was determined by loss of Doppler echo. Patients with larger glands (>500mm3) responded to the treatment similar to those with smaller glands (<500mm3) in the population of moderate to severe hyperparathyroidism. Taken together, the final result of this clinical study reveals a discrete effect of cinacalcet with maxacalcitol on parathyroid growth and PTH secretion in patients with secondary hyperparathyroidism. The biological mechanism should be clarified to understand this therapeutic evidence.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2165

**Inadequate Strategy for Treating Very Low PTH Levels in Haemodialysis Patients Leads to Poor Survival Rates: Results from the French ARNOS Cohort** Guillaume Jean,[1] X. Moreau-Gaudry,[2] Denis Fouque.[3] *[1]Hémodialyse, Centre de Rein Artificiel, Tassin, France; [2]Hémodialyse, AGDUC, Montélimar, France; [3]Néphrologie Hémodialyse, Hôpital Edouard Herriot, Lyon, France.*

**Introduction**: A very low parathyroid hormone (PTH) level (VLPL) is associated with an increased risk of vascular calcification, and mortality in haemodialysis (HD) patients. The aim of the study was to assess the frequency, the associated factors, and the prognosis of non-surgical VLPL in a cohort of prevalent HD patients.

**Methods**: In July 2005, a cross-sectional study was performed on the French ARNOS cohort in 1348 prevalent HD patients from 24 dialysis centres in the Rhône-Alpes area. Patients with a baseline PTH level < 50 pg/ml (VLPL, Group 1) and ≥ 50 pg/ml (Group 2) were compared and a 42-month survival analysis was performed. Patients with prevalent or incident parathyroidectomy (PTX) were excluded.

**Results**: We studied 1138 prevalent HD patients. As compared to patients of Group 2 (n = 1019), patients with VLPL (Group 1, n = 119) had lower serum albumin levels (34.5 ± 5 vs. 36.4 ± 5 g/L, p < 0.0001), less protein intake (nPCR 0.99 ± 0.28 vs. 1.1 ± 0.28 g/kg/d, *P* = 0.01), higher calcaemia (2.30 ± 0.2 vs. 2.26 ± 0.2 mmol/l, *P* = 0.01) and were more frequently treated with calcium carbonate (67% vs. 54%, p< 0.001). Patients with VLPL had a higher mortality rate (HR: 1.4 (1.07–1.8), *P* = 0.006) after adjustments for age, gender, diabetes, and dialysis vintage. The odds ratios of mortality for patients with VLPL remained higher in all calcaemia and serum albumin quartiles. Only 3/119 patients in Group 1 did not receive any PTH-lowering therapies (i.e. calcium carbonate (67%), alfacalcidol (38%), cinacalcet (10.1%), and dialysate calcium ≥ 1.5 mmol/l (94%)).

**Conclusion**: In this observational French cohort, VLPL was observed in 10% of prevalent HD patients and was associated with poor survival rates. The real consequences of this iatrogenic adynamic bone disease remain hypothetical, but it may be related to the risk of developing vascular calcification. It is hypothesized that a more adequate strategy, using fewer PTH-lowering therapies in cases of VLPL, may help in improving the poor prognosis.

Disclosure of Financial Relationships: Employer: my wife works for Merk laboratory; Consultancy: fresenius medical care, genzyme; Ownership: noneResearch Funding: Amgen; Honoraria: Shire, Amgen, Fresenius, Genzyme; Patent: none; Scientific Advisor: Fresenius medical care, Amgen, Genzyme; Other Relationship: none.

## SA-PO2166

**Secretion of FGF23 Is Regulated by PTH** Ignacio Lopez,[1] Maria Encarnacion Rodriguez Ortiz,[2] Yolanda Almaden Peña,[2] Addy Rosa Montes de Oca Gonzalez,[1] Carmen Pineda,[1] Victoria Shalhoub,[3] Mariano Rodriguez,[2] Escolastico Aguilera-Tejero.[1] *[1]Medicina y Cirugía Animal, Universidad of Cordoba, Spain; [2]Research Unit, Neprology Department, Hospital Universitario Reina Sofia, Cordoba, Spain; [3]Amgen Inc, Thousand Oaks, CA.*

The secretion of FGF23 is stimulated by phosphate and calcitriol. FGF23 increases urinary phosphorus excretion and decreases calcitriol production by kidneys. In addition to the kidney, the parathyroid glands also respond to FGF23 by decreasing PTH secretion. As a consequence of reduced PTH levels, calcium and calcitriol concentrations tend to decrease and, phosphorus levels tend to increase. It remain to be explored if PTH may directly affect FGF23 secretion. We hypothesized that, in addition to phosphorus and calcitriol, FGF23 secretion is also regulated by PTH.

Wistar rats were parathyroidectomized (PTX) and randomly allocated to 4 groups of 8 rats each. Sham: underwent a sham surgery and were not implanted pumps for PTH infusion; PTX: received constant infusion of vehicle using miniosmotic Alzet Pump; PTX1: received a physiological dose of PTH, and PTX3 received three times the physiological dose of PTH through the miniosmotic pump. Blood was collected on days 10, 20 and 28 day for measurements of: plasma ionized calcium (iCa), phosphorus (P), and FGF23. Urine was also obtained at the time of sacrifice for measurement of fractional excretion of calcium (FECa) phosphorus (FEP).

table1

| | Ca2+ (mM) | P (mM) | FGF23 (pg/ml) | EFCa (%) | EFP (%) |
|---|---|---|---|---|---|
| Sham | 1.23±0.05b | 2.04±0.2b | 241.4±61.6b | 0.80±0.17 | 19.3±4.0 |
| PTX | 0.59±0.07a | 3.75±1.09a | 115.7±35.3a | 1.14±0.19 | 13.3±1.4 |
| PTX1 | 1.18±0.09b | 1.71±0.29b | 238.0±41.3b | 1.02±0.17 | 29.3±2.5b |
| PTX3 | 1.46±0.18ab | 1.19±0.57b | 363.5±66.3ab | 1.13±0.15 | 50.2±8.0ab |

Mean±SE, a p<0.05 vs Sham, b p<0.05 vs PTX

The results show that PTH is necessary to maintain normal circulating FGF23 levels. In PTX rats, which are markedly hyperphosphatemic, plasma FGF23 concentration declines. Moreover, PTH supplementation increases FGF23 concentrations in a dose-dependent manner despite the concomitant decrease in serum P.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2167

**Calcineurin (CN) Activation Promotes Apoptosis of Glomerular Podocytes Both In Vitro and In Vivo** Liming Wang,[1] Jae-Hyung Chang,[1] Seung-Yeol Paik,[2] Yuping Tang,[1] Robert F. Spurney.[1] *[1]Medicine, Duke Medical Center, Durham, NC; [2]Medical School, Chung-ang University, Seoul, Korea.*

A reduction in glomerular podocytes is a characteristic feature of several glomerular disease processes in humans. While the etiology of podocyte loss is likely multifactorial, apoptosis is an important mechanism of podocyte depletion in glomerular diseases. Both angiotensin II (ANGII) and endothelin-1 (ET-1) cause podocyte apoptosis by activation of G protein coupled receptors (GPCRs). To investigate the signaling pathways activated by these GPCRs that cause apoptosis, we treated a podocyte cell line with ANGII or ET-1 in the presence or absence of the CN inhibitor FK506. Podocyte apoptosis induced by either ANGII or ET-1 was prevented by FK506. Because ANGII and ET-1 activate Gq coupled GPCRs, we used protein transduction to introduce a constitutively active Gq α-subunit (GqQ>L) into podocytes by tagging Gq>L with the TAT HIV protein sequence [Gq(+)]. We found that treatment with Gq(+) enhanced podocyte apoptosis in a CN dependent fashion. In support of a role for CN in this apoptotic effect, podocyte apoptosis was similarly induced by a constitutively active CN protein tagged with the TAT protein sequence [CN(+)]. Induction of apoptosis required NFAT (nuclear factor of activated T cells) induced gene transcription because apoptosis caused by either Gq(+) or CN(+) was blocked by a cell permeable peptide inhibitor (VIVIT) that blocks NFAT activation without affecting CN phosphatase activity. To determine if CN inhibition attenuated podocyte apoptosis in vivo, we treated a mouse model of type 1 diabetes mellitus (Akita mice) with FK506 or vehicle for 1 week. In 4-week old mice, apoptosis was significantly increased in Akita mice compared to wild type controls and treatment with FK506 significantly reduced podocyte apoptosis in the Akita animals. These data suggest that Gq signaling stimulates CN and, in turn, CN activity promotes podocyte apoptosis both in vitro and in vivo by mechanisms that require NFAT dependent gene transcription. A component of the beneficial effects of CN inhibitors in glomerular diseases may be mediated by inhibiting podocyte apoptosis.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2168

**The Role of Smooth Muscle Protein SM22α in Podocyte Injury** Caroline B. Marshall,[1] Ron D. Krofft,[1] Mary Blonski,[1] Jeffrey W. Pippin,[1] Charles E. Alpers,[2] Stuart J. Shankland.[1] *[1]Dept. of Medicine, Division of Nephrology, University of Washington, Seattle, WA; [2]Dept. of Pathology, University of Washington, Seattle, WA.*

Podocytes are considered terminally differentiated cells in the mature kidney under basal conditions. Upon injury, podocytes may proceed along several pathways, including de-differentiation and proliferation, persistent cell cycle arrest, hypertrophy, apoptosis, and necrosis. There is mounting evidence that transdifferentiation into a dysregulated phenotype may also be a potential cell fate. Previously, we reported that the transcript of actin-binding protein SM22α, an early marker of smooth muscle differentiation, is upregulated nearly 70-fold in glomeruli of rats with passive Heymann nephritis (PHN). In

contrast, the SM22α transcript is absent in normal adult rat glomeruli. This study's purpose was to define SM22α's expression during kidney development and its role in glomerular diseases characterized by podocyte injury and proteinuria. During glomerulogenesis and podocyte differentiation, SM22α was expressed in glomeruli. This expression disappeared with glomerular maturation. Along with SM22α induction in PHN, confirmed at both mRNA and protein levels, SM22α was also induced across a broad range of proteinuric diseases, including experimental animal models (PAN, ADR nephropathy, passive nephrotoxic nephritis, diet-induced obesity) and human diseases (collapsing glomerulopathy, diabetic nephropathy, FSGS, IgA nephropathy, minimal-change disease, membranous nephropathy, MPGN). Crescentic glomerulonephritis (CGN) was induced in SM22α +/+ and SM22α -/- mice by IP injection of sheep anti-rabbit glomeruli antibody 12.5 mg/20 g b.w. x 2 doses (n=12-15/group), with mice sacrificed at 7d and 14d. Compared to SM22α -/- mice, SM22α +/+ mice had worse histopathologic disease. In addition, there was greater apoptosis (cleaved caspase-3 immunostaining) and fewer podocytes (WT-1 immunostaining) in diseased SM22α +/+ mice. Furthermore, there was decreased activation of Erk1/2 in diseased SM22α +/+ mice. We conclude that the *de novo* expression of smooth muscle protein SM22α is maladaptive in the podocyte response in CGN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2169**

**Angiotensin (Ang) II Promotes Podocyte Autophagy: Role of Oxidative Stress** Madhuri Adabala, Anju Yadav, Shitij Arora, Divya Salhan, Swapna Sayeneni, Ashwani Malhotra, Pravin C. Singhal. *Feinstein Institute for Medical Research, North Shore LIJ Health System, Great Neck, NY.*

Podocyte injury plays a critical role in the development of glomerulosclerosis both in human and animal models of renal diseases. Since, podocytes are terminally differentiated, they are not capable of utilizing ongoing protein synthesis into their cell cycle progression. Thus, they have to have an efficient system to handle damaged organelles and oxidized proteins. We have previously demonstrated that Ang II promotes podocyte apoptosis both *in vivo* and *in vitro* (Am J Physiol 28:F173-180, 2002). In the present study, we evaluated the effect of Ang II on the induction of autophagy and apoptosis in conditionally immortalized mouse podocytes (CIMPs).

To evaluate the effect of Ang II on podocytes, CIMPs were incubated in media containing either buffer or Ang II ($10^{-8}$ to $10^{-6}$M) for 36 hours followed by morphologic evaluation for autophagy (electron microscopy, and monodansylcadaverine staining) and apoptosis (TUNEL assay). Cells treated under identical conditions were also probed for LC-3-2 and beclin-1 expression by immunoblotting . To evaluate the role of autophagy in the promotion of Ang II-induced apoptosis, Ang II treated cells were evaluated in the presence or absence of 3-mehyl adenine (3MA, an inhibitor of autophagy, 1 mM)/rapamycin (a promoter of autophagy, 100ng/ml) followed by apoptotic evaluation.

Ang II promoted both autophagy and apoptosis in CIMPs. Ang II promoted mitochondrial reactive oxygen species (ROS) generation; whereas, antioxidants partially inhibited Ang II-induced podocyte apoptosis and autophagy. Interestingly, 3MA facilitated Ang II-induced podocyte apoptosis; on the other hand, rapamycin, inhibited Ang II-induced podocyte apoptosis. Ang II also enhanced podocyte LC3-2 and beclin-1 expression in a dose and time dependent manner. Hydrogen peroxide ($H_2O_2$) at lower concentrations, enhanced both LC3-2 and beclin-1 expression in CIMPs.

These findings indicate that severity of oxidative stress determines occurrence of autophagy vs. apoptosis. It appears that Ang II may be invoking autophagy to provide protection against its proapoptotic effect.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2170**

**TGF-beta Effect on Bioenergetics and Reactive Oxygen Species Generation in Mouse Podocytes** Yoshifusa Abe,[1, 2] Toru Sakairi,[1] Craig Cano Beeson,[3] Jeffrey B. Kopp.[1] *[1]NIDDK, NIH, Bethesda, MD; [2]Pediatrics, Showa University School of Medicine, Tokyo, Japan; [3]Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

TGF-beta contributes to progressive glomerulosclerosis, including diabetic nephropathy and focal segmental glomerulosclerosis (FSGS). TGF-beta over-expression in transgenic mice induces podocyte apoptosis and FSGS. To determine whether TGF-beta alters podocyte bioenergetics, we studied transformed mouse podocytes, using a label-free assay system, Seahorse XF24, which measures oxygen consumption rate (OCR) and extracellular acidification rates (ECAR). In the presence of glucose, pyruvate and glutamine as substrates, TGF-beta (3 ng/mL) increased basal OCR 147~174% and oxidative capacity 107~196% relative to control after 24, 48, and 72 hours. After 48 hours exposure of TGF-beta, glycolytic capacity was higher than control. ATP content was 119% (P<0.01) and 130% (P<0.01) relative to control after 48 and 72 hours exposure, respectively. We investigated the effect of TGF-beta on fatty oxidation under conditions of maximal mitochondrial function (using the mitochondrial uncoupler FCCP) and found that palmitate-driven OCR was 168% (P<0.001) relative to control. These data indicate that TGF-beta increases mitochondrial oxygen consumption and ATP generation in the presence of diverse energy substrates. These effects of TGF-beta were not due to increased cell number (as shown by cell counts and calcein AM stain). Reactive oxygen species (ROS) were increased 150% by TGF-beta treatment for 48 hours (P<0.0001). Active caspase 3/7, a pro-apoptotic enzyme, was increased after TGF-beta treatment, 139% (P<0.05), 215% (P<0.001), and 143% (P<0.001), after 24, 48, and 72 hours. With regard to basal OCR, glycolytic capacity, and ROS generation, primary mouse podocyte showed similar in direction and magnitude. We propose that TGF-beta alters bioenergetics in mouse podocytes, and these changes are associated with increased ROS and a pro-apoptotic state.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2171**

**TGF-β1 Induces Podocyte Injury Via the Src Kinase Fyn-Dependent Upregulation of the Phosphorylated Ion Channel TRPC6** Jianming Ye,[1] Qiuxia Lin,[2] Lixia Yu,[1] Hua Liao,[3] Xiaohong Dong,[1] Jianhua Feng.[1] *[1]Department of Nephrology, First People's Hospital of Kunshan, Kunshan, Jiangsu, China; [2]Department of Tissue Engineering, Institute of Basic Medical Sciences and Tissue Engineering Research Center, Academy of Military Medical Sciences, Beijing, China; [3]Department of Anatomy, Southern Medical University, Guangzhou, China.*

Purpose

We explored the potential role of TRPC6 on TGF-β1-induced podocyte apoptosis and the underlying proximal signaling to further elucidate the mechanism by which TGF-β promotes podocyte injury and the development of glomerulosclerosis.

Methods and Results

1) Flow cytometry showed that podocyte apoptosis increased significantly following 5ng/ml of TGF-β1 stimulation for 24h. Immunofluorescence staining displayed that TGF-β1 induced obvious alterations of F-actin.

2) In TGF-β1-stimulated podocyte, the cytosolic free Ca2+ upregulated markedly, which was evidently inhibited by the specific TRPC6 knockdown. TRPC6 knockdown alleviated TGF-β1-induced podocyte apoptosis.

3) TGF-β1 caused significant increment of TRPC6 protein, especially the phosphorylated form. The Src kinase Fyn increased obviously in TGF-β1-stimulated podocyte, displaying the increment of active form pY418 and the reduction of the inactive form pY530.

4) Immunoprecipitation assay revealed that Fyn interacts with TRPC6 in podocyte. Notably, the specific knockdown of Fyn markedly blocked the phosphorylation of TRPC6 and the increment of cytosolic free Ca2+ following TGF-β1 stimulation.

5) Western blot showed that TGF-β1 induced significant activation of p-smad3, p-ERK and RelA/p65. Importantly, Western blot and immunofluorescence staining revealed the obvious translocation of RelA/p65 to nuclei in TGF-β1-stimulated podocyte, which was reduced by the treatment of ERK inhibitor U0126. Both U0126 and NF-kb inhibitor PDTC obviously inhibited the increment of TRPC6 protein and the flux of cytosolic Ca2+ induced by TGF-β1.

Conclusion

We provide evidences: 1) Fyn interacts with TRPC6 in podocyte; 2) TGF-β1 induces TRPC6 increment via the activation of smad3-ERK-NF-kB pathway; 3) Fyn-dependent upregulation of the phosphorylated TRPC6 might be responsible for TGF-β1-induced podocyte injury.β

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2172**

**Regulation of Podocyte Survival and Endoplasmatic Reticulum Stress by Fatty Acids** Jonas Sieber,[1] Maja Lindenmeyer,[3] Kapil Dev Kampe,[1] Kirk N. Campbell,[5] Clemens D. Cohen,[3,4] Peter H. Mundel,[5] Andreas Werner Jehle.[1,2] *[1]Department of Biomedicine, University Hospital, Basel, Switzerland; [2]Internal Medicine, Kantonsspital Bruderholz, Basel, Switzerland; [3]Institute of Physiology, University of Zurich, Zurich, Switzerland; [4]Division of Nephrology, University of Zurich, Zurich, Switzerland; [5]Department of Medicine, University of Miami, Miller School, Miami.*

**Introduction/Objectives:** Type 2 diabetes mellitus is associated with elevated free fatty acids (FFAs). The objectives of this study were to elucidate the role of palmitic acid, palmitoleic acid, and oleic acid in the regulation of podocyte cell death and endoplasmic reticulum (ER) stress.

**Methods:** Conditionally immortalized murine podocytes were differentiated for at least 11 days and FFA were complexed to BSA. Apoptosis and necrosis were quantified by staining with Annexin V and propidium iodide. BiP, CHOP and activated Caspase 3 levels were assessed by immunoblotting. CHOP knockdown was applied by shRNA using a lentiviral system.

**Results:** We show that palmitic acid increases apoptosis and necrosis of podocytes in a dose- and time-dependent fashion. Palmitic acid induces podocyte ER stress leading to an unfolded protein response (UPR) as reflected by the induction of the ER chaperone BiP and proapoptotic transcription factor CHOP. Of note, the monounsaturated palmitoleic and oleic acid can attenuate the palmitic acid-induced upregulation of CHOP, thereby preventing cell death. Similarly, gene silencing of CHOP protects against palmitic acid induced podocyte apoptosis. The clinical relevance of our results is underscored by the observation that the gene expression of BiP is significantly upregulated in microdissected glomeruli from patients with diabetic nephropathy.

**Summary/Conclusion:** Our results unveil the antagonistic effects of palmitic acid versus monounsaturated FFAs on podocyte survival, ER-stress and UPR. They support an important role of CHOP in the regulation of podocyte cell death by FFAs.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2173**

**Erythropoietin Receptor Agonist Protects Podocytes Against Apoptosis and Modulates Podocyte-Endothelial Cell Crosstalk** Keizo Matsushita, Lijun Ma, Haichun Yang, Agnes B. Fogo. *Pathology, Vanderbilt University Medical Center, Nashville, TN.*

Background: Enhanced survival of podocytes not only has direct benefit on maintaining the capillary wall barrier but also indirectly affects glomerular endothelial cells (GEN) by secreted angiogenesis factors. We assessed effects of long lasting EPO-R agonist, darbepoetin, on TGF-β-induced podocyte injury and on podocyte-GEN interaction.

Methods: Primary cultured mouse podocytes were pretreated with darbepoetin (100μg/ml) for 3 hr before injury with TGF-β (5 ng/ml) for 24 hrs. VEGF secreted in podocyte supernatant was measured by ELISA. Markers of podocyte differentiation (synaptopodin) and apoptosis (Bax) were examined by Western blot. The migration of GEN in response to conditioned medium from intact or injured podocytes with or without darbepoetin was estimated with wound-healing assay.

Results: TGF-β significantly increased expression of Bax and decreased expression of synaptopodin in podocytes. Treatment with darbepoetin restored expression of Bax and synaptopodin. Further, TGF-β significantly increased phosphorylation of Akt, and darbepoetin partially prevented p-Akt activation.

We assessed angiogenesis factors secreted in medium of podocytes. TGF-β significantly increased secretion of VEGF. darbepoetin had no effect on this TGF-β-induced VEGF secretion.

GEN migration was induced by media from TGF-β injured podocytes ($p<0.05$). Media from podocytes protected from TGF-β injury with darbepoetin induced less migration than that without darbepoetin ($p<0.05$).

Conclusion: Our data confirm a protective role of darbepoetin in TGF-β-induced podocyte injury, and suggest that darbepoetin also affects podocyte-GEN crosstalk by mechanisms other than VEGF secretion.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2174**

**tPA Activates Mitogenic Signaling Involving LDL Receptor-Related Protein 1-Mediated p90RSK and GSK3β Pathway** Ling Lin, William Brian Reeves, Kebin Hu. *Department of Medicine, Penn State University College of Medicine, Hershey, PA.*

The numbers of interstitial fibroblasts closely correlate with the extent of kidney damage. In renal fibrosis, interstitial fibroblasts have an increased proliferative phenotype, leading to expansion of the interstitium. However, the underlying mechanisms remain largely unknown. Here we define the intracellular signaling events by which tissue plasminogen activator (tPA) promotes interstitial fibroblast proliferation. tPA promoted the proliferation of renal interstitial fibroblasts independent of its protease activity. The mitogenic effect of tPA required $Tyr^{4507}$ phosphorylation of the cytoplasmic tail of its receptor LDL receptor-related protein 1 (LRP-1). tPA triggered sequential proliferative signaling events involving Erk1/2, p90RSK, GSK3β phosphorylation, and cyclin D1 induction. Blockade of Erk1/2 activation or knockdown of p90RSK suppressed tPA-induced GSK3β phosphorylation, cyclin D1 expression, and fibroblast proliferation. In contrast, expression of constitutively active Mek1 mimicked tPA in inducing GSK3β phosphorylation and cyclin D1 expression. Ectopic over-expression of an uninhibitable GSK3β mutant eliminated tPA-induced cyclin D1 expression. In the murine obstructive injury model, tPA deficiency reduced renal GSK3β phosphorylation after 7 and 14 days of obstruction. These findings show that tPA induces $Tyr^{4507}$ phosphorylation of LRP-1, which in turn leads to the downstream phosphorylation of Erk1/2, p90RSK, and GSK3β, followed by the induction of cyclin D1 in murine interstitial fibroblasts. This study implicates tPA as a mitogen that promotes interstitial fibroblast proliferation, leading to expansion of these cells.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2175**

**INK4a Knockout Mice Exhibit Increased Fibrosis under Normal Conditions and in Response to Unilateral Ureteral Obstruction** David H. Lee, Jesse M. Wolstein, Jennine Michaud, Vanessa C. Buot, Matthew D. Plotkin. *Nephrology, New York Medical College, Valhalla, NY.*

The two proteins encoded by the INK4a locus, $p16^{INK4a}$ and $p19^{ARF}$, have been shown to contribute to cell cycle arrest and senescence. However, the role of these proteins in controlling cell proliferation in normal kidney and kidney repair and the fibrotic response to injury is unknown. We performed unilateral ureteral obstruction (UUO) to induce fibrosis in 2-3 month old WT C57/B6 and INK4a knockout mice. By quantitative RT PCR, $p16^{INK4a}$ levels were increased 6-fold in WT mice 7 days after UUO and $p19^{ARF}$ remained undetectable. Kidney sections were examined to determine levels and localization of $p16^{INK4a}$, apoptosis, fibrosis, and senescent cells. INK4a knockout mice displayed mesangial cell proliferation, increased matrix deposition and medullary myofibroblast differentiation under normal conditions. Following UUO, INK4a knockout mice displayed 10-fold increased tubular and interstitial cell proliferation, 75% decreased collecting duct apoptosis by TUNEL staining, 2-fold greater collagen and fibronectin deposition, and no cell senescence by SA-ß-galactosidase staining compared to WT mice. Both INK4a knockout mesangial cells grown *in vitro* and kidney lysates from knockout mice following injury showed elevated levels of IL-6 by ELISA compared to WT samples. In addition, INK4a knockout proximal tubule cell cultures displayed increased mesenchymal cell markers compared to WT cells when exposed to TGF-ß. These results confirm that $p16^{INK4a}$ controls mesenchymal cell proliferation and matrix production and mitigates fibrosis in response to injury and suggest that the mechanism involves a role in limiting inflammation and cell proliferation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2176**

***Grem1* Deletion Alters Cell Proliferation *In Vitro* and *In Vivo* in a UUO Model of Fibrosis** Simon P. Curran,[1,2] Alan J. Watson,[1] Derek P. Brazil.[3] *[1]Nephrology Dept., St. Vincent's University Hospital, Dublin, Ireland; [2]Diabetes Research Centre, University College Dublin, Ireland; [3]Centre for Vision and Vascular Science, Queen's University Belfast, United Kingdom.*

Gremlin1 is a Bone Morphogenetic Protein antagonist that displays increased expression in models of renal fibrosis. It has been shown that allelic deletion of *grem1* attenuates Diabetic Nephropathy in mice. Previous reports have suggested that Gremlin1 can drive cell proliferation and promote apoptosis. We explored the role of this protein in cell proliferation to shed light on it's role in renal fibrosis.

Mouse embryonic fibroblasts (MEFs), wild type (+/+), *grem1*+/- and *grem1*-/-, were used to study *in vitro* markers of proliferation. An MTT assay found that *in vitro* there was increased proliferation in *grem1*-/- MEFs. A scratch wound assay indicated a trend for greater wound closure after serum stimulation in *grem1*-/- MEFs.

Unilateral ureteric obstruction was performed on +/+ and *grem1*+/- mice and kidney sections were examined for markers of renal damage and cell proliferation. An increase in kidney weight was seen in the contralateral kidney in both the sham and UUO mice at day 3 which reached statistical significance in the +/+ but not the *grem1*+/- animal. Histological staining for Ki-67 detected an increased number of proliferating cells in +/+ versus *grem1*+/- at baseline. The total number of proliferating cells increased in both +/+ and *grem1*+/- and this response was greater in +/+ mice.

These results suggest that Gremlin1 has a definite role in proliferation in both fibroblasts and kidney cells in vivo. The observed differences *in vitro* and *in vivo* may reflect cell specific effects of Gremlin1 deletion in embryonic fibroblasts compared to kidney epithelial cells. These data suggest that future efforts to modify Gremlin1 activity in areas of cellular injury may provide benefit to the fibrotic kidney.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2177**

**Interactions between Dead and Viable Proximal Tubular Cells (PTC) Influence Cell Survival, Growth, and Proliferation: Implications for Recovery after Acute Kidney Injury (AKI)** Vimal Patel,[1] Lanfei Feng,[1] Donald Massenburg,[1] Angelika Antoni,[3] Joyce Rauch,[4] Wilfred Lieberthal,[2] Jerrold S. Levine.[1] *[1]Medicine, University of Illinois, Chicago, IL; [2]Medicine, SUNY at Stony Brook, Stony Brook, NY; [3]Medicine, Kutztown University, Kutztown, PA; [4]Medicine, McGill University, Montreal, Canada.*

During AKI, PTC sustain sublethal injury or die by apoptosis or necrosis. Recovery depends on survival of injured cells and regeneration, which in turn requires cell growth (increased cell mass) and cell proliferation. We used BU.MPT cells, a conditionally immortalized PTC line, to examine the effects of receptor-mediated interaction between dead and viable BU.MPT cells. We show that apoptotic and necrotic PTC targets have profound, directionally opposite effects on survival, growth, and proliferation of viable PTC responders.

(1) Survival: Apoptotic PTC targets inhibited Akt activity in viable BU.MPT responders and induced apoptotic death of ~80% responders by 48 hrs. A constitutively active Akt construct blocked apoptosis of BU.MPT cells by only ~50%, implying that apoptotic PTC targets induce death via both Akt-dependent and -independent pathways. In contrast, necrotic PTC activated Akt and promoted BU.MPT cell survival.

(2) Proliferation: Apoptotic PTC targets inhibited ERK1/2 activity and markedly slowed cell cycle progression of actively proliferating BU.MPT cells. In contrast, necrotic targets activated ERK1/2 and stimulated cell cycle progression of quiescent BU.MPT cells.

(3) Growth: Apoptotic PTC decreased activity of mTOR and its downstream target p70S6K, inhibiting growth of viable BU.MPT cells. Inhibition of mTOR seemed to occur via at least two apoptotic target-dependent pathways, inhibition of Akt and activation of AMPK.

All effects on cell fate occurred in a dose-dependent manner, and required physical contact between dead and viable PTC. Effects were independent of engulfment, requiring only receptor-mediated recognition. Our data show that signaling events, induced by recognition of apoptotic and necrotic PTC, have distinct effects on survival, proliferation, and growth of viable PTC, events that are important to recovery from AKI.

Disclosure of Financial Relationships: nothing to disclose

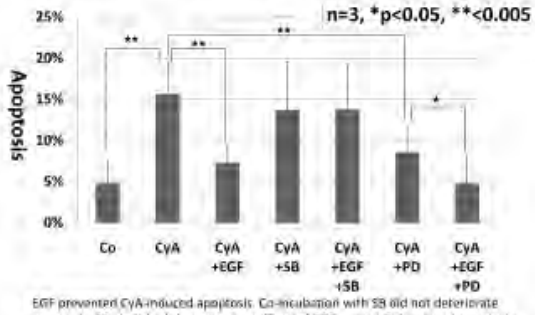
**SA-PO2178**

**Crosstalk between Smads and p38MAP Kinase and ERK and Its Modulation by Epidermal Growth Factor Regulate Apoptosis in Cyclosporine A-Induced Renal Tubular Injury** Hideyuki Iwayama,[1] Norishi Ueda.[2] *[1]Neonatology and Pediatrics, Nagoya City University, Nagoya, Aichi, Japan; [2]Nagoya University, Nagoya, Aichi, Jordan.*

We examined whether there is a crosstalk between Smads and MAPK kinases (MAPKs) and its modulation by epidermal growth factor (EGF) in cyclosporine A (CyA)-induced renal tubular cell (RTC) injury. HK-2 cells, human renal proximal tubular cells, were exposed to CyA (0.42-42μM) for 0-24 hrs in a serum-free condition. Apoptosis, measured by Hoechst 33258 staining, ocurred at 24 hrs after exposure to CyA. CyA induced apoptosis

in a time- and dose-dependent manner. Nuclear translocation (NT) of phospho(p)-Smad2/3, determined by immunofluorescence staining and Western blotting, occurred at 3 hrs after exposure to CyA (42μM), preceding apoptosis, increasing up to 24 hrs. CyA increased expression of p-p38MAPK, determined by Western blotting, at 1 hr and returned to control level at 12 hrs. CyA increased expression of p-ERK at 1 hr, increasing up to 24 hrs compared to control. EGF (20 ng/ml) activated both p-p38MAPK and p-ERK in control cells. EGF prevented CyA-induced NT of p-Smad2/3 and apoptosis. Co-incubation of HK-2 cells with a specific inhibitor of p38MAPK, SB202190 (SB:20μM), deteriorated CyA-induced NT of p-Sma2/3 but not apoptosis. SB202190 abolished the protective effects of EGF against CyA-induced NT of p-Smad2/3 and apoptosis. An inhibitor of ERK, PD98059 (20μM) did not affect the effect of CyA-induced NT of p-Smad2/3, but enhanced the protective effect of EGF on CyA-induced NT of Smad2/3 and apoptosis. Our data demonstrate a crosstalk between Smad2/3 and p38MAPK and ERK and that EGF inhibits Smad2/3 signaling and apoptosis through activation of p38MAPK and ERK in CyA-induced RTC injury.

**Figure1. Effect of EGF on CyA-Induced apoptosis and its modulation by an inhibition of ERK or p38MAPK**




EGF prevented CyA-induced apoptosis. Co-incubation with SB did not deteriorate apoptosis. SB abolished the protective effects of EGF against CyA-induced apoptosis. PD enhanced the protective effect of EGF on CyA-induced apoptosis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2179**

**Pharmacological Activation of Epac1 Protects Against Cisplatin-Induced Nephrotoxic Injury in Renal Tubular Epithelial Cells** Geurt Stokman,[1] Yu Qin,[1] Ingeborg M. Bajema,[2] Bob Water,[1] Leo Price.[1] *[1]Toxicology, LACDR, Leiden University, Leiden, Netherlands; [2]Pathology, LUMC, Leiden University, Leiden, Netherlands.*

Nephrotoxicity is the primary reason for discontinuation of cisplatin administration in cancer treatment. Toxicity is associated with disruption of adhesion of proximal tubular epithelial cells and apoptosis. Activation of the small GTPase Rap1 by Exchange Protein directly Activated by cAMP 1 (Epac1) promotes cell adhesion and supports adhesion-mediated cell survival signaling. Here we demonstrate the anti-apoptotic effect of Epac-Rap signaling pathway activation on in vitro cisplatin-induced cell death.

Proximal tubular epithelial cells were exposed to cisplatin, in combination with the Epac1 selective cAMP analogue 8-pCPT-2'-O-Me-cAMP (007). Activation of Rap1 was detected using Ral-GDS-RBD-pull down analysis. Cell death was measured by caspase 3 activity and cell cycle analysis. Cell-cell adhesion was quantified based on expression patterns of beta-catenin and zona occludens-1. Epac1 and Rap1 knockdown was performed by siRNA transfection. A panel of lung and breast cancer cell lines were tested for their responsiveness to 007 in cisplatin-induced cell death.

A 24 hour incubation of a proximal tubular epithelial cell line with cisplatin induced severe cellular apoptosis. This was accompanied by extensive disassembly of adherens and tight junctions. 007 treatment reduced cisplatin-induced apoptosis and cell junction disassembly – an effect that was dependent on expression of Epac1 and Rap1. In contrast, cell lines derived from lung and breast cancer tumors did not express Epac1 and were not protected against cisplatin-induced cell death by 007.

Activation of Epac-Rap signalling and stabilisation of tubular epithelial cell adhesions offers a potential strategy to reduce cisplatin-induced nephrotoxicity and widen the therapeutic window for cisplatin treatment without impairing efficacy towards cancer cells.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2180**

**Consideration of Protein Expression Changing with Pitavastatin Therapy in Chronic Puromaycin Aminonucreoside Nephropaty** Tokushi Nakajima, Koichi Kanozawa, Hajime Hasegawa. *Departement of Renal and Hypertension, Kasagoe, Saitama, Japan.*

< Back ground >

In puromaycin aminonucreoside nephrosis rats which is one of human nephritic syndrome model. In hyperlipidemia, statins have many evidences to protect from cardiovascular disease and renal failure. In nephritic syndrome, hyperlipidemia is one of main symptom. Excessive oxidants play roles in the pathogenesis of glomerular injury. Several studies have demonstrated protective effects of pitavastain against renal injury. To clarify the protective effects of pitavastatin, we investigated changes in chemical markers of lipid peroxidation and renal histology, and protein array in rats with chronic puromycin aminonucleoside nephrosis (C-PAN).

< Methods >

C-PAN was induced by intraperitoneal injections of PAN (130 mg/kg on day 1 and 60 mg/kg on day 14). Rats administered normal saline served as controls (n=5). C- PAN rats were divided into two groups (each group: n=4). Blood and urinary samples were collected every week. Animals were sacrificed at the end of experiment for histological and protein array analysis.

< Results >

In C-PAN+Pit rats, urinary excretion of albumin and 8-isoPGF2 were significantly decreased compared with that in C-PAN rats ($p<0.01$), whereas levels of urinary thiobarbituric acid-reactive substances (TBARS) did not differ between the two groups. On histological examination, the scores for glomerular injury and area positive for 4-HNE were significantly lower in C-PAN+Pit than in C-PAN rats ($p<0.01$). Infiltration of macrophages was also suppressed in C-PAN+Pit rats. By protein microarray analysis, mammalian target of rapamaycin (mTOR) protein is significantly upregulated (1500 field).

< Conclusion >

Pitavastatin treatment of C-PAN was able to decrease albuminuria and to ameliorate glomerular injury. Levels of several markers related to oxidative stress and lipid peroxidation were reduced after treatment, accompanied by reduction of macrophage infiltration and decrease in apoptotic cells in the glomeruli. These findings indicate that pitavastatin has protective effects against the glomerular injury observed in C-PAN nephrosis, and the effect is strongly connect with mTOR protein activity.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2181**

**Akt Regulates Albumin Uptake Via Dab2 Phosphorylation** Elif Erkan,[1] Kenneth R. Hallows,[2] Jeffrey Lee,[2] Kelly Koral.[1] *[1]Pediatrics, University of Pittsburgh; [2]Internal Medicine, University of Pittsburgh.*

Proteins involved in endocytosis have the potential to initiate signaling events. In addition to its role in clathrin assembly and sorting, Disabled 2 (Dab2) is implicated in cell positioning, adhesion and differentiation. Albumin endocytosis in proximal tubule epithelial cells occur via receptor mediated endocytosis with the involvement of clathrin, clathrin associated sorting proteins (CLASPs) and transmembrane receptor megalin. We examined whether there is a link between endocytosis and a kinase implicated in cell survival, protein kinase B (Akt). The proline rich domain (residues 600-730) of Dab2 and protein kinase B (Akt) co- immunoprecipitate and co-precipitate in GST pull-down experiments. The physiological relevance of this interaction was examined in albumin uptake studies. Overexpression of Akt in human kidney proximal tubule cells (HKC-8) with plasmids encoding wild type and constitutively active (CA) Akt increased internalization of albumin. Expression of dominant negative of Akt (Akt-T308A/S473A) or pretreatment of cells with the specific Akt inhibitor, API-2, caused a significant decrease in albumin uptake. Furthermore, inhibition of Dab2 by silencing RNA decreased albumin uptake in HKC-8 cell transfected with CA-Akt ,validating the role of Dab2-Akt interaction in regulation of albumin uptake and suggesting that Akt operates upstream from Dab2. *In vitro* phosphorylation experiments demonstrated that Akt phosphorylates the M15 portion (residues 335-610) of Dab2. In summary, we have demonstrated an interaction between Dab2 and Akt, that modulates albumin uptake in a proximal tubular cell line. We believe Akt is a new member of the endocytic machinery and may be involved in trafficking and sorting events by interacting and phosphorylating CLASPs in proximal tubule cells. Delineation of protein-protein interactions surrounding activation of Akt and identifying network of proteins involved in albumin uptake will enable us to understand the physiology of proximal tubule function and develop strategies to halt proteinuria induced progression by targeting key proteins of the endocytic pathway

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2182**

**Regulation of the Ste20-Like Kinase SLK by Phosphorylation** Artem Luhovy, Andrey V. Cybulsky. *Medicine, McGill University, Montreal, QC, Canada.*

Expression and activation of SLK is increased during kidney development and recovery from ischemic acute kidney injury, which recapitulates aspects of kidney development. SLK promotes apoptosis and may, therefore, regulate cell growth during development, injury or healing. We propose that activation of SLK may be due to upregulation of expression, which may then favor homodimerization and autophosphorylation. The aim of this study was to determine the role of phosphorylation in the regulation of kinase activity. We mutated serine and threonine residues in the putative activation segment of the SLK catalytic domain, and expressed wild type (WT) and mutant proteins in COS-1 or glomerular epithelial cells. Compared with SLK WT, the S189A mutant and the T183A/S189A double mutant showed trivial in vitro kinase activity, while kinase activity was reduced significantly by the T183A mutation. Expression of SLK WT increased activation-specific phosphorylation of c-Jun N-terminal kinase (JNK) and p38 kinase, reflecting enhanced signaling via stress kinase pathways. In contrast, activation of JNK and p38 by SLK T183A, S189A, and T183A/S189A was impaired. Similarly, expression of SLK WT stimulated activator protein-1 (AP1) reporter activity, but activation of AP1 by the three SLK mutants was ineffective. To test if homodimerization of SLK affects phosphorylation, the cDNA encoding the SLK catalytic domain (amino acids 1-373) was fused with a cDNA for a modified FK506 binding protein, Fv (SLK-Fv). After transfection, addition of AP20187 (an FK506 analog) induced regulated dimerization of SLK-Fv. AP20187-stimulated dimerization enhanced the kinase activity of SLK-Fv WT. In contrast, kinase activities of SLK-Fv T183A/S189A and another activation site mutant, T193A, were weak, and were not enhanced after dimerization. Surprisingly,

phosphomimetic mutants of SLK-Fv, including T183D, T183E, S189D, and S189E showed weak kinase activity, which was unaffected by dimerization. Finally, apoptosis and necrosis were increased after expression of SLK-Fv WT, but not T183A/S189A. Thus, phosphorylation of T183, S189 and T193 plays a key role in the activation and signaling of SLK, and could represent a target for novel therapeutic approaches to renal injury.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2183**

**IL-17A Induces Granulocyte Colony-Stimulating Factor Via ERK1/2 but Not p38 and JNK in Human Renal Proximal Tubular Epithelial Cells** Yuki Hirai, Masayuki Iyoda, Takanori Shibata, Yoshihiro Kuno, Tadao Akizawa. *Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan.*

Introduction: We investigated the potential role of interleukin (IL)-17A in the induction of granulocyte colony-stimulating factor (G-CSF), a critical granulopoietic growth factor, in renal proximal tubular epithelial cells. Methods: We used human renal proximal tubular cells (HK-2). The expression of G-CSF was evaluated by enzyme-linked immunosorbent assay (ELISA) and real-time RT-PCR. Activation of mitogen-activated protein kinase (MAPK) was assessed by immunoblotting. Results: We found that IL-17A stimulation of the cells led to a dose-dependent (27.54±0.69 vs. 41.86±1.39, pg/ml, IL-17A (10 ng/ml) vs. IL-17A (100 ng/ml)) and time-dependent (4.10±0.48 vs. 41.86±1.39, pg/ml, 6h vs. 24h stimulation with IL-17A (100 ng/ml)) increase in G-CSF release. This effect was dependent on mRNA transcription and protein translation (51.21±1.47 vs. 5.88±0.29 vs. 8.01±0.26, pg/ml, IL-17A (100 ng/ml) vs. IL-17A (100 ng/ml) + actinomycin D (1 μg/ml) vs. IL-17A (100 ng/ml) + cycloheximide (10 μg/ml)). Real-time RT-PCR demonstrated that G-CSF mRNA expression reached a maximum level 6 h following IL-17A stimulation. IL-17A also enhanced TNF-α or IL-1β-mediated G-CSF release in the cells (3.50±0.41 vs. 26.19±1.21 vs. 335.90±7.41, pg/ml, TNF-α (1 ng/ml) vs. IL-17A (100 ng/ml) vs. TNF-α (1 ng/ml) + IL-17A (100 ng/ml)). Additionally, we observed that IL-17A induced MAPK (ERK1/2 but not p38 MAPK or JNK) activation (Western blotting analysis showed the phosphrylation of ERK1/2 started at 5 min returned to baseline levels from 120 min in IL-17A-stimulated proximal tubular cells), and that pharmacological inhibitors of MEK1/2 (U0126) but not p38 MAPK (SB203580) or JNK (SP600125), significantly blocked the IL-17A-mediated G-CSF release (32.05±0.77 vs. 17.56±1.44, pg/ml, IL-17A (100 ng/ml) + DMSO vs. IL-17A (100 ng/ml) + U0126 (10μM)). Conclusion: We demonstrate the previously unappreciated ability of IL-17A in the induction of G-CSF in renal proximal tubular cells. It is proposed that IL-17A may play an important role in neutrophil recruitment via stimulating G-CSF in tubular injury.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2184**

**High Glucose Degradation Products PD-Fluids Overcome the Protective Effect of Apoptosis Inhibitors on Inflammation Induced Cell Death** Alberto Benito Martin, Beatriz Santamaria Pérez, Alvaro C. Ucero, Jesus Egido, Alberto Ortiz. *Experimental Nephrology Department, Fundacion Jimenez Diaz, Madrid, Spain.*

***Background*:** Peritoneal dialysis is complicated by peritonitis episodes that cause loss of mesothelium. Proinflammatory cytokines increase mesothelial cell apoptosis. However, there is scarce information about the influence of the cytokine-peritoneal dialysis solution interplay on mesothelial cell death.

***Methodology***: Human mesothelial cells were cultured from effluents of stable peritoneal dialysis patients and from omentum of non-dialysis patients. The cytokine combination of tumor necrosis factor alpha and interferon gamma increased the rate of mesothelial cell apoptosis and a polymeric nanoconjugate Apaf-1 inhibitor protected from apoptosis. Culture in the presence of high glucose, high glucose degradation product (GDP) containing PD solutions increased cell death induced by this cytokine combination. This increment was not observed when high glucose, low GDP PD solutions were added. Addition of high glucose, high GDP PD solutions resulted in an increased Bax/BclxL ratio. A BH4 peptide that behaves like BclxL protected from spontaneous or staurosporin-induced mesothelial cell apoptosis but did not protect from apoptosis induced by the cytokine combination in the presence of high glucose, high GDP PD solutions. Furthermore, the polymeric nanoconjugate Apaf-1 inhibitor also protected from high glucose, high GDP PD solution-induced apoptosis, but was unable to prevent apoptosis induced by the cytokine combination in the presence of high glucose, high GDP PD solutions.

***Conclusion*:** Cooperation between tumor necrosis factor alpha and interferon gamma contributes to mesothelial injury. The lethal effect is exacerbated by high glucose, high GDP PD solutions. Lowering the GDP content of PD solutions, but not the use of drugs targeting the apoptotic machinery, reverses cytokine induced apoptosis in the presence of inflammation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2185**

**AATF/Che-1 in DNA-Damage Response and Its Function in Carcinogenesis of Epithelial Cells in the Kidney** Katja Hoepker, Henning Hagmann, Safiya Khurshid, Bernhard Schermer, Thomas Benzing. *Department of Nephrology, University Hospital Cologne, Cologne, Germany.*

In every eukaryotic cell genomic integrity is continuously being challenged by DNA damage. An increased rate of DNA damage and mutations has been implicated as key causal events in the process of carcinogenesis. In response to genotoxic stress eukaryotic cells activate DNA damage checkpoint pathways to protect genomic integrity. Damaged cells with alarmed checkpoints can be eliminated by apoptosis, silenced by cellular senescence or can survive and resume cell cycle progression upon DNA repair and checkpoint termination.

We and other groups have identified AATF/Che-1, a transcriptional regulator and RNA polymerase II binding protein, as a key protein involved in DNA damage response pathways.

Here we show that under normal conditions AATF/Che-1 resides in the cytoplasm where it interacts with Myosin Regulatory Light Chain 3 (MRLC3). This interaction is regulated through the basophilic MAPKAP kinase 2 (MK2). DNA damage caused by genotoxic stress results in the activation of MK2 via the p38 pathway and phosphorylation of AATF/Che-1. This abrogates the interaction with MRLC3 and releases AATF/Che-1 from its cytoplasmic pool, allowing it to travel to the nucleus where it promotes transcription of target genes. We further show that AATF/Che-1 plays a critical role in resistance to apoptosis after DNA-damage. Suppression of AATF/Che-1 by siRNA increases the rate of apoptotic cells after genotoxic stimuli. Whereas RNAi knock down of MRLC3 leads to decreased apoptotic response. This "apoptosis antagonizing effect" of AATF/Che-1 was found to be largely p53 dependent.

Perspective: Renal cell carcinoma is a notoriously difficult to treat entity and largely resistant to conventional chemotherapy. Here we show that AATF/Che-1 is a key player in DNA-damage response regulated by the MK2 pathway. After genotoxic stress AATF/Che-1 delicately modulates the balance of p53 and other genes involved in DNA damage response preventing the cell from undergoing apoptosis. Being a part of the pharmacologically targetable MK2-pathway AATF/Che-1 may have implications in chemo-sensitizing therapy of renal cell carcinoma.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2186**

**Armcx2 Is Expressed in Late Stages of Nephrogenesis and Is Lost in Renal Cell Carcinoma** Stephan Grouls,[1] Ellen Krautkrämer,[1] Luis Eduardo Becker,[1] Jochen Reiser,[2] Martin G. Zeier.[1] *[1]Nephrology, University of Heidelberg, Heidelberg, Germany; [2]Nephrology and Hypertension, University of Miami, Miami.*

Background:

ARMCX2 is a member of the armadillo repeat protein family. Proteins containing arm repeat motifs, i.e. beta-catenin, are involved in fundamental processes such as proliferation, migration, and differentiation. Those processes play a key role not only during development but also in tumorigenesis. Therefore we wanted to investigate the potential role of ARMCX2 expression during nephrogenesis and in renal cell carcinoma.

Methods:

We analyzed the expression pattern of ARMCX2 in human renal cell lines, primary renal epithelial cells and renal cell carcinoma cell lines. Furthermore, we performed immunohistochemistry for the detection of ARMCX2 in sections of normal human kidney and renal cell carcinoma.

Results:

In normal human kidneys ARMCX2 was expressed exclusively in epithelial cells of the kidney including podocytes. These in vivo data were in consistence with our in vitro data which revealed strong ARMCX2 expression in primary tubular epithelial kidney cells and podocytes. During nephrogenesis ARMCX2 was earliest detectable in late stages. While there was no ARMCX2 expression in the s-shaped body stage strong expression was present in glomerular and tubular epithelial cells in the maturing glomeruli stage. However, ARMCX2 expression was downregulated in renal cell carcinoma cell lines and in biopsies of renal cell carcinoma. ARMCX2 was mainly localized in the perinuclear region and in filament-like structures in the periphery where it was colocalized with focal adhesions.

Conclusion:

We conclude that ARMCX2 is expressed in epithelial cells of the adult kidney and late during nephrogenesis. These findings indicate a role of ARMCX2 in the differentiation of kidney epithelial cells. The association with focal adhesions underlines the assumption that ARMCX2 plays a role in the final process of cell differentiation of renal epithelial cells. Furthermore, our results demonstrate that ARMCX2 is downregulated in renal cell carcinoma cell lines indicating a role as possible tumorsuppressor.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2187**

**SC35-Dependent Sequestration of Phospho-BADser112 in Nuclear Speckles Is a Novel Cell Survival Mechanism** Kirk N. Campbell,[1] Ritu Gupta,[1] Katsuhiko Asanuma,[2] Peter H. Mundel.[3] *[1]Nephrology and Hypertension, University of Miami Miller School of Medicine, Miami, FL; [2]Nephrology, Juntendo University School of Medicine, Tokyo, Japan; [3]Molecular Medicine, University of Miami Miller School of Medicine, Miami, FL.*

Speckles are nuclear organelles enriched in small nuclear ribonucleoproteins (snRNP) and numerous other splicing and transcription related proteins and kinases. SC35 is a non-snRNP serine/arginine-rich RNA binding protein important in splicing and spliceosome assembly that is constitutively expressed in speckles. A role for SC35 has also been identified in regulating cell cycle progression and genomic stability. We previously identified dendrin as a glomerular slit diaphragm component that relocates to the nucleus under disease conditions to promote podocyte apoptosis. Here we show that the binding of Yes-associated protein 2 (YAP2) to dendrin increases the steady-state protein expression of SC35 by inhibiting its degradation via the nuclear ubiquitin-proteasome system. We also show that phospho-BADser112 accumulates and co-localizes with SC35 in nuclear speckles induced by binding of YAP2 to dendrin. Gene silencing of SC35 abrogates the accumulation of phospho-BADser112 in speckles and enhances staurosporine-induced apoptosis in HEK293 cells cotransfected with YAP2 and dendrin.

In summary, we have identified a novel pro-survival mechanism where the binding of YAP2 to dendrin increases steady-state protein expression of SC35 culminating in the sequestration of phospho-BADser112 in nuclear speckles.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2188**

**Nucleosphosmin: An Apoptosis Promoting Co-Factor for Bax in ATP Depleted Renal Epithelial Cells** Zhiyong Wang, Jonathan M. Gall, Andrea Havasi, John H. Schwartz, Steven C. Borkan. *Renal Section, Boston Medical Center, Boston, MA.*

Nucelosphomin (NPM), a nuclear co-factor that regulates ribosomal protein synthesis, has recently been shown to complex with Bax, a key cause of stress-induced mitochondrial injury and cell death. We hypothesized that NPM acts as a co-factor that promotes Bax toxicity and apoptosis. To test this hypothesis, human renal proximal tubule cells were subjected to ATP depletion sufficient to induce apoptosis. At baseline, NPM localized almost exclusively to the nucleus. ATP depletion caused marked cytosolic NPM accumulation before mitochondrial injury or apoptosis occurred and without altering total cell NPM content. Injury induced Bax activation, evidenced by exposure of the 6A7 epitope and also increased the interaction between NPM and active Bax. Expression of either flag-tagged wild type NPM or a mutant lacking the nuclear localizing sequence (NPMΔNLS) increased both cytosolic NPM accumulation and stress-induced cell death. In contrast, over-expression of a NPM mutant lacking the nuclear export sequence (NPMΔNES) neither increased its cytosolic accumulation or promoted cell death after stress. Prior exposure of cells to leptomycin B, a nuclear pore inhibitor, decreased stress-induced cytosolic NPM accumulation in a dose-dependent manner. Furthermore, RNAi-mediated Bax knockdown completely prevented the toxic effects of NPMΔNLS expression during stress. These results show that with ATP depletion, NPM undergoes regulated nuclear export to the cytosol, interacts with active Bax and promotes Bax-mediated apoptosis. Thus NPM is a functional Bax co-factor that promotes apoptosis during renal epithelial cell stress.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2189**

**Inactivation of Mxi1 Decreases Insulin-Like Growth Factor Binding Protein-3 in Polycystic Kidney** Jong Hoon Park, Je Yeong Ko, Kyung Hyun Yoo. *Department of Biological Science, Sookmyung Women's University, Seoul, Korea.*

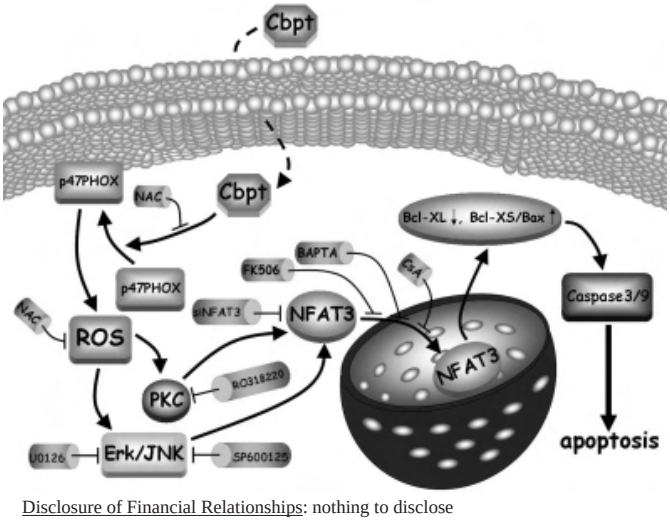
The Mxi1, an antagonist of c-Myc, is a member of Mad family that participate in cell proliferation and differentiation. According to the reports that have been published, overexpression of c-Myc is related to molecular mechanism of autosomal dominant polycystic kidney disease (ADPKD). Previously, multiple tubular cysts were observed in kidney of Mxi1-deficient mice aged 6 months or more. From these backgrounds, microarray analysis of Mxi1+/+ and Mxi1-/- MEFs was performed to identify the mechanism of cyst formation. We obtained some genes associated with cyst formation and selected insulin-like growth factor binding protein-3 (IGFBP-3), which has role for growth suppressor. Because increase of cell proliferation is necessary for early stage of cyst formation, we selected IGFBP-3 as a candidate gene. In our results, expression of IGFBP-3 was decreased in Mxi1-/- MEFs and level of this gene was regulated by Mxi1 expression in Mxi1 MEFs. Also, IGFBP-3 was reduced in not only Mxi1-/- MEFs but also Mxi1-/- mice and proliferation pathways related to IGFBP-3 were regulated in kidney of Mxi1-/- mice compared to controls. To determine whether inactivation of Mxi1 induces cell proliferation, we performed proliferation assay in both Mxi1 MEFs and Mxi1 mice. The cell viability was regulated by Mxi1 in Mxi1 MEFs and number of PCNA-positive cells was increased in Mxi1-/- mice when compared with Mxi1+/+ mice. Moreover, we found that IGFBP-3 level was significantly decreased in surrounding of cyst in Mxi1-/- mice. In conclusion, these findings suggest that inactivation of Mxi1 has a positive effect on cyst formation via down-regulation of IGFBP-3, so this mechanism is the potential target for polycystic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2190**

**Activation of a Nuclear Factor of Activated T-Lymphocyte-3 (NFAT3) by Oxidative Stress in Carboplatin-Mediated Renal Apoptosis** Heng Lin,[2] Shu-Hui Juan,[1] Wei-Shiung Lian,[3] Hsiao-Fen Li.[3] *[1]Physiology, Taipei Medical University, Taipei, Taiwan; [2]Institute of Pharmacology and Toxicology, Tzu Chi University, Hualien, Taiwan; [3]Academia Sinica, Institute of Biomedical Sciences, Taipei, Taiwan.*

Although carboplatin is currently used as a therapeutic drug for ovarian, breast, and non-small cell lung cancers, it has serious side effects including renal and cardiac toxicity that results in cell death. Herein, we examined the effect of carboplatin on murine renal tubular cell (RTC) apoptosis both *in vivo* and *in vitro* and its underlying molecular mechanisms associated with activation of the nuclear factor of activated T-lymphocytes-3 (NFAT3; a nuclear transcription factor). We demonstrated that carboplatin initiated an intrinsic apoptotic pathway of activating caspase-3 and -9, accompanied by a decrease in the ratio of Bcl-XL/Bax and a significant increase in Bcl-XS. Carboplatin increased NFAT activation in NFAT-luciferase reporter transgenic mice, RTCs, and in cells exogenously overexpressing NFAT3 that exacerbated cell death. Furthermore, the addition of either N-acetylcysteine (NAC, an antioxidant) or NFAT inhibitors, including FK-506 (tacrolimus), cyclosporin A (CsA, a calcineurin inhibitor), and BAPTA-AM (a calcium chelator) and successfully reversed the cell apoptosis induced by carboplatin, which was further confirmed using siNFAT3. Additionally, NAC blocked NFAT3 activation by inhibition of NADPH oxidase activation, and ERK/JNK and PKC pathways, resulting in the decrease in cell apoptosis, and the therapeutic effect of NAC was verified in vivo.The results presented herein show that carboplatin-mediated ROS might signal calcineurin and NFAT3 activation in RTCs, whereas NAC and NFAT inhibitors reversed carboplatin-mediated RTC apoptosis, suggesting that oxidative stress-mediated NFAT3 activation is essential for carboplatin-mediated RTC apoptosis.




Disclosure of Financial Relationships: nothing to disclose

**SA-PO2191**

**Ouabain Protects Renal Tubular Cell from Shiga Toxin Induced Apoptosis** Xiao Liu,[1] Evgeniya Burlaka,[1] Rachel Vieux,[1] Diana Karpman,[2] Anita Aperia.[1] *[1]Department of Woman and Child Health, Karolinska Institutet, Stockholm, Sweden; [2]Department of Pediatrics, Clinical Sciences Lund, Lund University, Stockholm, Sweden.*

Ouabain can protect renal tubule cells from apoptosis by triggering a Na, K-ATPase (NKA) / inositol 1,4,5-triphosphate receptor (IP3R) interaction and activating transcription factor NF-kB signaling pathway (Juan, JASN 2006). As shiga toxin (Stx) can cause apoptosis of human and murine renal tubular cells, we aimed to examine if ouabain protects from Stx-induced apoptosis. The study were performed in rat proximal tubule cells (RPTC) primary culture treated for 24h with Stx in the presence of ouabain or vehicle. Apoptotic degree was estimated by TUNEL assay, genomic DNA fragmentation, and flow cytometry detecting Annexin V. Immunoprecipitation, immunostaining and NF-kB TransAM assays were used to determine the signal pathway of the ouabain/NKA.

The experiment data indicated that 24h treatment with 5 nM ouabain, which has no effect on intracellular sodium (Juan, JASN 2006), almost completely rescued the RPTC from apoptosis provoked by Stx. Furthermore, NKA/IP3R immunoprecipitation, NF-kB nuclear translocation and NF-kB activity quantification measurement, as well as specific anti-apoptotic Bcl-xL protein detection showed that ouabain's protective effect in RPTC was mediated through the ouabain/NKA/IP3R signaling cascade which directly transduce the downstream activation of NF-kB into the increase of anti-apoptotic protein Bcl-xL expression. Taken together, our results provide an important research model and suggest that ouabain might be a novel therapeutic option to reduce toxic tubular injury in the development of renal disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2192**

**HIV-Associated Nephropathy (HIVAN): Role of Autophagy** Divya Salhan, Shitij Arora, Anju Yadav, Dileep Kumar, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.*

Autophagy is a highly regulated process to provide protection against oxidative stress-mediated cell injury. Oxidative stress-induced damaged organelles and proteins are usually degraded by the induction of autophagy. Inefficient or loss of autophagic process may allow accumulation of oxidized proteins and damaged organelles intracelluraly, which may be detrimental for cell survival. In *in vitro* studies, oxidative stress has been reported to contribute HIV-1-induced podocyte injury by the induction of podocyte apoptosis. We studied the role of oxidative stress and ongoing autophagy in a mouse model of HIVAN.

Age and sex matched control and Tg26 mice (8 weeks old) in groups of six were evaluated for ongoing oxidative stress by immunolabeling of frozen renal cortical sections with redox sensitive probe dihydroethidium (DHE). In addition, electron microscopic studies were carried out to study number of autophagosomes in renal cortical sections of control and Tg26 mice. Renal cortical sections were immunolabeled for expression of LC3-2, a gene associated with autophagosome formation. To establish a cause and effect relationship between autophagy and development of HIVAN, four weeks old Tg26 mice in groups of six were treated with either buffer or rapamycin (5 mg/kg/every other day), a stimulator of autophagy for two weeks, followed by evaluation of renal histology.

Tg26 mice showed enhanced generation of ROS both by glomerular and tubular cells. However, renal cortical section of Tg26 mice showed lower number of autophagosomes when compared with control mice. HIVAN mice showed higher number of both glomerular and tubular apoptotic cells. Interestingly, rapamycin, an autophagy promoter attenuated renal lesions in Tg26 mice.

We conclude that development of HIVAN is associated with the generation of oxidative stress but at the same time an attenuated response of autophagosome formation. The later may contribute to ongoing apoptosis of tubular and glomerular cells and progressive renal injury. Our hypothesis was further supported with the findings that the stimulation of autophagy by rapamycin slowed down the development of HIVAN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2193**

**HIV-1 Compromises Redox Activity Induced Stress Response Program in Tubular Cells** Divya Salhan, Dileep Kumar, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.*

HIV-associated nephropathy is associated with microcystic dilatation of tubules and tubular cell apoptosis. HIV-1 has been demonstrated to induce apoptosis in tubular cells by multiple mechanisms. Since HIV-1 has been reported to induce oxidative stress in a variety of cells, we evaluated the role of redox activity and associated stress response program in HIV-1-induced tubular cell apoptosis.

For *in vitro* models of HIV-1 infection, we have used mouse proximal tubular cells (MPTECs)-transduced with NL4-3 HIV (NL4-3/MPTEC, NL4-3 is an HIV-1 construct containing all the genes except *gag* and *pol*) and primary human proximal tubular cells pulsed with HIV-1 (HIV/HRPTEC). Both NL4-3/MPTEC and HIV/HRPTECs were evaluated for occurrence of apoptosis by morphologic assays. ROS generation as well ROS kinetics was studied in NL4-3/MPTECs and HIV/HRPTECs. In addition, proteins were extracted from NL4-3/MPTECs and HIV/HRPTECs, Western blots were prepared and probed for phospho-p66ShcA, Akt, Foxo3A, MnSOD and catalase.

Both NL4-3/MPTECs and HIV/HRPTECs showed enhanced apoptosis when compared with control cells. Both NL4-3/MPTEC and HIV/HRPTEs showed enhanced ROS generation. Both antioxidants and free radical scavengers inhibited apoptosis in both NL4-3/MPTECs and HIV/HRPTECs. Interestingly, NL4-3/MPTEC showed only mild increase in mRNA expression of MnSOD and catalase; whereas, HIV/HRPTECs showed only moderate increase in MnSOD and no increase in catalase. Both NL4-3/MPTECs and HIV/HRPTECs not only showed enhanced phosphorylation of p66 (ShcA) but also of Akt. However, both NL4-3/MPTEC and HIV/HRPTECs did not show any decrease in phospho-Foxo3A.

HIV-1 infected tubular cell apoptosis is mediated through ROS generation. Since there was no significant alteration in phosphorylation of Foxo3A despite ongoing oxidative stress and only modest increase in antioxidant molecules such as MnSOD and catalase, it appears that HIV-1-induced tubular cell apoptosis may be a consequence of the compromised redox activity-induced stress response program.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2194**

**Id1 Protects Mesangial Cells from Apoptosis Induced by TGFβ by Inhibiting USF2 Activity** Alex Yuri Sato,[1,2] Eliane Antonioli,[1] Alexandre Holthausen Campos.[1] *[1]IIEP-Albert Einstein Hospital, Sao Paulo, Brazil; [2]Dept. of Physiology, ICB-USP, Brazil.*

The late phase of diabetic glomerulopathy is characterized by death of mesangial cells (MC) and fibrosis. The identification of novel elements involved in cell death in MC would facilitate the comprehension of the pathophysiology of glomerular diseases. The Inhibitor of DNA Binding genes (Id) have been implicated in cell survival control and are expressed in MC. This study aims to investigate the role of Id1 in MC apoptosis.

Human MC were treated with TGF-β1(2ng/ml), BMP4(1ng/ml), BMP7(5ng/ml) or vehicle for different periods of time, and total RNA was extracted. To identify Id1/DNA interactions, we performed chromatin immunoprecipitation (ChIP). DNA fragments obtained were sequenced, and analyzed with bioinformatics tools. For apoptosis and gene reporter assays, Id1 overexpression in MC was followed by chromatin morphology analysis, caspase-3 and luciferase activity assays. qPCR was performed to measure mRNA expression levels of potentially regulated genes. BMP4 and 7 rapidly and potently up-regulated Id1 in MC (21 and 9 fold-change respectively, N=4, $p<0.01$). ChIP assay showed that Id1 interacts with conserved binding sites of the transcription factor USF2, with high specificity (dissimilarity ≤ 0.52%; hit by chance ≤ 0.001). Gene reporter assays showed that both BMP4 and Id1 reduce (-50% and -30%, N=6, $p<0.01$, respectively), whereas TGFβ1 increases USF2 activity (+40%, N=6, $p<0.01$). qPCR demonstrated that TGFβ-1 treatment increased USF2 expression (2.5 fold-change, N=4, $p<0.05$). Bioinformatics analysis disclosed USF2 binding sites in the promoter region of BAX, a pro-apoptotic gene. In fact, TGFβ-1 and USF2 stimulated BAX expression (1.8 and 1.5 fold-change, respectively, N=4, $p<0.05$), while Bcl2 levels remained unaffected. In addition, TGF-β1 increased whereas BMP-7 and Id1 decreased apoptosis in hMC (+25%, -35%, and -68% N=6, $p<0.01$ respectively).

In this study we demonstrated that Id1 is upregulated by BMPs and antagonizes TGFβ1-induced death by inhibiting USF2 activity in human MC. Our results point to a potentially novel molecular path involved in the pathogenesis of glomerular diseases.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2195**

**The LIM Protein Hic-5 Mediates Extracellular Matrix Dependent Changes in Mesangial Cell Phenotype Independent of TGF-β** Nick Hornigold,[1] Andrew F. Mooney.[2] *[1]CRUK Clinical Research Centre, St James's University Hospital, Leeds, West Yorkshire, United Kingdom; [2]Renal Unit, St James's University Hospital, Leeds, West Yorkshire, United Kingdom.*

Glomerular scarring is the final common pathway by which all glomerular diseases lead to renal failure, during which the mesangial cell assumes the phenotype of an activated myofibroblast.

Recently, we have reported the role of Hic-5 in glomerular scarring. Hic-5 is upregulated in mesangial cells following attachment to collagen I, and this is associated with increased susceptibility to apoptosis and increased synthesis of collagen I precursors.

Due to the established role of TGF-β in glomerulosclerosis, and because TGF- β has been shown to induce Hic-5 epxression in other cell types, we examined whether this growth factor mediated the pro-sclerotic phenotype of mesangial cells following collagen I attachment and Hic-5 upregulation.

Using a rat mesangial cell line, Hic-5 protein levels can be greatly reduced either by using siRNA, or by cell attachment to collagen IV rather than collagen I. In both cases Hic-5 downregulation leads to a large reduction in collagen I mRNA expression at 16 hours. Treatment of cells with exogenous TGF- β had no effect on collagen I expression in Hic-5 positive cells, but led to a slight upregulation of collagen I in Hic-5 negative cells, after 48 hours. Mesangial cells may express TGF- β, and it may also be present in growth media. Therefore we examined the effects of blocking TGF- β signalling, either with a blocking antibody or using the SMAD3 inhibitor SIS3. TGF- β blockade by either means did not affect collagen I or Hic-5 levels, either in Hic-5 positive or negative cells. Addition of exogenous TGF- β increased mesangial cell apoptosis in a dose-dependent manner, but this effect was attenuated in Hic-5 negative cells.

Taken together, these data show that mesangial cell attachment to collagen I leads to a Hic-5 dependent pro-sclerotic phenotype independent of TGF- β. The demonstration of a TGF- β independent mechanism of pro-sclerotic mesangial cell phenotype regulation has important implications for the understanding of glomerulosclerosis

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Disclosure of Financial Relationships: nothing to disclose

**SA-PO2196**

**Role of Glutamine Transport and Metabolism in the Development of Renal Hypertrophy** Hassane Amlal. *Internal Medicine, University of Cincinnati, Cincinnati, OH.*

Renal hypertrophy develops in several conditions associated with increased ammoniagenesis including $NH_4Cl$ loading-induced metabolic acidosis and hypokalemia. However, the role of glutamine transport and its metabolism through glucosamine vs. ammoniagenesis pathways in renal hypertrophy is poorly understood. To address this issue, we examined renal mass in rat models with and without increased ammoniagenesis. Experiment #1, rats were treated for 2 days, 2 and 5 weeks with normal diet (controls), $K^+$-free diet (KD), 280 mM $NH_4Cl$ loading (acidosis) and KD + acidosis. Experiment #2, ovariectomized (OVX) rats treated with vehicle or 17b-estradiol (E2) for 3 and 6 days. Our results indicate that as early as 2 days of treatment, $NH_4^+$ excretion increased from an average of (in mM/day) 0.32 in control to 0.74 ($P<0.01$) in KD, 1.77 ($P<0.001$) in acidosis and 2.48 ($P<0.0001$) in KD + acidosis. After 2 weeks, renal mass (kidney wt/BW) increased slightly by 4% in KD and 12% in acidosis, and was sharply increased by 62% ($P<0.01$) in KD + acidosis compared to controls. After 5 weeks renal mass was also significantly increased in KD alone and acidosis alone vs. controls ($P<0.05$). Interestingly, while $NH_4^+$ excretion did not increase in E2- vs. vehicle-treated OVX rats at 3 or 6 days, renal mass significantly increased by 37% after 6 days in E2 vs. vehicle rats ($P<0.001$). The proximal tubule glutamine transporter (SN1 or SNAT3) expression was upregulated after 2 days by 51% in KD, 156% in acidosis and 195% in KD + acidosis vs. controls. Interestingly, SN1 expression also increased by 60% ($P<0.02$) and 121% ($P<0.001$) after 3 and 6 days of E2 vs. vehicle treatment, respectively. Lastly, the increased renal mass correlated with the upregulation of cyclin-dependent kinase (CDK) inhibitors $p21^{Cip1}$ but not $p27^{KiP1}$ in 2 weeks KD + acidosis rats (4-fold) and 6 days of E2-treated rats (+ 51%, $P<0.01$ vs. vehicle). In conclusion, we propose that the upregulation of glutamine transporter SN1 and the

metabolism of glutamine through ammoniagenesis (KD, acidosis and KD + acidosis) or glucosamine pathway (E2) is likely responsible for renal hypertrophy in these conditions via the upregulation and activation of CDK inhibitor $p21^{Cip1}$.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2197**

**Heme Oxygenase (HO) Activity Modulates Subpressor Angiotensin II (SP-AngII)-Induced Hypertension (HTN) and Renal Injury** Kiran B. Chandrashekar,[1] Arnaldo F. Lopez-Ruiz,[1] Ramiro Juncos,[1] David E. Stec,[1] Karl A. Nath,[2] Ruisheng Liu,[1] Luis A. Juncos.[1] *[1]Nephrology/Physiology, Univ of Mississippi Medical Center, Jackson, MS; [2]Nephrology, Mayo Clinic, Rochester, MN.*

AngII causes HTN and promotes renal injury,but concurrently induces adaptive enzymes like HO.We examined the role of HO in modulating SP-AngII-induced renal inflammation and injury.Male SD rats were infused with either SP-AngII(200ng/kg/min) or vehicle for 14 days.We asked,whether,

1) Preventing SP-AngII-induced HO,by chronically inhibiting HO(SnPP;30mol/kg,i.p every 3rd day) exacerbate SP-AngII-induced renal injury?

2) Reversing SP-AngII-induced HO, by late HO inhibition(SnPP;50mol/kg,i.p on day 12) worsen this injury?

3) Further inducing HO chronically(CoPP;30mol/kg,i.p every 3rd day) prevent this injury?

4) Late HO induction(CoPP;50mol/kg,i.p on day 12) ameliorate this injury?

| | SBP mmHg | Renal TNFα-pg/ml | Renal IL6 pg/mg | Plasma Creatinine mg/dl | NGAL Ul/mg creat | HO activity nmol/mg/hr |
|---|---|---|---|---|---|---|
| Veh. | 122± 1 | 1.5 ± 0.4 | 8.5 ± 1.7 | 0.5 ± 0.02 | 0.3 ± 0.01 | 0.9 ± 0.02 |
| SP-AngII | 167 ± 2* | 11.7 ± 3.1* | 20.9 ± 4.3* | 0.9 ± 0.03* | 4 ± 0.5* | 2.1 ± 0.04* |
| SP-AngII + Chr.SnPP | 174 ± 2* | 26.6 ± 2.7# | 33.9 ± 3.1# | 1.2 ± 0.06 # | 15.9 ± 1.4# | 0.8 ± 0.02# |
| SP-AngII + Late SnPP | 173 ± 1* | 24.1 ± 1.3# | 24.4 ± 0.8 | 1.1 ± 0.06# | 13.7 ± 1.2# | 0.6 ± 0.12# |
| SP-AngII + Chr.CoPP | 123 ± 4# | 10.3 ± 1.9* | 15.6 ± 1.1* | 0.6 ± 0.03# | 5.1 ± 0.6* | 2.9 ± 0.26*# |
| SP-AngII + Late CoPP | 154 ± 10 | 12.0 ± 2.9* | 23.1 ± 2.5* | 0.8 ± 0.04 | 5 ± 0.8* | 3 ± 0.21*# |

Data: Mean ± SEM, * P < 0.05 vs. CT, # P< 0.05 vs. SP-AngII (n=5 or 6 per group)

Both chronic and late HO inhibition exacerbated SP-AngII-induced renal injury without increasing BP.Chronic HO induction prevented SP-AngII-induced HTN,while blunting the injury.Late HO induction ameliorated these parameters.We conclude that SP-AngII-induced HO dampens renal inflammation and injury independent of its blood pressure effects. Likewise, further induction of HO prevents SP-AngII's hypertensive effects and ameliorates renal dysfunction.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2198**

**Syndrome of Rapid Onset End-Stage Renal Disease (SORO-ESRD): A New Unrecognized Pattern of CKD Progression to ESRD** Macaulay A. Onuigbo,[1] Macaulay A. Onuigbo.[2] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Midelfort Clinic, Mayo Health System, Eau Claire, WI.*

Background

By most estimates, we have an increasing worldwide ESRD epidemic. This is despite at least two decades of intensified reno-protection strategies including extensive applications of RAAS blockade. The current consensus is that CKD-ESRD progression is a continuous, progressive and predictable loss of eGFR in CKD patients, inexorably leading to ESRD.

Objective

Our recent experience in a Mayo Health System Hypertension Clinic, and new reports associating CKD-ESRD progression following AKI, led us to hypothesize that CKD-ESRD progression may be unpredictable, after all.

Methods

In June 2009, we completed an 82-month prospective patient-level data analysis of CKD-ESRD progression in 100 high-risk CKD patients.

Results

All Caucasians. M:F = 52/48, age 71.5 (25-92) years. Enrollment CKD stages - II (1), III (25), IV (57) and V (16). 17 patients progressed to ESRD. CKD-ESRD progression was unpredictable by age nor by eGFR. ESRD was superseded by AKI in 15/17 (88%) patients. AKI was secondary to hypotension/shock (7), sepsis (2), following cardio-thoracic surgery (2), and following lymphoma, contrast nephropathy, urinary obstruction and dementia/failure to thrive, in 1 each. We have coined a new term, syndrome of rapid onset end-stage renal disease (SORO-ESRD) to represent this unrecognized syndrome of abrupt onset, unanticipated and precipitous but irreversible renal failure in CKD patients following medical and surgical events that precipitated AKI, with subsequent accelerated loss of GFR and finally irreversible ESRD.

Conclusion

The current popular theory of CKD-ESRD progression was debunked by our study. The majority of ESRD progression from CKD observed in our cohort was triggered unpredictably by AKI. Ishani et al recently demonstrated that 25.2% of patients who developed ESRD in a Medicare "Seniors" cohort similarly had experienced prior AKI. Larger studies are warranted. If confirmed, this finding will demand major paradigm shifts in current concepts of reno-protection and related "A-V Fistula first" programs.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2199**

**Urine Podocin:Uromodullin mRNA Ratio Is a Candidate of Biomarker in Patients with Glomerulonephrits** Yuji Sato,[1] Shouichi Fujimoto.[2] *[1]Div. of Circulatory and Body Fluid Regulation, Dept of Int Med, University of Miyazaki, Miyazaki, Japan; [2]Div. of Dialysis, University of Miyazaki Hosp., Miyazaki, Japan.*

If we could monitor the disease activity of glomerulonephritis (GN) by analyzing non-invasive methods (eg. urine biomarker), it would be clinically useful. We previously demonstrated urine podocyte mRNAs marked progression of glomerular disease using human diphtheria toxin receptor transgenic rat (JASN 20:1041, 2009), and podocyte-specific mRNAs (NPHS1 and NPHS2) were excreted in the urine of healthy controls and of patients with GN (JASN 20:309A, 2009). However, urine aquaporine2 (Aqp2) mRNA was not suitable as a candidate of kidney-specific reference gene to correct urine podocyte-specific mRNAs in human different from rats.

In this study, we examine whether the quantitative measurement of urine podocyte-specific mRNAs can distinguish between healthy controls and patients with GN, and is useful for the evaluation of the disease activity.

Human urine was centrifuged and pellet was collected. RNA was extracted using RNeasyMiniKit protocol and reverse transcribed to cDNAs. Real-time PCR was performed using TaqMan probes (for NPHS1, NPHS2, Aqp2 and uromodullin(UMOD)). Human kidney RNA transcribes was used as standard cDNAs. The %crescent formation was calculated at least 10 glomeruli on the PAS stained section of patients with IgA nephropathy.

Urine NPHS2:UMOD mRNA ratio can distinguish between healthy controls and patients with GN (membranous nephropathy, IgA nephropathy and diffuse crescentic glomerulonephritis). This mRNA ratio also showed significant positive correlation with %crescent formation (r=0.87) but not correlated with %segmental sclerosis (r= -0.15) on kidney biopsy specimens of patients with IgA nephropathy. Urine NPHS2:Aqp2 ratio and urine protein:creatinine ratio did not correlate with this %crescent formation (r=0.37).

We propose urine UMOD mRNA is a good kidney-specific reference gene in a setting of evaluation of urine podocyte-specific mRNAs. Increased urine NPHS2:UMOD mRNA ratio can reflect active glomerular lesion of IgA nephropathy and the change of this ratio may be a useful biomarker for the evaluation of the disease activity.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2200**

**FT011, a New Anti-Fibrotic Drug, Attenuates Progressive Renal Injury in 5/6 Renal Ablation Model** Yuan Zhang,[1] Robyn S. Kelly,[1] Alison Joy Cox,[1] Sih Min Tan,[1] Steven C. Zammit,[2] Spencer J. Williams,[2] Henry Krum,[3] Richard E. Gilbert,[4] Darren J. Kelly.[1,5] *[1]University of Melbourne, Australia; [2]Bio-21, University of Melbourne, Australia; [3]Monash University, Australia; [4]University of Toronto, Canada; [5]Fibrotech Therapeutics, Australia.*

To test the hypothesis that treatment with FT011 will attenuate the functional and structural manifestations of progressive kidney disease.

Progressive kidney disease is associated with glomerulosclerosis and tubulointerstitial fibrosis and obliteration of the microvasculature, that all correlate with declining renal function and development of proteinuria. FT011 (Fibrotech Therapeutics Pty Ltd, Australia) is an anti-fibrotic drug in preclinical development for diabetic nephropathy.

Subtotal nephrectomized (STNx) rats were randomly assigned to receive either FT011 (100mg/kg bid gavage) or vehicle for 12 weeks (n=10). Sham-operated rats were used as controls. In addition to renal function and histopathology examination, immunohistochemistry staining for ED-1 and RECA-1 were used to evaluate interstitial macrophage accumulation and the glomerular and peri-tubular capillary endothelial cell density, respectively. Changes of mRNA expression of VEGF and collagen IV were quantitated in situ hybridisation. STNx rats treated with FT011 were associated with less proteinuria (366±42 Vs 180±38 mg/day), glomerulosclerosis (1.38±0.18 Vs 0.69±0.07), tubulointerstitial fibrosis (9.53±2 Vs 5.34±1.25 %/area), macrophage infiltration (28±3.6 Vs 17±3 number/filed) and collagen IV mRNA expression (87±25 Vs 48±9 density/AU) along with improved GFR (0.38±0.14 Vs 1.19±0.14 ml/minute) when compared with vehicle treated STNx rats. In addition, treatment with FT011 was associated with a reduction in the loss of glomerular and peri-tubular capillary endothelial cells (RECA-1 1.24±0.27 Vs 6.34±0.87 %/gcs; and 0.08±0.04 Vs 0.23±0.04 %/interstitial area), and increased in VEGF mRNA expression (84.46±5.1 Vs 126.4±8.5 density/AU).

Treatment with FT011 attenuates structural and functional manifestations of experimental progressive kidney disease. While the precise mode of action is still uncertain, FT011 represents a new treatment for chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2201**

**NRK-49F Cells as a Model for Studying the Pro-Fibrotic Effects of All-*trans* Retinoic Acid: Roles for Retinoid Nuclear Receptors and Carrier Proteins** Alexandra C. Rankin, Bruce M. Hendry, Qihe Xu. *Department of Renal Medicine, King's College London, London, United Kingdom.*

Purpose: All-*trans* retinoic acid (tRA) is reported to have both anti- and pro-fibrotic effects in animal models of renal disease, the mechanisms of which are poorly understood. We have established a dose-dependent pro-fibrotic effect of tRA in a normal rat kidney fibroblast cell line (NRK-49F). Here we explore the effects of tRA on fibrosis-related genes, and the roles of the retinoid nuclear receptors and carrier proteins in the pro-fibrotic effects of tRA in NRK-49F cells.

Methods: NRK-49F cells were treated with tRA +/- TGF-β1 for 48 h. Picro-Sirius red (PSR) stain was used to quantify total collagen deposition. A PCR array and standard RT-qPCR were used to determine changes in expression of fibrosis-related genes, retinoid receptors and carrier proteins in cells treated with tRA +/- TGF-β1. Matrix metalloproteinase (MMP) activity was assessed using an activity assay. Roles of the receptors in the fibrogenic response to tRA were explored using retinoid receptor agonists and antagonists.

Results: tRA dose-dependently increased PSR staining that was further increased by TGF-β1. Despite this net pro-fibrotic effect, there was down-regulation of some fibrogenic markers, while some matrix degradation enzymes were also down-regulated. Furthermore, MMP activity was reduced following tRA or TGF-β1 treatment, however no additive effect was seen with dual treatment. All 7 retinoid receptors and 2 carrier proteins were expressed in NRK-49F cells and their levels changed following treatment with tRA and TGF-β1. Pan-RAR/RXR agonists increased PSR staining in the presence of TGF-β1, but pan-RAR/ RXR antagonists did not prevent the increase in PSR staining seen with tRA+TGF-β1.

Conclusions: tRA has both anti- and pro-fibrotic effects in NRK-49F cells at the molecular level. Its net pro-fibrotic effect may partly be due to reduced matrix degradation. Although activation of either RARs or RXRs was sufficient to induce a pro-fibrotic response, RAR and RXR pathways may not be necessary for the pro-fibrotic effect.

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Disclosure of Financial Relationships: nothing to disclose

## SA-PO2202

**Up-Regulation of SL CO4C1 Uremic Toxin Transporter Expression and Function by Statins for Therapeutics of Chronic Kidney Disease** Takehiro Suzuki,[1] Takafumi Toyohara,[1] Yasutoshi Akiyama,[1] Yoichi Takeuchi,[1] Eikan Mishima,[1] Masayuki Tanemoto,[1] Hiroshi Sato,[2] Masaaki Nakayama,[1] Sadayoshi Ito,[1] Tomoyoshi Soga,[3] Takaaki Abe.[1] *[1]Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan; [3]Institute for Advanced Biosciences, Keio University, Tsuruoka, Japan.*

[Purpose] Chronic kidney disease (CKD) is strongly associated with cardiovascular events and prognosis. The reduction of accumulated uremic toxins protects against the development of hypertension and renal damage in patients with CKD, but there is no established treatment. We have revealed that 1) human kidney-specific organic anion transporter SLCO4C1 is a responsive molecule for excreting uremic toxins (PNAS 2004), 2) the overexpression of human SLCO4C1 in rat kidney promotes renal excretion of uremic toxins and reduced hypertension, cardiomegaly and inflammation in renal failure (JASN 2009). Our purpose is to develop the new therapeutic strategy for CKD based on the uremic toxin excretion by the transporter.

[Methods] Analyzing the transcriptional regulation of human SLCO4C1 and exploring the drugs to enhance the expression and the function of SLCO4C1 in vivo.

[Results] Human SLCO4C1 transcription is regulated by xenobiotic responsive element (XRE)-like motifs. Various statins act as nuclear aryl hydrocarbon receptor (AhR) ligands and up-regulate SLCO4C1 transcription by AhR nuclear translocation and binding XRE. Statin promoted SLCO4C1 expression and SLCO4C1 substrate uptake in kidney derived ACHN cells. Luciferase reporter assay of SLCO4C1 promoter by statins revealed transcriptional enhancement in a dose-responsive manner. In addition, co-administration of statins and corticosteroids exerted additive effects on SLCO4C1 expression. Finally administration of statin to 5/6-nephrectomized renal failure rats exerted increased renal uremic toxin excretion.

[Conclusions] These data suggest that SLCO4C1 up-regulation by statins might provide a novel transporter-based therapeutic strategy for CKD patients to reduce major life-threatening events. Statins could be effective drugs for CKD remedy (JASN 20: 2546-2555, 2009).

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2203

**Increases in Intrarenal Prorenin and Renin Are Mediated by Angiotensin II in Chronic Anti-Thymocyte Serum (ATS) Nephritis Rats on High Salt Intake (HSI)** Yanjie Huang,[1] Tatsuo Yamamoto,[3] Hiroyuki Suzuki,[1] Akashi Togawa,[1] Hideo Yasuda,[1] Yoshihide Fujigaki,[1] Akihiko Kato,[2] Akira Nishiyama,[4] Naoki Ikegaya,[5] Akira Hishida.[1] *[1]First Dpt. Med., Hamamatsu Univ. Sch. Med., Hamamatsu, Shizuoka, Japan; [2]Div. Blood Purification, Hamamatsu Univ. Sch. Med., Hamamatsu, Shizuoka, Japan; [3]Dpt. Health & Nutritional Sciences, Hamamatsu Univ., Hamamatsu, Shizuoka, Japan; [4]Dpt. Pharm., Kagawa Med., Kagawa, Japan; [5]Med. Care Center, Shizuoka Univ., Shizuoka, Japan.*

Previously we reported that HSI increased intrarenal prorenin and renin in chronic ATS nephritis despite significant suppression of circulating RAS activity. To elucidate whether increased intrarenal prorenin and renin on HSI were mediated through the action of angiotensin II, we investigated the circulating and intrarenal RAS activities in 1) rats with chronic ATS nephritis induced by uninephrectomy and two consecutive injections of ATS fed on HSI (0.5% NaCl solution as drinking water; UAH), 2) UAH rats treated with olmesartan medoxomil (10 mg/kg/day; UAH+O), and 3) UAH rats treated with hydralazine hydrochloride (5 mg/kg/day; UAH+H). Increased blood pressure was noted only in UAH on day 21. Increases in urinary protein excretion, intrarenal TGF-b1 expression, and kidney fibrosis were observed in UAH and UAH+H, however, their levels were significantly less in UAH+O. Immunoreactivity for total renin and non-proteolytically activated prorenin was observed mainly in distal tubules and some proximal tubules in UAH and UAH+H despite significant suppression of their expression in the juxtaglomerular apparatus, and was markedly less in UAH+O. Membrane-bound (pro)renin receptor ((P)RR) was increased in UAH and UAH+H, and was decreased by olmesartan. No significant changes were noted in the levels of intrarenal AT1-receptor among each group. These data suggest that the increase in membrane-bound (P)RR, which was regulated, at least partly, through the action of angiotensin II, was involved in the augmentation of intrarenal binding of prorenin and renin, resulting in progression of kidney fibrosis in chronic ATS nephritis on HSI.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2204

**Serologic Profiles of Fvb.ROP Os/+ Mice with Reduced Nephron Number, Glomerular Hypertension, and Renal Failure** N. Stanley Nahman,[1,4] Robert Faulhaber-Walter,[2] Kathleen O. Heilig,[3, 4] Youli Wang,[3] Dilip K. Deb,[3] Jurgen B. Schnermann,[2] Victor Lopez De Mendoza,[4] Charles W. Heilig.[3, 4] *[1]Medicine, Charlie Norwood VA Medical Center & Medical College of Georgia, Augusta, GA; [2]Kidney Disease Branch, NIDDK, NIH, Bethesda, MD; [3]Medicine, University of Chicago, Chicago, IL; [4]Medicine, University of Florida COM Jacksonville, Jacksonville, FL.*

We have reported that Fvb.ROP Os/+ mice (Os) are a new & susceptible model of reduced nephron number (RNN) with rapid onset and progression of glomerulosclerosis (GS) and renal failure (Lab Invest 90:83, 2010). RNN was associated with glomerular hypertension and hyperfiltration (FASEB J 21:595.28, 2007). Herein, we assessed the serologic profiles of 4-5 week old Os mice and a control, fvb.ROP +/+ (WT). Compared to WT at this early time point, Os had a significant increase in serum BUN and creatinine (BUN: 93±9 mg/dl vs 29±4 for Os vs WT, respectively, and creatinine: 0.42±0.04 mg/dl vs 0.23±0.03 for Os vs WT, respectively, $p < 0.05$ for both). Os exhibited a decrease in total protein and albumin (total protein: 2.8±0.07 g/dl vs 3.3±0.03 for Os vs WT, respectively, and albumin: 1.8±0.04 g/dl vs 2.0±0.03 for Os vs WT, respectively, $p < 0.05$ for both). Plasma oncotic pressure (vapor pressure osmometry) was significantly decreased in Os (7.2±0.21 vs 8.9±0.12 for Os vs WT, respectively, $p < 0.05$). There were no differences in glucose, Na+, K+, Cl-, Ca++, phosphorus or uric acid at this time point. These data are consistent with the histologic demonstration of severe GS in Os when compared to WT (Lab Invest). Os also demonstrated increased expression of the glucose transporter GLUT1, and the prosclerotic cytokine VEGF. Thus, Os mice with the Fvb background, marked albuminuria & rapidly progressive GS exhibit early and rapid onset of azotemia, hypoproteinemia, hypoalbuminemia and decreased plasma oncotic pressure. These changes correlate with proteinuria and renal failure in these mice, providing clinical confirmation of the effects of the severe glomerulopathy in this new model. These changes may be used to monitor the progression of the disease in subsequent studies.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2205

**Effect of Caloric Restriction and a Ketogenic Diet on Proteinuria in Münich-Wistar-Frömter Rats** Astrid Klooster, Lianne Messchendorp, Henri G. D. Leuvenink, Harry Van Goor, Reinold O. B. Gans, Gerjan Navis, Stephan J. L. Bakker. *Kidney Center, University Medical Center Groningen, Groningen, Netherlands.*

Caloric restriction (CR) induced at young age delays onset of age-related proteinuria. This might act through stimulation of ketogenesis. Ketogenesis increases the number of mitochondria and thereby increases metabolic efficiency. It is unknown whether CR and/ or ketogenesis reduce established proteinuria. We studied CR and a ketogenic diet in an animal model of established proteinuria.

Münich-Wistar-Frömter (MWF) rats (n=56) and Wistar rats (n=14) were nephrectomised at 22 wks of age. At 26-wks, MWF rats were divided in 4 groups: control diet ad libitum (CON), control diet CR (CCR) (caloric intake 60% of CON), ketogenic diet ad libitum (KAL) and ketogenic diet CR (KCR) (caloric intake 60% of KAL). Differences between groups were tested with ANOVA and post-hoc Tukey.

At baseline weight (mean±SD) was significantly higher in Wistar rats than in MWF rats (453±34 vs. 372±26 g, $p<0.001$). Urinary protein excretion (UPE) was lower in Wistar than in MWF rats (median [IQR]: 5[4-10] vs. 57[42-78] mg/24 h, $p<0.001$). The same was true for plasma creatinine (38±4 vs. 42±5 mol/L, $p=0.004$) and mean arterial pressure (MAP) (115±14 vs. 131±9 mmHg, $p<0.001$). At baseline, there were no differences between the MWF groups.

After 14 wks of diet weight and UPE were significantly lower in CR than in ad libitum fed MWF groups (table 1). Plasma creatinine was lower in CCR, KAL and KCR compared to CON. There was no significant difference between the CR groups. Blood pressure was elevated in KAL compared to CON. This was reversed by CR.

In conclusion, CR reverses established proteinuria in MWF rats. It also prevented the rise in plasma creatinine and increase in blood pressure. These beneficial effects can not be attributed to stimulation of ketogenesis.

Table 1. Characteristics after 14 weeks of diet

| | CON | CCR | KAL | KCR |
|---|---|---|---|---|
| Weight (g) | 443±44 | 303±11 a,b | 468±32 | 320±12 a,b |
| UPE (mg/24 h) | 132[96-155] | 32[25-45] a,b | 144[109-167] | 38[19-52] a,b |
| Kreatinine (μmol/L) | 69±13 | 46±5 a | 54±13 a | 41±9 a,b |
| MAP (mmHg) | 152±18 | 135±15 a,b | 167±16 a | 135±8 a,b |

a = $p<0.05$ compared to CON; b = $p<0.05$ compared to KAL

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2206**

**Fibrosis in Wild Type and CTGF-Transgenic Mice with Aristolochic Acid Nephropathy Is Associated with Tubular Epithelial Cells and Not with Interstitial Myofibroblasts** Maria Fragiadaki, Abi S. Witherden, Charles D. Pusey, George Bou-Gharios, Roger M. Mason. *Renal Medicine, Imperial College London, London, United Kingdom.*

Aristolochic acid (AA) causes progressive renal interstitial fibrosis in humans and has been used successfully to induce fibrosis in rodents. Murine AA nephropathy (AAN) can serve as a model to study the involvement of specific cells and candidate genes in the development of interstitial fibrosis. We induced AAN (5mg/kg AAdaily, 5days) in either male wild-type (WT) or transgenic mice that overexpress connective tissue growth factor (CTGF) under the control of the *Col1a2* promoter (TG-mice). AA-treated WT and CTGF TG-mice developed the same degree of tubular damage and interstitial extracellular matrix and collagen deposition, as detected by Masson's trichrome and picrosirius–red staining. CTGF expression in cortex was measured by qPCR and western blot and was significantly increased in AA TG mice compared with AA control mice. CTGF was expressed predominantly in tubular epithelial cells. .Increased expression of CTGF in TG-mice was associated with increased kidney vascularisation, detected by staining for α-smooth muscle actin (α-SMA), a marker for vascular smooth muscle cells and myofibroblasts. Interestingly, α-SMA expression was confined to renal blood vessels and was not detected in the interstitium in either AAN or control animals. However, HSP-47, a collagen chaperone and intracellular marker for fibrillar collagen synthesis, was expressed in many tubular epithelial cells in both WT and TG-mice treated with AA, but was not expressed in control mice. Injured tubular epithelial cells continued to express E-cadherin, a marker of epithelial phenotype, in AAN. Conclusions: (1) Injured tubular epithelial cells show no evidence of giving rise to interstitial myofibroblasts by epithelial mesenchymal transition but adopt some mesenchymal phenotype characteristics *in situ* (HSP-47 expression, *Col1a2* promoter activation) and appear to be responsible for synthesising and depositing interstitial collagen. (2) Overexpression of CTGF in murine AAN does not enhance fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2207**

**Increased Lipolysis in Visceral and Subcutaneous White Adipose Tissue in 5/6 Nephrectomized Rats** Qiugen Zhou, Lili Hu, Fan Fan Hou. *Nephrology, Nanfang Hospital, Guangzhou, Guangzhou, Guangdong, China.*

Protein energy wasting, a state of decreased stores of body protein and fat, is a risk factor for mortality in advanced chronic kidney disease (CKD). Little is known about the mechanism underlying loss of fat in CKD. In the present study, we tested whether abnormality in lipolysis which is a process of fat catabolism occurs in a rat model of chronic renal failure. Our data showed that lipolysis in both visceral and subcutaneous white adipose tissue (WAT) were increased in 5/6 nephrectomized rats compared with control rat as revealed by increased release of glycerol in ex vivo fat pads and cultured primary adipocytes. In addition, increased phosphorylation of hormone sensitive lipase (HSL) and binding of adipose triglyceride lipase (ATGL) with comparative gene identification-58 (CGI-58) protein were observed in WAT from model rats, suggesting the activity of HSL and ATGL, two key lipases in lipolysis, enhanced in fat in the chronic renal failure rats. Moreover, phosphorylation of protein kinase A and mitogen-activated protein kinase, which is linked to activation of HSL and ATGL, were increased in WAT. While the differentiation capacity of preadipocytes isolated from WAT was comparable in the model and control rats as revealed by intracellular accumulation of triacylglycerol. Collectively, our results showed that increased lipolysis occurred in 5/6 nephrectomized rats, which was associated with increased activity of HSL and ATGL. This information may provide new light for better understanding the protein energy wasting and ectopic lipid accumulation in chronic renal failure.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2208**

**Serum Proteomics for Biomarkers of FSGS Recurrence Post-Transplant** Megan M. Lo, Prasad Devarajan, Michael R. Bennett. *Cincinnati Children's Hospital, OH.*

Introduction: Focal Segmental Glomerulosclerosis (FSGS) recurrence after transplant causes significant morbidity and shortens graft life. Currently, there are no predictors of recurrence, hindering prevention and treatment. As recurrence often occurs immediately after transplant, persistent circulating factors have been implicated. We hypothesized that FSGS recurrence patients (Group R) have these factors, while non-FSGS patients, FSGS non-recurrent patients, and FSGS patients in remission (Group C) do not. Our goal was to use serum proteomics to identify such factors.

Methods: Serum was collected from patients with FSGS and those receiving an allograft for other causes. Samples were analyzed by surface enhanced laser-desorption ionization time-of-flight mass spectrometry (SELDI). Protein peaks with a signal-to-noise ratio over 5 in at least 20% of spectra were analyzed. Peaks were compared between Groups R and C by Mann-Whitney rank sum test.

Results: There were 6 FSGS recurrence, 6 FSGS non-recurrence, and 8 non-FSGS patients, ages 8 to 19 years, with 7 females. 7 peaks were significantly different ($p<0.05$) with intensity ratios >3 in Group R (see Table 1). The most promising peaks are at 11.5kDa (22-fold change), 3.8kDa (6 to 35-fold change), and 4.1kDa (highest ROC at 0.93). These peaks were present both pre- and post-transplant.

Table 1

| M/Z (Da) | Chip Type | Group R | Group C | p-value | ROC | R:C ratio |
|---|---|---|---|---|---|---|
| 4097 | Q10 | 50.77 | 12.54 | 0.001 | 0.93 | 4.05 |
| 4383 | Q10 | 34.33 | 9.45 | 0.04 | 0.75 | 3.63 |
| 7140 | H50 | 109.22 | 22.66 | 0.002 | 0.89 | 4.82 |
| 11,482 | H50 | 41.05 | 1.83 | 0.01 | 0.82 | 22.41 |
| 3809 | CM10 | 207.71 | 5.99 | 0.002 | 0.83 | 34.70 |
| 3810 | IMAC | 103.52 | 16.28 | 0.01 | 0.83 | 6.36 |

M/Z: mass to charge ratio. ROC: receiver-operator characteristics. R:C: ratio Group R to Group C intensity ratios.

Conclusion: We have identified a proteomic signature in the serum of patients with FSGS who recur post-transplant, consisting primarily of peaks at 11.5, 4.1, and 3.8 kDa. Using these biomarkers prior to transplant may help predict recurrence and develop strategies to prevent recurrence.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2209**

**Possible Involvement of Proteinuria-Elicited ADMA Accumulation in Accelerated Atherosclerosis in Chronic Kidney Disease** Yusuke Kaida, Seiji Ueda, Yosuke Nakayama, Ryotaro Ando, Kei Fukami, Seiya Okuda. *Division of Nephrology, Department of Medicine, Kurume University, Kurume, Fukuoka, Japan.*

***Background.*** Albuminuria is widely recognized as a strong indicator of cardiovascular disease (CVD). There is an increasing body of evidence that endothelial dysfunction due to impaired NO generation is linked to albuminuria. In addition, it has been recently reported that there is a close relationship between proteinuria and ADMA in patients with chronic kidney disease (CKD). These observations led us to speculate that ADMA could link albuminuria to CVD in CKD patients. However, the underlying mechanisms for the possible association between ADMA and albuminuria remain to be elucidated.

***Methods.*** We investigated the relationship between ADMA levels and proteinuria in adriamycin (ADR)-treated rats, an animal model of nephritic syndrome. We also examined the expression levels and activity of DDAH and PRMT in the kidney of this animal. Further, we examined the effects of albumin on ADMA-DDAH axis in cultured human renal proximal tubular epithelial cells (RPTEC).

***Results.*** A positive correlation between ADMA and proteinuria was observed in the ADR-treated rats ($n=28$, $r^2=0.46$, $p<0.01$). Although the expression levels of DDAH were not changed 14 days after the treatment of ADR, the enzymatic activity of DDAH was significantly decreased in the kidney of ADR-treated rats. The expressin levels of PRMT significantly increased in the kidney of ADR-treated rats. In vitro, albumin time- and dose-dependently increased ADMA accumulation in cultured media of RPTEC. Albumin also decreased DDAH activity and increased the expression levels of PRMT in RPTEC. Albumin-elicited dysregulation of DDAH and PRMT was completely abolished by pretreatment of an anti-oxidant, N-acetyl cystein.

***Conclusion.*** These results suggest that albuminuria could increase ADMA accumulation via suppression of renal DDAH activity and PRMT overexpression, partly explaining a link between albuminuria and CVD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2210**

**Selective Estrogen Receptor Modulator Attenuates Albumin-Induced Apoptosis in Renal Proximal Tubules through Inhibition of Mitochondrial Stress** Yuko Nishi, Minoru Satoh, Naruya Tomita, Tamaki Sasaki, Naoki Kashihara. *Division of Nephrology and Hypertension, Department of Internal Medicine, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

[Background] Females are less susceptible than males to developing chronic kidney disease. However, the mechanisms responsible for the reduction have not yet been clarified. Recent studies have shown that mitochondrial oxidative stress is involved in proteinuria-induced apoptosis of proximal tubular cells. Estrogen has been shown to affect mitochondrial function by reducing oxidative stress. Therefore, we investigated whether the stimulation of estrogen receptor using estradiol or a selective estrogen receptor modulator, raloxifene, could attenuate albumin-induced tubular injury through reducing mitochondrial oxidative stress.

[Methods and Results] In vitro study, human renal proximal epithelial cells (hPTECs) were cultured with human fatty acid-bearing albumin (0, 5, 10, 20 mg/ml). This resulted in a dose- and duration-dependent cell viability loss, as determined by lactate dehydrogenase release and mitochondrial viability, mitochondrial membrane potential loss, and induction of apoptosis, as revealed by externalization of plasma membrane phosphatidylserine. Prestimulation of estrogen receptor with 1mM raloxifene successfully attenuated albumin-induced apoptosis in hPTECs. The effect was blocked by co-incubation with an estrogen receptor antagonist (ICI 182,780). In the in vivo study, ICR-derived glomerulonephritis (ICGN) mice underwent ovariectomy, and were treated with raloxifene (50μg/kg/day) for 6 weeks. Ovariectomy induced the apoptotic cell death in renal proximal cells (terminal deoxynucleotidyl transferase dUTP nick-end labeling staining) and decreased cytochrome-c oxidase activity in mitochondria. Electron microscopy of the ovariectomized ICGN mice revealed morphologically abnormal mitochondria, and these changes were suppressed following treatment with raloxifene with upregulation of the mitochondrial thioredoxin system.

[Conclusion] Stimulation of estrogen receptor attenuated programmed cell death in renal proximal tubules induced by albumin-overload as a result of amelioration of mitochondrial dysfunction.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2211**

**Protective Effects of Shichimotsukokato on Anti-Thy-1-Induced Irreversible Nephritis in Rats** Takahiko Ono,[1] Kohei Kamikado,[2] Tatsuya Morimoto.[2] *[1]Department of Nephrology, Shimada Municipal Hospital, Shimada, Japan; [2]Division of Molecular Medicine, University of Shizuoka, Shizuoka, Japan.*

Purpose: Oxidative stress is involved in the progression of chronic kidney disease (CKD). Shichimotsukokato (SKT), an herbal prescription in Japan, possesses an anti-oxidative activity and protective effects against hypertension in experimental studies. We investigated the mechanisms of the effects by long-term administration of SKT on irreversible nephritis induced by the anti-thymocyte antibody.

Methods: Six-week-old male Wistar rats were subjected to uninephrectomy, and to injection of rabbit anti-rat thymocyte serum to generate irreversible nephritis. These rats were then randomly assigned to a sham operation group (n = 6), a control vehicle (n = 5), and SKT (TJ-46) group (500 mg/kg, nearly 5 times of clinical dose) (n = 5). Drinking water with SKT was continued to give for 15 weeks.

Results: Blood pressure in the SKT group (127 ± 1 mmHg, $p < 0.05$ vs. vehicle) was significantly lower as well as sham group, comparing with the vehicle (138 ± 2 mmHg). Although SKT slightly reduced urinary protein, SKT markedly ameliorated renal function evaluated with urea nitrogen clearance (vehicle, 0.50 ± 0.06 ml/min; SKT, 0.74 ± 0.06 ml/min, $p < 0.05$). Compared with vehicle, SKT treatment lowered the glomerular enlargement and total glomerular cells number both by 80% ($p < 0.05$, respectively), and lowered extracellular matrix area by 75% ($p < 0.05$). SKT treatment also suppressed tubular injury score (vehicle, 3.0 ± 0.2; SKT, 2.1 ± 0.1, $p < 0.05$), and maintained CD31-positive peritubular capillary networks (vehicle, 3.7 ± 0.3 / 10 tubules, n = 5; SKT, 5.5 ± 0.5, $p < 0.05$). Furthermore, SKT recovered the superoxide dismutase (SOD) activity, a marker of anti-oxidative system, to the levels of sham group (vehicle, 272 ± 6 U/mg protein; SKT, 337 ± 21, $p < 0.05$).

Conclusion: These results suggest that SKT may be useful for the treatment of CKD during the progress to nephrosclerosis, through the mechanisms of anti-oxidative activity and maintenance of perivascular capillary networks.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2212**

**Comparative Research of Serum Cystatin C and Other Endogenous Markers in Glomerular Filtration Rate Measurement in Chronic Kidney Disease Patients with Mild to Moderate Impairment of Kidney Function** Xiaoying Du,[1] Qiang He,[1] Yu Chen,[2] Linfa Li,[3] Jianyong Wu,[1] Jianghua Chen.[1] *[1]Kidney Disease Center, the First Affiliated Hospital, College of Medicine,, Zhejiang University, Hangzhou, Zhejiang, China; [2]Clinical Laboratory, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China; [3]Nuclear Medicine,The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.*

To evaluate the diagnostic value of serum Cys C in GFR measurement in comparison with other endogenous markers in CKD patients with mild to moderate impairment of GFR.107 patients (64 males and 43 females, aged 23-87 years) with chronic kidney disease stages 2-3 (GFR 30-89 ml/min/1.73m2) were enrolled. On the day when the patients had performed 99mTc-DTPA clearance, the patients' blood had been withdrawn before breakfast and the serum samples been stored below -80°. One year later, all the frozen samples were thawed out and Cys C,β2-MG),RBP,creatinine were determined on the same day. The correlation coefficients,sensitivities and diagnostic accuracy between the different markers and $GFR_{DTPA}$ were determined. The correlation coefficients between $GFR_{DTPA}$ and serum concentrations were -0.811 for Cys C , -0.699 forβ2-MG , -0.69 for creatinine , -0.419 for RBP . ROC analysis indicated that the accuracy of Cys C was similar to that of creatinine andβ2-MG, while the diagnostic accuracy of RBP was significantly lower than that of Cys C. Sensitivity analysis showed that Cys C (98.1%) and β2-MG (98.1%) were better than creatinine (86.9%), while the sensitivity of RBP was very low (56.5%). We made the conclusion that:1. Retinol-binding protein is not an adequate marker of glomerular filtrateion rate. 2. The sensitivity of cystatin C and β2-microglobulin was better than that of creatinine, indicating that serum cystatin C and β2-microglobulin can reflect the decrease of GFR much earlier and more rapidly than serum creatinine in patients with mild to moderate kidney dysfunction.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2213**

**Lipoxin $A_4$ and Benzo-Lipoxin $A_4$ Protects Against Renal Fibrosis** Emma Borgeson,[1] Eoin P. Brennan,[1] Karen Nolan,[1] Aidan Ryan,[1] Debra F. Higgins,[1] Patrick Guiry,[2] Neil G. Docherty,[3] Catherine Godson.[1] *[1]Diabetic Research Centre, Conway Institute, UCD, Dublin, Ireland; [2]Centre for Synthesis and Chemical Biology, UCD, Dublin, Ireland; [3]Department of Physiology, Trinity College Dublin, Dublin, Ireland.*

Tubulo-interstitial fibrosis and inflammation are hallmarks of renal pathology and chronic kidney disease (CKD). Lipoxin $A_4$ ($LXA_4$) is an endogenously produced eicosanoid and there is growing evidence that $LXA_4$ promotes resolution of inflammatory responses and may be antifibrotic. We have explored the potential of $LXA_4$ and a synthetic benzo-$LXA_4$ to regulate renal fibrosis using *in vivo* and *in vitro* models. Using a rat renal fibroblast cell line (NRK49F) we found that $LXA_4$ (1 nM, 30') pretreatment modulated TGFβ1 (10 ng/ml) induced proliferation and fibroblast activation (α-smooth muscle actin, thrombospondin and CTGF expression). Interestingly, we also find that $LXA_4$ (1 nM, 30') protects human kidney epithelial cell line from mesenchymal transition (EMT) in response to TGFβ1, attenuating TGFβ1-induced Jagged-1 and N-cadherin expression. These responses were associated with upregulation of a specific cohort of miRNAs. The effect of $LXA_4$ on fibroblast activation and EMT suggested antifibrotic potential in renal disease. We have investigated the effect of pretreatment with vehicle, $LXA_4$ (15μg/250g, 30' prior ligation) or benzo-$LXA_4$ (45 μg/250g, 30' prior ligation) on the UUO model of renal fibrosis in the male Wistar rat. Three days post ligation animals were sacrificed and ligation significantly increased hydronephrosis in all obstructed kidneys relative to controls. Upregulation of collagen-1α2, CTGF, Jagged-1, MCP-1 and TNF-α gene expression was seen in the ligated verses contralateral kidneys. $LXA_4$ and benzo-$LXA_4$ significantly ($p<0.05$) attenuated collagen-1α2, Jagged-1, MCP-1 and TNF-α expression. Ligation also caused a significant increase in renal collagen deposition [+2.5 fold], as analysed by sirius red staining, and benzo-$LXA_4$ pretreatment caused a marked decrease of collagen deposition in obstructed kidneys relative to contralateral ($p<0.05$). In conclusion we propose that $LXA_4$ and synthetic analogs of $LXA_4$ represent a novel class of antifibrotic agents.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2214**

**Suppression of Podocyte Albumin Transcytosis by a NADPH Oxidase Inhibitor** Satoshi Kinugasa, Akihiro Tojo, Toshiro Fujita. *Department of Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan.*

Background: We investigated whether NADPH oxidase inhibitor suppresses albumin endocytosis by podocytes and reduces glomerular albumin filtration in nephrotic syndrome rats.

Methods: We used male sprague-Dawley rats as controls (n=19), and administered puromycin aminonucleoside (PAN) to rats to serve as a nephrotic syndrome model (n=24). Some rats were treated with apocynin (16mg/kg/day orally, n=8). Production of reactive oxygen species was detected by cerium chloride (CeCl3) histochemistry, and urinary H2O2 was measured, along with immunohistochemistry (IHC) for p47phox. Following intravenous injection of Evans blue-labeled human serum albumin (EB-HSA), a clearance study and immunogold scanning electron microscopy (immunogold SEM) for HSA were performed to detect glomerular albumin filtration.

Results: CeCl3 histochemistry showed enhanced production of reactive oxygen species (ROS) in the podocyte of PAN rats, which was much reduced in apocynin-treated rats. IHC stained positive for p47phox in the podocyte cytoplasm of PAN rats, providing evidence that the ROS was produced by NADPH oxidase. In PAN nephrotic rats, glomerular filtration of EB-HSA excretion showed a132-fold increase compared to control rats ($p<0.01$ vs. control), which was significantly decreased in the apocynin-treated group (41.0±4.6 vs. 12.8 ± 5.8 mg/day, $P<0.01$ vs. PAN). With immunogold SEM, many more gold particles were observed inside podocyte vesicles compared to controls (0.3±0.2 vs.33.3±6.0 vs. control), suggestive of enhanced albumin endocytosis. With apocynin treatment, the number of gold particles in the podocyte vesicles was significantly decreased (6.6±2.3 particles/cell, $p<0.01$ vs. PAN). Similarly, particles observed on the podocyte apical membrane was greatly increased in PAN rats (5.2±0.8 vs. 113.8±21.3 particles/image, $p<0.01$ vs. control), which may represent albumin exocytosis, and significantly decreased in apocynin-treated rats (26.2±3.4 particles/image, $p<0.01$ vs. PAN).

Conclusion: The NADPH oxidase inhibitor apocynin suppressed podocyte albumin endocytosis and ameliorated glomerular albumin filtration in nephrotic syndrome rats, which may lead to a novel strategy for nephrotic syndrome.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2215**

**Spironolactone Reverses Experimental Uremic Cardiomyopathy** George Budny, Steven T. Haller, Jiang Tian, Sankaridrug Periyasamy, Deepak K. Malhotra, Joseph I. Shapiro. *Medicine, University of Toledo Medical Center, Toledo, OH.*

Spironolactone has been noted to attenuate the development of cardiac fibrosis and diastolic dysfunction seen with experimental renal failure. We have also established that spironolactone and its major metabolite, canrenone, in addition to their well established roles as mineralocorticoid receptor antagonists also serve as competitive inhibitors of the binding of cardiotonic steroids to the plasmalemmal Na/K-ATPase. We performed the following studies to determine whether spironolactone might actually reverse established experimental uremic cardiomyopathy. Male Sprague Dawley rats weighing between 250-300g were used for these studies. Rats we subjected to partial nephrectomy (PNx) as we have previously described. This maneuver produces sustained hypertension by 2 weeks. Four weeks after surgery when we have previously determined the cardiac fibrosis is well established, the rats were divided into 4 groups. The first, second and third groups received spironolactone (in doses 5mg/kg; 20 mg/kg; 80mg/kg respectively) orally through gavage tube daily for one week. In the fourth group, the exact volume of vehicle was administered. After one week of treatment animals were sacrificed and their organs harvested. The PNx animals showed an increased systolic BP and cardiac fibrosis as previously reported. We found that spironolactone (in doses 20 mg/kg/day and 80 mg/kg/day, but not 5 mg/kg/day) decreased systolic blood pressure and attenuated the diastolic dysfunction as assessed by analysis of ventricular pressure-volume loops. Spironolactone decreased the cardiac fibrosis compared to PNx as assessed by quantitative morphology determined with Sirius red staining and collagen expression measured with Western blot in a dose dependent fashion. Our results suggest that not only does spironolactone therapy ameliorate the development of experimental uremic cardiomyopathy but that spironolactone therapy can reverse cardiac fibrosis which has already been established. If these data are confirmed clinically, spironolactone may have potential utility in the treatment of patients with uremic cardiomyopathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2216**

**Phosphate Handling in Experimental Chronic Kidney Disease** Bonnie W. Y. Shum,[1] Kristin M. McCabe,[1] Navid Shobeiri,[1] David P. Beseau,[1] Michael A. Adams,[1] Rachel M. Holden.[2] *[1]Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada; [2]Division of Nephrology, Queen's University, Kingston, ON, Canada.*

In chronic kidney disease (CKD), altered phosphate disposition (e.g. hyperphosphatemia) is considered to be important in the initiation of vascular calcification; the latter, is a key risk factor for the high cardiovascular mortality seen in this patient population. In the present study, we sought to develop a quantitative assessment of systemic phosphate handling in a rat CKD model. Sprague-Dawley rats (275-300g) were given a diet containing 0.25% adenine (CKD; n=10) or no adenine (control; n=6) for 3 weeks. Pulse pressure, tissue calcium, the phosphate regulating hormone fibroblast growth factor-23 (FGF-23), and serum phosphate were measured with the latter assessed before (t=0min) and after (0-60min) an intravenous infusion of sodium phosphate (100mM in saline, 4mL/kg, over 1-2min). In addition, to determine the impact of renal phosphate clearance, the procedure was repeated after the kidneys were removed in some animals. Kidney function, assessed by serum creatinine, was significantly compromised in the CKD group (114.26±37.61μmol/L) compared to controls (38.26±5.18μmol/L, $p<0.01$). There was a significant correlation between serum phosphate and creatinine levels ($r^2=0.67$, $p<0.001$). The rate of phosphate clearance was not altered by nephrectomy, but was greater in rats with CKD (-0.18±0.087mM/min) than in controls (-0.10±0.03mM/min). Furthermore, the phosphate clearance rate also correlated significantly with both serum creatinine ($r^2=0.64$, $p<0.01$) and FGF-23 ($r^2=0.92$, $p<0.0001$). These novel findings indicate that short-term handling of phosphate occurs independently of the kidneys, and the rate of phosphate clearance increases with severity of CKD. The strong correlation with elevated FGF-23 suggests a mechanistic link with enhanced phosphate uptake into extracellular compartments in CKD is possible.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2217**

**Suramin Is a Potent Anti-Fibrotic Agent in Obstructive Nephropathy** Shougang Zhuang. *Department of Medicine, Rhode Island Hospital and Warren Alpert Medical School of Brown University, Providence, RI.*

Suramin, a compound that inhibits multiple cytokines/growth factors binding to their receptors, has been used to treat some tumors in patients and prevent skeletal muscle fibrosis in experimental animals. In this study, we examined the effect of suramin on progression of renal fibrosis induced by unilateral ureteral obstruction (UUO). UUO or sham-operated mice were randomly assigned to suramin or vehicle and were killed on day 7 after UUO or sham operation. Suramin at a single dose after surgery significantly attenuated renal interstitial fibrosis as demonstrated by reduced collagen deposition and repressed protein expression of fibronectin and type I collagen. Suramin also inhibited the injury-induced expression of alpha-smooth muscle actin and proliferating cell nuclear antigen (PCNA) as well as accumulation of renal interstitial fibroblasts. Furthermore, suramin reduced infiltration of leukocytes and gene expression of multiple cytokines including transforming growth factor-beta-1 (TGF-beta1) and TGF-β receptor Type II. *In vitro*, treatment with suramin inhibited activation and proliferation of cultured renal interstitial fibroblasts, as evidenced by dose and time -dependent blockade of expression of alpha-smooth muscle actin, fibronectin and PCNA and reduction of cell numbers. Finally, we observed that suramin inhibits activation of STAT3, a critical transcription factor implicated in renal fibrosis both *in vivo* and *in vitro*. Collectively, these findings demonstrate that suramin is a potent anti-fibrotic agent and suggest its therapeutic potential in the treatment of kidney fibrotic diseases.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2218**

**Impaired Glomerular and Tubular Antioxidative Defense Mechanisms in Nephrotic Syndrome** Anna Granqvist, Ulf A. Nilsson, Kerstin Ebefors, Borje Haraldsson, Jenny C. Nystrom. *Medicine, University of Gothenburg, Molecular and Clinical Medicine, Gothenburg, Sweden.*

**Background:** The molecular mechanisms behind acquired nephrotic syndrome (NS) are still largely unknown. One possible explanation to the development of proteinuria is oxidative damage to the glomerular cells. Our hypothesis was that the oxidative defense is weakened in NS and we focused on measurements of the oxidative – antioxidative status in the glomerular and tubular parts of the nephron.

**Method:** Gene expression was analyzed in renal biopsies from patients with NS and healthy controls (n=8). In addition, to compare the acute and chronic phases of the disease, we studied puromycin-treated nephrotic rats (n=8). Gene data was combined with measurements on urine and plasma for several different markers of oxidative stress.

**Result:** In the biopsy material the expression of enzymes involved in the antioxidative defense was higher in the tubulointerstitial compartment than in the glomerular cells (both in control and NS). Real-time PCR analysis revealed a decreased glomerular expression in nephrotic kidneys for the antioxidant enzymes catalase (by 42%) ), glutathione peroxidase-3 (by 62%) and -4 (by 40%). The tubular gene expression was down-regulated for catalase (by 27%), glutathione peroxidase -3 (by 29%), thioredoxin reductases -1 and -2 (by 48% and 18% respectively). The altered gene expression seen in NS was accompanied by an increase in lipid peroxidation, a major indicator for oxidative stress, in the urine ($p\leq 0.001$).

In rats, serum concentrations of ascorbyl free radicals, measured with electron spin resonance (ESR), were elevated in the acute phase of the disease, suggesting increased oxidative stress in the circulation ($p\leq0.01$). In addition we saw an increase in the plasma antioxidant capacity ($p\leq0.05$) combined with a decreased oxidation of proteins in sera ($p\leq0.001$) from nephrotic rats, but not from humans.

**Conclusion:** In conclusion, there is a marked down-regulation of several antioxidative enzymes in nephrotic kidneys, especially in glomerular structures. Our data suggest that oxidative damage to glomerular cells may contribute significantly to the course and prognosis of nephrotic syndrome.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2219**

**Dietary Salt Restriction May Ameliorate the Increased Renal Expression of α-Smooth Muscle Actin and Fibronectin in Adriamycin Nephrosis** Joon-Sung Park, Chor Ho Jo, Sua Kim, Chang Hwa Lee, Gheun-Ho Kim. *Department of Internal Medicine, Hanyang University, Seoul, Republic of Korea.*

**Purpose:** Adriamycin (ADR) induces severe glomerular proteinuria in rats, and proteinuria is a major factor leading to renal fibrosis probably via epithelial mesenchymal transition (EMT). This study was undertaken to test the hypothesis that renal fibrosis may be modulated by dietary salt restriction in a rat model of ADR nephrosis.

**Methods:** Male Sragues-Dawley rats were randomly divided into normal-salt controls (n=4), normal-salt ADR (NS-ADR, n=5), and low-salt ADR (LS-ADR, n=5). ADR was intravenously given into the femoral vein as a single bolus (7.5 mg/kg). Five weeks later, kidneys were harvested for histopathologic studies, immunohistochemistry and western immunoblot analysis.

**Results:** At the end of the animal experiment, remarkable proteinuria was induced in both NS-ADR (234 ± 37 mg/d/100 g BW) and LS-ADR (210 ± 27 mg/d/100 g BW) groups in contrast to controls (2 ± 0 mg/d/100 g BW). Renal histopathology revealed that tubulointerstitial injury was remarkable in both NS-ADR (cortex, 3.7 ± 0.3; medulla, 3.7 ± 0.2) and LS-ADR (cortex, 2.8 ± 0.9; medulla, 2.6 ± 0.7) rats compared with controls (cortex, 0.1 ± 0.1; medulla, 0.1 ± 0.1). Compared with controls, the expression of α-smooth muscle actin (αSMA) in NS-ADR rats was increased in both cortex (5.5 ± 1.3 *vs.* 0.6 ± 0.1 %area, $P<0.05$) and medulla (7.8 ± 2.9 *vs.* 0.2 ± 0.0 %area, $P<0.05$). The abundance of fibronectin was also increased in NS-ADR rats in both cortex (6.71 ± 1.32 *vs.* 1.01 ± 0.36, $P<0.05$) and medulla (2.52 ± 0.23 *vs.* 1.01 ± 0.10, $P<0.05$). Compared with NS-ADR, the expression of medullary α-SMA (2.3 ± 1.4 %area, $P<0.05$) and fibronectin (1.59 ± 0.22, $P<0.05$) was significantly ameliorated in LS-ADR rats. Notably, both expression levels of α-SMA (cortex, $r^2=0.46$, $P<0.01$; medulla, $r^2=0.43$, $P<0.05$) and fibronectin (cortex, $r^2=0.76$, $P<0.01$; medulla, $r^2=0.63$, $P<0.01$) were correlated with proteinuria.

**Conclusions:** In rats with ADR nephrosis, the process of EMT and interstitial fibrosis appears to be associated with proteinuria. Dietary salt restriction may ameliorate renal expression of α-SMA and fibronectin in ADR nephrosis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2220**

**Definition of the Pericyte Transcriptome during Kidney Fibrosis by Polysome Immunoaffinity Purification from Whole Kidney** Ivica Grgic, Matthew A. Lalli, Jeremy S. Duffield, Benjamin D. Humphreys. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Renal fibrosis, an abnormal repair process in the kidney leading to progressive renal failure, is the main cause for end stage renal disease and remains a major unresolved problem in clinical medicine. We have shown using genetic fate mapping that pericytes and perivascular fibroblasts are the progenitor population for myofibroblasts which represent the main effector cells in the pathogenesis of tubulointerstitial fibrosis responsible for increased synthesis and deposition of extracellular matrix. Currently, our knowledge about kidney pericytes in health and disease remains very limited. This can be attributed to the challenge of studying single cell populations in highly complex tissues such as the kidney. To better understand the biological properties of renal pericytes and to dissect their role in states of disease we have applied *Translating Ribosome Affinity Purification* (TRAP) to isolate RNA from a genetically defined cell population *in vivo*. We generated a transgenic mouse line that expresses a GFP-tagged ribosomal fusion protein (eGFP-L10a) under the control of the collagen 1α1 promoter. Expression of eGFP-L10a was present in kidney medulla, strictly confined to cells in the tubulointerstitium staining positive for pericyte markers. These cells underwent dramatic expansion during the unilateral ureteral obstruction (UUO) fibrosis model and acquired αSMA expression, consistent with differentiation into myofibroblasts. Using the TRAP approach, we affinity purified GFP-tagged polysomes from healthy and fibrotic kidneys with anti-GFP antibodies, resulting in pericyte-specific mRNA isolation from whole kidney in a one-step procedure. We have used this RNA to generate the pericyte transcriptome by gene microarray during differentiation into myofibroblasts in kidney fibrosis. Polysome affinity purification represents a novel, powerful tool to isolate cell-specific RNA from genetically defined kidney cell populations *in vivo* and our dataset will inform our understanding of these critical cells during fibrotic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2221**

**Chronic Metabolic Acidosis Increases Renal Oxygen Consumption Rates** Daniel F. Balkovetz,[1,2] Cynthia Miracle,[3,4] Phillip H. Chumley.[1] *[1]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL; [2]Medicine, Birmingham VA Medical Center, Birmingham, AL; [3]Medicine, University of California San Diego, San Diego, CA; [4]Medicine, Veterans Affairs San Diego Healhcare System, San Diego, CA.*

A major factor in the progression of chronic kidney disease (CKD) is chronic metabolic acidosis (CMA). In rodents, CMA is associated with worsening of renal function and normalization of acid-base parameters with bicarbonate attenuated this renal disease. Correction of CMA in humans also slows the rate of CKD progression. The mechanism by which CMA accelerates CKD progression is not known. We have reported that CMA decreases levels of claudin-2 in proximal tubule. Claudin-2 mediates passive paracellular reabsorption Na+ selectively in the proximal tubule. Decreased claudin-2 expression would result in increased delivery of Na+ to the distal nephron to be reabsorbed by transcellular, active, transport mechanisms. This increase in active renal transport mechanisms in the kidney during CMA would also increase renal oxygen consumption rates. Increased renal oxygen consumption is critical for progression of CKD. To test this hypothesis, we measured renal oxygen consumption rates in whole kidney and in mitochondria isolated from the kidney of control and acidotic rats. CMA was induced in the rats using the protocol of NH4Cl (280 mM) added to the drinking water. In whole kidney, oxygen consumption rates normalized to GFR were significantly greater in the acidotic rats compared to control animals. Oxygen consumption rates in mitochondria isolated from control and CMA kidneys were greater in the mitochondria isolated from acidotic kidneys. These data are consistent with the hypothesis that CMA accelerates of CKD progression, in part, by increasing renal oxygen consumption rates. Future work will focus on the role of renal claudin-2 expression on the relative shift from passive paracellular to active transcellular transport in the renal tubule on the increased oxygen consumption rates. This work will help define the mechanism by which CMA increases renal oxygen consumption and CKD progression rates.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2222**

**Random Urinalysis Proteinuria Predicts Renal Function Change, a Pilot Study** Steven J. Rosansky,[1, 2] James W. Hardin,[2] Martin W. Durkin,[1] Kathlyn Sue Haddock.[1] *[1]Dorn Research Institute, WJBD VA Hospital, Columbia, SC; [2]School of Public Health, USC, Columbia, SC.*

**Purpose:** Examine the association of serum creatinine level (C) , the presence of proteinuria and renal function change over time, measured by change in C, in patients with CKD Class 2/3.

**Methods**: Male subjects from a VA Hospital data sample with at least: one (C) of ≥1.3 mg/dl; 2 creatinine values ≥ year apart; ≥ one reported urinalysis(UA) ; entry interval,1989 to 2003.Followup data obtained through 12-31-2008.The same serum creatinine methodology was used throughout the study (modified Jaffe). We investigated whether UA proteinuria influenced growth curve analysis (average creatinine changes per month) for a sample of 5558 patients with last names beginning A, B or C. We defined 3 groups of patients based on initial C: group 1 (n=4,014) ≥1.3mg/dl, group 2 (n=1040) 1.3 to 1.7 mg/dl and group 3 (n=504) > 1.7 mg/dl. Initial MDRD eGFR was calculated using the 4 variable model. We defined a binary variable for proteinuria, present if any random UA protein ≥30mg/dl. Growth curve analysis included creatinine group, proteinuria and their interaction.

**Results:** Mean values : age 62; follow-up 8.4 years; C's 21.1; UAs 7.9. For the 3 groups initial C (mg/dl) /MDRD (eGFR ml/min/1.73m$^2$) were:1.14/74.6, 1.47/51.83, 2.59/33.7. Creatinine group, proteinuria, and their interaction were all significant ($p < .001$); no statistical difference between group 2 and group 1. Group 3 maintained significantly higher creatinine level changes (average difference over the study interval of 0.35, $p<0.001$). For the total population, there was a tendency for creatinine levels to decline (average monthly decline 0.004, $p<0.001$), but proteinuric patients across all groups showed a tendency for creatinine levels to increase (average monthly growth 0.010, $p<0.001$). There were no significant associations over time of C with age or race.

**Conclusion**: In these CKD Class 2/3 patients over an average 8 year follow-up C's for non-proteinuric patients remain stable, while for proteinuric patients (any UA with ≥30 mg/dl protein) creatinine levels increase by 0.12 mg/dl per year, or an average of 1 mg/dl over the 8.4 year follow-up.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2223**

**Association of Chronic Kidney Disease with Skeletal Muscle Mitochondrial Dysfunction** Puya G. Yazdi, Jia-Ying John Yang, Pavan Gollapudi, Nosratola D. Vaziri. *Medicine, University of California, Irvine, Irvine, CA.*

Advanced chronic kidney disease (CKD) is associated with impaired exercise capacity, skeletal muscle dysfunction, and oxidative stress. Mitochondria are the primary source of production of energy and generation of reactive oxygen species (ROS). The present study was designed to test the hypothesis that skeletal muscle dysfunction and oxidative stress in CKD are, in part, due to impaired energy production and heightened ROS generation by mitochondria. Mitochondrial state 3 respiration (measured as the maximal rate of ADP conversion to ATP, using pyruvate and malate as substrates) and mitochondrial complex enzyme activities were determined in the gastrocnemius muscle of male SD rats 12 weeks after 5/6 nephrectomy (CKD) or sham-operation (control). The CKD group exhibited proteinuria, hypertension, azotemia, and reduced weight gain. This was associated with significant reduction (-39%, $p<0.05$) of state 3 mitochondrial respiration and a significant increase in the mitochondrial complex I plus III enzyme activity (18% , $p<0.05$). The latter is the first step in oxidative phosphorylation by catalyzing oxidation of reduced nicotinamide adenine dinucleotide (NADH→NAD+), a process which is linked to production of ROS. We further found a mild reduction in Complex IV activity (-13%) that was accompanied by a decrease in abundance of Complex IV subunit IV (-15%) in the CKD rats. Finally we found a mild reduction of Porin abundance (-8%), which points to diminished mitochondrial abundance in the skeletal muscle of CKD rats compared with the controls. Thus, the study demonstrated the deleterious effects of uremia on the skeletal muscle mitochondrial abundance and activity which can, in part, account for the CKD-induced reduction of exercise capacity.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2224**

**High Dose ARB Promotes Survival and Parietal Epithelial Cell to Podocyte Transition in 5/6 Nephrectomy Rats** Dan Gao,[1, 2] Vittorio Albertazzi,[1] Li-Jun Ma,[1] Haichun Yang,[1] Kang-Hsien Fan,[3] Yu Shyr,[3] Agnes B. Fogo.[1] *[1]Department of Pathology, Vanderbilt University; [2]Department of Nephrology, First Affiliated Hospital of ZhengZhou University, ZhengZhou, China; [3]Cancer Biostatistic Center, Vanderbilt-Ingram Cancer Center.*

Angiotensin receptor blocker (ARB) can regress existing sclerosis induced by 5/6 nephrectomy (Nx) if given short term from 8 to 12 wks. We now further investigated 1) long term effects on regression and survival and 2) potential parietal epithelial cell (PECs)-podocyte transition in regression.

Adult male Sprague Dawley rats underwent 5/6 Nx. Glomerulosclerosis (0-4 scale) was assessed by renal biopsy (Bx) at 8 weeks. Rats were then divided into two groups with equal average systolic blood pressure (SBP), 24-h urinary protein (Uprot) and sclerosis index (SI), and treated with high dose ARB (n=21, losartan, 200 mg/L/DW) or no treatment (CONT, n=21) till 30 wks after Nx.

ARB lowered SBP and proteinuria vs CONT, increased survival rate (median survival time 27.1 wks vs 19.7 wks, log-rank test, p=0.013). Protective effects of ARB on survival rate were greater in those animals with Bx SI>1.1 (as assessed by original data and 5000 bootstrap re-sampling analysis, median survival time: ARB 22.4 wks vs CONT 17.3 wks, log-rank test, p=0.018) compared to those with Bx SI≤1.1 (median survival time: ARB >30 wks vs CONT 20.6 wks, log-rank test, p=0.068). ARB significantly maintained WT1-positive podocyte numbers at sacrifice compared to CONT (2.4±0.3 vs 1.7±0.2, $p<0.05$). Interestingly, claudin 1 staining (PEC marker) was increased in capillary loop area at sacrifice (0-4 scale, ARB 1.1±0.1 vs CONT 0.6±0.1, $p<0.05$), indicating parietal-like cells were in anatomical location of podocytes.

We conclude that high dose ARB improves survival in this CKD model, and the beneficial effect is further potentiated when the intervention occurred at moderate stages of glomerulosclerosis. We postulate that the beneficial effects of ARB on survival are in part linked to maintained podocyte numbers contributed to by parietal epithelial cell to podocyte transition.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2225**

**Protein Restriction Plus Ketoacids Reduces the Severity of Renal Disease in 5/6 Nephrectomized Rats: An Association with KLF15 Levels** Xiang Gao, Changlin Mei. *Kidney Institute of PLA, Department of Medicine, Kidney Institute of PLA, Department of Medicine, Changzheng Hospital, Second Military Medical University, Shanghai, China.*

**Objective**: To investigate the efficacy and mechanism of kruppel-like factor 15 (KLF15) on renal fibrosis in chronic kidney disease (CKD).

**Methods**: 5/6 nephrectomy SD rats were randomly divided into three groups, respectively fed with normal protein (NPD), low protein (LPD) or low protein plus ketoacids (LPD+KA). The functional and morphological alterations of the remnant kidneys, oxidative stress and inflammatory level were measured. Then we examined the levels of KLF15 in remnant kidney. In vitro we observed the effect of TGF-β, oxidative stress and inflammatory on KLF15 in rat mesangial cells (RMCs) and explored the underlying mechanism. For further study RMCs and HEK293 cells were transfected with a KLF15 cDNA expression vector and the level of extracellular matrix was measured.

**Results**: Progressive injury was found in the remnant kidneys of NPD group. Protein restriction ameliorated above changes, and the role was more obvious in LPD+KA group after 5/6 nephrectomy. Oxidative products as well as chronic inflammation were significantly increased in LPD group compared with LPD+KA group. KLF15 was expressed in both glomeruli and interstitium. The levels drastically decreased in remnant kidney of 5/6 nephrectomized rats, while restriction of protein intake partially restored the levels of KLF15, and LPD+KA increased more effectively than LPD. In vitro we found that levels of KLF15 in RMCs were decreased by TGF-b1, TNF-a, as well as $H_2O_2$, and overexpression of KLF15 in RNCs and HEK293 significantly decreased extracellular matrix expression.

**Conclusion**: LPD+KA plays a more renal protective role than LPD in CKD rats. The effect may be mediated by ketoacids ameliorating oxidative stress and inflammation injury in remnant kidney tissue. The expression of KLF15 can be affected by oxidative stress and inflammation, and it may also have a role in extracellular matrix regulation in renal fibrosis in CKD. The improvement of renal disease by dietary treatment was associated with a partial restoration of KLF15 expression in remnant kidney.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2226**

**Very Advanced Chronic Kidney Disease (CKD) Can Still Be Arrested by Combined Losartan (L) and Hydrochlorothiazide (H) Treatment** Simone R. Costa, Carla P. Valente, Claudia R. Sena, Camilla Fanelli, Bianca H. Ventura, Denise M. Malheiros, Roberto Zatz, Clarice K. Fujihara. *Univ of Sao Paulo, Brazil.*

We showed earlier (AJP 292:F1810) that LH treatment started 1 month after 5/6 renal ablation (Nx) arrests renal injury for at least 7 months. However, such early intervention is unrealistic. We investigated if LH would still afford renoprotection when started 4 months after Nx, when renal injury is comparable to that seen in very advanced human CKD. Adult male Munich-Wistar rats underwent Nx, remaining untreated until 4 months after Nx, when tail-cuff pressure (TCP, mmHg), albuminuria (ALB, mg/d), plasma triglycerides (TG, mg/dL), serum creatinine (Scr, mg/dL), % glomerulosclerosis (%GS), % cortical interstitium (%INT) and tubulointerstitial (TI) proliferating cell nuclear antigen (PCNA+, cells/mm$^2$) were measured in 14 pretreatment controls (**$Nx_{pre}$**). The remaining 53 rats were distributed among **Nx** (untreated); **$Nx_L$** (L, 50 mg/kg/d) and **$Nx_{LH}$** (L+H, 6 mg/Kg/d). Results 3 months later (Mean±SE, $^a$p<0.05 vs. $Nx_{pre}$, $^b$p<0.05 vs. Nx; $^c$p<0.05 vs. $Nx_L$):

| | TCP | ALB | %GS | %INT | TI PCNA+ | $S_{Cr}$ | TG |
|---|---|---|---|---|---|---|---|
| $Nx_{pre}$ | 208±3 | 147±17 | 28±4 | 4.1±0.5 | 194±34 | 1.4±0.1 | 79±2 |
| Nx | 216±5 | 256±47$^a$ | 55±4$^a$ | 6.8±0.7$^a$ | 264±43 | 2.4±0.2$^a$ | 91±6 |
| $Nx_L$ | 209±3 | 174±24$^b$ | 46±5$^a$ | 6.2±0.4 | 191±24 | 2.0±0.3$^a$ | 91±12 |
| $Nx_{LH}$ | 163±4$^{abc}$ | 43±5$^{abc}$ | 25±4$^{bc}$ | 4.0±0.7$^b$ | 78±8$^{abc}$ | 1.4±0.1$^{bc}$ | 66±6$^{abc}$ |

At baseline (Group $Nx_{pre}$), severe renal injury and renal insufficiency (with $S_{Cr}$ almost threefold higher than normal) were evident. Untreated Nx rats mimicked severe human CKD, with high mortality (75%), marked hypertension and fast increase of ALB, $S_{Cr}$, GS, %INT and TI hyperplasia. Unlike L, LH arrested or reversed all abnormalities and reduced mortality (27%). Conclusions: 1) very advanced CKD can still be arrested; 2) as with early treatment, very late L may require a diuretic for best renoprotection; 3) at odds with established concepts: a) H can still be effective in very advanced CKD; b) H does not necessarily worsen, and may even ameliorate, dyslipidemia in CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2227**

**Amniotic Fluid Stem Cells and CKD** Laura Perin,[1,3] Sargis Sedrakyan,[1,3] Stefano Da Sacco,[1,3] Liron Shiri,[1,3] Astgik Petrosyan,[1,3] Kevin V. Lemley,[2,4] Roger E. De Filippo.[1,3] *[1]Urology, Childrens Hospital Los Angeles, Los Angeles, CA; [2]Nephrology, Childrens Hospital Los Angeles, Los Angeles, CA; [3]Laboratory for Organ Regenerative Research and Cell Therapeutics, Institute of Urology, Keck School of Medicine, University of Southern California, Los Angeles, CA; [4]Clinical Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, CA.*

Alport Syndrome is characterized by a hereditary form of glomerulonephritis, wherein an abnormal level of glomerular basement membrane (GBM) is produced, gradually leading to interstitial fibrosis and eventual loss of renal function. At present, there is no definitive therapy to delay progression to ESRD for patients with Alport Syndrome. Stem-cell based therapies may provide alternative therapeutic opportunities. Amniotic fluid stem cells (AFSCs) are well established to have pluripotential capabilities and represent a possible alternative approach to current therapies for CKD. Systemic injection of the cells provides beneficial effects; we found decreased levels of albuminuria, creatinine and BUN as well as significant increase in the life-span of the treated mice as compared to their untreated controls. Additionally, treated mice present lower number of macrophage infiltration, lower myofibrostast trasformation in the kidney interstitial space, accompanied with downregulated expression of chemokines such as MPC-1, IP-10 and cytokines such as IL-1, TNFα and TGFβ. Data from AFSC treated mice also show altered expression of other molecules/proteins associated with ECM turnover and fibrosis. In this study we demonstrate that AFSCs are capable of slowing down the progression of Alport disease by incurring structural and functional benefits to the kidney. These cells may present an alternative approach to treat various medical conditions where currently therapeutic options are either limited or inadequate.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2228**

**Molecular Characterization of a Murine Model of Nephrolithiasis and Kidney Failure Using Untargeted Plasma Metabolite Profiling** Qun Chen,[1] Hyeong-Cheon Park,[2] Michael S. Goligorsky,[3] Steven S. Gross.[1] *[1]Weill Cornell Medical College, NY, NY; [2]College of Medicine, Seoul, Korea; [3]New York Medical College, Valhalla, NY.*

We found that Xanthine oxidoreductase (XOR) nullizygous mice develop severe renal dysfunction and early death, in association with the accumulation of crystalline purine deposits in the renal tubules and parenchyma. Despite knowledge of XOR's substrates (xanthine and hypoxanthine) and ultimate product (urate), purine-independent actions of XOR on intermediary metabolism have been inferred and indirect consequences of XOR inactivation are likely. Metabolomic technologies offer the potential to broadly survey changes in the expression levels of thousands of structurally diverse metabolites in complex biological mixtures. The present study employed untargeted plasma metabolite profiling in attempt to discover systemic changes that result from XOR deletion or inhibition. Using untargeted LC-MS in conjunction with a chemoinformatic platform for data acquisition, filtering and statistical analysis, we surveyed >3700 metabolites (50 – 1000 Da) for differential expression in plasma of wild-type vs. xor-nullizygous mice. Notably, of 150 plasma metabolites that were significantly altered by > 2-fold in xor-null mice (up or down), 110 changes were recapitulated in wild-type mice after treatment with allopurinol, a pharmacological inhibitor of XOR. We discovered that XOR inhibition results in 6-fold reduction in circulating levels of the XOR cofactor molybdopterin as well as profound changes in circulating phospholipid profiles. The lower level of molybdopterin suggests that XOR may regulate the expression of its cognate cofactor and altered phospholipid levels may be explained by a prior report showing that XOR-knockout results in a developmental defect in adipogenesis. Anatomical and functional studies confirmed the plasma metabolite evidence of renal dysfunction in XOR-null mice. These studies suggest unrecognized XOR activities and demonstrate the power of untargeted plasma metabolite profiling for systemic discovery of both direct and indirect consequences of gene mutations and drug treatments.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2229**

**Anti-Angiogenic Proteins as Premonitory Diagnostic Markers of Chronic Allograft Dysfunction** Eliza Moskowitz-Kassai,[1] Lina Mackelaite,[1] Jun Chen,[1] Darshana M. Dadhania,[2] Steven S. Gross,[2] Praveen Chander,[1] Veronica Delaney,[1] Manikam Suthanthiran,[2] Michael S. Goligorsky.[1] *[1]New York Medical College, Valhalla, NY; [2]Weill Cornell Medical College, NY, NY.*

Our previous "shotgun" mass spectroscopy studies demonstrated the de novo appearance in the urine of CAD patients of 180 proteins/peptides, of which 39 were not detectable in the urine from patients with stable graft function and 21 were unique for CAD grades 1-3. We have recently documented the appearance of one anti-angiogenic peptides, endorepellin, in the urine of patients occurred at the expense of the parent molecule, perlecan. Here, we analyzed three additional anti-angiogenic peptides previously detected in the urine of CAD patients, endostatin, pigment epithelium-derived factor (PEDF) and Kruppel-like factor-2 (KLF-2). In healthy subjects and patients with CAD 0, endostatin excretion was confined to the level of detection. In contrast, Tukey's Multiple Comparison Test (MCT) indicated that there were significant differences ($p<0.05$) between the subgroups of patients with CAD 3 and CAD 0, CAD 1, and healthy patient controls. PEDF excretion in healthy controls and CAD0 patients was at the lower level of detection. MCT indicated that there were significant differences ($p<0.05$) between the subgroups of patients with CAD 3 and CAD 0, CAD 2, and healthy controls. KLF-2 urinary excretion by healthy controls and CAD0 patients was also at the lower level of detection. MCT showed that there were significant differences ($p<0.05$) between the subgroups of patients with CAD 3 and CAD 2 and healthy controls. ROC curve analyses demonstrated a highly discriminative profile for all three biomarkers offered 83% sensitivity and 90% specificity in distinguishing patients with CAD-0 from groups CAD-1-3. PEDF and KLF-2 levels in the urine were elevated in patients with CyA nephrotoxicity detected by biopsy. In conclusion, these findings indicate the diagnostic potential of urinary detection of endostatin, PEDF and KLF-2 and are suggestive of the mechanistic role played by anti-angiogenic substances in the developing vasculopathy and vascular rarefaction in patients with CAD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2230**

**Effect of Chronic Kidney Disease on the Expression of Thiamin and Folic Acid Transporters** Farhan J. Bukhari,[2] Hamid Moradi,[1] Hyun Ju Kim,[1] Nosratola D. Vaziri,[1] Hamid M. Said.[2] *[1]Medicine/Nephrology, University of California, Irvine, Irvine, CA; [2]Medicine, Long Beach VA Medical Center, Long Beach, CA.*

**Objectives:** Chronic kidney disease (CKD) is associated with significant cardiovascular, neurological and metabolic complications. Thiamin and folate are essential for growth, development and normal cellular function, and their uptake is mediated by regulated transport systems. While plasma folate and thiamin levels are generally normal in patients with CKD, they commonly exhibit features resembling vitamin deficiency states. Earlier studies have documented impaired intestinal absorption of several B vitamins in experimental CKD. In this study we explored the effect of CKD on expression of folate and thiamin transporters in the key organs and tissues.

**Method:** Sprague-Dawley rats were randomized to undergo 5/6 nephrectomy or sham-operation and observed for 12 weeks. Plasma folate and thiamin concentrations and gene expression of folate (RFC, PCFT) and thiamin transporters (THTR-1 and THTR-2) were determined in the liver, brain, heart and intestinal tissues using real-time PCR.

**Results:** Plasma folate and thiamine levels were comparable among the CKD control groups. However expressions of both folate (RFC and PCFT) and thiamin (THTR-1, THTR-2) transporters were markedly reduced in the small intestine, heart, liver and brain of the CKD animals. Similarly, expression of the mitochondrial folate (MFT) and thiamin pyrophosphate transporters (MTPP) was significantly reduced in the CKD animals.

**Conclusion:** CKD results in marked down-regulation in the expression of folate and thiamin transporters in the intestine, heart, liver and brain. These events can lead to reduced intestinal absorption and impaired cellular homeostasis of these essential micronutrients despite their normal plasma levels.

**Table:** Thiamin and Folate transporters percent difference in CRF compared to sham-operated animals.

| | RFC | PCFT | THTR-1 | THTR-2 | MFT | MTPP |
|---|---|---|---|---|---|---|
| INTESTINE | -47%** | -74%* | -47% | -64%** | | |
| LIVER | -48%* | -67%** | -57%* | -68%* | -55%** | -43%** |
| HEART | -40%* | -44%* | -30%** | -43%* | | |
| BRAIN | -32%* | -60% | -28% | -46% | | |
| *=<0.05 | | | | | | |
| **=<0.01 | | | | | | |

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2231

**Ezetimibe Alone or in Combination with Pitavastatin Prevents Kidney Dysfunction in 5/6 Nephrectomized Rats Fed High-Cholesterol** Yusaku Mori, Tsutomu Hirano. *Diabetes, Metabolism, and Endocrinology, Showa university school of medicine, Shinagawa, Tokyo, Japan.*

Purpose: We investigated the renoprotective effect of ezetimibe, an inhibitor of cholesterol absorption, in 5/6 nephrectomized rats and compared with a statin.

Methods: The nephrectomized rats or sham-operated rats were fed either 1% cholesterol or normal chow containing ezetimibe or pitavastatin for 12 weeks.

Results: The nephrectomized rats exhibited kidney dysfunction (creatinine clearance (CCr, ml/min/kg) 7.5±0.8 vs. control 2.7±0.8; urine protein excretion (UPE, mg/dl/creatinine) 24.7±0.9 vs. control 1.4±0.2), hypercholesterolemia, and pathological changes (glomerulosclerosis and interstitial fibrosis). Pitavastatin failed to improve hypercholesterolemia in the nephrectomized rats but it significantly ameliorated renal dysfunction (CCr 3.4±0.3; UPE 11.5±3.3) and pathological changes. Ezetimibe conferred no effect on either cholesterol or kidney damage. The high-cholesterol diet increased serum cholesterol even further and worsened the kidney damage (CCr 1.5±1.1 vs. control 6.8±0.7; UPE 36.4±11.4 vs. control 5.3±2.6). Ezetimibe attenuated the hypercholesterolemia and significantly ameliorated the kidney dysfunction (CCr 2.4±0.6; UPE 30.1±6.1) and pathological changes, while pitavastatin failed to improve the kidney damage (CCr 2.1±1.0; UPE 34.6±13.8). The combination of ezetimibe and pitavastatin additively ameliorated the kidney damage (CCr 3.0±0.4; UPE 24.4±9.8). Macrophage infiltration area on glomeruli which was caused by high cholesterol diet was ameliorated by ezetimibe (5.8±2.9 vs. without ezetimibe 11.2±1.8%), and that area was closely associated with serum cholesterol levels. The kidney dysfunction and morphological changes were significantly associated with serum cholesterol levels and the gene expression of connective tissue growth factor in the remnant kidney.

Conclusion: These results suggest that ezetimibe and pitavastatin confer their renoprotective effects in different ways, the former by cholesterol reduction via inhibition of cholesterol absorption, and the latter by a pleiotropic effect independent of the cholesterol reduction.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2232

**Effect of Indoxyl Sulfate on Apoptosis, Reactive Oxygen Species Production in Neutrophils and Synthesis of MCP-1 and Monocytes** Edgar Ferreira Cruz, Silman Manfredi, Gori Mouro, Marie Beata Redublo Quinto, Caren Cristina Grabulosa, Marcelo Costa Batista, Miguel Cendoroglo, Maria Dalboni. *Medicine/Nephrology, Universidade Federal de São Paulo, Sao Paulo, Brazil.*

Introduction: Indoxyl sulfate is a uremic toxin that accelerates the progression of chronic kidney disease (CKD). Serum levels of indoxyl sulfate are increased in dialysis patients.

Aim: The aim of this study was to evaluate the effect of the uremic compound indoxyl sulfate (IS) on apoptosis, reactive oxygen species production (ROS) and synthesis of MCP-1 in polymorphonuclear cells and mononuclear cells.

Methods: We selected 35 healthy subjects (20 men and 15 women. Neutrophils (PMN) and monocytes (PBMC) were incubated in the absence and presence of IS. Apoptosis was evaluated by annexin V- FITC and the expression of CD95-FITC. To measure ROS production we used DCFH-DA and DAF. Apoptosis, CD95 expression and ROS was analysed mean intensity of fluorescence (MIF) by flow cytometry. MCP-1 was measured by immunoassay (ELISA).

Results: We observed that the viability of PMN was lower after incubation with indoxyl sulfate (18 ± 11 vs 33 ± 14, p <0.001). Moreover, apoptosis (47 ± 20 vs. 36 ± 11, p <0.001), expression of CD95 (110 ± 29 vs 48 ± 20, p <0.001) and hydrogen peroxide (43,234 ± 31,488 vs. 8,663 ± 5,138; p <0.001) and nitric oxide (27,077 ± 9,688 vs 2,278 ± 2,965, p <0.001) were higher in PMN incubated in the presence of indoxyl sulfate compared with PMN incubated in the absence of toxin. The expression of MCP-1 was lower in monocytes incubated with indoxyl sulfate (1,105 ± 663 vs. 1,673 ± 1,233, p <0.01).

Conclusions: Thus, in accordance with our results, we suggest that IS is a uremic toxin that increases the generation of hydrogen peroxide and nitric oxide, substances that are potentially inducing or contributing to injury in vascular endothelium. Indeed, this toxin might be associated with atherosclerosis. Additionally, the IS induces apoptosis of PMN and decreased synthesis of MCP-1, suggesting that this toxin may also contribute to low response to challenge infectious and consequently susceptibility to infections observed in the CKD patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2233

**Can Podocytes Undergo Epithelial-to-Mesenchymal Transition?** Tet-Kin Yeo, Charbel C. Khoury, Sheldon C. Chen. *Medicine/Nephrology & Hypertension, Northwestern University, Chicago, IL.*

Transforming Growth Factor-beta (TGF-β) is a major cytokine known to induce podocyte dysfunction leading to glomerulosclerosis and albuminuria. TGF-β is also a strong inducer of epithelial-to-mesenchymal transition (EMT). While EMT has been suggested to be a mechanism of podocyte disease, this remains controversial. To obtain a more comprehensive understanding, we determined the mRNA and protein expression profiles of cultured podocytes when treated with TGF-β.

Expression profile of podocyte culture in the presence of TGF-β

| Category | Increased Expression | Decreased Expression | Unchanged Expression |
|---|---|---|---|
| Cell Surface Proteins | Integrins α5, β1, B3 | Integrin α3 | Integrin αv |
| | | Nephrin | |
| | | P-Cadherin | |
| Cytoskeletal Proteins | Vimentin | | FSP1 |
| | α-SMA | | |
| Matrix Proteins | Fibronectin | | |
| | Collagens 1α2, 3α1, 5α2 | | |
| Polarity Proteins | | Crumbs 3 | |
| | | PALS1 | |
| | | PATJ | |
| | | Protein Kinase C, iota | |
| Transcription Factors | Zeb2 | | Zeb1 |
| | Snail | | |
| Matrix Metalloproteases | Mmp 2, 9 | | |
| Protease Inhibitors | PAI-1 | | |
| | Timp | | |

Exposure of the podocytes to TGF-β (2 ng/mL) for 24 or 48 hr resulted in a decrease of slit diaphragm proteins as well as an increase in phosphorylation of Nephrin. Also notable was a marked decrease in the Crumbs apical cell polarity complex. These changes correlated with alterations in functional characteristics including the loss of cellular arborization and actin stress fibers, as well as increased cell motility. However TGF-β-treated cells failed to upregulate FSP-1. Nonetheless, the sum of these changes, along with the increased expression of other known mesenchymal markers, suggests that the podocyte undergoes EMT. The phenotypic changes of podocyte EMT might be attributable to an increase of the transcription factor Zeb2, an E-box repressor known to participate in EMT. Zeb2 itself might be increased because of loss of inhibition by members of the microRNA-200 family. This marks the first time that Zeb2 has been correlated with components of podocyte EMT.

We provide a more complete profile of EMT, including the repression of Crumbs polarity complex, and identify Zeb2 and others as novel signaling pathways involved in the podocytopathy that contributes to TGF-β-related kidney diseases, ranging from obstruction to diabetic nephropathy to nephritis.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2234

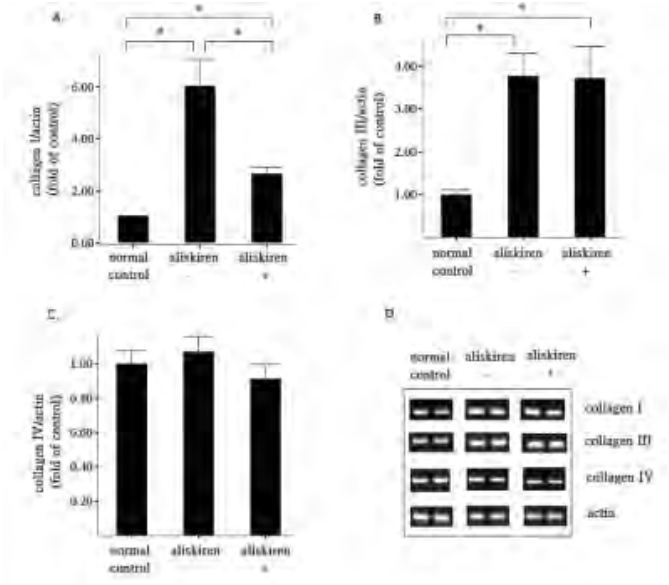
**Revisiting Renin-Angiotensin System in Chronic Renal Ischemia/ Renovascular Hypertension with Direct Rennin Inhibitor – A Study from a 2K1C Mice Model** Chiao-Yin Sun. *Nephrology, Chang Gung Memorial Hospital, Keelung, Taiwan.*

Chronic renal ischemia (CRI)leads to renal fibrosis. The aim of this study is to define the renal RAAS alteration and the effect of direct renin inhibition by aliskiren in kidney with CRI. A two kidney-one clip model was used in this study.Aliskiren significantly lowered the blood pressure in mice with renal artery constriction (92.1 ± 1.1 vs. 81.0 ± 1.8 mmHg, P< 0.05). Renal renin (0.20 ± 0.10 vs. 1.00 ± 0.06, P<0.05) and AT2 receptor (0.46 ± 0.24 vs. 1.00 ± 0.00, P<0.05) expression decreased after CRI. Renin (0.35 ± 0.06 vs. 1.00 ± 0.06, P<0.05) expression decreased and angiotensinogen (3.46 ± 1.08 vs. 1.00 ± 0.14, P<0.05) expression increased in un-clipped kidney. (Pro)renin receptor was decreased by direct renin inhibition (clipped kidney: 0.52 ± 0.06 vs.0.95 ± 0.05, P< 0.05; un-clipped kidney: 0.59 ± 0.03 vs. 0.91 ± 0.14, P<0.05).

Summary of RAAS profile changes in study animal.

| | Ischemic kidney (vs. normal control) | Compensatory kidney (vs. normal control) |
|---|---|---|
| Renin | ↓ | ↓ |
| (pro)renin receptor | — | — |
| Angiotensinogen | — | ↑ |
| AT1 receptor | — | — |
| AT2 receptor | ↓ | — |
| | Ischemic kidney (aliskiren+ vs. aliskiren-) | Compensatory kidney (aliskiren+ vs. aliskiren-) |
| Renin | ↑ | ↑ |
| (pro)renin receptor | ↓ | ↓ |
| Angiotensinogen | — | — |
| AT1 receptor | — | ↓ |
| AT2 receptor | ↑ | — |

CRI dcreased klotho expression, increased Bax, caspase-3, TGF-β1, and CTGF expression significantly, which can be all reversed by direct renin inhibition with aliskiren. The relative changes of collagen expression were showed in Figure 1.




Histology exam revealed that aliskiren significantly decreased renal fibrosis induced by CRI. In our study, we demonstrated that aliskiren significantly reversed renal fibrosis, apoptosis, and *klotho* expression after CRI possibly via angiotensin II dependent and independent pathways.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2235

**Discovery and Validation of A1BG as a Differential Urinary Biomarker in Pediatric Nephrotic Syndrome** Nuntawan Piyaphanee, Michael R. Bennett, Kimberly Czech, Mark Mitsnefes, Prasad Devarajan. *Nephrology, Cincinnati Children's Hospital, Cincinnati, OH.*

We set out to discover a non-invasive biomarker that could distinguish steroid resistant nephrotic syndrome (SRNS) vs steroid sensitive nephrotic syndrome (SSNS).

Methods: Urine and clinical data were collected from patients with idiopathic nephrotic syndrome and healthy controls. Using surface enhanced laser desorption ionization time of flight (SELDI-TOF) mass spectrometry, we identified a strongly upregulated 13.8 kDa fragment of α-1B-glycoprotein (A1BG fr) in urine in SRSS and/or FSGS. To validate our findings, A1BG was detected by western blot. Creatinine was measured and transformed to GFR by the new Schwartz formula and classified to CKD stage. P-values were determined by unpaired t-test and Mann-Whitney Rank Sum analysis.

Results: By western blot, the 13.8 kDa A1BG was present in 7 of 13 patients with FSGS; the fragment was absent in SSNS (n=15) and controls (n=10). The A1BG fr+ patients had higher serum creatinine and lower GFR than the A1BG fr- patients. CKD stage was also higher, but not statistically significant (table1).

Table1 Clinical data and renal function of A1BG fr+ and A1BG fr- patients

| Variable | A1BG fr+ (n=7) | A1BG fr- (n=21) | P-value |
|---|---|---|---|
| Age (years; mean±SD) | 13.1±2.9 | 7.9±4.9 | 0.003 |
| Sex Male:Female | 2.5:1 | 1.6:1 | NS |
| Race(%)African American/Caucasian | 50.0/50.0 | 42.9/42.9 | NS |
| Cr (mg/dl; median [IQR]) | 1.4 (0.68-3.23) | 0.5 (0.40-0.60) | 0.006 |
| GFR (ml/min/1.73m2; mean±SD) | 54.66±36.31 | 102.43±40.47 | 0.011 |
| CKD stage (median [IQR]) | 3 (1.25-3.75) | 1 (1.0-2.0) | 0.069 |

Conclusions: The A1BG fragment was absent in patients with SSNS or normal subjects, and therefore is potentially useful as a non-invasive biomarker for prediction of FSGS with unfavorable renal function.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2236

**Enalapril Reduces Albuminuria Via Inhibiting Multiple Disease Pathways but Not Apoptosis in a Rat Model of Crescentic Glomerulonephritis** Mandy M. Smith, Eric C. Roux, Steven R. Ledbetter, Hong Ling. *Cardiovascular Metabolic Renal Science, Genzyme Corp, Framingham, MA.*

Crescent glomerulonephritis (CGN), a debilitating kidney disease, could lead to renal failure within weeks or months. Several disease pathways may be implicated, including TGF-β expression, infiltration of macrophages, and podocyte derangement. However, the potential therapeutic role along with the modulation of these pathogenic events by ACEi is not fully elucidated. The aim of this study was to examine the effects of enalapril in a rat model of anti-GBM CGN. The model was induced by one bolus injection of sheep anti-rat GBM serum, followed with enalapril (200 mg/L in drinking water) for 6 weeks. Following the dosing of anti-serum, rats developed albuminuria, glomerular crescents, sclerosis and tubulointerstitial damage. Enalapril significantly reduced albuminuria, with almost normalized renal function and histological appearance. Consistent to these observations, TGF-β gene expression was remarkably attenuated, along with reduction of PAI-1, collagen III genes, suggesting an inhibition of fibrogenesis in this model. Molecules involved in macrophage recruitment and proliferation, osteopontin and c-fms, were upregulated (2-20 fold), in untreated rats. Treatment with enalapril partially abrogated the up-regulation of OPN and c-fms mRNA, which was associated with a significant reduction of serum MCP-1 level and inhibition of macrophage accumulation within the kidney. Moreover, distressed podocytes, a key element for the integrity of glomerular filtration barrier, was rescued by enalapril, as evidenced by restored expression of nephrin and podocin, accompanied with reduced urinary nephrin excretion (344 vs.102 ug/mg Cr). Apoptosis related proteins, Bcl-xl, Apaf-1 and c-caspase 3, however were not altered nor affected by enalapril. Our results indicate an activation of multiple disease pathways which contributed to disease progression was retarded by enalapril. The study sheds light on the mechanisms of action of ACEi and set a model system for discovery of novel therapeutics to CGN and other chronic kidney diseases.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2237

**Is Decreased Renal ACE2 Expression Specific for Diabetic Nephropathy?** Sonoo Mizuiri,[1] Hiromichi Hemmi,[2] Moriatsu Miyagi,[1] Michitsune Arita,[2] Atsuhiko Mutou,[1] Yoshinari Hattori,[1] Yasunori Suzuki,[1] Yoshihide Tanaka,[1] Yasushi Ohashi,[1] Ken Sakai,[1] Kazutoshi Shibuya,[3] Atsushi Aikawa.[1] *[1]Nephrology, Toho University School of Medicine, Tokyo, Japan; [2]Molecular Biology, Toho University School of Medicine, Tokyo, Japan; [3]Surgical Pathology, Toho University School of Medicine, Tokyo, Japan.*

Angiotensin-converting enzyme 2 (ACE2) generates angiotensin 1-7, which may protect the kidney by attenuating the effects of angiotensin II. Decreased renal ACE2 expression in diabetic nephropathy is reported. This is possible that this may occur other renal diseases. Renal ACE and ACE2 expression was assessed in 13 patients with primary membranous nephropathy (MN), 17 patients with minimal change nephrotic syndrome (MCNS) and 20 healthy kidney transplant donors (controls) by immunohistochemistry and in situ hybridization. The percentage and intensity of labeled area were evaluated using a computer-based image analyzer. Blood pressure, proteinuira, serum albumin, eGFR at biopsy was also evaluated. There were no significant differences in blood pressure and eGFR across groups. Proteinuira (g/day) was higher in MCNS [6.2 (4.8-10.9)] than MN [3.7 (2.8-7.1)], but the difference was not significant. There were no differences in renal ACE and ACE2 protein expression in MCNS compared with controls. There were significantly decreased tubulointerstitial ACE2 protein [ 28.7 (22.5-32.1) vs. 47.5 (41.5-53.6), P<0.001] and glomerular ACE2 protein [0.9 (0.2-2.0) VS. 6.9 (1.8-12.0), P<0.01] and increased tubulointerstitial ACE protein [36.8 (31.9-43.9) vs. 21.2 (14.6-31.7), p<0.05] expression (density/pixel ) in MN compared with controls but no difference in glomerular ACE protein expression. The differences in ACE and ACE2 mRNA expression were not statistically significant across the 3 groups. None of clinical parameters significantly correlated with renal ACE, ACE2 expression in all subjects (n=50). However, significant correlation between tubulointerstitial ACE2 protein and eGFR (r=0.7, p<0.05) was observed in MN.

**Conclusion:** Membranous nephropathy is associated with decreased renal ACE2 expression and decreased renal ACE2 expression may be a common feature in progressive kidney disease.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2238

**Non-Diabetic Renal Disease in Diabetic Patients: A Learning Experience from over 3,500 Renal Biopsies with Diabetic Glomerulosclerosis** LaTonya J. Hickson,[1] Ziad El-Zoghby,[1] Sanjeev Sethi,[2] Mary E. Fidler,[2] Samih H. Nasr,[2] Lynn D. Cornell.[2] *[1]Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [2]Anatomic Pathology, Mayo Clinic, Rochester, MN.*

The frequency of renal diseases other than diabetic nephropathy has been estimated to be 12-81% in diabetics based on limited data or small sample size.

Aim: To determine the prevalence of non-diabetic (non-DM) renal disease in biopsies with diabetic changes

Methods: Our renal biopsy database was queried for "diabetic glomerulosclerosis" (DGS) from July 1994 to February 2010. Each report was reviewed for non-DM renal disease and DM findings. Arteriosclerosis/arteriolar hyalinosis, secondary FSGS, and interstitial fibrosis/tubular atrophy were considered part of DM.

Results: Of ~57,400 native biopsies, 3575 (6.2%) had DGS. Among these, 92.3% had diffuse and/or nodular DGS while 7.7% had early, mild changes. Non-DM renal disease was present in 972 biopsies (**27.2% of total**). Tubulointerstitial was more common than glomerular disease (**18.7% vs 8%**). Tubulointerstitial findings consisted of acute tubular necrosis (60%), interstitial nephritis (36%), and other diseases (2.7%) including light chain cast nephropathy (0.7%) and pyelonephritis (0.6%). 18 cases (0.5%) had atheroembolic disease. Non-DM glomerular disease was present in 286 (**8% of total**) including: IgA (21.7%), pauci-immune crescentic glomerulonephritis (16.8%), membranous (16.4%), post-infectious (16.4%), membranoproliferative (7.7%), thrombotic microangiopathy (4.5%) and other diseases (16.4%). The "other" 49 cases were other immune complex(11), primary FSGS with collapse(7), fibrillary(6), lupus(6), amyloid(5), anti-GBM(5), minimal change(5), light chain deposition(2), type III collagen(1), and dense deposit disease(1). One-fourth (24.5%) of glomerular diseases had concurrent tubulointerstitial disease. The majority of non-DM glomerular diseases were found in those with advanced DGS compared to earlier diabetic changes (87% vs 13%).

Conclusion: Non-DM renal disease is not infrequent in biopsies with DGS. Even in advanced diabetic nephropathy, kidney biopsy is an important diagnostic tool which allows for appropriate intervention.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2239**

**Cytochrome P450 Polymorphisms Do Not Affect Response of Lupus Nephritis (LN) to Cyclophosphamide (CTX) Therapy** Brad H. Rovin, Johan Winoto, Haikady Nagaraja, Huijuan Song. *Ohio State University, Columbus, OH.*

The addition of CTX to corticosteroids significantly improves the prognosis of severe kidney involvement in systemic lupus erythematosus (SLE). However, not all patients respond to CTX. It has been suggested that genetic variations that reduce the metabolism of CTX reduce its effectiveness. CTX is metabolized and activated by the cytochrome P450 (CYP) system and in particular the CYP enzymes 2B6 and 2C19. Both CYP2B6 and CYP2C19 have variant alleles (designated CYP2B6*5 and CYP2C19*2) that attenuate or eliminate enzymatic activity. This investigation was done to determine the impact of CYP2B6*5 and CYP2C19*2 on the renal response in LN patients treated with CTX.

Patients with SLE (n=197), unclassified autoimmune disease (n=40), and healthy controls (n=202) were genotyped for the poor metabolizer alleles CYP2B6*5 and CYP2C19*2. The associations between these alleles and achievement of complete or partial response, development of end-stage renal disease, and time to remission were determined.

The frequencies of the variant alleles CYP2B6*5 and CYP2C19*2 were 6.9 % and 14.2% respectively. CYP2C19*2 genotypes were more frequent among African Americans than European Americans (33 vs 22%, P=0.018), and CYP2B6*5 genotypes were more frequent among European American SLE patients than healthy controls (20 vs 9%, P=0.043). Among LN patients treated with CTX (n=36), there were no differences between those with or without these genotypes in regard to the frequency of complete or partial remissions or the time to remission. Partial responders with a variant CYP allele used a higher cumulative CTX dose than wild-type patients (P=0.032), but unexpectedly variant complete responders tended to have a lower cumulative CTX dose than wild-type patients (P=0.067).

**Conclusion**: This single center retrospective analysis failed to show an association between the common variant alleles CYP2B6*5 and CYP2C19*2 and treatment outcomes in LN. This suggests that genotyping for these CYP450 variants will not be useful in individualizing treatment for severe LN. However, CYP2B6*5 may predispose to the development of SLE.

Disclosure of Financial Relationships: Scientific Advisor: Questcor, Centocor, Genetech, Osprey, Glaxo.

**SA-PO2240**

**Chronicity Index as a Prognostic Factor in the Outcome of Severe Lupus Nephritis** Elerson Costalonga, Lectícia Jorge, Victor Sato, Rui Toledo Barros, Viktoria Woronik. *Nephrology, Hospital das Clinicas, School of Medicine, University of Sao Paulo, Sao Paulo, Brazil.*

**Introduction:** Factors which predict renal outcomes in patients with severe lupus nephritis (LN) are poorly understood, as these patients are often excluded from treatment trials. **Aim:** To access clinical and kidney biopsy features which predict renal outcome in LN patients with eGFR < 30ml\min\1.73 m²(estimated by MDRD simplified formula) at biopsy time. **Methods:** Analyses of 311 patients with systemic lupus erithematosus submitted to kidney biopsy at our center from 1999 to 2007 were carried out. Eighty four patients with eGFR < 30ml\min\1.73 m² were selected. All patients were treated with intravenous cyclophosphamide and prednisone for at least 3 months. Multivariate logistic regression analyses were performed to access variables which were associated with an eGFR > 30ml\min\1.73 m² at one year follow up. **Results:** Baseline clinical and histological features are showed in table 1.

Baseline Features

| | |
|---|---|
| Age(yr) | 29(23-39) |
| Female | 83% |
| eGFR(ml\min\1.73 m²) | 17(11-23) |
| Proteinuria(g\day) | 3,7(1,8-6) |
| Hematuria | 78% |
| Hemoglobin(g\dL) | 8,5(7,5-10) |
| C3 (mg\dl) | 60(40-80) |
| Histological Features | |
| WHO LN Classes | |
| LN II | 5% |
| LN III+IV | 75% |
| LN V | 13% |
| LN VI | 7% |
| Crescents | 61% |
| Diffuse Interstitial Fibrosis | 33% |
| Activity Index | 4(3-6) |
| Chronicity Index | 5(2,8-7) |

Results expressed as median and IQR

At one year follow up, eGFR increased in an average rate of 12ml\min (CI 6.6 – 17.7ml\min) and 43% of subjects reached an eGFR < 15ml\min. Chronicity index (OR:0.62, CI 0.43-0.9; p<0.01) was independently associated to renal outcome after adjustment for initial eGFR(p=0.08), diffuse interstitial fibrosis(p=0.27) and proliferative LN(p=0.7). Based on the ROC curve (AUC 0.83, CI 0.73-0.91), the best chronicity index cutoff value to identify patients who improved renal function was 3 (sensibility 62%; specificity 90%; predictive positive value of 80%). **Conclusion:** Chronicity index can predict renal outcomes in patients with severe lupus nephritis and can be used as a tool to guide therapeutic decision in this group of patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2241**

**Elevated Double Stranded DNA Antibodies Are Associated with Future Diagnosis of Proliferative Lupus Nephritis** Jessica J. Lee, Kevin C. Abbott, Stephen W. Olson. *Department of Nephrology, Walter Reed Army Medical Center, Washington, DC.*

Arbuckle et al (*NEJM* 2003) found that 55% of systemic lupus erythematosus (SLE) cases had elevated double stranded DNA antibodies (dsDNA-Ab) prior to diagnosis. There have been no subsequent studies to further delineate a potential subpopulation of SLE that has a stronger association with elevated dsDNA-Ab. Elevated dsDNA-Ab are associated with lupus nephritis (LN) at diagnosis but are not present in all cases. We hypothesize that proliferative lupus nephritis (PLN) cases have elevated and rising dsDNA-Ab prior to diagnosis.

**Methods:** Twenty-three cases of biopsy proven PLN were identified using the Walter Reed Army Medical Center renal biopsy database. The Department of Defense Serum Repository (DoDSR) identified an age, sex, race, and age of serum matched SLE disease control for each study subject. Up to three chronologic serum samples for each study and disease control subject from prior to the date of biopsy diagnosis were sent from the DoDSR to Quest diagnostics® for measurement of plasma dsDNA-Ab levels. The Fisher's exact test was used for data analysis.

**Results:** A greater percentage of LN cases had elevated dsDNA-Ab levels (>10 IU/ml) prior to diagnosis compared to matched diseased (SLE) controls (78% vs. 5%; p<0.0001). dsDNA-Ab levels greater than 40 IU/ml were 100% specific for future LN (70% vs. 0%; p<0.0001). Subgroup comparisons of disease versus disease controls at less than 1 year (82% vs. 8%; p=0.0001), 1-4 years (53% vs. 0%; p=0.0008), and >4 years (33% vs. 0%; p=0.04) prior to biopsy diagnosis remained significant. Increasing dsDNA-Ab >1 IU/ml per year was 100% specific for future diagnosis of proliferative LN (70% vs. 0%; p<0.0001).

**Conclusion:** Elevated and rising dsDNA-Ab are strongly associated with the future diagnosis of PLN, but not the diagnosis of SLE without nephritis. PLN has the highest risk for end stage renal disease. Our novel data could provide a diagnostic and prognostic tool to identify SLE patients at high risk for PLN. This information could allow for more tailored follow up plans and potential earlier intervention for maximal preservation of renal function.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2242**

**Anti-C1q IgG Levels Are Unreliable as Biomarkers of Lupus Nephritis Flare** Joshua E. Bitter, Haikady Nagaraja, Udayan Y. Bhatt, Chack-Yung Yu, Brad H. Rovin, Lee A. Hebert, Daniel J. Birmingham. *Ohio State University College of Medicine, Columbus, OH.*

The recognition of biomarkers that reliably identify, or more importantly forecast, a lupus nephritis (LN) flare would greatly improve LN management. The presence of anti-C1q is strongly associated with active LN, which has led to the belief that circulating anti-C1q IgG levels can also serve as a valid LN flare biomarker. This study tested this premise. Anti-C1q IgG levels were measured by standardized ELISAs in 112 SLE patients (72 with LN) enrolled in the Ohio SLE Study (OSS), a longitudinal study of active SLE patients tested at regular bimonthly visits (mean of 44 months), and 40 age/race matched unrelated controls. The OSS samples selected were at the patients' first LN flare (N=37), or at baseline if they never experienced a LN flare. Patients who were anti-C1q-positive and who experienced LN flares with available plasma samples at 8, 6, 4, and 2 months before (-8, -6, -4, -2) and at the time of LN flare (together termed a flare interval) were further tested at these times. For the cross-sectional analysis, anti-C1q IgG was present in 51% of the OSS patients vs. 5% of the controls (P < 0.001), in 62% of the LN patients vs. 30% of the nonrenal SLE patients (P = 0.002), and in 73% of the LN patients with at least 1 LN flare vs. 51% of LN patients with no LN flare during the entire OSS period (P = 0.088). For the longitudinal analysis, 24 LN flare intervals were identified from the anti-C1q positive LN patients. Though there was a trend for an increase in mean levels at -2 and at flare (compared to -8, -6, and -4), it did not reach significance (P = 0.159 by repeated measures ANOVA of least squares means). Furthermore, analysis of each of the 24 individual flare intervals revealed that anti-C1q IgG levels increased in only 6/24 at -2, and only 9/24 at flare. In conclusion, these data show a significant association between the presence of anti-C1q IgG and SLE, and specifically with LN, in agreement with previous reports. However, changes in anti-C1q levels over time in a given patient are neither reliable forecasters nor markers of LN flare.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2243**

**Rituximab for Severe Lupus Nephritis: A Systematic Review and Meta-Analysis** Kiran Pokhrel, Gautham Viswanathan, Smita Padala. *Nephrology Division, Tufts Medical Centre, Boston, MA.*

Current treatment options for severe lupus nephritis (LN) are limited by high failure rates and adverse effects. Rituximab, an anti-CD20 monoclonal antibody has recently been used in patients with LN. Although the LUNAR trial evaluating Rituximab as an initial induction agent in LN did not show any benefit, evidence regarding the role of Rituximab in refractory LN (persistence of active LN despite immunosuppressive therapy) is lacking. We systematically reviewed the available evidence of Rituximab in refractory LN and performed a meta-analysis.

**Methods** MEDLINE, Pubmed and Cochrane library were searched for studies analyzing the efficacy of Rituximab in LN. Data on renal response was extracted. Results were expressed as weighted mean difference (WMD) for continuous outcomes and as

proportions for categorical outcomes with 95% confidence intervals (CI) using random effects model.

**Results** Nine studies reported the effect of Rituximab in 189 patients with LN, 41 of whom had refractory LN. The mean age of patients with refractory LN was 28yrs and median duration of LN was 3yrs. Mean cumulative cyclophosphamide dose before Rituximab therapy was 6.8g and 80% had class IV LN. Mean reduction in proteinuria was 2.02 g/24h (95% CI 2.96-1.08). The table shows the different renal responses to Rituximab. Serious adverse events including infection occurred in 8 patients including 3 deaths.

**Conclusion** Although there was a significant reduction in proteinuria and an overall renal response of 57% in refractory LN, the analysis was limited by observational studies with few patients. Randomized trials are needed to better evaluate the efficacy of Rituximab in refractory LN.

Renal response to Rituximab in refractory LN

| | Proportion | 95% CI |
|---|---|---|
| Complete Response (CR) | 0.27 | 0.16, 0.43 |
| Partial Response (PR) | 0.30 | 0.18, 0.46 |
| Overall Response (CR+PR) | 0.57 | 0.41, 0.70 |
| | WMD | 95% CI |
| Decrease in proteinuria (g/24h) | 2.02 | 1.08, 2.96 |
| Increase in GFR (ml/min/1.73 $m^2$) | 2.46 | 0.93, 3.99 |

CR: Normal serum creatinine and albumin, inactive urinary sediment and 24h urinary protein <0.5g PR: >50% improvement in all renal parameters GFR: Glomerular filtration rate

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2244

**Efficacy of Low-Dose Tacrolimus (TAC) in Induction Therapy of Lupus Nephritis (LN)** Satoshi Takahashi, Keiju Hiromura, Hiroko Hamatani, Noriyuki Sakurai, Hidekazu Ikeuchi, Akito Maeshima, Takashi Kuroiwa, Yoshihisa Nojima. *Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.*

A previous pilot study showed that TAC was an effective option for induction treatment of diffuse proliferative lupus nephritis (Mok-C, Kidney Int 68:813, 2005). Recently, a placebo-controlled double-blind study demonstrated that TAC was effective in patients with LN who had moderate and persistent disease activity despite of steroid therapy (Miyasaka-N, Mod Rheumatol 19:606, 2009). Based on this study, TAC was approved for LN in Japan. However, the approved maximal dose is 3 mg/day that is almost half of the dose used in the Mok's study. In the current study, we retrospectively evaluated the efficacy of low-dose TAC for induction therapy of active LN. Thirteen adult patients (2 male and 11 female) who were treated with TAC for induction therapy in our department since Mar-07 to Mar-09 were examined. Eight patients were treated for a flare-up of LN and 6 of them had a history of receiving other immunosuppressants. Mean serum creatinine level and urinary protein/creatinine ratio before treatment was 0.7±0.2 mg/dl and 2.5±2.1 g/gCr, respectively. Twelve patients received or had received renal biopsy: 8 with class IV, 2 with class III+V, 1 with class IV+V and 1 with class V, according to ISN/RPS classification. The mean initial dose of prednisone and TAC was 34.6±14.5 and 2.7±0.6 mg/day (0.69±0.32 and 0.05±0.01 mg/kg/day), respectively. All patients reached complete remission (CR) (mean intervals, 7.1±7.1 months), except for 2 patients who discontinued TAC (1 for hypertension and 1 for worsening SLE). Two patients experienced a flare-up after achieving CR. The mean TAC blood concentration which was measured 12 hrs after the last administration was lower in patients with a flare-up compared to those with sustained CR (1.5±1.5 vs 5.1±1.5 ng/ml, p=0.041). These data showed that low-dose TAC was also effective in induction therapy of active LN, although lower TAC blood concentration may be associated with a poor outcome.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2245

**The Association of Disease Activity and Metabolic Syndrome in Lupus Nephritis** Masako Kochi,[#1] Kentaro Kohagura,[#1] Yusuke Ohya,[#1] Kunitoshi Iseki.[#2] *[1]Cardiovascular Medicine and Nephrology and Neurology, University of the Ryukyus, Nishihara-cho, Okinawa, Japan; [2]Dialysis Unit, University of the Ryukyus, Nishihara-cho, Okinawa, Japan.*

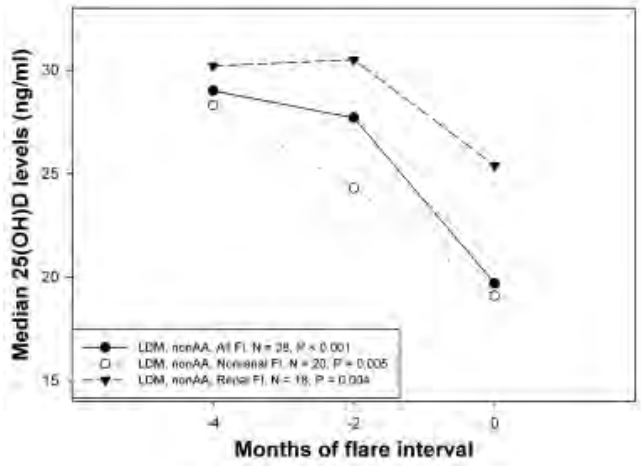
Patients with lupus have been reported to have higher prevalence of metabolic syndrome (Mets). Components of Mets such as hyper triglyceridemia (TG) were shown to be associated with the disease activity of lupus. However, it has not been clear whether Mets is associated with the activity and proteinuria in lupus nephritis. We examined 23 patients with lupus nephritis (91% female) and analyzed the association of Mets and parameter of disease activity of lupus such as anti-double-stranded DNA antibody (anti-dsDNA Ab) and urine protein (UP). The diagnosis of Mets was based on the Japanese definition including waist circumference. The mean (SD) was: age 36.3 (14.3) years, UP 3.2 g/gCr (range 0.1-9.6), and eGFR 79.7 (37) ml/min/1.73$m^2$. There were six patients (26%) with Mets. UP and anti-dsDNA Ab were significantly higher in the Mets group than those in non Mets group. We found the significant positive relationship between the number of components of Mets and UP ($\rho$=0.502, $P$=0.012) as well as between the parameter of disease activity of lupus such as anti-dsDNA Ab and UP. Moreover, there was significant positive correlation between the number of Mets and anti-dsDNA Ab. Finally, multiple logistic analysis adjusted for age and the dose of steroid used showed that the presence of Mets was significantly associated with higher risk for the presence of higher UP (above the highest quartile): the adjusted odds ratios (95% CI) were 176.9 (1.2 to 26557.2). In conclusion, the presence of Mets was associated with both anti-dsDNA Ab and UP. The intervention for Mets might be effective to prevent CKD progression of Lupus nephritis.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2246

**Evidence That Abnormally Large Seasonal Declines in Vitamin D Status Can Trigger SLE Flare in Non-African Americans** Daniel J. Birmingham,[1] Lee A. Hebert,[1] Huijuan Song,[1] Haikady Nagaraja,[1] William T. Noonan,[2] Brad H. Rovin,[1] Chack-Yung Yu.[1] *[1]Ohio State University College of Medicine, Columbus, OH; [2]Abbott Laboratories, Abbott Park, IL.*

Cross-sectional studies have suggested a role for vitamin D levels in SLE flare. However this has not been tested through serial vitamin D measurements leading up to flare. This study did so by measuring serum 25-OH vitamin D (25(OH)D) levels at 4 and 2 months before and at flare (-4, -2, 0: a flare interval) in 46 SLE patients (35 with nephritis) experiencing 82 flares (31 renal). The patients were from the Ohio SLE Study, a longitudinal study of SLE patients tested bimonthly. Our results showed no serial differences in 25(OH)D levels when considering all flare intervals. However, when analyzing only the flares that occurred during the 6 lowest daylight months (LDM, Oct-Mar), significant decreases in 25(OH)D were found, but only in non-African Americans (nonAA, 21 European Caucasians, 3 Asians) (38 flares, P < 0.001, Fig). This decrease (mean 17% from -4 to 0, P < 0.001) occurred in both renal (19%, N = 18, P = 0.004) and nonrenal (15%, N = 20, P = 0.005) flares.




To account for independent seasonal effects of LDM, 25(OH)D levels were measured in samples from 15 of these 24 patients who had a 4-month no-flare interval occurring in the same months as their flare interval and within 2 years of the flare. The mean decrease in 25(OH)D levels during the no-flare interval (11%) was not quite significant (P = 0.056), and was less than the mean decrease that occurred in the matching flare intervals (25%, P = 0.018 by paired t test). In conclusion, our data suggest that greater than usual seasonal decreases in 25(OH)D can trigger SLE flare in nonAA. AA typically have chronically low 25(OH)D levels with less seasonal variation. Thus, the effects of vitamin D on SLE flare may be muted in AA.

Disclosure of Financial Relationships: Research Funding: Research support from Abbott Laboratories.

## SA-PO2247

**A Combinatorial Urine Biomarker That Identifies Renal Interstitial Inflammation (INF) in Patients with Lupus Nephritis (LN)** Brad H. Rovin, Xiaolan Zhang, Huijuan Song, Haikady Nagaraja, Tibor Nadasdy. *Ohio State University, Columbus, OH.*

Kidney pathology is important for choosing therapy and monitoring disease progress in LN. Because it is not practical to biopsy an SLE patient at every renal flare, a non-invasive test that accurately reflects renal pathology is highly desirable. The objective of this study was to identify urine biomarkers that can be used as surrogates for specific pathologic kidney lesions. Here we report the results of biomarker discovery for INF in LN.

INF was semi-quantitatively graded as not present (0), mild (1, occupying less than 25% of the interstitium), or moderate-severe (2) in 45 diagnostic LN kidney biopsies. Three proteins known or postulated to be relevant to interstitial kidney injury were measured by ELISA in urine samples collected at biopsy and correlated to the level of INF. These proteins were MCP-1, L-FABP, and hepcidin. The ability of these markers to discriminate between levels of INF was tested by receiver operating characteristic (ROC) analysis.

Regression analysis demonstrated significantly increased expression of uMCP-1 (P<0.0004, $r^2$=0.26), uL-FABP (P=0.004, $r^2$=0.17), and uHepcidin (P=0.004, $r^2$=0.17) with severity of INF, however correlation coefficients were low. While all three markers were significantly higher in grade 2 INF than grade 0, only uMCP-1 was higher in grade 1 compared to grade 0. ROC curves were generated for each protein to determine if they could predict the level of INF. The area under the ROC curve (AUC) for uMCP-1 was 0.79, uL-FABP was 0.81, and uHepcidin was 0.78. As predictors these proteins misclassified INF in 47-62% of the biopsies. Using discriminant analysis, a linear equation combining all three proteins was derived that gave a combinatorial biomarker score. This score was used to plot an ROC curve to discriminate between grade [0+1] and grade 2 INF. It showed an AUC of 0.91, sensitivity of 90%, specificity of 80%, and misclassification rate of only 20%.

Conclusion: Combining several weak urine biomarkers can yield a more robust biomarker that can discriminate between different levels of severity of INF in LN without a biopsy.

Disclosure of Financial Relationships: Scientific Advisor: Questcor, Centocor, Genetech, Osprey, Glaxo.

**SA-PO2248**

**Biomarkers for Global and Renal Disease Activity in Juvenile Systemic Lupus Erythematosus (jSLE)** Michael R. Bennett, Rina Mina, Joshua David Pendl, Hermine Brunner, Prasad Devarajan. *Cincinnati Children's Hospital.*

Background: Preliminary data show that the Lupus Nephritis Renal Panel (LNRP), composed of transferring (Tf), ceruloplasmin(CP), acid-1-glycoprotein (AAG), neutrophil gelatinase-associated lipocalin (NGAL), prostaglandin-D synthethase (L-PDGS) and monocyte chemotactic protein 1 (MCP1), is associated with worsening lupus nephritis in jSLE. Cell-bound complement activated products (CB-CAP) which consist of erythrocyte, reticulocyte and platelet-bound complement components (E-C4d, E-C3d E-fBb, E-CR1, R-C4d, R-C3d, R-fBb and P-C4d) have been shown to reflect ongoing global disease activity in studies limited to adults with SLE.

Objectives: Evaluate the association of the LNRP and CB-CAP with global and renal disease activity in jSLE.

Methods: Clinical, laboratory and biomarker data were collected from 12 jSLE patients during initial and follow-up visits. Levels of LNRP standardized to urine creatinine and CB-CAP were measured by enzyme-linked immunosorbent assay and flow cytometry respectively. Physician-rated change in patient's disease course between visits (global/renal disease worsening: yes/no) served as the criterion standard.

Results: Using Wilcoxon Rank Sum Test and Spearman's correlation, statistically significant association was seen between levels of all urine biomarkers and the renal domains of 1-3 disease activity indices each (Renal SLAM, SLEDAI and BILAG), and the criterion standard. Levels of Tf, CP, L-PGDS and AAG were seen to be elevated three months prior to renal worsening with the change in the biomarker level associated with the change in the renal disease activity scores. No significant correlation between extra-renal disease activity and urine biomarkers was seen. Of the CB-CAP, only the E-CR1 and R-C4d were associated with current global/extra-renal disease activity. Traditional biomarkers for global and renal worsening correlated poorly with disease course or activity.

Conclusions: LNRP and CB-CAP are associated with renal and extra-renal disease activity in jSLE, respectively. The LNRP is predictive of worsening jSLE renal disease. Further studies assessing the predictive properties of combining these biomarkers are in progress.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2249**

**Clinical Impact of Serial Renal Biopsies in Patients with Lupus Nephritis** Smaragdi Marinaki,[1] Chrysanthi Skalioti,[1] Irene Synodinou,[1] Argyrios Georgalis,[1] Lydia Nakopoulou,[2] Charalambos Moutsopoulos,[3] John Boletis.[1] *[1]Nephrology and Transplantation Unit, Laiko Hospital, Athens, Greece; [2]Pathology Department, Medical School of Athens, Athens, Greece; [3]Pathophysiology Department, Medical School of Athens, Athens, Greece.*

OBJECTIVE:To evaluate the role of serial renal biopsies in the monitoring and therapeutic guiding in patients with lupus nephritis.

METHODS:We retrospectively analyzed the clinical course and the histological findings in patients with lupus nephritis, who underwent repeat renal biopsies between 2000 and 2009. Seventeen patients (16 women,1man) with a mean age of 24.8±5.4 years were evaluated. The mean SLE duration before renal involvement was 34±42 months.

Twenty eight repeat indication biopsies were performed in 17 patients with: relapse (n:10), ongoing activity/partial remission (n:11), aggravation (n:7).

Proliferative lupus nephritis (class III and IV-ISN/RPS2003) was the first diagnosis in 13/17(76.4%) patients. Eleven patients underwent 2 kidney biopsies (median time between 1st and 2nd biopsy 21 months), four patients had 3 biopsies (median time between the 2nd and the 3rd biopsy, 31 months); one patient had 4 and another one 5 renal biopsies.

There was no difference in patients' clinical characteristics at the time of the repeat biopsies. There was no difference in the mean activity scores whereas the mean chronicity score increased significantly (2.1±1.9and4±2.6) between 2nd and 3rd biopsy. Conversion to another class of nephritis occurred in 9/17(53%), and 2/4 patients(50%) between 1st to 2nd and 2nd to 3rd biopsy, respectively. Among these patients, 7/9(77%), and 1/2(50%) had conversion to a higher class.

In all the cases but one, therapy was intensified after biopsy, either by increasing the dose and/or the duration of therapy (8/27,29.6%) or by switching to other immunosuppressives (20/27,74%).

At the end of follow-up, 7/17 patients (25%) were in complete remission, 9/17 (32%) in partial remission and one patient was on haemodialysis.

In conclusion, given the poor clinicohistological correlation in lupus nephritis and the need for long term toxic immunosuppression, serial renal biopsies may have a central role in guiding therapeutic decisions..

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2250**

**Evaluation of MMF as Induction Agent in Pediatric SLE Subjects: An Indian Experience** Sandip K. Bhattacharya, Saubhik Sural. *Department of Nephrology, Peerless Hospital & B.K.Roy Research Centre, Kolkata, West Bengal, India.*

**Purpose :** MMF has been proved to be as good as Cyclophosphamide (Cyc) in adult population with lupus nephritis but very few studies have been conducted in pediatric patients. The purpose of this study is to evaluate the outcome of pediatric lupus patients treated with Enteric Coated Mycophenolate Sodium (ECMPS) as induction agent.

**Method :** It is a single centre retrospective cohort study. Cohorts were evaluated at presentation (including Renal Biopsy). They were followed up at 6 months, 12 months and 24 months with urine, renal function , serology and complement.

**Results :** The population consisted of 20 patients, mean age 12.8 ± 3.2 years . All were female of Indian origin. 14 patients had class IV and 6 patients had class III lesion. Mean albuminuria was 2.6± 1.2 gm/day and mean GFR was 48.3 ± 18.4 ml/min at presentation. All the patients received ECMPS 30 mg/kg/day in 2 divided dosages for 1 year followed by 1.5 mg/kg/day of Azathioprine (Aza) along with prednisolone 1 mg/kg to start with and slowly tapered to 5 mg/day and maintained thereafter.

17 patients reached complete remission (CR) by 8 months and 3 patients achieved partial remission (PR). Two patients from PR group and 1 patient from CR group had proteinuric relapse on Aza and did not respond to reinstitution of ECMPS. Pulse Cyc also failed in these patients.

Three patients had diarrhea and managed with dose reduction of ECMPS. Two patients with ECMPS and 1 patient with Aza had leucopenia. None required discontinuation of therapy. Serious infective episode was not observed in any individual. 17 patients are still in remission after 28 months from initiation of therapy.

**Conclusion :** MMF is a good therapeutic option as induction agent in pediatric SLE patients with minimal adverse reaction. Aza is effective as maintenance drug after MMF induction for 1 year. Long term follow up is required to prove its efficacy in preventing ESRD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2251**

**The Low-Dose Cyclosporine A Treatment Improves SLE Disease Activity of Japanese Patients with Diffuse Proliferative Lupus Nephritis** Yuji Kamijo, Makoto Higuchi. *Department of Nephrology Internal Medicine, Shinshu University School of Medicine, Matsumoto, Nagano, Japan.*

Cyclosporine A (CyA), a representative calcineurin inhibitor, would be a useful medicine for the treatment of lupus nephritis accompanied with severe proteinuria. In contrast to its strong effects against proteinuria, the beneficial effects of CyA against SLE disease activity had been obscure. To elucidate it, we investigated the effects of low-dose CyA treatment (2.5 mg/kg/day) in 11 Japanese patients (Male 1, Female 10), which having uncontrolled diffuse proliferative lupus nephritis (WHO type IV) with relatively high SLE disease activity. The high level of serum makers of SLE disease activity and disease activity index in all patients were significantly improved within 1 month, as well as amelioration of their proteinuric state. The required amounts of corticosteroid were decreased in these patients. These favourable effects were continued for 2 years without serious adverse effects. Kidney function was not changed in the patients having enough kidney function prior to CyA therapy (serum creatinin level < 1.1 mg/dl, and eGFR > 45 ml/min/1.73m$^2$), while two of three patients having obvious kidney impairment with chronic tubulointerstitial changes exhibited aggravation of kidney dysfunction. The current study suggests that low-dose CyA treatment could ameliorate the high SLE disease activity as well as improvement of proteinuria in Japanese patients. This treatment would be safe and useful for the SLE patients having enough kidney function.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2252**

**Treatment of Class IV Lupus Nephritis with Rituximab & Mycophenolate Mofetil (MMF) with No Oral Steroids Is Effective and Safe** Marie B. Condon, Megan Griffith, H. Terence Cook, Jeremy B. Levy, Liz Lightstone, Tom Cairns. *Imperial College Kidney and Transplant Institute.*

**Purpose:** To assess whether treatment with Rituximab & MMF without oral steroids is effective in severe (class IV) lupus nephritis.

**Methods:** Patients presenting with biopsy-proven lupus nephritis ISN class IV were treated with 2 doses of Rituximab (1g) + methyl prednisolone (500mg) 2 wks apart, & maintenance MMF (to MPA trough levels 1.2-2.4mg/l). They received NO oral steroids. Pts with cerebritis, requiring dialysis, and those already on oral steroids were excluded.

Complete remission (CR) was defined as stable creatinine, normal albumin and urine PCR <50mg/mmol OR histological resolution on repeat biopsy (= no active lesions on LM & no new deposits on EM).

Partial remission (PR) as stable creatinine, albumin 30g/l, nephrotic to non nephrotic proteinuria OR >50% fall of urine PCR if baseline non-nephrotic

**Results:**

21 patients (5 male, mean age 35+/-13.8 yrs) were treated with mean follow up of 35+/-13.8 months. Mean albumin was 22+/-6g/L, mean creatinine was 129+/-52umol/L.

16/21 (76%) have achieved CR, (15 clinical, 1 histological). Mean time to CR 11.8+/-9.3 months. A further 2 (9.5%) are in PR, mean time to PR 6+/-4.24 months, 1 of whom has received further rituximab, the other is poorly compliant with treatment.

3 patients (14.2%) have not achieved remission, 2 have been switched to cyclophosphamide, 1 has so far achieved remission.

1 patient relapsed at 36 months.

The treatment was generally well tolerated. 4 patients required hospital admission: 1 coronary stents, 1 cellulitis, 1 patient 3 admissions (2 with peripheral oedema and 1 with diarrhea). One had multiple admissions with peripheral vascular disease and died post surgery. In 1 patient (in CR) MMF was changed to tacrolimus due to leucopenia.

**Conclusions:** Exclusion of steroids from maintenance therapy by using rituximab and methylprednisolone induction followed by MMF alone in class IV lupus nephritis, leads to high rates of sustained complete remission and low toxicity.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2253

**Enteric-Coated Mycophenolate Sodium (EC-MPS) for the Treatment of Lupus Nephritis – MyLupus Study** David R. W. Jayne, Margit Zeher. *For MyLupus Study Group.*

**Purpose of the study**: The MyLupus study was designed to compare the non-inferior efficacy and safety of EC-MPS using two different oral prednisolone regimens (standard SD or reduced dose RD) for the induction of remission of a lupus nephritis (LN) flare.

**Methods**: A 24-week (W) exploratory, multi-center, open-label study was conducted in patients with a LN flare (ISN/RPS Class III or IV documented by renal biopsy within 24 months of study entry). All patients received EC-MPS 1440 mg/day for the first 2W, followed by 2160 mg/day for the remaining 22W, and methylprednisolone iv. (0.5 g/day) for the first 3 days. Patients were randomized to either SD or RD (starting dose of 1.0 mg/kg/day and 0.5 mg/kg/day for 2W, respectively, followed by pre-specified dose reduction). Primary end-point was the proportion of patients with complete response (urine protein/creatinine [P/C] ratio <0.5, urine sediment normalized, and serum creatinine <10% normal value) of LN after 24W. Partial response was defined as urine P/C ratio reduced by at least 50% from baseline and serum creatinine stable (<10% of baseline value) or improved.

**Results**: 81 patients were enrolled, 42 in SD and 39 in RD group. Baseline characteristics were comparable between groups. After 24 W, a similar proportion of patients achieved complete response in both groups (19% SD vs. 18% RD), although non-inferiority was not demonstrated. A higher proportion of patients achieved partial response in SD (48% SD vs. 33% RD). Index scores decreased in both groups from 4 to 24W (British Isles Lupus Assessment Group [BILAG] index: mean change from baseline -8.6 SD vs. -9.4 RD, Systemic Lupus Erythematosus Disease Activity Index [SLEDAI]: -10.4 SD vs. -9.4 RD). There was greater improvement in glomerular filtration rate (mL/min/1.73m$^2$) in RD (mean change from baseline 9.0 SD vs. 15.2 RD). There were 2 deaths, both in SD group. The incidence of serious adverse events was 16.7% (SD) vs. 10.3% (RD).

**Conclusions**: This exploratory study suggests that EC-MPS in combination with steroids is a viable therapy for LN. The overall clinical results indicate that RD of CS may offer benefits in terms of tolerability compared to SD of CS while maintaining efficacy.

Disclosure of Financial Relationships: Consultancy: Roche, GSKResearch Funding: Roche, ASPREVA.

## SA-PO2254

**Corticosteroid Therapy beyond 6 Months Does Not Decrease Relapses in ANCA Disease** JulieAnne G. McGregor, Susan L. Hogan, Yichun Hu, Caroline E. Jennette, Ronald J. Falk, Patrick H. Nachman. *Division of Nephrology and Hypertension, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Purpose: To assess the impact on patient outcomes and complications of corticosteroid therapy beyond 6 months (mo.) in an inception cohort of patients with biopsy-proven with Antineutrophil Cytoplasmic Antibody (ANCA) disease.

Methods: 147 patients were selected if diagnosed between 1/2000- 1/2009 and received induction therapy with corticosteroids and cyclophosphamide. Patients who presented with end stage kidney disease (ESKD), died within 6 mo., or had no response to treatment were excluded.

Patients were divided into 3 groups based on whether they were on 0, 5 or >5mg/day of prednisone at 6 mo. after therapy induction. Groups were analyzed for disease relapse, death, ESKD, new onset diabetes mellitus (DM) and infection. Comparisons between groups were done by Kruska-Wallis, Wilcoxon or Fisher Exact tests. Time to relapse was evaluated by Kaplan-Meier analysis with a log-rank test to compare groups.

Results: There were no statistically significant differences between the 3 groups in age, sex, ANCA specificity, disease category, frequency of risk factors of relapse, serum creatinine, length of therapy with cyclophosphamide (mean 8.7 mo.±5.9) or any maintenance immunosuppressant (mean 15.3 ± 23.1 mo.). The mean dose of prednisone in the >5mg group was 21.8±15.7mg/d.

Outcomes in ANCA Vasculitis by Corticosteroid Treatment Groups

| Steroid Dose at 6 months | 0 mg.(n= 69) | 5 mg/d.(n=17) | >5mg/d.(n=61) | P values |
|---|---|---|---|---|
| Relapse | 31(44.9%) | 4(23.5%) | 26(42.6%) | 0.269 |
| DM | 13(18.8%) | 6(35.3%) | 19(31.2%) | 0.165 |
| DM | 13(18.8%) | 25(32.1%)* | | 0.089 |
| Absolute Number of Infections | 2.20±1.49 | 1.94±1.09 | 2.43±1.55 | 0.533 |

*Prednisone 5mg. and >5mg. groups combined

Relapse-free survival, ESKD and death were not different between the 3 groups. The odds ratio of DM in patients receiving corticosteroids of any dose beyond 6 mo. was 2.03 (95%CI 0.94-4.38).

Conclusion: Corticosteroid therapy beyond 6 mo. does not improve outcomes or the frequency of relapse in patients with ANCA disease but is associated with a trend towards an increased risk of DM.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2255

**Microparticle Tissue Factor Activity Is Increased in PR3-ANCA Vasculitis Patients with Active Disease** Madelyn Burkart,[1] Sam L. Glover,[2] JulieAnne G. McGregor,[1] Elisabeth Berg,[1] Yichun Hu,[1] Susan L. Hogan,[1] Raj Kasthuri,[2] Ronald J. Falk,[1] Donna O. Bunch.[1] *[1]UNC Kidney Center, University of North Carolina; [2]McAllister Heart Institute, University of North Carolina, Chapel Hill, NC.*

Microparticles are small, membrane-bound vesicles shed into circulation in response to cellular activation or apoptosis. Microparticles can participate in many biological processes including inflammation and vascular reactivity and are known to contain tissue factor activity and participate in thrombosis. We studied the role of microparticles as a biomarker of disease activity in patients with ANtineutrophil Cytoplasmic Autoantibodies (ANCA) disease. Platelet free plasma was obtained from whole blood of 25 healthy controls and 84 ANCA disease patients (36 MPO and 48 PR3) during active disease (BVAS >1) and remission (BVAS ≤1). Microparticles were isolated and analyzed by flow cytometry according to methods established in 2009 by the Vascular Biology Scientific Subcommittee of the International Society on Thrombosis and Haemostasis. To capture microparticles not identified by Annexin V only, staining for HLA CI, a protein on all nucleated cells, was done. Cell of origin was determined with antibodies to endothelial, platelet, neutrophil, and red blood cell antigens. Trucount tube beads were utilized to enumerate microparticles. In our patient cohort, we detected no differences in the number of total, endothelial-derived, platelet-derived, red blood cell-derived, or neutrophil-derived microparticles of any subclass between patients classified as active or in remission. However, microparticles from PR3-ANCA patients with active disease had statistically higher tissue factor activity (0.097 ± 0.113 pg/ml) compared to healthy controls (0.008 ± 0.018 pg/ml; P=0.001). Thrombotic and bleeding events are common in PR3-ANCA patients with active disease. It will be important to correlate tissue factor activity temporally with clotting and bleeding events and the presence of anti-plasminogen antibodies. Microparticle tissue factor activity is a new marker of disease activity and may be more useful than microparticle number as a measure of disease activity in patients with ANCA vasculitis.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2256

**Protocolised Versus Non-Protocolised Rituximab Treatment for Refractory ANCA-Associated Vasculitis** Rachel B. Jones, Rona M. Smith, Mary-Jane C. Guerry, Fausta Catapano, Afzal N. Chaudhry, David R. W. Jayne. *Vasculitis and Lupus Clinic, Addenbrookes Hospital, Cambridge, United Kingdom.*

Rituximab is effective new therapy for refractory ANCA-associated vasculitis (AAV). However, the majority of patients relapse after rituximab, needing further treatment courses. Relapses occur from six months onwards and are associated with organ damage and high corticosteroid exposure. Relapse avoidance is desirable; however biomarkers that accurately predict relapse are not available.

We performed a single centre cohort study comparing six monthly, protocolised rituximab re-treatment and non-protocolised rituximab re-treatment according to clinical need for refractory AAV.

72 patients received a protocolised rituximab regimen; 1g x2 followed by 1g x1 every 6 months for 2 years (5g total) with early immunosuppression and corticosteroid withdrawal. 34 received non-protcolised rituximab; either 1g x2 or 375mg/m$^2$ x4 only repeated if relapse occurred.

Overall 75% patients had Wegener's granulomatosis. At first rituximab median disease duration was 55 months; prior cyclophosphamide exposure was 14g. Rituximab indication was relapsing disease in 82% of protocol and 83% of non-protocol patients; the remainder had grumbling disease whilst receiving continuous high dose corticosteroids or immunosuppression.

Median follow-up was 31 (4-56) months, protocol patients vs 22 (6-84) months, non-protocol patients. Response to rituximab occurred in 70/72 (97%) protocol patients (93% full remission, 4% partial), and 33/34 (97%) non-protocol patients (82% full remission, 15% partial). In protocol patients only 4/72 (6%) were still receiving immunosuppression at 6 months and by 24 months 26% had withdrawn from prednisolone (4.75mg/day median). At 2 years relapse had occurred in 16/72 (22%) protocol patients vs 24/34 (71%) non-protocol patients and by the end of follow-up 21/72 (29%) protocol patients, 26/34 (76%) non-protocol patients (p<0.01). Serious infections occurred in 10/72 (31%) protocol patients and 6/34 (26%) non-protocol patients.

Six monthly protocolised rituximab re-treatment is effective for relapse prevention, allows immunosuppression withdrawal and appears safe in refractory AAV.

Disclosure of Financial Relationships: Honoraria: Lecture fees from Roche.

## SA-PO2257

**Renal Survival in PR-3 ANCA and MPO-ANCA Associated Systemic Vasculitis during Long-Term Follow Up** Anoek A. E. de Joode, Johannes S. Sanders, Coen A. Stegeman. *Nephrology, UMCG, Groningen, Netherlands.*

**Purpose**: We evaluated renal survival in a large cohort of patients with PR3- ANCA- and MPO-ANCA-associated vascilitis (AAV).

**Methods**: All patients diagnosed with AAV in our hospital from january 1990 until december 2009 were evaluated from first contact until last visit or death. Primary end-point was ESRD defined as in need for renal replacement therapy (RRT), renal transplantation or death due to renal failure.

**Results**: We evaluated 235 patients: 178 were PR3-ANCA and 57 MPO-ANCA positive. They were all treated with cyclophosphamide and prednisolone, from 1996 onwards patients were switched to azathioprine after induction of remission. Mean follow-up was

7,5 years in the PR3- and 7 years in the MPO-group. Renal involvement at diagnosis was present in 137 of 178 (77%) patients in the PR3-group and in 48 of 57 (84%) patients in the MPO-group.In the PR3-group 25 (14%) patients needed RRT at diagnosis. Of these 25 patients, 5 (20%) died within 3 months and 5 (20%) had to continue RRT. Renal function recovered within three months in 15 (60%) patients, in 4 of these 15 RRT had to be restarted later. Additionally 8 other patients developed ESRD, all but one related to renal relapses of disease activity. Overall, 17 (10%) patients with PR3-AAV developed ESRD during long-term follow up. At 5 and 10 years after diagnosis, 91% and 89% of surviving patients were independent of RRT. In the MPO-group, 16 (28%) patients needed RRT at diagnosis (p=0,043 vs PR3-group). Of these 16 patients, 6 (38%) died, and 5 (31%) had to continue RRT. Renal function recovered in 5 (31%) patients (p=0.11 vs PR-3 group), in 1 of these RRT had to be restarted later. Another 2 patients developed ESRD, without relation to renal relapse. Overall 8 (14%) patients with MPO-AAV reached ESRD during long-term follow-up. At 5 and 10 years after diagnosis 86% of surviving patients were independent of RRT.

**Conclusion**: In this single center retrospective study we found that patients with MPO-AAV were significantly more often in need of RRT than patients with PR3-AAV at diagnosis and were less likely to recover renal function. Patients who did not need RRT at diagnosis did rarely develop ESRD during follow-up.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2258

**Alveolar Haemorrhage in ANCA Associated Vasculitis: A Retrospective Analysis from the European Vasculitis Study Group** Alina L. Casian,[1] Annelies Evaline Berden,[2] David R. W. Jayne.[1] *[1]Addenbrooke's Hospital; [2]Leiden Medical Centre.*

Alveolar haemorrhage (AH) is a pauci-immune capillaritis and potential life-threatening feature of ANCA associated vasculitis (AAV): previous studies report 50% mortality in severe presentations.

**Aims:** To characterise the prevalence and outcomes (end-stage renal failure and mortality) of AH further. We undertook a retrospective analysis in 387 patients with AAV from 3 RCTs of the European Vasculitis Study Group: NORAM (non-renal vasculitis), CYCAZAREM (creatinine < 500 micromol/l) and MEPEX (creatinine >500 micromol/l).

**Methods:** Data was retrieved from the EUVAS database and analysed by SPSS and Access. AH was defined as haemoptysis and/or pulmonary infiltrates scored by the Birmingham Vasculitis Activity Score. No patients with severe, ventilator dependent AH were included in these trials.

Re**sults:** 93/387 (24%) AAV patients had AH at presentation. Haemoptysis was present in 16%, whilst the remainder had isolated pulmonary infiltrates. AH developed subsequently in a further 4%. Renal involvement accompanied 78/93 (84%) of those presenting with AH and 160/294 (54%) of those without AH (p=0.0001). Mean presenting creatinine in those with and without AH was 385 micromol/l and 387 micromol/l respectively (p>0.005). 19/93 (20%) patients presenting with AH developed ESRF vs 49/294 (17%) who did not present with AH (p=0.44).

Of 161 with PR3-ANCA, 52 (32%) had AH vs 41/131 with MPO-ANCA and AH (31%) (p=0.9); 4/22 (18%) ANCA negative patients had AH (p=0.6).

Mortality at 1 year was 15% in the AH group vs 11% in patients without AH (p=0.27). 12 of the 24 (50%) MEPEX AH patients who died were treated with plasma exchange and 12 (50%) with methylprednisolone. Causes of death were sepsis in 53% and respiratory failure in 27%.

**Conclusions:** Alveolar haemorrhage was present in 24% of AAV patients at presentation and developed subsequently in a further 4%. The frequency of AH was not influenced by ANCA subtype. AH was associated with the presence but not the severity of renal vasculitis, consistent with the 2 manifestations having a common pathogenesis. AH was not associated with a higher mortality at 1 year, which may reflect the exclusion of severe AH.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2259

**Low Cyclophosphamide (CYC) Dose and Plasma Exchange (PLEX) a Less Toxic Induction Treatment of ANCA Associated Vasculitis (AAV)** Wladimir M. Szpirt, Elizabeth Krarup, Martin Egfjord. *Nephrology P, Rigshospitalet, Copenhagen, Denmark.*

The AAV treatment related adverse events are the greatest threat influencing the induction treatment outcome more than active vasculitis. The use of PLEX in AAV is not commonly accepted in patients (pts.) with plasma creatinine below 500 µmol/L (<500) despite growing evidence of the involvement of ANCA in the pathogenesis of vasculitis. We combined low dose of CYC and PLEX in order to minimize the infection/sepsis rate mostly due to over-immunosuppression. A prospective cohort study of all AAV pts. referred to our centre between 2000 and 2010 was performed. All pts. had AAV based on positive ANCA titre and a compatible clinical syndrome, whereas PLEX use was determined on active lesions on renal biopsy and ANCA titres. Standard therapy for all pts. consisted of prednisolone 1 mg/kg/day and a low dose of oral CYC (100 mg/day in pts. <65 years and 50 mg/day in pts. >65). All pts. received PCP prophylaxis. Azathioprine or Mycophenolate Mofetil was given for maintenance of remission after 4 months. 128 pts. were admitted and 116 followed for a mean of 3 years (range 0.5-9,0; 391 patient years). 58% were male, 76% were PR3-ANCA and 24% were MPO-ANCA positive. 44% had an initial creatinine >500. 39% were aged >65 and 61% <65 years. 103 pts. received PLEX (mean 7 (range 5-11)). Only 3 pts (2,4%) died during CYC treatment 1 sepsis, 1 AMI, 1 lung haemorrhage, all on HD. Totally 9 pts. (7,0%) died initially within 12 months of active vasculitis (5 pts. from >500) and 9 more pts. died within 7 years without signs of active vasculitis. (Total mortality of 14%). 17 pts (13%) had infection within the first 4 months, 8 (47%) being leucopenic; 5>500. UTI (27%), pneumonia (18%) predo-minated, whereas 1 died of septicaemia - 2 (9%). 36 relapses occurred during the 9 years of follow up, 12 during the first year after induction, 14 (11%) developed ESRD. Pts. aged <65, <500 had significantly better dialysis free survival. Thus, the use of induction therapy with low dose CYC and PLEX resulted in a high remission rate with good preservation of renal function and low rate of complications providing evidence of possible sparing effect of PLEX.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2260

**Severe Lymphopenia Is Frequent and Predictive of Infectious Complications in ANCA Vasculitis** Remi Goupil,[1] Annie-Claire Nadeau-Fredette,[1] Stephan Troyanov,[1] Soumeya Brachemi.[2] *[1]Medicine, Hôpital du Sacré-Coeur de Montréal, Montréal, QC, Canada; [2]Medicine, CHUM, Montréal, QC, Canada.*

Infections can be severe and life-threatening consequences of immunosuppressive therapy of ANCA-associated vasculitis (AAV). Few tools help clinicians manage the intensity of treatment to minimize these events. We sought to determine risk factors of infectious complications.

We identified AAV vasculitis patients by reviewing all ELISA ANCA measurements since February 2004. Demographics, clinical and laboratory assessment at diagnosis and during follow-up, treatment and infectious complications were collected from medical records.

Of the 39 AAV patients identified, 36 received immunosuppressive therapy. The mean age was 56 ± 15 years, 44% were females. Twenty-one and 15 patients were c and p-ANCA positive, respectively, 3 displaying both antibodies. The Birmingham vasculitis activity score at onset (BVAS) was 7.1 ± 3.1 with an initial eGFR of 35 ± 31 mL/min/1.73m2. A third required dialysis. All patients received corticosteroids (24 with pulse methylprednisolone), 29 cyclophosphamide, 2 methotrexate and a third plasmapheresis. The median follow-up duration was 37 months. During that time, 25% (9) of patients died, 53% (19) had an infectious complication of which 11 were hospitalized and 5 died as a consequence. Sixteen percent experienced neutropenia, 80.6% lymphopenia (< 1,0x10(9)/L) and 36% severe lymphopenia (<0.3x10(9)/L). Predictors of infections were the need for dialysis, lymphopenia and severe lymphopenia. Predictors of infections leading to death correlated with the severity of the disease and included the need for dialysis, BVAS score at onset, pulmonary haemorrhage and use of plasmapheresis. Severe lymphopenia was also associated with death from infectious complications with an odds ratio of 9.8 (95% CI 1.0-99.9, p=0.05). Neutropenia was not predictive of this outcome.

In conclusion, lymphopenia, particularly severe lymphopenia, is strongly associated with infectious complications during the treatment of AAV. Monitoring therapy to minimize lymphopenia may reduce infectious side effects of these potent therapies.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2261

**A Multi-Centre Survey of Plasma Exchange Practices in ANCA Associated Vasculitis** Alina L. Casian,[1] Michael W. Walsh,[2] David R. W. Jayne.[1] *[1]Vasculitis, Addenbrooke's Hospital, Cambridge, United Kingdom; [2]Epidemiology and Biostatistics, McMaster University, Hamilton, Canada; [3]Dr.Walsh & Dr.Casian are joint first authors, .*

Background: The optimal prescription of plasma exchange (PLEX) for the treatment of ANCA associated vasculitis (AAV) is uncertain. We performed a multi-centre, international survey to assess practice patterns in the use of plasma exchange for AAV.

Methods: We assessed the provision of PLEX in 48 hospitals from Europe, North America, and Australia/New Zealand using an internet based survey. Centres were selected by contacting locally identified experts in AAV and PLEX. The survey assessed practice patterns in terms of PLEX modality, frequency, and replacement fluid use. We conducted a survey of indications for use of PLEX in a subgroup of 34 centres.

Results: All 34 centres surveyed for PLEX indications reported using PLEX routinely for AAV with nephritis: 6 centres (18%) would consider PLEX in patients with creatinine >200 micromol/l, 15 (44%) would PLEX if creatinine >250 micromol/l and 13 (38%) centres use PLEX for rapidly rising creatinine. 26/34 (76%) reported using PLEX routinely for alveolar hemorrhage.

25 of 48 (52%) centres used only centrifugation, 10 (21%) used used filtration only, 10 (21%) used a combination and 3(6%) did not comment. 5 (10%) of centres aim to provide <7 PLEX sessions, 19 (40%) 7 sessions, and 8 (17%) >7 sessions for AAV. 11 (23%) of centres modified the number of PLEX treatments according to disease activity but no centres modified PLEX according to ANCA levels; the remaining 5 (10%) centres did not comment. The volume of exchanges was fixed in 27 of 48 (56%) centres (most commonly 1 plasma volume) while 15 of 48 (31%) used body weight to guide volume. The commonest replacement fluid was albumin alone (36 centres; 75%) in patients not at risk of bleeding. In patients at risk of bleeding, 56% of centres used a combination of plasma and albumin, while in patients with active bleeding 48% of centres used plasma only.

Conclusions: The way in which patients with AAV receive PLEX varies significantly between centres. Further research is required to determine whether the method of PLEX prescription influences its efficacy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2262**

**PEXIVAS: Design of a Randomised Controlled Trial of Plasma Exchange and Glucocorticoid Dosing in Severe ANCA Associated Vasculitis** Alina L. Casian,[1] Michael W. Walsh,[2] Peter A. Merkel,[3] David R. W. Jayne.[1] *[1]Vasculitis, Addenbrooke's; [2]Biostatistics, McMaster University; [3]Vasculitis, Boston University.*

The outcomes of ANCA associated vasculitis(AAV) are frequently poor for patients presenting with severe renal disease or lung hemorrhage and drug-related toxicity contributes to mortality and morbidity. Plasma exchange(PLEX) removes potentially pathogenic ANCA and was shown by a meta-analysis of all PLEX trials in vasculitis to improve renal recovery in severe renal vasculitis. However it is unclear whether this benefit is sustained and PLEX reduces mortality or is effective in lung hemorrhage. The expense and potential complications of PLEX demand stronger evidence before recommending its routine use. Current glucocorticoid(GC) dosing contributes to adverse events, especially in those with uremia or respiratory failure. Recent optimisation of other immunosuppressives in AAV has re-focused attention on whether GC can be reduced without compromising efficacy.

**Primary Objectives: 1)** The effect of PLEX (in addition to immunosuppressives and GC) on death and end-stage renal disease (ESRD)**, 2)** To determine whether a reduced GC regimen is non-inferior to standard GC with respect to death and ESRD, and superior with respect to adverse events (especially infections)**.**

**Secondary Objectives:** the effects of the 2 interventions on disease activity, quality of life, cost-effectiveness, disease-related damage, vasculitis biomarkers.

**Design:** 500 Patients with new or relapsing severe AAV (recent eGFR< 50 mls/min or/ and pulmonary haemorrhage), >=15 years of age, from 97 centres in 15 countries will be recruited over 5 years and followed-up for a maximum of 7, minimum 2 years. PEXIVAS is an open RCT; randomisation is centralized into 4 groups with 125 in each: 1) PLEX + standard GC 2) PLEX+ reduced GC 3) no PLEX + standard GC 4) no PLEX + reduced GC. All patients will receive induction therapy with cyclophosphamide or rituximab and maintenance azathioprine.

**Conclusions:** PEXIVAS aims to clarify the role of PLEX in severe AAV and whether GC dosing can be safely reduced. The multi-national collaboration will promote best practice in vasculitis management

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2263**

**Outcomes of ANCA Associated Vasculitis (AAV) in Multidisciplinary Vasculitis Clinic & General Nephrology Clinics in North-West England** Nina Brown,[1] Ajay Prabhakar Dhaygude,[2] Donal J. O'Donoghue,[3] Philip A. Kalra,[3] Michael Venning,[1] Laurence Richard Solomon,[2] Paul Brenchley.[1] *[1]MRI; [2]Preston Hospital; [3]Hope Hospital.*

**Introduction and Aims:** The impact of specialist versus generalist care on outcomes of patients with AAV is uncertain. Evidence suggests improved outcomes for patients treated by a specialist. EULAR recommendations state "*patients with primary small and medium vessel vasculitis be managed in collaboration with, or at centres of expertise"*. In Northwest England AAV patient care is in general nephrology clinics (Neph OP, centres A, B) or in a dedicated multidisciplinary vasculitis clinic (Vasc OP, centre C) with input from nephrologist, rheumatologist, immunologist and neurologist. We compare outcomes (infection and relapse rates) in these clinics.

**Methods:** A retrospective study of immunosuppression, rate of relapse and infections for 133 patients attending vasculitis clinic in the year 2007- 2008 at centres A, B and C (infection defined as requiring hospital admission/IV antibiotics or herpes zoster; relapse defined as disease activity in a patient in remission requiring increased immunosuppression). We recruited >95% patients from centres A and C but 30% patients from centre B -missing AAV cohorts from some centre B-based Neph OPs

**Results:** (Cyp= cyclophosphamide).

| Centre | Pt nos. | F/U pt months | Mean cum Cyp dose (grams) | Median cum Cyp dose (grams) | Relapse/ 100 pt months | Infections/ 100 pt months | Malignancies |
|---|---|---|---|---|---|---|---|
| A | 59 | 2891 | 5.45 | 3.5 | 0.86 | 0.55 | 3 |
| B | 31 | 1581 | 6.6 | 4.0 | 1.01 | 0.44 | 0 |
| C | 43 | 3612 | 26.19 | 11.3 | 0.44 | 0.28 | 2 |

**Conclusions:** Patients treated in Vasc OP had higher cumulative dose of Cyp, lower relapse rates and fewer infective complications.

**Discussion:** Patients in Vasc OP had longer follow-up and higher median dose of Cyp, but a lower rate of adverse events in terms of infection rates and cancer. Despite lower ascertainment from the various Neph OP in Centre B, the outcome data are comparable to centre A. Within the limitations of retrospective study, we believe these are the first data to suggest better outcomes for patients attending Vasc OP compared with Neph OP.

With assistance from Shelly Harris

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2264**

**ANCA-Associated Glomerulonephritis in the Very Elderly** Andrew S. Bomback, Gerald B. Appel, Jai Radhakrishnan, Shayan Shirazian, Leal C. Herlitz, Michael B. Stokes, Vivette D. D'Agati, Glen S. Markowitz. *Columbia University College of Physicians & Surgeons.*

**BACKGROUND:** Antineutrophil cytoplasmic autoantibody (ANCA)-associated pauci-immune glomerulonephritis (GN) is the most common renal biopsy finding in very elderly patients biopsied for acute kidney injury, with a biopsy incidence of 33%. Appropriate treatment strategies in this age group currently are undefined because it is unclear whether the benefits of immunosuppression exceed the risks.

**METHODS:** This retrospective cohort study evaluated all cases of pauci-immune GN in individuals aged ≥ 80 years, diagnosed by kidney biopsy from 2001-2008. We obtained available patient data from time of diagnosis until death or last clinical encounter and used logistic regression and Cox proportional hazards models to evaluate outcomes of end stage renal disease (ESRD) and death.

**RESULTS:** Seventy-eight cases were identified, with a mean age of 83.3 years. p-ANCA and c-ANCA positivity were seen in 71.8% and 19.7% of patients, respectively. Follow-up data were available for 69 of 78 patients (88.5%), ranging from 1 week to 67 months. Patients treated with immunosuppression had a significantly lower incidence of ESRD 1 year after biopsy (36.2%) compared with untreated patients (72.7%; p=0.03). Only peak serum creatinine before biopsy and use of immunosuppressive therapy influenced progression to ESRD. Although differences in 1-year mortality rates between these groups were not statistically significant (46.9% vs. 63.6%; p=0.3), in multivariate models extending out to >2 years of follow-up, immunosuppressive therapy was associated with a lower risk for death (HR 0.33, 95% CI 0.11-0.97) and death or ESRD (HR 0.16, 95% CI 0.06-0.42). ROC curves suggested that this benefit could be most expected in patients presenting with a creatinine ≤4.5 mg/dl.

**CONCLUSION:** In very elderly patients with ANCA-associated pauci-immune GN, the benefit of immunosuppressive therapy is experienced early (within the first 6 months) in the form of reduced risk for ESRD and later (after 1 year) in the form of prolonged survival and, in particular, prolonged dialysis-free survival.

Disclosure of Financial Relationships: Consultancy: QuestcorResearch Funding: Questcor, Novartis, Alexion, Teva; Honoraria: Questcor, Novartis, Alexion.

**SA-PO2265**

**Podocalyxin Positive Glomerular Epithelial Cells in the Urine Correlate with a Positive Outcome in Glomerular Diseases** Janina Müller-Deile, Philipp Kümpers, Hermann G. Haller, Mario Schiffer. *Nephrology, Hannover Medical School, Hannover, Germany.*

**Background:** Parietal epithelial cells (PECs) and podocytes are the two epithelial cell types in the glomerulus. In contrast to podocytes, PECs have the ability to proliferate lifelong and they have the ability to transdifferentiate into other cell types. We previously published that excretion of podocalyxin-positive PECs in the urine correlates with disease activity in different glomerular diseases. Here we investigated whether urinary excretion of podocalyxin-positive cells might be a prognostic marker for proteinuria development and kidney function. **Methods:** We analyzed the excretion of podocalyxin-positive cells in the urine of patients with FSGS, MGN and MPGN. Follow up serum creatinine and total urine protein of these patients was analyzed ~ 3 years after urinary cell count. Differences in serum creatinine and urine protein between the two visits were expressed as delta-values. **Results:** FSGS- and MPGN-patients with excretion of podocalyxin-positive cells in the urine had a negative delta serum creatinine three years later (FSGS: ∅ -18µmol/l; MPGN: ∅ -18µmol/l). In contrast to that FSGS- and MPGN-patients without cell excretion showed a positive delta serum creatinine three years later (FSGS: ∅ +53µmol/l; MPGN: ∅ +63µmol/l). Interestingly in patients with MGN we detected a positive delta serum creatinine regardless of cell excretion. All patients with excretion of podocalyxin-positive cells in the urine had negative delta total urine protein three years later, reflecting a reduction in protein excretion between the two visits. FSGS and MPGN-patients showed a negative correlation between of podocalyxin-positive cells in the urine and delta serum creatinine. (Corr.-coefficient: FSGS: -0,4 ; MPGN:-0,5). Moreover all patient groups with podocalyxin-positive cells had lower delta total urine protein compared to patients with the same renal disease but no significant cell excretion. **Conclusion:** Our data indicate that patients excreting high amounts of podocalyxin-positive cells in their urine have a better outcome regarding kidney function compared to patients without excretion of podocalyxin-positive cells.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2266**

**Risk Factors for Hepatitis B Virus Associated Nephropathy** Jianfang Cai, Hang Li, Xuemei Li, Xue-Wang Li. *Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Science, Beijing, China.*

**Objective** To retrospectively evaluate the potential risk factors for hepatitis B virus associated nephropathy (HBV-AN) and its pathological types.

**Methods** 86 cases of HBV-AN and 160 controls with renal biopsy obtained during 2001 to 2009 were enrolled in this retrospective case-control study. HBV-AN was diagnosed as biopsy-proven glomerulonephropathy in chronic HBV carriers with other secondary glomerulonephropathy excluded and glomerular deposition of HBsAg or HBcAg. Chronic HBV carriers without glomerular deposition of HBsAg or HBcAg were recruited as controls. In pathology, HBV-AN was presented as membranous nephropathy (HBV-MN, 50cases), membranoproliferative glomerulonephrtitis (HBV-MPGN, 18 cases) and IgA nephropathy (HBV-IgAN, 18 cases). Upper limit of normal (ULN) of alanine amiotransferase (ALT) was defined as 19U/L in women and 30U/L in men. Step forward multivariate logistic regression was used.

**Results** In chronic HBV carriers, HBV-AN was associated with male (OR 1.85; 95% CI 0.96-3.56; p=0.065), liver cirrhosis(OR 2.59; 95% CI 0.92-7.28; p=0.072), HBV-DNA ≥$10^5$copies/µl (OR 2.17; 95% CI 1.10-4.28; p=0.025) and ALT ≥1.5*ULN (OR 1.91; 95% CI 1.07-3.42; p=0.030). When the patients with MN and MPGN, in whom glomerular HBeAg deposition is common, were excluded from the controls, the associations were strengthened and listed as follows, male (OR 2.78; 95% CI 1.16-4.49; p=0.017), liver cirrhosis(OR 4.23; 95% CI 1.20-14.95; p=0.025), HBV-DNA ≥$10^5$copies/µl (OR 2.74; 95% CI 1.14-5.55; p=0.005) and ALT ≥1.5*ULN (OR 2.14; 95% CI 1.14-4.03; p=0.018). In patients with

HBV-AN, compared with those with HBV-IgAN, HBV-MN was associated with male (OR 5.92; 95% CI 1.65-21.12; P=0.006), ALT ≥1.5*ULN (OR 3.18; 95% CI 0.90-11.25; P=0.073) and HBV-DNA ≥$10^5$copies/μl (OR 4.38; 95% CI 1.19-16.10; P=0.026), while HBV-MPGN was associated with male (OR 7.12; 95% CI 1.16-43.80; P=0.034) and HBV DNA ≥$10^5$ copies/μl (OR 10.71; 95% CI 1.09-105.50; P=0.042).

**Conclusions** In chronic HBV carriers, Male, liver cirrhosis, HBV-DNA ≥$10^5$ copies/μl and elevated ALTcopies/μl are associated with HBV-AN. In patients with HBV-AN, male and HBV-DNA ≥$10^5$ copies/μl are related with HBV-MN and HBV-MPGN.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2267

**Both Obesity and Low Glomerular Number in Biopsy Are Independent Risk Factors for the Development of Glomerular Hypertrophy** Nobuo Tsuboi, Yasunori Utsunomiya, Tetsuya Kawamura, Tatsuo Hosoya. *Division of Kidney and Hypertension, The Jikei University School of Medicine, Tokyo, Japan.*

Studies have suggested that glomerular hypertrophy represents one of the structural components of glomerular hyperfiltration, which is an established risk factor for the development of glomerulosclerosis and the loss of renal function. We have recently reported that a low glomerular density (GD, number of non-sclerotic glomeruli per renal cortical area) in renal biopsy is associated with an increased glomerular volume (Gv) and also a progression of IgA nephropathy (IgAN) (NDT2009, CJASN2010). To date, however, it has not been fully elucidated regarding by which factors the $G_v$ is defined, because clinically and/or histopathologically relevant factors associated with $G_v$ are multi-factorial and are often overlapping within each individual. To examine the essential determinants of $G_v$, the present study analyzed the relationship between the clinicopathological factors and the $G_v$ in biopsy specimens. We included proteinuric patients (n=64) without a histological diagnosis of primary or secondary immunological nephropathies, diabetic nephropathy, nephrotic syndrome and acute kidney injury. The Gv in biopsy specimens was calculated by measuring the glomerular area with a computed imaging analyzer. In univariate analyses, the $G_v$ correlated with mean arterial pressure (MAP, r=0.31) and body mass index (BMI, r=0.46), and inversely correlated with GD (r=-0.46). On the other hand, the $G_v$ did not show any correlation with age, eGFR or the degree of global glomerulosclerosis. In a multivariate analysis, only BMI (t=3.44) and GD (t=-3.14) were still independent factors that associated with Gv. Furthermore, almost the same results were obtained by analyzing a patient cohort of IgAN (n=124).

Conclusion: Our results suggest that both obesity and low GD independently contribute to the development of glomerular hypertrophy. Also, these results indicate that a mismatch between obesity-related increased metabolic demands and reduced nephron number, either acquired or later in life, may be an important determinant for the development of glomerular hypertrophy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2268

**Renal Involvement in Monoclonal Gammopathies** Ana Carina Ferreira, Fernanda Carvalho, Dulce Carvalho, Maria Joao Galvao, Helena Viana, Manuel A. Ferreira, Fernando Nolasco. *Nephrology, H Curry Cabral, Lx, Portugal.*

Monoclonal gammopathies (MG) are frequently associated with organ dysfunction. The aim of this retrospective study was to evaluate the histological findings of kidney biopsies (KB) and clinical data in patients (pts) with MG, with or without multiple myeloma (MM).

In the last 30 years, 181 KB were performed: 54.7% females, 95.8% caucasian, mean age 64.5±10 years. At the time of the KB, MG was known in 49%, and a history of MM was present in 24.5%. The MG were: IgG in 17 pts, IgA in 8, IgM in 3, IgD in 2, λ in 60%, k in 30%.

KB was performed for nephrotic syndrome (35.4%), acute kidney injury (22.7)%, rapidly progressive renal failure (15.5%), chronic kidney disease (9.9)%, sub nephrotic proteinuria (8.8%), nephritic syndrome (2.8%), and hematoproteinuria (1.1%).

The predominant lesions were AL amyloidosis (40.3%), myeloma kidney (38.1%), and light chain deposition disease (LCDD - 11%). Other histological features found: heavy chain deposition disease, chronic interstitial nephrities, IgA nephropathy, acute tubular necrosis, segmental and focal glomeruloesclerosis, chronic glomerulopathy, crescentic glomerulonephrities, AA amyloidosis, membranoproliferative glomerulonephritis, post infectious glomerulopathy, minimal change disease, diabetic nephropathy, malignant hypertension.

Clinical findings

| | AL amyloidosis (n=73) | Myeloma Kidney (n=69) | LCDD (n=20) |
|---|---|---|---|
| Scr (mg/dl) | 1.9±1.5 | 6.5±3.5 | 5.8±6.1 |
| Proteinuria (g/day) | 7.3±5.8 | 2.4±4.4 | 5.2±3.2 |
| Haemodialysis (%) | 6.3 | 90 | 56 |
| Known MM (%) | 12.3 | 39 | 25 |
| Known MG (%) | 27.4 | 62.3 | 40 |
| Presence of Myeloma kidney (%) | 5.5 | 100 | 50 |
| Main light chain (%) | λ in 79.5 | No predominance | No predominance |

In conclusion, MG are associated with a variety of renal disorders, not all related to monoclonal chain. The majority of pts submitted to KB didn't have MG previously identified and the majority of pts with myeloma kidney didn't have previous MM diagnosis. MG related renal disorders may be coupled. MGUS seems to be linked with renal disorders. Light chain is the most frequent chain related to these renal diseases. KB is the gold standard to establish diagnosis and ascertain therapeutic in pts with MG and renal involvement.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2269

**HIV-Associated Nephropathy in the Twenty First Century: Presentation, Outcome and Prognosis Factors** Naïke Bigé,[1] Fanny Lanternier,[2] Prochore Kamgang,[3] Eric Daugas,[4] Kaoutar Jidar,[5] Marie Noelle Peraldi,[6] Olivier Lortholary,[2] Laure-Helene Noel,[7] Guillaume Bollee.[1] *[1]Nephrology, Necker Hospital, APHP, Paris, France; [2]Infectious Diseases, Necker Hospital, APHP, Paris, France; [3]Nephrology, La Pitie Hospital, APHP, Paris, France; [4]Nephrology, Bichat Hospital, APHP, Paris, France; [5]Nephrology, Saint Louis Hospital, APHP, Paris, France; [6]Infectious Diseases, Bichat Hospital, APHP, Paris, France; [7]Pathology, Necker Hospital, APHP, Paris, France.*

HIV associated nephropathy (HIVAN) is the first cause of end stage renal disease (ESRD) among HIV-infected patients. The purpose of our study was to describe the presentation and outcome of HIVAN and to identify factors associated with progression to ESRD in the era of highly active antiretroviral therapy.

**Methods:**

We retrospectively studied 57 patients with biopsy-proven HIVAN diagnosed between 2000 and 2009 in four teaching hospitals in Paris, France. Clinical features at diagnosis and over follow-up were analyzed.

**Results:**

African ancestry (86.8%), severe renal dysfunction (median eGFR 20 ml/min/1.73m²), high grade proteinuria (median 4.1g/L), highly replicative HIV infection (median viral load 4.5 log) and severe immunodeficiency (median CD4 count 127/mm3) were typically present. Nevertheless, about quarter of patients displayed less typical presentation. Data on follow-up were available in 51 patients, of whom 30 (58.8%) 21 reached ESRD (median renal survival 40 months). Time from diagnosis of HIV infection to those of HIVAN <1 year ($p<0.05$) and percentage of sclerotic glomeruli at diagnosis ($p<0.05$) were significantly associated with progression to ESRD. Forty-eight patients (96.1%) received HAART during follow-up. Viral suppression was associated with better outcome, as the proportion of patients reaching ERSRD was 53.3% in those with undetectable viral load at last follow up compared to 90.5% in others ($p<0.05$).

**Conclusion**

HIVAN remains a severe complication of HIV infection, leading to ESRD in more than half of patients. Our study demonstrates the favourable impact of viral suppression on the course of HIVAN and highlights the crucial importance of early diagnosis.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2270

**Henoch-Schönlein Purpura in Adults: A Single Centre Review** Arvind Ponnusamy, Jyothi Kondlapudi, Krish S. Raman, Philip A. Kalra. *Renal, Salford Royal NHS Trust Foundation, Salford, United Kingdom.*

**Introduction:** Henoch-Schönlein purpura (HSP) is a leukocytoclastic small-vessel vasculitis involving small vessels with the deposition of immune complexes containing immunoglobulin A and neutrophil and eosinophilic infiltration. It is a multisystem characterized by the association of skin, joint, and gastrointestinal manifestations

**Method:** A retrospective analysis of 31 patients was undertaken between 2000 till 2008. We reviewed clinical symptoms, laboratory data, renal pathology and immunological data (Ig A/C3 ratio) at the time of presentation. These parameters were correlated to outcome (End Stage Renal Disease, Chronic Kidney disease Stage 3-5). We divided the group into progressers and non-progressers. Progressers were those with chronic kidney disease (Stage 3 – 5)at the last follow up.

**Results:** Average age onset [Mean±SD] was 50.6±22 and the median follow up period was 21 (range 1 to 91) months. Males represented 66% of the cohort. Clinical presentation included purpura (100%), proteinuria (>3gm) (26%) and acute kidney injury (AKI). One patient who presented with AKI requiring dialysis subsequently recovered their renal function. Baseline proteinuria was [Mean± SD] 3.1 gm±3.4 and the follow up proteinuria was 0.97±2.1. Mean creatinine at presentation was 1.3gm/dl±0.9. Crescents were found in 45% of patients that were biopsied. 8.6% died during the follow up. Mean Ig A levels were 3.98± 1.4. The mean Ig A /C3 ratio was 2.8±1.15.

| Mean | Progressers | Non-Progressers | p-value |
|---|---|---|---|
| Creatinine | 1.9+1.3mg/dl | 0.9+0.2mg/dl | 0.005* |
| Proteinuria | 3.8+ 4.9 | 2.7+ 2.9 | 0.270 |
| IgA C3 ratio | 2.76+1.3 | 2.8+1.2 | 0.82 |

**Baseline characteristics of HSP patients separated according to renal functional outcome category**

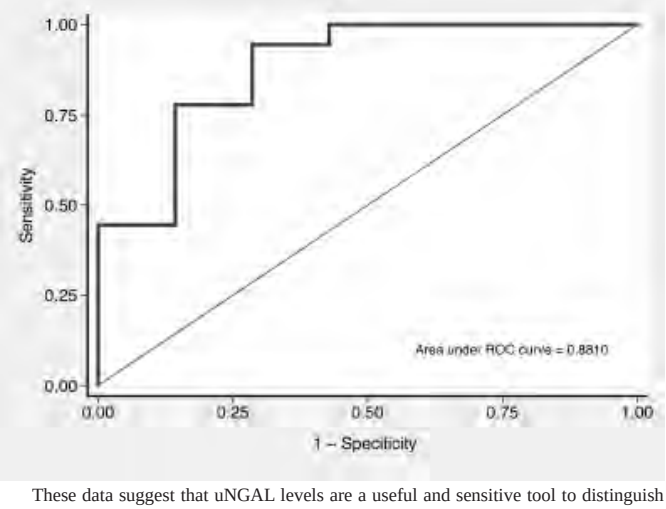
CONCLUSION: The presence of higher creatinine at presentation is an indicator of progression in HSP patients. Mean proteinuria in the progressers group was higher than the non-progresses although this was not statistically significant. None of our patients reached end stage renal disease however there was mortality rate of 8%. Mean IgA/C3 ratio does not appear to be a marker of progression.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2271**

**Urinary NGAL Is a Useful Clinical Biomarker of HIV-Associated Nephropathy** Sumit Mohan,[1] David Sola-Del Valle,[2] Jen-Tse Cheng,[1] Neal A. Paragas,[2] Meghan E. Sise,[2] Vivette D. D'Agati,[3] Jonathan M. Barasch.[2] *[1]Dept of Medicine, Columbia University & Harlem Hospital; [2]Dept of Medicine, Columbia University; [3]Dept of Pathology, Columbia University, New York, NY.*

HIV-associated nephropathy (HIVAN) is a progressive form of chronic kidney disease (CKD) characterized by collapsing focal segmental glomerulosclerosis and microcytic tubular dilatation that often leads to ESRD. Urinary Neutrophil Gelatinase Associated Lipocalin (uNGAL) is known to be expressed in AKI, but has also been observed in some progressive forms of CKD. We have previously reported upregulation of uNGAL and abundant NGAL mRNA expression in the microcystic tubules of HIVAN (JASN 20: 1687,2009). Here we examine the utility of uNGAL levels as a noninvasive biomarker for HIVAN by performing a comparative study of HIV-positive patients with diverse proteinuric glomerulopathies. Among 25 HIV-positive patients,, 18 (72%) had a diagnosis of HIVAN and 7 had other glomerulopathies (4 MPGN, 1 MGN, 1 amyloid and 1 malarial GN). The HIVAN and non-HIVAN glomerulopathy patients did not differ with respect to age (43.5 vs 44.8 yrs, p=0.6), ethnicity (100 vs 94% black, p=0.65), serum creatinine (sCr: 4.2 vs 2.8 mg/dL, p=0.11), estimated GFR (31.5 vs 46.6 mL/min, p=0.86), degree of proteinuria (9.4 vs 7.9 g/day, p=0.34) or prevalence of hypocomplementemia (6 vs 29%, p=0.18) while patients with HIVAN were less likely to have HCV coinfection (11 vs 57%, p=0.03). The HIVAN patients had higher uNGAL than the HIV patients with other glomerulopathies (387±338 vs. 94±101 µg/g uCr, p=0.02). A cutpoint of 121.5 µg uNGAL/g uCr demonstrated 94.4% sensitivity and 71.3% specificity for the diagnosis of HIVAN, with an area under the ROC curve of 0.88 (see figure).




These data suggest that uNGAL levels are a useful and sensitive tool to distinguish HIVAN from other proteinuric glomerulopathies in HIV-infected patients.

Disclosure of Financial Relationships: Scientific Advisor: Amgen.

**SA-PO2272**

**A Prospective Study of HIV-Associated Albuminuria: Interim Report** Karmini Sampath,[1] Colleen Hadigan,[2] Leon L. Lai,[3] Jeffrey B. Kopp.[1] *[1]NIDDK, National Institutes of Health, Bethesda, MD; [2]NIAID, National Institutes of Health, Bethesda, MD; [3]Washington Hospital Center, Washington, DC.*

HIV-associated microalbuminuria may be due to incipient HIV-associated nephropathy, metabolic syndrome and medication-associated tubular dysfunction. We are carrying out a prospective study to define the period prevalence of HIV-associated albuminuria, enrolling 250 subjects at the NIH Clinical Center and Washington Hospital Center. Inclusion criteria include HIV infection and age >18 years. Exclusion criteria include diabetes, serum creatinine >1.4 g/dL, urine protein/creatinine (P/C) >0.5 g/g, pregnancy and recent cancer or active opportunistic infection. In the baseline phase, we measured random urine albumin/creatinine (A/C) ratio 3 times over 6-9 months. Microalbuminuria was defined as a geometric mean A/C ratio of 25-355 mg/g in females and 17-250 mg/g in males. Macroproteinuria was defined as P/C >0.5 g/g. Tubular proteinuria was defined as the presence of 2 of the following: glycosuria, phosphaturia, or uricosuria. In the extension phase involving subjects with microalbuminuria, urine A/C was measured at 12, 18, 24 and 36 months after entry. 142 subjects have completed the baseline phase. Mean age was 44 years, with a range of 23 to 67; racial background included 69 European descent (17 Hispanic), 68 African descent, and 5 Asian descent. 18/146 (9%) had microalbuminuria; no subject had incident macroproteinuria. Of those with microalbuminuria, 7 had glomerular albuminuria, 6 had tubular albuminuria (3 on tenofovir) and 5 were not categorized. Among those with microalbuminuria, the mean urine A/C was 47 mg/g and the range was 17 to 146 mg/g. Among the 128 subjects without microalbuminuria, 113 (89%) had all three A/C values below the microalbuminuria threshold. Among the 18 subjects with microalbuminuria, 12 (69%) had all 3 values within the microalbuminuria range; for the remaining 5 subjects, 4 had 1 normal value and 2 had 2 normal values. We conclude that 1) microalbuminuria is more common in HIV disease compared to the general population and 2) a single determination of albuminuria is reasonably predictive of albuminuria status.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2273**

**Urinary Angiotensinogen Originates from Kidney and Reflects Kidney Injury Regardless of the Extent of Proteinuria** Junseok Jeon, Ajin Cho, Hye Ryoun Jang, Jung Eun Lee, Wooseong Huh, Dae Joong Kim, Ha Young Oh, Yoon-Goo Kim. *Nephrology Division, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.*

The origin and clinical significance of urinary angiotensinogen (AGT) excretion are not known in patients with nephrotic syndrome. We studied the main source and clinical significance of urinary AGT in patients with various degrees of proteinuria.

A total of 119 GN and diabetic nephropathy (DN) patients [minimal change disease (MCD) in 22, focal segmental glomerulosclerosis (FSGS) in 18, membranous nephropathy (MN) in 30, IgA nephropathy (IgAN) in 27, DN in 22 patients] with proteinuria were included and 9 thin basement membrane disease (TBM) patients were used as control. Urinary and plasma AGT was measured using sandwich ELISA and intrarenal AGT expression was measured using western blotting.

The mean age of patients (M:F=72:47) was 44.5 years. Sixty patients had nephrotic syndrome. Urinary AGT excretion was markedly increased in GN and DN patients [Ln (urine AGT/Cr): mean±SD, 1.04±1.40 in TBM, 2.86±2.00 in MCD, 4.76±2.02 in FSGS, 4.74±1.35 in MN, 3.45±1.47 in IgAN, 5.08±1.79 in DN]. Although estimated urinary AGT/Cr (ug/g) calculated using fractional excretion of albumin was higher in nephrotic syndrome, it was less than 0.3% of measured urinary AGT/Cr in all patients (% of estimated among measured urinary AGT/Cr, mean±SE, 0.04±0.01% in non-nephrotic proteinuria, 0.20±0.07% in nephrotic proteinuria, P=0.04). Urinary AGT/Cr was positively correlated with AGT/β-actin in patients with non-nephrotic proteinuria ($P = 0.007$, $r = 0.385$). Urinary AGT excretion was positively correlated with proteinuria ($P < 0.001$, $r = 0.452$) and negatively correlated with eGFR ($P < 0.001$, $r = -0.438$) in all patients but there was no correlation between plasma AGT and proteinuria or eGFR.

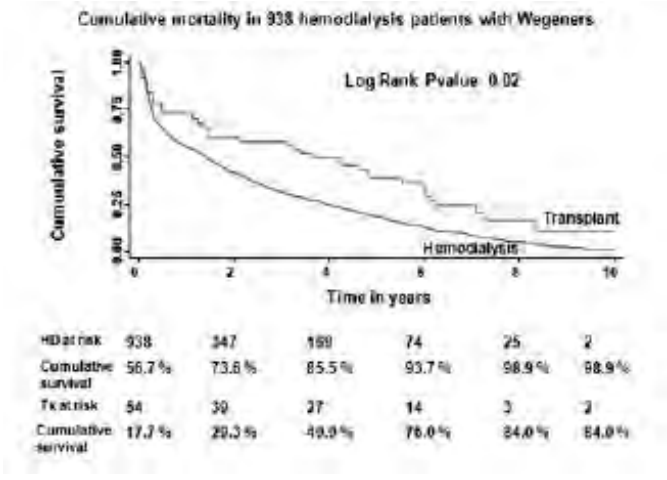
Regardless of the extent of proteinuria, more than 99.5% of urinary AGT originates from the kidney, suggesting that urinary AGT consistently reflects the activity of intrarenal RAS. In GN and DN patients, urinary AGT excretion was enhanced and seems to reflect the degree of renal injury independent of systemic RAS.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2274**

**Wegener's Granulomatosis: Survival on Dialysis and Transplant** Duvuru Geetha,[1] Pooja C. Oberai,[1] Rulan S. Parekh.[2] *[1]Johns Hopkins University; [2]Hospital for Sick Children.*

Renal morbidity is common in Wegener's granulomatosis (WG) and end stage renal disease occurs in up to 30% of patients with kidney transplantation as an important therapeutic option. Using data from the United States Renal Data System from 1996 to 2005, we performed a non concurrent cohort study of dialysis patients with WG compared to those with a primary glomerulonephritis (GN) and studied time to wait list for kidney transplant, and survival on dialysis, waitlisted and post transplant.Using the WG study population, we randomly selected primary GN patients in a 2:1 ratio matched by age and year of dialysis initiation. Logistic regression analysis was used to model the time listing for renal transplantation, censored for death and transplantation adjusting for gender, race and comorbidities. The study population consisted of 938 WG and 1715 GN patients. The WG patients had a mean age of 66 years, 43 % were female and 93% were White. Median time to wait list for renal transplant was 1.2 (95% CI: 1.1, 1.3) years for patients with Wegener's granulomatosis and 1.5 (95% CI: 1.4, 1.6) years for those with primary GN. The risk of death was 59% lower when ESRD patients with WG were transplanted. In multivariate analysis, lower age and absence of cardiac failure were protective of risk of death. These results indicate that time to wait list for kidney transplant is comparable for patients with WG and primary GN. Long term survival in WG patients with ESRD is better for those that undergo transplantation compared to those that are on dialysis.




Patients with WG and ESRD should therefore be offered pre-emptive renal transplantation or be wait listed sooner.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2275

**Profile and Prognosis of Rapidly Progressive Glomerulonephritis in a Tertiary Care Hospital in Queensland** Dwarakanathan Ranganathan,[1] Dev Krish Jegatheesan,[1] Thaminda Liyanage,[1] Leo P. Francis,[1] Sree Krishna Venuthurupalli.[2] *[1]Renal Medicine, Royal Brisbane & Women's Hospitals, Herston, QLD, Australia; [2]Renal Medicine, Rockhompton Base Hospital, Rockhompton, QLD, Australia.*

BACKGROUND: Rapidly progressive glomerulonephritis (RPGN) is an important cause of end-stage renal failure. RPGN can be grouped according to immunofluorescence patterns as linear type I (anti-glomerular basement membrane); pauci-immune type II (anti-neutrophil cytoplasmic antibody) and granular type III (immune complex disease). This retrospective study looked at the profile and prognosis of RPGN patients seen in a tertiary care setting over the last ten years. METHODS: Electronic records and case notes of all patients diagnosed with biopsy-proven (crescentic) RPGN between January 2000 to November 2009 at the Royal Brisbane and Women's Hospital were reviewed. Patient demographics, laboratory parameters, patient survival and the need for immunosuppressive and or renal replacement therapy were noted. Type I disease patients were excluded from analysis. RESULTS: 34 patients were diagnosed with type II disease, with a mean age of 57.7 years and mean haemoglobin at presentation of 96.0 ± 18.2 g/L. Those who required long-term dialysis presented with significantly higher mean serum creatinine levels (764.0 ± 454.9 VS 231.4 ± 138.7 µmol/L; p<0.05), but this was not the case for patients who eventually died (606.0 ± 385.2 VS 566.8 ± 478.0 µmol/L; p=0.83). 17 patients were diagnosed with type III disease, with a mean age of 38.1 years; mean haemoglobin of 106.8 ± 21.2 g/L. Those who required long-term dialysis presented with significantly higher mean serum creatinine levels (283.7 ± 81.2 VS 91.8 ± 44.6 µmol/L; p<0.05) but this was not the case for patients who eventually died (141.7 ± 140.1 VS 163.4 ± 109.3 µmol/L; p=0.769). CONCLUSIONS: This study suggests that early referral would most likely reduce the need for long-term dialysis and may improve renal and patient survival.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2276

**Acute Post-Infectious Glomerulonephritis in the Elderly** Samih H. Nasr,[1] Mary E. Fidler,[1] Anthony M. Valeri,[2] Lynn D. Cornell,[1] Sanjeev Sethi,[1] Michael B. Stokes,[2] Glen S. Markowitz,[2] Vivette D. D'Agati.[2] *[1]Pathology, Mayo Clinic, Rochester, MN; [2]Pathology and Nephrology, Columbia University, New York, NY.*

Acute post-infectious glomerulonephritis (APIGN) is primarily a childhood disease that occurs after upper respiratory tract infection or impetigo. There have been no clinicopathologic studies addressing APIGN in the elderly. We report 109 cases of APIGN in the elderly (≥65 years of age) diagnosed by renal biopsy at 2 large North American referral centers between 1995-2010. The M:F ratio was 2.8:1. Sixty-one percent (61%) of patients had immunocompromised background, most commonly diabetes (49%) and malignancy (14%). The most common site of infection was skin (28%), followed by pneumonia (16%) and urinary tract infection (13%). Staphylococcus was by far the most common causative agent found in 46% of patients followed by streptococcus (16%) and unusual gram negative organisms. Hypocomplementemia was present in 72% of patients. The mean peak creatinine was 5.1 mg/dl; 67% of patients had a peak creatinine >4.0 mg/dl and 46% required acute dialysis. Twenty-six percent (26%) of patients developed new onset congestive heart failure at presentation. The most common light microscopic patterns were diffuse (53%), focal (28%), and mesangial (13%) proliferative glomerulonephritis. IgA-dominant APIGN was seen in 17% of patients. Of the 72 patients with ≥3 months of follow up (mean 29 months), 22% achieved complete recovery, 44% had persistent renal dysfunction, and 33% progressed to ESRD. Outcomes were worse than in adult counterparts 16-64 years of age. Correlates of reaching ESRD were presence of diabetes, higher creatinine at biopsy, dialysis at presentation, presence of diabetic glomerulosclerosis, and greater tubular atrophy and interstitial fibrosis.

In conclusion, the epidemiology of APIGN is shifting as the population ages. Elderly males, diabetics, and patients with malignancy are particularly at risk. Skin, lung and urinary tract are more common sites of infection than upper respiratory tract, and staphylococcus is 3-fold more frequent than streptococcus. Prognosis is guarded with less than a quarter of patients fully recovering renal function.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2277

**The Validation Study of Oxford Classification: The Significance of Crescent** Ritsuko Katafuchi,[1] Toshiharu Ninomiya,[2] Koji Mitsuiki,[3] Masaharu Nagata,[2] Hideki N. Hirakata.[3] *[1]Kidney Unit, National Fukuoka-Higashi Medical Center, Koga, Fukuoka, Japan; [2]Department of Medicine and Clinical Science Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [3]Kidney Unit, Fukuoka Red Cross Hospital, Fukuoka, Japan.*

**BACKGROUND**: An international IgA nephropathy (IgAN) group has proposed an Oxford classification, consisting mesangial hypercellularity score(M), and the presence or absence of segmental glomerulosclerosis(S), endocapillary hypercellularity(E), and tubular atrophy/interstitial fibrosis(T).

**AIM**: To clarify the prognostic significance of crescent as well as M, E, S, T in our cohort study.

**MATERIALS**: 702 patients with IgAN with 12 months or more follow-up were included.

**METHODS**: The prognostic significance of M, E, S, T and crescent was investigated by the Cox's proportional hazards model. Endpoint was the development of end-stage renal failure (ESRF) defined as initiation of renal replacement therapy.

**RESULTS**: Median age was 30 years old. The mean value of estimated glomerular filtration rate (eGFR) was 82 ml/min/1.73m$^2$. During the median 62-month follow-up, 85 patients developed ESRF. M, S and E did not show any significant influence on kidney survival. The risk of ESRF increased gradually with higher grade of T (hazard ratio [HR] 2.63, p=0.009 in grade 2 and HR 8.06, p<0.001 in grade 3). Patients with crescent had a significantly greater risk for ESRF than those without (HR 2.07, p=0.01). When the effect of crescent on kidney survival was examined in two group divided according to inclusion criteria in the study of Oxford classification (i.e. eGFR of more than 30 ml/min per 1.73m$^2$ and urinary protein: creatinine ratio of more than 0.5), the presence of crescent was associated with increased risk of the development of ESRF in 286 patients who did not meet the Oxford criteria (HR 4.10, p=0.004), but not in 416 patients who met it (HR 1.23, p=0.529).

**CONCLUSION**: The prognostic significance of crescent was evident in our cohort study. The reason why the prognostic value of crescent was not evident in Oxford classification is likely that the patients with eGFR of less than 30 ml/min per 1.73m$^2$ were excluded in their study as they suggested.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2278

**Cigarette Smoking Abrogates a Renoprotective Effect of Renin-Angiotensin-Aldosterone System (RAAS) Blockade in Patients with IgA Nephropathy** Ryohei Yamamoto,[1] Yasuyuki Nagasawa,[1] Tatsuya Shoji,[2] Noritaka Kawada,[1] Masaru Horio,[1] Toshiki Moriyama,[1] Atsushi Yamauchi,[3] Yoshiharu Tsubakihara,[2] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Department of Kidney Disease and Hypertension, Osaka General Medical Center, Osaka, Japan; [3]Department of Nephrology, Osaka Rosai Hospital, Sakai, Osaka, Japan.*

**Aim**: To evaluate predictive power for renoprotective effect of RAAS blockade in patients with IgA nephropathy (IgAN).

**Design and Setting**: Multicenter retrospective cohort study in three major nephrology centers in Osaka, Japan.

**Patients**: 726 IgAN patients aged at least 20 yr.

**Covariates**: Baseline characteristics at kidney biopsy and therapeutic interventions initiated within 1 year of kidney biopsy (RAAS blockers and corticosteroid).

**Outcome**: Slope of eGFR (ΔeGFR, mL/min/1.73m$^2$/yr)

**Statistics**: Effect modifications between RAAS blockade and potential predictors of effectiveness of RAAS blockade were assessed incorporating their interaction terms into multivariate linear regression models to evaluate their predictive powers for renoprotective effect of RAAS blockers.

**Results**: Clinical characteristics were as follows; age 37 ±13 yr, male 42.7%, systolic/diastolic blood pressure 122 ±17/75 ±13 mmHg, eGFR 79 ±24 mL/min/1.73m$^2$, urinary protein 0.8 ±1.0 g/day, current smokers 24.9%, RAAS blockade 48.8%, corticosteroid 30.0%, and observational period 7.6 ±4.4 yr. Multivariate linear regression model revealed that urinary protein (per g/day, β -1.40 [95%CI -2.07 to -0.72, P=0.013) and current smokers(β -1.07 [-1.79 to -0.35], P=0.024) were significantly associated with ΔeGFR. Interestingly, smoking status was identified as significant predictors of effectiveness of RAAS blockers (P=0.027 for interaction), along with urinary protein (P=0.007 for interaction). Effectiveness of RAAS blockers were blunted in current smokers and the patients at lower level of urinary protein, especially, less than 1g/day.

**Conclusion**: Smoking status was identified as the predictor of renoprotective effect of RAAS blockers in IgAN, along with urinary protein.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2279**

**Reduced eGFR in IgAN Predicts Poor Response to Corticosteroid Treatment**
Pietro A. Canetta, Ana Huerta, Andrew S. Bomback, Jai Radhakrishnan, Gerald B. Appel. *Division of Nephrology, Columbia University College of Physicians & Surgeons, New York, NY.*

**INTRODUCTION:**
A number of studies suggest that 6 months of corticosteroids can reduce proteinuria and prevent a decline in renal function in IgAN, especially in patients who do not achieve disease control with RAAS inhibition alone. However, defining which patients will benefit from treatment remains a challenge. We studied a series of patients with IgAN treated with steroids to evaluate factors predicting progression of disease despite treatment.

**METHODS:**
We reviewed clinical, histopathologic, and laboratory data in 20 patients with IgAN treated with corticosteroids at the Center for Glomerular Diseases at Columbia University. Inclusion criteria were biopsy-proven IgAN (scored according to the Oxford-MEST classification) and proteinuria >1 g/day despite RAAS inhibition. Patients were treated with a course of every other day prednisone, starting at 2 mg/kg for 1 month then tapering off over 5-6 months. Response was defined as a >50% decrease in proteinuria to <1 g/day, with <25% increase in serum creatinine from baseline to 12 months of follow-up. Logistic regression was performed to evaluate factors predicting failure to respond.

**RESULTS:**
Of 20 patients, 9 had progression of disease despite corticosteroid treatment. There was no significant difference between responders and non-responders with respect to age, race, gender, time since diagnosis, proteinuria, or serum albumin. Non-responders had significantly lower eGFR than responders (33 vs. 68 ml/min/1.73m$^2$, p = 0.002), higher tubular atrophy/interstitial fibrosis score and higher percentage of globally sclerotic glomeruli. On multivariate logistic regression, the only factor that predicted failure to respond to treatment was an eGFR <45 ml/min/1.73m$^2$ (p = 0.003).

**CONCLUSION:**
In this uncontrolled series of patients with IgAN treated with every other day corticosteroids, the key predictor of failure to respond to treatment was an eGFR <45 ml/min/1.73m$^2$. IgAN patients with significant reductions in GFR may not benefit from 6 months of corticosteroids.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2280**

**Urinary KIM-1 and NGAL Excretion in Immunoglobulin A Nephropathy**
Hilde P. Peters,[1] Femke Waanders,[2] Esther Meijer,[2] Jan A. J. G. van den Brand,[1] Harry Van Goor,[2] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; [2]Kidney Center, University Medical Center Groningen, Groningen, Netherlands.*

The variable course in patients with Immunoglobulin A nephropathy (IgAN) warrants accurate tools for prediction of progression. Urinary kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) are solid markers for the detection of active tubulointerstitial damage in various renal diseases.

We evaluated the prognostic value of KIM-1 and NGAL in patients with IgAN.

**Methods**
We included patients (n=65, 72% male, age 39 (range 17-70) yrs) with IgAN who were evaluated for severe proteinuria between 1995-2007. Healthy controls were matched for age and gender. KIM-1 and NGAL were measured in urine by ELISA. Univariate and multivariate cox regression analysis was used to assess predictors of ESRD.

**Results**
Median plasma creatinine was 142 (range 70-362) mol/L, and proteinuria 2.2 (0.4-24.2) g/day. During follow-up (median 75 (3-146) months), 23 patients (35%) developed ESRD. In patients with IgAN median urinary KIM-1 excretion was 1.7 [interquartile range 0.8-3.2] ng/min and NGAL excretion was 47 [21.7-104.0] ng/min, both significantly higher than in healthy controls (KIM-1 excretion 0.6 [0.4-0.9] ng/min and NGAL excretion 15.7 [10.5-20.1] ng/min).

KIM-1 and NGAL correlated with proteinuria (r=0.40 and 0.35 respectively, p<0.01) and with each other (r=0.53, p<0.01) but not with serum creatinine or GFR. By univariate analysis excretion of KIM-1, NGAL and IgG, proteinuria and serum creatinine were significantly associated with ESRD. Multivariate analysis showed that serum creatinine and KIM-1 excretion were the only significant independent predictors of ESRD. The hazard ratio for KIM-1 excretion was 1.2 corresponding to a 20% increase in the risk of developing ESRD for each ng/min increase in urinary KIM-1 excretion.

**Conclusion**
Urinary KIM-1 and NGAL excretion is increased in patients with IgAN and correlates with proteinuria but not with GFR. Serum creatinine and KIM-1 excretion, but not proteinuria, are independent predictors of ESRD in patients with IgAN.

Disclosure of Financial Relationships: nothing to disclose

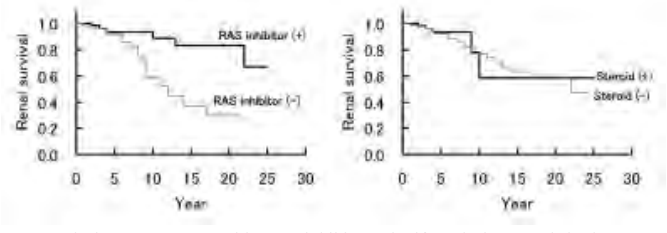
**SA-PO2281**

**Renin-Angiotensin System Blockade Improves the Long-Term Renal Survival Better Than Steroid Therapy in IgA Nephropathy**
Kensuke Asaba, Akihiro Tojo, Satoshi Kinugasa, Kikuno Hanamura, Toshiro Fujita. *Division of Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan.*

Purpose: Patients with IgA nephropathy may gradually progress to end-stage renal disease (ESRD), and the long-term randomized controlled trial using dialysis induction as an outcome is difficult. We used the start of dialysis as the end point and examined the long-term renal survival in IgA nephropathy.

Methods: We evaluated retrospectively the effects of renin-angiotensin system (RAS) inhibitors and steroid therapy on the renal survival rate by Kaplan-Meier method in 159 patients with biopsy-proven IgA nephropathy diagnosed in the last 25 years.

Results: By multivariate analysis, lower eGFR and higher score of glomerulosclerosis index at renal biopsy and treatment without RAS inhibitors were independent risk factors for ESRD. Eight (9%) of 93 patients treated with RAS inhibitors and 16 (24%) of 66 patients treated without RAS inhibitors developed ESRD, respectively. Kaplan-Meier renal survival curve was significantly better in the former than in the latter (P = 0.002). Five (8%) of 60 patients treated with prednisolone and 19 (19%) of 99 patients treated without prednisolone developed ESRD, respectively, and the renal survival was not different significantly between these two groups (P = 0.673). To investigate the additional effects of steroid therapy to the RAS blockade, we compared 47 patients treated with both RAS inhibitors and prednisolone and 46 patients treated with RAS inhibitors but without prednisolone, and found no difference in the renal survival (P = 0.420).




Conclusions: Treatment with RAS inhibitors significantly improved the long-term renal survival better than steroid therapy in IgA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2282**

**Impact of Body Mass Index on the Clinical Course of IgA Nephropathy**
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In the present study we evaluated the influence of body mass index (BMI) on clinical and histological parameters of IgA nephropathy.

We studied n=178 patients with biopsy proven primary IgA nephropathy, followed up for a mean of 5.9 ± 6.0. The rate of deterioration of renal function was estimated by the slope of the curve of reciprocal serum creatinine against time. According to the BMI at the time of renal biopsy, patients were divided into the following groups: normal BMI (<25, n=104), overweight (25-29.99, n=54) and obese (≥30, n=20).

Age, initial renal function, proteinuria, blood pressure under treatment and the number of antihypertensive agents taken at the time of renal biopsy were similar among normal, overweight and obese patients (ns). The rate of progression however differed significantly between patients with normal BMI (-0.092 ± 0.116), overweight (-0.188 ± 0.366) and obese patients (-0.211 ± 0346 dl*mg$^{-1}$*year$^{-1}$, p=0.020). Similarly, BMI as a continuous variable correlated with the rate of deterioration of renal function (r=0.232, p=0.002).

An increased BMI is associated with a faster decline of renal function in patients with IgA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2283**

**Elevated Serum Uric Acid Is Associated with Reduced Renal Function and Severity of Histological Injury in IgA Nephropathy**
Jingyuan Xie,[1,2] Krzysztof Kiryluk,[2] Zhaohui Wang,[1] Hong Ren,[1] Xiaoxia Pan,[1] Weiming Wang,[1] Yifu Li,[2] Ali G. Gharavi,[2] Nan Chen.[1] *[1]Nephrology Department, Ruijin Hospital, Shanghai Jiaotong University, Shanghai, China; [2]Medicine, Div. Nephrology, Columbia University, New York, NY.*

Our objective was to determine the role of serum uric acid (UA) in the pathogenesis and progression of IgA nephrolophy (IgAN). For this purpose, we analyzed a cohort of 1,360 biopsy-diagnosed primary IgAN cases and 1,093 healthy controls; a subgroup of 458 cases was followed for an average of 2.7 years (1mo-20 yrs). All participants were recruited in Shanghai, east China. Hyperuricemia was defined by gender-specific criteria of serum UA >450 umol/L in males and >340 umol/L in females. Statistical analyses were performed in SPSS v18.0.

Hyperuricemia was 3x more common in cases compared to controls (38% *vs.* 11%, p=5.7x10$^{-50}$). After age, gender, and disease status adjustments, higher serum UA levels were strongly associated with reduced eGFR; beta= -8.0 (8 mL/min/1.73m2 decline in

eGFR per 1 mg/dL increase in UA, $p=3.9x10^{-124}$). This association remained significant after additional adjustments for serum triglycerides, cholesterol, hemoglobin, and albumin levels (beta=-7.0, $p=3.9x10^{-50}$). When case and control groups were analyzed separately, the age and gender-adjusted association was strongest within the case group; beta=-9.3 ($p=1.6x10^{-87}$) in cases vs. beta=-2.6 ($p=2.9x10^{-11}$) in controls. Among cases, increased UA levels were also independently associated with a greater degree of histological injury by HAAS criteria after adjustment for age, gender, blood pressure, serum albumin, hematuria, proteinuria, and eGFR (p=0.007). In the fully adjusted Cox proportional hazards model, eGFR at the time of biopsy, hypertension, and age were the most important predictors of progression to ESRD. Age-and gender- adjusted UA level was associated with disease progression (p=0.001), but this effect lost significance after accounting for eGFR in the model.

In summary, our data indicate that elevated serum UA level is independently associated with decreased renal function and severity of histological injury in IgA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2284

**Relocation of Dendrin to the Podocyte Nucleus in Kidney Biopsy Specimens and Urine Samples in Patients with IgA Nephropathy** Fumiko Kodama, Katsuhiko Asanuma, Rin Asao, Miyuki Takagi, Yasuhiko Tomino. *Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Bunkyo-ku, Tokyo, Japan.*

An often insidious progression to end-stage kidney disease includes development of glomerulosclerosis that is associated with podocytopenia. It has been reported that podocytopenia occures with increasing disease severity in patients with IgA nephropathy (IgAN). Dendrin, proline-rich protein, is localized at the slit diaphragm (SD) insertion site in podocytes. We showed that dendrin relocates to the nucleus of injured podocytes in experimental nephritis and nuclear dendrin promotes podocyte apoptosis. It is not known whether dendrin translocates from the SD to the podocyte nucleus in human IgAN. We investigated the presence of nuclear dendrin in human kidney biopsy specimens with IgAN and the association of relocated dendrin to podocyte nucleus with disease progression. Fourteen adult patients with IgAN, diagnosed by renal biopsy, were enrolled. The patients were divided into four groups by the Japanese predicted prognosis classification system . The pathological parameters of Shigematsu et al. were analyzed. Immunostaining of kidney sections and urinary sediments from IgAN patients and three minimal change nephrotic syndrome (MCNS) patients was performed for dendrin, podocaryxin, annexin V and DAPI. Dendrin relocated dendrin to the podocyte nucleus was detected in glomeruli of kidney specimens with IgAN. A positive correlation was detected between acute extracapillary change and the number of dendrin positive nuclei. Nuclear dendrin was observed in urinary podocytes from IgAN patients. Moreover, the number of dendrin positive nuclei per urinary podocyte from IgAN patients was significantly higher than that from MCNS patients. Urinary annexin V positive podocytes were also detected in IgAN. **(Conclusion)** An increasing number of dendrin positive nuclei suggests the acute phase of glomerular inflammation in patients with IgAN. The apoptotic podocytes were shed into the urine in IgAN patients. It is suggested that dendrin relocated to podocyte nuclei enhances podocyte apoptosis for acute inflammation in IgAN and leads to podocytopenia.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2285

**A Randomized Multicenter Trial of Valsaratan in Normotensive IgA Nephropathy with Mild Proteinuria** Young-Il Jo,[1] Ju-Young Moon,[2] Sang-Ho Lee,[2] Sang-Woong Han,[3] Dongho Yang,[4] Sug Kyun Shin.[5] *[1]Nephrology, Konkuk University Hospital, Seoul, Republic of Korea; [2]Kyung-Hee Univ EWN Medical Center, Seoul, Republic of Korea; [3]Hanyang University Guri Hospital, Guri, Republic of Korea; [4]Bungdang CHA Hospital, Sungnam, Republic of Korea; [5]NHIC Ilsan Hospital, Goyang, Republic of Korea.*

1. Background; Proteinuria, even though it is mild, causes renal damage that accelerates the progression of IgA nephropathy (IgAN) towards ESRD and decreased by ACE inhibition. However, prospective controlled studies of the clinical benefit of angiotensin II receptor blocker (ARB) in the treatment of normotensive IgAN with mild proteinuria are lacking.

2. Purpose; This study was designed to investigate the antiproteinuric effects of a ARB, valsartan, in normontensive IgAN patients with mild proteinuria.

3. Methods; Sixty-one IgAN patients (M:F 22:39, age 41.0±12.7) were recruited from 5 centers in this randomized, prospective, multicenter study. Inclusion criteria were persistent proteinuria with spot urine protein-to-Cr ratio (UPCR) of 0.3-1.5 (mg/mg), BP <140/80mmHg without antihypertensive medication, and eGFR-MDRD >60 mL/min/1.73m$^2$. Eligible subjects were randomized assigned to administration of valsartan 40 mg/day or 80 mg/day.

4. Results; There were no significant differences in baseline parameters including UPCR, eGFR, serum K, and BP between two groups. Proteinuria gradually and significantly decreased from 4 weeks after administration of valsartan 80mg, but partially ameliorated by valsartan 40mg (see Figure). BP, eGFR, and serum K levels did not significantly change during 12 weeks in both groups. There were no serious adverse events that caused withdrawal of treatment.

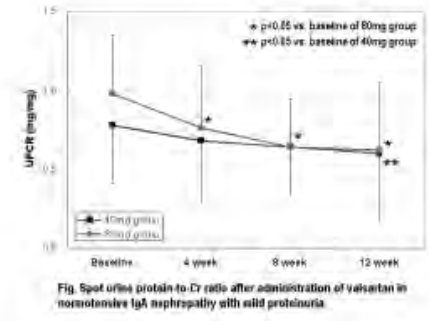



Fig. Spot urine protein-to-Cr ratio after administration of valsartan in normotensive IgA nephropathy with mild proteinuria

5. Conclusion; These results suggest that valsartan (40 or 80mg) could significantly decrease proteinuria without any serious adverse events in normotensive IgAN patients with mild proteinuria.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2286

**Evaluation with Oxford Classification: Correlations between Pathological Features and Clinical Features in Patients of IgA Nephropathy with Crescents** Jiong Tian, Rong Lv, Huiping Wang, Jianghua Chen. *Kidney Disease Center, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China.*

Oxford classification for IgA nephropathy (IgAN) was presented in Kidney International 2009 by International IgA nephropathy Network. However the predict value of the novel classification for the renal prognosis needs more validation in different cohorts. Besides, the significance of crescent is not included in Oxford classification and remains to be clarified.A total of 83 patients (age 34.7±13 years, 54% male vs 46% female) diagnosed primary IgAN during Jan, 2005-Dec, 2006 were included in the present study. Blindly and randomly 10 glomeruli per slide were selected for re-observation by renal pathologist according to the four pathological variables: mesangial hypercellularity, segmental glomerulosclerosis, endocapillary hypercellularity and tubular atrophy/interstitial fibrosis. Patients were divided into various groups based on the semi-quantitative scores. Rapid renal function decline was defined by slope > 1.6ml/min/ per 1.73m$^2$ per year calculated by eGFR. At the time of biopsy the daily protein was 3.0±2.8g and declined to 1.3±1.5 g after a follow-up for a median of 36 months. 5 patients progressed into ESRD or doubling of serum creatinine within the time of follow-up. Both endothelial proliferation and tubular atrophy were associated with rapid renal function decline significantly whereas mesangial hypercellularity or segmental glomerulosclerosis was not. The proteinuria in patients with endothelial hypercellularity was more than that in patients without the pathological feature (2.7±1.3 vs 1.5±1.5 p=0.001). Differences of proteinuria in three groups divided according to percentage of crescent (<10%,10-20%,>20%) were significant (1.37±1.1 vs 2.8±1.1 vs 3.6±2.3, p<0.05 ). The percentage of crescent was not related to renal outcome either using rapid renal function decline parameter or creatinine increase >25% of baseline. Crescent formation was related to proteinuria in IgAN patients but not to the renal prognosis in the present cohort. Larger cohorts and longer follow-up are needed to evaluate the Oxford classification in Asian patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2287

**Clinical Remission of IGA Nephropathy (IGAN)** David K. Packham. *Melbourne Renal Research Group, Melbourne, Victoria, Australia.*

Aim

To establish the frequency of clinical remission of IGAN in adult patients and the significance of associated vasculitic rash (VR).

Introduction

Reported cases of spontaneous remission in IgAN vary between 0-33%. This discrepancy may reflect differences in the definition of remission and particularly resolution of significant haematuria.

We report the incidence of clinical remission in an adult cohort of patients followed for a minimum of 24 months post-biopsy. The significance of VR is discussed.

Methods

Retrospective analysis of 99 adult patients with biopsy proven IgAN and a minimum follow up (FU) of 24 months. Biopsied between 1991-2005.

Patient demographics, the presence of recent history of VR, creatinine (Cr), protein excretion (24 hr UPr) and glomerular red blood cell counts (grbcc), were recorded at biopsy and at six monthly intervals thereafter. Grbcc <18x103/ml were regarded as normal. Resolution of glomerular haematuria was defined as at least three sequential normal grbcc over a minimum of eighteen months without subsequent abnormal grbcc. Remission of IgAN was only diagnosed in patients with resolution of glomerular haematuria in the absence of renal impairment (Cr > 120mmol/L) or significant proteinuria (>0.15g/24 hrs).

Results

Median follow up was 72 months (24-162). 20% had resolution of haematuria. However, 63% of these had either renal impairment, significant proteinuria or both. Thus, only 7 patients (7%) fulfilled our definition of clinical remission (remitters). 7 (7%) of patients in the whole cohort reported VR but 4 of these (57%) were subsequently remitters. Thus if VR was absent, only 3% of patients went into clinical remission.

Conclusions

1 Clinical remission of IgAN occurred in 7% of patients followed for a median of 72 months.

2 If patients presenting with a history of VR are excluded, clinical remission rate was 3%.

3 Patients with VR are over-represented amongst remitters. They may represent adult Henoch Schönlein purpura and have a different natural history.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2288**

**Urinary Kidney Injury Molecule-1 and Neutrophil Gelatinase-Associated Lipocalin Correlate with Clinical and Pathologic Findings in IgA Nephropathy** Se-Bin Song, Dongyoung Lee, Ju-Young Moon, Kyung-Hwan Jeong, Tae Won Lee, Chun-Gyoo Ihm. *Department of Nephrology, Kyung Hee University, School of Medicine, Seoul, Republic of Korea.*

Introduction: Urinary neutrophil gelatinase-associated lipocalin (uNGAL) and Kidney injury molecule-1 (uKIM-1) have been known as a predictor of kidney injury but there was a lack of study on its roles in chronic glomerulonephritis. This study was performed to exam the levels of uNGAL and uKIM-1 and to assess whether they had an association with a pathologic finding and clinical activity in patients with IgA nephropathy.

Method: uNGAL and uKIM-1 were measured using commercial human ELISA kits on 40 patients with IgA nephropathy (15 males (37.5%) with a mean age of 36.2±12.9 years) and 10 healthy volunteers (5 males (50%) with a mean age of 37.3±9.6 years).

Results: The patients with IgA nephropathy had higher uNGAL (26.9±35.4 ng/mgCr vs. 9.1±7.8 ng/mgCr, p=0.006) and uKIM-1 (1.17±1.51 ng/mgCr vs. 0.29±0.20 ng/mgCr, p=0.001) compared with those of healthy volunteers. On univariate regression analysis, uNGAL was correlated with serum creatinine (r=0.652), eGFR (r=-0.393), and albumin (r=-0.423) and uKIM-1 was correlated with pathologic grading (H.S Lee grading) (r=0.335), tubulointerstitial inflammation (r=0.353), and hemoglobin (r=-486). In a model of multivariate regression analysis, uNGAL had an association with serum creatinine (p=0.006) and eGFR (p=0.005) and uKIM-1 did with H.S. Lee grading (p=0.004) and tubulointerstitial inflammation (p=0.011).

Conclusion: uNGAL was associated with residual renal function and uKIM-1 had high correlation with pathologic severity such as tubulointerstitial inflammation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2289**

**Normoalbuminemia May Predict IgA Nephropathy in Primary Glomerulopathy with Nephrotic-Range of Proteinuria in Chinese Patients** Fude Zhou, Min Chen, Ming Hui Zhao, Hai Yan Wang. *Renal Division, Peking University First Hospital, Beijing, China.*

**[Purpose]** Massive proteinuria is often associated with hypoalbuminemia in glomerulopathy. However, patients may have normal level of serum albumin in spite of heavy proteinuria in many circumstances. The current study aimed to investigate factors affecting serum level of albumin in primary glomerulopathy patients with nephrotic-range of proteinuria with special attention paid to different pathological types.

**[Methods]** The renal histopathology data of adult patients (age ≥18 year-old) with primary glomerulpathy and nephrotic-range of proteinuria, who received native renal biopsies in Peking University First Hospital from 1998 to 2007, were retrospectively analyzed. Demographic data, interval between the onset of disease and renal biopsy, blood pressure, 24 h proteinuria, serum albumin level, serum lipid profile, serum creatinine concentration, eGFR, histological diagnosis were collected and analysed. The receiver operating characteristic (ROC) curve analysis was employed to determine the specificity and senstivity for predicting certain type of glomerulopathy.

**[Results]** There were totally 780 patients with primary glomerulopathy and nephrotic-range of proteinuria recruited for further analysis in this retrospective study. Compared with patients with hypoalbuminemia (serum albumin lower than 30g/L), patients without hypoalbuminemia were significantly younger (36.8±11.6 vs. 41.0±15.8 years old, P=0.000), and had significantly lower level of proteinuria (5.34±2.06 vs. 8.63±4.26g/24hr, P=0.000). Patients without hypoalbuminemia had significantly higher proportion of IgA nephropathy (66.0% vs. 17.2%, P=0.000). The independent predictors of hypoalbuminemia in nephrotic-range of proteinuria patients included age, gender, interval between onset of the disease and renal biopsy, proteinuria level and pathological type of glomerulopathy. A serum level of albumin ≥ 35g/L could predict IgA nephropathy with the specificity of 95.8%, and the specificity increased with age.

**[Conclusion]** Among patients with primary glomerulopathy and nephrotic-range of proteinuria, normoalbuminemia is a useful predictor for the diagnosis of IgA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2290**

**Hyperuricemia as a Marker for Progression of IgA Nephropathy** Su-Ji Kim, Junghoon Kim, Sujin Seok, Hyo-Wook Gil, Jong-Oh Yang, Eun-Young Lee, Sae-Yong Hong. *Internal Medicine, Soonchunghyang University, Cheonan, Korea.*

Because of variable clinical and histopathological manifestation of immunoglobulin A nephropathy (IgAN), it is very difficult to predict the disease progression. In recent studies, hyperuricemia which is common in hypertension and vascular disease has also been thought to contribute to the renal dysfunction and the histological changes, including renal arterioleosclerosis, tubular atrophy and interstitial fibrosis. In the present study, we investigated the clinical significance of uric acid level at the time of biopsy as a marker for the progression of IgAN.

**Methods:** A total 193 patients (men 103, women 90, 14 to 71 years) with biopsy-proven IgAN were studied. Mean follow up duration after renal biopsy were 5.8 years. Progression of renal disease defined as an elevation of serum creatinine above 1.2 mg/dL or over 20% elevation from baseline. Hyperuricemia defined as a level of serum uric acid ≥ 7.3 mg/dL in men or ≥ 5.3 mg/dL in women according to the consensus from our hospital, upper level of one standard deviation of normal control group. The control group consisted of 6245 participants, who were clinically well and without hypertension, diabetes, hematuria or proteinuria at Soonchunhyang health promotion center.

**Results:** The hyperuricemia group (*n*=50) showed higher systolic blood pressure, body mass index, serum creatinine, total cholesterol, triglyceride, a greater amount of proteinuria and lower glomerular filtration rate than normal hyperuricemia group (*n*=143). Hyperuricemia increased risk for progression of IgAN (OR 4.53, 95% CI 1.31-15.66) after adjusted for age, gender, body mass index, hypertriglyceridemia, hypercholesterolemia and proteinuria. The disease progression group (*n*=26) had a greater frequency of hyperuricemia, hypertension, proteinuria and the nephrotic range of proteinuria than non progression group (*n*=119). The survival curves for renal function in relation to hyperuriceima showed that the hyperuricemia group had a higher rate of disease progression in IgAN.

**Conclusion:** We conclude that hyperuricemia at the time of diagnosis might be an important marker for progression of IgAN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2291**

**Relationship between Urinary Podocalyxin Level and Histological Activity in Patients with IgA Nephropathy** Rin Asao,[1] Katsuhiko Asanuma,[1] Fumiko Kodama,[1] Miyuki Takagi,[1] Yukihiko Takeda,[1] Isao Ohsawa,[1] Yoshiaki Hirayama,[2] Satoshi Horikoshi,[1] Masanori Hara,[3] Yasuhiko Tomino.[1] *[1]Nephrology, Juntendo University, Tokyo, Japan; [2]Reagents Development, Denka Seiken Co., Ltd, Niigata, Japan; [3]Pediatrics, Yoshida Hospital, Niigata, Japan.*

[Introduction)

Podocalyxin is present on apical cell membrane of podocytes and is shed into urine from injured podocytes. Urinary podocalyxin is associated with severity of active glomerular injury in children with glomerular diseases. In the present study, we examined the relationship between level of urinary podocalyxin and glomerular injury in adult patients with IgA nephropathy.

[Materials and Methods)

Urine samples voided in the morning were obtained from 42 patients with IgAN (13 males and 29 females; mean age 32.9 yr) during the period from October 2007 to July 2009. Patients with corticosteroid treatment and/or tonsillectomy were excluded from this study. All renal biopsy specimens were analyzed histologically. The pathologic parameters of IgAN were analyzed according to Shigematsu's classification (Pathol Int. 1997 Apr;47(4):194-202) and the Oxford classification of IgAN. Levels of urinary podocalyxin (PCX) were measured by sandwich ELISA.

[Results)

In the histological analysis based on Shigematsu's clasiffication, there was a significant correlation between levels of urinary PCX and severity of acute extracapillary changes (r=0.6006 p<0.01), but levels of urinary podocalyxin were not related to severity of chronic glomerular and interstitial changes. There was a significant correlation between levels of urinary protein and severity of chronic endocapillary and extracapillary changes (r=0.5677 p<0.01, r=0.4752 p<0.01, respectively). There was no statistically significant correlation with levels of urinary protein and severity of acute glomerular changes. In the histological analysis based on the Oxford classification, there was no correlation between levels of urinary PCX and histological renal findings.

[Conclusion)

It appears that urinary podocalyxin is a useful marker for predicting the histological activity in patients with IgA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2292**

**Combination of Tonsillectomy and Corticosteroid Suppressed Recurrence of Urinary Protein and Occult Blood in Patients with IgA Nephropathy, Compared with Corticosteroid Alone** Atsushi Takahashi,[1] Ryohei Yamamoto,[1] Yasuyuki Nagasawa,[1] Hirotsugu Iwatani,[1] Kenichiro Iio,[1] Maki Shinzawa,[1] Arata Horii,[2] Atsushi Yamauchi,[3] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Otolaryngology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [3]Nephrology, Osaka Rosai Hospital, Sakai, Osaka, Japan.*

*Background:* Although several recent studies suggest that tonsillectomy with corticosteroid (TLX+CS) may have a renoprotective effect on immunogloblin A nephropathy (IgAN), it remains to be elucidated whether TLX+CS is more effective than corticosteroid therapy alone (CS). *Methods:* We retrospectively reviewed 59 IgAN patients who received TLX+CS and 37 patients who did CS between 2000 and 2007 in two nephrology centers. Outcomes were time from initiation of TLX+CS or CS to disappearance (- or ± by dipstick) and recurrence of urinary protein (UP) or occult blood (UOB). Cumulative incidence rate of outcomes in TLX+CS and CS were estimated and compared using Kaplan-Meier method and Log-rank test. Cox proportional hazard (CPH) analysis was used to determine the factors associated with remission and recurrence of urinary abnormalities. *Results*: No significant

difference between TLX+CS and CS groups was observed in baseline characteristics before treatment, including age, gender, urinary protein/creatinine ratio (0.64 (0.39-1.38) vs. 0.78 (0.48-1.45), p=0.58), eGFR (77(58-88) vs. 72 (56-86) ml/min/1.73m$^2$, p=0.83), and systolic blood pressure (116 (105-130) vs. 114 (107-123) mmHg, p=0.37). Cumulative remission rate of UP and UOB were not significantly different between TLX+CS and CS (UP; p=0.73 for Log-rank test and UOB; p=0.08). On the contrary, cumulative recurrence rate of UP and UOB were significantly higher in CS, compared with TLX+CS (UP; p = 0.007 for Log-rank test and UOB; p=0.002). Univariate CPH analysis identified CS (vs. TLX+CS) as significant predictors of recurrence of UP and UOB (UP: hazard ratio 4.17 [95% confident interval 1.49-14.3] and UOB (12.5 [2.33-100]). *Conclusion*: Tonsillectomy with corticosteroid was more effective than corticosteroid alone in preventing recurrence of IgAN.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2293

**Serial Measurement of Galactose-Deficient IgA1 (Gd-IgA1) in Children** Margaret Colleen Hastings,[1] John T. Sanders,[1] Zina Moldoveanu,[2] Jan Novak,[2] Bruce A. Julian,[2] Robert J. Wyatt.[1] *[1]University of Tennessee Health Science Center, Memphis, TN; [2]University of Alabama-Birmingham, Birmingham, AL.*

Gd-IgA1 is a key factor in the pathogenesis of IgA nephropathy (IgAN). Serum Gd-IgA1 levels are elevated in adult IgAN patients. However, serum Gd-IgA1 levels and their stability over time in pediatric IgAN patients, healthy children, and children with other glomerular diseases have not been assessed. To address this issue, serial serum samples from 15 children with biopsy-proven IgAN, 33 healthy controls, and 12 non-IgAN disease controls were analyzed for Gd-IgA1. Children with Henoch-Schonlein purpura were excluded. Serum samples from children (2-19 years) were obtained at the time of study entry and then at 6-month intervals, with 54 children sampled at 6 months and 38 children at 12 months. Serum Gd-IgA1 levels were determined by lectin ELISA using an *N*-acetylgalactosamine-specific lectin from *Helix aspersa*. Mixed linear modeling was used to determine statistical significance. At study entry, mean age (in years) of the healthy controls, disease controls, and patients with IgAN were 12.9±2.8, 11.2±3.5, and 11.2±3.5, respectively, with male:female ratios 1.5, 0.7, and 0.7. African Americans comprised 67% of healthy controls, 67% of disease controls, and 27% of patients with IgAN. Median (range) serum Gd-IgA1 levels (units/ml) at entry, 6 months, and 12 months for healthy controls were 284 (117-1009), 238 (129-910), and 215 (96-427); for disease controls 316 (176-716), 218 (116-987), and 280 (115-769); and for patients with IgAN 635 (315-1517), 684 (180-1516), and 647 (543-1342). When serum Gd-IgA1 levels from subsequent visits were compared with the levels at the initial visit within each group, no statistically significant difference was identified. Serum Gd-IgA1 levels were elevated in the patients with IgAN group compared with those for healthy children and disease controls ($p<0.0001$), adjusting for age, gender, and ethnicity. In summary, in this small pediatric cohort, serum Gd-IgA1 levels were elevated in patients with IgAN and appear to be stable over 12 months of follow-up in all cohorts, including children with IgAN or non-IgAN glomerular disease and healthy children.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2294

**Increased Urinary KIM-1 and Tubular KIM-1 Expression in IgA Nephroapathy** Soon Hyo Kwon, Moo Yong Park, Jin Seok Jeon, Jin Kook Kim, Dong-Cheol Han, Seung D. Hwang. *Internal Medicine, Soon Chun Hyang University, Korea.*

**Background:** Tubulo-interstitial fibrosis is one of the important prognostic factors in IgA nephropathy (IgAN). Kidney injury molecule-1 (KIM-1) is a specific marker of the proximal tubular injury. This molecule is increased in various renal diseases. It suggests KIM-1 could represent degree of tubular injury in IgAN.

**Method:** To investigate the role of KIM-1 in predicting the kidney injury of IgAN. We prospectively enrolled 50 patients with IgAN and 27 normal controls (NC). Spot urine KIM-1 standardized by urine creatinine were measured by ELISA and tubular KIM-1 was stained by immunohistochemistry. We classified pathologic classification of IgAN with Oxford classification. Quantification of tubular KIM-1 expression was defined as the count of the stained tubules.

**Results:** The concentration of urinary KIM/Cr of IgAN was significantly higher than that of normal control.($p<0.0001$)(1.32±0.18 vs 0.56±0.19) There was no significant difference of urinary KIM-1 level in the extent of severity of tubular lesion. (p=0.37)[T0= 1.7±1.7(n=26): T1=1.1±1.0(n=27): T2=0.6±0.4(n=7)] There was significant increase of tubular KIM-1 expression in T1, T2 lesions.($p<0.0001$)(2.2±2.0 vs 7.9±3.8; 6.5±0.7) Urinary KIM-1 was not correlated with proteinuria and tubular KIM expression.

**Conclusion:** KIM-1 is upregulated in IgAN. Tubular KIM-1 more predictable to estimate tubular injury than urinary KIM-1. There is a need for a study about clinical implication of high level of urinary KIM-1 in T0 lesion.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2295

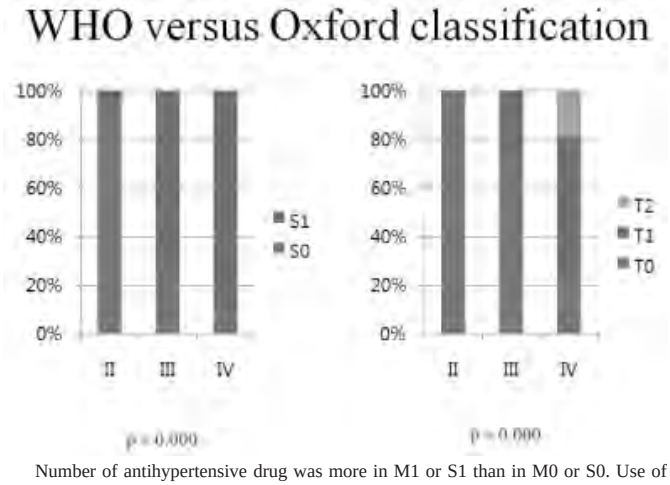
**Comparision between Oxford Classification and WHO Classification of IgA Nephropathy** Seokhui Kang,[1] Cheol Whee Park,[1] Young Soo Kim,[1] Chul Woo Yang,[1] Bumsoon Choi,[1] Young Jin Choi,[3] Byung Ha Chung.[1] *[1]Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's Hospital, Seoul, Korea; [2]Department of Pathology, Seoul St. Mary's Hospital, Seoul, Korea.*

IgA nephropathy is most common cause of glomerulonephritis. Prognostic factors of IgA nephropathy are pathologic finding, serum creatinine, hypertension, proteinuria. Althought pathologic classification of IgA nephropathy is important, most classifications for IgA nephropathy are complicated, difficult. Oxford classification for IgA nephropathy was reported in 2009. We compared clinical outcmes of different pathologic classification (Oxford, WHO classification). A retrospective study comprised of 103 patients of IgA nephropathy who underwent renal biopsy between 2000 and 2006.

Baseline characteristics

| | Mean ± SD |
|---|---|
| Total number of patients | 103 |
| Age (years) | 34 ± 11 |
| BMI (kg/m2) | 22 ± 3 |
| Taking antihypertensive drug | 28 (26%) |
| Proteinuria (g/day) | 2.69 ± 2.87 |
| Creatinine (mg/dl) | 1.06 ± 0.43 |
| MDRD GFR (ml/min/1.73m2) | 80 ± 25 |

WHO classification showed class I as 1%, II as 16.5%, III as 50.5%, IV as 32%. Oxford classification showed M1 as 96.2%, S1 as 67.3%. E1 as 15.4%, T1 as 32.7% and T2 as 5.8%. The higher class of WHO classification, S and T class of Oxford classification was higher.




Number of antihypertensive drug was more in M1 or S1 than in M0 or S0. Use of immunosuppressants was more patients with T1 or T2 than T0. Patients with E1 had more decline of proteinuria. In CKD stage 2 and 3, patients with E1 had less decline of GFR than E0. In CKD stage 2, patients with T1 and T2 had more decline of GFR than than T0. Oxford classification is well correlated with other classification regarding IgA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2296

**Oxford Classification of IgA Nephropathy: Is It Prognostic in Different Cohort of Patients?** Katia Lopez-Revuelta,[1] Dolores Martin-Rios,[2] Elena Corchete,[1] Mrenee Gomez,[1] M. Carmen Guerrero.[3] *[1]Nephrology Unit, Hospital Universitario Fundacion Alcorcon, Alcorcon, Madrid, Spain; [2]Preventive Medicine; [3]Pathology Unit, .*

Oxford classification of IgA nephropathy needs to be validated in different cohorts of patients.

Purpose: To study the clinico-pathological associations of Oxford IgA classification at biopsy and follow-up in a non-selected cohort of patients.

Methods: Retrospective cohort study of all IgA nephropathy biopsies (≥8 glomeruli) in one adult center between 05/1998-05/2009 reclassified using Oxford classification. Demographic, clinical and laboratory data at biopsy, yearly and at the end of follow-up were recorded. Primary endpoint was progression of renal disease (PRD): ESRD and/or 50% drop in eGFR. Follow-up period: months from biopsy until endpoint, dead or last follow-up. Statistical analysis included Kaplan Meier, Cox regression and linear regression models to assess predictive role of pathological data on renal function loss.

Results: 62 patients (86% male), median (SD) age 50(17)yr were included, 20% liver disease, 20% Schönlein-Henoch, 56% AKI. Clinical data are shown in Table 1.

Table 1. Clinical characteristics at biopsy and follow-up

| | At time of biopsy | At follow-up |
|---|---|---|
| Median (IQR) s Cr(mg/dl) | 1.66(1.97) | 1.66(1.59) |
| Median(IQR) eGFR (ml/min/1.73m2) | 43 (52) | 47(37) |
| %Stage 1-2,3,4,5 | 32,31,24,13 | 26,43,10,21 |
| %HTA | 68 | 99 |
| Median(IQR)uProt/cr (mg/mg) | 1.54(1.66) | 0.51(1.83) |

Median (IQR) follow-up (mo): 33(62)

Twenty two % reached study endpoint, and 10% died. Main histological data were: 97% M1, 48% E1, 40%S1, T1-2 40-3%, 11% had >25% crescents. Independent predictors of PRD: sCr at biopsy p<0.40, uPCR at last follow-up p<0.001, HR (95% CI) T1-2/0 5.69(1.36-23.78) p<0.01, E1/0 4.88 (1.02-23.34) p<0.05, liver disease 6.68 (1.35-33.22) p<0.20. Independent predictors of yearly sCr increment were E (p< 0.032) and sCr at biopsy (p<0.006).

Conclusion: New Oxford IgA classification helps to stablish risk of renal disease progression in patients with severe renal failure at the time of biopsy. Endocapillary hypercellularity (E) and interstitial fibrosis are independent predictors of renal outcome. Confirmation of these observations need larger studies.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2297

**IgA Nephropathy Associated with End-Stage Liver Disease Has Features of Membranoproliferative Glomerulonephritis and May Not Improve after Liver Transplantation** Donna S. Sanders,[1] Mohamed Mokhtar Desouki,[3] Stephen Osaguona,[1] Wayne R. Fitzgibbon,[1] Juan Carlos Q. Velez.[1] *[1]Medicine, MUSC, Charleston, SC; [2]Lifeline Vascular Center, Wichita, KS; [3]Pathology, MUSC, Charleston, SC.*

IgA nephropathy (IgAN) presents as primary or secondary glomerulonephritis. The most common cause of secondary IgAN is end-stage liver disease (ESLD). Because of the bleeding risks intrinsic to ESLD, subjects with IgAN secondary to ESLD (ESLD-IgAN) are rarely biopsied. Thus, the histopathological features of this entity, compared to primary IgAN, are poorly recognized. Moreover, the prognosis after liver transplantation (OLT) is not well known. Therefore, we reviewed non-transplant kidney biopsy of all IgAN cases diagnosed at our institution from 1994 to 2009 (n=57), in order to compare the histology of ESLD-IgAN with primary IgAN. We found 8 cases (14%) of ESLD-IgAN. In comparison to primary IgAN (n=49), we observed a significant increase in membranoproliferative (MPGN) changes [glomerular basement membrane (GBM) double contour] in ESLD-IgAN (p<0.001). Similarly, we found an increase in C1q by immunofluorescence (p<0.005) as well as in reduplication of the GBM and subendothelial and mesangial deposits by electron microscopy (p<0.01). Subjects with ESLD-IgAN were all male, older (52 ± 8 vs. 36 ± 9) and had a higher serum creatinine at the time of the biopsy (4.7 ± 2.6 vs. 2.3 ± 0.5 mg/dl). Positive Hepatitis C viral (HCV) serology did not account for the MPGN features [3 of 5 HCV (-) cases had MPGN features vs. 1 of 3 HCV (+) cases]. Three patients with ESLD-IgAN underwent OLT. At 6 months post-transplant, none of the patients had resolution of hematuria or proteinuria or improvement in kidney function. One patient had a biopsy 4 months post-OLT showing persistence and worsening of the IgAN findings. In conclusion, ESLD-IgAN commonly has histological features of MPGN that are not accounted by positive HCV serology. Liver transplantation does not uniformly result in reversal of ESLD-IgAN as has been previously reported. Prospective studies are needed to improve our understanding of this entity and design outcome-oriented strategies.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2298

**Tumor Necrosis Factor-α Gene (TNFA) G-308A Polymorphism Is Not Associated with IgA Nephropathy (IgAN): A Meta-Analysis** Ioannis Stefanidis,[1] Theodoros Eleftheriadis,[1] Christos Bantis,[4] Peter R. Mertens,[2] Vassilios Liakopoulos.[3] *[1]Nephrology, University Thessaly, School of Medicine, Larissa, Thessaly, Greece; [2]Nephrology, Otto von Guericke University Magdeburg, School of Medicine, Magdeburg, Germany; [3]Nephrology Ahepa Hospital, School of Medicine, Aristoteleion University, Thessaloniki, Greece; [4]Nephrology, Hippokrateion Hospital, School of Medicine, Aristoteleion University, Thessaloniki, Greece.*

Tumor necrosis factor α (TNF-α) is an inflammatory cytokine, pathogenetically involved in IgAN. A polymorphism in the promoter region, at position -308 (G/A) of TNFA is associated with increased TNF-α production. However, previous association studies of this polymorphism and IgAN rendered contradictory findings. Therefore, a meta-analysis of these association studies was conducted.

Five studies involving 723 cases of IgAN and 910 healthy controls were analyzed. Three included European and two east Asian populations. The meta-analysis examined the association of allele A with IgAN or with progressive IgAN relative to allele G. Associations were indicated as odds ratios (OR) with the corresponding 95% confidence interval (ci). Heterogeneity between studies was tested using the Q-statistic.

Meta-analysis suggested large heterogeneity between studies (p=0.024, $I^2$=64%) and no significant association between G-308A transition and the risk of developing IgAN: random effects OR=0.78[95%ci(0.54, 1.13)]. Sensitivity analysis (exclusion of one European study with controls not in HWE) showed no heterogeneity (p=0.15, $I^2$=44%) and no significant association: fixed effects OR=0.93[95%ci(0.72, 1.20)] and random effects OR=0.90[95%ci(0.64, 1.27)]. Both, subgroup analysis for the Asian and the European population, and sensitivity analysis in the Caucasian population, produced no significant association. For studies investigating the role of the polymorphism in IgAN progression, no significant association overall and in subgroups was detected.

There was no significant association between TNFA G-308A polymorphism and the development or progression of IgAN. Analysis is based on a relatively small number of studies and participants and any inferences have to be cautious.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2299

**Skin-Autofluorescence Is Associated with Amount of Proteinuria and Tubulointerstitial Damage in Patients with IgA Nephropathy** Kenichi Tanaka, Yoshihiro Tani, Jun Asai, Yuki Kusano, Hodaka Suzuki, Yoshimitsu Hayashi, Koichi Asahi, Tsuyoshi Watanabe. *Nephrology and Hypertension, Fukushima Medical Univerity, Fukushima City, Japan.*

**Background.** Tissue accumulation of advanced glycation end-products (AGE) is thought to be a contributing factor to the progression of vascular complications in renal disease. Our recent study showed that skin AGE accumulation is related to renal function and cardiovascular disease in chronic kidney disease (CKD) patients (*Nephrol Dial Transpl; in press, 2010*). The present study aimed to evaluate relationships of skin AGE accumulation to clinical parameters such as proteinuria and histopathological damage in IgA nephropathy (IgAN) patients in early stage of CKD.

**Methods.** Subjects in this cross-sectional analysis comprised 50 untreated biopsy-proven IgAN patients (median age, 54.0 years; median eGFR, 66.3 ml/min/1.73 $m^2$; CKD stage 1, n=12; stage 2, n=16; stage 3, n=22). Patients with diabetes or CKD stage over 4 were excluded. AGE accumulation in skin was assessed by skin-autofluorescence (AF) using an AF reader at the time of renal biopsy. Histopathological changes were classified into four glomerular grades and three tubulointerstitial grades. Relationships between skin-AF, clinical parameters, and histopathological grade were evaluated.

**Results.** Skin-AF significantly correlated with age (r=0.45, *P*<0.01), systolic blood pressure (r=0.36, *P*<0.01), diastolic blood pressure (r=0.45, *P*<0.01), amount of proteinuria (r=0.35, *P*=0.01), duration of proteinuria (r=0.39, *P*<0.01), eGFR (r=-0.60, *P*<0.01), glomerular grade (r=0.43, *P*<0.01), and tubulointerstitial grade (r=0.43, *P*<0.01). Multiple regression analysis revealed significant correlations of skin-AF with age (β=0.46, *P*<0.01), amount of proteinuria (β=0.26, *P*=0.02), and tubulointerstitial grade (β=0.30, *P*=0.01) ($R^2$=0.54).

**Conclusions.** Tissue AGE accumulation measured as skin-AF was independently related to amount of proteinuria and tubulointerstitial damage in IgAN patients in early stage of CKD. Non-invasive AF readers may provide useful additional markers for clinical risk assessment in daily practice, reflecting renal histopathological change in these patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2300

**Medium Dose of Cyclosporine Combined with Methlprednisolone in IgA Nephropathy: Prospective Randomized Controlled Trial** Hong Liu, Xiaoqiang Ding, Yi Fang, Xialian Xu, Xiaoyan Zhang, Yihong Zhong. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

**Background.** IgA nephropathy (IgAN) is the most common form glomerulonephritis in worldwide. Approximately 30% of patients will progress to end-stage renal disease (ESRD) after 10-20 years. High dose cyclosporine A (CsA) can reduce proteinuria but have no renal protective effect. In this study, we aim to evaluate the effects of a combined therapy with medium dose of CsA and methprednisolone on clinic outcomes.

**Methods.** It is a prospective randomized controlled trial.43 IgAN patients aged 18-70 years with proteinuria >1g/24hours and eGFR≥30ml/min.1.73$m^2$ . Patients were divided into two groups: (1)full-dose steroid treatment group (methlprednisolone 0.8mg/kg for 8 weeks gradually reduced to maintenance doses 4mg/d), and (2)combined therapy group (methprednisolone 0.6mg/kg and CsA 3mg/kg/d as the initial dosage, then maintained with 4mg/d and 25-50mg/d, respectively. ) The primary end point was reduction of proteinuria by 50% or more. Secondary end point was a 50% increase of serum creatinine level or 25% decrease in eGFR.

**Results:** After 12 months treatment, 24 hr urinary protein excretion declined from 3.23±3.31g to 0.37±0.23g (*P*=0.002) in combined therapy group. Patients in steroid group also experienced a significantly remission on proteinuria (0.38±0.27g/24h vs 2.87±2.20 g/24, P=0.0001). The complete remission rate was 52.63% and 47.37% in combiened group and steroid group respectively. Patients undertook combined therapy achieved significantly improvement on eGFR after 9 months (91.0±32.02 vs 78.75±23.12ml/min.1.73$m^2$, P=0.02)while the steroid group patients achieved significantly increased of eGFR after 3 months(100.10±28.10 to 86.46±26.12 ml/min.1.73$m^2$ (P=0.011). The incidence of a transient drop of eGFR (<25%) was 27.27% in combined therapy group and 4.76% in steroid group .

**Conclusion:** Full dose of steroid treatment and the combined therapy with medium dose of CsA and steroid both can remarkably reduce proteinuria and ameliorate renal function in patients with IgAN without sever adverse events, however, the combined trerapy didn't show any additional benefit on outcomes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2301**

**Prognostic Factors in Childhood IgA Nephropathy with Focal Mesangial Proliferation Treated with Angiotensin Converting Enzyme Inhibitors**
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Objective: It is generally considered that the prognosis of childhood IgA nephropathy (IgAN) is unpredictable. In Japan since the beginning of the 1990s, generally in pediatric area, angiotensin converting enzyme inhibitors (ACEIs) have been used for children with IgAN showing focal mesangial proliferation (FMP). Details of the effectiveness and prognostic factors of them remain unclear. The purpose of this study is to clarify them in childhood IgAN with FMP treated with ACEIs.

Methods: We analyzed retrospectively consecutive 65 children with newly diagnosed childhood IgAN with FMP treated with ACEIs from August 1987 to October 2007 and observed for more than 2yrs after the start of ACEIs treatments.

Results: Mean observation period was 4.1±1.9 years. Among 65 children, 28 children (43.1%) showed proteinuria remission after 2yrs of ACEIs treatments. There was no significant difference in clinical findings (onset mode, blood pressure, urinary protein excretion, and serum IgA level) and in pathological findings (scleroses, crescents, adhesions, and mesangial proliferation) depending on proteinuria remission after 2 yrs of ACEIs treatments.

The prognostic factor related to proteinuria remission after 2 yrs of ACEIs treatments was the ratio of glomeruli showing crescents at diagnosis in multivariate analyses (OR 0.66, 95%CI 0.41-0.88, p=0.02). The cut off point of the ratio in ROC curve was 8.8%.

Conclusions: The proteinuria remission rate in childhood IgAN with FMP after 2 yrs of ACEIs treatments was 43.1%. If the patients show the ratio of glomeruli showing crescents of over 8.8%, we may have to consider another treatment option, because such patients are likely to be refractory to ACEIs treatments.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2302**

**Urinary Angiotensinogen Reflects Clinico-Pathological Findings in IgA Nephropathy**
Chun-Gyoo Ihm, Se-Bin Song, Dongyoung Lee, Ju-Young Moon, Kyung-Hwan Jeong, Tae Won Lee. *Division of Nephrology, Kyung Hee University, School of Medicine, Seoul, Republic of Korea.*

**Introduction**: Urinary angiotensinogen (uAGT) provides a specific index of intrarenal renin-angiotensin system (RAS). However, its role is not evident in IgA nephropathy (IgAN). This study was performed to exam the levels of uAGT in patients with IgAN and to find the relation of uAGT to clinicopathologic findings and mRNAs of renin, angiotensin converting enzyme (ACE) and ACE2 from renal biopsy tissue.

**Methods**: Forty patients with IgAN and 15 healthy volunteers were included. Samples of serum and random morning urine were obtained. The AGT concentration was measured with commercial human ELISA kits.

**Result**: The patients with IgAN had higher uAGT (113.1 ng/mgCr vs 9.5 ng/mgCr) and lower GFR (82.6 mL/min/1.73m$^2$ vs 116.6 mL/min/1.73m$^2$) compared with those of healthy volunteers. When patients were grouped by their uAGT levels of above (Group A) and below 100 ng/mgCr (Group B), group A showed significantly lower eGFR (65 vs 87 mL/min/1.73 m$^2$) and higher urine protein/creatinine ratio (PCR) (2.91 vs 0.78 g/gCr) and higher degree of hematuria (3.5 vs 2.8 grade) compared to group B. uAGT levels correlated with serum AGT levels (r=0.33), amount of proteinuria (r=0.45), diastolic blood pressure (r=0.32), tubulointerstitial inflammation (r=0.52) and tubular atrophy (r=0.52) in pathologic findings. Also uAGT correlated negatively with Δurine PCR at 0-3mo (r=0.41) regardless of treatment. However, it had no correlation with renin, ACE and ACE2 mRNAs from renal biopsy tissue.

**Conclusion**: uAGT can be considered a good index of clinic-pathological activity in IgAN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2303**

**IgA-Dominant Postinfectious Glomerulonephritis**
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**Background:** Pathologic findings in postinfectious glomerulonephritis (PIGN) are various, and the subtypes may have different clinical significances. IgA-dominant PIGN was recently reported as a unique subtype of PIGN, but its clinicopathologic features were not clearly characterized. We present demographic, clinical and renal biopsy findings from 7 patients with IgA-dominant PIGN.**Methods:** All renal biopsy specimens (n=1119) processed from 2005 to 2009 by the Department of Pathology in Hanyang University Hospital, Seoul, Korea were reviewed. Seven patients with IgA-dominant PIGN were identified, and their clinical data were analyzed. **Results:** The patient age ranged from 31 to 84 years, and males were 6 out of 7. All glomeruli showed diffuse endocapillary proliferation, infiltration of neutrophils, granular IgA and C3 deposits along the peripheral capillary walls and in the mesangium, and subepithelial 'humps' with intramembranous and mesangial electron dense deposits. All patients had renal insufficiency (mean serum creatinine ± SD,3.1±1.0 mg/dL), hematuria and heavy proteinuria. End-stage renal disease was developed in 2 patients, chronic renal failure was stationary in 2, and azotemia was improved in 3. Two patients had recent methicillin-resistant *Staphylococcus aureus* infection, and one recent Rickettsial infection. Only one was diabetic, and none of our patients had any underlying renal disease. **Conclusions:** We demonstrate that IgA-dominant PIGN is various in its causes of preceding infection, demographic profiles, course and prognosis. It is often associated with overt staphylococcal infections, and the underlying infection may be subclinical or rickettsial. Both non-diabetic and diabetic patients can be subject to this form of PIGN. The prognosis could be despaired in some cases with PIGN, and other bad prognostic factors than underlying diabetic nephropathy and renal insufficiency should be identified

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2304**

**The Oxford IgA Nephropathy Classification Predicting 1-Year Renal Outcome**
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**Background:** There had been no international consensus for pathological classification for IgA nephropathy until Oxford classification was presented in 2009. Oxford classification was reported to predict the average rate of renal function decline. But it did not show the association of renal outcome with the pathologic lesions at specific time-point. The present study tested whether Oxford classification is useful to predict renal outcome of patients with IgA nephropathy at 1-year after biopsy.

**Methods**: The diagnosis of IgA nephropathy was made between 2006 and 2009 on 181 adult patients. We retrospectively re-analyzed the cohort according to Oxford classification system for IgA nephropathy and collected clinical parameters within 1 months of date of biopsy and during 1-year follow-up. We also included the global sclerosis for clinical relevance in the patients.

**Results:** The subjects consisted of 90 males and 91 females. At the time of renal biopsy, the median age was 37 years (18-77 years). There were a median number of 17 glomeruli per biopsy. By Oxford classification, 52.5% and 48.7% of patients had mesangial hypercellularity or segmental glomerulosclerosis. Tubular atrophy/interstitial fibrosis was seen in 63 cases (34.8%). Severe pathologic lesions by Oxford classification were correlated with high grade WHO classification. Mesangial score, segmental glomerulosclerosis, tubular atrophy/interstitial fibrosis, and global sclerosis were strongly associated with eGFR at the time of biopsy. By multivariate analysis, the rate of renal function decline at 1-year was significantly associated only with tubular atrophy/interstitial fibrosis.

**Conclusions:** The study demonstrates tubular atrophy/interstitial fibrosis has an independent value in predicting 1-year renal outcome of patients. Other pathologic lesions of Oxford classification and global sclerosis were correlated with renal function at the time of biopsy but not at 1-year.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2305**

**Graft-Versus-Host Disease and Membranous Nephropathy in Patients of Post-Allogeneic Haematopoietic Stem Cell Transplantation**
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We studied the clinical and pathological features and pathogenesis of the patients with post-allogeneic haematopoietic stem cell transplantation (Allo-HSCT) membranous nephropathy (MN) and investigated the relationship between the Allo-HSCT MN and graft-versus-host disease (GVHD). Five patients with Allo-HSCT MN which proved by renal biopsy were involved in this study. The clinical manifestations, laboratory data, and morphology, immunefluorescence and electron microscopy of the renal pathology of those patients were analyzed. Moreover, the IgG subclasses in glomerular deposits and the co-deposition of nephrin and IgG4 were studied. Finally, We detected the Anti-PLA2R(M-type phospholipase A2 receptor) autoantibodies in serum samples from patients with Allo-HSCT MN. The following items are the clinical and pathological features of the 5 patients: (1) All of the 5 patients have no history of kidney disease. (2) All patients were combined with the syndrome of chronic GVHD at the time of proteinuria presence, and 4 patients had a history of acute GVHD, after an effective anti-GVHD treatment, the patients' proteinuria all remissioned. (3) The autoantibodies for some of the patients were positive and were accompanied by the deposition of C4 and C1q in kidney, suggesting the existence of autoimmune phenomenon. (4) The IgG subclasses showed that all patients were positive for IgG1 and IgG4, and negative for IgG3. The staining intensity of IgG1 and IgG4 were the same in two cases, but in three cases the dominant subclass of IgG deposition was IgG4. Moreover, the distribution of IgG4 was as same as nephrin.(5) The Anti-PLA2R autoantibodies were negative other than one case with positive result.

Our data suggest that the MN of Allo-HSCT patients is a form of cGVHD. It is characterized by coincidence of proteinuria and cGVHD, remission of proteinuria after anti GVHD immunosuppressant therapy, and the Anti-PLA2R autoantibodies were negative in most of the patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2306**

**Efficacy, Durability, and Immunological Analyses Following Rituximab Therapy in Idiopathic Membranous Nephropathy: A 2-Year Study**
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**Introduction:** Pharmacokinetic (PK) analysis in patients with Membranous Nephropathy (MN), treated with Rituximab (RTX) dosed at 1g x 2 showed that RTX levels were 50% lower compared to non-proteinuric patients. This study tested the hypothesis that weekly doses of RTX would result in more effective B cell depletion, a higher remission rate while maintaining the same safety profile.

**Methods:** Twenty patients (11 failed prior therapy) with MN and proteinuria (P) >5g/24h received RTX (375mg/m$^2$ x 4), with retreatment at 6 mo regardless of P response. Analysis of B and T cell subsets were performed to clarify the role of RTX on lymphocyte subpopulations. A detailed PK analysis was also repeated.

**Results:** Baseline P=11.9±4.9g/24h decreased to 4.2±3.8g/24h and 2.0±1.7g/24h at 12 and 24 months, respectively (p<0.001) while creatinine clearance increased from 72.4±33 at baseline to 90.2 ±32 ml/min/1.73m$^2$ at 24 months (p=0.02). Of 18 patients who completed 24-mos follow up, 4 are in complete remission, 12 are in partial remission, 1 has a limited response (>50% drop in P but >3.5g/24h) and 1 relapsed. Serum RTX levels were similar to those obtained with 2 doses of RTX.

**Conclusion:** Four weekly course of RTX is effective in reducing P in a significant number of patients with MN, however, P reduction was similar to RTX 1g x 2. B cell depletion was more profound and persisted longer when compared to our previous study. We found no baseline B cell abnormalities nor any quantitative abnormalities in B cell subsets that helped predict response to RTX therapy.

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**SA-PO2307**

**PLA2R Specific Autoantibodies in Patients with Membranous Glomerulonephritis (MN) – The Effect of Rituximab**
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Background

Idiopathic membranous glomerulonephritis (IMN) is a leading cause of the nephrotic syndrome in adults. Autoantibodies (AAb) against M-type phospholipase A2 receptors (PLA2R) might be involved in disease induction. PLA2R in the kidney are exclusively expressed on glomerular podocytes. Detection and titration of AAb in patients with IMN may help to guide therapy and the monitoring of therapeutic effectiveness.

Methods

In 12 patients with biopsy proven MN, PLA2R-specific AAb titers were measured in serum by indirect immunofluorescence using HEK293 cells which were transiently transfected with full-length PLA2R isoform 1 cDNA. In addition to supportive therapy (ACE-i, ARB, diuretics, statins, anti-coagulation) or immunosuppressive therapy (3 patients with glucocorticoids, 2 with glucocorticoids + MMF, 1 with glucocorticoids + CNI) all patients were treated with 375 mg/m² rituximab (RTX). 10 patients had at least 1 immunosuppressive therapy before RTX therapy.

Results

PLA2R-specific AAb were detected in 7 of 12 patients tested. Following RTX therapy, anti-PLA2R-AAb levels decreased in 4 patients within 1 week (w), 2 of these patients had no detectable AAb after 3 and 6 months (m), respectively. 1 patient had a decrease in AAb levels after 3m, 2 had constant levels over 3m.

In 3 patients anti-PLA2R-AAb levels increased 3m to 6m after RTX therapy. In 1 of these patients, anti-PLA2R-AAb levels never decreased after RTX. In the other 2 patients anti-PLA2R-AAb levels decreased after RTX therapy and increased again after 3m and 6m.

Proteinuria decreased in all patients with decrease in AAb titers. Patients with an increase in AAb titers had a paralleled increase in proteinuria.

Conclusions

58.3% of our patients with biopsy proven MN had anti-PLA2R AAb in serum. Changes in AAb titer upon RTX therapy were paralleled by changes in proteinuria.

These results indicate that anti-PLA2R AAb may help to guide and monitor the follow-up of patients with IMN following RTX. A prospective study in a large cohort, however, is necessary to validate these preliminary findings.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2308**

**Glycan Analysis of Serum IGG4 in Idiopathic Membranous Nephropathy Using Lectin Microarray**
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IgG4, the major IgG subclass in glomerular deposits and of anti-phospholipase A2 receptor (PLA2R) antibody (Ab) in idiopathic membranous nephropathy (IMN), is incapable of activating the classical complement pathway. However, experimental and clinical investigations indicate that complement dependent mechanism may play roles in podocytes damage. These suggest the possibility that the alternative or mannan binding lectin pathways may be involved in the pathogenesis of the disease. In this study, we analyzed serum IgG4 glycan profiles using lectin microarray to examine whether IgG4 in IMN have unique glycan structure, which is responsible for activating the complement pathway.

Serum samples were obtained from 6 patients with IMN, 4 patients with benign nephrosclerosis (BNS), 2 patients with minimal change disease (MCD), and 6 healthy individuals (normal control, NC). From each sample, IgG4 was enriched by immunoprecipitation with anti-IgG4 Ab. For glycan analysis, IgG4 were labeled with Cy3 and were subjected to lectin microarray in serial dilutions. The glycan profiles were analyzed using the dilution, which showed the comparable concentration of IgG4. Signal intensity was standardized using the lectin, which showed a good linearity in the dose-response curve in all of the samples.

From 10µl of sera, 0.5-1.0µg of IgG4 were obtained without contamination of IgG1or IgM. Compared to the NC group, the IMN group showed slightly higher signals for core fucose (Fucα1-6GlcNac) binders (e.g. PSA and LCA) and α2-6 Sialic acid binder (e.g. SNA and SSA). However, those IgG4 glycan profiles found in IMN were also found in the BNS and the MCD group.

In summary, although serum IgG4 in IMN patients showed a particular glycan structure compared to the NC group, this structure was also found in other proteinuric diseases. Further studies, analyses of glycan profiles of IgG4 anti-PLA2R Ab, or IgG4 eluted from the kidneys of IMN are required to understand the role of mannan binding lectin pathway in IMN.

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**SA-PO2309**

**Efficacy and Safety of Mycophenolate Mofetil for Patients with Idiopathic Membranous Nephropathy: A Systematic Review**
Yan Song, Jianyong Wu, Jianghua Chen. *Kidney Disease Center, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.*

Mycophenolate mofetil (MMF), an immunosuppressive drug acting by impairing *de novo* purine synthesis in T and B lymphocytes, was recently off-label used for treating idiopathic membranous nephropathy (IMN). We searched PubMed database to perform a systematic review of relevant English-language articles until September 2009. Articles accessing the efficacy and safety of MMF for treating IMN were included in this review. Sixteen articles including 153 patients, composed of 3 randomized controlled trials (RCTs), 1 clinical controlled trial, 5 observational studies, 5 case series, and 1 case report, were included in this review. Results were described qualitatively instead of being pooled because of heterogeneity of the included studies. Most patients were steroisds/immunosuppressants-resistant. Two RCTs showed that MMF therapy (concomitant with steroids) was as effective as the comparative immunosuppressants to reduce proteinuria. However, one RCT showed that MMF monotherapy had no benefit to proteinuria comparing with conservative treatment. Most observational studies without controls and case series/reports showed MMF was efficient in decreasing proteinuria. Adverse effects were not well described in most studies. MMF can effectively reduce proteinuria and improve/stabilize renal function in most reported studies. However, the evidence did not show that the drug is superior to conventional immunosuppressants. Adverse effects of MMF for treating IMN were not well described in most studies. Double blind RCTs with larger number of patients and longer term follow-up are required to evaluate the efficacy and safety of MMF monotherapy in IMN patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2310**

**Tacrolimus Monotherapy for Idiopathic Membranous Nephropathy: Long Term Outcomes**
Marie B. Condon, Nicholas R. Thomas, Neill D. Duncan, Tom Cairns, Megan Griffith. *Imperial College Kidney and Transplant Institute.*

Nephrotic syndrome secondary to idiopathic membranous nephropathy (IMN) is associated with poor renal out come. We have previously reported effective induction of remission with tacrolimus (Tac) monotherapy. We now report the long term outcomes of Tac monotherapy.

Longterm data was available on 29 nephrotic patients (20 male, 49+/-15.1yrs) with biopsy proven IMN commenced on tacrolimus monotherapy. 5 patients were converted from CyA and 3 from oral pred. In addition all received standard treatment with ace inhibitors/ARB. Complete remission (CR) was defined as a normal serum albumin with proteinuria <0.5g/day, partial remission (PR) as normalisation of serum albumin (>33g/l) with residual proteinuria,

Mean follow up was 45.3+/-20.28 months. Mean serum albumin at presentation was 21.3+/-6.4g/L. Remission was achieved in 24 patients (82.7%) at an average of 18.3+/-13.2 months: 18 (75%) achieved CR and 6 (25%) achieved PR.

3/24 patients achieving remission on Tac relapsed whilst on Tac at 34+/-22.7 months post remission, they have been switched to alternative treatment regimes.

5/24 in CR had Tac withdrawn. Time on Tac 49.6+/-17.6mths. Pre withdrawal biopsies were done on 2 patients, both showed resolving lesions with no new deposits. 3/5 have relapsed at 5+/-1.73 months post Tac withdrawal, including those biopsied. 2 of the 5 patients remain in remission off Tac at 7 and 18 months respectively.

16/24 remain on Tac (mean duration 44.8+/-20.8 months) their mean current creat is 124.7+/-48.0umol/L, which is not a significant difference from their baseline mean creat of 108.3+/-41.2umol/L (p=0.047 paired t-test).

5 patients did not achieve remission; 2/5 have also failed to achieve remission with alternative immunosuppressive regimes, suggesting resistant disease, 1/5 achieved PR with the addition of Mycophenolate mofetil.

Tac is an effective treatment of IMN, but longterm treatment is often required, and patients relapse both on and off treatment. Additional work on predictors of relapse including biopsy features is required. Additional agents may be needed to prevent relapse, and this is currently being investigated (Clinical trials.gov ref no. NCT00843856)

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2311

**ACTH Treatment of Nephrotic Patients with Membranous Nephropathy – Long Time Follow Up** Anna-Lena Berg. *Department of Nephrology, University Hospital, Lund, Sweden.*

**Background**

In 1994, we published the novel finding that ACTH at pharmacological dosage has a considerable lipid-lowering effect in healthy subjects. Short-term treatment with ACTH decreased ApoB-containing lipoproteins also in patient groups with various lipid disorders. In one of the follow-up studies of the ACTH-specific lipid-lowering effect I also found an ACTH-specific antiproteinuric effect in patients with membranous nephropathy. Synthetic adrenocorticotrophic hormone (ACTH 1-24) has been given to nephrotic patients with membranous nephropathy in about 15 years at the department of nephrology, university hospital in Lund, Sweden.

**Methods**

ACTH was given subcutaneous first 1 mg weekly during four weeks then increased to 0.75 mg and later in some patients to 1 mg twice a week. The treatment continued at this dose until two months after clinical remission and then it was lessened during three months. The patients were individually treated during about 9 to 15 months. 30 of the treated patients have been observed in this follow up study.

**Result**

The results in 30 patients after 8 (3-13) years are presented (medians). Before treatment albumin in urine was 7.92 (3.71-16.32) g/L, in serum 19 (10-27) g/L and serum creatinine was120 (46-310) micromol/L and at the end of the follow-up period the values were 0.19 (0-1.30) g/L, 38 (32-48) g/L and 128 (42-388) micromol/L respectively. Five of the 30 patients had relapsed during the follow up period and were treated again with ACTH. One patient developed end stage kidney disease and received a kidney transplant.. The side effects during ACTH treatment were mild and reversible.

**Conclusion**

Most of the patients seem to have a lasting decrease of albumin in the urine and a lasting increase in the serum after treatment. Serum creatinine was unchanged after the follow up period.

Speculations

Glucocorticosteroids has unproven effect on membranous nephropathy. ACTHs effect in membranous nephropathy might be mediated by melanocortinreceptors, (MCR 1-5). MCR 1 and 3 are known to be located in white blood cells and MCR 1 in the kidney. MCR 2 acts in the adrenals for production of cortisol.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2312

**Anti-PLA2R-Antibodies in a Patient Recurrent Membranous Nephropathy after Renal Transplantation** Elion Hoxha,[1] Kai Fechner,[2] Rolf A. Stahl.[1] *[1]Department of Internal Medicine III, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Institute for Experimental Immunology, EUROIMMUN AG, Lübeck, Germany.*

Introduction

Up to one third of patients with membranous nephropathy (MN) develop end stage renal disease (ESRD). Following renal transplantation MN recurs in up to 40% of patients. Recurrence of disease is often seen at a very early stage, even days after transplantation. The recently discovered autoantibodies (AAb) against M-type phospholipase A2 receptor (PLA2R) which may be responsible for the induction of idiopathic MN, could play a major role in recurrent MN after renal transplantation.

Methods

In three patients with biopsy proven MN, PLA2R-specific AAb titers were measured from serum collected before and after renal transplantation. AAb titers were measured by indirect immunofluorescence (IIF). HEK293 cells were transiently transfected with full-length PLA2R isoform 1 cDNA and used as substrates for IIF.

Results

Anti-PLA2R-AAb could be detected in serum from one patient before renal transplantation. Three days after transplantation the patient developed proteinuria, which increased to 9 g/24h. After renal biopsy showed recurrent MN, the patient was treated with 375 mg/m² rituximab in addition to the standard immunosuppressive therapy with mycophenolate mofetil, cyclosporine and corticosteroids. Following rituximab, anti-PLA2R-AAb levels fell from 1:100 to 1:10 at 3 months and remained at 1:10 after 5 months. Proteinuria decreased from 9.0 g/24h to 3.1 mg/24h after 5 months.

The remaining two patients had no detectable anti-PLA2R-AAb in serum before transplantation or during the follow-up time, 3 months and 1 year respectively. In this time, both these patients showed no proteinuria and no recurrence of disease.

Conclusions

Anti-PLA2R-AAb can be persistent in patients with endstage renal disease due to membranous nephropathy. These AAb could be responsible for the recurrence of MN in the renal transplant. Measurement of anti-PLA2R-AAb titers of patients with MN on dialysis prior to renal transplantation may discover a potential risk for recurrence of disease in the transplant and may modify immunosuppressive therapy for these patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2313

**Successful Remission Induction of Nephrotic Syndrome in a Patient with M-Type Phospholipase A2 Receptor (PLA2R) Autoantibodies with Immunoadsorption Therapy Followed by Rituximab** Mario Schiffer, Jan T. Kielstein, Bernhard M. W. Schmidt, Hermann G. Haller, Marion Haubitz. *Nephrology, Hannover Medical School, Hannover, Germany.*

**Background:** Recently a close association of idiopathic membranous nephropathy and autoantibodies against the M-type phospholipase A2 receptor (PLA2R) was described. However, even though 70-80% of patients are positive for anti-PLA2R-antibodies a real causative relationship has not been conclusively demonstrated. Here we describe a case where the Ponticelli-Scheme was not successful for remission induction, however immunoadsorption therapy and rituximab treatment led to remission induction which correlated with the reduction of anti-PLA2R-antibody levels.

**Case report:** A 37 year old patient was diagnosed with idiopathic membranous nephropathy (II-IV) in October 2005 after the patient had developed a pulmonary embolism. Proteinuria was 22 g/day and renal function was normal. Treatment with prednisolon and cyclosporin led only to a transient and insufficient reduction in proteinuria (13g/d) and renal function detoriated. Cyclosporin A was stopped and steroids and oral cyclophosphamide (in monthly alteration, according to the Ponticelli-Schema) was initiated. Renal function was stabilized but only a partial and transient improvement was seen (proteinuria 6g/d). In January 2010 the patient presented with a proteinuria of 17g/d MDRD-clearance was 80 ml/min at that time. The patient was diagnosed to be highly positive for anti-PLA2R-antibodies and was treated with 5 cycles of immunoadsorption therapy with a Globaffin®adsorber (Fresenius Medical Care, Germany) which contains synthetic peptide-GAM® as ligand known to be very effective in binding of IgG4 and in addition he received 4 cycles of rituximab (375mg/m2). Proteinuria 2 months after treatment was 8.3g/d and further decreased to 5g/d 3months after treatment (at the time of abstract submission). **Conclusion:** This case indicates a good correlation with anti-PLA2R-antibodies and proteinuria and demonstrates a successful partial remission induction by removal of anti-PLA2R-antibodies using immunoadsorption and rituximab.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2314

**Maintenance of Hemoglobin Levels with Once-Monthly C.E.R.A. in Patients with Chronic Kidney Disease Not on Dialysis: The PRADO Study** Jean-Philippe Ryckelynck,[1] Vincent L. M. Esnault,[2] Bertrand Knebelmann,[3] Maurice Laville,[4] Christophe Pompon,[5] Bernard Richalet.[6] *[1]Nephrology, CHU Clemenceau, Caen, France; [2]Nephrology, CHU Pasteur, Nice, France; [3]Nephrology, CHU Necker, Paris, France; [4]Nephrology, CHU E. Herriot, Lyon, France; [5]Roche, Neuilly-sur-Seine, France; [6]Nephrology, CH Memorial France Etats-Unis, Saint-Lo, France.*

**Objective:** Methoxy polyethylene glycol-epoetin beta is a Continuous Erythropoietin Receptor Activator (C.E.R.A.) approved for once-monthly treatment of renal anemia in chronic kidney disease patients. This prospective, single-arm, open-label, multicentre study investigates direct switch from shorter acting Erythropoiesis Stimulating Agents (ESA) to once-monthly C.E.R.A. in a real-life setting.

**Material and Methods:** Predialysis patients with stable ESA treatment and adequate iron status were directly converted from SC epoetin β or darbepoetin α to SC C.E.R.A. once-monthly for a periode of 48 weeks (16 for dose ajustment, 8 for evaluation and 24 for follow-up). We here report on the primary endpoint: Hb stability between 10 and 12g/dL during evaluation (weeks 16-24).

**Results:** 127 patients were included in the present analysis. 58% female, age 71±11 years, 36% diabetics, baseline eGFR 24±8 ml/mn/1.73m². ESA treatments at inclusion were: epoetin β in 62% and darbepoetin α in 38% of patients, with median weekly doses of 3000 IU and 17.5 µg respectively. Baseline ferritin was 226±157 µg/L and TSAT was 24±10%. The Hb level was 11.2±0.5 g/dL during the run-in period (week -4, -2 and D0) and 11.7±0.8g/dL during the evaluation period (week 16, 20 and 24). 50% of the patients were in the target between 10 and 12g/dL during the evaluation period. 10 patients (9%) with at least one missing Hb during evaluation were not considered as in the target. 87% were between 10 and 13g/dL The monthly dose of C.E.R.A. was 125±20µg at D0 and 103±60µg at week 20. The safety profile was comparable to published data. No serious adverse event has been considered as related to C.E.R.A.

**Conclusion:** These preliminary results in a real-life setting show that Hb stability can be achieved with once-monthly SC C.E.R.A. in predialysis patients directly converted from shorter acting ESA.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2315**

**Is Aliskiren Combined with ARBs or ACEIs in Type 2 Diabetes and CKD Stages 3 and 4 Renoprotective?** Hiromichi Suzuki, Tsutomu Inoue, Yusuke Watanabe, Tsuneo Takenaka, Hirokazu Okada. *Nephrology, Saitama Medical University, Irumagun, Saitama, Japan.*

**Background**: Although the renoprotective effects of Aliskiren have been clearly demonstrated in CKD stage 2 patients with hypertension, type 2 diabetes, and nephropathy treated with losartan, questions still remain whether these effects are also applicable to CKD patients in stages 3 and 4.

**Purpose:** The aim of this preliminary study was to evaluate the renoprotective effects of Aliskiren in CKD patients in stages 3 and 4 with hypertension and type 2 diabetes.

**Subjects and Methods:** Thirty two patients (female/male: 10/22; 62±7 years old) were enrolled. All were diabetic patients who were on an angiotensin receptor blocker (ARB) therapy alone or in combination with an angiotensin converting enzyme inhibitor (ACE), had more than 1 g daily proteinuria (1.8 ± 0.6 g/gcr daily), and estimated GFR less than 60 ml/min/1.73 m2 (28.3 ± 5.7 ml/min/1.73 m$^2$). Patients received Aliskiren 150 mg daily for 3 months. If proteinuria was not reduced to less than 1 g daily**,** or not decreased from baseline to 50%, dosage was increased up to 300 mg. A reduction of estimated GFR (eGFR) and increase in serum potassium level were evaluated during 6 months, as well as a reduction in the ratio of protein to creatinine, as measured in an early morning urine sample every month.

**Results**: Thirteen patients were withdrawn from the study, because of increases in potassium levels greater than 5.5 mEq/L in 3, of decreases in eGFR more than 30% in 2, and of both in 8 patients. In 9 patients, daily proteinuria was reduced to less than 1 g and/or 50% without increases in potassium levels and reduction of eGFR was observed. In 10 patients, daily proteinuria was decreased from baseline ranging from 30% to 42% with mild reduction of eGFR and increases in potassium levels. Both systolic and diastolic blood pressures of all patients were significantly reduced (144/80 ± 11/8 to 126/72 ± 12/9 mmHg) ($p<0.01$).

**Conclusion:** Add-on therapy with Aliskiren to ARBs or ACEIs should be cautiously used in diabetic CKD patients in stages 3 and 4.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2316**

**Trends in Health-Related Quality of Life in Children with Mild to Moderate CKD** Susan R. Mendley,[1] Arlene C. Gerson,[2] Stephen R. Hooper,[3] Alison G. Abraham,[2] Matthew Matheson,[2] Debbie S. Gipson,[4] Marc Lande,[5] Bruce Z. Morgenstern,[6] Shlomo Shinnar,[7] Bradley A. Warady,[8] Susan L. Furth.[9] *[1]Univ of MD; [2]Johns Hopkins Univ; [3]Univ of NC; [4]Univ of MI; [5]Univ of Rochester; [6]Phoenix Children's Hosp; [7]Montefiore Med Ctr; [8]Children's Mercy Hosp; [9]Children's Hosp of PA.*

We and others have shown that children with CKD have inferior health-related quality of life (HRQoL) when compared to normals and that this deficit is most pronounced in children on dialysis. However, data on the natural history of changes in HRQoL are lacking and questions as to which children are at greatest risk and which specific domains are most sensitive to declining GFR remain unanswered.

Methods: We evaluated annual change in HRQoL as assessed by Quality of Life Core Scales 4.0 (PedsQL) in 485 participants in the NIH-sponsored CKiD cohort study over a median follow-up of 2.9 years. Annual HRQoL change was stratified by baseline iohexol GFR category (>50, 41-50, 31-40, ≤30 ml/min/1.73 m$^2$) and the proportions experiencing change greater than 5 points were compared between GFR groups.

Results: Subjects had a median age of 11 yr and median iGFR of 45. Overall parent and child-reported HRQoL was 78, compared to published norms of 81 (parent) and 83 (child). While median Physical Function scores in CKiD (84, parent and child) were not different from norms of 83 (parent) and 87 (child), pairwise comparison of the highest and lowest GFR strata showed a significantly larger proportion of children with GFR ≤30 experienced a decline in the Physical Function subscale of at least 5 points ($p<.05$ parent and child). There were no substantial differences in changes in the PedsQL subscales of Emotional, Social and School Function across the GFR strata.

In summary, parent and child-reported declines in Physical Function are significant in those with the most advanced CKD, while other domains appear stable over the duration of observation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2317**

**Diabetes Impairs the *In Vitro* and *In Vivo* Activity of Progenitor Cells in CKD: Implications for Autologous Cell Therapy** Darren A. Yuen, Yanling Zhang, Andrew Advani, Kim Connelly, Karim Ladak, David Kepecs, Suzanne Advani, Kerri Thai, Richard E. Gilbert. *St. Michael's Hospital, Toronto, Canada.*

**Background:** Diabetic nephropathy, the leading cause of CKD, is characterized by microvascular loss. While several studies have shown that bone marrow derived progenitor cells can ameliorate kidney damage in a range of disease models, donor cells from healthy animals have almost invariably been administered. However, in the human context, autologous cells derived from an unhealthy donor would instead be used. As such, we sought to compare the *in vivo* and *in vitro* efficacy of endothelial progenitor cells (EPCs) derived from diabetic and healthy donors. **Methods:** We cultured EPCs derived from streptozotocin-diabetic and non-diabetic F344 rats, examining the angiogenic effects of their secretory products using a capillary tube formation assay. The renoprotective effects of EPCs were then assessed by the tail vein administration of: saline, 1 x $10^6$ EPCs derived from diabetic donors, or 1 x $10^6$ EPCs derived from healthy donors, into subtotally nephrectomized (SNX) F344 rats, 4 wks post-SNX. Urine protein and glomerular capillary density were analyzed 4 wks later. **Results:** Conditioned medium from non-diabetic EPCs induced robust endothelial tube formation, while that from diabetic EPCs was substantially reduced ($p < 0.05$). In contrast to the renoprotective effect of non-diabetic EPCs, those derived from diabetic animals were ineffective, leading to worsening proteinuria (Table 1) and capillary rarefaction in SNX rats (non-diabetic EPCs vs diabetic EPCs: 0.55 ± 0.02 vs 0.38 ± 0.06 AU, $p < 0.05$). **Conclusions:** Diabetic EPCs display reduced angiogenic activity *in vitro*, in association with impaired renoprotective capacity *in vivo*. Since the human context would require autologous EPCs to be administered in order to avoid immunosuppression, *ex vivo* strategies to restore diabetic EPC angiogenicity need to be explored.

| | SNX - saline | SNX - Healthy EPC | SNX - Diabetic EPC |
|---|---|---|---|
| Urinary protein (mg/day) | 111 ± 23 | 35 ± 8*† | 118 ± 27 |

* $p < 0.05$ vs SNX - saline, † $p < 0.05$ vs SNX - Diabetic EPC

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2318**

**Anemia Treatment among Patients with Chronic Kidney Disease Not on Dialysis (CKD-NOD) and Moderately Severe Anemia** H. Lester Kirchner,[1] Ze Cong,[4] Robert M. Perkins,[1] James E. Hartle,[2] Ion D. Bucaloiu,[2] Jeffrey Petersen.[4] *[1]Center for Health Research, Danville, PA; [2]Nephrology/Geisinger, Danville, PA; [3]Amgen, Inc, Thousand Oaks, CA.*

**Background:** Iron and Erythropoiesis-Stimulating Agent (ESA) use among patients with CKD-NOD and moderately severe anemia has not been well characterized. **Methods:** Patients with prevalent CKD-NOD and anemia (new-onset hemoglobin (hgb) < 10 g/dl) and receiving ESA, IV and/or oral iron, PRBC transfusion, or who had a confirmatory hgb < 10 g/dl within 6 months were identified (2004-2009) from the database of a tertiary medical center. Summary statistics of demographics, baseline labs, and iron and ESA use were analyzed. **Results:** From 18688 CKD-NOD patients, we identified 1546 with anemia [8.3%, mean (SD) age 74 (12.0), 97% white, 40% male, mean (SD) follow up of 3.4 (1.5) years].

Iron and ESA use among patients with CKD-NOD and anemia

| | Iron Mono Therapy | | Iron Dual Therapy | | No iron Therapy |
|---|---|---|---|---|---|
| | IV only (n=93) | Oral only (n=381) | IV First (n=29) | Oral First (n=60) | (n = 983) |
| Baseline eGFR, ml/min/1.73m$^2$, mean (SD) | 38.5 (15.8) | 42.2 (12.5) | 36.8 (13.4) | 34.7 (13.2) | 40.9 (14.2) |
| Baseline HGB, g/dl, mean (SD) | 9.1 (0.9) | 9.1 (0.9) | 9.2 (0.6) | 9.2 (0.8) | 9.2 (1.1) |
| Baseline TSAT, %, mean (SD) | 16.6 (15.5) | 22.2 (12.5) | 14.2 (4.0) | 18.4 (10.7) | 24.5 (15.6) |
| ESA during follow up, n (%) | 45 (48.4) | 115 (30.2) | 19 (65.5) | 37 (61.7) | 230 (23.4) |

Almost 50% of patients received no treatment, 15% ESA alone, 22% iron alone, and 14% both. Baseline TSAT was higher among iron users than non-users (mono vs. none: $p<0.01$, dual vs. none, $p<0.01$). ESA use was more common among iron users than non-users (mono vs. none, OR 1.67, 95% CI 1.31-2.21; dual vs. none, OR 5.56, 95% CI 3.53-8.75, $p < 0.001$ for both). **Conclusions:** A significant proportion of patients with CKD-NOD and new onset moderately severe anemia are not treated with iron or ESA. Of those treated, nearly half received iron therapy alone with oral iron usage more common. The optimal modality and timing of iron and ESA treatment require further investigation.

Disclosure of Financial Relationships: Research Funding: Merck, Daiichi-Sankyo, Pfizer, Takeda Pharmaceuticals, Amgen.

**SA-PO2319**

**Treatment of Osteoporosis by RANKL Inhibition with Denosumab in Women at High Cardiovascular Risk and with Renal Impairment Does Not Accelerate Vascular Calcification** Ogo I. Egbuna,[1] A. M. Cheung,[2] S. Siddhanti,[1] A. Wang,[1] N. Daizadeh,[1] M. Anthony,[1] L. Grazette,[1] Paul D. Miller.[3] *[1]Amgen Inc., Thousand Oaks, CA; [2]University Health Network and University of Toronto, Toronto, Canada; [3]Colorado Center for Bone Research, Lakewood, CO.*

The relationship between OPG/RANK/RANKL and vascular calcification is unclear. We report the effects of denosumab (DMAb), a mAb that inhibits RANKL, on abdominal aortic calcification in women from the FREEDOM trial at high risk of cardiovascular (CV) events. These effects were also examined by renal function status as patients with renal impairment are at increased risk for vascular calcification.

Postmenopausal women with osteoporosis (N=7868) were randomized to receive placebo (Pbo) or DMAb for 3 years. Women at high risk for CV events (N=2363) were identified using modified RUTH criteria at baseline and categorized by level of renal function (< or ≥45 mL/min using Cockcroft-Gault [CG] and MDRD equations). Abdominal aortic calcification was scored for L1-L4 anterior and posterior segments on lumbar spine x-rays and total score was the sum of these 8 measures. Change in total score from baseline to 3 years was calculated for women with complete data (N=1045) and categorized as no change or increased score (worsening). Proportion of DMAb and Pbo women with a change score >0 overall and by renal function was compared between groups with logistic regression.

Baseline demographics and CV risk factors were similar between groups. Proportion of women with an increase in calcification score was not different between Pbo and DMAb groups, regardless of renal function level or method of renal function estimation (CG [Table] or MDRD [not shown]).

Treatment of osteoporosis with DMAb in women at high CV risk did not increase the likelihood of more extensive vascular calcification compared with Pbo after 3 years, regardless of renal function.

**Table: Odds of Increased Total Aortic Calcification Severity Score in DMAb vs Pbo Groups From Baseline to Year 3 by Baseline Estimated Creatinine Clearance (CrCl) Level**

| | Subjects With Change >0 | Odds Ratio* | |
|---|---|---|---|
| | n (%) | Est (95% CI) | P value |
| **All subjects** | | | |
| Pbo (N=501) | 109 (21.8) | | |
| DMAb (N=544) | 118 (21.7) | 1.02 (0.76, 1.36) | 0.92 |
| **Baseline CrCl <45 mL/min** | | | |
| Pbo (N=64) | 16 (25.0) | | |
| DMAb (N=69) | 17 (24.6) | 0.97 (0.44, 2.15) | 0.94 |
| **Baseline CrCl ≥45 mL/min** | | | |
| Pbo (N=437) | 93 (21.3) | | |
| DMAb (N=475) | 101 (21.3) | 1.02 (0.74, 1.40) | 0.90 |
| Baseline CrCl by treatment group interaction† | | | 0.91 |

N = Number of randomized women who received ≥ 1 dose of investigational product, belonged to the cardiovascular high risk subset, and had observed data at all 8 abdominal aortic segments at baseline and at the time point of interest. n = Number of women with an increase in aortic calcification from baseline (score change >0). *Based on a logistic regression model adjusting for baseline total aortic calcification severity score; an odds ratio > 1 represents higher risk for increased aortic calcification in DMAb-treated women when comparing between treatment groups. †Adding baseline CrCl level variable and treatment-by-baseline CrCl level interaction to the logistic regression model.

Disclosure of Financial Relationships: Employer: Amgen Inc.; Ownership: Amgen Inc.

## SA-PO2320

**Effect of Atorvastatin on NGAL and Cystatin C in Chronic Kidney Disease: A Post-Hoc Analysis of the LORD Trial** Robert G. Fassett,[1] Iain Robertson,[2] Helen G. Healy,[1] Dominic P. Geraghty,[2] Madeline J. Ball,[2] John William Cardinal,[4] Jeff Coombes.[3] *[1]Renal Medicine, Royal Brisbane and Women's Hospital and The University of Queensland, Brisbane, Queensland, Australia; [2]Human Life Sciences, University of Tasmania, Launceston, Tasmania, Australia; [3]Human Movement Studies, The University of Queensland, Brisbane, Queensland, Australia; [4]Pathology Queensland, Queensland Health, Brisbane, Queensland, Australia.*

Aims The effect of atorvastatin on serum neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C and their relationships to kidney function were assessed in patients with stages 2-4 CKD.

Methods We conducted a post-hoc analysis using stored blood samples of 88 patients from the LORD Trial, a randomised, double-blind, placebo-controlled trial that assessed the effects of atorvastatin on kidney function. Patients received either atorvastatin 10 mg/day (48) or placebo (40) and were followed for a mean of 2.9 years. The primary aims were to determine the effects of atorvastatin on NGAL and cystatin C and relationships between MDRD eGFR and serum NGAL and cystatin C. Analysis was based on intention to treat and included all patients with at least one follow-up sample. Serum NGAL was measured at baseline and study conclusion using an immunoturbidometric method, cystatin C using a competitive immunoassay.

Results There was a strong negative association between initial NGAL levels and initial MDRD eGFR (P<0.001), but no association between initial NGAL level and subsequent MDRD eGFR (P=0.88). The rate of change of MDRD eGFR was not associated with initial NGAL or cystatin C levels. The rate of change of NGAL increased in the placebo group (mean 4.6 ng/mL/year; SD 56.6) but decreased in the atorvastatin group (mean -7.4 ng/mL/year (SD 128.4))(P=0.047). There was a strong association between incident cystatin C level and incident MDRD eGFR (P<0.001)

Conclusions This study reveals NGAL is a biomarker of existing CKD but baseline NGAL did not predict CKD progression. Atorvastatin reduced serum NGAL but the significance and mechanism require further investigation. Atorvastatin had no significant effects on cystatin C. Registration: http://www.anzctr.org.au ACRN012605000693628.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2321

**Sevelamer Treatment in Stage III-IV Pre-Dialysis Patients with Secondary iperPTH and Chronic Inflammatoy Status: Impact on Clinical Features and Biochemical Pattern** Luca Di Lullo, Fulvio Floccari, Pasquale Polito. *Department of Nephrology and Dialysis, San Giovanni Evangelista Hospital, Tivoli, Roma, Italy.*

INTRODUCTION AND AIMS: CV involvement, chronic inflammatory status and secondary hyperPTH are complications emerging on first stages of CKD. Fibroblast growth factor (FGF23) is a new sensitive biomarker being increasingly used to monitor the efficacy of treatments aimed to prevent calcium/phosphorus dismetabolism. We studied the clinical efficacy of sevelamer chlorohydrate on echocardiographic and serum parameters in stage III and IV of CKD patients with signs and symptoms of secondary hyperPTH and CV comorbidities.

METHODS: 200 CKD patients (120M/80F, mean age 50.7 +/- 4.3 years, 100/170 [58.8%] with anemia at the baseline visit) were studied. All patients with CKD related anemia were divided into two subgroups and treated with darbepoietin or alpha-epopoietin for 12 months before entering the study. Efficacy of 12-month daily 1600 mg oral dose of sevelamer has been evaluated by laboratory tests (FGF23, PCR, phosphoremia, calcemia), echocardiography(e.g.: EF, LVEDV and Wilkins Score on mitral calcifications) at 0, 6 and 12 months of therapy.

RESULTS: After 12 months of treatment, a mean reduction of 27.2% of serum phosphate (mg/dl), 33.1% of FGF23 (pg/ml) and 43.6% of PCR (mg/dl) and a dramatic improvement of cardiovascular tissue deposition (79.3% mean reduction of Wilkins score) were observed in all patients. By contrast, hemodynamic exams improved slightly (EF 4.1%, LVEDV -2.4%). The degree of improvement of serum phosphorus, FGF23, PCR, EF and Wilkins score after 12 months of sevelamer therapy was comparable among anemic and not anemic subgroups.

CONCLUSIONS:treatment for hyperPTH in stage III-IV of CKD patients has typically focused on the use of oral calcium-based phosphate binders. Sevelamer,non-calcium and aluminium-free agent has accumulated consistent body of evidence on the effects on all-cause of cardiovascular mortality. Our study confirms published data on serum mineral levels control efficacy of this phosphate binder drug, and draws the attention on its role in reducing chronic inflammation markers (FGF23, PCR) and the sclerotic process of cardiovascular apparatus (Wilkins score).

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2322

**Effect of the Vitamin D Receptor Agonist Paricalcitol on Biomarkers of Vascular Reactivity in CKD 3 and 4** Amy B. Pai,[1] Darren W. Grabe,[1] George Eisele.[2] *[1]Albany College of Health of Health Sciences, Albany, NY; [2]Albany Medical College, Albany, NY.*

Non-classical effects of vitamin D receptor agonists (VDRA) may explain, in part, mortality benefits in observational analyses. Reduced expression of cell adhesion molecules has been observed with VDRAs *in vitro*. This study investigates the effect of the VDRA, paricalcitol (PCT) on soluble cell adhesion molecules in patients with CKD 3 and 4.

This is a preliminary analysis of an 8 week, randomized, single-blind, controlled trial of PCT versus placebo(NCT00915876). Eligible patients are randomized to 1 mcg of PCT or placebo daily. Study visits are at Baseline, Week 4 and Week 8. Soluble intracellular adhesion molecule (sICAM ) and vascular adhesion molecule (sVCAM) were assayed simultaneously in serum using customized human cytokine multiplex suspension panels. Serum sICAM and sVCAM are reported as median (range), all other data are reported as mean ± SD. The proportion of patients achieving a >20% reduction from baseline was determined by Fishers exact test.

Demographic characteristics of the 18 patients (9 PCT and 9 placebo) were well matched. Baseline eGFR (ml/min/1.73m2) for PCT and placebo groups at baseline were 29.8 ± 7.4 and 35.8 ± 11.3, respectively. Serum sICAM concentrations (ng/mL) at baseline were 1275.9 (654.8-2377.7) in PCT treated patients vs. 271.9 (249.6-1276.2) in patients receiving placebo. There were no significant changes in sICAM at Week 4 in either group, however at Week 8, sICAM was reduced from baseline in the PCT group 278.3 (225.1-1715.3) vs. placebo 254.2 (223.8-1204.3). Similarly, there was a greater proportional reduction in sVCAM concentrations (ng/mL) from baseline to Week 8 in the PCT group [1605.7 (400.2-2622.8) to 495.1 (456.4-2316.2)] vs. placebo treated patients [842.7 (362.9-1879) to 648.5 (308.6-2327)]. More patients receiving PCT (55% vs. 12.5%) had a ≥ 20% reduction in sICAM and sVCAM after 8 weeks of treatment (p=0.061).

This is the first clinical investigation of the effects of a VDRA on biomarkers of vascular reactivity. These preliminary data suggest that treatment with the VDRA paricalcitol may reduce principal mediators of vascular monocyte adhesion.

Disclosure of Financial Relationships: Research Funding: Abbott Laboratories.

## SA-PO2323

**Enzyme Replacement Therapy Improves Serum Asymmetric Dimethylarginine Levels and Coronary Microvascular Dysfunction in Fabry Disease** Hideki Fujii,[1] Keiji Kono,[1] Shinichi Nishi,[1] Masafumi Fukagawa.[1, 2] *[1]Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Japan; [2]Division of Nephrology and Metabolism, Tokai University School of Medicine, Isehara, Japan.*

B**ACKGROUND**

Fabry disease (FD) is a rare disease and one of the causes of progressive renal and cardiac dysfunction. FD results from an X-linked recessive lysosomal storage disorder caused by a defect in the gene encoding lysosomal α-galactosidase A. Though the accumulation of globotriaosylcelamide (GL-3) leads to renal and cardiac manifestation, the detailed mechanisms remain unclear. Coronary endothelial dysfunction seems to be one of the causes of cardiac complications in FD. The aim of our study was to access coronary flow reserve (CFR) and the effect of enzyme replacement therapy (ERT) on coronary microvascular dysfunction.

**METHODS**

Recently, we performed a screening test of FD for more than 1,000 subjects and found seven FD patients. Present study included three FD patients (two male and one female patient) among them, who have never received ERT. We measured serum asymmetric dymethylarginine (ADMA) levels as a marker of endothelial dysfunction and performed ehocardiography and measurement of CFR by adenosine-triphosphate stress transthoracic Doppler echocardiography for all the study patients before starting ERT, and 3, 6, 12 months after starting ERT.

**RESULTS**

All the study patients could receive ERT without any side effects. At baseline, two of them had impaired CFR, increased left ventricular mass index (LVMI) and elevated serum ADMA levels. Twelve months after starting ERT, CFR was increased in all the patients and LVMI and serum ADMA levels were decreased in two patients. In one patient who

showed most greatly improved CFR, serum brain natriuretic peptide level also decreased remarkably. Furthermore, serum ADMA levels were significantly correlated with CFR ($r = -0.698$, $p < 0.05$).

**CONCLUSIONS**

The results of our study suggest that ERT prevents the progression of cardiac abnormalities possibly by improving coronary microvascular dysfunction and ADMA may be a useful surrogate marker in FD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2324**

**Sodium Bicarbonate Supplementation in Chronic Kidney Disease: Evaluation of Dose-Response and Changes in Muscle Strength** Matthew K. Abramowitz, Michal L. Melamed, Amanda C. Raff, Carolyn A. Bauer, Thomas H. Hostetter. *Albert Einstein College of Medicine, Bronx, NY.*

Background: Metabolic acidosis is associated with protein-energy wasting in patients with chronic kidney disease (CKD). In severe acidosis, sodium bicarbonate ($NaHCO_3$) supplementation is prescribed routinely. However, the role of alkali treatment of mild acidosis remains unclear. In CKD patients with mild acidosis, we evaluated the change in serum bicarbonate at specific doses of $NaHCO_3$ and the effect on muscle strength of alkali supplementation.

Methods: Sixteen adult subjects with estimated GFR 16 – 45 ml/min/1.73 $m^2$ and serum bicarbonate 20 – 24 mEq/L were treated during successive 2 week periods with placebo followed by escalating doses of oral $NaHCO_3$ (0.3, 0.6, and 1.0 mEq/kg/day, based on ideal body weight). Subjects were blinded to treatment status. At each visit handgrip strength was tested using a hydraulic hand dynamometer and the time required to complete 10 repetitions of a sit-to-stand (STS) test was measured. Random-effects models were created to examine the association of $NaHCO_3$ dose with outcomes at each 2 week interval.

Results: The mean age was 64 years, 10 were women, 5 were Hispanic, 11 were non-Hispanic black, mean BMI was 30.7 kg/$m^2$, 15 had hypertension, 13 had diabetes mellitus, and 2 had congestive heart failure. Mean (± SD) eGFR was 34.8 ± 8.9 ml/min/1.73 $m^2$ and mean serum bicarbonate was 22.9 ± 2.5 mEq/L at baseline. After 2 weeks of placebo, or $NaHCO_3$ 0.3, 0.6, or 1.0 mEq/kg/day, serum bicarbonate was 22.9 ± 2.3, 23.9 ± 2.5, 24.7 ± 2.2, and 25.6 ± 2.5 mEq/L, respectively ($p<0.001$ for trend). Each 0.1 mEq/kg/day increase in $NaHCO_3$ dose was associated with a 0.3 mEq/L (95% confidence interval, 0.2 – 0.4 mEq/L) higher serum bicarbonate. The time required for the STS test decreased after 6 weeks of $NaHCO_3$ supplementation (24.3 ± 1.8 vs. 25.7 ± 1.6 seconds, $p=0.02$). No change was seen in handgrip strength ($p=0.18$). Higher $NaHCO_3$ doses were not associated with increased systolic or diastolic blood pressure or edema.

Conclusions: $NaHCO_3$ supplementation produces a dose-dependent increase in serum bicarbonate and may improve muscle strength in patients with CKD and mild acidosis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2325**

**Sodium Bicarbonate Supplementation Decreases Electronegative LDL in Patients with Chronic Kidney Disease under Conservative Management** Felipe Rizzetto Santos,[1] Deise Monteiro Carvalho,[2] Denise Mafra,[3] Dulcinéia Saes Parra Abdalla,[4] Maurilo Leite.[1] *[1]Universidade Federal do Rio de Janeiro, Brazil; [2]Hospital Federal de Bonsucesso; [3]Universidade Federal Fluminense; [4]Universidade de Sao Paulo.*

The mortality of patients with chronic kidney disease (CKD) is mostly related to cardiovascular disease and associated with diverse factors, including metabolic acidosis (MA). Serum concentrations of electronegative LDL (LDL(-)), a minimally oxidized LDL, in CKD patients on dialysis, are higher than healthy individuals. This modified LDL is related to acute coronary syndrome. In this study we measured serum levels of LDL(-) in CKD patients stage 4, and further addressed the effect of oral sodium bicarbonate supplementation ($NaHCO_3$, 1mEq/kg/day), on LDL(-) in this population. Methods: Twenty pre-dialysis patients ($Cl_{Cr}$: 20.0±6.0 ml/min, age 46.1±11 years; 10 men), from Hospital Federal de Bonsucesso, were studied and compared with 21 healthy individuals (age 50.7±15.7 years; 9 men). Thereafter, CKD patients were randomly divided into 2 groups: group 1 received daily doses of $NaHCO_3$, while group 2 received placebo, all for a period of 20 weeks. Blood samples were collected before and after this period for creatinine and urea. Serum LDL(-) was measured by ELISA. Results: The concentrations of LDL(-) (µg/l) were higher in CKD patients as compared to healthy individuals (0.30±0.15 vs 0.12±0.09, respectively, $P<0.001$), and was positively correlated with serum levels of creatinine ($r=0.58$, $P<001$) and urea ($r=0.5$, $P<004$). Levels of LDL(-), before and after oral $NaHCO_3$, decreased in group 1 (0.40±0.16 and 0.27±0.15, respectively, $P<0.01$), whereas in group 2 there was no significant change (0.36±0.13 and 0.38±0.15, respectively). Conclusion: CKD patients under conservative management have high levels of LDL(-), which seem to increase as renal function decreases. Oral bicarbonate supplementation for 20 weeks decreased LDL(-) levels in these patients. These results suggest that oral alkali supplementation in CKD patients stage 4 may be beneficial to decrease LDL modification, which attenuates the development of cardiovascular disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2326**

**Effect of Homocysteine Lowering on Benefits and Harms in People with Kidney Disease: A Systematic Review** Meg J. Jardine,[1] Amy Kang,[1] Sagar U. Nigwekar,[2] Sophia Zoungas,[1] Toshiharu Ninomiya,[1] Sankar D. Navaneethan,[3] Martin P. Gallagher,[1] Giovanni F. M. Strippoli,[4] Alan Cass,[1] Vlado Perkovic.[1] *[1]The George Institute for International Health; [2]Rochester General Hospital; [3]Glickman Urological and Kidney Institute, Cleveland; [4]Consorzio Mario Negri Sud.*

**Aim:** To systematically review all randomized trial data examining the effect of homocysteine lowering on cardiovascular events in people with kidney disease.

**Background:** High homocysteine levels are associated with an increased risk of cardiovascular events. Trials of homocysteine lowering in the general population have not shown a reduction in the risk of cardiovascular events, but people with kidney disease have higher homocysteine levels, higher rates of cardiovascular disease and potentially different mechanisms of cardiovascular disease.

**Methods:** MEDLINE, EMASE, the Cochrane Library from 1950 to December 2009, conference proceedings and trials websites were searched without language restriction. Studies of homocysteine lowering therapy with a minimum of 100 patient years of follow-up, reporting outcomes for people with kidney disease including end stage kidney disease (ESKD), functioning kidney transplant (KT) and chronic kidney disease (CKD), were included. The primary outcome was cardiovascular mortality. Analyses were conducted using a random effects model. Heterogeneity was assessed using the Cochran Q test and the $I^2$ test.

**Results:** Data was extracted from ten trials (9113 participants) conducted in populations with CKD (2 trials), CKD or ESKD (2 trials), ESKD (5 trials) and KT (1 trial). Homocysteine lowering had no effect on cardiovascular mortality (relative risk 1.00, 95% confidence interval (CI) 0.81-1.25), all-cause mortality (RR 0.98, 95%CI 0.89-1.08), myocardial infarction (RR 0.94, 95%CI 0.80-1.12), stroke (0.91, 95%CI 0.69-1.20) access thrombosis (RR 0.97, 95%CI 0.84-1.11) or adverse events (RR 0.99, 95%CI 0.91-1.08). There was no evidence of heterogeneity across trials for any endpoint (all p for heterogeneity ≥0.2).

**Conclusions:** Homocysteine lowering does not prevent cardiovascular mortality, myocardial infarction, stroke or access thrombosis in people with chronic kidney disease.

Disclosure of Financial Relationships: Employer: The George Institute for International Health

Sydney South West Area Health ServiceResearch Funding: My employer has received an unrestricted grant from CSL Biotherapies.

**SA-PO2327**

**Effects of Cinacalcet with Low Dose Vitamin D Sterols in Hemodialysis Patients with Secondary Hyperparathyroidism; Results from the ADVANCE Study** Carmel M. Hawley,[1] Pablo A. Urena,[2] William G. Goodman,[3] Frank Petavy,[4] Bastian Dehmel,[5] Jurgen Floege.[6] *[1]Univ. of Queensland, Princess Alexandra Hospital, Australia; [2]Clinique du Landy, Saint Ouen, France; [3]Amgen Inc.; [4]Amgen Europe GmbH, Switzerland; [5]Amgen Europe GmbH, Switzerland; [6]RWTH University, Aachen, Germany.*

The ADVANCE study (NCT00379899) investigated the progression of vascular and cardiac valve calcification in 360 prevalent hemodialysis patients with sHPT randomized to either cinacalcet plus vitamin D (VitD) sterols or flexible doses of vitD sterols alone for 52 weeks. As per protocol, patients in the cinacalcet arm were to receive low dose vitD (≤6µg paricalcitol equivalent/week) but *actually received* a mean dose of 8-11µg/week after initiation of treatment.

Progression of calcification and laboratory parameters were analysed in a subset of patients in the cinacalcet arm treated per protocol at week2 ("per protocol" group). Week2 was chosen as the cut-off for this analysis assuming that the investigators needed this time to adapt their patients' vitD regimen.

Overall, 70/115(61%) cinacalcet patients were treated per protocol at week2. The mean(SD) doses for vitD at week2 in the per protocol and control groups were 4.7(1.9) and 12.8(10.2)µg/week, respectively.

Baseline patient characteristics were similar between the two groups. Differences in calcification (Agatston) scores are reported in the Table.

Reductions in iPTH(pg/mL), Ca(mg/dL) & P(mg/dL) were significantly greater in the per protocol group vs. control: $p=0.033$;$p<0.001$;$p=0.024$ respectively.

Table 1

| Median % change (Q1, Q3) in calcification | Per protocol | Control | P value* |
|---|---|---|---|
| Coronary Artery | n=70 | n=119 | p=0.017 |
| | 17.8(-1.8, 54.7) | 31.3(7.6, 81.1) | |
| Aorta | n=54 | n=102 | p=0.166 |
| | 23.8(2.2, 39.8) | 33.1(3.8, 69.4) | |
| Aortic valve | n=37 | n=51 | p=0.017 |
| | 6.0(-16.8, 34.4) | 51.5(-9.9, 123.4) | |
| Mitral valve | n=33 | n=64 | p=0.087 |
| | 6.7(-22.0, 117.5) | 54.4(-3.9, 177.7) | |

*Generalised Cochran-Mantel-Haenszel

Cinacalcet plus low doses of vitD improves laboratory control and may attenuate the progression of calcification compared to the control group as shown in a sub-analysis of ADVANCE study patients.

Disclosure of Financial Relationships: Research Funding: I have received travel and research grants from Amgen.

**SA-PO2328**

**Anemia Knowledge Is Limited among Patients with Kidney Disease Receiving ESA Therapy** Kerri L. Cavanaugh,[1] Neil S. Sanghani,[1] Kenneth A. Wallston,[1] Mae Thamer,[2] Dennis J. Cotter,[2] Russell Rothman,[1] T. Alp Ikizler.[1] *[1]Vanderbilt University; [2]MTPPI.*

FDA has put forth safety warnings regarding use of erythropoietin-stimulating agents (ESA) to treat anemia associated with kidney disease. Little is known about risks for low anemia management knowledge in patients receiving therapy.

From January until May 2010, chronic kidney disease (CKD) or Hemodialysis (HD) patients receiving ESAs were enrolled from one center. We measured anemia knowledge with the newly developed Patient Anemia Knowledge in Kidney Disease (PAKKD) survey (Score:0-100%). We also measured health literacy and risk numeracy. ESA use and clinical variables were abstracted from the medical record. Associations with anemia knowledge were examined with Spearman's correlations and regression analyses.

In 99 patients (50% CKD/50% HD), mean(SD) age was 56(16) years, 43% male, 47% black, 50% had a diagnosis of diabetes, 23% had limited health literacy, 65% had poor risk numeracy skill. Median hemoglobin was 11 mg/dl, in both CKD and HD subjects. Duration of ESA therapy was longer in HD patients (42 mo) compared to CKD (19 mo). Anemia knowledge score was a mean(SD) 58%(21%), however <10% of subjects could describe how therapy may be harmful, and <50% correctly answered multiple choice items regarding potential harm. When asked if there may be *serious* risks to ESA therapy, 68% were not sure, and 11% disagreed. Lower anemia knowledge was associated with older age (rho (r):-0.28, p<0.01), fewer years of education (r:0.26; p=0.01), limited health literacy (r:0.23, p=0.02), and lower risk numeracy score (r:0.34, p<0.01). In adjusted analyses only lower risk numeracy (p<0.01) remained significantly associated with anemia knowledge. No association was found with type of ESA or duration of use. The most common source of information about anemia management for patients was their physician (43%), but 33% reported receiving no information about ESAs.

Even patients with long-standing kidney disease receiving ESA therapy may not understand the rationale or the risks related to this important element of their care. Improving patient education about ESA risks, while addressing limited health literacy and numeracy, is needed.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2329**

**Conversion to Methoxy Polyethylene Glycol-Epoetin-b (C.E.R.A.) with Administration of Iron Carboxymaltose in Anemia Associated with K/DOQI Stage 3B and 4 Chronic Kidney Disease** Jose L. Gorriz,[1] Monica Climente-Marti,[2] Veronica Escudero,[1] Amelia Garcia-Hervas,[1] Victor Jiménez-Torres,[2] Luis M. Pallardo.[1] *[1]Servicio de Nefrologia, Hospital Unuiversitario Dr Peset, Valencia, Spain; [2]Servicio de Farmacia, Hospital Unuiversitario Dr Peset, Valencia, Spain.*

**Aim:** To evaluate the effectiveness of the treatment with methoxy-polyethylene glycol-epoietin-βb (CERA) in the correction of renal anaemia after treatment start or change with epoietin-beta or darbepoietin-alpha and to estimate the conversion factor to CERA from epo-β and darbe-α.

**Methods:** Prospective study. 21 patients CKD stages 3b and 4. In 4 CERA was initiated; in 10 (48%), there was a change from darbepoietin-a, and, in 7 patients, from epoietin-β (33%) with previous optimal response during the last 3 months and stable doses.

Patients with darbepoetin doses: 40, 60, 80, 100 µg/month were converted to 30, 60, 80 and 100 µg/month, respectively, of C.E.R.A.. Patients with epoetin-β doses: 8000-11000, 12000-15000, 16000-18000, 20000 IU/month were converted to 30, 50, 60 and 75 µg/month, respectively. All patients received iron carboxymaltose to achieve optimum ferritin levels (300-500 ng/mL or TSAT > 20). A C.E.R.A. dose reduction of 52.0±13.9% (45.1–59.0) was applied versus Summary of Product Characteristics (SPC) recommendations, and of 33.0±13.2% (26.5–39.6) versus over the expert ones. Mean initial C.E.R.A. dose was: 66.9±2.9 (95% CI 54.2–79.6) µg/month.

**Results:** Initial and final values of Hb (g/dL) were 11.2±1.0 and 11.1±1.4 (Median-diference: 0.03, 95% CI -0.58–0.64, p=0.924); ferritin 213.8±111.1 and 601.9±388.3, p=0.001); and TSI 27.7±19.6 and 30.0±14.8 (p=0.58). The conversion factors in our study were: 1 µg C.E.R.A.= 1.17 µg darbepoetin-a (1.1-1.25); 1 µg C.E.R.A.: 293±36.5 IU epoetin-β (248.0–338.7).

**Conclusions:** Conversion from darbepoetin-a and epoetin-β to C.E.R.A. with adequate replenishment of iron stores achieves a 52% reduction in CERA dose over the dose recommended in the SPC and a 33% reduction over other recommended dose schedules without significant changes in hemoglobin.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2330**

**Effects of Modality Change and Transplant on Health-Related Quality of Life in Dialysis Patients** Patricia Lynn Painter,[1] Lynda A. Frassetto,[2] Joanne Krasnoff,[3] Kirsten L. Johansen.[3] *[1]School of Nursing, University of Minnesota, Minneapolis, MN; [2]Dept of Medicine, University of Miami, Miami, FL; [3]Dept of Medicine, Univerisity of Califormia-San Francisco, San Francisco, CA.*

There are reports of improved health-related quality of life (HRQOL) with increased frequency of hemodialysis (HD) and with transplant (TX). It is unclear whether there is a relationship between changes in exercise capacity ($VO_{2peak}$) and HRQOL with changing treatment modality in ESRD. Three groups of patients with ESRD were studied in a pre-post design 6 months apart. Groups were patients treated with conventional hemodialysis (CHD) who stayed on CHD (n=13; age 45.6± 12.8; 11m/ 2 f); changed to daily HD (n=10; age 43.9±12.7; 9m /1f); changed to living donor TX (n=20; age 42.5 ± 13.4; 17m/ 3f). Healthy controls (n=34; age 47.7 ± 8.5; 28m / 6f) were tested once for comparison. HRQOL was assessed using the SF-36 questionnaire and $VO_{2peak}$ was measured using a symptom-limited exercise test. Analysis of co-variance was used to determine differences among the groups at baseline, at 6 months and in changes on the HRQOL scale scores from baseline to 6 month follow-up. The CHD group was the patient reference group. At baseline, all patient groups were lower in 3 of 4 physical scales and the physical composite scale (PCS) of the SF-36 than the controls (p<.05), whereas at 6 months, HRQOL scales for the TX were similar to controls but the CHD and SDD groups remained lower in 3 of 4 physical scales and the PCS. There were no changes in the physical scales in either CHD or SDD, whereas change in TX was significant compared to CHD on 3 of 4 physical scores, the vitality score and the PCS. $VO_{2peak}$ increased only in the TX group (p=.02). Significant correlations were observed between change in $VO_{2peak}$ and change in physical function scale (r=0.51;p<.001, and in the change on the vitality scale (r=0.39;p=0.01). TX but not changing to SDD improves HRQOL and $VO_{2peak}$ in ESRD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2331**

**Desensitisation in Patients with Chronic Kidney Disease (CKD) and Allergy to Allopurinol** Antonio Di Felice, Emiliana Ferramosca, Antonio Santoro. *Division of Nephrology Dialysis and Hypertension, St. Orsola-Malpighi University Hospital, Bologna, Italy.*

Hyperuricemia is frequent in patients with CKD and it is on the one hand a severe risk-factor for gout or nephrolithiasis, on the other it presents a potential toxicity on the vascular endothelium and therefore can accelerate the progression of the nephropathy. Allopurinol is a low-cost widely-prescribed urate-lowering agent and is the only one available for oral administration; to date uricosuric drugs are not available in commerce and i.v. urate-lowering drugs are very expensive and not indicated for routinary employment. However, allopurinol hypersensitivity syndrome, a form of cutaneous adverse reaction with high risk of mortality and morbidity, is frequent in this population and precludes further administration.

In this study we report our experience with desensitisation using a schedule of titrated dosages in a group of patients with hyperuricemia and renal impairment who interrupted their therapy because of various cutaneous eruptions due to hypersensitivity to allopurinol.

*Methods*. We considered 11 patients, 7 male and 4 females, 52-77 years old, serum creatinine range 1,3-3.2 mg/dl , GFR range 23-67 ml/min; 3 of them suffered from gout. All of them showed pruritic micro and macropapular rash during treatment with allopurinol at standard dose of 150-300 mg/day, that was then withdrawn. After regression of cutaneous lesions, a treatment was started with oral suspension of allopurinol at increasing dosage, starting from 50 micrograms/day, increasing every 3 days until 100 mg/day within 30 days.

*Results*. Basal uric acid levels were 10.75 ± 1.75 mg/dl (range 9,4-11,7); after 3-6 months treatment urate levels were 6.73±0.72 (range 5.8-7.6) (p=<0.05). No patient showed recurrence of cutaneous reactions. Renal function was unmodified.

In *conclusion*, our data suggest a good efficacy and safety of slow oral desensitisation with low-increasing dosage in patients with allergy to allopurinol and renal disease, who cannot be treated with uricosurics or i.v. urate-lowering drugs. Lowering uric acid levels is important in renal patients not only for gout or nephrolythiasis risk, but also for the progression of renal damage.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2332**

**Effect of 25 Hydroxy Vitamin D on Endothelial Function in Predialysis CKD** Nihil Chitalia,[1] Elanor W. Au,[1] Anatoli Kiotsekoglou,[2] Juan Carlos Kaski,[2] Debasish Banerjee.[1] *[1]Renal Medicine, St. George's Hospital, London, United Kingdom; [2]CV Sciences, St. George's University of London, London, United Kingdom.*

**Introduction**

Non traditional CV risk factors such as vitamin D deficiency have been implicated for the heightened CV risk in CKD patients. In large observational studies, hypovitaminosis D relates to CVD in CKD patients. However the relationship of vitamin D levels with early markers of CVD are unknown in pre-dialysis CKD. This study investigated the relationship of vitamin D with endothelial function and carotid Intima Media Thickness (IMT), independent of traditional risk factors, in predialysis CKD patients.

**Methods and Results**

44 non-diabetic, pre-dialysis CKD patients were investigated: age 56±11 years, hypertension 84%, males 59%, current smokers 11%, BMI 25±4 kg/m$^2$, total cholesterol 4.9±1.2mmol/L, eGFR 37±16 ml/min/1.73m$^2$. Serum 25(OH)D levels were 54±34 nmol/L. Brachial artery Flow Mediated Vasodilatation (FMD) was measured in the vascular laboratory under standardized conditions. IMT of the common carotid artery was measured bilaterally 2 cm proximal to the carotid bulb. Serum 25 hydroxy vitamin D [25(OH)D] levels were assessed by electrochemiluminescence immunoassay. All data were analysed using SPSS v.17.

FMD correlated with serum 25 (OH) D (r=0.44,p<0.005) (figure 1) and hsCRP (r=-0.46,p<0.05). IMT also correlated with hsCRP (r=0.503, p<0.01), but did not correlate with 25 (OH)D levels (r=0.189,p=0.26). Adjusted for traditional risk factors (age, male gender, blood pressure, BMI, cholesterol and smoking), 25 (OH) D was directly related to FMD (Ad.β=0.48, 95%CI 0.13-0.61,p<0.005) on multiple regression analyses.

**Conclusion**

This study demonstrates for the first time a relationship between 25(OH)D levels and endothelial function in predialysis CKD. This suggests vitamin D deficiency as a possible risk factor for CV complications in CKD.

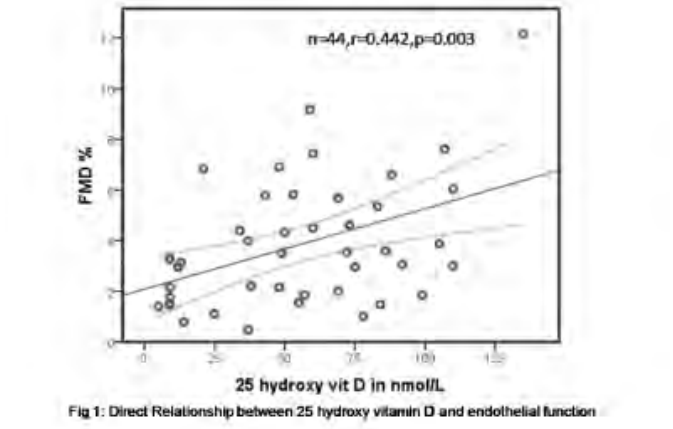



Fig 1: Direct Relationship between 25 hydroxy vitamin D and endothelial function

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2333

**The Long-Term Effects of Melatonin on Sleep and Quality of Life in Hemodialysis Patients** Marije Russcher,[1] Birgit Koch,[1] Ernst C. Hagen,[1] Carlo A. Gaillard,[1,2] Elsbeth Nagtegaal,[1] Pieter M. Ter Wee.[2] *[1]Clinical Pharmacy, Internal Medicine, Meander Medical Center, Amersfoort, Netherlands; [2]Nephrology, VU Medical Center, Amsterdam, Netherlands.*

Purpose

The pineal hormone melatonin plays a major role in circadian sleep-wake rhythm. Previously, we reported that the nocturnal endogenous melatonin rise is absent in hemodialysis (HD) patients and that short-term daily use of exogenous melatonin improves sleep onset latency (SOL), actual sleep time (AST) and sleep efficiency (SE) in HD-patients. The aim of the present study was to investigate long-term effects of exogenous melatonin on sleep and quality of life (QoL) in HD-patients.

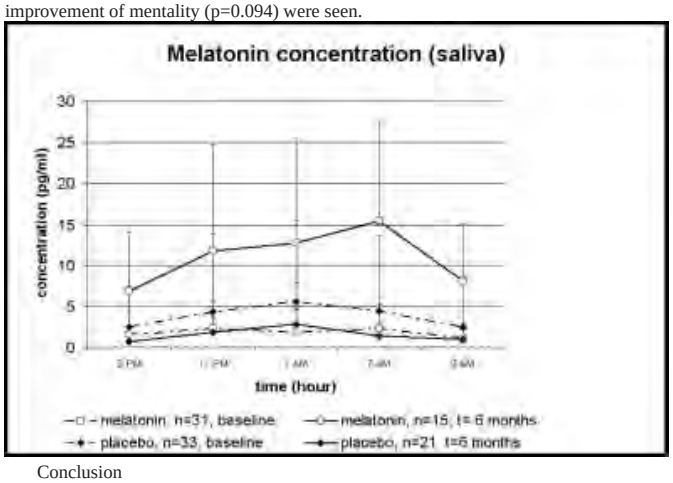
Methods

We conducted a 1 year randomized double-blind placebo-controlled trial with melatonin 3 mg in 70 HD-patients (ClinicalTrials.gov: NCT00388661). Objective sleep measurements were taken at 0, 3, 6, 9 and 12 months by means of actigraphy. QoL parameters were measured by the Medical Outcomes Study Short Form-36 questionnaire. Melatonin concentration curves were sampled in saliva at 0 and 6 months. Statistical analysis was performed by generalized estimating equation.

Results

Administration of exogenous melatonin resulted in higher endogenous melatonin levels (figure).

At 3 months, the previously shown beneficial effect of the short-term use of exogenous melatonin on SOL was confirmed (p=0.023). A trend in improvement of SE (p=0.105), AST (p=0.057) and actual awake time (AAT) (p=0.150) was observed. In contrast, at 12 months none of the measured sleep parameters differed significantly from placebo. At the end of the study period a positive effect on social functioning (p=0.032) and a trend in improvement of mentality (p=0.094) were seen.




Conclusion

In this study the short term beneficial effects of melatonin on sleep were confirmed. However, there is no indication that these beneficial effects persist in long-term usage of melatonin.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2334

**Contribution of Common Medications and Comorbidities to Physical Activity and Exercise Capacity in Chronic Kidney Disease** Erin Howden, William G. Petchey, Nicole M. Isbel, Jeff Coombes. *CCRE – Cardiovascular and Metabolic Health, University of Queensland, Australia.*

Chronic kidney disease (CKD) is associated with inactivity and reduced exercise capacity. We sought to determine whether common medications and co-morbities contribute to physical activity and exercise capacity.

**Methods** Cross sectional analysis of 101 pts with stages 3/4 CKD (eGFR 25-60ml/min) was performed. Physical activity (PA) was assessed using a standard questionnaire and classified as sedentary (<600 MET mins per week, SG) or active (>600, AG). Exercise capacity and endurance were assessed by graded treadmill test ($VO_2$peak) and six minute walk test (6MW).

**Results** Pts were 59.2±9.9 yrs (eGFR 39±9.3ml/min, 60% male). Compared to the AG (n=34), the SG (n= 67) had lower exercise capacity (22.1±4.8 vs. 25.5±9.0ml/kg/min), 6MW distance (445±106 vs. 493±110m) and peak systolic blood pressure (177±23 vs. 188±23mmHg; p<0.05 for all). The SG were taking less antihypertensive medications (2.33 vs. 2.68/pt), diuretics (0.4 vs 0.5/pt), statins (0.6 vs 0.8/pt) and allopurinol (0.14 vs. 0.24/pt), had less hypertension (93% vs. 97%), hyperlipidemia (66% vs. 74%), previous cardiac events (40% vs. 47%), peripheral vascular disease (16% vs. 18%) and more diabetes (50% vs. 44%). Multivariate regression analysis identifed predictors of exercise capacity in all patients (table1).

**Conclusion** Antihypertensive medications (including heart rate limiting) do not have an association with the level of PA or exercise capacity of CKD patients. Common comorbidities such as obesity, diabetes and hyperlipidemia are independent predictors of exercise capacity.

Table 1.Associations and regression analysis of VO2peak

| Variable | Univariate | | Multivariate (VO2) (n=93, adjusted $R^2$ 0.421) | |
|---|---|---|---|---|
| | r | p | β | p |
| BMI | -0.403 | <0.001 | | |
| Waist (cm) | -0.402 | <0.001 | -0.365 | <0.001 |
| Hip (cm) | -0.354 | <0.001 | | |
| Hyperlipidemia | | <0.001 | -0.237 | 0.009 |
| Cardiac history | | 0.014 | | |
| Diabetes | | <0.001 | -0.306 | 0.047 |
| No. Diuretics | | 0.004 | | |
| Hemoglobin | 0.442 | <0.001 | 0.345 | <0.001 |

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2335

**Cross-Sectional, Observational Study To Evaluate the Effectiveness and Safety of Mircera® for Treatment of Anemia in Patients with Chronic Kidney Disease Stage III/V with or without Dialysis in Conversion or Correction, in Routine Clinical Practice** J. M. Portolés,[1] N. J. Vega,[2] G. Fernández-Fresnedo,[3] R. Romero,[4] S. Bea,[5] M. J. Camba.[6] *[1]Hospital Fundación Alcorcón, Spain; [2]Hospital Dr. Negrín, Gran Canaria, Spain; [3]Hospital Marqués de Valdecilla, Santander, Spain; [4]Policlínica Souto Boo, Santiago de Compostela, Spain; [5]Centro de HD Gamapal, Valencia, Spain; [6]CH Ourense, Spain.*

**Background:** In randomised controlled trials (RCTs), Mircera® once-monthly dosing, has shown non-inferiority in efficacy and safety to others ESAs. However, clinical practice differs from RCTs.

**Methods:** A cross-sectional observational study performed in 12 nephrology centers from patients who completed 6 months of Mircera® for correction or maintenance. We present preliminary analysis.

**Results:** A total of 171 patients were evaluated. Mean age: 66.5(14.5) years, 63.7% male. At baseline, the percentage of patients with CKD stages III, IV and V was 6.4%, 25.7% and 67.8%, respectively. 61.4% on hemodialysis. Etiologies: vascular (24%), DM (21.1%), interstitial (7%), polycystic (5.8%), unknown (15.2%) and others (27%). Mean Charlson index 4.64(2.06), weight 72.7(15.09)kg and BMI 26.6. 96% of patients previously received another ESA: 21.4% epoetin alfa (mean weekly dose 11,612.90IU, once or more per week in 100%), 57.2% epoetina beta (5,798 IU/week, once a week in 73.8%) and 19.3% darbepoetina alfa (34 IU/week, once a week in 85%). At time of conversion to Mircera®, mean Hb level was 11.7(0.6)g/dL, and the proportion of patients with Hb values <11g/dL was 31%. Mean initial dose was 123.2(70.14)µg/month. At month 6, mean Hb was 11.8(0.6)g/dL and only 24% of patients had Hb values <11g/dL. In subgroup who initiated Mircera® as first treatment, mean Hb levels increased significantly for 10.4(0.88)g/dL to 11.6(1.34)g/dL (p<0.005). There were 17 minor adverse events not related to study drug. No discontinuation or dose reduction due to adverse drug reaction were identified. No patient died during the study.

**Conclusion:** Mircera® is a safe and effective option regardless the previous type of ESA or dosing schedule and simplifies anemia treatment secondary to CKD in routine clinical practice.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2336**

**Once-Monthly C.E.R.A. Maintains Stable Hemoglobin Levels in Patients with Chronic Kidney Disease Not on Dialysis (CKD NOD) Previously Treated with Darbepoetin alfa: An Italian Multicenter Experience** Bruno Cianciaruso. *Nephrology Division, University Federico II, Naples, Italy.*

Purpose: Once-monthly (QM) continuous erythropoietin receptor activator (C.E.R.A.) is approved for the maintenance of hemoglobin (Hb) levels in patients (pts) with CKD NOD. This Phase IIIb multicenter, single-arm, open-label study investigates direct switching from darbepoetin alfa (DA) to C.E.R.A. QM in pts NOD in a real-life clinical setting.

**Methods:** Adult pts with CKD NOD receiving a range of DA schedules as maintenance therapy were switched to subcutaneous C.E.R.A. QM over a 16-week titration period, followed by a 12-week evaluation period. Three Hb stability criteria (A, B and C) were applied through the study: (A) maintaining mean Hb levels within target range (10.0-12.0 g/dL); (B) maintaining mean Hb within ±1 g/dL of baseline Hb. The proportion of patients with (A) and (B) was defined as a challenging end point; (A) or (B) was defined as overall stability; (C) was defined as fulfilling overall stability without Hb ≥0.5 g/dL outside of 10.0-12.0 g/dL. The study also examined safety.

**Results:** Pts (n=87) had a mean age of 68.2 (36-87) years, with 55% having hypertension and 32% having diabetes at baseline; most pts were pre-treated with DA either once weekly (56%), once every 2 weeks (33%) or QM (5%). During titration and evaluation, respectively, mean (SD) Hb levels were 11.8 (0.9) and 11.5 (0.7) g/dL. Stable Hb was maintained in 60% of pts who fulfilled the more challenging end point (A+B), while 84% of pts maintained the more clinically relevant overall stability criterion (A or B), and 82% of pts achieved criterion C during evaluation. Mean Hb fluctuation was 0.46 g/dL during titration and 0.38 g/dL during evaluation. There was a mean of 2.0 dose changes per pt in 28 weeks of treatment; 59% of all dose changes were dose decreases with a median C.E.R.A. dose of 90 μg during the evaluation period.

**Conclusion:** These preliminary results show that C.E.R.A. QM is able to maintain stable Hb levels in pts with CKD NOD previously treated with DA (Q2W-QM), and indicate that lower than recommended C.E.R.A. doses may be effective in maintaining Hb stability in this pt population.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2337**

**Low-Protein Diet Supplemented with α-Ketoacids Delays Progression of Diabetic Nephropathy by Anti-Oxidative Stress in a KKAy Mice Model** Dongmei Liu, Xiang Gao, Changlin Mei. *Kidney Institute of PLA, Division of Nephrology, Chang Zheng Hospital, Second Military Medical University, Shanghai, China.*

There are controversies over whether low-protein diet (LPD) could delay the progression of chronic kidney disease (CKD) and reduce mortality. The aim of the present study was to see whether low-protein diet supplemented with ketoacids could slow down the progression of DN by observing progression of DN in a mice model of early diabetic kidney fed with different diets. The possible mechanisms were also discussed.

8-week-old male KK-Ay mice were selected as the type 2 diabetes model, and randomly divided into 3 groups: normal protein diet (NPD) group, low protein diet (LPD) group, and low protein diet supplemented with ketoacids (LPD+ KA) group. C57BL/6J mice were used as the control group.

The mice were fed with the NPD for the first 4 weeks, and then with the designated food for additional 12 weeks. Blood and urine samples were collected at 12,16, 20 and 24 weeks and 24-h urine for calculation of the urine protein/creatinine ratio.

Compared with the control group, significant pathological changes of DN, a visible increase in mesangial matrix, and significant thickening of glomerular basement membrane were observed in 24-week-old mice of NPD group. The changes in LPD and LPD + KA groups were not so severe as those in NPD group, and the mildest in LPD + KA mice. Lower body weight, lower serum albumin level, and increased urine protein/creatinine ratio were observed in LPD group, as compared with those of LPD + KA group.

SOD, MDA, oxybolt and nitrotyrosine in the renal tissue homogenate were detected. Compared with the control group, MDA, nitrotyrosine and oxybolt increased and SOD decreased significantly in KKAy mice ($P < 0.05$). Related parameters of LPD + KA group were significantly better than those of NPD and LPD groups.

In conclusion, compared with simple low-protein diet, low-protein diet supplemented with ketoacids delayed kidney damage in KKAy mice through reducing renal oxidative stress state. Low-protein diet supplemented with ketoacids may therefore prove to be a suitable diet for diabetic patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2338**

**Iron-Deficit Correction after the Administration of a Single Dose of Ferric Carboxymaltose (FCM) in Patients with Chronic Renal Disease (CKD) in Stages 3B and 4 K/DOQI in Treatment with Erythropoiesis-Stimulating Agents** Jose L. Gorriz,[1] Monica Climente-Marti,[2] Amelia Garcia-Hervas,[1] Veronica Escudero,[1] Victor Jiménez-Torres,[2] Luis M. Pallardo.[1] *[1]Servicio de Nefrologia, Hospital Universitario Dr Peset; [2]Servicio de Farmacia, Hospital Universitario Dr Peset.*

**Objective:** To evaluate the effectiveness and the safety of the administration of a single dose FCM in the correction of iron deficiency in patients with CKD stages 3b and 4 K/DOQI.

**Methods:** Longitudinal, prospective study including 21 patients CKD 3b and 4, who were treated with C.E.R.A and show suboptimal iron stores: ferritin < 300ng/mL and/or transferrin saturation (TSAT) < 20%. One dose of FCM was administrated. If ferritin < 300: 1000 mg; if ferritin 300-500: 500 mg.

The main ferritin values and TSAT were compared at the beginning and at the end (2 months after administrating FCM). 90.5% of the patients (19/21) were given 1000mg of FCM, whereas 2 patients were given 500mg. The median CERA dose was 66.9±27.9 (IC95% 54.2-79.6) mcg/month.

**Results:** Two months after treatment with one FCM dose, the proportion of patients with optimal ferritin values incremented from 14.3% (3/21) (IC95% -0.7-29.3) to 90.5% (19/21) (IC95% 77.9-103.0) with an RR 6.33 (IC95% 2.20-18.22) and for TSAT from 58.8% (10/17) (IC95% 35.4-82.2) to 83.3% % (15/18) (IC95% 66.1-100.6) with an RR 1.42 (IC95% 0.90-2.22). Complete response (optimal Ferritin and TSAT): 61.9% of patients (13/21). Partial response (optimal ferritin or TSAT): 38.1% (8/21). Patients with ferritin >500ng/mL: 38.1% (8/21) (IC95%17.3-58.9%). IST higher than 50%: 5.6% (1/18) (IC95% -5.0-16.1). Patients with Hb >11g/dL: 61.9% (13/21) at the beginning and 42.9% (9/21) at the end. Two patients (9.5%) reached Hb levels above 13.0g/dL by the end of the trial.

**Conclusions:** The administration of a single dose of FCM increases the proportion of patients with optimal ferritin and TS values; after 2 months, 61.9% of patients showed a complete response in the correction of iron deficiency. 38.1% of patients reached higher-than-optimal ferritin levels in the same period. The proportion of patients with optimal Hb levels did not change significantly.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2339**

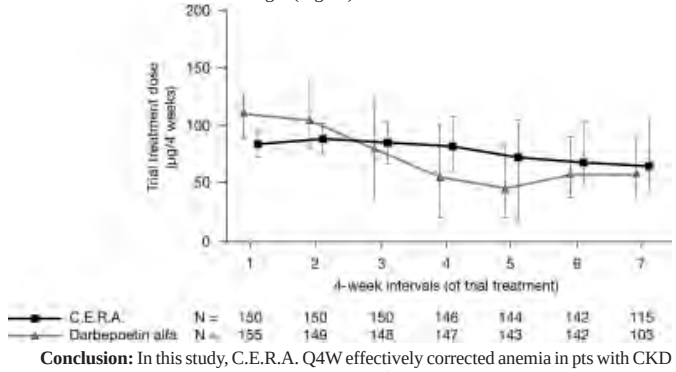
**Fewer Dose Changes and Hemoglobin (Hb) Values above the Target Range Following Once-Monthly (Q4W) C.E.R.A. Treatment in Anemic Patients (pts) with Chronic Kidney Disease (CKD)** Francesco Locatelli. *Alessandro Manzoni Hospital, Via dell'Eremo, Lecco, Italy.*

**Purpose:** The Phase III CORDATUS trial showed that once-monthly (Q4W) continuous erythropoietin receptor activator (C.E.R.A.) effectively corrects anemia in pts with CKD not on dialysis. These data were analyzed with respect to the relationship between dose and Hb values above the target range.

**Methods:** Adult pts with stage 3/4 CKD received either subcutaneous C.E.R.A. Q4W (starting dose of 1.2 μg/kg) or darbepoetin alfa (DA) either weekly or once every 2 weeks according to local labeling. After response (a single Hb value ≥10.0 g/dL and an increase from baseline ≥1.0 g/dL), Hb was to be maintained within the range of ±1.0 g/dL of the response level and between 10.0-12.0 g/dL.

**Results:** Of the randomized pts, 150 received C.E.R.A. and 155 DA. High response rates were achieved with both C.E.R.A. (94.1%) and DA (93.5%). Median times to Hb response were 43 days (C.E.R.A.) and 29 days (DA); fewer C.E.R.A.-treated pts than DA-treated pts had Hb values >12.0 g/dL in the first 8 weeks (25.8% and 47.7% respectively; $p<0.0001$). DA-treated pts required more dose adjustments to achieve and maintain Hb: 21% of the DA pts required ≥5 adjustments, compared with 11% of the C.E.R.A. pts.

Median C.E.R.A. doses changed little from baseline to evaluation period (6.6%) compared with a 35.6% decrease with DA, indicating a need for more modification of DA dose in order to maintain Hb in target (Figure).




**Conclusion:** In this study, C.E.R.A. Q4W effectively corrected anemia in pts with CKD not on dialysis. A more gradual increase in Hb and fewer Hb values above the target range were observed with C.E.R.A. than with DA. The results also indicate that a lower rate of Hb increase than proposed in current guidelines (1-2 g/dL per month) can correct anemia and significantly lower the number of Hb values exceeding the upper target.

Disclosure of Financial Relationships: Consultancy: I'm a member of an advisory board of Abbott, Affimax,Amgen,Genzyme,Merck,Shire, Takeda, Roche, GlaxoSmithKline and member of a safety committee of Sandoz.

**SA-PO2340**

**Evolution of Calciphylaxis before and after Introduction of Bisphosphonates. Our Experience** Ana Ramos, Jose-Vicente Torregrosa, Aleix Cases, Marta Arias, Josep Campistol. *ICNU, Hospital Clinic, Barcelona, Spain.*

Calciphylaxis is a rare and serious disease, with a very high morbi-mortality. It is a vascular pathology affecting almost exclusively patients with end stage renal failure (ESRF) either on hemodialysis (HD) or renal transplanted (RT) (prevalence of 1-4%). There are few

effective treatments. Bisphosphonates experimentally have showed interesting effects on vascular calcifications thorough an inhibition of cytokines and proinflammatory mediators, so they could be usefulness in this setting.

We describe our experience comparing the last 7 patients presenting with calciphylaxis before introduction of bisphosphonates (Group I) and our first 7 patients with calciphylaxis treated with bisphosphonates (Group II).

METHODS: Group I (before 2002): 7 patients (3 on HD / 4 RT) mean age: 50±13 years. 4 men/3women, mean time on HD: 4.8±1.5 years. 4 with history of PTH>800 pg/ml, steroids in 4. Group II (after 2002): 7 patients (5 on HD / 2 RT) mean age: 61±7 years, 3 men/4women, mean time on HD: 6.7±5.9 years. 5 with history of PTH>800 pg/ml, steroids in 5. All of them (14) showed an important cutaneous involvement in the inner part of both thighs diagnosed of calciphylaxis (cutaneous biopsy in 12 of them). Group I: in all patients on HD the frequency was increased, 3 patients (1 TR) were parathyroidectomized (PTx). Group II: all of them received bisphosphonates: Alendronate 70 mg/weekly in one case, Risendronate 35 mg/weekly in four cases and Ibandronate 3 mg/ev + Ibandronate 150 mg/ monthly in two cases. No negative effects were observed.

RESULTS: Group I =5 cases required an amputation of the leg, 1 case died and in 1 case the lesion improved and resolved (RT patient with high PTH that was PTx). Group II =all of them showed a progressive improvement of the cutaneous lesions with complete resolution after 6 months. There were no significant changes in blood levels of Ca and P in patients receiving bisphosphonates. Renal function remained stable.

CONCLUSIONS: Bisphosphonates could constitute a new and attractive alternative as a treatment of calciphylaxis.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2341

**Toward a Rational Basis for Phosphate Binder Prescribing: Estimating the Binding Capacity of Available Binders** Raymond D. Pratt,[1] Michael D. L. Smyth.[2] *[1]Shire Pharmaceuticals, Wayne, PA; [2]Shire Pharmaceuticals, Basingstoke, United Kingdom.*

**Purpose:** Self-adjustment of phosphate binder (PB) dose to suit meal phosphate content has recently been shown to improve the management of hyperphosphatemia in CKD patients. We calculated the binding capacity of available PBs to provide information that may help to tailor dosage to the patient's needs. **Methods:** We examined dietary phosphate absorption in hemodialysis patients and healthy individuals, and calculated the binding capacities of PBs based on data from a metabolic study of lanthanum carbonate and sevelamer carbonate and the published results of other trials in healthy volunteers; these studies directly measured phosphate binding in the gastrointestinal tract. Results were compared with estimates based on urinary phosphate excretion. **Results:** A metabolic study in hemodialysis patients showed that absorption of ingested phosphate ranged from 60–86% depending upon vitamin D status. In healthy controls, 80% of phosphate was absorbed. This was consistent with the results of the lanthanum/sevelamer study in volunteers (N = 18), in whom 76% of phosphate was absorbed. Therefore, published data from healthy volunteers were used to calculate the binding capacities of PBs (Table).

| | Phosphate bound, mg (Dose [mg] per meal, N) | | |
|---|---|---|---|
| Binder | Metabolic Study | Urinary Excretion | Phosphate bound per tablet, mg |
| Lanthanum carbonate | 135 (1000[a], 18) | 79–156[b] (1000[a], 6–48) | 135 vs 79–156 |
| Calcium acetate | 177 (1000[a], 10) | 132 (1000[a], 6) | 30 vs 22[c] |
| Calcium carbonate | 116 (1000[a], 10) | 31 (1000[a], 6) | 46 vs 12[d] |
| Sevelamer | 63 (2400, 18) | 36 (1000, 6) | 21 vs 29[e] |

[a]Elemental La or Ca. Calculations are vs controls or [b]change from baseline. [c]Based on PhosLo®. [d]Based on ultra strength TUMS®. [e]Sevelamer carbonate vs sevelamer hydrochloride, respectively.

**Conclusions:** Data from direct phosphate binding studies in healthy volunteers generally gave similar results to calculations of binding capacity using urinary phosphate excretion. These data may be useful to begin tailoring PB dosage to the patient's dietary phosphate intake.

Disclosure of Financial Relationships: Employer: Shire Pharmaceuticals.

## SA-PO2342

**Medication Regimen Complexity among Patients with Chronic Kidney Disease Stages 3-5D** Katie E. Cardone, Magdalene M. Assimon, Darius Mason, Amy B. Pai, Darren W. Grabe. *ANephRx, Albany College of Pharmacy and Health Sciences, Albany, NY.*

Background: Medication regimen complexity should be evaluated in patients with chronic kidney disease (CKD). The Medication Regimen Complexity Index (MRCI) is a tool that has been validated in patients with respiratory disease, and accounts for dosage forms, administration frequency, and special instructions that may complicate a medication regimen and lead to poor adherence or medication-related problems (MRPs). The typical patient with ESRD takes approximately 12 medications, putting them at high risk for MRPs. MRCI may have utility in assessing regimens of those with CKD. The objective of this study was to evaluate the MRCI for typical medication regimens in patients with CKD 3-5D.

Methods: This is a cross-sectional study in which patients with CKD 3, 4, & 5D (on hemodialysis), at least 18 y.o. & seen at least twice at the study clinic were included. Home medication regimens were determined for each patient at enrollment. Number of medications (NM), pill burden (PB) and MRCI were calculated for each regimen. Student's t test was used to compare means between groups. Pearson's correlation was used to identify relationships between MRCI and number of comorbid conditions (NCC), PB, & NM. A 5% significance level was used.

Results:

| | CKD 3-4 (n=31) | HD (n=40) |
|---|---|---|
| DEMOGRAPHIC | | |
| Age (yr) | 65.8±14.7 | 64.4±15.6 |
| Male (%) | 43.3 | 52.5 |
| GFR (ml/min/1.73 m2) | 32.5±14.0 | |
| HD vintage (yr) | | 3.4±2.8 |
| NCC* | 8.6±3.1 | 12.3±3.2 |
| MEDICATION REGIMEN | | |
| NM | 12.9±3.7 | 14.3±5.0 |
| PB* | 16.7±5.2 | 22.4±10.8 |
| MRCI* | 25.6±11.4 | 33.8±13.5 |

Mean±SD; *p<0.05

MRCI positively correlated with NM (r = 0.82) and PB (r = 0.86) in the CKD group and positively correlated with NM (r = 0.90), PB (r = 0.76) and NCC (r = 0.73) in the HD group, $p < 0.05$ for all correlations.

Conclusion: MRCIs are higher in patients receiving HD compared to those with CKD 3-4. This finding is associated with more comorbid disease states and accordingly, a greater number of medications and pill burden, well-established risk factors for MRPs. These data indicate the importance of evaluating the predictive value of MRCI on costly outcomes such as adverse drug events and hospitalizations.

Disclosure of Financial Relationships: Research Funding: Merck & Co.

## SA-PO2343

**Adding Dietary Fruits and Vegetables Reduces Kidney Injury in Subjects with Moderately Reduced GFR** Nimirit Goraya,[1, 2] Jan Simoni,[3] Kristine Broglio,[4] Donald E. Wesson.[1, 2] *[1]Internal Medicine, Texas A&M College of Medicine, Temple, TX; [2]Internal Medicine, Scott and White Healthcare, Temple, TX; [3]Surgery, Texas Tech University Health Sciences, Lubbock, TX; [4]Statistics, CenterTexas A&M University, College Station, TX.*

Subjects with hypertensive nephropathy (HN) have progressive GFR decline despite blood pressure reduction and ACE inhibition (Arch Int Med 2008). Dietary alkali ameliorates kidney injury and GFR decline in HN with severely reduced (Kid Int 2010) and moderately reduced (Kid Int 2010) eGFR. Because diets in industrialized societies are largely acid-inducing, we explored if dietary addition of base-inducing fruits + vegetables reduces kidney injury in HN with moderately reduced eGFR (CKD stage 2 = 60-90 ml/min). Subjects with macroalbuminuric HN and eGFR 60-90 ml/min (n=40) and those with eGFR > 90 ml/min (n=26) had blood pressue reduction with regimens including ACE inhibition. eGFR 60-90 compared to eGFR > 90 had baseline potential renal acid load (PRAL), a measure of dietary acid intake (60.4 ± 19.4 vs. 62.8 ± 14.5 mmol/day, respectively, $p = 0.582$) and baseline 8 hour urine net acid excretion (8h NAE) (24.6 ± 5.0 vs. 24.7 ± 2.9 meq, respectively, $p = 0.924$) that were not different. Thirty days of fruits + vegetables added in an amount calculated to reduce PRAL by 50% in eGFR 60-90 subjects reduced 8h NAE to 16.5 ± 5.1 meq, $p < 0.0001$. This dietary intervention in eGFR 60-90 subjects also reduced urine excretion of albumin (422 ± 152 to 388 ± 122 mg/g creatinine [cr], $p < 0.0001$), N-acetyl-β-D-glucosaminidase (2.68 ± 0.73 to 2.60 ± 0.70 U/g cr, $p < 0.0001$) and transforming growth factor β (63.8 ± 14.5 to 57.3 ± 13.7 ng/g cr, $p < 0.0001$), consistent with reduced kidney injury. Time control eGFR subjects (n=40) showed no change in any of the 3 kidney injury parameters. The data show that adding fruits + vegetables to diets of subjects with macroalbuminuric hypetensive nephropathy and CKD stage 2 eGFR improves urine parameters of kidney injury and support exploring this dietary intervention as an adjunctive kidney protective strategy to blood pressure reduction and ACE inhibition.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2344

**Efficacy and Safety of Long-Term Intravenous (I.V.) Ferric Carboxymaltose (FCM) in the Treatment of Iron Deficiency Anemia in Non-Dialysis Dependent Chronic Kidney Disease (ND-CKD) Patients** Joseph Benjamin. *Dept of Nephrology, Temple University, Philadelphia, PA.*

**Introduction:** The purpose of this study was to evaluate the safety and efficacy of I.V. ferric carboxymaltose (FCM) when used as long-term therapy of iron deficiency anemia (IDA) in ND-CKD patients.

**Methods:** This was an open-label, uncontrolled, extension (up to 44 wks) of a previously conducted study comparing I.V. FCM to oral iron in anemic ND-CKD patients. FCM was administered as 15mg iron/kg bodyweight I.V. over 15 minutes to a maximum of 1000mg iron in subjects with TSAT <25% and ferritin <300ng/ml (dose was reduced to 500mg iron if TSAT <30% & ferritin < 500ng/ml). Hb and iron parameters were measured every 4 wks. Clinical & laboratory findings, and adverse events (AEs) were monitored. Efficacy (clinical success) was defined as % of subjects with Hb >11.0g/dL, TSAT 30-50%, and ferritin 100-800ng/ml at any single visit. In addition, the mean change from baseline to the highest value for Hb, TSAT and ferritin were summarized.

**Results:** A total of 28 sites enrolled 145 subjects (104 subjects [71.7%] completed the study;140 were included for efficacy analysis; 127 for safety). The mean baseline values were: Hb 10.36g/dL, TSAT 17.2% and ferritin 115ng/ml. Clinical success was achieved in 51.4% (72/140) of subjects. Similar proportions of subjects receiving FCM (52.3%) or oral iron (50.0%) in the previous trial achieved clincial success in the present study. The mean change from baseline to highest Hb was 1.89g/dL, baseline to highest TSAT was 20.4%, & baseline to highest ferritin was 745ng/ml. During the study, at least one AE was experienced by 66.1% (84/127) of subjects. Most of these were classified as mild to moderate in severity. At least one serious AE was reported in 18.1% (23/127) of subjects, including

2 deaths (intestine perforation due to diverticulitis 98 days after last dose of FCM, upper GI bleed following exploratory laparotomy 46 days after last dose). No serious AEs were determined to be related to FCM.

**Conclusion:** In this study of ND-CKD patients, ferric carboxymaltose was shown to be effective & well tolerated for the long-term treatment of IDA regardless of previous treatment.

Disclosure of Financial Relationships: Consultancy: American RegentResearch Funding: Luitpold Pharmaceutical, inc.

### SA-PO2345

**Insulin Resistance in ESRD** Sara Kazempour,[1] Maytham Omran,[4] Jeremy J. Turner,[3] Frederick W. K. Tam,[2] Gary S. Frpst,[1] Anne Dornhorst,[1] Andrew H. Frankel.[2] *[1]Imperial College; [2]Imperial College; [3]Norfolk & Norwich University Hospital; [4]Imperial College.*

Insulin resistance (IR) is known as a contributing factor to cardiovascular disease (CVD), and CVD is the main cause of high morbidity and mortality rates in the end-stage renal disease (ESRD) population. Although it is likely that multiple factors contribute to CVD risk, understanding changes in IR is likely to be of importance in preventing adverse outcomes. Inflammation and adiponectin are also known to influence both insulin sensitivity and CVD, and may therefore be important regulating factors in prevention against CVD.

**Aims.** To examine changes in IR, inflammation and adiponectin levels in chronic kidney disease (CKD) stages 3, 4, 5 and maintenance haemodialysis (MHD) and to establish whether these changes are predictors of cardiovascular events (CVE) and mortality.

**Methods.** Assessment of IR (HOMA-IR model), inflammation status assessed by high-sensitivity CRP (hs-CRP) and adiponectin levels were available for 55 patients with CKD (stages 3-5) and 51 patients on MHD together with 40-month CVE and mortality data.

**Results.** The mean IR as measured by HOMA-IR increased through the CKD stages 3-5 before significantly falling in the MHD group (1.78±1.30 in CKD 3, 2.73±2.52 in CKD 4, 3.57±2.33 in CKD 5 and 1.50±1.46 in the MHD group, p=0.001). The mean adiponectin and hs-CRP were similar among the CKD and MHD groups.

Regression analysis for CVE risk revealed that hs-CRP was significantly associated with increased CVE in the CKD group with a hazard ratio (HR) of 1.092 (CI: 1.024, 1.165; p=0.002). Calculations for risk of mortality showed that high adiponectin and hs-CRP levels were both predictive of mortality in the MHD group (HR: 1.029, CI: 1.005, 1.054; p=0.020 and HR: 1.018, CI: 1.000, 1.038; p=0.05 respectively).

**Conclusions.** Our study clearly shows that IR increases as renal function deteriorates but is significantly improved with haemodialysis. In this cohort changes in IR were not predictive of CVE or mortality. However, an increased hs-CRP was predictive of CVE in the CKD group while increased adiponectin and hs-CRP levels were both predictive of mortality among the MHD group.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2346

**A Cost Analysis of Effecting Whole Population Improvement in Blood Pressure Control in CKD** Hannah Kilbride, Jean Irving, Paul E. Stevens, Kushan Karunaratne, Helen Hobbs, Richard W. C. Kingston, Christopher K. T. Farmer. *Kent Kidney Care Centre, East Kent Hospitals, Canterbury, Kent, United Kingdom.*

Introduction

Inclusion of renal indicators in primary care incentive payments, the Quality and Outcomes Framework (QOF), has led to a significant improvement in blood pressure (BP) control.

Aim

To undertake a cost analysis of the improvement in BP control following implementation of renal indicators in the QOF.

Methods

7474 of 36519 individuals with serum creatinine estimations 2 y pre- and 2 y post-QOF had stage 3-5 CKD and comprised the study cohort. Demographic, GFR, BP and anti-hypertensive prescription data were recorded. Hypertension was defined as a mean BP >140/85 or on antihypertensive drugs pre-QOF. The annual cost of antihypertensives was calculated based on primary care prescription data. Generic prescribing costs were also calculated.

Results

5871/7474 (79%) were hypertensive pre-QOF. BP control improved from 147/79 ± 14/8 to 140/76 ± 22/12 mmHg post-QOF (p<0.001). The table details annual prescription data, actual costs and generic costs pre- and post- introduction of renal indicators in the QOF.

| | | Pre-QOF | | | Post-QOF | |
|---|---|---|---|---|---|---|
| Prescription item | N | Actual cost, $ | Generic cost, $ | N | Actual cost, $ | Generic cost, $ |
| Diuretic | 624 | 39,051 | 8,828 | 1,780 | 56,408 | 24,929 |
| Beta-blocker | 390 | 15,880 | 5,859 | 1,166 | 53,167 | 17,244 |
| Alpha-blocker | 213 | 12,052 | 11,013 | 430 | 24,666 | 22,129 |
| Ca channel blocker | 407 | 66,571 | 8,815 | 1,080 | 179,884 | 23,132 |
| ACEI | 2,904 | 124,853 | 75,578 | 2,186 | 86,742 | 56,318 |
| ARB | 1,166 | 278,028 | 278,028 | 1,451 | 342,532 | 342,532 |
| Other | 30 | 4,301 | 8,733 | 45 | 8,187 | 13,100 |
| Total prescription costs | | 540,735 | 449,774 | | 751,585 | 565,854 |

Conclusion

Improved BP control was effected through increased antihypertensive prescribing, a 40% increase in prescribing costs. Incentive payments to primary care will have further increased costs but a significant proportion of these could be offset by generic prescribing. The increased cost is approximately $36.4/patient treated/y which, given the reduction in BP achieved, is well within the cost effective threshold of $30K per quality adjusted life year (QALY) outlined in UK NICE guidance.

Disclosure of Financial Relationships: nothing to disclose

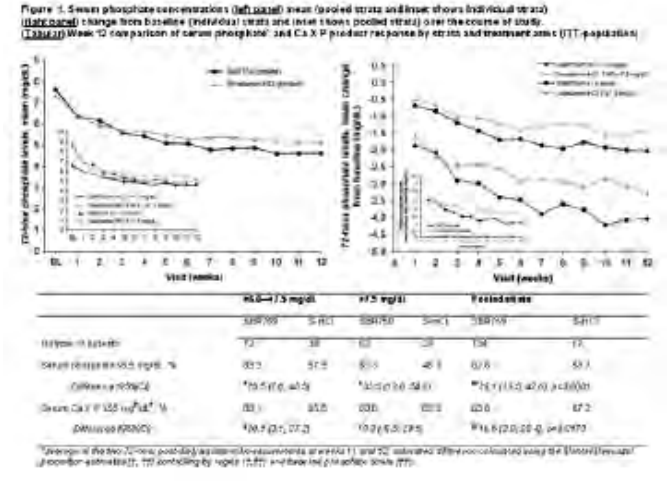
### SA-PO2347

**SBR759 Is More Effective Than Sevelamer Hydrochloride in Sustaining Serum Phosphate Reduction in Hemodialysis Patients** J. B. Chen, S. S. Chiang, H. C. Chen, S. Obayashi, M. Nagasawa, M. Hexham, A. Balfour, N. Cretin, Takashi Akiba, Masafumi Fukagawa. *For Study A2202.*

**Purpose**: SBR759, a novel calcium-free, polymeric, iron(III)-based oral phosphate binder (OPB) for the treatment of hyperphosphatemia was compared with sevelamer-HCl (S-HCl).

**Methods**: This was a 12 week, open-label, dose-titration study in Asian hemodialysis (HD) patients with hyperphosphatemia. Patients (N=203) were stratified (baseline P< or ≥7.5mg/dL) and randomized to starting doses of 3.0 or 4.5g SBR759 (N=135) or 2.4 or 4.8g S-HCl (N=68). Daily doses were up-titrated every 2 weeks to reach target serum P levels ≤5.5mg/dL at 72-h post-dialysis. Primary end point was the proportion of patients with mean target P at week 12.

**Results**: Age, dialysis conditions, and baseline P levels were comparable between SBR759 and S-HCl groups. A significantly higher proportion of SBR759 patients achieved target P levels at week 12 compared to S-HCl (83% vs 54%, p<0.0001; pooled). P levels in pooled strata were numerically lower from week 3 to 12 with SBR759 than with S-HCl and better in both strata at week 12. Greater reductions in P levels throughout the study with SBR759 were clinically meaningful and statistically significant vs S-HCl at week 12 (-0.95 vs -0.71mg/dL, p=0.0024) (Fig.1).



Calcium (Ca) levels did not change from baseline and CaXP was ≤55mg$^2$/dL$^2$ in a significantly higher proportion of patients on SBR759 (p=0.0173). Similar incidences of (serious)/adverse event (S/AE) were seen with SBR759 and S-HCl (5.2/90.3% vs 4.4/94.1%), but overall discontinuation rates were lower with SBR759 (11.9% vs 20.6%). Diarrhea reported as AE was more frequent with SBR759 whereas constipation and abdominal distension affected more patients on S-HCl. No SAE was drug-related.

**Conclusion**: SBR759 was superior to standard of care OPB S-HCl in achieving target serum P levels in HD patients and was well-tolerated.

Disclosure of Financial Relationships: Research Funding: Novartis Pharma K K; Other Relationship: Travel expenses.

### SA-PO2348

**SBR759 Is Well-Tolerated with Good Gastrointestinal Tolerability Profile Compared to Sevelamer Hydrochloride** Takashi Akiba,[1] J. B. Chen,[2] H. C. Chen,[2] S. Obayashi,[2] M. Nagasawa,[2] M. Hexham,[2] A. Balfour,[2] N. Cretin,[2] Masafumi Fukagawa,[2] S. S. Chiang.[2] *[1]Tokyo Women's Medical University, Japan; [2]For Study A2202.*

**Purpose**: SBR759 is a novel, calcium-free, polymeric, iron (III)-based oral phosphate binder for the treatment of hyperphosphatemia. 12wk safety results from a phase II, open-label, dose-titration study in Japanese and Taiwanese hemodialysis patients are reported here.

**Methods**: Patients (N=203) with elevated phosphate (P) levels were stratified according to baseline P levels (< or ≥7.5mg/dL) and randomized 2:1 to starting doses of 3.0 or 4.5g SBR759 (N=135) or 2.4 or 4.8g sevelamer hydrochloride (S-HCl) (N=68). Daily doses were up-titrated every 2 wk to reach target serum P level ≤5.5mg/dL at 72h post-dialysis. Safety was evaluated on the basis of adverse events (AE), serious AE (SAE) and changes in laboratory values. Efficacy was assessed by number of patients achieving target serum P at wk 12.

**Results:** Efficacy of SBR759 was superior to S-HCl in achieving target serum P at wk 12 (83% vs 54%; p<0.0001). Similar incidences of SAE/AE were seen with SBR759 and S-HCl (5.2/90.3% vs 4.4/94.1%); no SAE was drug-related. Overall discontinuation rates were lower with SBR759 (11.9% vs 20.6%) as well as discontinuation due to AE (3.7%

vs 13.2%). Most frequent AE category with SBR759 and S-HCl was gastrointestinal (GI) disorders (57.5% vs 64.7%). GI AE intensity was mostly mild with SBR759 (mild 45.5%, moderate 11.2%; severe 0.7%) whereas with S-HCl more moderate and severe AEs were reported (mild 30.9%; moderate 27.9%; severe 5.9%). Diarrhea AEs were more frequent with SBR759 (19.4% vs 10.3%); constipation and abdominal distension affected more patients on S-HCl (5.2% and 25.0% vs 3.0% and 25.0%, respectively). Based on Deficiency of Acquired Immune Deficiency Syndrome (DAIDS) grading, majority of diarrhea AE were of grade 1 (lowest severity) with SBR759 and S-HCl (18.7% vs 10.3%).

**Conclusion:** SBR759 is well-tolerated with good GI tolerability profile compared to S-HCl.

Disclosure of Financial Relationships: Consultancy: Consultant fee from Chugai RocheResearch Funding: Grant/Research support from Novartis.

## SA-PO2349

**One Dose Iron Repletion with Iron Isomaltoside 1000 (Monofer®) in Pre-Dialysis CKD Patients** Philip A. Kalra,[1] Sunil Bhandari,[2] Lars L. Thomsen.[3] *[1]Renal Medicine, Salford Royal Hospital, Salford, United Kingdom; [2]Renal Medicine, East Yorkshire Hospitals, East Yorkshire, United Kingdom; [3]Clinical R&D, Pharmacosmos A/S, Holbaek, Denmark.*

Purpose: Patients with chronic kidney disease (CKD) often need treatment with intravenous iron preparations. The present study analysed the use of high single dose infusion of iron isomaltoside 1000 to treat anemia in a pre-dialysis subgroup of patients recruited in a previously presented open-label, multicentre safety study (1).

Methods: 21 pre-dialysis patients with CKD and anemia (mean age 69y; range 43-83y; 8 F , 13 M, mean (SD): serum creatinine 325µmol/L (131), Hb 102g/L (8), TSAT 16.9% (11.5), Ferritin 191ug/L (134)) were included in the 8 week study. 19 patients were not currently treated with parenteral iron and 2 patients switched from an existing parenteral iron treatment. 7 patients were on ESA therapy which was maintained without dose change during the study. Iron isomaltoside 1000 was administered as a Total Dose Infusion (mean dose: 953.5mg (range 650-1550mg)) diluted in 100-500 ml 0.9 % saline and infused over 30-60min at baseline. No initial test dose was given.

Results: One solitary possible treatment related adverse reaction was observed. This was an anginal episode in an 80 year old male patient with a known medical history of angina pectoris. The reaction was classified as serious and possibly related to the trial medication by the investigator. However, it occurred 10 days after the patient had received 1400 mg iron isomaltoside 1000. No acute anaphylactoid/anaphylactic or delayed allergic reactions were observed. There were no clinically significant changes in routine clinical laboratory tests or vital signs. The mean maximal change from baseline in Hb was 8.5 g/L (SD: 8.8: p=0.0003). The mean maximal change from baseline in serum ferritin was 500 µg/L (SD: 208::p < 0.0001).

Conclusion: One dose iron repletion with Iron isomaltoside 1000 administered intravenously to pre-dialysis CKD patients in this study was safe and well tolerated and improved markers of iron deficiency anemia.

1) Poster No. M560, World Congress of Nephrology, May 22-26 2009, Milan, Italy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2350

**The Effect of Once-Monthly C.E.R.A. Administration on Iron Status and Hemoglobin Concentrations in Dialysis Patients with Chronic Kidney Disease** Kostas C. Siamopoulos. *(on Behalf of the ML20952 Study Group) Department of Nephrology, University Hospital of Ioannina, Ioannina, Greece.*

**INTRODUCTION AND AIMS:** Continuous erythropoietin receptor activator (CERA) is a novel agent with unique receptor activity, allowing once-monthly (Q4W) administration for effective maintenance of stable hemoglobin (Hb) in patients (pts) with chronic kidney disease (CKD). We examined the effect of Q4W CERA on iron status, assessed by ferritin/transferrin saturation (TSAT), and on Hb concentrations.

**METHODS:** This prospective, multicentre, single arm, open label study assessed the efficacy, safety and tolerability of Q4W CERA for the maintenance of Hb levels in dialysis patients with CKD. Pts (n=188) in Greece (≥18y) receiving adequate dialysis and IV epoetin or darbepoetin with stable baseline (bl) Hb (10.5-12.5g/dL) entered a 16-week (wk) CERA dose titration phase (DTP) followed by an 8wk efficacy evaluation period (EEP). At DTP (wk16) and EEP (wk24) efficacy parameters and exposure were analyzed by bl tertiles of ferritin and TSAT. The iron administration protocol remained unchanged during the whole course of the study.

**RESULTS:** The table below shows that CERA Q4W was effective in maintaining Hb levels, irrespective of ferritin/TSAT bl levels (ANOVA test).

| | Bl mean (SD) Hb (g/dL) | Wk 16 mean (SD) Hb (g/dL) | Wk 24 mean (SD) Hb (g/dL) |
|---|---|---|---|
| Ferritin µg/L | | | |
| ≤295 | 11.6 (0.56) | 11.9 (0.87) | 11.6 (1.01) |
| >295-≤529 | 11.6 (0.54) | 11.8 (1.02) | 11.7 (1.15) |
| >529 | 11.6 (0.60) | 11.7 (1.06) | 11.4 (1.20) |
| TSAT % | | | |
| ≤25.5 | 11.6 (0.57) | 11.8 (0.87) | 11.7 (1.19) |
| >25.5-≤35.5 | 11.6 (0.54) | 11.8 (1.09) | 11.5 (1.08) |
| >35.5 | 11.6 (0.60) | 11.7 (0.99) | 11.5 (1.11) |

Mean TSAT at wk16 was 40(SD=18.8)% and wk24 43(30.0)% were significantly higher (*p<0.05;* sign ranked test*)* compared to bl 35(16.1)%. This could be correlated with the trend of decreasing CERA doses over time, especially in the low TSAT subpopulation.

**CONCLUSIONS:** Irrespective of baseline ferritin and TSAT levels, Q4W CERA administration in dialysis pts provided stable Hb maintenance. Interestingly, the analysis showed that during the 24wks of the study the mean TSAT levels significantly increased compared to baseline.

Disclosure of Financial Relationships: Research Funding: Roche, Novarits; Honoraria: Roche, Novarits.

## SA-PO2351

**Effect of Iron Repletion on Platelet Counts (PLT) in Iron Deficient (ID) Non-Dialysis-CKD Patients** Anatole Besarab, Jerry Yee, Stan Frinak. *Nephrology & Hypertension, Henry Ford Health System.*

ID produces "relative" thrombocytosis that may contribute to thrombotic complications noted in erythropoietin treated patients. A database analysis of 40,000 hemodialysis (HD) pts found PLT to be inversely related to severity of ID. Post-hoc analysis of the DRIVE Study showed a reduction in PLT after administration of 1000 mg of ferric gluconate over 8 dialysis sessions to HD patients.

We dosed non-dialysis CKD outpatients with 500 or 1000 mg of low molecular weight iron dextran over 2-4 hr to correct ID, [TSAT < 20%, ferritin <100 ng/mL] and analyzed hematologic responses of Hb, iron indices, and PLT in 132 subjects without intercurrent events who were receiving once-monthly darbepoetin (DA) and 32 pts "unresponsive to oral iron therapy" not on DA (table1).

Results are presented as mean (sem). Age and eGFR of the DA treated (+) and DA-naive (-) subjects were 69.9 yr and 68.3 yr and 34.4 (1.4) and 36.5 (1.7) mL/min/1.73 $m^2$ respectively, both p >0.05.

| parameter | Dose status/Time | - 1 mo | 0 (basal) | + 1 mo | +2 mo |
|---|---|---|---|---|---|
| Hb (g/dL) | DA+ | 10.2 (0.1) | 10.3 (0.2) | 11.4 (0.2) | 11.5 (0.2) |
| Hb (g/dL) | DA- | 10.1 (0.2) | 10.0 (0.2) | 10.7 (0.2) | 10.9 (0.2) |
| PLTS (x 1000) | DA+ | 295 (12) | 296 (9) | 268 (12) | 255 (7) |
| PLTS (x 1000) | DA- | 289 (19) | 308 (18) | 281 (17) | 264 (13) |
| TSAT (%) | DA+ | 16.3 (1.0) | 13.2 (0.6) | 24.4 (1.2) | 25.3 (1.3) |
| TSAT (%) | DA- | 15.5 (1.1) | 14.0 (1.0) | 24.5 (1.9) | 25.6 (2.0) |
| Ferritin (ng/mL) | DA+ | 56 (5) | 39 (5) | 167 (16) | 155 (15) |
| Ferritin (ng/mL) | DA- | 66 (8) | 53 (7) | 237 (21) | 203 (18) |
| DA dose (mcg/mo) | DA+ | 116 (10) | 170 (11) | 157 (12) | 115 (11) |
| DA dose (mcg/mo) | DA- | 0 | 0 | 0 | 0 |

MANOVA : iron dose in DA + pts determined magnitude of change from baseline in PLT, DA dose, Hb and ferritin (all p<0.05). PLT decrease was greatest in those with basal counts >300 K, varied inversely with initial basal PLT (R=0.70,, p<0.05)) and was independent of basal ferritin, TSAT or DA dose.

In summary, correction of ID lowers PLTS and improves erythropoiesis. The PLT effect is independent of DA. Clinical significance of PLT reduction to other outcomes requires additional study.

Disclosure of Financial Relationships: Consultancy: Amgen, Hoffman la Roche, Akebia, Affymax, Rockwell International; Ownership: Vasc AlertResearch Funding: Abbott, Roche, Fibrogen, Luitpold; Honoraria: Affymax, Amgen, ASN, Ash Access Technology, Bioconnect, FALLON MEDICA, FMC, Genentech, HemoSphere, Hoffman la Roche, Hospira, Indiana University, John Hopkins Univ, Luitpold Pharm, Merck and Co, National Kidney Fnd, NKF of Michigan, NKF of Georgia, New York Soc of Nephrology, QUINTILES, Renal Advantage, Rockwell Medical, Scientic Consulting Group (NIH) Soc of Nephrology of Puerto Rico, Speedel, St. Michael's Hosp. (Toronto), St. John's Hosp. (Detroit), Takeda, University of Cincinatti, University of Miami, University of Missouri, VascAlert, Walter Kluger (Publisher) Winthrop Univ., Watson Pharma; Scientific Advisor: Amgen, Affymax, Akebia, Rockwell International.

## SA-PO2352

**The Impact of Intravenous Ferric Carboxymaltose on Renal Function: An Analysis of the FAIR-HF Study** Piotr Ponikowski,[2] Iain C. Macdougall,[1] Stefan D. Anker.[3] *[1]Kings College Hospital, London, United Kingdom; [2]4th Military Hospital, Wroclaw, Poland; [3]Charité, Campus Virchow-Klinikum, Berlin, Germany.*

Background: Renal dysfunction commonly complicates the natural course of chronic heart failure (CHF) and predicts poor outcome. Currently applied CHF therapies have either no effect on, or even worsen renal function. The FAIR-HF study demonstrated that treatment with intravenous ferric carboxymaltose (FCM) in iron deficient CHF patients is well tolerated and improves symptoms and quality of life. Here we report the effects of FCM on renal function in FAIR-HF. Methods: We enrolled 459 CHF patients (NYHA class II/III, LVEF 32%) with iron deficiency (ferritin <100 µg/L, or between 100-300 µg/L if transferrin saturation <20%); 304 patients were randomly assigned to FCM and 155 to placebo; treatment was continued for 24 weeks. Renal function was assessed at baseline and at Week 4, 12 and 24 visits as estimated glomerular filtration rate (eGFR) using the MDRD formula. Results: At baseline, renal function did not differ between groups (63.8±21.2 vs 64.8±25.3 mL/min/1.73$m^2$, FCM vs placebo). FCM significantly improved NHYA class and self-assessed patient global assessment from week 4 (P<0.001) and the effect was independent of baseline eGFR and anemia status (P>0.42). Treatment with FCM was associated with improved renal function across all patients (table; P>0.2 for interaction with baseline renal function, age, sex, CHF severity, underlying CHF aetiology, anemia status). More patients in the FCM group demonstrated an eGFR improvement of at least 5mL/min/1.73$m^2$ (week 4: 38% vs 34%, week 12: 33% vs 26%, week 24: 35% vs 25%, FCM vs placebo respectively).

Effect of FCM on renal function

| | eGFR change (mL/min/1.73m$^2$) | | | |
|---|---|---|---|---|
| | FCM | Placebo | eGFR difference (ml/min/1.73m$^2$)* | P for treatment |
| Week 4 | 3.7±0.9 | 0.9±1.2 | 2.8±1.5 | 0.054 |
| Week 12 | 2.3±0.9 | -0.7±1.1 | 3.0±1.5 | 0.049 |
| Week 24 | 3.2±0.7 | -0.6±1.4 | 4.0±1.7 | 0.017 |

*difference between FCM and placebo (LSM mean±SE)

Conclusions: Intravenous FCM improved CHF symptoms and quality of life regardless of baseline renal function. Iron treatment was also associated with improved renal function.

Disclosure of Financial Relationships: Consultancy: Vifor PharmaResearch Funding: Vifor Pharma; Honoraria: Vifor Pharma; Scientific Advisor: Vifor Pharma.

## SA-PO2353

**Early Improvement in Blood Pressure and Renal Cytokines after Bariatric Surgery** Sukhpreet Singh Dubb,[1] Marco Bueter,[1] Abhijit Singh Gill,[1] Lia Joannou,[2] Ahmed Ahmed,[3] Andrew H. Frankel,[4] Frederick W. K. Tam,[4] Carel W. Le Roux.[1] *[1]Department of Investigative Medicine, Imperial College, London, United Kingdom; [2]Department of Chemical Pathology, Imperial College, London, United Kingdom; [3]Department of Academic Surgery, Imperial College, London, United Kingdom; [4]Imperial Kidney and Transplant Institute, Imperial College, London, United Kingdom.*

**Introduction:** We hypothesised that metabolic related inflammatory cytokines monocyte chemotactic protein 1 (MCP-1), macrophage migration inhibitory factor (MIF) and chemokine (C-C motif) ligand 18 (CCL-18) are associated with obesity related glomerulopathy (ORG) and measured changes in these cytokines in patients with weight-loss following bariatric surgery.

**Methods:** Blood pressure (BP) measurements, urine and blood samples were collected from 34 morbidly obese patients prior to and 4 weeks after bariatric surgery. Serum biochemistry, urine albumin and creatinine were measured. Glomerular filtration rate (GFR) was assessed by serum cystatin C. Urinary and serum levels of MCP-1, MIF, CCL-18 and hemofiltrate CC chemokine 15 (CCL-15, a non-metabolic control cytokine) were measured using ELISA.

**Results:** Weight loss averaged 9.3 ±0.8 kg 4 weeks after surgery. Systolic BP decreased from 142.9 ±3.2 to 128.1 ±2.1 mmHg (p<0.001), and diastolic BP decreased from 87.1 ±1.5 to 79.2 ±1.1 mmHg (p<0.001). Serum creatinine decreased from 73.6 ±2.0 to 68.1 ±1.7 umol/L postoperatively (p<0.05), however there were no significant differences in urinary albumin/creatinine ratio (p=0.47) or serum cystatin C (p=0.47). Bariatric surgery significantly improved the inflammatory status of patients with a reduction of serum CRP (25.2 ±4.4 to 8.1 ±1.1 mg/L, p<0.001). Furthermore there were reduced levels of urinary MIF (203.1 ±26.6 to 90.4±12.1 ng/mmol Cr, p<0.001), urinary MCP-1 (26.1±2.6 to 16.7±2.3 ng/mmol, p<0.001), urinary CCL-18 (96.8±29.2 to 22.0±7.5 ng/mmol Cr, p<0.05) and serum CCL-18 (423.1 ±106.5 to 96.1 ±23.7 ng/ml, p<0.001). Urinary CCL-15, serum MIF, MCP-1 and CCL-15 changes were not statistically significant.

**Conclusion:** This study demonstrates the early effects of surgically induced weight loss upon BP and urinary cytokines.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2354

**The Effect of a Multidisciplinary Care Clinic on Outcomes in Pediatric Chronic Kidney Disease** Salma A. Ajarmeh,[1] Lee Er,[2] Ognjenka Djurdjev,[2] Janis M. Dionne.[1] *[1]Pediatric Nephrology, BC Children's Hospital, University of British Columbia, Vancouver, BC, Canada; [2]BC Provincial Renal Agency, Vancouver, BC, Canada.*

**Objectives**: To describe the effect of multidisciplinary care (MDC) on the clinical outcomes of children with chronic kidney disease (CKD) in British Columbia.

**Methods**: The MDC clinic started at BC Children's Hospital in 2006. In this cross-sectional retrospective study, we analyzed the data of all patients seen in 2003 (n=73) and in 2009 (n=125). Patient demographics and laboratory results were analyzed.

**Results**: Patient demographics were similar though CKD stage is significantly lower in 2009 (P=0.02). Hemoglobin level was significantly higher in 2009 (130 g/L vs. 122 g/L, P=0.002) and varied by both cohort and CKD stage. For bone mineral metabolism, 78% of the patients reached KDOQI calcium target for age in 2009 compared to 55% in 2003 (P=0.001). Calcium level varied by cohort and CKD stage (P<0.01). Phosphate and PTH varied only by CKD stage. Albumin level was significantly higher in 2009 (44 g/L vs. 40 g/L, p=0.0001) and varied by CKD stage and cohort. Blood pressure control was better in 2009 with 17% hypertensive on evaluation compared to 30% in 2003 (P= 0.045). There were no significant differences in physical growth or renal disease progression between groups. Patients were significantly more likely to see a social worker or pharmacist in 2009 and a dietician if they had CKD stages 3-5 (P<0.05). Allied health support was essential as the median number of medications per patient in 2009 was 4.0 (max 10) and 30% were receiving caloric supplements. Hospitalization events were comparable but the total length of stay in 2009 was shorter than in 2003 (median 0.2 vs 3.0 days, P=0.012).

**Conclusions:** The multidisciplinary care clinic improved the outcome of children with CKD especially in anemia, bone metabolism and blood pressure control.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2355

**Irbesartan Prevented the High Glucose Induced Endothelial Mesenchymal Transition** Rining Tang, Hou-Yong Dai, Jie Ni, Kun Ling Ma, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China.*

**Background---:** Substantial evidence suggests that high glucose causes endothelial cells damage, while the potential mechanism remains to be clarified. The aim of this study was to investigate the influence of high glucose on endothelial to mesenchymal transition (EndMT) and it's relevance with the activation of renin angiotensin system.

**Methods:** Primary human aortic endothelial cells (HAEC) were divided into three groups: normal glucose group (NG), high glucose group (HG) and the Irbesartan (1μM) treated group (HG+Irb). The concentration of angiotensin II in the supernatant was detected by radioimmunoassay. The pathological changes were investigated by fluorescence microscope, electron microscope. Immunofluorescence staining was performed to detect the co-expression of CD31 and markers of fibroblasts such as fibroblast specific protein1 (FSP1). Expression of FSP1 and α-SMA were detected by RT-PCR and Western Blot.

**Results:** Treatment of HAEC to HG led to a significant increasing expression of FSP1 protein and angiotensin II in dose and time dependent manner. Incubation of HAEC with HG resulted in a fibroblast-like phenotype and an increasing microfilament and rough endoplasmic reticulumin in the cytoplasm. And the expression of FSP1, α-SMA was significantly increased in the HG group. These changes were inhibited by treatment with Irbesartan ($P$<0.05). Double staining of HAEC showed co-localization of CD31 and FSP1, and some cells acquisition of a spindle-shaped morphology and loss of CD31 staining. Irbesartan, however, attenuated the expression of EndMT ($P$<0.05).

**Conclusions:** These findings suggested a novel mechanism that angiotensin II might mediate the HG induced endothelial damage through EndMT, which provided a new insight about the early application of ARB to protect the blood vessels and finally to prevent the organ failure in diabetes.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2356

**Hemodialysis Does Not Reverse Cognitive Dysfunction and Cerebral Metabolic Changes in Patients with Chronic Renal Insufficiency** Anita Blanka Tryc,[1,5] Güldan Alwan,[1] Martin A. Bokemeyer,[2] Annemarie Goldbecker,[1,5] Hartmut Hecker,[3] Karin Weissenborn,[1] Marion Haubitz.[4] *[1]Clinic for Neurology, Hannover Medical School, Hannover, Germany; [2]Institute for Neuroradiology, Hannover Medical School, Hannover, Germany; [3]Institute for Biometry, Hannover Medical School, Hannover, Germany; [4]Centre for Internal Medicine, Clinic for Nephrology, Hannover Medical School, Hannover, Germany; [5]Centre for Integrated Research and Treatment in Transplantation Medicine, Hannover Medical School, Hannover, Germany.*

**Background:** The diagnosis of uremic encephalopathy is normally considered if patients with end-stage renal disease present with neuropsychiatric symptoms. However, cognitive deficits may occur in patients with chronic renal insufficiency (CRI) long before any overt neurological symptoms can be observed. We hypothesized that cognitive dysfunction in patients with CRI both, treated and untreated by hemodialysis, may correspond to metabolic changes in distinct brain regions. **Methods:** Magnetic resonance spectroscopy ($^1$H-MRS) of the parieto-occipital white matter, the occipital gray matter, the basal ganglia and the pons, conventional magnetic resonance imaging of the brain and a comprehensive neuropsychological assessment was performed in 38 patients with CRI compared to 63 healthy controls, adjusted for age and education. Fifteen patients were on hemodialysis, twenty-three suffered CRI stage 4 to 5. **Results:** MRS alterations were predominantly found in the white matter. Concentrations of creatine containing compounds (Cr) were decreased in dialyzed and non-dialyzed patients. Choline concentration (Cho) and combined N-acetylaspartate and N-acetylaspartylglutamate concentration (NAx) were reduced only in dialyzed patients. Disturbance in memory and learning ability as well as attention deficits were observed in both patient groups. Of note, attention deficits were more severe in dialyzed patients. MRS results correlated with attention deficits in dialyzed patients. **Conclusions:** Hemodialysis does not warrant cognitive capability in patients with chronic renal insufficiency. Instead a negative impact of hemodialysis on cognitive function must be considered.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2357

**A Randomized, Crossover, Multicenter Study of Once-Biweekly Administration of Epoetin-α Compared with Darbepoetin-α in CKD Patients Not Receiving Dialysis** Young-Il Jo,[1] Dongho Yang,[2] Sug Kyun Shin.[3] *[1]Nephrology, Konkuk University Hospital, Seoul, Republic of Korea; [2]Bundang CHA Hospital, Sungnam, Republic of Korea; [3]NHIC Ilsan Hospital, Goyang, Republic of Korea.*

1. Background: There is now increasing evidence that epoetin-α (EPO), despite its relatively short serum half-life, can be administrated at extended dosing intervals. However, there is no randomized, cross-over trial concerning comparison of once-biweekly (Q2W) EPO with darbepoetin-α (DA) in CKD patients not receiving dialysis.

2. Introduction: We designed this study to investigate whether Q2W EPO is as effective as Q2W equal dose of DA in correcting of anemia in CKD patients not receiving dialysis.

3. Methods: Seventy-four pre-ESRD subjects with renal anemia (M:F 31:43, age 60.6±12.2, eGFR-MDRD 22.9±9.9 mL/min/1.73m$^2$, DM 58.1%) were equally randomized to EPO or DA group and treated with Q2W subcutaneous EPO or DA for 2 months. After 4 or 8 weeks of washout period, subjects of EPO or DA group switched to another regimen for 2 months.

4. Results: No significant differences were observed in baseline parameters before DA or EPO therapy, including Hb levels, serum ferritin, and transferrin saturation. Hb levels of post-erythropoiesis stimulating agents (ESA) therapy significantly increased compared with those of pre-ESA therapy (DA, 9.5±0.9 *vs.* 10.7±1.2 g/dL, *p<0.001*; EPO, 9.6±0.9 *vs.* 10.3±1.2 g/dL, *p=0.002*). Percent increase of Hb levels of DA therapy was significantly higher than that of EPO therapy (112.6±13.5 *vs.* 107.3±9.8%, *p=0.018*) although there was no significant difference in Hb levels of post-ESA therapy between two groups. During washout period, Hb levels were slightly decreased both two groups, but there was no significant difference in % decrease of Hb levels between two groups. Erythropoietin resistance index did not show significant difference between two groups (DA *vs.* EPO, 7.9±1.7 *vs.* 8.1±1.9 IU/kg weight/g Hb, *p=0.683*). There were no any serious adverse effects that led to permanent withdrawal.

5. Conclusion: These findings of our study indicate that Q2W high dose of EPO therapy may be effective as Q2W DA therapy in the management of renal anemia in CKD patients who are not on dialysis.

Disclosure of Financial Relationships: nothing to disclose

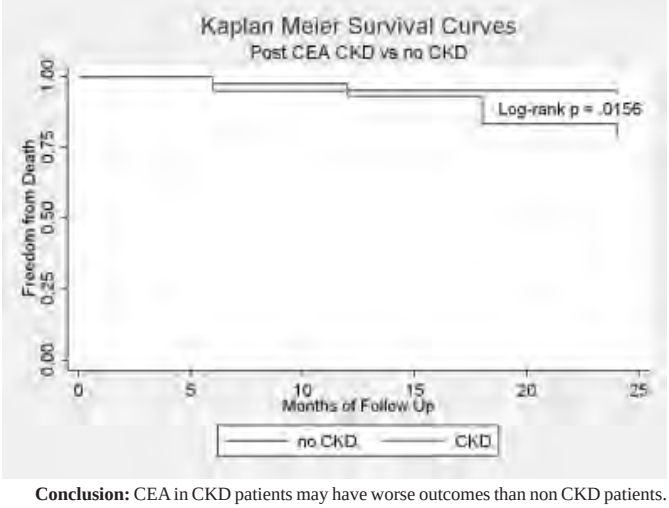
## SA-PO2358

**Chronic Kidney Disease Negatively Impacts Survival in Patients Having Carotid Endarterectomy Surgery** Laura Hesemann,[1] Sri Divya Muppala,[1] Gautam M. Phadke,[1] Georges Saab,[2] Kunal Chaudhary.[1,3] *[1]Department of Medicine, University of Missouri, Columbia, MO; [2]Division of Nephrology, Washington University, St Louis, MO; [3]Nephrology Division, Harry S Truman VA Hospital, Columbia, MO.*

**Introduction** : There are multiple etiologies for Cardiovascular accidents(CVA) , and among them extracranial carotid artery disease accounts for approximately 25% of ischemic strokes. It has been shown that carotid revascularization by carotid endarterectomy(CEA) can decrease the risk of CVA in appropriately selected population with carotid artery disease. Carotid revascularization has been shown to be safe and clinically effective in many large multicentered randomized clinical trials. However, most of these large trials have predominately excluded the patients with kidney failure. The aim of the study was to find out whether underlying Chronic Kidney Disease(CKD) influences outcomes in patients undergoing CEA.

**Methods:** We retrospectively analyzed data of one hundred sixty-eight patients who had undergone CEA at the University of Missouri and Harry S Truman Veterans Hospital between years 2002 and 2008.

**Results:** After adjusting for age, race, body mass index, coronary artery disease, statin use, ACE inhibitor/ARB use , beta blocker use and Diabetes mellitus, each 10ml/min/1.73 m$^2$ increase in GFR was associated with a 49% (95% CI;23%-66%, p =0.001) lower risk of death after CEA. At 24 months of follow up patient survival was greater in non CKD patients as compared to CKD patients (95% vs 80% ; log rank p= 0.016).




**Conclusion:** CEA in CKD patients may have worse outcomes than non CKD patients. Providers should take this in account when making decisions regarding this surgery. Larger clinical studies will be needed to verify this conclusion with certainty.

Disclosure of Financial Relationships: nothing to disclose

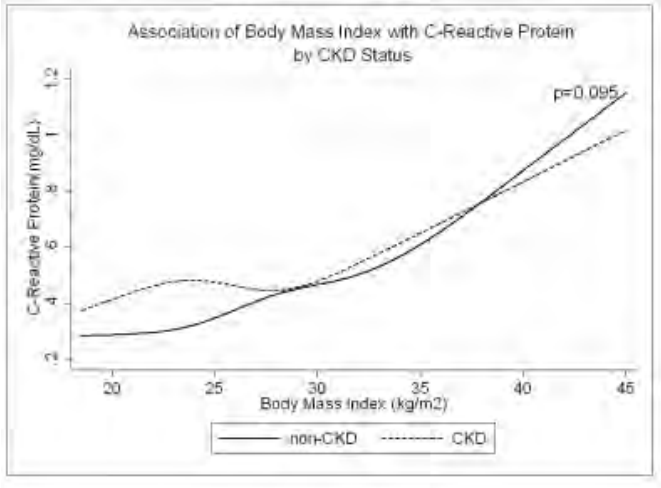
## SA-PO2359

**Does Chronic Kidney Disease Modify the Association between Body Mass Index and Cardiovascular Disease Risk Factors?** Nisha Bansal, Eric Vittinghoff, Laura C. Plantinga, Chi-Yuan Hsu. *Medicine, University of California, San Francisco, San Francisco, CA.*

Background: Several studies have shown that excess weight is "paradoxically" associated with better cardiovascular (CVD) outcomes and mortality in chronic kidney disease (CKD). One potential explanation for this "inverse" relationship is that the usual positive correlation between severity of CVD risk factors -- such as worse lipid levels, inflammation, blood pressure and fasting glucose levels -- and higher body mass index (BMI) is reversed among those with CKD. To test this hypothesis, we determined the relationship between BMI and CVD risk factors in patients with and without CKD.

Methods: This was a cross-sectional study of NHANES 1999-2006. Subjects with BMI <18.5 kg/m$^2$ were excluded. CKD was defined as glomerular filtration rate < 60 ml/min/1.73 m$^2$. Covariates were: age, race, gender and use of relevant prescription medications. Outcome variables were: total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides (TG), C-reactive protein (CRP), systolic blood pressure (SBP), diastolic blood pressure (DBP), and fasting glucose (FG).

Results: There were 1,895 and 32,431 patients with and without CKD, respectively. Those with CKD were older, more likely to be female or white, and had higher BMI. The shapes of the association between BMI and total cholesterol, LDL cholesterol, HDL cholesterol, TG, CRP, SBP, and FG were similar in those with or without CKD. The figure below illustrates the association between BMI and CRP. In a sensitivity analysis excluding patients taking relevant prescription medications, our results did not differ substantially.




Conclusions: CKD did not modify the relationship between higher BMI and CVD risk factors. Thus, inverse associations between BMI and CVD risk factors cannot explain why CKD patients with higher BMI have better outcomes.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2360

**Chronic Kidney Disease Impairs the Hematopoietic Stem Cell Compartment** Matthieu Monge,[1,2] Melissa van Pel,[3] Michiel Siebelt,[4] Marina M. Aleksinskaya,[1] Hetty C. de Boer,[1] Jacques Duijs,[1] Coen van Solingen,[1] Ziad Massy,[2] Ton J. Rabelink,[1] Anton Jan Van Zonneveld.[1] *[1]Nephrology and Einthoven Laboratory for Vascular Experimental Medicine, LUMC, Leiden, Netherlands; [2]INSERM ERI12, Amiens University, Amiens, France; [3]Department of Immunohematology and Bloodtransfusion, LUMC, Leiden, Netherlands; [4]Orthopaedic Research Laboratory, ErasmusMC, Rotterdam, Netherlands.*

Chronic kidney disease (CKD) is marked by sustained parathyroid hormone (PTH) increase. This results in specific bone disorders (CKD-MBD), mainly due to increased osteoblast activity. Osteoblasts are key regulators of the hematopoietic stem cell (HSC) niche. We investigated the effect of increased osteoblast activity on HSC homeostasis.

Ten C57Bl/6 mice underwent surgically-induced CKD (thermocauterisation-nephrectomy), and were compared to sham operated controls. 12 weeks after CKD-induction, bone structure was analyzed by micro-CT scan to confirm CKD-MBD and bone marrow cells (BMC) were harvested. HSC and hematopoietic progenitor cells (HPC) (long- and short-term repopulating cells respectively) were analyzed phenotypically by flowcytometry and functionally by cobblestone area forming cell (CAFC) assay.

Throughout the study urea levels were increased in the CKD group compared to controls (30.5mM±2.8 vs 10.2mM±1.3 , p<0.01). Bone analysis showed increased trabecular bone volume (BV, 515µ$^3$±64 vs 400µ$^3$±89, p<0.001), decreased span incurvation (1.9AU±0.1 vs 2.1AU±0.1, p<0.01), and decreased cortical thickness (150µ±9 vs 158µ±9, p<0.05 ), compatible with a chronic exposure to high levels of PTH.

In CKD mice, long-term repopulating Lin$^{neg}$Sca1$^{pos}$cKit$^{pos}$ (LSK) CD135$^{neg}$CD34$^{neg}$ HSC were significantly decreased compared to controls (0.0028%±0.001 vs 0.0049%±0.001, p<0.05), whereas HPC (LSK CD135$^{neg}$CD34$^{pos}$) remained unaltered. However, CAFC analysis showed a decrease (0.5±0.1 vs 1.4±0.7 per 10$^5$ BMC, p<0.05) in both HSC and

HPC frequencies, indicating a functional defect in their repopulating capacities. Also, total LSK cells number correlated positively to BV.

Our data show that CKD is associated with functional changes in the hematopoietic compartment, due to increased osteoblasts activity, as indicated by the bone changes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2361**

**Evolution of Left Ventricular Geometry from CKD through ESRD: Results from the CRIC Study** Nisha Bansal, Martin Keane, Dawei Xie, Kelvin Tao, Crystal A. Gadegbeku, Alan S. Go, Akinlolu O. Ojo, Mahboob Rahman, Jackson T. Wright, Patrice Delafontaine, L. Lee Hamm, John W. Kusek, Leigh Rosen, Chi-Yuan Hsu. *Chronic Renal Insufficiency Cohort.*

Background: Left ventricular (LV) hypertrophy is a powerful and independent predictor of mortality in incident end-stage renal disease (ESRD) patients. However, little is known about the longitudinal change in LV geometry as patients transition from chronic kidney disease (CKD) to ESRD, which will identify opportunities for intervention during the natural history of disease. To our knowledge, no prior study performed research echocardiograms among advanced CKD patients who subsequently transitioned to ESRD.

Methods: We examined participants enrolled in the Chronic Renal Insufficiency Cohort (CRIC). In pair-wise analyses we compared protocol echocardiograms performed at advanced CKD and incident ESRD.

Results: One hundred sixty two participants (age 55.2 ± 12.1 years, 46% female and 62% African-American) had echocardiograms performed at advanced CKD and again at incident ESRD. Mean estimated GFR at advanced CKD was 17 ± 4 ml/min/1.73 $m^2$. The mean time between the two echocardiograms was 23 ± 11 months. There was no change in LV geometry between advanced CKD and incident ESRD [Table 1]. However, ejection fraction (EF) decreased. The prevalence of myocardial infarction increased from 24% to 35% (p=0.02) and congestive heart failure from 14% to 24% (p=0.02).

Conclusions: There was further decrement in EF accompanied by an increase in clinical cardiac events during the transition from advanced CKD to ESRD. However, much of the cardiac structural disease observed among incident ESRD patients was already present during advanced CKD. Interventions to prevent and treat cardiovascular disease must start at earlier stages of CKD.

Table 1

| Echocardiographic Measurement | Advanced CKD | Incident ESRD | P-Value |
|---|---|---|---|
| LV mass index (g/m^2.7) | 76 ± 28 | 75 ± 24 | 0.8 |
| LV EF (%) | 53 ± 8 | 50 ± 9 | 0.006 |
| EF < 35% (%) | 1 | 7 | 0.007 |
| LV geometry | | | 0.8 |
| Normal (%) | 7 | 5 | |
| Concentric remodeling (%) | 5 | 7 | |
| Eccentric hypertrophy (%) | 20 | 25 | |
| Concentric hypertrophy (%) | 68 | 63 | |

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2362**

**Association of Impaired Endothelium-Dependent and Nitroglycerin-Induced Vasodilation with Risk of Chronic Kidney Disease (CKD)** Jing Chen,[1] L. Lee Hamm,[1] Fred E. Husserl,[3] Emile Mohler,[2] Robin Arora,[1] Islam Enver Khan,[1] Arnold B. Alper,[1] Myra A. Kleinpeter,[1] Rajesh G. Shenava,[1] Eric E. Simon,[1] Jiang He.[1] *[1]Medicine, Tulane University, New Orleans, LA; [2]Medicine, University of Pennsylvania, Philadelphia, PA; [3]Medicine, Ochsner Health System, New Orleans, LA.*

Previous clinical studies reported that impaired endothelium-dependent vasodilation was common in patients with end stage renal disease. We studied endothelium-dependent and nitroglycerin-induced vasodilation in 171 patients with CKD and 183 controls without CKD from community. CKD was defined as estimated glomerular filtration rate (eGFR) <60 ml/min/1.73$m^2$ or presence of albuminuria. Brachial artery endothelium-dependent vasodilation [flow mediated dilation (FMD)] to reactive hyperemia following 5 min of forearm ischemia and the response to sublingual nitroglycerin (NTG) were measured in the subjects using a standard protocol. Compared to those without CKD, patients with CKD were older (56 vs. 53 yrs), more likely to be male (57% vs. 45%), less likely to have graduated from high school (59% vs. 81%), or consume alcohol (29% vs. 58.2%). Race and cigarette smoking were comparable between CKD patients and controls. Mean systolic blood pressure (132 vs. 122 mmHg), body mass index (32 vs. 29 kg/$m^2$), fasting glucose (120 vs. 102 mg/dL), and triglyceride (144 vs. 106 mg/dL) were higher while HDL (50 vs. 58 mg/dL) and LDL cholesterol (102 vs. 118 mg/dL) were lower in CKD patients than in controls. After adjustment for these risk factors, multivariable-adjusted median (inter-quartile range) of FMD was 5.4% (2.6%, 8.7%) in CKD patients and 8.8% (5.8%, 11.9%) in controls (p<0.0001 for group difference). In addition, multivariable-adjusted median (inter-quartile range) of nitroglycerin-induced vasodilation was 14.2% (9.4%, 19.5%) in CKD patients and 20.9% (14.6%, 26.5%) in controls (p=0.005 for group difference). These data indicated that impaired endothelium-dependent and nitroglycerin-induced vasodilation were associated with CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2363**

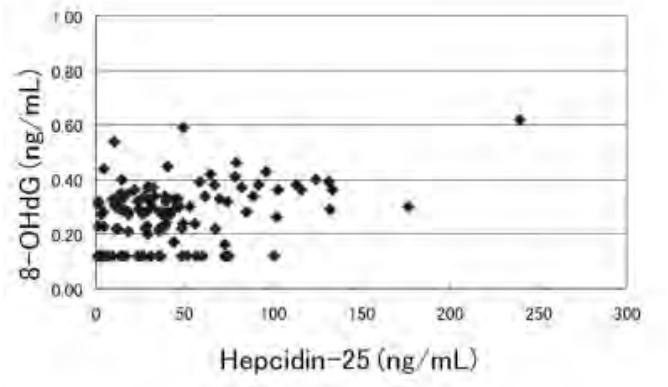
**Association between Hepcidin and Oxidative Stress in Chronic Kidney Disease** Yukio Maruyama,[1] Keitaro Yokoyama,[1] Hiroyasu Yamamoto,[1] Masaaki Nakayama,[1] Tatsuo Hosoya.[1] *[1]Division of Kidney and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan; [2]Reseach Division of Dialysis and Chronic Kidney Disease, Tohoku University Graduate School of Medicine, Sendai, Japan.*

INTRODUCTION AND AIMS. Among patients with chronic kidney disease (CKD), both increased oxidative stress and higher serum ferritin levels are risk factors for CVD, and correlate with one another. Hepcidin controls circulating iron levels by regulating the absorption from the intestine as well as the release from macrophages. Although it is clear that hepcidin is a key mediator of anemia of chronic disorders, associations between hepcidin and oxidative stress in CKD are still unknown.

METHODS. One hundred seventeen CKD patients not receiving renal replacement therapy (62±15 years and 85 males) participated in this cross-sectional study. The concentration of serum hepcidin were measured using surface-enhanced laser desorption ionization time of flight mass spectrometry (SELDI-TOF MS), and serum 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of DNA oxidative injury, were measured using competitive ELISA kit.

RESULTS. The serum hepcidin and serum 8-OHdG levels were 32.8 (0.7 to 240) ng/mL and 0.27±0.11 ng/mL, respectively. There were strong positive associations between serum hepcidin and serum ferritin levels (rho=0.68, P<0.01). Interestingly, serum hepcidin levels were positively associated with serum 8-OHdG levels (rho= 0.34, P<0.01). The serum 8-OHdG level was independently associated with serum hepcidin level in a multiple regression model.

CONCLUSIONS. It is the first report to show a positive association between increased oxidative stress and serum hepcidin levels among CKD patients. It is suggested that increased oxidative stress induces defective iron utilization and increased body iron storage through hepcidin synthesis.




Disclosure of Financial Relationships: nothing to disclose

**SA-PO2364**

**Aortic Valve Calcification in Patients with Stage 5D Chronic Kidney Disease** Tatsuhiro Yaginuma, Ichiro Ohkido, Hiroyasu Yamamoto, Keitaro Yokoyama, Yasunori Utsunomiya, Tatsuo Hosoya. *Division of Kidney and Hypertension, Department of Internal Medicine, The Jikei University, School of Medicine, Minato, Tokyo, Japan.*

Background: There are many cases of recurrent calcification in patients with chronic kidney disease (CKD) and hemodialysis (HD) at an early time point after aortic valve replacement (AVR). While atherosclerotic plaque is thought to be one of the main factors of aortic valve calcification, mineral metabolism (calcium and phosphate) might contribute to aortic valve calcification. However, additional mechanisms of plaque formation remain uncertain. Therefore, we performed histopathological analysis to determine whether mineral metabolism had been associated with the development of aortic valve calcification in CKD patients with HD.

Methods: We performed histopathological analysis in CKD patients due to non diabetic mellitus (DM) with HD who underwent AVR for aortic stenosis (AS) (N=4) and normal kidney function patients without DM who underwent AVR (N=6) in the hospital from 2006 through 2009.

Results: We found the same expression of markers related to calcification such as Sodium/Phosphate co-transporter type-III (Pit-1), Cbfa1/Runx2 and Osteopontin (OPN) in CKD patients with hemodialysis as compared with normal kidney function patients. In addition, we found robust CD34 expression on calcified valves within both groups.

Conclusions: These findings collectively suggest that mineral metabolism might not contribute to the mechanism of aortic valve calcification in CKD patients with hemodialysis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2365**

**Right Ventricular Diastolic Function in Dialysis Patients Could Be Affected by Vascular Access** Luca Di Lullo, Fulvio Floccari, Pasquale Polito. *Department of Nephrology and Dialysis, San Giovanni Evangelista Hospital, Tivoli, Roma, Italy.*

BACKGROUND. Tricuspidal annulus plane excursion (TAPSE) measurement is a prognostic index for stratification of cardiac mortality risk in CRF patients with heart failure. It can be easily measured in all patients irrespective of heart rate and rhythm; its usefulness can therefore be extended to those patients in whom tachycardia or atrial fibrillation limits findings from mithral valve Doppler velocity curve. It has been demonstrated adding significant prognostic information to NYHA clinical classification.

AIM. Aim of the study was to examine effects of acute pre-load reduction by hemodialysis (HD) on TAPSE, with focus on the effects of vascular access typology

PATIENTS AND METHODS. Thirty chronically uremic patients (age 51 +/- 10 years, dialytic age 24 +/- 8 months), without overt heart disease, underwent conventional 2D and Doppler ECHO immediately before starting and 15 minutes after ending a mid week hemodialysis session. Twenty patients had distal radio-cephalic artero-venous fistula (AVF), whether ten of them had permanent CVC. Fluid volume removed by HD was 2706 +/- 1047 g/session.

RESULTS. Hemodialysis led to reduction in TAPSE, LVEDV, LVESV, RVEDV, RVESV, peak early (E wave) transmitral flow velocity and ratio of early to late Doppler velocities of diastolic mitral flow.

AVF patients showed significantly greater RV diameters versus CVC patients, whether TAPSE appeared higher in second ones. Only AVF patients group showed TAPSE values below 15 mm.

CONCLUSIONS. Right ventricle diastolic function plays a crucial role in determining clinical outcome in patients with CHF; reduction in TAPSE means reduction in ventricular diastolic distensibility, leading to diastolic dysfunction.

Our data confirm effects of terminal uremia on right ventricle function (chamber dilation, impaired diastolic function), showing that these abnormalities are more frequent in AVF patients versus CVC patients. It is reasonable to explain these clinical features as the effect of pre – load increase operated by AVF.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2366**

**Cardiovascular Autonomic Tone and Vascular Stiffness in Chronic Kidney Disease Stage 5 Patients** Manpreet Kaur,[1] Charanjit Lal,[2] Ashok Kumar Jaryal,[1] Dipankar M. Bhowmik,[2] Dinu Chandran,[1] Kishore Kumar Deepak.[1] *[1]Physiology, All India Institute of Medical Sciences, New Delhi, Delhi, India; [2]Nephrology, All India Institute of Medical Sciences, New Delhi, Delhi, India.*

Arterial baroreflex sensitivity (BRS) is markedly reduced in Chronic Kidney Disease stage 5 (CKD-5) patients. It could be due to vascular stiffening and/or autonomic neuropathy. We investigated baroreflex function and its relation to arterial stiffness and autonomic tone (Heart rate variability, HRV; and blood pressure variability ,BPV)in CKD-5 patients. 15 CKD-5 patients (Age - 40.9 +/- 11 years) and 15 age matched healthy controls were studied. Simultaneous and continuous lead II ECG recordings for HRV and non- invasive beat to beat blood pressure recordings for BPV were done using short term recording protocol. HRV was assessed using time and frequency domain analysis and BRS by sequence method using HRV and BPV data. Vascular stiffness was measured by carotid femoral pulse wave velocity (PWV) and Augmentation index (AI) using applanation tonometry. There were significant differences in BRS, PWV, AI and HRV in CKD-5 patients as compared to controls. BRS was markedly reduced in CKD-5 patients (8.36 +/- 2.1 vs. 17.89 +/- 1.35 ms/mmHg ; p=0.001). Patients showed significantly increased vascular stiffness indices : PWV (9.27 +/- 0.81 vs 7.8 +/- 0.4 m/s; p=0.003) and AI (23.87 +/- 5.26 vs. 16.2 +/- 2.15 % ; p=0.001). PWV and AI were not found to be correlated with BRS. HRV indices in both time and frequency domain were reduced in patients (p=0.001) and directly correlated with BRS (r = 0.479 p=0.03). Values are expressed as Mean +/- SEM. In summary, CKD-5 patients have a decreased BRS, reduced HRV and increased vascular stiffness indices. Our results suggest that, probably increase in arterial stiffness is not primarily responsible for the reduced BRS in CKD-5 patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2367**

**Effect of Exercise (EXE) on Renal and Cardiac Functions in Animals with Nx5/6 Nephrectomy** Rafael Da Silva Luiz,[1] Kleiton Silva,[1] Rodolfo Rampaso,[1] Luciana Teixeira,[1] Ednei Antônio,[2] Jairo Montemor,[2] Danilo Bocalini,[2] Luiz Moura,[1] Leonardo dos Santos,[2] Paulo Tucci,[2] Nayda P. Abreu,[1] Nestor Schor.[1] *[1]Nephrology Division, UNIFESP, São Paulo, São Paulo, Brazil; [2]Cardio-Physiology and Pathophysiology Laboratory, UNIFESP, São Paulo, São Paulo, Brazil.*

The aim was to evaluate the effects of chronic aerobic EXE on renal and cardiac functions. Adult Wistar rats were studied as control(C,n=8), control+EXE(E,n=8), Nx5/6sedentary(NxS,n=8) and Nx5/6+EXE(NxE,n=8). EXE sessions were performed by swimming with overload of 70% of the maximum load reached for 30min, applied 5days/week/5weeks. Creatinine clearance (CrCl), 24h proteinuria and sclerosis index, cardiac function through echodopplercardiography(ECHO), hemodynamic and muscle papillary contractility (in vitro) were evaluated. CrCl were lower for NxS and NxE vs C and E(0.90±0.11, 1.10±0.09 vs 2.01±0.15, 1.57±0.13mL/min,p<0.05), respectively. Proteinuria was lower in NxE than for NxS (51.4±9.9 vs 96.9±9.9 mg/24h p<0.05). From 100 glomeruli evaluated, NxS presented higher index of alterations vs NxE (16% vs 2%,p<0.05). ECHO disclosed that the weight LV of NxS increased vs C,E and NxE(1.15±0.06 vs 0.98±0.03, 0.91±0.02 and 1.08±0.04 $cm^2$,p≤0.05), respectively. The systolic pressure in the LV was higher in NxS vs C(134 vs 112mmHg,p≤0.05). The maximum strength of contraction developed isometrically at Lmáx was lower in NxS vs NxE, E and C(2.56±0.12 vs 4.56±0.58 , 5.00±0.28, 5.29±0.37g/$mm^2$, p<0.05). Cardiac weight was higher in NxS and NxE vs E and C (4.0±0.1, 3.6±0.3 vs 3.0±0.1, 2.8±0.1mg, respectively, p<0.05) and the weight of LV of NxS was greater than the NxE, E and C (3.2±0.1 vs, 2.8±0.2, 2.3±0.1, 2.1±0.1 mg,respectively,p<0.05). It is suggested that despite the mild effect of EXE on CrCl (~20%:NxE>NxS), there was a significant reduction on proteinuria (~50%) and an important reduction of glomeruli sclerosis in NxE. Also the EXE attenuates the cardiac dysfunction in rats in this model. Thus, it is suggested that these animals would have a diminished progression of renal disease and lower cardiovascular impact of Nx5/6, which indicates that this EXE, could contribute to a better evolution in renal and cardiac functions.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2368**

**Abdominal Obesity Is a Relevant Risk Factor of Subclinical Cardiac Damage in a Cohort of Patients Affected by Chronic Kidney Disease** Carlo Maria Alfieri,[1] Simone Vettoretti,[1] Riccardo Floreani,[1] Cosimo Cafforio,[1] Carla Bonanomi,[2] Gian Battista Danzi,[2] Piergiorgio Messa.[1] *[1]U.O. Nephrology and Dialysis, Fondazione IRCCS CA' GRANDA Ospedale Maggiore Policlinico, Milan, Italy; [2]U.O. Cardiology, FONDAZIONE IRCCS CA'GRANDA Ospedale Maggiore Policlinico, Milan, Italy.*

**Background**: We evaluated whether the coexistence of chronic kidney disease (CKD) and metabolic syndrome (MS) may influence the presence of subclinical cardiac damage.

**Methods**: we evaluated 168 patients with CKD and free from previous cardiovascular (CV) events. Albuminuria (A/C) was determined on the first voiding on three different days and on 24h collection.We used echocardiography to determine left ventricular mass index (LVMI, $g/m^{2.7}$) and midwall Fractional Shortening (mFS) as a precocious index of systolic dysfunction. Left ventricular hypertrophy (LVH) was defined as LVMI>51 $g/m^{2.7}$ and MS according to ATPIII criteria.

**Results**: age 62±15 yrs; males 64%; MS+ 56%; type 2 diabetes 51%; SBP 140±22 mmHg; DBP 80±13 mmHg; waist circumference 100±13 cm; eGFR 60±32 ml/min; logA/C 1,25±0,86. Patients were equally distributed between CKD stages (stage 1=25%;2=25%;3=25%;4=25%). MS+ subjects had a higher prevalence of type 2 diabetes (DM+ 68% in MS+ vs 34% in MS- ;p<0,0001), higher SBP (MS+143±18 mmHg; MS-130±24 mmHg, p=0,0002) while eGFR and logA/C did not differ between two groups. MS+ had higher LVMI (MS+ 57,4±14,5 and MS- 42,5±12,5 $g/m^{2.7}$; p<0,0001) and lower mFS (MS+ 12,2±4% and MS- 16,7±5,3%; p<0,0001). LVMI and mFS correlated with: age, eGFR, logA/C and with all the variables that define MS. In a logistic regression, LVH correlated only with waist circumference (WC), SBP and HDL (RR 1,59 p< 0,0001; RR 1,138 p=0,038 and RR 1,28 p=0,002 for 5 cm increase of WC, 5 mmHg increase of SBP and 5 mg/dl decrease of HDL), while eGFR and logA/C did not.

**Conclusions**: although patients affected by CKD are generally considered to have a high CV risk, the coexistence of MS may increase the risk of subclinical cardiac damage beyond the severity of renal impairment and other relevant risk factors. Moreover, in this population waist circumference was the most important risk factor correlated with subclinical cardiac damage.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2369**

**Skin Autofluoresence Is Associated with Important Cardiovascular Risk Factors in Patients with CKD Stage 3** Natasha J. Mcintyre, Richard J. Fluck, Chris W. McIntyre, Maarten W. Taal. *The Department of Renal Medicine, The Royal Derby Hospital, Derby, Derbyshire, United Kingdom.*

**INTRODUCTION**: Tissue advanced glycation end products (AGE) accumulation is a measure of cumulative metabolic stress. Assessment of tissue AGE by skin autofluoresence (AF) correlates well with cardiovascular (CV) outcomes in diabetic, transplant and dialysis patients and may be a useful marker of CV risk in earlier stages of CKD.

**METHODS**: 1741 patients with estimated GFR 59-30ml/min/1.73$m^2$ were recruited from Primary Care Practices for the Renal Risk In Derby ($R^2$ID) Study. Detailed medical history was obtained and each participant underwent clinical assessment as well as urine and serum biochemistry tests. Skin AF was assessed (mean of 3 readings) as a measure of skin AGE deposition using a cutaneous AF device (AGE Reader®, DiagnOptics, Groningen, The Netherlands).

**RESULTS**: Univariate analysis revealed significant correlations between AF readings and several potential risk factors for CV disease including age, eGFR, cholesterol, haemoglobin, albumin, glucose, diastolic BP, pulse wave velocity, waist to hip ratio, uric acid and deprivation score.

Skin AF readings were also significantly higher among males, diabetics, patients with microalbuminuria, a history of smoking or evidence of self reported cardiovascular disease and also those with CKD Stage 3b.

Multivariable linear regression analysis identified independent determinants of higher skin AF (Table 1; $R^2$=0.16 for equation).

Table 1. Independant determinants of higher skin AF

| | β | p value |
|---|---|---|
| Age (yrs) | 0.153 | <0.0001 |
| Diabetes | 0.137 | <0.0001 |
| Haemoglobin (g/dL) | -0.138 | <0.0001 |
| eGFR (ml/min/1.73m²) | -0.102 | <0.0001 |
| Ever smoked | 0.119 | <0.0001 |
| Previous CVD | 0.085 | <0.0001 |
| Microalbuminuria | 0.072 | 0.002 |
| IMD score (Deprivation) | 0.066 | 0.004 |

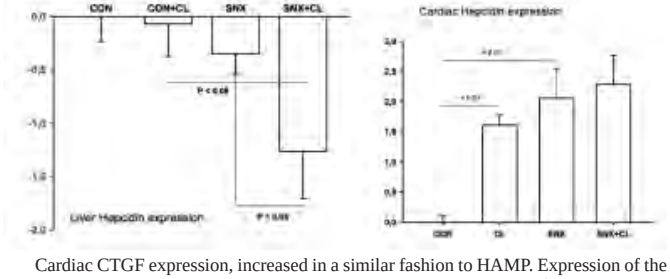
**CONCLUSION**: Increased skin AF is independently associated with multiple CV risk markers in CKD 3. Long-term follow up will be conducted to assess the value of skin AF as a predictor of cardiovascular risk in this population.

Disclosure of Financial Relationships: Research Funding: Research funding from Roche; Honoraria: Chairing honoraria from LINC medical.

## SA-PO2370

**Myocardial Hepcidin Expression Is Associated with Cardiac Injury in Experimental Cardiorenal Failure** Lennart G. Bongartz,[1] Jaap A. Joles,[1] Marianne Christina Verhaar,[1] Maarten Jan M. Cramer,[2] Roel Goldschmeding,[3] Pieter Doevendans,[2] Branko Braam,[4] Carlo A. Gaillard.[5] *[1]Nephrology, UMC Utrecht, Netherlands; [2]Cardiology, UMC Utrecht, Netherlands; [3]Pathology, UMC Utrecht, Netherlands; [4]Nephrology, Univ. of Alberta, Canada; [5]Nephrology, VUMC, Amsterdam, Netherlands.*

In response to increased iron stores and inflammation hepcidin leads to degradation of ferroportin, the iron efflux channel. Hepcidin is expressed in hearts of rats exposed to hypoxia or inflammation. We hypothesized that cardiac expression of hepcidin in experimental combined heart and kidney failure is also increased. We subjected male Lewis rats to subtotal nephrectomy (SNX) or sham (CON). In wk 9, we performed coronary ligation (CL) or sham-surgery into 4 groups (n=10/grp): CON, SNX, CON+CL, and SNX+CL. Myocardial and hepatic expression of hepcidin (HAMP), CTGF, a marker of fibrosis and the erythropoietin receptor (EPOR) was measured by q-RT-PCR. Biochemical and hemodynamic parameters and cardiac and renal function confirmed the existence of cardiorenal failure. At 15 weeks hepatic expression of HAMP was unchanged or decreased as compared to CON whereas cardiac HAMP expression was increased in CL, SNX and SNX+CL.




Cardiac CTGF expression, increased in a similar fashion to HAMP. Expression of the EPOR did not change. Interestingly, tempol and molsidomine treatment further increased cardiac HAMP expression. In contrast to liver, cardiac HAMP expression is increased by cardiac (local) and renal (remote) injury, which is not associated with modulated EPOR expression. Since destruction of cardiomyocytes may change iron distribution, we speculate that hepcidin expressed in (surviving) cardiomyocytes regulates distribution of iron and iron homeostasis in damaged hearts. The increase in cardiac HAMP upon tempol and molsodomine suggests that ROS does not cause increased HAMP in SNX±CL.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2371

**Beneficial Effects on Arterial Stiffness and Pulse-Wave Reflection of Combined Enalapril and Candesartan in Chronic Kidney Disease** Marie Frimodt-Møller,[1] Anne-Lise Kamper,[2] Svend Strandgaard,[1] Svend Kreiner,[3] Arne Høj Nielsen.[1] *[1]Department of Nephrology, Herlev University Hospital, Herlev, Denmark; [2]Department of Nephrology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; [3]Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark.*

Cardio-vascular disease (CVD) is highly prevalent in patients with chronic kidney disease (CKD). Inhibition of the renin-angiotensinsystem (RAS) in hypertension causes differential effects on central and brachial blood pressure (BP), which has been translated into improved cardio-vascular (CV) outcome. *Objective*: To examine if a more complete inhibition of RAS by combining an angiotensin converting enzyme inhibitor (ACEI) and an angiotensin receptor antagonist (ARB) compared to monotherapy has an additive effect on central BP and pulse-wave velocity (PWV), which are known markers of CVD. *Methods:* 67 CKD patients (mean GFR 30, range 13-59 ml/min/1.73m²) participated in an open randomized study of 16 weeks of monotherapy with either enalapril or candesartan followed by 8 weeks of dual blockade aiming at a total dose of 16 mg candesartan and 20 mg enalapril o.d. Pulse-wave measurements were performed at week 0, 8, 16 and 24 by the SphygmoCor device. *Results:* Significant additive BP-independent reductions were found after dual blockade in aortic PWV (-0.3 m/s, P<0.05) and in augmentation index (-2 %, P<0.01) compared to monotherapy. Furthermore PP-amplification was improved (P<0.05) and central systolic BP reduced (-6 mmHg, P<0.01). *Conclusions*: Dual blockade of the RAS resulted in an additive BP-independent reduction in pulse-wave reflection and arterial stiffness compared to monotherapy in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2372

**Carotid Intima Media Thickness (cIMT) in Children with Chronic Kidney Disease (CKD): Results from the Chronic Kidney Disease in Children (CKiD) Cohort Study** Tammy M. Brady,[1] Michael F. Schneider,[2] Joseph T. Flynn,[2] Christopher Cox,[2] Thomas R. Kimball,[2] Mark Mentser,[2] Colin T. White,[2] Susan L. Furth,[2] Bradley A. Warady,[2] Mark Mitsnefes.[2] *[1]Johns Hopkins University, Baltimore, MD; [2]CKiD Study Group.*

OBJECTIVE: To determine the association between selected cardiovascular and demographic exposures and cIMT in children with stage 2-4 CKD.

METHODS: Carotid artery ultrasound (u/s) was obtained 1 year after enrollment in 96 children enrolled in the CKiD study. Standardized protocol imaging was done locally by B-mode u/s and read centrally. Since cIMT was normally distributed, a multivariate linear regression analysis was conducted to quantify the relationship each exposure had with cIMT.

RESULTS: The median age was 12.4 y; median GFR 42.9 ml/min/1.73m2 (IQR: 29.0 to 53.8); median cIMT 0.43 mm (IQR: 0.38 to 0.48). Multivariate analysis which included age, sex, race, pubertal status, birth weight (BWT), CKD etiology, GFR, Ca x P, BMI percentile, LDL-c, baseline and concurrent SBP index (SBPi = SBP/95th %ile BP), and use of antihypertensive medications revealed that each 0.1 unit increase in a child's SBPi at baseline (approximately 1 year prior to cIMT measurement) was associated with a 0.02 mm increase in cIMT (95% CI: -0.001 to 0.03; p=0.07). cIMT in children taking antihypertensive medications was 0.04 mm thicker (95% CI: 0.004 to 0.07, p=0.03) than those not receiving antihypertensive agents, and was 0.05mm smaller in children with a BWT < 2.5kg (-0.09 to -0.007, p=0.02) compared to those with BWT≥2.5kg. All other exposures, including concurrent SBPi, were not associated with cIMT.

CONCLUSION: Children with CKD have impaired carotid artery structure. SBP 1 year prior to cIMT measurement, use of BP medications and higher BWT is associated with increased cIMT. This suggests that despite treatment, hypertensive children with CKD may continue to be at risk for increased cIMT. Further studies are needed to better understand the mechanisms of increased cIMT in this population, and to further explore the relationship between BWT and cIMT.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2373

**The Accuracy of Myocardial Perfusion Imaging in Patients with CKD** Hilana H. Hatoum,[1] Aileen May Arguelles,[1] Kavitha Kesari,[1] Ali K. Owda,[1] Fadi S. Rzouq.[2] *[1]Michigan State University/MRMC; [2]University of Washington.*

Background: Myocardial perfusion imaging (MPI) using sestamibi has gained considerable importance in the evaluation of coronary artery disease (CAD). Nonetheless, little is known about the accuracy of MPI in patients with chronic kidney disease (CKD). We sought to evaluate the accuracy of MPI in patients with CKD with coronary angiography (CA) as the gold standard.

Method: Patients who were admitted to our hospital with suspected acute coronary syndrome were involved in the study. Those who had a sestamibi-MPI followed by CA were the final study group. Characteristics of the patients and their MPS and angiography results were collected and concordance between the two studies were analyzed (we identified the lesions reported in the CA and reviewed the MPS to see if they detected the exact lesions). Then, we identified the true positive (TP), false positive (FP), and the false negative (FN) results on MPS (keeping in mind that a single MPS can have a combination of the TP,FP, and FN results based on the number of lesions in detected in CA). Next, we compared the proportions of TP, TN, and FN between the patients who had a normal kidney function (GFR >60ml/min) Vs those with CKD(GFR <60ml/min).

Results: 150 patients had complete data that was analyzed. 103 had normal kidney function (group A) and 47 had CKD (group B). TP results were reported in 56% Vs 73% in patients in group A Vs B (z value of 1.8, P 0.08), FP results were reported in 27% Vs 15% in patients with group A Vs B (z value of -1.65, P 0.049), and FN results were reported 33% Vs 42% in patients with group A vs B (z value 1.13, P 0.13).

Conclusion: Our analysis showed that MPI is as accurate in patients with CKD as in normal patients. While the TP and FN results were not statistically significant between the 2 groups, patients with CKD had statistically significant lower percentage of FP results (15% Vs 27% and thus a better specificity of the study (79% Vs 71%)), making MPI an even more accurate and dependable study in patients with CKD. These findings need to be confirmed by larger studies but we think that the use of MPI to evaluate for CAD is very reasonable in patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2374**

**Retinal Arterioles and Venules Narrow with Progressive Renal Failure** Qi-Lun Ooi,[1] Foong Kien Newk-Fon Hey Tow,[1] Rajeev Deva,[1] Mohd Afzal Alias,[1] Ryo Kawasaki,[2] Tien Y. Wong,[2,3] Deb J. Colville,[1] Anastasia F. Hutchinson,[1] Judith A. Savige.[1] *[1]Medicine (Northern Health), The University of Melbourne, Melbourne, VIC, Australia; [2]Centre for Eye Research Australia, The University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, VIC, Australia; [3]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore.*

**Background and Objectives:** Retinal microvascular narrowing reflects systemic small vessel disease such as stroke and possibly diastolic dysfunction. This study examined the effects of chronic kidney disease (CKD) on retinal vascular caliber, and the role of traditional risk factors in these changes.

**Design, setting, participants and measurements:** This was a cross-sectional study of 126 patients with CKD 3-5 (eGFR< 60 ml/min/1.73 $m^2$) and 126 hospital patients with CKD 1-2. Retinal vessel diameters were measured from digital fundus images (Canon CR5-45NM non-mydriatic camera) by a trained grader using a computer-assisted grading method (University of Wisconsin, Maddison, WI, USA) and Knudtson's formula. These were summarized as the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE).

**Results:** Patients with CKD 3-5 had a smaller mean CRAE (139.4 ± 17.8 um versus 148.5 ± 16.0 um, p<0.001) and CRVE (205.0 ± 30.7 um versus 217.4 ± 25.8 um, p = 0.001) than patients with CKD1-2. CRAE and CRVE decreased progressively as renal function deteriorated from CKD stage 3 to stage 5 (p for trend = 0.075 and 0.044 respectively). Hypertension (OR 3.94, CI 1.52 - 10.2, p = 0.005) and CKD (OR 2.84, CI 1.25 - 6.46, p = 0.013) were independent determinants of arteriolar narrowing but only CKD was an independent risk factor for venular narrowing (OR 3.59, 1.46 - 8.85, p = 0.005) after adjusting for age, gender, diabetes, dyslipidemia and smoking history. Patients with CKD5 and diabetes had a larger mean CRAE (141.4 ± 14.9 um versus 132.9 ± 14.2 um, p=0.05) and CRVE (211.1 ± 34.4 um versus 194.8 ± 23.8 um, p=0.05) than non-diabetics with CKD5.

**Conclusions:** Retinal small vessel narrowing in CKD 3-5 depends on the severity of renal impairment. Studies of retinal vessel caliber must control for impaired renal function.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2375**

**Low 1,25-Dihydroxyvitamin D Is Associated with Vascular Calcification, but Not Vascular Structure or Function in Chronic Kidney Disease** William G. Petchey, Trevor Watkins, Erin Howden, Carmel M. Hawley, Nicole M. Isbel. *CCRE-Cardiovascular Disease and Metabolic Disorders, Brisbane, Australia.*

**Background:** Vascular calcification (VC) and function (VF) may be modulated by vitamin D. In pre-dialysis chronic kidney disease (CKD) circulating 1,25-dihydroxyvitamin D (1,25-OHD) concentrations are still homeostatically maintained and it is not clear whether substrate (25-hydroxyvitamin D, 25-OHD) or active 1,25-OHD is more important, and whether either is associated with either VC or VF.

**Aim:** To examine the relationship between 25- and 1,25-OHD with VC and VF in CKD.

**Methods:** Patients with stage 3-4 CKD (eGFR 25-60ml/min/1.73$m^2$) were recruited. Vascular parameters were assessed by; abdominal aortic calcification score (AAC) on lateral lumbar radiograph (n=41); carotid-femoral pulse wave velocity (PWV; arterial stiffness); ultrasound measured flow-mediated brachial artery reactivity (FMD; endothelial function), and common carotid imtima-media thickness (cIMT; atheroma burden).

**Results:** Study characteristics of participants (n=104); Age 59±10 years, eGFR 39±9ml/min/1.73$m^2$, 60% male, 44% diabetic, 25-OHD 82±32 nmol/L, 1,25-OHD 87±33 pmol/L. 1,25-OHD was lower in patients with aortic calcification (107 vs 83 pmol/L, p=0.03), but concentrations did not correlate with PWV (rho=-0.06, p=0.6). Moreover, 25-OHD did not correlate with either VC or PWV, and neither 25- or 1,25-OHD correlated with FMD or cIMT. Adjusting for age and systolic BP, the risk of vascular calcification was reduced by 7% for every 1pmol/L increase in serum 1,25-OHD (OR 0.93, 95% CI: 0.87-1.0 p=0.05). No relationship with calcium, phosphate or PTH was observed.

**Conclusion:** The presence of vascular calcification was associated with lower levels of 1,25-OHD, but not 25-OHD, independent of age and systolic blood pressure in patients with pre-dialysis CKD. Neither vitamin D moiety was related to vascular stiffness, atheroma burden or endothelial function at the concentrations observed. Whether 1,25-OHD may be protective against development of vascular calcification *de novo* in CKD needs prospective study.

Disclosure of Financial Relationships: Research Funding: Roche Pharmaceuticals Pty Ltd.

**SA-PO2376**

**Left Ventricular Mass Is Greater in Stage 2-4 Chronic Kidney Disease Patients Than in Controls** Laima Siddiqi,[1,2] Peter J. Blankestijn.[1,2] *[1]Nephrology and Hypertension, University Medical center, Utrecht, Netherlands; [2]On Behalf of Smart Heart Study Group, University Medical Center, Utrecht, Netherlands.*

Cardiovascular (CV) mortality and morbidity are major problems in patients with chronic kidney disease (CKD). Almost all CKD patients are hypertensive. Hypertension is considered an important CV risk factor.LVM is an accepted surrogate endpoint for assessing CV risk. MRI is considered the gold standard. Aim: Compare LVM assessed by MRI in CKD patients and age matched controls and to identify factors associated with LVmass. Hypothesis: CKD patients have higher LVM than age matched controls. Further, for any given blood pressure CKD patients have higher LVmass than healthy controls.

Methods: 228 CKD (stage 2-4) patients and 30 healthy volunteers received cardiac MRI to assess LV variables. Blood pressure was the mean of office blood pressures. Patients were recruited from the outdoor clinics for cardiovascular diseases.

Results: In CKD patients arterial blood pressure was 148/85 ± 18/10 mmHg, eGFR 62 ± 15 ml/min, Hb 8.7 ± 0.8 mmol/l (14.5 ± 0.7 g/dL), BMI 26 ± 3.8 kg / $m^2$. LVM index in controls and CKD patients were 38.5 ± 8.2 and 52 ± 12 g/ $m^2$ respectively (P < 0.01), after adjusting for blood pressure and haemoglobin. Healthy volunteers had an arterial blood pressure 126/76±13/9 mmHg, normal kidney function, BMI 23 ± 3 kg / $m^2$.

Univariate analysis showed that LVM is significantly correlated only to systolic blood pressure and haemoglobin level (P < 0.005). In CKD patients the mean LVM were 98 ± 26 and in healthy volunteers 76 ± 9.6 g (P < 0.01); Further analysis showed that for any given blood pressure patients have a higher LVMindex than healthy volunteers.

Conclusion: CKD patients have a greater LVM than healthy volunteers, independent of the level of blood pressure and haemoglobin. Moreover, for any given blood pressure CKD patients have a higher LVM than healthy volunteers. This suggests that there are kidney specific risk factors such as sympathetic nerve activity that play a role in CV damage in this population.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2377**

**Risk Factors for Vascular Events in CKD Patients Not on Dialysis Receiving Once-Monthly (Q4W) C.E.R.A. or Once-Weekly (QW) or Once Every 2 Weeks (Q2W) Darbepoetin Alfa: Post Hoc Analysis of the CORDATUS Study** Simon D. Roger. *Department of Renal Medicine, Gosford Hospital, Gosford, NSW, Australia.*

**Purpose:** CORDATUS was a large randomized study demonstrating non-inferiority of Q4W methoxy polyethylene glycol-epoetin beta (a continuous erythropoietin receptor activator [C.E.R.A.]) to QW or Q2W darbepoetin alfa for anemia correction in CKD patients not on dialysis. This post hoc analysis has been conducted to investigate the effect of vascular risk factors on correction of anemia.

**Methods:** CKD patients not on dialysis (n=307) were randomized to Q4W C.E.R.A. or darbepoetin alfa QW or Q2W for a 20-week correction period, followed by an 8-week evaluation period. The primary efficacy analyses (Hb response defined as an increase in Hb ≥1 g/dL from baseline and a concentration ≥10.5 g/dL; non-inferiority [NI] test for Hb change from baseline in group comparison) were repeated in subgroups defined by presence of vascular disease (ischemic heart disease [IHD], peripheral vascular disease [PVD], cerebrovascular disease [CVD]) or vascular risk factors (hypertension [HTN], hyperlipidemia [HLP], diabetes [DM]).

**Results:** Hb response rates were similar for Q4W C.E.R.A. vs QW/Q2W darbepoetin alfa, irrespective of the presence of vascular disease or risk factors. Similarly, NI test for Hb change from baseline was significant for each category analyzed, with the exception of 'absence of HTN', in which there were only 10 patients.

| | Hb response rate (%) | | NI for the difference between groups in Hb change from baseline to evaluation | |
|---|---|---|---|---|
| **Risk factor** | **C.E.R.A.** | **Darbepoetin alfa** | **Difference in mean Hb (g/dL)** | **p value** |
| HTN | 94.0 | 93.9 | -0.015 | <0.0001 |
| No HTN | 100.0 | 85.7 | -0.941 | 0.8033 |
| HLP | 94.3 | 93.5 | -0.015 | <0.0001 |
| No HLP | 93.6 | 93.6 | -0.941 | 0.0026 |
| DM | 94.2 | 95.1 | 0.010 | <0.0001 |
| No DM | 94.0 | 91.8 | -0.078 | <0.0001 |
| IHD | 86.0 | 91.7 | -0.046 | 0.0014 |
| No IHD | 97.3 | 94.3 | 0.001 | <0.0001 |
| PVD | 89.5 | 91.3 | 0.285 | 0.0001 |
| No PVD | 94.8 | 93.9 | -0.088 | <0.0001 |
| CVD | 85.7 | 100.0 | 0.414 | 0.0148 |
| No CVD | 94.5 | 92.8 | -0.031 | <0.0001 |
| HLP, DM, IHD, PVD or CVD | 93.8 | 93.3 | -0.044 | <0.0001 |
| No HLP, DM, IHD, PVD or CVD | 95.7 | 94.7 | 0.122 | 0.0050 |

**Conclusion:** Q4W C.E.R.A. is as effective as QW/Q2W darbepoetin alfa in correcting Hb levels in CKD patients not on dialysis, regardless of existing vascular disease and risk factors.

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A$12,500
ROCHE
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BMS
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GEORGE INSTITUTE A$21,250
AMGEN
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COVANCE
A$101,469
SHIRE
A$188,789

BI
A$13,770; Honoraria: Sandoz A$3,200
Roche A$4,800
Vifor A$4,800
Solvay A$2,400
Novartis A$750
Amgen A$300.

## SA-PO2378

**A Renal Arterial Resistive Index Superior to 0.65 Is Associated with Interstitial Fibrosis and Arteriolosclerosis and Is Predictive of Renal Function Decline** Naïke Bigé,[1] Pierre Levy,[2] Patrice Callard,[4] Jean-Manuel Faintuch,[3] Pierre M. Ronco,[1] Jean-Jacques Boffa.[1] *[1]Nephrology, Tenon Hospital, APHP, Paris, France; [2]Biostatistics, Tenon Hospital, APHP, Paris, France; [3]Radiology, Tenon Hospital, APHP, Paris, France; [4]Pathology, Tenon Hospital, APHP, Paris, France.*

The clinical interest of arterial resistive index (RI) in the management of chronic kidney disease (CKD) is questionable. Our purpose was to analyze parameters associated with RI in order to emphasize its predictive value.

Patients and Methods

RI was measured before renal biopsy in 58 patients between 2006 and 2007. Data were collected prospectively. On renal biopsy, we determined the percentage of interstitial fibrosis and sclerotic glomeruli and the presence of arteriolosclerosis. Standardized ultrasonographic exam included measurement of maximal kidney length, diastolic and systolic velocities on three points and calculation of mean RI according to the Pourcelot formula. MDRD eGFR at 12 and 18 months was retrospectively collected for 42 and 34 patients.

Results

Median values for age, eGFR, percentage of interstitial fibrosis and sclerotic glomeruli and RI were respectively: 46 years (21-87), eGFR 59mL/min/1,73m² (5-130), fibrosis 10% (0-90), 13% (0-96), 0.63 (0.31-1.00). Forty-seven patients (82%) had glomerulonephritis, 5 (5.1%) tubulointerstitial nephropathy and 2 (3.4%) vascular nephropathy. In a univariate analysis, RI was positively correlated with age ($p<0.001$), pulse pressure ($p<0.05$), and negatively with renal length ($p<0.05$) and eGFR ($p<0.005$). RI was higher in patients with intima/media ratio ≥ 1 ($p<0.05$). In a multivariate analysis, only age ($p<0.01$) and eGFR ($p<0.05$) were correlated with RI. Patients with baseline RI >0.65 were older, had lower baseline eGFR, more interstitial fibrosis ($p<0.05$) and arteriolosclerosis ($p<0.005$) and more renal function decline at 12 and 18 months than those with baseline RI<0.65 ($p<0.05$).

Conclusion

RI is a non invasive marker of interstitial fibrosis and arteriolosclerosis and is predictive of renal function decline. Its measurement could help to identify patients with high risk of CKD progression to improve their nephroprotective treatment.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2379

**Cardiomyopathy in Patients with Chronic Renal Failure Is Accompanied by Thickening of Intramyocardial Arterioles** Kerstin Benz,[2] Solveig Knauth,[1] Kerstin U. Amann.[1] *[1]Pathology, University of Erlangen-Nürnberg, Erlangen, Germany; [2]Pediatrics, University of Erlangen-Nürnberg, Germany.*

Background:

In patients with chronic renal failure (CRF), cardiovascular morbidity and mortality are major clinical problems with cardiac death as the main cause of death. In animal and human studies specific uremic cardiovascular alterations were identified, i.e. coronary artery sclerosis and reduced myocardial capillarisation. Since myocardial ischemia in CRF patients is also seen in the absence of stenosis of epicardial vessels, changes of intramyocardial arterioles are under discussion.

Patients:

Using morphometry and immunohistochemistry we investigated the heart (both ventricles, septum) and different sites of the vascular bed (aorta asc. and desc., a. carotis communis, int.,ext., a. mesenterica int., a. iliaca communis, int., ext. in 36 autopsy cases (mean age: 62 years) . Cases were divided in 17 CRF and 19 age-matched non-renal control patients.

Results:

In CRF patients, relative heart weight, right and left ventricular wall thickness were significantly increased compared to controls. Interestingly, significantly higher wall thickness of intramyocardial arterioles (8.57±3.61 vs. 4.97±1.53 $_{\mu}$m), wall-lumen-ratio (0.59±0.21 vs. 0.36±0.16) and myocardial fibrosis was seen in CRF patients compared to controls. Detailed analysis of various vascular beds showed intima thickening and in several vessels also higher plaque areas, increased intima calcification and plaque neovascularisation in patients with CRF compared to controls. Atherosclerosis was significantly advanced in aorta, iliacal and mesenterial arteries whereas media calcification was more pronounced in the aorta of CRF patients compared to controls.

Conclusion:

We found thickening of intramyocardial arterioles in both ventricles in CRF patients confirming studies in experimental renal failure. This microarteriopathy could play an important role for cardiac blood and oxygen supply and may account for the higher rate of cardiac death in CRF patients. Our findings in extracardiac vessels point to different regulation of vascular thickening and vessel calcification at various sites of the vascular bed.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2380

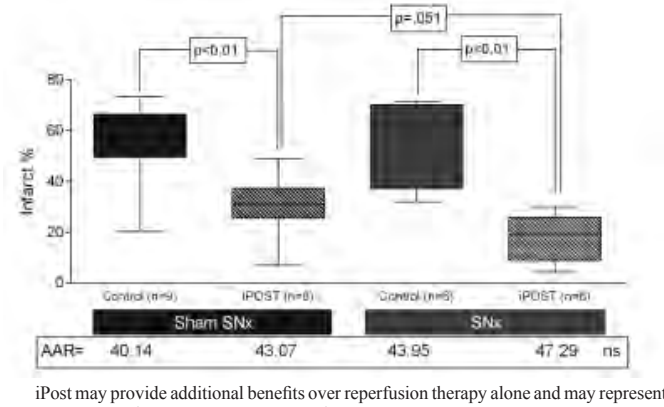
**Experimental Uremia Is Not a Barrier to Myocardial Protection by Post-Conditioning** Conor Byrne, Kieran Mccafferty, Julius Edward Kieswich, Martin J. Raftery, Magdi Yaqoob. *WHRI, Queen Mary, University of London, London, United Kingdom.*

CKD is associated with both increased incidence of acute myocardial infarction (AMI) and an increased post AMI mortality. Moreover, reperfusion therapy does not appear to improve outcomes for patients with advanced CKD. Adjuvant myocardial protective strategies may further limit infarct size and thus improve outcome for patients with CKD.

We have previously demonstrated that ischemic preconditioning reduces myocardial infarct size in subtotally nephrectomised rats (SNx) (see F-PO1228, 2009). However, preconditioning strategies are only applicable to predictable events such as elective surgery or percutaneous coronary intervention (PCI). In non-uremic animals infarct size can be reduced by interrupting reperfusion with brief episodes of ischemia, termed Post-conditioning (iPost). We investigated whether iPost would be efficacious in the context of uremia.

Male Wistar rats underwent a 2 stage SNx or a sham procedure. 4 weeks later we induced AMI by ligating the LAD for 25 mins. we allowed reperfusion for 10 secs before reoccluding the LAD for 10 secs, this was repeated 5 times. After 2 hours reperfusion the animals were sacrificed and infarct size determined.

iPost was associated with a relative reduction in infarct size of 50% in non-uremic animals ($p=0.008$) and 70% in uremic animals ($p=0.002$)(Fig.1). The area at risk was similar for all groups. iPost appeared more beneficial in uremic animals, this was of borderline statistical significance ($p=0.051$) Median serum creatinine for the four groups was; 32.9, 29.0, 128, and 125 (in same order as shown in Fig.1). Hematocrit was significantly lower in the SNx groups versus the shams (40,43 32 and 23%, same order as above).




iPost may provide additional benefits over reperfusion therapy alone and may represent our best chance of reducing the high rate of post AMI mortality in CKD.

Disclosure of Financial Relationships: nothing to disclose

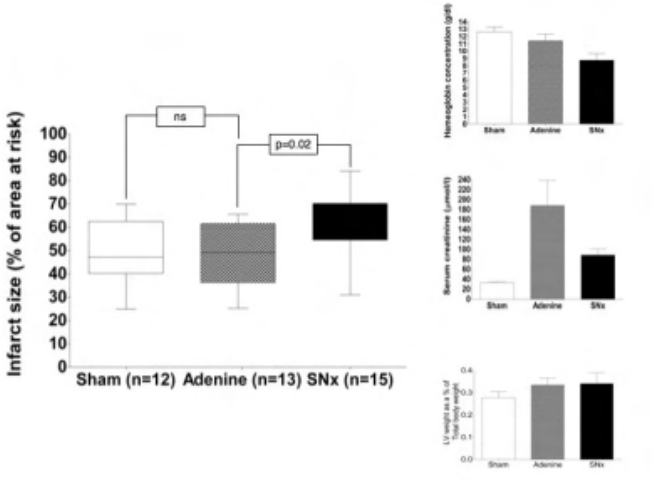
## SA-PO2381

**Myocardial Infarct Size in Two Different Rodent Models of Chronic Uremia** Conor Byrne, Kieran Mccafferty, Julius Edward Kieswich, Martin J. Raftery, Magdi Yaqoob. *WHRI, Queen Mary, University of London, London, United Kingdom.*

Cardiovascular disease is the leading cause of death in patients with CKD. It has previously been suggested that this is due to a reduced ischemia tolerance in the uremic heart. Reversible left anterior descending artery (LAD) ligation was performed in 2 rat models of chronic uremia; sub-total nephrectomy (SNx) and adenine induced uremia (Ad).

Male Wistar rats were fed a diet containing 0.75% adenine for 4 weeks or underwent a 2 stage sub-total nephrectomy or a sham procedure. The animals were allowed 4 weeks to develop a uremic phenotype. Myocardial infarction was induced by occluding the left anterior descending artery for 25 min followed by 2 hrs reperfusion at the end of which the animals were sacrificed and infarct size determined.

Serum creatinine was significantly higher in the animals with adenine induced renal failure (median 145 v 86 v 34.7 µmol/l; p=0.0001). Despite this there was no difference in infarct size between rats with adenine induced uraemia or sham animals fed control diet (52.2% +/- 11.8 v 50.0% +/- 8.8).However, there was a significant increase in infarct size in the rats that underwent SNx (61.2% +/-12.8; p=<0.05). The SNx rats had significantly higher mean arterial blood pressures (MAP) compared with the other groups (141 +/- 17 v 150 +/- 12.4 v 143 +/- 19.7 mmHg), they also had the lowest mean haemoglobin of the 3 groups (11.4 +/- 1.84 v 13.8 +/- 1.85 v 8.7 +/- 1.68 g/dl).




In these animal models of chronic kidney disease, the degree of uremia per se does not appear to have a significant impact on myocardial infarct size. Hypertension and degree of anaemia may well play a more significant role in determining infarct size. Further work is required to identify the factors contributing to larger infarct size in the SNx model.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2382

**Ischemic Preconditioning Protects the Heart in the Adenine Model of Severe Chronic Uremia** Kieran Mccafferty, Conor Byrne, Julius Edward Kieswich, Martin J. Raftery, Magdi Yaqoob. *William Harvery Research Institute, Queen Mary university London, London, United Kingdom.*

Ischemic preconditioning (IPC) is a process by which brief episodes of ischemia and reperfusion to an organ render that tissue resistant to subsequent ischemic injury. Comorbidities such as diabetes and senescence attenuate the effects of IPC. We have previously shown that the uremic heart can be preconditioned (ASN 2009 PO1228). However, the sub-total nephrectomy model(SNx) is a model of mild-moderate CKD. It is still unknown whether severe CKD limits the effects of IPC.

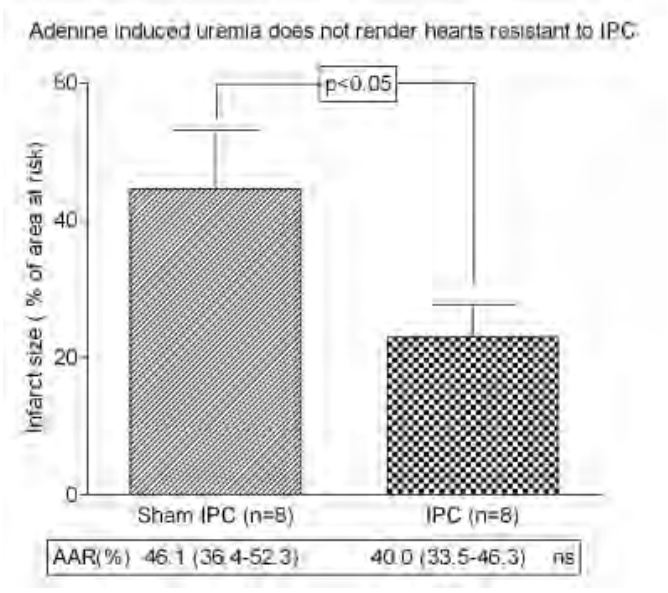
Male Wistar rats were given 4 weeks of 0.75% Adenine diet to induce severe uremia. Animals then underwent either an IPC protocol of 3cycles of 5 minutes ischaemia/reperfusion to the left anterior descending artery (LAD), or sham IPC protocol before undergoing an acute myocardial infarction (AMI) of 25 minutes LAD ligation and 2 hours reperfusion.

Results

| | Control | Adenine | SNx | p |
|---|---|---|---|---|
| Weight (g) | 419 (35) | 223 (20) | 389 (29) | 0.0001 |
| Creatinine (umol/l) | 32.1 (4.8) | 252.6 (52.4) | 133.5 (79.7) | 0.0001 |
| Hematocrit (%) | 41.6 (3.1) | 29.8 (7.3) | 28 (5.7) | 0.0001 |

Values shown as Mean (SD) and analyzed using ANOVA

Adenine animals are anemic, growth restricted, have twice the plasma creatinine of the SNx model, and 7 times the creatinine of a non uremic control group. (Data shown from previous work)




Despite severe renal dysfunction IPC animals had a 33% reduction in infarct size following IPC compared to Sham IPC animals.

Conclusions

These data in conjunction with our past work in the SNx model, suggests preconditioning affords protection across the spectrum of CKD.

CKD patients have poor outcomes following AMI and have been routinely excluded from trials in IPC. Our work suggests CKD patients should not be excluded from trials and have much to gain from future work in preconditioning.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2383

**1,25-Dihydroxyvitamin D Is Associated with Cardiac Structure but Not Function in Patients with CKD** William G. Petchey, Brian A. Haluska, Erin Howden, Rodel Leano, David W. Johnson, Nicole M. Isbel. *CCRE - Cardiovascular Disease and Metabolic Disorders, Brisbane, Australia.*

**Background** Patients with Chronic Kidney Disease (CKD) experience a greatly increased risk of cardiovascular events. Vitamin D status is inversely related to cardiovascular morbidity, both in the general and CKD populations. Animal models suggest this risk may be related to cardiac hypertrophy and dysfunction in states of hypovitaminosis D. CKD patients are at increased risk of hypovitaminosis D, due to an impaired ability to generate active 1,25-dihydroxyvitamin D (1,25-OHD), irrespective of substrate availability (25-hydroxyvitamin D, 25-OHD).

**Hypothesis** 1,25-OHD but not 25-OHD is negatively associated with cardiac mass and early diastolic dysfunction in patients with CKD.

**Methods** Patients with stages 3-4 CKD were recruited. Cardiac structure was assessed echocardiographically by: left ventricular (LV) mass (indexed to height$^{2.7}$, LVMI); septal and posterior wall thickness (SWT and PWT respectively). Diastolic dysfunction was defined as; mitral inflow deceleration time <150msec or >250msec, E/E' >15, or >10 with left atrial enlargement (>20cm$^2$).

**Results** Population characteristics (n=104); age 59±10 years, 60% male, eGFR 39±9ml/min, BP 135±14/77±7mmHg. Whilst there was no association between cardiac structure and 25-OHD, 1,25-OHD correlated negatively with SWT (r=-0.22, p=0.032), PWT (r=-0.22, p=0.029), and LVMI (r=-0.26, p=0.011), and the effect was independent of eGFR. Diastolic dysfunction was present in 60 subjects (58%), but did not correlate significantly with either 25-OHD or 1,25-OHD concentrations. In multivariate analysis, higher LVMI was independently associated with a history of coronary artery disease, LV preload (end diastolic volume), BMI, systolic BP and inversely with 1,25-OHD (adjusted $R^2$=0.40, p<0.0001). In this model, every 6.7pmol/L increase in serum 1,25-OHD predicted an average decrease in LV mass of 1g/m$^{2.7}$ (p=0.004).

**Conclusion** LV mass is negatively correlated with 1,25-OHD in patients with CKD, however there is no association with LV diastolic function. Whether 1,25-OHD supplementation can regress LV mass in pre-dialysis CKD patients should be the subject of future work.

Disclosure of Financial Relationships: Research Funding: Roche Pharmaceuticals Pty Ltd.

**SA-PO2384**

**Activation of Innate Immunity by LPS Is Associated with Systemic Inflammation and Endothelial Dysfunction in Patients with Chronic Kidney Disease** Andréa Marques Stinghen,[2] Aline Borsato Hauser,[1] Simone Cristina Mikosz Goncalves,[1] Bettina Gruber,[2] Roberto Pecoits-Filho.[1] *[1]Center for Health and Biological Sciences, Pontificia Universidade Católica do Paraná, Curitiba, Paraná, Brazil; [2]Basic Pathology Department, Universidade Federal do Paraná, Curitiba, Paraná, Brazil.*

Patients with CKD have high levels of systemic inflammatory markers and increased cardiovascular risk. Circulating levels of LPS may represent a trigger for the inflammatory response. The protein CD14 is an essential part of the innate immune response through TLR4, which is a molecule in the cellular signaling of the innate response to LPS. The biomarker of this phenomenon is soluble CD14 (CD14s). In this study we investigated the associations between plasma levels of CD14s, systemic inflammation and endothelial dysfunction in patients with CKD. Plasma were collected from patients at different stages of CKD evaluated according to the average of the clearances of urea and creatinine. The systemic inflammation and endothelial dysfunction was assessed by fibrinogen and sICAM-1 levels, respectively. CD14s (evaluated using ELISA), was used as a biomarker of LPS-mediated innate immunity response. The population consisted of 73 pre-dialysis patients (57±1.4 years old, 51% males) with GFR of 34±21mL/min. Plasma levels of sICAM-1 and CD14s were respectively 80±1.7 ng/mL and 2.6±0.1 µg/mL. There was a negative correlation between GFR with fibrinogen (ρ=-0.48, P<0.0001) and CD14s (ρ=-0.26, P<0.05). When patients were evaluated according to the median of their GFR, we observed higher levels of CD14s (2.9±0.1 µg/mL) in patients with GFR below 34±21mL/min, compared to patients with higher GFR (34-107±21 mL/min; P<0.05). We also observed a positive correlation between CD14s with systemic inflammation (fibrinogen)(ρ=0.32, P<0.005) and with endothelial dysfunction (sICAM-1)(ρ=0.33, P<0.005). Finally there was a positive correlation between fibrinogen and sICAM-1 (ρ= 0.32, P<0.001). Based on the analysis of associations between biomarkers in this pre-dialysis patients, systemic inflammation in CKD may be partially induced by activation from LPS through TLR4 and may additionally be associated with endothelial dysfunction.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2385**

**Relationships of Skin-Autofluorescence to Renal Function and Cardiovascular Disease in Patients with Diabetes and Chronic Kidney Disease** Yoshihiro Tani, Kenichi Tanaka, Jun Asai, Yuki Kusano, Hodaka Suzuki, Yoshimitsu Hayashi, Koichi Asahi, Tsuyoshi Watanabe. *Nephrology and Hypertention, Fukushima Medical University, Fukushima, Japan.*

Background

The accumulation of advanced glycation endproduct (AGE) is thought to play a role in the pathogenesis of chronic complications such as atherosclerosis and cardiovascular disease (CVD) in patients with diabetes and renal failure. An Autofluorescence (AF) reader non-invasively assesses AGE accumulation using skin-AF. We recently reported that skin-AF had an independent relationship to CVD in Japanese patients with ESRD (*Ther Apher Dial* 14;2010). The present study aimed to evaluate relationships of skin-AF to clinical parameters and CVD in pre-dialysis patients.

Methods

Subjects in this cross-sectional analysis comprised 379 patients with CKD and/or diabetes who visited Fukushima University Hospital between December 2008 and December 2009 (median age, 60.6 years; median eGFR, 61.2 ml/min/1.73m$^2$). Of the 379 patients, 154 (40.6%) had diabetes, 306 (80.7%) had CKD, 81 (21.3%) had both diabetes and CKD. AGE accumulation in skin was assessed by skin-AF using an AF reader.

Result

Skin-AF was increased as CKD stage advanced in patients with diabetes and/or CKD. Twenty percent of the variance in skin-AF among diabetic patients could be explained by the independent effects of eGFR (β=-0.27, *P*<0.01), serum albumin (β=-0.27, *P*<0.01). Twenty-seven of the variance in skin-AF among CKD patients could be explained by the independent effect of diabetes (β=0.17, *P*<0.01), age (β=0.23, *P*<0.01), eGFR (β=-0.24, *P*<0.01), serum albumin (β=-0.12, *P*=0.01). Gender, smoking, age, skin-AF, eGFR, hemoglobin, high-density lipoprotein cholesterol were significantly correlated with CVD history, and age (odds ratio 1.05, 95%CI 1.009-1.105, *P*<0.05), skin-AF (odds ratio 2.23, 95%CI 1.01-4.91, *P*<0.05), eGFR (odds ratio 0.97, 95%CI 0.94-0.99, *P*<0.05) were independently related to CVD history in the multiple logistic regression analysis.

Conclusions

Skin-AF was independently related to renal function and CVD history in Japanese CKD and diabetes patients. Non-invasive AF reader may provide potential marker for clinical risk assessment in these patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2386**

**Endothelial Dysfunction and Left Ventricular Diastolic Dysfunction Are Markers of Cardio-Renal Syndrome in the Early Stages of Chronic Kidney Disease** Masashi Kitagawa, Hitoshi Sugiyama, Hiroshi Morinaga, Tatsuyuki Inoue, Keiichi Takiue, Shinji Kitamura, Yohei Maeshima, Hirofumi Makino. *Medical and Clinical Science, Okayama University Graduate School, Okayama, Japan.*

[Purpose] Cardiovascular disease (CVD) is the major cause of mortality in patients with moderate to advanced chronic kidney disease (CKD). Endothelial dysfunction has been shown to be prevalent in mild to moderate CKD. A higher left ventricular (LV) mass and worse diastolic dysfunction in CKD patients is reported.

[Methods] This study observed 62 Japanese patients with different stages of non-diabetic CKD (mean age 54 ± 16 years old, 46.8% male). The relationships between the brachial artery flow-mediated dilation (FMD), the brachial-ankle pulse wave velocity (baPWV), intima-media thickness (IMT) of the common carotid artery, echocardiography, and albuminuria were all evaluated. Echo measurements included the left ventricular mass index (LVMI), ejection fraction (EF), peak early (E) and late (A) diastolic mitral filling velocity (E/A) ratio, peak early diastolic mitral annular velocity (e'), and E/e' ratio.

[Results] The level of FMD was found to significantly correlate with the estimated GFR ($r^2$=0.2711, p<0.0001). The level of FMD in CKD stage 2 significantly decreased in comparison to CKD stage 1. The e' and E/A ratio showed a positive correlation with the level of FMD, and LVMI negatively correlated with the level of FMD, however, EF did not. The estimated GFR demonstrated a significant positive correlation with the e' ($r^2$=0.3461, p<0.0001) and E/A ratio, it negatively correlated with LVMI. The e' demonstrated a significant negative correlation with baPWV and max IMT. The e' and E/A ratio in CKD stage 3 significantly decreased in comparison to CKD stage 1. In a multiple regression analysis, age, albuminuria and hypertension were independently associated with the e'.

[Conclusion] These data suggest that renal impairment in CKD patients is closely associated with either endothelial dysfunction, atherosclerosis, or LV diastolic dysfunction. Moreover, both endothelial dysfunction and LV diastolic dysfunction appear even in the early stages of CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2387**

**Elevated Angiopoietin-2 Independently Correlated with Arterial Stiffness in Chronic Kidney Disease** Fan-Chi Chang, Chih-Kang Chiang, Shuei-Liong Lin. *Renal Division, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan.*

Purpose

Accelerated arterial stiffness is recognized detrimental to cardiovascular outcome in chronic kidney disease (CKD). Given arterial remodeling taking place during CKD progression, we hypothesize that CKD causes arterial stiffness through disturbed angiotrophic growth factors vascular endothelial growth factor (VEGF) and angiopoietin (Ang).

Methods

We investigated 416 patients with CKD stages 3 to 5. Arterial stiffness was measured by noninvasive pulse wave velocity (PWV).

Results

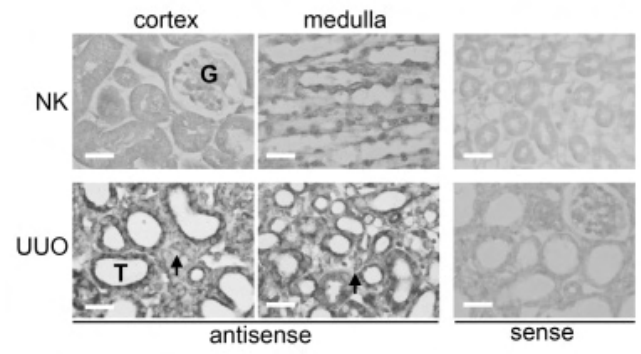
Plasma levels of Ang-2 and VEGF increased as estimated glomerular filtration rate (eGFR) declined (correlation coefficient -0.373, P < 0.0001 for Ang-2 and -0.148, P = 0.002 for VEGF). Plasma Ang-1 did not show significant change (P =0.207). The endogenous Ang antagonist, soluble Tie-2, decreased as eGFR declined (P <0.0001). Only Ang-2 was independently correlated with PWV by multivariate analysis.

Multivariate-adjusted regression analyses of plasma Ang-2 and PWV

| | Regression Coefficient (x10-5) | P | 95% CI (x10-5) |
|---|---|---|---|
| Univariate | | | |
| Ang-2 | 1.65 | <0.001 | 0.87–2.4 |
| Multivariate | | | |
| Model 1 | 1.25 | 0.001 | 0.54–1.96 |
| Model 2 | 0.96 | 0.008 | 0.25–1.67 |
| Model 3 | 0.86 | 0.023 | 0.12–1.61 |

Model 1, Ang-2+age; Model 2, Model 1+traditional risk; Model 3: Model 2+CaXP, medication including ACE inhibitor, ARB, statin, calcium channel blocker, β-blocker).

We further demonstrated increased Ang-2 expression in fibrotic kidneys following unilateral ureteral obstruction, principally in the injured tubular epithelial cells and interstitial fibroblasts.




Conclusions

Increased circulating Ang-2 was associated with arterial stiffness in CKD patients. The progressively fibrotic kidneys might increase circulating Ang-2 at least in part through Ang-2 upregulation in kidneys.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2388**

**Fibroblast Growth Factor-23 (FGF-23) Concentrations Predict Coronary and Aortic Calcification in Stage 3-5 CKD Patients** David C. Wheeler,[1] Ben Caplin,[1] Joanne Marks,[1] Nicholas A. Faure Walker,[1] Michael B. Rubens,[7] Leon J. Schurgers,[2] Willi Jahnen-Dechent,[5] Edward R. Smith,[3] W. Charles O'Neill,[4] Helen Colhoun,[6] John Cunningham,[1] Todd A. Dewees.[6] *[1]UCL Medical School, United Kingdom; [2]Univ of Maastricht, Netherlands; [3]Royal Sussex County Hospital, United Kingdom; [4]Emory Univ, Atlanta; [5]RWTH Aachen Univ, Germany; [6]Univ of Aberdeen, United Kingdom; [7]Royal Brompton Hospital, London, United Kingdom.*

The London Arterial Calcification, Kidney and Bone Outcomes (LACKABO) study set out to determine the association between cardiovascular (CV) risk factors, soluble biomarkers and arterial calcification in 289 patients with stages 2-5 CKD (eGFR <86 ml/min/1.73m2, range 8.3-85.5), of whom 21.11% had a prior diagnosis of CV disease. Coronary artery calcification (CAC) was quantified using electron beam CT scanning (Agatston protocol). A left censored (TOBIT) regression model was used. eGFR was weakly associated with CAC score adjusted for age and sex with 4% higher CAC score for every ml/min/1.73m² lower eGFR (p=0.023). FGF-23 levels did not significantly differ between those with or without prior CV disease. In cross sectional analysis adjusted for age and sex, higher FGF-23 levels were positively associated with urea, urate, phosphate, NT-proBNP, parathyroid hormone and osteoprotegerin and inversely associated with matrix GLA protein and eGFR (all p<0.05). However only eGFR was independently associated with FGF-23 such that for every unit decrease in eGFR, FGF-23 increased by 2% (p<0.0001) with a borderline relationship with urate. Overall the strongest independent relationships with logCAC score were seen with age (ß=0.20, p<0.0001), BMI (ß=0.14, p=0.004), and logFGF-23 (ß=1.02, p=0.007) with lower CAC score in women than men (ß for female vs. male = -1.38, p=0.045). A similar pattern of relationships was seen in patients without prior CV disease. Modelling for aortic calcification showed the same patterns, although the relationship with eGFR was stronger (p=0.0068). FGF-23 and urate maintained significance (p=0.0045 and 0.0983, respectively). **Conclusion:** In stages 2-5 CKD, FGF-23 is an independent biomarker of arterial calcification.

Disclosure of Financial Relationships: Employer: University College LondonResearch Funding: Abbott; Honoraria: Abbott, Amgen, Genzyme, Shire, Takeda; Scientific Advisor: Amgen, Takeda.

**SA-PO2389**

**Evaluation of Arterial Stiffness in Chronic Kidney Disease (CKD) Stage 2-5 by Pulse Wave Measurements and Ambulatory Arterial Stiffness Index (AASI)** Lene Boesby,[1] Thomas Elung-Jensen,[2] Svend Strandgaard,[1] Anne-Lise Kamper.[2] *[1]Department of Nephrology, Herlev Hospital, Herlev, Copenhagen, Denmark; [2]Department of Nephrology, Rigshospitalet, Copenhagen, Denmark.*

**Purpose:** To study arterial stiffness in CKD stage 2-5 by AASI as compared to Augmentation Index (AIx) and aortic pulse wave velocity (aPWV) obtained by applanation tonometry. The intra-patient reproducibility of AASI was also studied.

**Methods:** Patients were studied 2 days within 2 weeks. Double recordings of the radial pressure wave form and aPWV and 24-h ambulatory blood pressure measurements were done. Examination conditions were standardized and all measurements made by the same observer. AASI was calculated as 1 minus the regression slope of diastolic over systolic blood pressure. CKD stage was determined by estimated glomerular filtration rate (eGFR) using the MDRD7-formula. Spearman's correlation coefficient (SCC) was used for evaluating correlations. Day-to-day reproducibility was evaluated by the intra-class correlation coefficient (ICC).

**Results:** 68 patients (M50:F18), median age 63 years (range 30-79), with CKD stage 2 (n=17), stage 3 (n=22), stage 4 (n=20) and stage 5 (n=9) were studied. Thirteen had diabetes. Mean (±SD) AASI was 0.44±0.15, mean AIx was 28.2%±10.4 % and mean aPWV was 9.4 m/s ±1.0 m/s with no significant differences among the stages. The SCC between AASI and AIx was 0.320 (P=0.01), between AASI and aPWV it was 0.643 (P<0.0001) and between AIX and aPWV it was 0.346 (P=0.006). $ICC_{AASI}$ was 0.755 (95% CI: 0.630-0.841) with even greater reproducibility in CKD stages 4-5 (ICC>0.860).

**Conclusions:** The observed values of AASI in CKD patients were similar to those previously reported for the background population, while AIx and aPWV were higher. Despite good correlations between these parameters, the normal values of AASI found in the present study preclude its use as an index of vascular stiffness in CKD. However, intra-patient reproducibility of AASI in CKD stage 2-5 was high.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2390**

**Cardiovascular-Renal Interaction in Japanese Patients with Essential Hypertension** Junichi Yatabe,[1,2] Midori Sasaki Yatabe,[2,3] Tsuyoshi Watanabe,[2] Pedro A. Jose,[4] Hironobu Sanada.[1] *[1]Div. of Health Science Research, Fukushima Welfare Federation of Agricultural Cooperatives, Fukushima, Japan; [2]Dept. of Nephrology, Hypertension, Diabetology, Endocrinology and Metabolism, Fukushima Med. Univ., Fukushima, Japan; [3]Dept. of Pharmacology, Fukushima Med. Univ., Fukushima, Japan; [4]Center for Molecular Physiology Research, Children's National Medical Center, Washington, DC.*

Background: Long-term prognosis of hypertensive patients depends greatly on the presence of CKD and/or cardiovascular damage. The current study examined relations among indices of renal function and atherosclerosis to determine clinical manifestations of cardiovasucular-renal interaction in Japanese.

Methods: The study examined 368 essential hypertensive patients (164 male and 204 female, age 32-85). CKD group was defined by estimated GFR (eGFR) below 60 ml/min/1.73 m², calculated using the Japanese equation.

Results: Body weight was significantly less in CKD (n=80) than non-CKD (n=268) (non-CKD vs. CKD; 59.1±12.3 vs 55.9±10.8 kg, p<0.05). CKD group showed similar systolic blood pressure (BP) (143.6±17.1 vs 144.5±16.6 mmHg) with lower diastolic BP (85.0±12.6 vs 80.2±10.9 mmHg, p<0.01), resulting in greater pulse pressure (58.7±13.8 vs 64.3±13.8 mmHg, p<0.01). Augmentation index (AI) was significantly higher (84.0±14.1 vs 87.5±11.7 %, p<0.05) in CKD. Pulse wave velocity (PWV) was accelerated (1274±382 vs 1500±409 cm/s, p<0.05), and ankle-brachial index was slightly but significantly lower in CKD group (1.13±0.07 vs 1.11±0.08, p<0.05). Multiple linear regression analysis showed that eGFR independently correlated with diastolic BP, pulse pressure, AI and PWV. Human heart-type cytoplasmic fatty acid-binding protein (H-FABP) was significantly higher in CKD group (3.0±1.2 vs 3.8±1.3 ng/ml, p<0.05). However, cystatine C did not have significant correlation with H-FABP.

Conclusion: In Japanese hypertensive patients, those with low eGFR were associated with significantly worse indices of atherosclerosis. This may reflect the presence of cardiovascular-renal interaction in these patients. The results reinforce the importance of careful management in hypertensive patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2391**

**Physical Function and Subclinical Brain Disease in Stage 3-4 CKD** Stephen L. Seliger,[1] Joshua Francis Betz,[2] Gregory Steinbrenner,[1] Shari R. Waldstein,[3] Leslie I. Katzel.[1] *[1]Medicine, U Maryland Sch Med, Baltimore, MD; [2]Radiology, U Maryland Sch Med, Baltimore; [3]Psych, U MD Balt Co, Baltimore.*

**Purpose**: To determine the severity of physical functional impairment in older adults with CKD, and examine the association of subclinical vascular brain disease with functional impairment.

**Methods:** We performed cranial magnetic resonance imaging (MRI) and measured physical performance in 46 community-dwelling adults with stage 3-4 CKD (mean age = 71 yrs; 91% male; 39% Black) without dementia or stroke. Physical performance tests included the 6-minute walk (6MW) and timed get up and go (TGUAG) tests, grip strength, peak expiratory flow, and (among 28 subjects) peak aerobic capacity ($V0_2$peak) on graded treadmill. From MRI, we derived the white matter (WM) lesion burden and ventricle:brain ratio (subcortical atrophy). Observed performance was compared to expected performance based on normative data, and to performance in healthy older controls. Among CKD patients, age-adjusted correlations between functional and neuroanatomical measures were estimated.

**Results:** Mean eGFR was 36.5 mL/min/1.73m², body mass index 29.7 kg/m². 24% had >500mg/g proteinuria, and 67% were diabetic. Compared to normative (expected) performance and older controls, CKD patients had markedly reduced $V0_2$peak and performance on the 6MW and TGUAG tests (Table). Among CKD subjects, greater subcortical atrophy correlated with poorer performance on the 6MWT (r=0.6; p<.001) and TGUAG tests (r=0.5; p<.001), and greater WM lesion burden correlated with poorer performance on the 6MWT (r=0.35; p<.05), after adjusting for age.

| Test | Function Tested | CKD | Normative | Controls |
|---|---|---|---|---|
| Peak V02 (mL/kg/min)* | Aerobic capacity | 14.1 (4.0)* | >25.0 | 23.0 (6.6) |
| "Get Up and Go" (secs.) | Strength, balance | 11.3 (3.5)* | <10.0 | 6.0 (1.0) |
| 6-Min. walk (ft) | Submaximal Gait | 1,286 (344)* | 1,683 (194) | 1,986 (387) |
| Peak Expiratory Flow (L/min) | Respiratory Function | 373 (146) | 499 (44.7) | 441 (121) |
| Grip (kg) | Strength | 35.3 (9.5) | 35.1 (7.5) | 36,4 (11.7) |

* p<.05 vs older controls

**Conclusion:** Physical performance is markedly impaired in older adults with stage 3-4 CKD, and is associated with greater burden of subclinial brain disease.

Disclosure of Financial Relationships: Research Funding: 1)Amgen, Inc
2) Bracco, Inc
3) Roche, Inc.

**SA-PO2392**

**The Relationship between Age, Blood Pressure, Kidney Function and Outcomes among a National Cohort of Veterans** Michael J. Fischer,[1] Jessica W. Weiss,[3] Adam J. Batten,[2] Dan Bertenthal,[4] Indra Gupta,[2] Jeffrey Todd-Stenberg,[2] Ann M. O'Hare.[2] *[1]Jesse Brown and Hines VA; [2]VA Puget Sound; [3]Oregon Health & Science U.; [4]San Francisco VA.*

Hypertension and chronic kidney disease (CKD) are both common in the elderly. How the relationship between blood pressure (BP), estimated glomerular filtration rate (eGFR), and health outcomes varies across age groups is not known.

We identified all Veterans with at least one outpatient serum creatinine (SCr) within the Department of Veterans Affairs (VA) between 10/1/2000 - 9/30/2001 and at least one BP measurement up to one year before and 1 month after that SCr. We examined the cross-sectional association between eGFR (ml/min/m$^2$) and BP (mmHg) as a function of age (yrs) and the longitudinal association between BP and incident end-stage kidney disease (ESKD) and death as a function of both age and eGFR.

Among 1,875,516 Veterans, the prevalence of systolic hypertension (SBP > 140) ranged from 19% to 40%, diastolic hypertension (DBP > 90) from 6% to 3%, and eGFR < 60 from 3% to 38% in adults < 45 yrs compared with those ≥ 75 yrs. In logistic regression analysis, while there was a greater likelihood of both systolic and diastolic hypertension at progressively lower levels of eGFR for adults < 45 yrs ($p < 0.05$), this relationship was progressively attenuated at older ages and no longer present in adults ≥ 75 yrs ($p>0.05$). In Cox proportional hazard analyses across all age and eGFR strata, progressively higher levels of SBP but not DBP were significantly associated with a greater risk of ESKD ($p<0.05$). For all age and eGFR groups, there was a U-shaped relationship between SBP and death. However, the level of SBP associated with the lowest risk of death ranged from 120-139 mmHg in those aged 45-59 yrs to 150-159 mmHg in those aged ≥ 75 yrs. DBP was inconsistently associated with risk of death in all age groups.

In older compared with younger adults, the prevalence of systolic hypertension is higher but varies less as a function of eGFR. While SBP is strongly and linearly associated with risk of ESKD at all levels of eGFR regardless of age, its association with death was non-linear and varied substantially by age.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2393**

**Complete Recovery of Renal Function after Acute Kidney Injury Is Associated with Long-Term All-Cause Mortality in a Large Managed Care Organization** Jennifer A. DeGraauw,[1] John R. Holmen,[2] Jason Jones,[2] Sidney N. Thornton,[2] Michel B. Chonchol.[1] *[1]University of Colorado Denver Health Sciences Center, Aurora, CO; [2]Intermountain Health Care, Salt Lake City, UT.*

Purpose: Risk of long-term mortality among patients with complete recovery of renal function after acute kidney injury (AKI) has been poorly studied. This study aimed to determine the risk of death in hospitalized patients who had an episode of AKI with complete recovery of renal function.

Methods: We conducted a population-based cohort study of adult patients with no history of chronic kidney disease (CKD) and who developed AKI requiring hospitalization with complete recovery of renal function between January 1 1999 and December 31 2009. AKI was defined by the RIFLE classification, which requires at least a 50% increase in serum creatinine. Complete renal recovery was defined if the serum creatinine returned to a level less than 50% above baseline serum creatinine within 7 days after discharge. The primary outcome of interest was all-cause mortality occurring after hospital discharge. Patients were tracked for outcomes beginning at discharge and followed until March 31 2010. All subjects were required to have a minimum of 12 months follow-up data. These patients were matched for demographics and risk factors associated with death with individuals without AKI or CKD.

Results: We identified 1411 individuals with complete recovery of renal function after AKI and 1411 matched controls with no AKI during the index hospitalization. The median age of the enrolled participants was 65 years, 45% were women. Approximately 56% and 19% had a history of diabetes and myocardial infarction, respectively. After a median follow-up of 2.8 years, the deaths were 27% and 18% among those with and without AKI, respectively, corresponding to a unadjusted HR of 1.60 (95% CI, 1.38-1.86). After adjusting for potential confounders, the HR decreased to1.26 (95% CI, 1.07-1.48).

Conclusion: Subjects with an episode of AKI during hospitalization that have complete recovery of renal function appear to have an increased risk of all-cause mortality. Further studies are needed to confirm these findings.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2394**

**Survival of Patients on Dialysis by Incident Intake Volume in Hemodialysis and Peritoneal Dialysis Units** Lilyanna Trpeski,[1] Charmaine E. Lok,[2] Stanley S. Fenton.[2] *[1]Ontario Renal Network, Cancer Care Ontario, Toronto, ON, Canada; [2]Nephrology Department, University Health Network, Toronto, ON, Canada.*

Introduction: Survival on dialysis is dependent on patient, provider and other external factors. The effect of the volume of patients initiated onto dialysis in a facility may impact survival and has not been well studied.

Objective: To examine whether the intake volume of patients into a dialysis program influences overall patient survival on dialysis

Methodology: The study population consisted of patients who initiated dialysis between 1998 and 2005 in Ontario, Canada from The Renal Disease Registry (TRDR). 1,290 peritoneal dialysis (PD) and 5,587 hemodialysis (HD) patients were analyzed by Cox regression and adjusted for the variables in Table 1. Patients were censored on December 31 2009 (end of study), and when they were transferred to a non-TRDR site or received a transplant.

Results: Overall 6,877 patients received HD in 12 centers and PD in 10 centers. Facilities that had an average of 75 new patients/year (>643 total/8yrs) had better 3 year survival (65.4%) than those with an average of 40 new patients/year (52.4% ). Even when adjusted for patient demographics and comorbidities, dialyzing in a facility with a high volume of incident patients was associated with improved survival. The volume of incident patients did not affect survival in PD patients.

Table 1. Adjusted survival on dialysis by volume of incident patients, TRDR, Ontario, Canada

| Factors in Peritoneal Dialysis Patients | Hazard Ratio PD | Upper CI | Lower CI | Factors in Hemodialysis Patients | Hazard Ratio HD | Upper CI | Lower CI |
|---|---|---|---|---|---|---|---|
| Age 45-64 yrs | 2.093 | 2.48 | | Age 45-64 yrs | 1.815 | 1.98 | 1.66 |
| >65 years | 3.499 | 4.14 | 2.96 | >65 yrs | 2.857 | 2.9 | 2.43 |
| Ethnicity: Black | 0.634 | 0.82 | 0.49 | Ethnicity: Black | 0.498 | 0.59 | 0.43 |
| Indigenous | 1.701 | 3.82 | 0.76 | Indigenous | 0.762 | 1.14 | 0.51 |
| Other | 0.648 | 0.78 | 0.55 | Other | 0.823 | 0.88 | 0.57 |
| 120-160 incident patients | 1.041 | 1.39 | 0.78 | 300-534 incident patients | 0.961 | 1.06 | 0.87 |
| 160-247 incident patients | 1.042 | 1.37 | 0.79 | 534-643 incident patient | 1.048 | 1.15 | 0.95 |
| >247incident patients | 1.217 | 1.62 | 0.92 | >643 incident patients | 0.83 | 0.92 | 0.75 |
| Sex: female | 1.031 | 1.18 | 0.9 | Sex: female | 0.901 | 0.97 | 0.84 |
| diabetes | 1.818 | 2.09 | 1.58 | diabetes | 1.406 | 1.51 | 1.31 |
| coronary artery disease | 1.549 | 1.77 | 1.36 | coronary artery disease | 1.34 | 1.44 | 1.25 |

HD-Hemodialysis PD-Peritoneal Dialysis CI-confidence interval

Conclusion: Facilities with high volumes of patient dialysis initiation per year are associated with improved patient survival. The data suggests that the experience of larger patient volumes may be associated with processes that promote improved care and patient survival.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2395**

**In Dialysis Patients the Spatial QRS-T Angle Is a Significant Predictor of All-Cause and Cardiovascular Mortality** Arien Gaasbeek,[1] Mihaly K. De Bie,[1] Marion G. Koopman,[2] Pascal F. Van Dessel,[2] Cees A. Swenne,[1] Arthur A. Wilde,[2] Martin J. Schalij,[1] Ton J. Rabelink,[1] J. Wouter Jukema.[1] *[1]Leiden University Medical Center, Leiden, Netherlands; [2]Academic Medical Center, Amsterdam, Netherlands.*

**Introduction**

Cardiovascular disease largely contributes to mortality in dialysis patients. Assessing the predictive value of CV parameters is therefore warrented, but has proven difficult. In several non-dialysis patient groups, the spatial vectorcardiogram QRS-T angle, has shown to have predictive value for CV and all-cause mortality. The aim of this study was to assess the predictive value of an abnormal spatial QRS-T angle in dialysis patients.

**Methods**

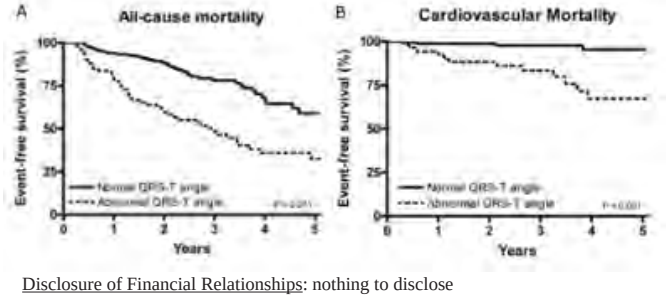
All patients who initiated dialysis therapy between 2002 and 2009 in the hospitals of Leiden (LUMC) and Amsterdam (AMC) at least 3 months on dialysis with a routine ECG available were included. The vectorcardiogram was approximated from the standard 12-lead ECG and the spatial QRS-T angle was calculated. Finally its predictive value for all-cause and cardiovascular mortality was assessed. An abnormal QRS-T angle was defined as > 130 in men and > 116 in women.

**Results**

A total of 273 patients (170 male, avg. age 56 ± 17) were included. During an avg. follow-up of 891 ± 601 days 88 (32%) patients died (19 due to cardiovascular causes). In patients with an abnormal spatial QRS-T angle both mortality and cardiovascular mortality were significantly higher in patients with an abnormal spatial QRS-T angle (fig1). Adjusted for all univariate predictors, an abnormal QRS-T angle was associated with a higher risk of death from all causes (HR 2.45, 95% CI 1.57 – 3.83) and also with a higher risk of cardiovascular mortality (HR 7.10, 95% CI 2.28 – 22.05)

**Conclusions**

In a population of chronic dialysis patients the spatial QRS-T angle is a significant and independent predictor of all-cause and cardiovascular mortality.




Disclosure of Financial Relationships: nothing to disclose

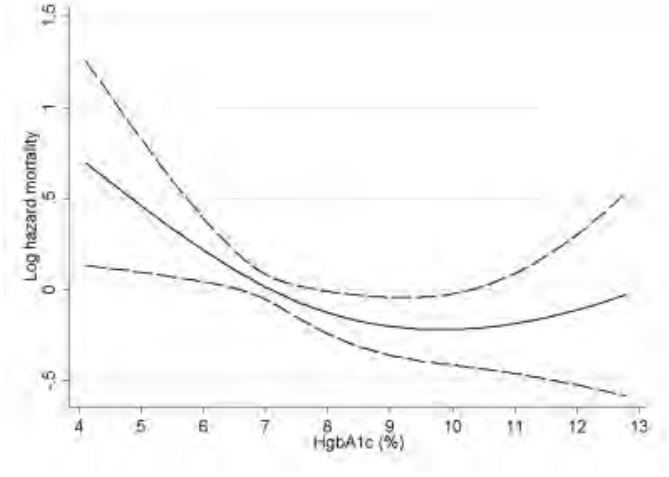
**SA-PO2396**

**Association of HgbA1c with Outcomes in Non-Dialysis Dependent CKD: Effect Modification by Hypoglycemia** Fregenet A. Alemu,[1] Jun Ling Lu,[2] Sandra M. Malakauskas,[1] Rasheed A. Balogun,[3] Kamyar Kalantar-Zadeh,[4] Csaba P. Kovesdy.[1,3] *[1]Salem VA Medical Center, Salem, VA; [2]Salem Research Institute, Salem, VA; [3]University of Virginia, Charlottesville, VA; [4]Harbor-UCLA, Torrance, CA.*

The impact of glycemic control and the role of hypoglycemic episodes on clinical outcomes in diabetic patients with non-dialysis dependent CKD (NDD-CKD) is unclear.

We examined the association of hemoglobin A1c (HgbA1c) with all-cause mortality, ESRD and slopes of eGFR in 660 diabetics with NDD-CKD. Associations were examined in Cox models, competing risk regression models and mixed effects models, with separate analyses in patients with and without documented severe hypoglycemia (blood sugar <50 mg/dl).

HgbA1c showed non-linear association with mortality, with an increase in mortality at HgbA1c levels <9% (Figure).




The higher mortality associated with lower HgbA1c was more accentuated in patients with hypoglycemia: adjusted hazard ratio of mortality associated with a 1% lower HgbA1c below 9% was 1.33 (95%CI: 1.06-1.67), p=0.015 vs. 1.19 (0.93-1.52), p=0.18 in patients with and without hypoglycemia, respectively. Higher HgbA1c was associated linearly with higher ESRD incidence in unadjusted analyses (unadjusted HR for 1% higher HgbA1c: 1.15 (1.07-1.23), p=0.006), with attenuation after adjustments (1.08 (0.97-1.20), p=0.17). Similar attenuation was observed for associations with slopes of eGFR (unadjusted and adjusted changes in eGFR/year associated with 1% higher HgbA1c: -0.36 (-0.57, -0.14), p=0.001 vs. -0.11 (-0.31, 0.08), p=0.26). The association of HgbA1c with ESRD and slopes of eGFR was independent of hypoglycemia.

Lower HgbA1c is associated with higher mortality at levels <9% in CKD patients who experience severe hypoglycemia, but may also be associated with attenuated progression of CKD. Glucose control may be beneficial in NDD-CKD if hypoglycemic episodes are avoided.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2397**

**Advanced Age Does Not Modify the Relationship between Low GFR and the Metabolic Complications of CKD** Paul E. Drawz,[1] Denise C. Babineau,[2] Mahboob Rahman.[1] *[1]Nephrology and Hypertension, Case Western Reserve University, Cleveland, OH; [2]Center for Clinical Investigation, Case Western Reserve University, Cleveland, OH.*

Background: Significant controversy exists as to clinical significance of a low glomerular filtration rate (GFR) in the elderly.

Purpose: To evaluate whether age modifies the relationship between low GFR and the metabolic complications of chronic kidney disease (CKD).

Methods: We performed a retrospective cross-sectional study within Veterans Integrated Service Network 10 of patients over 65 years of age with an outpatient GFR between 15 and 45 mL/min per 1.73m$^2$ in 2008 with at least one outpatient GFR less than 60 between 90 and 365 days prior to the index GFR. Anemia was defined as a hemoglobin <10 g/dL, hyperkalemia as a potassium >5.5 mEq/L, acidosis as a serum bicarbonate level <21 mEq/L, and hyperphosphatemia as a phosphorus level >4.6 mg/dL. Multivariable logistic regression was used to evaluate the association between age, GFR, hypertension, diabetes, cancer, medications and anemia, hyperkalemia, acidosis, and hyperphosphatemia. To evaluate whether age modifies the effect of low GFR on metabolic complications, an interaction term including age and GFR was included in each model.

Results: There were 6998 veterans included in the study. The average (SD) age was 80 (6.3), the average GFR was 37.4 (6.8), 4.9% had anemia, 3.7% hyperkalemia, 3.8% acidosis, and 6.3% had hyperphosphatemia. There was no significant interaction between age and GFR in the models including only age and GFR or in multivariable models (*P* value for interaction term in multivariate models: 0.70 for anemia, 0.20 for hyperkalemia, 0.24 for acidosis, and 0.23 for hyperphosphatemia). The adjusted odds ratio (95% CI) per 5 mL/min per 1.73m$^2$ decrease in GFR for anemia was 1.17 (1.08, 1.27), for hyperkalemia was 1.22 (1.12, 1.33), for acidosis was 1.52 (1.40, 1.64), and for hyperphosphatemia was 1.62 (1.45, 1.82) ($P < 0.001$ for all).

Conclusions: This study demonstrates that age does not modify the relationship between low GFR and anemia, hyperkalemia, acidosis, or hyperphosphatemia. Elderly patients with CKD should be monitored for metabolic complications.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2398**

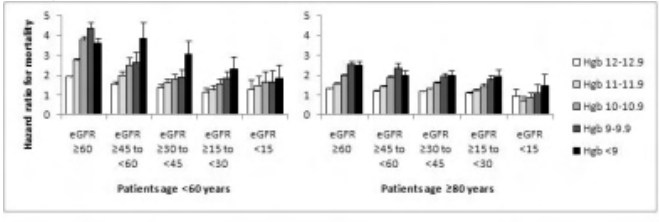
**Anemia in Chronic Kidney Disease (CKD): Differential Prevalence and Outcomes among Older Versus Younger Patients Stratified by GFR** Jessica W. Weiss,[1] Thy Do,[2] Indra Gupta,[2] Jeffrey Todd-Stenberg,[2] Adam J. Batten,[2] Dan Bertenthal,[3] Ann M. O'Hare.[2] *[1]Division of Nephrology, Oregon Health and Science University, Portland, OR; [2]Division of Nephrology, University of Washington, VA Puget Sound Healthcare, Seattle, WA; [3]Medicine, VA Medical Center, San Francisco, San Francisco, CA.*

Prior studies have described an association between anemia and death among patients with CKD but have not examined whether or how this relationship varies with age.

We conducted a retrospective analysis among national VA patients (1,751,819) ages 18-100; at least one outpatient serum creatinine between 10/1/2000 - 9/30/2001 and at least one outpatient hemoglobin (Hgb) within one year before or one month after that creatinine measurement were required. Within this cohort we examined the association of Hgb with time to death after stratification by age and eGFR.

The prevalence of anemia (Hgb <13 g/dL) increased with age and eGFR. In adjusted logistic regression analyses, a lower eGFR was associated with increased odds of anemia in all age groups, but the magnitude of this association was attenuated with increasing age. In Cox proportional hazards analysis, even mild decreases in Hgb were associated with an increased risk of death at most ages and most levels of eGFR. However, the strength of this association was attenuated with both increasing age and decreasing eGFR (p<0.001 for both eGFR and age interactions). The relative risk of death associated with a given level of Hgb was attenuated at lower levels of eGFR in all age groups, and varied more as a function of eGFR in younger versus older patients (Figure 1).

Our study suggests that the prognostic significance of a given Hgb level varies with both age and eGFR.



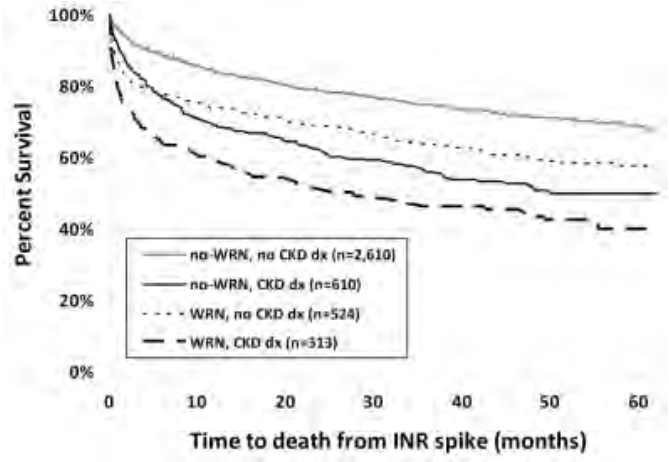

Adjusted hazard ratios for mortality in patients age <60 and >80. Hgb >13 g/dL is the reference group.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2399**

**Warfarin Related Nephropathy (WRN) Is a Risk Factor for Increased Mortality in Chronic Kidney Disease (CKD) and Non-CKD Patients** Sergey V. Brodsky, Brad H. Rovin, Tibor Nadasdy, Amy M. Lehman, Gyongyi Nadasdy, Anjali A. Satoskar, Udayan Y. Bhatt, Jon R. Von Visger, Lee A. Hebert. *The Ohio State University, Columbus, OH.*

Recently we demonstrated that warfarin therapy that results in an INR>3.0 can result in acute kidney injury (AKI). The mechanism involves glomerular hemorrhage and renal tubular obstruction by red blood cell (RBC) casts and perhaps other mechanisms. This study involves the 15,258 patients who had at least one order for warfarin therapy at our institution during 2005-2009. Of these, 6019 had at least 1 episode of INR>3.0. Of these, 4059 had serial serum creatinine (SC) measures in relation to the INR>3.0. Of these, 838 (21%) experienced an increase in SC>0.3 mg/dl within 1 week after INR>3.0 (WRN group). The remaining 3221 patients (79%) were designated no-WRN. The WRN group had a 5-year mortality rate of 42±3%, as compared to 27±2% for the no-WRN group (p<.001). The highest risk of death in the WRN cohort occurred within the 1st month after INR>3.0 (hazard ratio =2.15). For both WRN and no-WRN groups, the 5-year mortality rate was consistently higher in those with CKD compared to those with no-CKD (50.8±5.5% vs. 37.0±4.1% for the WRN cohort; 39.7±3.9% vs. 24.5±1.7% for the no-WRN cohort; p<.0001).




Compared to no-WRN patients, WRN patients tended to be older (63.7±14.7 years vs. 61.7±15.6 years, p=.025), diabetic (47% vs 37%, p<.0001), hypertensive (82% vs 72%, p<.001) and had a history of heart failure (62% vs 42%, p<.001). Preliminary models indicate that WRN still is a significant predictor of death even after adjusting for these factors.

We conclude that WRN is associated with increased mortality rate in the elderly, the diabetic, and those with CKD and cardiovascular diseases. However, the mechanisms of the association are not clear. Further study is needed to elucidate the pathogenesis and prevention of WRN.

Disclosure of Financial Relationships: nothing to disclose

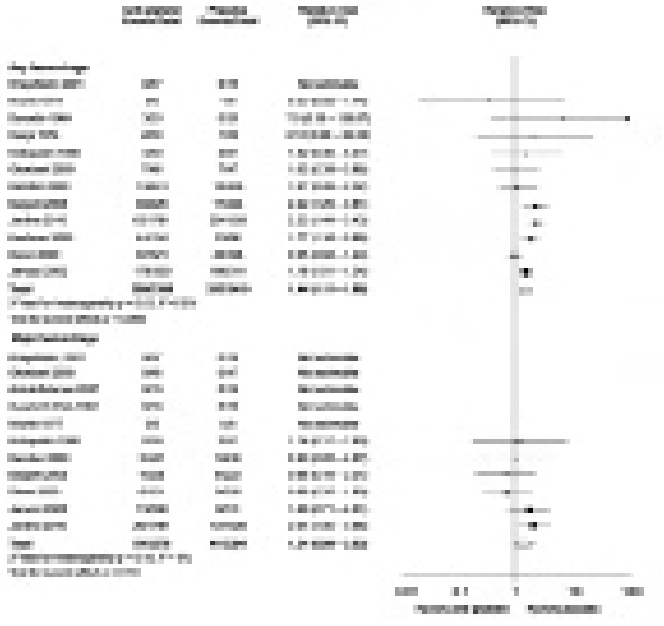
**SA-PO2400**

**Benefits and Harms of Anti-Platelet Therapy in Chronic Kidney Disease: A Systematic Review** Suetonia Palmer,[1] Lucia Di Micco,[2] Vlado Perkovic,[3] Meg J. Jardine,[3] Jonathan C. Craig,[4] Giovanni F. M. Strippoli,[4,5,6] Mona Razavian.[3] *[1]University of Otago Christchurch; [2]University of Naples; [3]George Institute for International Health; [4]University of Sydney; [5]Consorzio Mario Negri Sud; [6]Diaverum Medical Scientific Office, .*

Anti-platelet agents are widely used for prevention and treatment of cardiovascular disease in high-risk populations. We are conducting a systematic review of randomized trials to determine benefits and harms of anti-platelet treatment in people with chronic kidney disease.

Effects of anti-platelet agents in people with chronic kidney disease (GFR<60ml/min/1.73 $m^2$ or urine abnormalities), receiving dialysis, or with a kidney transplant were summarized as relative risks (RR) with 95% CI and pooled using random-effects models.

25 trials were identified (9486 patients). 22 placebo-controlled trials evaluated aspirin, dipyridamole, clopidogrel, sulphinpyrazone, picotamide, ticlopidine, tirofiban, or aspirin and dipyridamole combined. Anti-platelet agents did not reduce the risk of all-cause/cardiovascular death, or vascular access thrombosis. Compared with placebo, anti-platelet therapy reduced the risk of myocardial infarction (142 events; 2 trials, 5628 patients; RR 0.62, 95% CI 0.45-0.87), but not stroke (146 events; 4 trials, 7154 patients; RR 0.86, 95% CI 0.62-1.18). Anti-platelet agents increased the risk of bleeding, but risks of major hemorrhage were not significantly different.



The level of renal impairment was not an effect-modifier of risks of clinical outcomes.

In conclusion, anti-platelet agents reduce the risk of myocardial infarction in people with chronic kidney disease, while increasing risks of hemorrhage. Beneficial effects of anti-platelet therapy on mortality, cardiovascular death, and vascular access thrombosis in this population at high risk for cardiovascular disease are not proven.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2401**

**Risk Factors for Vascular Calcifications Preventing the Eligibility for a Kidney Transplant** Samira Sadowski,[1] Marie-Josee Hebert,[1] Francois Madore,[2] Agnes Rakel.[1] *[1]Research Center, Centre Hospitalier de l'Université de Montréal, Montreal, QC, Canada; [2]Nephrology Division, Hôpital Sacré-Coeur de Montréal, Montreal, QC, Canada.*

Vascular calcifications (VCs) are observed in about 50 to 65% of patients with end-stage renal disease (ESRD) and are thought to increase the risk of cardiovascular disease and mortality in this population. Patients with severe pre-transplant calcifications may be denied access to transplantation because of cardiovascular risk or technical contraindications to surgery. Several factors have been associated with VCs including hyperphosphatemia, high calcium phosphorus product and secondary hyperparathyroidism. However, the importance of these factors in the development of VCs severe enough to deny access to renal transplantation has not been clearly delineated.

The purpose of this study was to evaluate the association between the phosphocalcic product and the risk of severe VCs preventing the eligibility to kidney transplantation.

We conducted a case control study in a single-centered cohort of 1574 adults from our computerized transplant database, which included data on all patients evaluated for a renal transplant from January 1995 to June 2009. Cases were defined as patients who were refused for a kidney transplant because of severe VCs and controls were defined as patients who were accepted for a kidney transplant. The evaluation date for transplant eligibility was the case index date.

The study included 83 cases and 80 controls. The phosphocalcic product was above 4 $mmol^2/L^2$ in 44% of the cases and in 22% of the controls (crude OR: 2.77; 95% CI: 1.4–5.5). After adjusting for age, dialysis duration, history of smoking, diabetes as cause of ESRD and history of coronary artery disease, the phosphocalcic product above 4 $mmol^2/L^2$ remained a significant risk factor for transplant non eligibility (adjusted OR: 3.3; 95% CI: 1.1–9.8).

A high phosphocalcic product significantly increases the risk of non eligibility for a kidney transplant among adults with ESRD. Since the phosphocalcic product is a modifiable risk factor, nephrologists should be attentive to its level, in order to prevent long-term consequences of severe VCs and its impact on transplantation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2402**

**Mortality Associated with Hepatitis C and Chronic Kidney Disease among Community-Dwelling Adults: NHANES 1988-1994** Rajiv J. Gandhi, Robert N. Foley. *Department of Medicine, Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN.*

Purpose: Chronic hepatitis C virus (HCV) infection and chronic kidney disease (CKD) are both individually associated with increased mortality. The interaction of HCV and CKD on mortality, however, in non-referred, community-dwelling adults is unknown.

Methods: We compared blood pressure levels, estimated glomerular filtration rate (eGFR) (with the 4-variable MDRD and CKD-EPI equations), and urinary albumin-creatinine ratio (ACR) among adult participants age 20 years in the National Health and Nutrition Examination Survey (1988 to 1994, N = 15,540). Participants in the NHANES linked mortality files were then compared on mortality categorized by HCV and CKD status. All population estimates and statistical comparisons were weighted for the survey design employed.

Results: 2.3% of US adults were HCV-positive. Although HCV-positive and HCV-negative subjects had similar blood pressures and ACR, mean age (40.8 Vs. 44.9) was lower ($P < 0.05$) in subjects with HCV. Mean MDRD eGFR (101.6 Vs. 92.5) and CKD-EPI eGFR (106.4 Vs. 98.8) were higher ($P < 0.05$) in subjects with HCV. HCV-positivity showed a non-significant trend toward increased percentage of dead participants (22.2 Vs. 17.2). The unadjusted hazard ratios for all-cause mortality were 6.5 for CKD alone ($P < 0.01$; 95% CI 5.7, 7.6) compared with 1.5 for HCV-positivity alone ($P < 0.01$; 1.0, 2.6), and 6.9 when both conditions were present ($P < 0.01$; 3.4, 14.0). After adjusting for age, sex, and race, the corresponding hazard ratios for all-cause mortality were 2.5 ($P = 0.8$; 2.1, 2.8), 3.9 ($P < 0.05$; 2.3, 6.7), and 4.3 ($P = 0.1$; 1.8, 10.2).

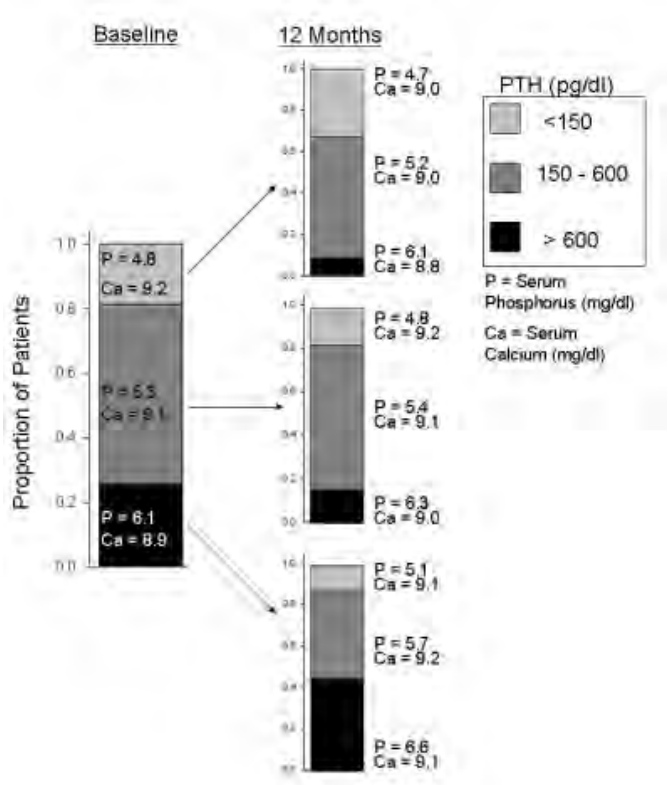
Conclusion: The risk for mortality attributable to HCV alone is higher than that for CKD alone. When both conditions are present, the risk increases slightly, suggesting that the addition of CKD does not significantly increase mortality beyond the risk associated with HCV alone. This may have implications regarding the utility of screening for CKD among HCV-positive individuals in the community.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2403**

**Exploring the Relationship between Temporal Trends in PTH, P and Ca in HD Patients** Ryan D. Kilpatrick,[1] Karminder S. Gill,[2] Geoffrey A. Block.[3] *[1]Biostatistics and Epidemiology, Amgen, Inc, Thousand Oaks, CA; [2]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Denver Nephrologists PC, Denver, CO.*

Objective: The pathogenesis of CKD-MBD is multi-factorial and there are important temporal interactions among biochemical parameters including PTH, Ca and P. Although the KDIGO guidelines recommend using dynamic trends in PTH to inform therapeutic decisions, most studies to date have focused only on static measures of biochemical indices. Methods: We conducted a retrospective cohort study using data from a large dialysis provider in 54,641 HD patients in 2007. PTH, Ca and P were provided as the last value of each month. The first monthly PTH in the dataset and the P and Ca measurement from the same month were considered baseline values. Follow-up values were those occurring 12 +/- 2 mos after baseline. PTH at both points was categorized into three groups: < 150, 150-600, and > 600 pg/dl, corresponding to 2 and 9 times the upper limit of normal stated in KDIGO. Results: Whether mediated by disease progression or changes in treatment, large changes in PTH over 12 mos were seen in many patients. Approx. 6% of patients shifted from <150 to >600 and 13% moved from 150-600 to > 600 cat. Changes in PTH category were associated with changes in P but not in Ca. Those showing an upward shift in PTH had higher P levels at 12 mos., those remaining in same PTH category had similar avg. P values at baseline and 12 mos, and those with a downward change in PTH had lower P values at 12 mos compared with baseline. Only minor changes in Ca suggest the relationship between PTH and P over time is unlikely related to change in vitamin D use. Conclusion: Large changes in PTH occur often in HD patients over 12 mos. and temporal changes in PTH are associated with changes in P but not Ca.

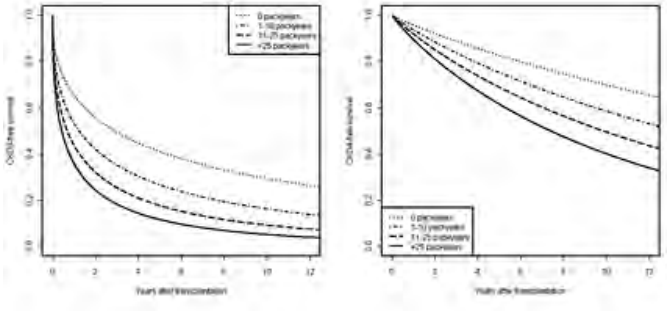



Disclosure of Financial Relationships: Employer: Department of Biostatistics and Epidemiology, Amgen, Inc.

**SA-PO2404**

**Past Smoking Determines Development of Chronic Kidney Disease after Lung Transplantation** Merel E. Hellemons, Stephan J. L. Bakker, Gerjan Navis. *Internal Medicine, University Medical Center of Groningen, Groningen, Netherlands.*

Smoking is risk factor for chronic kidney disease (CKD) and for pulmonary disease. CKD is a main complication after lung transplantation (LTx). We investigated whether past smoking was a risk factor for CKD and mortality after LTx in the patients that underwent LTx in our centre (1990-2008). Smoking cessation > 1 year was required to be eligible for LTx. Glomerular filtration rate (GFR; $^{125}$I-Iothalamate) was measured before LTx and regularly during follow-up. Of 326 patients (age 45 ± 12 yrs, 51 % male; GFR 99 ± 24ml/min/1.73m2) 134 were never smokers and 192 were former smokers with a median [IQR] of 17.5 [10-30] packyears (PCKY). Incidences of CKD stage III, IV and death at 5 yrs after LTx were 73, 21 and 35 %, respectively. Former smokers had a higher risk for CKD (stage III: HR [95% Confidence Interval (CI)] = 1.71 [1.25 – 2.33]); stage IV (HR 1.85 [1.04- 3.33]) but not mortality (HR 0.99 [0.71 – 1.38]) compared to never smokers. A dose-dependent association existed between PCKY and CKD stage III (HR per 10 PCKY 1.20 [1.03 – 1.37]) and IV (HR 1.47 [1.10 – 1.89]). Figure 1 shows that the increased risk persists on long term follow-up. Interaction analysis showed a higher risk for CKD per PCKY in subjects older at LTx. Thus, smoking prior to LTx is dose-dependently associated with a persisting higher risk for CKD after LTx, in spite of smoking cessation, in particular in older patients.



Disclosure of Financial Relationships: nothing to disclose

**SA-PO2405**

**Carotid Plaque, Carotid Intima Media Thickness, and Coronary Calcification Are Comparable Predictors of Prevalent Cardiovascular Disease: Report from the Chronic Renal Insufficiency Cohort (CRIC) Study** Gbemisola Adeseun, Dawei Xie, Xin Wang, Marshall M. Joffe, Emile Mohler, Raymond R. Townsend, Sylvia E. Rosas. *University of Pennsylvania, Philadelphia, PA.*

Despite the significant morbidity and mortality attributable to cardiovascular disease (CVD), risk stratification remains an important challenge in the chronic kidney disease population. We compared the relationship of non-invasive measures of atherosclerosis, including carotid intima-media thickness (IMT), carotid plaque, coronary artery calcification (CAC) and ascending and descending thoracic aorta calcification to prevalent self-reported CVD. Participants were enrolled in the carotid IMT ancillary study of the Chronic Renal Insufficiency Cohort Study who also had all of the above measures within an 18 month period. CVD, defined as history of myocardial infarction/revascularization, stroke, and peripheral vascular disease, was present in 20% of participants. C-statistics were used to ascertain the discriminatory power of each measure of atherosclerosis. The study population (n=155) was 60.5% male; 50% black and 46% white; with 22%, 40%, 26.5%, and 11.6% of individuals in eGFR strata <60, 45-60, 30-44, and <30ml/min/1.73m², respectively. In multivariate analyses, CAC (C-statistic 0.74, 95% CI: 0.64-0.83), carotid plaque (C-statistic 0.68, 95% CI: 0.60-0.76) and carotid IMT (C-statistic 0.64, CI: 0.55-0.72) were the best predictors of CVD when compared to ascending and descending thoracic aorta calcification (table 1); however, only the difference between CAC and measures of thoracic aorta calcification achieved statistical significance. CAC, carotid plaque, and carotid IMT were comparable predictors of prevalent CVD, but only CAC outperformed aortic calcification as a non-invasive measure of atherosclerosis.

The association between measures of atherosclerosis and prevalent CVD

| Measurement | C-statistic§ | CI |
|---|---|---|
| CAC | 0.74 | 0.64-0.83 |
| Plaque | 0.68 | 0.60-0.76 |
| Carotid IMT | 0.64 | 0.55-0.72 |
| Ascending aorta calcification | 0.62 | 0.54-0.71 |
| Descending aorta calcification | 0.60 | 0.52-0.68 |

§ Adjusted for age, gender, and race

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2406**

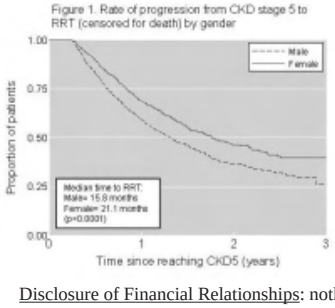
**Rates of Progression to Death or Renal Replacement Therapy after Reaching CKD Stage 5 in 1,971 Patients at 7 UK Renal Centers** Daniel Ford,[1,3] Dirk J. Van Schalkwyk,[1] David Ansell,[1] Charles Tomson,[1,2] Yoav Ben-Shlomo,[3] Damian G. Fogarty.[1,4] *[1]UK Renal Registry, Bristol, United Kingdom; [2]Southmead Hospital, Bristol, United Kingdom; [3]University of Bristol, United Kingdom; [4]City Hospital, Belfast, United Kingdom.*

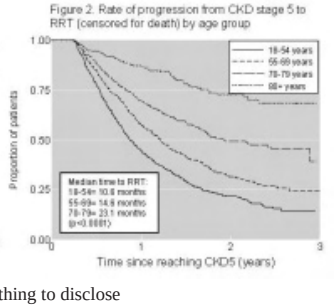
**Introduction** We have shown elsewhere that there are demographic differences between patients reaching CKD stage 5 (not on RRT) and patients commencing RRT. It is hypothesized that one reason for these differences could be differing rates of progression to RRT or death.

**Methods** All adult patients reaching CKD stage 5 (two eGFR results <15ml/min/1.73m², >90 days apart with no intervening eGFR results ≥15) during 2006-7 at 7 UK renal centers were included. Baseline demographic and clinical data were extracted direct from the centers' IT systems. Patients were followed up until December 2008. Rate of progression to RRT (censored for death) and to death (uncensored) were calculated using the Kaplan-Meier method. Groups were compared using a log-rank test for survivor function.

**Results** 1,971 patients reached CKD5 during 2006-7. 1,020 commenced RRT before the end of the study period. Significant differences in rate of progression to RRT were found by gender (males progressing faster, figure 1), age (younger patients progressing faster, figure 2) and primary renal disease (GN progressing fastest). Differences in rates of death were found by age (younger patients having better survival) and primary renal disease (GN & PKD having better survival) (all log-rank tests p<0.001). These differences persisted after adjustment for age, gender, ethnicity, center and primary renal disease.

**Conclusions** These differences in the rate of progression to RRT or death explain at least some of the differences in demographic make-up between CKD stage 5 and incident RRT patients.




Disclosure of Financial Relationships: nothing to disclose

**SA-PO2407**

**Both Hypokalemia and Hyperkalemia Predict Cardiovascular Risk during Blood Pressure Lowering Therapy in Patients with Diabetes and Nephropathy** Hiddo Jan Lambers Heerspink,[1] Gozewijn Dirk Laverman,[1] Julia Lewis,[2] Hans-Henrik Parving,[3] Dick De Zeeuw.[1] *[1]University Medical Center Groningen, Groningen, Netherlands; [2]Vanderbilt University, Naishville; [3]University Hospital Copenhagen, Copenhagen, Denmark.*

Both hypokalemia and hyperkalemia increase cardiovascular risk. Treatment with angiotensin receptor blockers (ARBs) increase serum potassium levels, while some report that treatment with calcium channel blocker (CCB) lower serum potassium. We examined the association between the on-treatment serum potassium and subsequent cardiovascular risk.

A post-hoc analysis in the merged RENAAL and IDNT trials was performed. Patients with diabetes and nephropathy were randomized to ARB treatment (losartan or irbesartan), CCB treatment (amlodipine in the IDNT trial), or placebo. The impact of the month 3 serum potassium level on CV outcomes was assessed by multivariate Cox analysis. Hypo-, and hyperkalemia were defined as a serum potassium concentration < 3.5 mmol/L and ≥ 5.5 mmol/L, respectively. The CV endpoint was the composite of myocardial infarction, stroke, hospitalization for heart failure, revascularization procedures, or CV death.

At month 3 hyperkalemia had developed in 131 (10.9%) patients on ARB treatment, 13 (2.7%) on CCB treatment and 47 (4.0%) on placebo (p<0.001 vs. ARB), Hypokalemia developed in 11 (0.9%) patients on ARB treatment, 13 (2.7%) on CCB treatment, and 12 (1.0 %) on placebo (p<0.008 vs. CCB). Both hypo- and hyperkalemia were independently associated with increased overall cardiovascular risk (table). Similar associations were observed for the myocardial infarction and stroke component of the CV endpoint.

ARB treatment is associated with increased likelihood of hyperkalemia while CCB treatment is associated with an increased likelihood of hypokalemia. Both hypo- and hyperkalemia are in turn associated with increased CV risk and may limit the CV protective efficacy of these drugs.

| Month 3 serum potassium concentration (mmol/L) | Hazard ratio CV endpoints (95%CI) | p-value |
|---|---|---|
| <3.5 | 1.79 (1.06 – 3.01) | 0.029 |
| 3.5 - 5.0 | reference | - |
| 5.0 - 5.5 | 1.17 (0.96 – 1.43) | 0.129 |
| ≥ 5.5 | 1.34 (1.01 – 1.77) | 0.039 |

Disclosure of Financial Relationships: Research Funding: Abbott Research Grant. Payments directed to institution.

**SA-PO2408**

**Comparison of Cardiovascular Prognosis by Three Serum Cystatin C Methods** Michael Shlipak,[1] Yongmei Li,[1] Joachim H. Ix,[2] Lars-Olof Hansson,[3] Anders Larsson,[3] Mary Whooley.[1] *[1]San Francisco VA Medical Center; University of California, San Francisco, San Francisco, CA; [2]University of California, San Diego, San Diego, CA; [3]University Hospital, Uppsala, Sweden.*

Cystatin C (cysC) is a promising new measure of glomerular filtration that has been proven superior to creatinine-based estimated GFR (eGFR-MDRD) in forecasting clinical outcomes. However, Siemens' cystatin C assay (cysC-S), used in many longitudinal studies, has had limited applicability because it requires a Siemens nephelometer. Two other companies, Gentian and Roche, have developed cystatin C assays (cysC-G and cysC-R) that can be measured using most clinical auto-analyzers. We compared the agreement of cysC-G and cysC-R with cysC-S in 948 participants from The Heart and Soul Study, a longitudinal study of persons with established coronary artery disease who were followed for an average of 8 years. We then compared associations of all 3 measures and eGFR-MDRD with clinical outcomes. The cysC-S had higher correlations with cysC-G (0.96) than cysC-R (0.93); agreement (K) across cysC-S quartiles was 0.73 for cysC-G and 0.64 for cysC-R quartiles. These differences in agreement had minimal impact on associations with clinical outcomes; the Table presents hazard ratios for the high vs. low quartile of each measure with mortality, cardiovascular events (CVD), and heart failure (CHF), adjusted for age, sex, race, BMI, diabetes, hypertension, smoking, HDL, and CRP. All cysC measures had stronger and more linear associations than eGFR-MDRD. In summary, 3 methods for cystatin C measurement had similar utility as predictors of clinical outcomes. For widespread clinical use as a GFR marker, however, the optimal cystatin C assay will need to demonstrate consistent accuracy and calibration against a reference standard.

Adjusted Hazard Ratios for Clinical Outcomes of High versus Low Quartiles using 3 Cystatin C Measures and eGFR-MDRD

| | Mortality | | CVD | | CHF | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | HR | 95% CI | HR | 95% CI |
| CysC-S | 3.2 | 2.1-4.8 | 2.6 | 1.6-4.2 | 5.3 | 2.4-12.1 |
| CysC-G | 3.1 | 2.1-4.7 | 2.6 | 1.6-4.2 | 5.2 | 2.4-11.3 |
| CysC-R | 3.1 | 2.1-4.7 | 2.4 | 1.5-3.9 | 4.6 | 2.0-10.3 |
| eGFR-MDRD | 1.6 | 1.1-2.3 | 1.9 | 1.2-2.9 | 3.2 | 1.6-6.6 |

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2409**

**Acute Renal Failure May Not Affect Mortality in Patients with Extreme APACHE II Scores** Jean-Sebastien Rachoin, Lawrence S. Weisberg, David R. Gerber, Christa Schorr, Daniel Fabius, Jean-Pierre El-Khoury. *Medicine, UMDNJ-RWJ Medical School, Cooper University Hospital, Camden, NJ.*

Acute renal failure (ARF) is associated with increased mortality in large cohorts of critically-ill patients. Indeed, mortality reduction is used to define success of interventions in patients with ARF. It is unclear, however, whether the impact of ARF on mortality is uniform across the range of severity of illness. We performed a retrospective observational study of all patients admitted to a medical/surgical ICU from 2003 through 2009. Patients were categorized by severity of illness using the admission APACHE II score. We defined ARF as that requiring renal replacement therapy (RRT). We analyzed mortality by APACHE II strata. 9441 patients were initially considered for inclusion, of whom 7202 had complete data available. The median [IQR] APACHE II score was 15[11-20]. 256 patients (3.6%) had ARF requiring RRT. 1268 of 7202 patients (17.6%) died in hospital. Using recursive partitioning analysis we found that APACHE II score cutoff points of 13 and 22 improved group homogeneity for mortality. In these three APACHE groups (<13, 13-22 and >22), mortality increased progressively: 3.9%, 16.2% and 52.5%, respectively (P<0.001). Among patients with ARF, 111patients (43.4%) died. ARF was significantly associated with increased mortality in all patients (OR 3.8 [3-4.9], P<0.001), and in patients with intermediate APACHE score (2.8 [1.9-4.2], P<0.001), but not in patients with low (P=0.112) or high (p=0.570) scores. After adjusting for other confounding variables (age, gender, diabetes, hypertension, CKD, CHF) the associations were unchanged. Thus, ARF appears to increase the risk of death in patients with intermediate severity of illness, but not in patients with low or high severity scores. Future clinical trials should take into account this finding.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2410**

**Pulse Wave Velocity (PWV) and Heart Rate Variability (HRV) Predict Cardiovascular (CV) Outcomes in Chronic Kidney Disease (CKD)** Preeti Chandra,[1] Robin L. Sands,[1] Brenda W. Gillespie,[1] Nathan W. Levin,[2] Peter Kotanko,[2] Margaret A. Kiser,[3] Fredric O. Finkelstein,[4] Alan L. Hinderliter,[3] David Sengstock,[5] Sanjay Rajagopalan,[6] Rajiv Saran.[1] *[1]Univ of MI, Ann Arbor, MI; [2]Renal Research Inst., NYC, NY; [3]Univ of NC, Chapel Hill, NC; [4]St. Raphael Yale Univ, New Haven, CT; [5]Oakwood Healthcare System, Dearborn, MI; [6]Ohio State Univ, Columbus, OH.*

The relationship between arterial stiffness, autonomic dysfunction and CV events has not been evaluated in CKD. We postulated that low HRV (a measure of autonomic dysfunction) would be associated with increased arterial stiffness (PWV) and that both would independently predict CV events in CKD.

The RRI-CKD Study is a 4-center prospective cohort study of CKD stages 3-5 (n=834). A subset underwent both HRV testing using 24-hour Holter monitoring and carotid-femoral PWV (n=240). Cox regression was used to test HRV parameters (time and frequency domain) and PWV as predictors of CV events, adjusted for history (h/o) of CV, age, gender, phosphorus, and albumin (i.e., CV base model). Selection of the CV base model was obtained by method of best subsets with the $R^2$ selection criterion.

Mean age was 60 ± 15, 50% were male, 78% white, 31% diabetic (DM), 89% hypertensive and 37% with h/o of CV events. 47 patients had at least one CV event (including 5 cardiac deaths). Several HRV measures (LF, VLF, TP, ASDNN) were negatively correlated with PWV, with or without adjustment for age, systolic blood pressure, DM, and BMI ($p < 0.05$). HRV measures were more strongly predictive of CV events than PWV in separate models, and when in the same model. Lower HRV and higher PWV were both associated with adverse CV events (Table).

Both HRV and PWV predict CV events in CKD independent of traditional risk factors. Their role as intermediate outcomes for clinical trials needs further evaluation in this high risk population.

| | | HRV | | PWV | |
|---|---|---|---|---|---|
| | Generalized Model $R^2$ | Hazard Ratio | p-value | Hazard Ratio | p-value |
| CV base model (includes hlo CV, age, gender, albumin, and phosphorus) | 0.13 | – | – | – | – |
| + Pulse Wave Velocity (PWV) | 0.15 | – | – | 1.15 | 0.0541 |
| + Low frequency* (LF) | 0.18 | 0.57 | 0.0009 | – | – |
| + LF + PWV | 0.19 | 0.62 | 0.0017 | 1.10 | 0.1287 |
| + Very low frequency* (VLF) | 0.16 | 0.61 | 0.0072 | – | – |
| + VLF + PWV | 0.18 | 0.66 | 0.0300 | 1.08 | 0.1928 |
| + Low to High Frequency ratio* (LF/HF) | 0.15 | 0.61 | 0.0222 | – | – |
| + LF/HF + PWV | 0.18 | 0.60 | 0.0163 | 1.15 | 0.0425 |
| + Total power* (TP) | 0.16 | 0.55 | 0.0092 | – | – |
| + TP + PWV | 0.17 | 0.58 | 0.0170 | 1.10 | 0.1273 |
| + Average of all 5-minute standard deviation of NN over 24 hours (ASDNN) | 0.16 | 0.32 | 0.0112 | – | – |
| + ASDNN + PWV | 0.17 | 0.36 | 0.0188 | 1.10 | 0.1111 |

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2411**

**Nationwide Frequency and Outcomes of Atrial Fibrillation in Patients with Chronic Kidney Disease in an Inpatient Population** Ankit Sakhuja, Abhishek Deshmukh, Nilay Kumar, Rahul S. Nanchal, Aaron T. Dall, Gagan Kumar. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

**Background**

Literature suggests inflammation may play a role in the pathogenesis of atrial fibrillation (AF). In a few smaller studies, the prevalence of AF has been shown to be greater in patients with chronic kidney disease (CKD) which is known to be a chronic inflammatory state. The clinical outcomes in patients with AF who have CKD are largely unknown. We sought to examine the frequency of AF in CKD and outcomes of patients with CKD who developed AF.

**Methods**

Using the Nationwide Inpatient Sample 2007, patients aged ≥18 years discharged with a diagnosis of AF and stage 1-5 CKD were identified through appropriate ICD-9, clinical modification codes. Patients with end stage renal disease were excluded. Outcome variables included in-hospital mortality, LOS and disposition on discharge. Multivariate regression analysis was used to adjust for age, sex, demographics and other clinically relevant co-morbid conditions.

**Results**

There were an estimated 32,759,253 adult discharges in 2007; out of which 3,324,689 had AF. The frequency of AF in patients with CKD and normal renal function was 455,047(25.3%) and 2,727,138(9.1%) respectively. After adjusting for the above mentioned variables, the odds for developing AF in CKD patients was 1.2 (95%CI 1.18-1.22) times higher than those with normal kidney function.

In-hospital mortality in patients admitted with the primary diagnosis of AF was 0.88% in patients with normal kidney function and 2.1% in CKD patients (adjusted OR 1.33, 95%CI 1.04-1.69). AF patients with CKD had significantly longer LOS (1.1 days longer, 95%CI 0.91-1.17days) and were more likely to be discharged to a nursing home compared to those with AF who did not have CKD (OR 1.45; 95%CI 1.31-1.60).

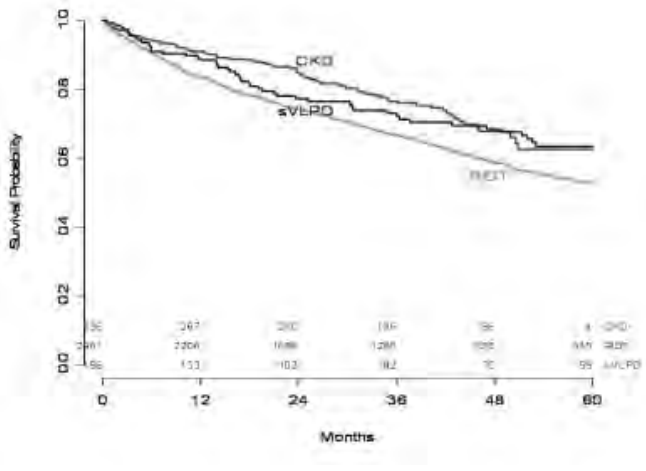
**Conclusion**This observational study showed that the frequency of AF is greater in patients with CKD. Presence of CKD was predictive of worse outcomes in terms of mortality and lengths of stay in patients discharged with the primary diagnosis of AF. Further prospective studies are needed to investigate causal relationships.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2412**

**Very Low-Protein Diet Plus Ketoanalogs (sVLPD) during CKD and Survival during Renal Replacement Therapy (RRT): A Controlled, Cohort Study** Vincenzo Bellizzi,[1] Paolo Chiodini,[2] Adamasco Cupisti,[3] Mauro Pezzotta,[4] Battista Fabio Viola,[4] Luca De Nicola,[2] Roberto Minutolo,[2] Giuliano Barsotti,[3] Giorgina B. Piccoli.[5] *[1]Salerno; [2]Napoli; [3]Pisa; [4]Brescia; [5]Torino, Italy.*

In CKD low protein-diets allow better control of metabolic disorders and delay dialysis start, but it has been suggested sVLPD may worsen survival after starting RRT. We evaluated whether prolonged sVLPD during CKD affects mortality during RRT by comparing time to all-cause death during RRT in patients previously followed in renal clinics either treated (sVLPD, n=158) or not (CKD, n=335) with sVLPD. As controls, we selected 2.961 coeval patients from Italian dialysis & transplantation registry (RIDT). Age (67±18, 66±14, 67±14 yrs) and CV disease (39, 31, 31%) did not differ in sVLPD, CKD and RIDT. Females were 54, 55 and 60% (p=0.037) in the three groups while diabetes was similar in CKD and RIDT (31 *vs.* 22%), but lower in sVLPD (13%; p<0.001). The median follow-up time in RRT (37 [18-103], 30 [15-43], 30 [12-55] mts [IQR]) did not differ. A better survival was detected in sVLPD and CKD [log-rank, 20.62; p<0.001].



These results persisted in the Cox model adjusted for age, gender, diabetes, CV disease; as compared with controls, the HRs [95% CI] for death were 0.74 [0.56-0.96] (p=0.024) and 0.68 [0.54-0.85] (p=0.001) for sVLPD and CKD. Due to the age interaction with survival in sVLPD (p=0.024), we re-evaluated survival after patients stratification in older (≥70 y) and younger (<70 y). In the latter, HRs for death reduced to 0.34 [0.16-0.73] and 0.58 [0.37-0.92] for both sVLPD and CKD while HRs resulted unchanged, 0.89 [0.67-0.18] and 0.72 [0.55-0.94], for elderly sVLPD or CKD. Thus, prescription of sVLPD during CKD improved survival after starting RRT. This survival advantage held particularly true in younger patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2413

**Renal Function Is Associated with Arterial Stiffness among African Caribbean Families of Tobago** Alan L. Patrick,[1,2] Hu Li,[2] Lewis Kuller,[2] Candace M. Kammerer,[2] Joseph M. Zmuda,[2] Iva Miljkovic,[2] Victor W. Wheeler,[1,2] Clareann H. Bunker.[2] *[1]Tobago Health Studies Office, Scarborough, Tobago, Trinidad and Tobago; [2]Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; [3]Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; [4]Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; [5]Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; [6]Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; [7]Tobago Health Studies Office, Scarborough, Tobago, Trinidad and Tobago.*

Purpose of study: To test the hypothesis that an increased serum creatinine concentration and a decreased eGFR may be related to increased arterial stiffness as measured by pulse wave velocity at the level of the peripheral arteries in this community-based sample of African ancestry population.

**Methods:** Serum creatinine and brachial ankle pulse wave velocity (baPWV) were measured using standard protocols on 7 large, multi-generation pedigrees (average family size: 50; range: 19 to 96; nearly 3500 relative pairs) a total of 402 participants, aged 18 to 103 years (male: 42.1 ± 16.9 (SD) years, female: 42.6 ± 17.3 (SD) years). eGFR was calculated using the four-variable MDRD equation for standardized serum creatinine. A multivariate regression model was built for males and females separately to assess significant predictors of baPWV.

**Results:** The average baPWV was 1438.2 ± 319.7 (cm/s) in men and 1412.9 ± 404.1(cm/s) in women. BaPWV increased across age groups. Among Tobago men, serum creatinine and eGFR were found to be independently associated with baPWV along with age, systolic blood pressure and triglyceride (β=-0.18, 0.2 and p=0.04, 0.006 respectively). Such significant association was stronger among Tobago women, after adjusting for age, systolic blood pressure (β= 0.05, -0.13 and p <0.0001, 0.003 respectively).

Conclusion: our data suggest that reduced kidney function, indicated by either increased serum creatinine level or decreased eGFR was an independent predictor for arterial stiffness in this Afro-Caribbean population in each gender.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2414

**High Non-Steroidal Anti-Inflammatory Drug Use among Persons with Chronic Kidney Disease in the U.S.** Laura C. Plantinga,[1] Vanessa Grubbs,[1] Chi-Yuan Hsu,[1] Elizabeth Hedgeman,[2] Bruce M. Robinson,[2] Nilka Rios Burrows,[3] Mark Eberhardt,[4] Neil R. Powe.[1] *[1]University of California, San Francisco, CA; [2]University of Michigan, Ann Arbor, MI; [3]Centers for Disease Control and Prevention, Atlanta, GA; [4]National Center for Health Statistics, Hyattsville, MD.*

Use of non-steroidal anti-inflammatory drugs (NSAIDs) has been associated with greater difficulty in controlling hypertension, hyperkalemia, and acute renal failure among persons with CKD. We characterized patterns of NSAID use among persons with CKD in the U.S. In the 1999-2004 National Health and Nutrition Examination Survey, 12,755 adult participants (≥20 years) were surveyed regarding their use of over-the-counter (OTC) and prescription NSAIDs (excluding aspirin and acetaminophen). CKD was defined as a urinary albumin:creatinine ratio of ≥30 mg/g (stages 1/2) or estimated GFR of 15-59 ml/min/1.73 $m^2$ (stages 3/4). All estimates were calculated using appropriate population-based weighting. Chronic use (*i.e.*, nearly every day for ≥30 days) of any NSAID was reported by 8.5%, 8.0%, and 8.1% of the U.S. population with no CKD, CKD stages 1/2, and CKD stages 3/4, respectively. Nearly all (>99%) of the chronically used NSAIDs were available OTC: ibuprofen (77.8%) and naproxen (24.6%) were used most often. Among those with CKD stages 3/4, almost 10% had a current NSAID prescription and, among chronic users, 63% had used NSAIDs for ≥1 year. Many of those with CKD who had prescriptions for NSAIDs also had prescriptions for ACEIs/ARBs (18%) or loop diuretics (18%); joint use of either with NSAIDs lessens their therapeutic actions and increases the risk of acute renal failure. After adjustment for demographics and comorbid conditions, including arthritis, chronic NSAID use was not significantly different between those with CKD and those without CKD (6.1% vs. 7.3%, *P*=0.143). Persons with CKD, were, however, less likely to report current NSAID prescriptions (5.1% vs. 6.9%, *P*=0.003). This study found high usage rates of NSAIDs among those with CKD in the U.S., reinforcing the benefits of assessing NSAID use and informing those with CKD about appropriate NSAID use.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2415

**Renal Impairment in Patients with Type 2 Diabetes Mellitus and Associated Antihyperglycemic Medication Adjustment: Evidence from an Electronic Medical Records Database** Kathleen Lang,[1] Juliana Meyers,[2] Sean D. Candrilli,[2] Birgit Kovacs.[1] *[1]BIPI, Ridgefield, CT; [2]RTI Health Solutions, Research Triangle Park, NC.*

**Objective:** This study evaluated the presence of renal impairment (RI) in patients (pts) with type 2 diabetes mellitus (T2DM) and the dose adjustment of oral antidiabetic drugs (OAD) for which a dose reduction is indicated in RI.

**Methods:** This retrospective analysis used the GE Centricity outpatient electronic medical record database. Subjects ≥18 years were included if they had evidence of T2DM (ICD-9-CM codes 250.X0, 250.X2) between 1/1/00 and 6/30/09 and had ≥12 months of data following identification. Renal function was evaluated using the MDRD formula-derived eGFR value, with pts classified as having moderate RI (eGFR 30-59 ml/min/1.73$m^2$), severe RI (eGFR 15-29ml/min/1.73$m^2$), or ESRD (eGFR <15ml/min/1.73$m^2$) based on the first observed serum creatinine (SC) result. Days between eGFR and physician diagnosis were calculated when a diagnosis was recorded. Dose adjustment after development of RI was reported for metformin and sitagliptin. The relationship between presence of RI and progression to ESRD was explored in a multivariate logistic regression.

**Results:** Of 344,770 pts identified with T2DM, 121,395 (35.2%) had evidence of RI. Of those, only 20% also had a physician diagnosis (ranging from 16% to 66% among those with moderate RI and ESRD). Physician diagnosis was recorded an average (SD) of 253.4 (584.5) and 86.9 (417.7) days after a confirmatory SC result among patients with moderate and severe RI, respectively. Among pts with moderate or severe RI, a corresponding clinical diagnosis was associated with significantly lower odds of progressing to ESRD (OR: 0.200; 95% CI: 0.188 to 0.213). Following the SC, 15.1% and 0.1% of pts treated with sitagliptin and metformin were receiving an appropriately adjusted dose for their level of RI.

**Conclusions:** RI is common but often undetected in pts with T2DM. Pts with accurate RI diagnosis have lower odds of progression to ESRD. Many RI pts are on inappropriate doses of OADs. Further analyses to understand the clinical and economic consequences of these findings are needed.

Disclosure of Financial Relationships: Employer: Boehringer_ingelheim Pharmaceuticals Inc.

## SA-PO2416

**The Association of Cognitive Function with Albuminuria and Kidney Function in the General Population** Hanneke Joosten,[1,2] Gerbrand J. Izaks,[2] Joris Slaets,[2] Henk Bilo,[3] Paul E. de Jong,[1] Ron T. Gansevoort.[1] *[1]Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, Groningen, Netherlands; [2]Department of Internal Medicine, Division of Geriatrics, University Medical Center Groningen, Groningen, Netherlands; [3]Department of Internal Medicine, Diabetes Center, Isala Clinics, Zwolle, Netherlands.*

Recent studies found various associations between a decreased glomerular filtration rate (GFR) or albuminuria with cognitive function. These studies were limited to elderly and vascular high-risk populations and did not take both CKD associated variables into account. We therefore aimed to analyze the association of estimated GFR (eGFR) as well as albuminuria with cognitive function in community-dwelling persons with a wide age range (35-82 year).

This study comprised 4095 subjects participating in the third survey of the PREVEND study (52% male, mean (SD) age 55 (12) year). Cognitive function was measured with the Ruff Figural Fluency test (RFFT) (scores 0-175) Mean (SD) RFFT score was 69 (26). Mean (SD) eGFR was 79 (15) ml/min/1.73$m^2$ (range 21-150). The prevalence of elevated albuminuria (≥30mg/24h) was 15%. Unadjusted, cognitive function was associated with both eGFR and albuminuria. After adjustment for age, sex and education level, no significant association was found between eGFR and RFFT (p=0.57). As an interaction of albuminuria was noted with age, adjusted analysis were performed per age group. This showed a significant association for albuminuria in the youngest tertile (35-48 years) (*p*=0.028), but not in older tertiles. Moreover, participants with normal albuminuria at baseline (1997-1998), who developed elevated albuminuria at the third survey (2003-2006) had a significantly lower mean RFFT score (SD) compared to participants with stable, normal albuminuria. Again, this was only significant in the youngest tertile (RFFT score 76 (22) versus 84 (22), respectively, *p*=0.04). In conclusion, elevated albuminuria was associated with worse cognitive function in this community-based cohort, being most explicit in the young. Thus, elevated albuminuria not only reflects renal damage, but also impaired cognitive functioning, especially at young age.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2417**

**Albuminuria as a Risk Factor for Peripheral Arterial Disease in a General Japanese Population: The Hisayama Study** Tomoko Usui,[1,2] Toshiharu Ninomiya,[2] Masaharu Nagata,[1,2] Yasufumi Doi,[2] Masayo Fukuhara,[1] Yutaka Kiyohara.[1] *[1]Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.*

**Background:**
Albuminuria has been acknowledged to be a risk factor for cardiovascular disease and death. However, there are limited studies investigating the influence of albuminuria on the risk of peripheral arterial disease (PAD). The aims of this study are to elucidate the relationship between albuminuria and the prevalence of PAD, and to assess the effect of albuminuria on the accuracy of the risk assessment of PAD in a general Japanese population.
**Methods:**
In a total of 3,061 community-dwelling subjects aged ≥40 years, we investigated the association of urinary albumin-creatinine ratio (UACR) levels with the prevalence of PAD. PAD was defined as ankle-brachial index <0.9. The odds ratio for the presence of PAD was estimated by using the logistic regression model. To compare the accuracy of the risk assessment for the presence of PAD between the models adjusted for potential risk factors with and without UACR levels, the receiver operating characteristic (ROC) curves were plotted.
**Results:**
Overall, 1.47% of the study participants had PAD. The age- and sex-adjusted prevalence of PAD increased linearly for UACR levels of <5.6, 5.6-10.8, 10.9-29.9, 30.0-299.9 and ≥300.0 mg/g, being 0.34, 0.80, 2.02, 2.50 and 2.53%, respectively (p for trend <0.001). The multivariate-adjusted odds ratio for the presence of PAD was 1.89 (95% confidence interval 1.14-3.12) for every 10-fold increment in UACR. The area under the ROC curve significantly increased when UACR levels was incorporated into a model with potential risk factors for PAD (0.80 vs. 0.76, p =0.02).
**Conclusion:**
Our findings suggest that greater UACR levels are associated linearly with higher prevalence of PAD, even within normoalbuminuric range, in the general Japanese population, and the information of UACR levels substantially improves the accuracy of the risk assessment of PAD beyond a model based on potential risk factors.
Disclosure of Financial Relationships: nothing to disclose

**SA-PO2418**

**Electrocardiogram Abnormalities and Cardiovascular Mortality in Patients with Chronic Kidney Disease** Mirela A. Dobre,[1] Andrei Brateanu,[1] Arash Rashidi,[2] Mahboob Rahman.[3] *[1]Huron Hospital, A Cleveland Clinic Hospital; [2]Nephrology, Fairview Hospital A Cleveland Clinic Hospital; [3]Nephrology and Hypertension, Case Western Reserve University.*

**Background**: The objective of the study was to evaluate whether electrocardiogram (EKG) abnormalities are predictors of cardiovascular mortality in patients with chronic kidney disease (CKD).
**Methods:** We used the Cardiovascular Health Study limited database from 1989 – 2005 to identify a cohort of patients with CKD at baseline (eGFR<60 mL/min/1.73m$^2$). The study population was categorized as having major, minor or no EKG abnormalities. Major EKG abnormalities included ventricular conduction delay, left ventricular hypertrophy, major Q-QS abnormalities, ST-T changes, atrial fibrillation and first degree atrio-ventricular block. Minor EKG abnormalities were defined as minor Q, QS waves, high R waves, minor isolated ST-T abnormalities, ST elevation, incomplete right bundle branch block, long QT, short PR interval, right axis deviation, or left axis deviation. The rates of cardiovascular events and mortality were compared between the three groups using proportional hazards regression analysis.
**Results:** 1192 patients had CKD at baseline. Of these, 452 (38.8%) had major, 346 (29.7%) had minor and 367 (31.5%) had no EKG changes. Patients with eGFR<60 were more likely to have EKG changes at baseline (adjusted risk ratio 1.23; 95% confidence interval [CI], 1.06-1.43) than those with higher eGFRs. 814 (68.3%) patients died during the mean (SD) follow-up of 10.3 (3.8) years. A cardiovascular cause of death was found in 184 (54.4%) patients with major EKG changes, 95 (28.1%) patients with minor EKG changes, and 59 (17.5%) patients with no EKG changes. The group of patients with major EKG changes had the highest risk for cardiovascular events and mortality, hazard ratio (HR), 2.0; 95% CI, 1.6-2.7, and 2.2; 95% CI, 1.6-3.0, respectively. In patients with minor EKG changes HR was 1.3; 95% CI, 1.0-1.7 and 1.5; 95% CI, 1.1-2.1, respectively.
**Conclusion:** In patients with CKD (eGFR<60 mL/min/1.73m$^2$), major EKG abnormalities are frequently present and predict a significantly higher risk of mortality and adverse cardiovascular outcomes.
Disclosure of Financial Relationships: nothing to disclose

**SA-PO2419**

**Alcohol, Tobacco and Illicit Drug Use and Progression of Chronic Kidney Disease (CKD)** Lydia Bazzano, Manjunath Balaram, Janet Cohan, Jeffrey C. Fink, Kenneth A. Jamerson, James P. Lash, Gail K. Makos, Nancy Robinson, Susan P. Steigerwalt, Kelvin Tao, Jacqueline R. Theurer, Dawei Xie, Chi-Yuan Hsu. *For the CRIC Study Investigators.*

Lifestyle factors associated with progression of CKD are of increasing interest. Most studies have not found strong associations between alcohol and tobacco use and progression of CKD. Less is known about illicit drug use. We obtained self-reported use of alcohol, tobacco (usual consumption) and illicit drugs (lifetime use) among 3,939 persons in the Chronic Renal Insufficiency Cohort (CRIC). Cox proportional hazards models adjusted for age, sex, race/ethnicity, education, diabetes, hypertension, BMI, systolic blood pressure, clinical site, eGFR and proteinuria at baseline were used to examine time to 50% reduction in eGFR from baseline and/or the development of ESRD by category of alcohol, tobacco and illicit drug use. Reference categories for alcohol, tobacco and illicit drug use were <once/year, not currently smoking, and never using illicit drugs. Alcohol, tobacco or lifetime use of illicit drugs did not increase the risk of CKD progression [Table]. However, few participants reported active use of illicit drugs in the last 30 days (marijuana n=152, methamphetamine n=2, inhaled cocaine n=28, injected cocaine n=0, heroin n=0). Alcohol and tobacco do not appear to be important factors in CKD progression. The impact of illicit drugs is less clear.

| *Variable* | *N* | *Frequency of use* | *Hazard Ratio (95% CI)* | *P-value* |
|---|---|---|---|---|
| Alcohol | 980 | <once/month | 1.00 (0.80, 1.25) | 0.99 |
| | 269 | once/month | 0.71 (0.47, 1.06) | 0.09 |
| | 432 | 2-4 times/month | 1.04 (0.76, 1.42) | 0.83 |
| | 355 | 1-2 times/week | 0.84 (0.58, 1.22) | 0.36 |
| | 438 | >2 times/week | 1.12 (0.79, 1.58) | 0.51 |
| Tobacco | 517 | current smoking | 1.26 (0.99, 1.60) | 0.06 |
| Marijuana | 1,293 | ever used | 1.04 (0.85, 1.26) | 0.74 |
| Methamphetamine | 146 | ever used | 1.01 (0.65, 1.57) | 0.96 |
| Cocaine (inhaled) | 424 | ever used | 0.96 (0.72, 1.27) | 0.76 |
| Cocaine (injected) | 45 | ever used | 0.99 (0.48, 2.02) | 0.98 |
| Heroin | 58 | ever used | 1.23 (0.72, 2.10) | 0.45 |

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2420**

**Impact of Chronic Kidney Disease on Risk of Incident Atrial Fibrillation (AF) and Subsequent Survival in Medicare Patients** Charles A. Herzog, Shuling Li. *CVSSC, USRDS, Minneapolis, MN.*

**Introduction:** Atrial Fibrillation (AF) is common in elderly pts and its prevalence increased in pts with chronic kidney disease (CKD). Few studies have examined CKD as a risk factor for incident AF in elderly pts.
**Methods:** We identified 1,092,649 pts (age 66+) prevalent on 12/31/06 in the 5% General Medicare database (prior AF and ESRD excluded) and followed through 12/31/08. CKD stage was identified from ICD-9 codes 585.1-585.5, 585.9. The risk of developing AF was assessed in a Cox model adjusting for demographics, and comorbidity. Unadjusted survival after incident AF was estimated by Kaplan-Meier method.
**Results:** The prevalent 2006 cohort was 41% male, 88% white, 23% age 66-69, 25% age 70-74, 21% age 75-79, 16% age 80-84, and 14% age 85+. By CKD stage: 94.9% no CKD, 0.5% stage I-II, 1.8% stage III-V, 2.9% stage unknown. By CKD stage incident AF occurred in 7.0% no CKD, 11.1% stage I-II, 12.4% stage III-V, 11.6% stage unknown. The Table shows predictors of AF (age 66-69, male, white, no CKD, no comorbid conditions is reference) with hazard ratio (HR) and survival of AF pts.
**Conclusion:** CKD stage is a predictor of incident AF and subsequent risk of death in elderly pts. Medicare pts with CKD and AF have a high (>30%) one year mortality.
Predictors of AF

| Variable | HR (95%CI) | P | | |
|---|---|---|---|---|
| CKD Stage I-II | 0.99 (0.91, 1.08) | 0.8992 | | |
| CKD Stage III-V | 1.12 (1.07, 1.16) | <.0001 | | |
| CKD (Stage unknown) | 0.97 (0.94, 1.01) | 0.1062 | | |
| Age 70-74 | 1.42 (1.38, 1.46) | <.0001 | | |
| Age 75-79 | 1.99 (1.94, 2.05) | <.0001 | | |
| Age 80-84 | 2.61 (2.55, 2.68) | <.0001 | | |
| Age 85+ | 3.56 (3.47, 3.65) | <.0001 | | |
| Female | 0.77 (0.76, 0.78) | <.0001 | | |
| Black | 0.64 (0.62, 0.66) | <.0001 | | |
| ASHD | 1.24 (1.22, 1.26) | <.0001 | | |
| CHF | 1.66 (1.62, 1.69) | <.0001 | | |
| Diabetes | 1.17 (1.15, 1.19) | <.0001 | | |
| Conduction disorder/Dysrhythmia | 1.71 (1.68, 1.75) | <.0001 | | |
| | **Survival after AF** | | | |
| | **CKD Stage** | | | |
| Month | **No CKD** | **I-II** | **III-V** | **Stage Unknown** |
| 1 | 93.2 | 89.4 | 88.5 | 87.1 |
| 6 | 84.9 | 76.2 | 73.2 | 71.7 |
| 12 | 79.3 | 68.3 | 64.4 | 63.4 |
| 24 | 70.7 | 54.9 | 52.9 | 51.1 |

Disclosure of Financial Relationships: Consultancy: Amgen, CorMedix; Ownership: Cambridge Heart, Boston Scientific, Johnson & JohnsonResearch Funding: Amgen, NIH (NIDDK); Honoraria: UpToDate; Other Relationship: RoFAR (Roche Foundation for Anemia Research) Board of Trustees Member.

**SA-PO2421**

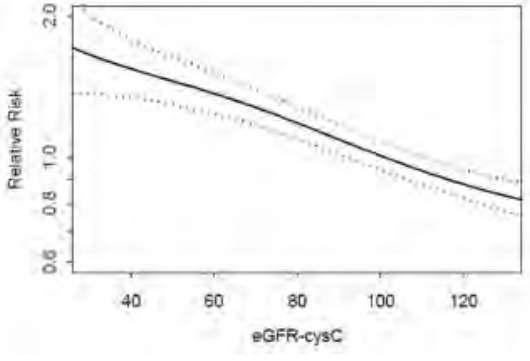
**Infectious Hospitalization among Older Patients with Chronic Kidney Disease** Lorien S. Dalrymple,[1] Ronit Katz,[2] Linda F. Fried,[3,4] Ian H. de Boer,[5] Bryan R. Kestenbaum,[5] Mark J. Sarnak,[6] Michael Shlipak.[7,8] *[1]Medicine, UC Davis, CA; [2]Biostatistics, University of Washington, WA; [3]Medicine and Epidemiology, University of Pittsburgh, PA; [4]VA Pittsburgh Health Care System, PA; [5]Medicine, University of Washington, WA; [6]Medicine, Tufts Medical Center, MA; [7]Medicine, VAMC San Francisco, CA; [8]Medicine, UC San Francisco, CA.*

**Purpose:** Moderate chronic kidney disease (CKD) may independently predispose patients to serious infectious complications. We characterized the association of kidney function with infectious hospitalizations in the Cardiovascular Health Study (CHS).

**Methods:** CHS participants with baseline serum cystatin C measures (n=5,157) were included, and kidney function was estimated by the CKD-EPI cystatin C equation (eGFR-cysC). Outcomes included hospitalizations with infection as the primary cause. We evaluated specific (pulmonary, genitourinary) and all-cause infectious hospitalization. Poisson regression was used to estimate rate ratios, adjusted for age, gender, race, BMI, diabetes, coronary artery disease, stroke, heart failure, cancer, chronic obstructive pulmonary disease, and serum albumin.

**Results:** The age-adjusted rate of infectious hospitalization (per 100 person-years) was 5.6, 4.2, 2.8 and 2.1 for eGFR-cysC < 45, 45-< 60, 60-< 90, and ≥ 90, respectively. Baseline level of kidney function was linearly and inversely associated with overall risk of infection.

Figure 1. Age-Adjusted Risk of Infectious Hospitalization by eGFR-cysC




The strength of association, however, differed by the type of infection: in multivariable models, each 10 ml/min/1.73 m² lower level of eGFR-cysC was associated with a 9% (95% CI: 6-12%) increased risk of hospitalization for all-cause infection, 9% (4–14%) increased risk of pulmonary infection and 16% (6-27%) increased risk of genitourinary infection.

**Conclusion**: Reduced kidney function is independently associated with risk of infectious hospitalization.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2422**

**Proteinuria Is Superior to eGFR as a Predictor of Cardiovascular Death in Patients with CKD Attending a Hypertension Clinic** Shona Methven,[1] Bruce Mackinnon,[2] Billy Sloan,[3] Lilian Murray,[1] Adel A. Alharf,[1] Matthew Walters,[1] Gordon T. Mcinnes,[1] Alan G. Jardine.[1] *[1]Dept of Medicine, Univeristy of Glasgow, United Kingdom; [2]Renal Unit, Glasgow Royal Infirmary, United Kingdom; [3]Dept of Public Health, University of Glasgow, United Kingdom.*

Proteinuria and reduced eGFR independently predict the development of end stage kidney disease. It is unclear which is the superior predictor of death in patients with hypertension and chronic kidney disease (CKD).

We studied 9981 attendees at a hospital hypertension clinic with a baseline measurement of kidney function. Those <18 years (n=39), eGFR <15ml/min/1.73m² (n=45) and not screened for proteinuria (n=1420) were excluded. A Cox Proportional Hazards model was constructed. Outcomes were cardiovascular (CV) and all cause mortality. Model covariates were eGFR (4 variable MDRD formula), dipstick measure of proteinuria, age, sex, blood pressure, vascular disease, smoking and diabetes.

Of 8477 patients, 23% had an eGFR of <60ml/min/1.73m² and 22% had proteinuria. Mean age was 50(±13) years, blood pressure 169/100(±29/15) mmHg, 52% male and 7% diabetic. During follow-up, 49% of those with eGFR<60ml/min/1.73m² died, 76% of whom died of CV disease.

Adjusted hazard ratios (aHR) for CV and all-cause mortality, divided according to CKD stage

| | CKD 1/2 | CKD 3A | | CKD 3B | | CKD 4 | |
|---|---|---|---|---|---|---|---|
| | p+ | p- | p+ | p- | p+ | p- | p+ |
| All-cause mortality | 1.19 (1.05-1.35) | 1.10 (0.99-1.22) | 1.40 (1.15-1.71) | 1.54 (1.31-1.82) | 1.80 (1.45-2.23) | 2.45 (1.69-3.54) | 4.65 (3.49-6.20) |
| CV Mortality | 1.22 (1.05-1.41) | 1.13 (1.00-1.28) | 1.52 (1.22-1.90) | 1.67 (1.39-2.02) | 1.95 (1.53-2.49) | 2.51 (1.64-3.84) | 4.89 (3.55-6.75) |

p+; proteinuria, p-; none, ref group; eGFR>60ml/min/1.73m2, p-

Proteinuria and eGFR entered as dichotomous variables (co-variates as before); proteinuria is a stronger predictor of both CV and all-cause mortality, aHR 1.38(1.24-1.54) vs 1.27(1.16-1.40) and 1.36(1.23-1.49) vs 1.22(1.13-1.33) respectively (though confidence intervals overlap).

In conclusion, proteinuria is a powerful predictor of CV and all cause mortality in patients with hypertension, and is essential in addition to eGFR for risk stratification in this population.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2423**

**Incidence of Symptomatic Stroke and Cancer in Chronic Kidney Disease Patients Treated with Epoetins** Enyu Imai,[1] Ryohei Yamamoto,[2] Hiromichi Suzuki,[3] Tsuyoshi Watanabe.[4] *[1]Nephrology, Nagoya University, Nagoya, Japan; [2]Osaka University; [3]Saitama Medical University; [4]Fukushima Medical University.*

Use of erythropoiesis-stimulating agents (ESA) was reported to increase cardiovascular diseases. TREAT study showed increasing stroke and cancer by use of darbepoetin in diabetic patients. Surveillance of epoetin adverse events of stroke and cancer was urgently conducted by the Japanese Society of Nephrology. The patients aged at least 18 years with CKD stage 4 and 5, eGFR < 30 mL/min/1.73m², who visited the outpatient department of the participating facilities between December 2009 and January 2010 with at least six month of prior medical treatment in the participating facilities, were eligible. Among 7415 patients with CKD stage 4 and 5, 3653 (49.3%), 879 (11.9%), and 2883 (38.9%) patients received no epoetin, Epoetin for less than 6 months, and epoetin for at least 6 months, respectively. In patients with no use of epoetin, use of epoetin for less than 6 months, and use of epoetin for at least 6 months, the numbers of patients with stroke were 38 (1.0%), 8 (0.9%), and 27 (0.9%), respectively, and those with newly diagnosed or exacerbation of malignancy were 88 (2.4%), 30 (3.4%), and 71 (2.5%), respectively, demonstrating insignificant associations between outcomes and duration of treatment with epoetin (P for trend = 0.666 in stroke and 0.836 in malignancy). Regarding stroke, age (per 10 years, odds ratio 1.40 [1.15 - 1.71], P = 0.001), male gender (2.17 [1.26 - 3.72], P = 0.005), and diabetes (2.02 [1.26 - 3.25], P = 0.004), not use of epoetin (0.89 [0.55 - 1.43], P = 0.621), were associated with stroke after adjusting for random effect of the facility. Their associations remained significant even after adjustment for the multiple covariates (age per 10 years, 1.43 [1.15 - 1.77], P = 0.001; male gender 2.00 [1.15 - 3.47], P = 0.014; diabetes 1.85 [1.14 - 3.00], P = 0.013), whereas not use of epoetin (0.74 [0.42 - 1.31], P = 0.301) . In conclusion, no significant increase in risk of symptomatic stroke and cancer development was observed by use of epoetin in a present clinical practice in Japan.

Disclosure of Financial Relationships: Consultancy: Daiichi-Sankyo, Kyowa-Hakkou-Kirin, KakenResearch Funding: Daiichi-Sankyo, Kyowa-Hakko-Kirin, Chugai, Banyu; Honoraria: Daiichi-Sankyo, Kyowa-Hakko-Kirin, Chugai, Takeda, Banyu, Novartis,; Scientific Advisor: Japanese Society of Nephrology, KDIGO, JASN, KI, NDT, Clincal Nephrology, Nature Review Nephrol,.

**SA-PO2424**

**Serum Phosphorus, Parathyroid Hormone and Calcium Levels and Risks of Death and Cardiovascular Disease in Chronic Kidney Disease: Systematic Review** Suetonia Palmer,[1] Andrew Hayen,[2] Petra Macaskill,[2] Fabio Pellegrini,[3] Grahame J. Elder,[4] Jonathan C. Craig,[2] Giovanni F. M. Strippoli.[2,3,5] *[1]University of Otago Christchurch; [2]University of Sydney; [3]Consorzio Mario Negri Sud; [4]Garvan Institute of Medical Research, Sydney; [5]Diaverum Medical Scientific Office.*

Clinical practice guidelines for care of individuals with chronic kidney disease recommend low targets for serum phosphorus, parathyroid hormone (PTH), and calcium.

We evaluated the association between serum phosphorus, PTH, and calcium levels and risks of death and cardiovascular disease in cohort studies of people with chronic kidney disease. 23 studies (218791 patients) contributed to meta-analyses. 19 (213238 patients) were conducted in dialysis, 3 (4845 patients) in chronic kidney disease not requiring dialysis, and one (708 patients) in transplant. 3 (3643 patients) evaluated incident patients commencing dialysis and 8 (204655 patients) were from registry data.

Six adequately adjusted studies reported risks of mortality by variable levels of serum phosphorus. We could not fit a summary model to describe the relationship between serum phosphorus and mortality as there was strong non-linearity in a single study. Visual inspection of studies with adequate adjustment for confounding suggested a J-shaped association between serum phosphorus and mortality. The risk of cardiovascular death associated with serum phosphorus (risk per 1 mg/dl increase, relative to 3.5 mg/dl, 1.13; 95% CI, 1.12-1.14) relied on a single adequately adjusted study. No relationships between all-cause or cardiovascular mortality and serum PTH or all-cause mortality and calcium were evident. The risk between serum calcium and cardiovascular mortality was reported in one adequately adjusted study (risk per 1 mg/dl increase, relative to 5 mg/dl, 1.02; 95% CI, 1.01-1.03).

Serum PTH levels are not associated with increased risks of cardiovascular and all-cause mortality in chronic kidney disease in adequately adjusted cohort studies. Associations between higher serum phosphorus and risks of all-cause mortality and both serum phosphorus and calcium with cardiovascular mortality require evaluation in randomized trials.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2425**

**Proteinuria and Kidney Function Independently Predict Cardiovascular Outcomes: Pooled Analysis of 7 Cohort Studies (EPOCH-JAPAN)** Masaharu Nagata,[1] Toshiharu Ninomiya,[1] Yutaka Kiyohara,[1] Yoshitaka Murakami,[2] Fujiko Irie,[3] Toshimi Sairenchi,[4] Tomonori Okamura,[5] Katsuyuki Miura,[6] Hirotsugu Ueshima,[6] EPOCH-JAPAN Group. *[1]Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Medical Statistics, Shiga University of Medical Science, Otsu, Japan; [3]Department of Health and Welfare, Ibaraki Prefectural Office, Mito, Japan; [4]Department of Public Health, Dokkyo Medical University School of Medicine, Mibu, Japan; [5]Department of Preventive Cardiology, National Cardiovascular Center, Suita, Japan; [6]Department of Health Science, Shiga University of Medical Science, Otsu, Japan.*

**Background:** Proteinuria and reduced kidney function are risk factors for cardiovascular (CV) disease. It is unclear whether these risk factors are mutually independent or synergistic.

**Methods:** Using individual pooled data of 7 prospective cohort studies in Japan, we assessed the effect of proteinuria (defined as dipstick 1+ or over) and estimated glomerular filtration rate (eGFR) on the risk of CV death in 39,406 participants aged 40 to 89 years without kidney failure at baseline. The hazard ratios were estimated by Cox proportional hazards model.

**Results:** During an average of 10-year follow-up, 1,928 subjects died from CV disease. Participants with proteinuria had a significantly higher age- and sex-adjusted CV mortality rate than those without it (10.8 vs. 4.4 per 1000 person-years). The age- and sex-adjusted CV mortality rate increased linearly for eGFR of ≥90, 60-89, 45-59 and <45 mL/min/1.73m$^2$, being 2.4, 5.4, 7.9 and 14.7 per 1000 person-years, respectively. After adjusting for the potential confounding factors, proteinuria and eGFR<45 mL/min/1.73 m$^2$ were associated with significant 1.7 and 2.3-fold higher risk of CV death, respectively, and the combination with a 4.0-fold higher risk of CV death, as compared to participants without these factors. There was no evidence of interaction between proteinuria and reduced eGFR (p for interaction=0.40).

**Conclusions:** Proteinuria and reduced eGFR are significant and independent risk factors for CV death in the Japanese population.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2426**

**Erythropoietin Resistance Predicts Mortality in Hemodialysis Patients from the Japan Dialysis Outcomes and Practice Patterns Study** Tetsuya Fujikawa,[1] Yumiko Ikeda,[2] Shunichi Fukuhara,[3] Takashi Akiba,[4] Tadao Akizawa,[5] Akira Saito.[6] *[1]Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Kanagawa, Japan; [2]Yokohama Minami Kyousai Hospital, Yokohama, Kanagawa, Japan; [3]Department of Epidemiology and Healthcare Research, Kyoto University Graduate School of Medicine, Kyoto, Japan; [4]Department of Blood Purification and Internal Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan; [5]Division of Nephrology, Showa University School of Medicine, Tokyo, Japan; [6]Department of Internal Medicine and Institute of Medical Science, Tokai University School of Medicine, Kanagawa, Japan.*

Our aim in this study is to investigate the availability of EPO resistance as an independent lethal predictor in hemodialysis patients. This study investigated 2105 Japanese hemodialysis patients participating in the Dialysis Outcomes and Practice Patterns Study III . Erythropoietin resistance index (ERI) was defined as the weekly weight-adjusted dose of EPO divided by the hemoglobin concentration. ERI in females was significantly higher than in males (9.73 ± 5.89 vs. 6.98 ± 5.31 IU/week/g per 100 ml, p<0.001). Of all patients, the number of all lethal events were 227 (10.8%), which included 113 events (5.4%) of cardiovascular disease (CVD), 48 (2.3%) of infection, 23 (1.1%) of cancer, and 5 (0.2%) of gastrointestinal bleeding. In multivariate analysis, ERI was independently significantly related to the all-cause mortality after adjusting the related factors (HR 1.04, 95% CI(1.02-1.07), p<0.001). In addition to ERI, this analysis identified age, sex, cause of chronic kidney disease, serum albumin, and congestive heart failure as independent significant factors related to all-cause mortality. In analysis for CVD mortality, ERI was an independent factor related to CVD related mortality (HR 1.05, 95% CI (1.02-1.08), p=0.002). This investigation concluded that ERI is an independent risk factor for all cause and CVD mortality in hemodialysis patients. Further study is needed to investigate the underlying mechanism of the relation between ERI and mortality.

Disclosure of Financial Relationships: nothing to disclose

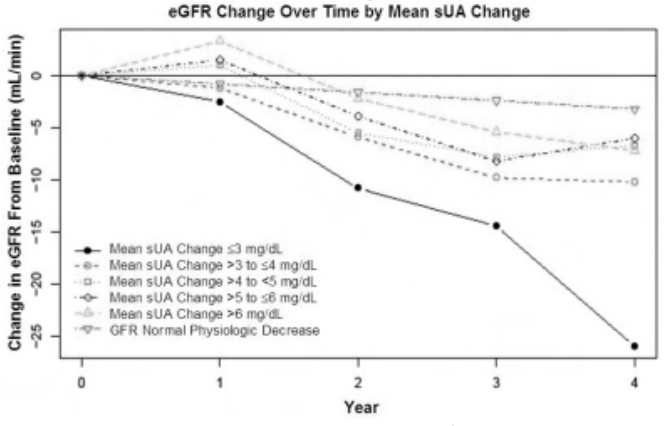
**SA-PO2427**

**Long-Term Impact of Urate-Lowering Therapy on Renal Function in Hyperuricemic Gout Subjects** Andrew Whelton,[1] Patricia MacDonald,[2] Lhanoo Gunawardhana,[2] Barbara J. Hunt.[2] *[1]UCRC, Inc., and The Johns Hopkins University School of Medicine, Baltimore, MD; [2]Takeda Global Research & Development Center, Inc., Deerfield, IL.*

**Objective:** To evaluate the effect of urate-lowering therapy (ULT) on renal function (estimated glomerular filtration rate [eGFR]) in hyperuricemic gout subjects treated daily with febuxostat (80 or 120 mg) or allopurinol (300 mg).

**Methods:** Subjects (1086) completing 2 phase 3 trials (FACT and APEX) were enrolled in the long-term EXCEL study and received ULT for ≤4 years. eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) equation. A repeated-measures linear model was used to assess the relationship between eGFR change and sUA change, with factors for year of treatment and baseline (BL) renal function (eGFR <60 vs ≥60 mL/min).

**Results:** Most subjects were male (96%) and Caucasian (79%); BMI was ≥30 kg/m$^2$ (63%) and mean age 51 yrs; 7% had BL eGFR <60. Greater sustained sUA decreases were associated with less renal function deterioration (p<0.001; Figure).




The model projects that every 1 mg/dL sUA reduction from BL will yield 1.25 mL/min less of a decrease in eGFR compared with no sUA reduction. Based on available data, the model is relevant for treatment duration up to 4 years and sUA change up to 10 mg/dL. BL renal impairment was also significant (p<0.05): given the same treatment duration and sUA change, subjects with renal impairment (BL eGFR <60 mL/min) had 4.7 mL/min less of an eGFR decrease compared to those with BL eGFR >60 mL/min.

**Conclusion:** In treated gout subjects, the magnitude of persistent sUA decrease significantly influences, in an inverse fashion, progression of renal impairment. These findings are consistent with our previously reported results from a 5-year (n=116) trial wherein greater sustained sUA decreases from BL were associated with less renal function deterioration.

Disclosure of Financial Relationships: Employer: Self; Consultancy: Speakers Bureau of Takeda and Pfizer

Consultant for Takeda, Lux and Canyon Pharmaceuticals; Honoraria: Lux, Takeda, Pfizer, NiCox, Canyon Pharmaceuticals; Scientific Advisor: Takeda Pharmaceuticals.

**SA-PO2428**

**Ischemia Reperfusion Injury as a Risk Factor for Native Kidney Dysfunction after Liver Transplantation** Bernd Krüger,[1,2] Anja Richter,[1] Vinay Nair,[3] Sridhar Reddy Allam,[1] Bernhard K. Krämer,[2] Barbara T. Murphy,[1,3] Bernd Schroppel.[1,3] *[1]Mount Sinai School of Medicine, Division of Nephrology, New York; [2]V. Medizinische Klinik, Universitätsklinikum Mannheim, Mannheim, Germany; [3]Mount Sinai School of Medicine, The Transplantation Institute, New York.*

Kidney dysfunction after liver transplantation (LT) is common and has detrimental impact on mortality and morbidity. The pathophysiology of acute and chronic kidney injury associated with LT is poorly understood. Here, we tested the hypothesis that recipients or livers with severe IR injury are at higher risk to develop long-term kidney dysfunction.

577 adult patients underwent LT between January 2000 and December 2004. After exclusion of retransplants and living donor transplants we included 316 decreased donor LT with available kidney function data. We established 3 groups of IR injury based on liver enzymes ((AST+ALT)/2) on day 2: immediate LT function: <25th percentile (<285 U/l), average LT function: 25th-75th percentile (285-986 U/l), and delayed LT function: >75th percentile (>986 U/l). Immunosuppressive maintenance therapy consisted of tacrolimus and/or mycophenolate.

At baseline chronic renal failure, i.e. eGFR ≤30 ml/min as suggested by Ojo et al., was not different between the 3 groups (p=0.06). Compared to pre-transplant kidney function, Δ eGFR post-transplant was gradually increased post LT in groups 1 to 3. This effect was maintained throughout 12 months follow-up, and remained significant after adjusting for HCV-status, donor and recipient age and race (p=0.01).

| | Δ eGFR from pretransplant [ml/min] | | | |
|---|---|---|---|---|
| | immediate LT function | average LT function | delayed LT function | p-value |
| 1 month | 3.6 ± 43.1 | -13.8 ± 39.8 | -20.7 ± 39.9 | 0.001 |
| 3 months | -8.7 ± 38.9 | -17.0 ± 37.0 | -26.8 ± 37.7 | 0.013 |
| 6 months | -12.2 ±35.8 | -18.8 ± 38.0 | -29.4 ± 38.8 | 0.023 |
| 12 months | -12.9 ± 38.3 | -18.4 ± 35.7 | -29.9 ± 36.9 | 0.026 |

Our results show that recipients of liver transplants that are subjected to severe IR injury develop kidney dysfunction post LT. Strategies to prevent acute liver injury at the time of transplantation can preserve native kidney function after LT.

Disclosure of Financial Relationships: nothing to disclose

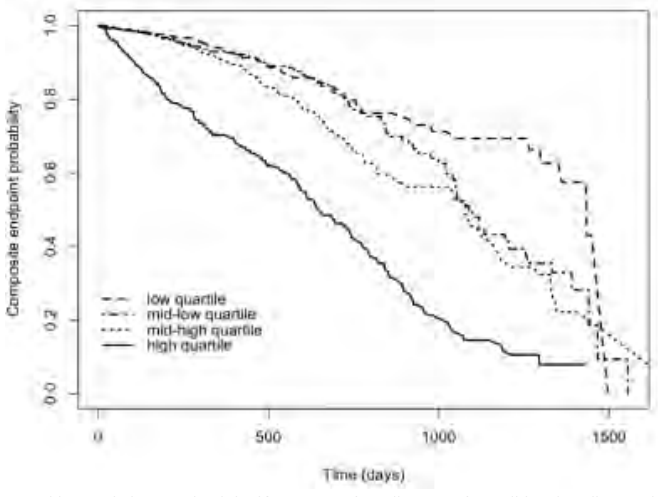
**SA-PO2429**

**Chronic Kidney Disease Progression and Mortality in Mild to Moderate Kidney Dysfunction According to Serum Phosphorous Levels** Antonio Bellasi, Marcora Mandreoli, Antonio Santoro. *On Behalf of the PIRP Study Group, Nephrology Unit-Policlinico S.Orsola-Malpighi, Bologna, Italy.*

The impact of serum phosphate abnormalities on Chronic Kidney Disease (CKD) progression and mortality among patients with mild to moderate kidney failure is still far from being established.

We determined the association of baseline phosphatemia with the composite endpoint of dialysis inception or all-cause mortality. We utilized the patient's records from the "Prevenzione Insufficienza Renale Progressiva" (PIRP) database, a large project sponsored by the Emilia-Romagna Institute of Health aimed at optimizing CKD patients care.

We identified all patients who underwent a glomerular filtration rate (GFR) and serum phosphorous assessment between 2004 and 2007. The patients were followed up to 4 years. Survival analyses estimated the relationship between serum phosphorous at baseline and outcomes. A total of 1716 female and male subjects with CKD stage 3-5 were identified. Elevated serum phosphorous was significantly associated with diabetes mellitus, but inversely associated with GFR, age and male sex (all p-trend<0.001). A graded increase in the risk of starting dialysis or dying was noted across quartiles of serum phosphorous (log-rank test p<0.001).




This association remained significant even after adjustment for traditional cardiovascular risk factors and CKD stage.Indeed, patients in the highest serum phosphorous quartile (>4.3 mg/dl) experienced a 117% increase in the risk of the occurrence of the composite endpoint when compared to patients with serum levels of phosphorous of 3.3-3.8 mg/dl (HR 2.17; 95%; CI 1.62-2.93; p<0.001).

These analyses lend support to the hypothesis that serum phosphorous levels at baseline might accelerate residual renal function deterioration and increase the risk of death in patients with mild to moderate renal function impairment.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2430**

**CanPREDDICT: Canadian Study for Predicting Dialysis, Death and Cardiovascular (CVD) Risk in CKD** Adeera Levin,[1] Francois Madore,[2] Claudio Rigatto,[3] Brendan J. Barrett,[4] Norman Muirhead,[5] Daniel T. Holmes,[1] Ognjenka Djurdjev.[1] *[1]UBC; [2]CHUM; [3]UM, Canada; [4]MU; [5]LHSC.*

CanPREDDICT is a prospective observational cohort study of 2500 pts known to nephrologists at 25 Canadian centers. Primary objectives are to describe the levels and temporal evolution of 5 known biomarkers (BM) for inflammation (CRP, IL-6), cardiac injury (Troponin I), heart failure ( ProBNP), and vascular health (ADMA); and establish whether these BM , can accurately discriminate pts at high or low risk of renal or CVD outcomes.

2542 CKD pts ( eGFR 15-45 ml/min) were enrolled: mean age is 68yrs, median eGFR is 28ml/min, 62% are male, 90% are Caucasian; 48% have DM; 34% have IHD and 27% have CHF. Table 1 presents BM associated with comorbidities at baseline:

Biomarkers associated with comorbidities at baseline

| Variables | Diabetes OR (95% C.I.) | Ischemic HD OR (95% C.I.) | Congestive HF OR (95% C.I.) |
|---|---|---|---|
| Age (5 yrs) | 1.02 (0.97-1.06) | 1.22 (1.17-1.29)* | 1.19 (1.11-1.28)* |
| Sex (Male vs. Female) | 1.25 (1.01-1.57)* | 1.69 (1.36-2.09)* | 0.86 (0.63-1.18) |
| Race (Other vs. Caucasian) | 1.26 (0.90-1.76) | 0.90 (0.63-1.27) | 0.71 (0.44-1.14) |
| DM | n/a | 1.94 (1.58-2.38)* | 1.18 (0.88-1.60) |
| eGFR (5mL/min) | 1.10 (1.01-1.17)* | 1.04 (0.98-1.11) | 1.17 (1.07-1.28)* |
| Po4 (0.1 mmol/L) | 1.11 (1.06-1.16)* | ns | ns |
| iPTH (log pmol/L) | ns | ns | 1.22 (1.01-1.47)* |
| Alb (g/L) | 0.96 (0.94-0.99)* | ns | ns |
| Hb (5g/L) | 0.94 (0.90-0.97)* | ns | ns |
| IL6 (>LLD - 6pg/mL vs. <LLD) | 1.56 (1.20-2.03)* | 1.35 (1.04-1.75)* | ns |
| IL6 (>6.0 pg/mL vs. <LLD) | 2.17 (1.66-2.83)* | 1.42 (1.09-1.84)* | ns |
| Troponin (>LLD vs. <LLD) | 1.61 (1.28-2.02)* | 1.79 (1.44-2.23)* | 2.36 (1.70-3.28)* |
| ProBNP (1000 pg/mL) | ns | 1.08 (1.04-1.12)* | 1.18 (1.10-1.26)* |

The addition of BM to multivariate models with traditional variables increased the AUC for DM (63% to 67%), IHD (69% to 73%) and CHF (67% to 75%) models.

The ability to identify pts who will progress wrt CVD or CKD is important in planning interventional trials. Use of BM to discriminate and identify high risk pts appears to be possible.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2431**

**Coronary Artery Calcification and Kidney Function: *Association or Causation*** Sejoong Kim, Jae Hyun Chang, Jiyoon Sung, Sun Young Na, Ji Yong Jung, Hyun Hee Lee, Wookyung Chung. *Internal Medicine, Gachon University of Medicine and Science.*

*Introduction:* Cardiovascular disease (CVD) is the most common cause of morbidity and mortality in patients with chronic kidney disease (CKD). Coronary artery calcification (CAC) is a phenomenon described in individuals with CKD, and its presence is associated with an increased risk of cardiovascular death. The aims of this study were to evaluate the association of renal function with CAC and whether CAC may predict the rate of change in renal function over time.

*Methods:* We retrospectively enrolled 918 Korean outpatients who had undergone multislice computed tomographic coronary angiography in Gachon University Gil Hospital from January 2008 to December 2009. Of these, 237 patients underwent followe-up more than 3 months. Decrease of renal function was defined as > 8% decrease per year in estimated glomerular filtration rate (eGFR)

*Results:* The mean age was 57±12 years, and the mean eGFR 82.5 ± 15.4 mL/min/1.73 $m^2$. Compared with CAC score (CACS) >100 group (n=136), CACS <100 group (n=782) were more likely to have younger age, and higher levels of eGFR, albumin, hemoglobin, and HDL-cholesterol. Hypertension and DM were less frequent among CACS<100 group compared with CACS >100 group. After the adjustment for age, gender, smoking, hypertension, diabetes, albumin, hemoglobin, calcium-phosphorus product, total cholesterol, and body mass index, eGFR was negatively associated with CACS >100 (odds ratio[OR], 0.983; 95% confidence interval[CI], 0.968-0.999; *P*=0.031). The mean follow-up duration of 237 followed-up patients was 9.6±4.9 months, and 62 (26.2%) patients had decrease of renal function. After the adjustment for the same confounding variables, CACS >100 was independently associated with decrease of renal function (OR, 2.351; 95% CI, 1.133-4.88; *P*=0.022).

*Conclusion:* This study suggests that CACS may be inversely related to baseline renal function and can also predict the aggravation of renal function.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2432**

**Physical Functioning in Chronic Kidney Disease: Contribution of Uremia** Eva Segura-Ortí,[2] Patricia L. Gordon,[1] Julie W. Doyle,[1] Kirsten L. Johansen.[1] *[1]UCSF; [2]Universidad CEU Cardenal Herrera, Valencia, Spain.*

The aim of the study was to determine the extent to which poor physical functioning and muscle atrophy among patients on dialysis is evident in the pre-dialysis phase of chronic kidney disease (CKD) and to explore the contributions of uremia to functioning in this cohort.

57 subjects completed the study (19 control, 53.2 ± 8.0 years; 22 CKD, 62.5 ± 10.1 years; 16 dialysis patients, 54.7 ± 7.2 years). Measures included: Cross-sectional area (CSA) of the quadriceps muscle contractile tissue by MRI; maximum voluntary contraction (MVC) and isokinetic peak torque during knee extension by dynamometer; physical activity (PA) using three-dimensional accelerometers; 6-min walk test; and SF-36. Characteristics were compared across groups using ANOVA. Univariate and multivariate linear regression was performed.

By ANOVA, muscle area was smaller and MVC was lower in dialysis patients than controls, but CKD patients were not different from controls. On the other hand, daily PA and 6-min walk were significantly lower than controls in both CKD and dialysis subjects. In univariate regression, eGFR was positively correlated with daily PA (r=0.54), muscle CSA (r=0.54), isometric MVC (r=0.47), and 6-min walk distance (r=0.32). Multivariable models showed association of eGFR, PA and subjects' age and BMI with muscle area (r2 = 0.63).

Multivariable correlates of physical function

| Dependent variable | eGFR | Age | BMI | PA |
|---|---|---|---|---|
| Muscle area | 0.26 | -0.34 | 0.46 | 0.38 |
| Isometric MVC | 0.34 | -0.34 | 0.34 | - |
| Isokinetic peak torque, 90° | 0.29 | -0.33 | - | - |
| 6-min walk | 0.37 | - | - | - |
| SF-36 PF | 0.32 | - | - | - |

All coefficients and models statistically significant.

For most variables related to functioning, eGFR was either more strongly associated than PA (e.g., MVC, 6-min walk, SF-36 PF) or independently associated after considering PA (e.g., muscle area). This study demonstrated that CKD patients' were less active than sedentary controls, with PA levels similar to those of dialysis patients. Decreased kidney function was associated with muscular atrophy and functional impairments in CKD patients even after controlling for low PA.

Disclosure of Financial Relationships: nothing to disclose

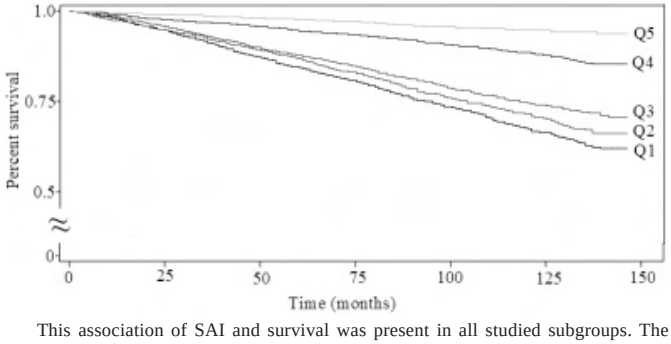
## SA-PO2433

**Social Adaptability Index Predicts Survival of Patients with Chronic Kidney Disease** Alexander S. Goldfarb-Rumyantzev,[1] Gurprataap Singh Sandhu,[1] Hongying Tang,[2] Preeti Rout,[1] Mark E. Williams,[1] Anna Barenbaum.[3] *[1]Division of Nephrology, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA; [2]Transplant Institute, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA; [3]Tel Aviv University, Tel Aviv, Israel.*

Background. While individual socioeconomic factors have been associated with clinical outcome, a composite index has not been developed. In this project we tested the hypothesis that Social Adaptability Index (SAI) based on employment, education, income, marital status and substance abuse is associated with survival in chronic kidney disease (CKD) patients.

Methods. This is a retrospective cohort study of patients (18 years or older) from NHANES -3 with CKD stage 2 or greater. Our primary variable of interest is SAI. Each component of SAI (employment status, education, marital status, income, and substance abuse) has been graded on the scale of 0 to 3. Age, sex, race, diabetes, co morbidity index, BMI, geographic location, hemoglobin, serum creatinine, serum albumin, serum cholesterol, and Hba1c were used as covariates. The outcome of the study is patient's mortality (time between the first interview by NHANES and death).

Results. We analyzed 13,400 subjects with mean age of 50.6 ± 20; 53.6% males; 44.4 % white; 29.7 % African American. Lower SAI is associated with greater stage of CKD. Higher SAI was associated with decreased mortality (HR 0.88, p <0.001, 95% CI 0.86-0.89). There was "dose dependent" association between SAI quintiles and survival.




This association of SAI and survival was present in all studied subgroups. The limitations of the study include retrospective design, potential misreporting and misclassification, reverse causality.

Conclusions. We demonstrated that Social Adaptability Index has a strong and clinically significant association with mortality in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2434

**Family Socioeconomic Status (SES) and Disease Severity in Children with CKD: Results from the Chronic Kidney Disease in Children (CKiD) Cohort Study** Guillermo Hidalgo,[1] Susan L. Furth,[2] Derek Ng,[3] Colin T. White.[4] *[1]Pediatrics, University of Illinois, Chicago, IL; [2]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA; [3]Bloomberg School of Public Health, John Hopkins, Baltimore, MD; [4]Pediatrics, BC Children's Hospital, Vancouver, BC, Canada.*

**Introduction:** The role of SES on disease severity in children with CKD is not well established. To examine this relationship, we described and compared subjects by SES strata at time of enrollment in the CKiD cohort using co-morbid conditions as a proxy for disease severity. Our hypothesis was that household (HH) income and level of maternal education are associated with uncontrolled hypertension, hyperphosphatemia, elevated Ca X P, increased iPTH and growth failure.**Methods:** Cross-sectional analysis used descriptive statistics to examine trends and Poisson regression to calculate prevalence ratios (PrR) for univariate and adjusted analyses. Adjusted models included age, race, type of CKD and GFR.

**Results:** There were 586 subjects at enrollment of which 572 had complete data for HH income and maternal education. The cohort consisted of 62% males and 23% AA. Their mean age was 11 y. ± 5 SD and mean iohexol GFR of 47.1 ± 19.0 mL/min/1.73m². Glomerular CKD diagnosis was found in 22% of subjects. In univariate analyses, higher income groups had a lower prevalence of all specified co-morbidities, when compared to lower income groups.

Table 1

| Co-morbid Condition | Income < $ 30K n=240 (42%) | Income $30 to $75K n=174 (30%) | Income >$75K n=158 (28%) | Test for trend p-value |
|---|---|---|---|---|
| SBP or DBP> 95th %tile | 24% | 23% | 17% | 0.43 |
| Hyperphosphatemia | 17% | 18% | 11% | 0.21 |
| Elevated Ca X P | 12% | 6% | 7% | 0.01 |
| Growth failure | 18% | 13% | 12% | 0.04 |
| > 3 co-morbidities | 11% | 6% | 5% | 0.01 |

These trends persisted in the adjusted analysis. Similar results were observed among strata of maternal education (<HS, HS to college, >college). **Conclusion:** The results show a relationship between lower SES and higher disease severity, as measured by prevalent comorbidities among the CKiD population. This high risk group warrants clinical attention when treating pediatric CKD.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2435

**Effect of Zinc Supplementation and Dietary Counseling on Nutritional and Antioxidant Status as Well as Quality of Life of the Patients with CKD** Nancy Sahni,[1] Rajindra Prasad,[2] Satya V. Rana,[3] A. K. Bhalla,[4] Vinay Sakhuja,[5] Krishan L. Gupta.[5] *[1]Deptts of Dietetics,; [2]Biochemistry; [3]Gastro-enterology; [4]Pediactrics; [5]Nephrology, PGIMER, Chandigarh, India.*

This study **aimed** at assessing the influence of Zinc (Z) supplementation/dietary counseling on the antioxidant and nutritional status and quality of life of patients with Chronic kidney disease (CKD). **Methods:** A total of 95 patients with CKD (mean age = 42.88±11.48yrs) and 40 apparently healthy adults as controls (mean age=42.42±2.23 yrs) were included. The patients were divided into moderate (s creat<5 mg/dl) and severe (s creat>5 mg/dl) CRF groups. In group I (n=45) dietary counseling alone was given to 25 moderate and 20 severe CRF patients whereas in group II (n=50) dietary counseling along with zinc supplementation was given to 30 moderate and 20 severe CRF patients. Statistical significance was calculated using student's t-test and paired t-test between two groups as well as before and after interventions respectively. All the patients were followed weekly for compliance for a period of one month. **Results:** All CKD patients showed ↓mean nutritional intake($p \leq 0.001$) [in terms of macronutrients such as energy, carbohydrates, fats and proteins as well as micronutrients such as vitamin A , vitamin C & zinc and essential amino acids such as cystiene and methionine], as compared to the control group. After one month of individualized diet counseling, both group-1 and group-2 patients showed significant ↑ in nutritional status evaluated anthropometrically (weight and BMI). Antioxidant enzymes viz. SOD and catalase as well as Serum GSH were significantly higher in group-2 patients ($p \leq 0.001$) as compared to group-1 patients ($p \leq 0.05$). Serum zinc levels did not change significantly after diet counseling alone (group-1), while there was a significant ↑ ($p \leq 0.001$) in group-2 patients on Zn supplementation. Quality of life of all CRF patients showed significant improvement. **Conclusion:** The role of diet counseling in removing the myths, taboos and food misconceptions along with zinc supplementation resulted in improving the nutritional and antioxidant status and quality of life of CRF patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2436

**Clinical Features and Prognosis of Extrapulmonary Tuberculosis in Patients with Chronic Kidney Disease** Jung Sub Kim, Ihm Soo Kwak, Soo Bong Lee, Sang Heon Song, Eun Young Seong, Jungmin Son, Il Young Kim, Harin Rhee. *Department of Internal Medicine, Pusan National University School of Medicine, Busan, Korea.*

There is an increased risk of tuberculosis (TB) owing to impaired cellular immunity in patients with chronic kidney disease. Extrapulmonary TB is more common in patients with chronic kidney disease with percentages varying from 38% up to more than 80%. We explored the clinical features of the extrapulmonary TB according to the renal function.

This study was performed retrospectively through the review of medical records of the patients at Pusan national university hospital. From January 2003 to December 2007, 342 patients aged ≥18 years were diagnosed with extrapulmonary TB. We classifed patients into two groups according to estimated glomerular filtration rate (eGFR) by MDRD formula as follows; eGFR< 60 ml/min/1.73m² (group I) vs.eGFR ≥ 60 ml/min/1.73m² (group II). Their clinical features, treatment outcomes, and mortality related to extrapulmonary TB about them were evaluated.

eGFR of Group I (N=30) and Group II (N=312) was 34±19ml/min/1.73m² and 102±26ml/min/1.73m². The pleura was the most frequent site in both groups (Group I, 30.0% vs. Group II, 28.2%, p=0.379). There was no treatment failure and recurrence in both groups. The mortality in Group I is higher than that of Group II (22.2% vs. 2.8%, p<0.01). In multivariate analysis, the eGFR<60 ml/min/1.73m² was an independent risk factor for mortality (HR=11.51 CI 2.512-52.741, p=0.002).

The mortality related with extrapulmonary TB is higher in patients with impaired renal function and the kidney function is an independent predictor. However, there is no difference in treatment failure and recurrence according to renal function. Therefore, if early diagnosis and prompt initiation of treatment is done, the mortality rate related to tuberculosis will decrease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2437**

**Predictors of Outcome in the Elderly Patients with Chronic Kidney Disease (CKD)** Maria Marin,[1] Rafi T. Ishaq,[3] Anacleto Baizas Diaz,[2] Joel Topf.[1] *[1]Department of Nephrology, St John Hospital and Medical Center, Detroit, MI; [2]Department of Internal Medicine, St John Hospital and Medical Center, Detroit, MI; [3]Department of Internal Medicine, Providence Hospital, Southfield, MI.*

CKD occurs in 49% of the elderly and increases the risk of cardiovascular disease, end-stage renal disease (ESRD) and death. Elderly patients represent the fastest growing group initiating dialysis. The aim of our study is to examine the determinants of outcome in an elderly population followed in a CKD clinic.

We retrospectively examined the charts of patients older than 65, with eGFR 15-45 mL/min at inclusion. CKD was defined as a persistent decrease in eGFR to < 60 mL/min over 3 months.

A total of 198 patients were included: 117 females and 81 males, average age 75.6 (range 65-96). There were 149 Caucasian and 48 African American subjects. The average GFR was 30 mL/min.

At a mean follow-up of 39.9 (± 24) months, 18 patients died and 19 progressed to ESRD. The GFR at baseline was lower in patients who progressed to ESRD (22.1 mL/min) than in those who died (28.2 mL/min) or those without an endpoint (31.2 mL/min) ($p<0.0001$). Men were more likely to die than women (14.8% vs 5.1%, $p=0.05$) and females were more likely to progress to ESRD (11.1% vs 7.4%, $p=0.05$). Patients who progressed to ESRD had a lower calcium and higher phosphorus, PTH and urinary protein levels at baseline. Patients with CKD due to diabetes mellitus had a faster loss of GFR and were more likely to progress to ESRD ($p=0.003$) than patients with other causes of CKD. In multivariable logistic regression, female patients ($p=0.006$) and those treated with erythropoietin ($p=0.01$) were more likely to progress to ESRD and patients with a history of peripheral vascular disease ($p=0.002$) or treated with erythropoietin ($p=0.052$) were more likely to die.

This study shows a higher rate of dialysis than previously reported. We show equal rates of death versus dialysis. Female gender and lower GFR at baseline were predictive of dialysis while male gender and history of peripheral vascular disease were predictors of death. This information will be helpful for practitioners to guide CKD care.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2438**

**Complement Factor H Related 5 (CFHR5) Nephropathy: An Endemic Cause of Renal Disease in Cyprus** Daniel P. Gale,[1] Elena Goicoechea de Jorge,[2] H. Terence Cook,[3] Rubén Martínez-Barricarte,[4] Andreas Hadjisavvas,[5] Adam Mclean,[1] Charles D. Pusey,[1] Alkis Mikis Pierides,[6] Konstantinos Voskarides,[7] Constantinos Deltas,[7] Andrew B. D. Palmer,[1] Santiago Rodriguez de Cordoba,[4] Matthew C. Pickering,[2] Patrick Maxwell.[8] *[1]Imperial College Kidney and Transplant Institute, Imperial College, London, United Kingdom; [2]Rheumatology Section, Imeprial College, London, United Kingdom; [3]Histopathology Department, Imperial College, London, United Kingdom; [4]Centro de Investigaciones Biológicas, Instituto Reina Sofía de Investigaciones Nefrológicas, Madrid, Spain; [5]Department of Electron Microscopy and Pathology, Institute of Neurology and Genetics, Nicosia, Cyprus; [6]Nephrology Department, Hippocrateon Hopsital, Nicosia, Cyprus; [7]Department of Biological Science, University of Cyprus, Nicosia, Cyprus; [8]Division of Medicine, University College, London, United Kingdom.*

We report an autosomal dominant disease in which microscopic and synpharyngitic macroscopic hematuria cosegregates with C3 glomerulonephritis (C3GN: membranoproliferative GN with C3 but not immunoglobulins deposited in the glomerulus).

There was genome-wide linkage in two unrelated families from Cyprus to the Complement Factor H/Complement Factor H Related (CFHR) 1-5 gene cluster, LOD 3.4. A duplication of exons 2 and 3 of *CFHR5* cosegregated with the disease and was present in one out of 1015 Cypriot controls. It was subsequently identified in over 100 Cypriot individuals with familial hematuria, C3GN or renal failure of unknown cause. Haplotype analysis confirmed a common ancestor in all 19 of the families studied, most likely c90 generations (2500 years) ago.

The mutant CFHR5 protein was detectable in the circulation and demonstrated reduced affinity for heparin and complement-lysed erythrocytes. Recombinant mutant CFHR5 exhibited reduced affinity for heparin and glomerular C3 when compared with the recombinant wild type protein.

This disease, designated 'CFHR5 Nephropathy,' accounts for a significant proportion of renal disease in Cyprus and implicates CFHR5 as an important regulator of complement within the kidney, suggesting that it may have therapeutic potential in diseases associated with complement deposition.

Disclosure of Financial Relationships: Honoraria: Alexion.

**SA-PO2439**

**Mutation Analysis of 18 Nephronophthisis-Related Ciliopathy Genes Using a DNA Pooling and Massively Parallel Resequencing Strategy** Gokul Ramaswami,[1] Edgar Otto,[1] Sabine Janssen,[1] Moumita Chaki,[1] Susan J. Allen,[1] Weibin Zhou,[1] Rannar Airik,[1] Toby W. Hurd,[1] Amiya K. Ghosh,[1] Corinne Antignac,[2, 3] Sophie Saunier,[3] Colin A. Johnson,[4] Friedhelm Hildebrandt.[1, 5] *[1]Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; [2]Department of Genetics, Hôpital Necker-Enfants Malades, Assistance Publique–Hopitaux de Paris, Paris, France; [3]INSERM U-983, Hôpital Necker-Enfants Malades, Université Paris Descartes, Paris, France; [4]Division of Molecular & Translational Medicine, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, United Kingdom; [5]Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI.*

Nephronophthisis-related ciliopathies (NPHP-RC) comprise a group of autosomal recessive cystic kidney diseases that includes nephronophthisis (NPHP), Senior-Loken syndrome (SLSN), Joubert syndrome (JBTS), and Meckel-Gruber syndrome (MKS). To date, mutations in 18 different genes have been described to cause NPHP-RC, rendering mutation analysis tedious and expensive. To perform mutation screening in NPHP-RC we devised a strategy of DNA pooling with consecutive massively parallel resequencing (MPR) in order to overcome the genetic locus heterogeneity. Genomic DNA of 120 patients with a severe NPHP-RC phenotype was divided into 5 pools (24 patients each) and used as templates to amplify all 376 exons of 18 NPHP-RC genes by PCR. PCR products were then subjected to MPR on an Illumina Genome Analyzer II platform. Identified mutations were subsequently assigned to their respective mutation carrier via CEL I endonuclease based heteroduplex screening and confirmed by Sanger sequencing. Using this technique led to the molecular diagnosis in 30/120 individuals (25%). A total of 43 different mutations were identified in the genes *NPHP4* (4), *IQCB1* (1), *CEP290* (11), *RPGRIP1L* (2), *TMEM67* (13), *AHI1* (1), *CC2D2A* (6), and *TTC21B* (5), 28 of which were novel findings. The combined approach of DNA pooling followed by MPR strongly facilitates mutation analysis in heterogeneous single-gene disorders. The lack of identified mutations in 75% of patients in our cohort indicates further genetic heterogeneity in NPHP-RC.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2440**

***INF2* Is a Major Gene of Autosomal Dominant Focal and Segmental Glomerulosclerosis** Olivia Boyer,[1] Genevieve Benoit,[1] Olivier Gribouval,[1] M. J. Tete,[1] Jacques Dantal,[2] Brigitte Gilbert-Dussardier,[3] Guy Touchard,[4] Alexandre Karras,[5] Claire Presne,[6] Christophe M. Legendre,[7] Jean-Pierre Grunfeld,[7] Dominique Joly,[7] Nabil Mohsin,[8] Thierry P. Hannedouche,[9] Philippe Rieu,[10] Marie-Claire Gubler,[1] Isabelle Broutin,[11] Geraldine Mollet,[1] Corinne Antignac.[1] *[1]Inserm U983, Hopital Necker, Paris, France; [2]Nephrology Department, CHU Hotel Dieu, Nantes, France; [3]Genetic Department, CHU La Miletrie, Poitiers, France; [4]Nephrology Department, CHU La Miletrie, Poitiers, France; [5]Nephrology Department, HEGP, Paris, France; [6]Nephrology Department, CHU, Amiens, France; [7]Nephrology Department, Necker Hospital, Paris, France; [8]Nephrology Department, Royal Hospital, Muscat, Oman; [9]Nephrology Department, CHU, Starsbourg, France; [10]Nephrology Department, CHU, Klemlin Bicetre, France; [11]Cristallography Lab, CNRS UMR 8015, Paris, France.*

Mutations in the *INF2* gene, encoding a member of the formin family of actin-regulating proteins, have recently been identified in familial focal and segmental glomerulosclerosis (FSGS) cases, thereby emphasizing the importance of an intact actin cytoskeleton in podocyte function. In order to better determine the prevalence of *INF2* mutations in autosomal dominant FSGS, we screened 54 families (78 patients) and detected mutations in 17% of them. All were missense variants localized in the N-terminal diaphanous inhibitory domain (DID) of the protein, a region which interacts with the C-terminal diaphanous autoregulatory domain (DAD), thereby competing for actin monomer binding and inhibiting depolymerization. Interestingly, six out of the seven altered different residues identified were localized in the corresponding INF2 region of a mDia1 DID subdomain reported to co-immunoprecipitate with IQGAP1. Ninety sporadic cases were also evaluated and no mutation was detected. In conclusion, *INF2* is a major gene of autosomal dominant FSGS. As IQGAP1 is known to interact with crucial podocyte proteins (nephrin, PLCε1), the identification of mutations which may alter the putative INF2-IQGAP1 interaction provides additional insights into the pathophysiological mechanisms linking formin proteins to podocyte dysfunction and FSGS.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2441**

**Total Exome Capture and NextGen Sequencing Reveal Mutation of *Cubilin* as a Single-Gene Cause of Proteinuria** Virginia Vega-Warner,[1] Bugsu Ovunc,[1] Edgar Otto,[1] Pawaree Saisawat,[1] Shazia Ashraf,[1] Hanan Fathy,[3] Gil Chernin,[1] Friedhelm Hildebrandt.[1,2] *[1]Department of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; [2]Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI; [3]Pediatric Nephrology Unit, El Shatty Children's Hospital, Alexandria, Egypt.*

Childhood nephrotic syndrome (NS) is a genetically heterogeneous disorder with prominent protenuria.

**Hypothesis.** The likely existence of dozens of rare recessive NS genes necessitates the ability to identify new NS-causing genes in single families.

**Methods.** We therefore performed homozygosity mapping (Affymetrix™ SNP 6.0 Array) with consecutive total human exome capture (THEC) (NimbleGen™ Sequence Capture Human Exome 2.1M Array) and NextGen sequencing (Illumina™) in two siblings (A2410) with intermittent nephrotic-range proteinuria.

**Results.** Total genome homozygosity mapping yielded 7 segments of homozygosity by descent that covered a cumulative physical distance of 130 Mb. THEC revealed 309 homozygous non-synonymous exonic (or splice) variants from normal reference sequence throughout the entire genome. 21 of the exonic variants occurred within the mapped homozygous candidate regions. One of those variants was a homozygous truncating mutation in exon 53 of the *CUBN* gene (c.8355delA; p.S2785fsX19), which was present in both affected siblings and absent from 180 normal control individuals (1000 Genome project). We thereby identified mutation of *CUBN* as the cause of proteinuria in this sibship. Recessive *CUBN* mutations are known to cause Imerslund-Gräsbeck syndrome, a hereditary from of megaloblastic anemia secondary to vitamin $B_{12}$ deficiency. Proteinuria occurs in 50% of cases in addition to anemia due to the dual role of cubulin as coreceptor for both, i) the intestinal vitamin $B_{12}$-intrinsic factor complex and, ii) the receptor complex that mediates tubular reabsorption of protein from primary urine in the proximal tubule.

**Conclusion.** Homozygosity mapping with consecutive THEC and NextGen sequencing allows identification of very rare single-gene causes of NS. In our case an unexpected tubular rather than glomerular pathogenesis was revealed.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2442**

**Identification of 34 Novel Mutations in 12 Known Bardet-Biedl Syndrome (BBS) Genes by DNA Pooling and Massively Parallel Resequencing in 90 Individuals** Sabine Janssen,[1] Gokul Ramaswami,[1] Jennifer M. Kasanuki,[1] Rannar Airik,[1] Toby W. Hurd,[1] Erica E. Davis,[3] Nicholas Katsanis,[3] Elise Héon,[4] Lauren A. Van der Kraak,[4] Susan J. Allen,[1] Edgar Otto,[1] Friedhelm Hildebrandt.[1] *[1]Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; [2]Molecular Medicine Unit, UCL Institute of Child Health, London, United Kingdom; [3]Center for Human Disease Modeling, Duke University Medical Center, Durham, NC; [4]Department of Genetics and Genome Biology, The Hospital for Sick Children, Toronto, ON, Canada.*

Bardet-Biedl syndrome (BBS) is a rare autosomal-recessive ciliopathy. The phenotype of this pleiotropic disease includes cone-rod dystrophy, postaxial polydactyly, truncal obesity, learning disabilities, hypogonadism and renal abnormalities, among others. To date, 12 genes *(BBS1-12)* have been described to cause BBS. Mutations in these genes can be identified in approximately 75% of individuals with BBS. The broad genetic locus heterogeneity renders mutational screening time-consuming and expensive. We screened individuals from 90 families with BBS for mutations in known *BBS* genes by DNA pooling and subsequent massively parallel resequencing (MPR). DNA of 90 individuals with BBS was pooled in 4 pools (3x24 individuals + 1x18 individuals). All 133 coding exons of 12 known *BBS* genes (*BBS1-12*) were amplified by conventional PCR. Subsequent MPR was performed on an Illumina Genome Analyzer II platform. Following mutation identification, the mutation carrier was assigned by CEL I endonuclease-based heteroduplex screening and confirmed by Sanger sequencing. In 27 out of 90 individuals (30.0%) 56 mutations in 11 different *BBS* genes were found, 26 of which were novel. In 13 out of 90 (14.4%) individuals 15 single heterozygous mutations were found, 8 of which were novel. Thus, DNA pooling combined with MPR offers a valuable strategy for mutation analysis of large patient cohorts, especially in genetically heterogeneous diseases such as BBS.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2443**

**Detection of CAKUT-Causing Genes Using Next Generation Exon Resequencing of 30 Candidate Genes in 45 Patients with Unilateral Renal Agenesis** Pawaree Saisawat,[1] Velibor Tasic,[2] Elijah O. Kehinde,[3] Virginia Vega-Warner,[1] Edgar Otto,[1] Friedhelm Hildebrandt.[1] *[1]Department of Pediatrics and of Human Genetics and Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI; [2]Department of Pediatric Nephrology, University Children's Hospital, Skopje, Macedonia, The Former Yugoslav Republic of; [3]Department of Surgery, Kuwait University, Safat, Kuwait.*

**Background.** Congenital abnormalities of the kidney and urinary tract (CAKUT) account for ~50% of all chronic kidney disease in children. Although many forms of CAKUT are likely caused by single-gene defects, only few causative genes have been identified. The broad genetic heterogeneity of CAKUT necessitates analysis of many candidate genes. We therefore applied a newly developed approach of next generation exon resequencing to 45 patients with unilateral renal agenesis from 43 different families.

**Methods.** We pooled genomic DNA of 20 individuals each and performed exon PCR in 30 CAKUT candidate genes (402 exons) simultaneously using next generation resequencing. We repeated pooling 3 times to cover 45 renal agenesis patients. Candidates were genes implicated in renal agenesis in humans or mice. Sequencing was performed using Illumina Genome Analyzer II.

**Result.** We detected 7 heterozygous missense mutations in 4 new CAKUT genes not previously implicated in non-syndromic CAKUT in humans: 1 each in *EMX2, FREM2* and *SPRY1* and 4 in *FRAS1*. All mutations were absent from 96 healthy controls and had a polyphen score of >2.0, except 2 *FRAS1* mutations with a score of 1.69. Whereas recessive *truncating* mutations in *FRAS1* and *FREM2* cause Fraser syndrome in human and mice, we detected heterozygous *missense* mutations in both genes in non-syndromic CAKUT. Likewise, homozygous *EMX2* mutations cause CAKUT in mice, and heterozygous *truncating* mutations cause human schizencephaly. However, we found heterozygous *missense* mutations as causing non-syndromic CAKUT. While *Spry1*-null mice develop CAKUT, no human phenotype of *SRPY1* mutation is known.

**Conclusions.** We thus generate evidence that heterozygous missense mutations in *FRAS1, FREM2, EMX2* or *SPRY1* may be a new cause of non-syndromic CAKUT in humans.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2444**

**Total Exome Capture and Next Generation Sequencing Reveals Mutations of *NAT10, ANGEL1* and *TOP1MT* in Patients with CAKUT** Pawaree Saisawat,[1] Elijah O. Kehinde,[2] Velibor Tasic,[3] Rannar Airik,[1] Virginia Vega-Warner,[1] Edgar Otto,[1] Friedhelm Hildebrandt.[1] *[1]Departments of Pediatrics and of Human Genetics and Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI; [2]Department of Surgery, Kuwait University, Safat, Kuwait; [3]Department of Pediatric Nephrology, University Children's Hospital, Skopje, Macedonia, The Former Yugoslav Republic of.*

**Introduction:** Congenital abnormalities of the kidney and urinary tract (CAKUT) account for 50% of all chronic kidney disease in children. Although non-syndromic CAKUT does not frequently cluster in families, there is strong evidence from mouse models that recessive monogenic causes of non-syndromic CAKUT exist. By total genome homozygosity mapping we demonstrated the presence of homozygosity peaks in patients with non-syndromic CAKUT. To identify new recessive CAKUT-causing genes we established a new method of total human exome capture (THEC) of 180,000 exons with consecutive next generation exon sequencing.

**Method:** We performed in solution total exome capture using NimbleGen's SeqCaP EZ Exome™ protocol in 3 patients with unilateral renal agenesis from 3 different families who showed homozygous candidate regions by homozygosity mapping. Sequencing was performed using Illumina Genome Analyzer II.

**Result:** We detected 1 homozygous truncating mutation p.K1020KfsX17 in the *NAT10* gene (family A1077) and 1 homozygous missense mutation each in *ANGEL1* (p.R398C) (family A796) and *TOP1MT* (p.D319H) (family A899). Mutations in *ANGEL1* and *TOP1MT* had a maximum polyphen-2 score of 1 and were absent from 120 multi-ethnic healthy controls. NAT10 protein was reported to regulate cytokinesis, and *NAT10* depletion leads to cell cycle arrest.

**Discussion:** Mutations in *NAT10, TOP1MT* or *ANGEL1* may represent new causes of non-syndromic CAKUT in humans. We are currently studying loss of function phenotypes for these genes. THEC can be an efficient tool to detect new CAKUT-causing genes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2445**

**Clinical and Immunohistochemical Analyses of Japanese Families with Genetically-Defined Autosomal-Recessive Alport Syndrome** Kazumoto Iijima,[1] Masafumi Oka,[1,2] Yuya Hashimura,[1] Ohtsuka Yasufumi,[2] Hiroshi Kaito,[1] Yoshikazu Sado,[3] Kunimasa Yan,[4] Koichi Nakanishi,[5] Norishige Yoshikawa,[5] Hironobu Nagasako,[6] Kandai Nozu,[1] Masafumi Matsuo.[1] *[1]Pediatrics, Kobe Univ., Kobe, Japan; [2]Pediatrics, Saga Medical Sch., Saga, Japan; [3]Shigei Medical Research Institute, Okayama, Japan; [4]Pediatrics, Kyorin Univ., Mitaka, Japan; [5]Pediatrics, Wakayama Medical Univ., Wakayama, Japan; [6]Department of Pediatrics, Kagoshima Univ., Kagoshima, Japan.*

**Background.** Autosomal recessive Alport syndrome (ARAS) is caused by *COL4A3* or *COL4A4* mutations. Clinical and immunohistochemical characteristics of ARAS are still unclear because the mutation detection rates were low in previous studies.

**Methods.** To improve the detection rates of *COL4A3* and *COL4A4* mutations, we utilized combined several molecular techniques: 1) Direct sequencing of genomic DNA, 2) semi-quantitative PCR amplification using capillary electrophoresis of genomic DNA, and 3) RT-PCR and direct sequencing analysis of RNA. Thus, we examined clinical and immunohistochemical characteristics in Japanese families with genetically-defined ARAS.

**Results.** We examined *COL4A3* and/or *COL4A4* mutations in 14 patients (3 -25 years old) in 12 families with possible ARAS. We detected homozygous or compound heterozygous mutations in *COL4A3* or *COL4A4* in all the patients. We found 19 mutations, and 17 were novel. The median age of ESRD was 19 years (25 years in X-linked Alport syndrome (XLAS) male patients in the previous study). Six patients over 15 years of age showed no expression of type IV collagen alpha 3-5 chains ((IV) α3-5) in GBM. Four of them developed ESRD, 2 had heavy proteinuria, and 4 of 6 patients had hearing loss.

However, 2 patients over 15 years of age showed the weaker but definite expression of ((IV) α3-5) in GBM, mild urinary abnormalities with normal renal function and no hearing loss although they had no distinct characteristics of mutations in *COL4A3* or *COL4A4.*

**Conclusions.** The renal prognosis of ARAS patients is worse than that of XLAS male patients. And, the expression pattern of ((IV) α3-5) in GBM may be correlated with the prognosis of ARAS

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2446

**Novel Dominant Renin Gene Mutations Associated with Hyperuricemia, Anemia and Chronic Renal Failure** Vincent Moriniere,[1] Fabien Nevo,[2] Olivier Gribouval,[2] Chantal Loirat,[3] Guillaume Bollee,[4] Patrick Niaudet,[4] Marie-Claire Gubler,[2] Corinne Antignac.[2, 5] *[1]MARHEA, Necker Hospital, Paris, France; [2]Inserm U983, Necker Hospital, Paris, France; [3]Nephrology Department, Robert Debre Hospital, Paris, France; [4]Nephrology Department, Necker Hospital, Paris, France; [5]Genetic Department, Necker Hospital, Paris, France.*

Recently, two different heterozygous mutations in the first exon of the *REN* gene encoding renin have been described in patients with autosomal dominant chronic tubulointerstitial nephritis (CTIN), hyperuricemia and anemia responding to EPO in childhood (Zivná M et al, 2009). The mutations resulted in the deletion (p.Leu16del) or the amino acid exchange (p.Leu16Arg) of a single leucine residue in the peptide signal sequence.

Given the phenotypic similarities with familial hyperuricemic nephropathy due to UMOD mutations, we screened *REN* exon 1, encoding the pepdide signal of preprorenin in 141 unrelated individuals with CTIN without UMOD mutations. Autosomal dominant inheritance and hyperuricemia and/or gout were present in 97 and 90 cases respectively. In 3 cases, we identified 3 novel missense mutations involving the signal sequence, not found in ~100 controls and segregating with the phenotype in the families. All these mutations (p.Leu16Pro, p.Trp17Arg et p.Cys20Arg) are predicted to be probably damaging (Polyphen scores 2.2, 3.7 et 3.6 respectively) and to drastically decrease the hydrophobicity of the peptide signal.

As previously described, the 3 patients present with CTIN (discovered at 9, 26 and 29 years), hyperuricemia (and gout at 26 years in a female patient) and anemia in childhood in two cases, the anemia being present before the development of renal failure. All had a family history of CTIN and gout.

Altogether, these data prove that *REN* mutations can be responsible for a small percentage of familial cases of CTIN and screening of *REN* exon 1 should be undertaken in these families, especially when a history of anemia in childhood is clearly documented. It remains to be understood the exact mechanism leading to chronic renal failure in these patients and whether mutations in other parts of the gene might also be responsible for the same renal disease.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2447

**Heterozygous Deletions in the SLC12A3 Gene as a Cause of Gitelman Syndrome: Analysis of a Large Cohort** Rosa Vargas-Poussou,[1] Diana Kahila,[1] Annabelle Venisse,[1] Pascal Houillier,[2] Anne Blanchard,[3] Olivier Devuyst,[4] Xavier Jeunemaitre.[1] *[1]Genetics, APHP Hôpital Européen Georges Pompidou, Paris, France; [2]Physiology, APHP Hôpital Européen Georges Pompidou, Paris, France; [3]Centre d'Investigations Cliniques, APHP Hôpital Européen Georges Pompidou, Paris, France; [4]Nephrology, Université Catholique de Louvain, Bruxelles, Belgium.*

Gitelman Syndrome (GS) is an autosomal recessive salt losing tubulopathy caused in the vast majority of cases by mutations in the SLC12A3 gene, encoding the thiazide-sensitive NaCl cotransporter. Between 18 to 40% of patients with clinical GS are found to carry only one mutant allele after SLC12A3 screening. It has been suggested that large genomic rearrangements could account for these unidentified alleles. We screened by direct sequencing a large cohort of 451 GS that allowed the detection of two mutations in 321 patients (71%), but only one or none in 80 (18%) and 50 (11%) patients, respectively. We then performed a search for large rearrangements by multiplex ligation dependent probe amplification (MLPA) in a subset of patients either simple heterozygous (n=53) or with no punctual mutation (n=25). Nine different deletions (E1_E7del, E2_E3del, E4_E5del, E19_E23del and E24_E25del) and two duplications (E1_E3dup and E1_E4dup) were found in 24 out of the 53 heterozygous patients (45%) but none in the non-mutated group. Each copy number variation was confirmed by a second technique (QMPSF, gap-PCR and direct sequencing or micro arrays in one case). Large rearrangements accounted for at least 6% of all mutations detected in our cohort. The breakpoints of 6 deletions were characterized, showing that non-allelic homologous recombination by Alu sequences as well as non-homologous end-joining are most likely responsible for intragenic deletions. In 5.5 % of our GS patients we have excluded mutations and large rearrangements in SLC12A3 gene, even after the analysis of the CLCNKB gene, which raises the question of the diagnosis of GS and/or of the possible genetic heterogeneity of this tubulopathy.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2448

**Characterization of the Genetic Complexity of Meckel Syndrome (MKS) Using Targeted Exon-Enriched Next-Generation Sequencing** Katharina Hopp,[1] Christina M. Heyer,[2] Susan A. Henke,[3] Jamie L. Sundsbak,[2] Vicente E. Torres,[2] Sandro Rossetti,[2] Peter C. Harris.[2] *[1]BMB, Mayo Clinic; [2]Nephrology Research, Mayo Clinic; [3]Genome Center, Mayo Clinic, Rochester, MN.*

Meckel Syndrome (MKS) is an embryonic lethal, apparent autosomal recessive disorder characterized by polycystic kidney disease, central nervous system defects, polydactyly and liver fibrosis; phenotypic parameters that place it in the group of ciliopathies. Up to now five genes have been clearly implicated, highlighting a high level of genetic heterogeneity. Mutation screening of these genes (*MKS1, MKS3, CEP290, CC2D2A,* and *RPGRIP1L*), revealed two mutated alleles in only 52% of our MKS cohort (46 families) while a further 12 families had one potential mutation in MKS or one other ciliopathy gene. To explore the full genetic complexity and to re-sequence all implicated MKS genes, we have performed targeted exon-enrichment on pedigrees with unclear etiology. Using *RainDance* droplet-PCR, DNA samples were enriched for the coding region of 31 ciliopathy associated genes and sequenced on the *IlluminaGAII*. We found a likely pathogenic *MKS3* change (G250R) that was missed by Sanger sequencing in a pedigree that had already one known *MKS3* change (R549C). Segregation analysis shows appropriate inheritance of both changes, categorizing this pedigree as a *MKS3* family. In accordance with possible complex inheritance we found a pathogenic splicing change in a novel MKS gene, *B9D1*. The fetus inherited the IVS4+2T>C change and a possible pathogenic *CEP290* change (R2210C). *B9D1* cDNA analysis of fetal cells shows skipping of exon 4 causing a frameshift and no WT product, suggesting a second deleted allele. *B9D1* is one of three proteins containing a B9 domain, others being *MKS1* and *B9D2*. The protein has been proposed to be important in cilia development of *C. elegans* but has not been found mutated in any ciliopathy prior this case. To identify other possible disease genes, we are widening the screen to 707 ciliome genes using the *SureSelect* technology. Both studies will not only provide comparative information of two novel exon-enrichment technologies, but will reveal a much clearer view of the complex inheritance of MKS.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2449

**Genetics of Renal Hypodysplasia in the Chronic Kidney Disease in Children Cohort Study (CKiD)** Rosemary Thomas,[1] Simone Sanna-Cherchi,[2] Patricia L. Weng,[2] Bradley A. Warady,[3] Susan L. Furth,[4] Frederick J. Kaskel,[1] Ali G. Gharavi.[2] *[1]Ped Nephrol, Children's Hospital at Montefiore, Bronx, NY; [2]Nephrol, Columbia University, New York, NY; [3]Ped Nephrol, Children's Mercy Hospital, Kansas City, MO; [4]Ped Nephrol, Children's Hospital of Philadelphia, Philadelphia, PA.*

**Introduction:** Malformations of the kidney and lower urinary tract are the most common cause of End Stage Renal Disease in children. *TCF2* and *PAX2* are transcription factors with crucial roles in kidney development.

**Objective:**

1) Determine the prevalence of mutations in *TCF2* and *PAX2* genes in the North American CKiD population with RHD.

2) In patients with no mutations in *TCF2* or *PAX2* genes, explore the contribution of rare copy number variations using high-density single nucleotide polymorphism arrays.

**Methods:** Genomic DNA was obtained from the NIH biorepository. We performed direct sequencing of *TCF2* and *PAX2* exons and intron boundaries. We then performed simultaneous amplification of multiple short exonic fragments (MLPA) to assess the prevalence of *TCF2* whole/partial gene deletions, as these are not found by sequencing alone. Findings on MLPA were then verified by QPCR.

**Results:** We received 73 DNA samples of children with RHD. By sequencing, we confirmed 6 likely pathogenic variants.Three in *TCF2*: a premature termination signal (p.R181X), a missense mutation (p.S148L), and a frameshift (Y352fsX352); the first two were noted previously to be pathogenic . Three in *PAX2:* one splice site mutation (IVS4-1G>T), one missense mutation (p.G24E), and one frameshift mutation (G24fsX28); the last one was previously reported to be pathogenic. Using MLPA, we found one child with a whole gene deletion in *TCF2*. All the cases were Caucasian. 10% of total cohort (14% of the Caucasians) were noted to have mutations.

**Conclusions:** A proportion of patients with non-syndromic RHD carry mutations in either *TCF2* or *PAX2* genes. These patients should be evaluated for complications (e.g. diabetes for TCF2 mutations, colobomas for *PAX2*) and referred for genetic counseling.

A genome-wide screen for novel pathogenic structural variants in patients without mutations in *TCF2* or *PAX2* genes is ongoing.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2450

**Familial Interstitial Nephritis Is Linked to Chromosome 1q21-23** Peter J. Lavin,[1] Rasheed A. Gbadegesin,[1] Jason J. Eckel,[1] Gentzon Hall,[1] Guanghong Wu,[1] Alison Byrd,[1] Alison Homstad,[1] Peter J. Conlon,[2] Michelle P. Winn.[1] *[1]Duke University Medical Center, NC; [2]Beaumont Hospital, Ireland.*

Chronic tubulo-interstitial nephritis (TIN) leads to progressive decline in renal function, involving patho-physiological processes that are implicated in the progression of most types of renal disease. Here, we present six kindreds with familial interstitial nephritis (FIN) and report a locus for the disease on chromosome 1q21-23 in one kindred.

Affected individuals were initially identified by the Department of Nephrology,

Beaumont Hospital, Ireland. Affected families had two or more members with biopsy-proven primary TIN in the absence of renal cysts or hyperuricemia. All family members were offered screening to determine their affection status by measurement of serum creatinine, serum uric acid levels and qualitative urinalysis. Genomic DNA was isolated from blood using a standardized salting out procedure. The maximal attainable logarithm of the odds (LOD) scores for each pedigree, using full pedigree and affected-only models, was calculated via computer simulation with the SIMLINK 4.1 program. Genome-wide linkage scan was performed with the Illumina Infinium II HumanLinkage-12 genotyping beadchip. Two-point and parametric multipoint LOD scores were calculated by using the VITESSE and MERLIN statistical programs. Microsatellite markers were genotyped in areas suggestive of linkage on the genome-wide screen. The largest kindred, with 12 affected individuals spanning 3 generations, had a maximal predicted LOD score of 4.5 when calculated with SIMLINK. Genome-wide linkage analysis yielded a two-point LOD score of 2.62 at chromosome 1q23.1 and significant multipoint LOD score of 3.38 at D1S2721, D1S394 and D1S2635. Recombination events among affected individuals, as detected by haplotype analysis, established an 18-centiMorgan minimal candidate region flanked by markers D1SPJL and D1S2844. These data support a gene locus for FIN at chromosome 1q21-23. Fine mapping to further refine the minimal candidate region is underway. Identification of the mutated gene at this locus may provide further insight into the disease mechanisms of interstitial nephritis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2451**

**Identification of a Novel Disease Gene in Dominantly Transmitted Glomerulocystic Kidney Disease** Monica C. Tucci,[1] Xiang-Yang Lou,[2] Lisa M. Guay-Woodford.[1] *[1]Pediatric Nephrology / Genetics, University of Alabama Birmingham, Birmingham, AL; [2]Biostatistics, University of Alabama Birmingham, Birmingham, AL.*

Glomerulocystic kidney disease (GCKD) is a relatively rare form of PKD with both sporadic and familial occurrence. In previous studies, we have identified a large, four-generation African-American family with autosomal dominant GCKD. Using a set of directed linkage studies, we determined that this GCKD phenotype results from mutation in a novel locus distinct from the ADPKD genes, *PKD1* and *PKD2*, as well *BICC1*, the human orthologue of the gene disrupted in the mouse *jcpk* model. The current study was designed to expand our initial phenotypic characterization of this family and perform a genome-wide linkage study to localize and identify the novel disease-susceptibility gene.

**Methods/Results:** We characterized all available individuals who were 14 years or older by evaluating blood pressure and renal function, and we assessed renal structure using sonography. Of the 19 family members evaluated, 13 were classified as affected based on renal ultrasound, renal biopsy, or progression to ESRD. Using the Illumina Cyto-SNP12 assay (294,655 SNPs), we genotyped each of the 19 individuals and performed a genome wide association study (GWAS) using the statistical software PLINK to identify SNPs strongly associated with disease transmission. The significance was set at a p-value threshold greater than $-\log_{10}(p)=3.5$. Analyses to date do not support linkage to *UMOD* on chromosome 16 but have identified potential candidate intervals on chromosomes 4,6,9,11,18.

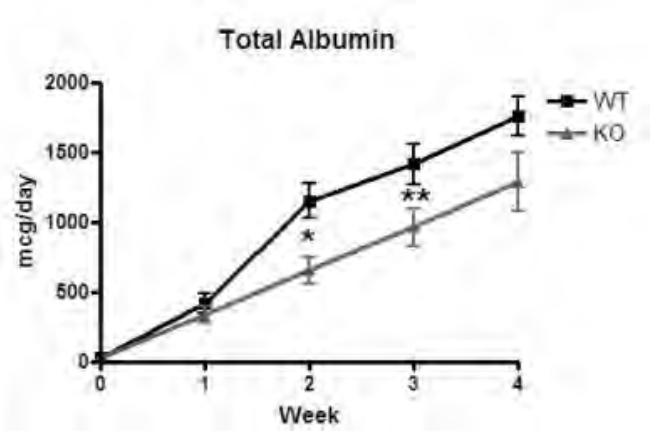
**Conclusions/Ongoing Studies:** We have characterized a large, four-generation African-American family with autosomal dominant GCKD. Using a GWAS-based strategy, we have identified several candidate genomic intervals for this novel GCKD locus. Current efforts are directed towards building haplotype blocks within these intervals and using linkage analysis to identify and delimit a single candidate interval. We propose that identification of this novel GCKD gene will add to the current knowledge of renal cystic disease genetics and provide new insights into pathogenic pathways.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2452**

**A Role for TRPC6 in Angiotensin II-Induced Albuminuria in Mice** Jason J. Eckel,[1] Peter J. Lavin,[1] Rasheed A. Gbadegesin,[1] Laura M. C. Barisoni,[2] Matthew A. Sparks,[1] Michelle P. Winn.[1] *[1]Medicine, Duke University Medical Center, Durham, NC; [2]Nephrology, New York University, New York, NY.*

Mutations in the canonical transient receptor potential cation channel 6 (*TRPC6*) are responsible for familial forms of adult onset focal segmental glomerulosclerosis (FSGS). However, speculation exists at to the precise mechanism whereby *TRCP6* mutations cause kidney disease. We used a TRPC6-deficient mouse line to examine the role of TRPC6 in the kidney. We found no difference in baseline blood pressures, measured by radiotelemetry, between TRPC6-deficient and wild type mice. Additionally, there was no difference in urinary albumin excretion between these two groups at baseline. Mice were then infused with angiotensin II for 28 days to determine whether the absence of TRPC6 would alter susceptibility to hypertension and renal injury. TRPC6-deficient mice had significantly less albuminuria especially during the early phase of the infusion despite similar increases in blood pressure.




Cell membrane currents were analyzed by whole-cell patch-clamping in primary cultures of podocytes from wild type and TRPC6-deficient mice. There was an augmented response to angiotensin II in podocytes from wild type mice, and these currents were abolished in podocytes lacking TRPC6. Our findings suggest that TRPC6 promotes albuminuria, perhaps by promoting angiotensin II-dependent calcium transients. The absence of TRPC6 protects against angiotensin II-dependent albuminuria without affecting blood pressure suggesting that specific blockade of *TRPC6* may be beneficial in proteinuric kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2453**

**Identification of PAR6γ as a New Interaction Partner of PLCε1, the Protein Mutated in Nephrotic Syndrome Type 3** Shazia Ashraf,[1] Toby W. Hurd,[1] Virginia Vega-Warner,[1] Friedhelm Hildebrandt.[1,2] *[1]Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; [2]Howard Hughes Medical Institute, .*

**Objectives:** Steroid resistant nephrotic syndrome (NS) is a malfunction of the kidney glomerular filter that leads to proteinuria, hypoalbuminemia, edema, and renal failure. Using positional cloning, we have identified recessive mutations in the *phospholipase C epsilon 1* (*PLCE1*) gene as causing early onset NS with end stage kidney disease (1). We also demonstrated the interaction of the PLCε1 protein with IQGAP1 (1), BRAF (2), β-arrestin 2 and ERK1/2. We hypothesized that identifying novel interaction partners of PLCε1 would help clarify the mechanism by which *PLCE1* mutations cause NS.

**Methods:** To search for additional members of the PLCε1-associated protein complex, we performed GST pull down assay in rat glomerular lysates with purified GST-PLCε1 fusion protein, represented by exons 4-6. Proteins thus obtained were subsequently analysed with two-dimensional gel electrophoresis and compared to proteins from a GST-only negative control. Proteins differentially pulled down by GST-PLCε1 were identified by liquid chromatography/mass spectroscopy (LC-MS) analysis.

**Results:** One of the proteins showed identity with PAR6-gamma (PAR6γ), an adaptor protein that engages in many protein-protein interactions that are spatiotemporally regulated to control cell polarity. We then confirmed by *in vivo* analysis that overexpressed myc-tagged PAR6γ coimmunoprecipitates with overexpressed flag-tagged PLCε1 in HEK293T cells demonstrating that PAR6γ can form a protein complex with PLCε1. We examined and confirmed the interaction both ways endogenously by coimmunoprecipitation in rat glomerular lysates, the most relevant tissue source for glomerular expression. We also mapped the interaction of PAR6γ to exons 4-6 of PLCε1, encoding the RAS-GEF domain.

**Conclusion:** We identified PAR6γ as a novel interaction partner of PLCε1 in glomeruli, strongly suggesting a functional role of PLCε1 in the regulation of cell polarity.

(1) Hinkes B et al. *Nat Genet* 38:1397-405, 2006
(2) Chaib H et al. *Am J Physiol Renal Physiol* 294:F93-9, 2008

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2454**

**Characterisation of a Novel Mouse Model of Joubert Syndrome** Ann Marie Hynes, Lorraine Eley, Roslyn Jane Simms, Colin Miles, John A. Sayer. *Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Joubert syndrome (JBTS) is an inherited ciliopathy leading to a cerebellum-retinal-renal syndrome. Recent genetic advances have allowed positional cloning and identification of several JBTS genes. *CEP290 (alias NPHP6)* encodes nephrocystin-6, a centrosomal protein. Mutations in *CEP290* account for 7% of patients with JBTS5. Here we create and characterise a novel murine model of JBTS, carrying a truncating mutation in the *Cep290* gene.

A murine ES cell line containing a *Cep290* "gene trap" was identified. Re-sequencing confirmed this cell line harboured a β-galactosidase reporter gene (β-*geo*) inserted into the endogenous *Cep290* locus leading to a truncating mutation. ES cells were cultured before injecting into murine blastocysts to create chimaeric mice. Chimeras were bred to produce

viable, healthy heterozygous mutant mice. The β-*geo* reporter gene, being placed under transcriptional control of the *Cep290* regulatory elements, allowed determination of the spatiotemporal expression pattern of *Cep290* in heterozygous mutant mice, using X-gal staining of tissue in adult and embryos. Heterozygotes were bred to give rise to homozygote *Cep290*$^{-/-}$ animals which were viable for up to 9 months of age. Histological examination of retinal and renal tissues was performed, alongside immunofluorescent antibody staining and fluorescent microscopy.

In *Cep290*$^{+/-}$ animals, specific X-gal staining representative of *Cep290* expression was observed in a range of tissues (including the kidney, eyes and cerebellum) at various embryological time points. Interestingly, in renal tissues *Cep290* expression was noted at the corticomedullary junction. Homozygous mice developed corticomedullary cysts, in contrast to wild type and heterozygous littermates. Retinal degeneration and hydrocephalus was also observed in the homozygous mice over the first month of life.

The development of *Cep290-/-* mouse has provided a novel model of JBTS with a cerebro-retinal-renal phenotype, consistent with the human disease phenotype. We aim to use this mouse to dissect out disease mechanisms and test novel therapeutic agents in this disease.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2455

**Uromodulin Controls Centrosome Integrity in Cells of the Thick Ascending Limb of the Loop of Henle** Lorenzo Battini, Kim Lee, Carles Martinez-Romero, Lin Geng, G. Luca Gusella. *Medicine, Division of Nephrology, Mount Sinai School of Medicine, New York, NY.*

Mutations of the uromodulin gene, UMOD, are responsible for familial juvenile hyperuricemic nephropathy (FJHN) and medullary cystic kidney disease type 2 (MCKD2). Both diseases are autosomal dominant tubulointerstitial nephropathies, characterized by hyperuricemia, gout, hypertension, cysts formation, and end-stage renal disease. The biological function of uromodulin in cystogenesis is not completely understood, partly for the lack of suitable in vitro models. We generated a novel cell line, TIRE131, by infecting normal human primary epithelial cells with a lentiviral vector expressing the human telomerase. TIRE131 showed no expression of alkaline phosphatase, low levels of aquaporin-2, and high expression of uromodulin. TIRE131 cells formed monolayers with weak transepithelial resistance, but showed resistance to hyperosmolar culture conditions comparable to that of (IMCD3) cells. These morphological and functional properties are consistent with the origin of TIRE131 cell line from the thick ascending limb of the loop of Henle (TAL). To investigate the role of uromodulin in the cystogenic process, we used lentiviral-mediated expression of specific siRNAs to knockdown the gene. Inhibition of uromodulin expression in TIRE131 cells rapidly induced centrosome amplification and genomic instability, similarly to what observed after PKD1 knockdown in IMCD3 and MDCK, suggesting overlapping mechanisms in the cystogenic process triggered by the two genes.However, despite the constitutive expression of polycystin-1 in TIRE131 cells, PKD1 knockdown had no effects on the centrosome integrity or cell growth profiles. IMCD3 and MDCK cells were equally unaffected by UMOD knockdown. Overall, these data show that another cystic gene, uromodulin, is involved in the control of centrosome integrity. Furthermore, these results indicate a variable susceptibility of different renal cell types to cystic genes, supporting the observed prevalence of cyst formation along different tracts of the nephron or collecting ducts, and suggest that adult renal cytoarchitecture is controlled by different genes in different cell types.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2456

**Diabetes Accelerates Cystogenesis in the Adult Conditional *ift88* Knockout Mouse** Kelli Margot Sas, May Y. Amria, P. Darwin Bell. *Medicine, Medical University of South Carolina, Charleston, SC.*

Recently we have found that deletion of cilia in the adult mouse exacerbates renal hypertrophy and accelerates cystogenesis in response to unilateral nephrectomy. In this model, hypertrophic signaling in the absence of cilia may initiate cyst formation, at least in part through inappropriate activation of the mTOR pathway. Diabetes, the leading cause of end stage renal disease, also results in structural and functional hypertrophy in the kidney. The goal of this study was to determine if the presence of diabetes would modify the time course of cyst formation in the adult mouse with deletion of cilia. To examine the role of cilia in diabetes, we utilized a conditional floxed allele for the *ift88/Tg737* gene to produce cilia (+) or cilia (-) adult mice. Mice were administered streptozotocin at 50 mg/kg for 5 days to induce diabetes. Blood glucose concentrations ranged from 250 mg/dl to 600 mg/dl and were not different between mice that had cilia and mice in which cilia had been removed. After 7 weeks, mice underwent MRI to determine kidney volume and cystic burden and kidneys were removed for histological analysis. MRI and histological analyses identified accelerated cystogenesis mainly in the collecting ducts of diabetic cilia (-) mice, as well as exaggerated structural hypertrophy. Cilia (-) mice without diabetes had little to no cystogenesis or structural hypertrophy at the 7 wk time point. In diabetic cilia (-) mice, there was a marked increase in cellular proliferation, especially in collecting ducts/distal tubules as well as in glomeruli. There was also enhanced mTOR activity in kidneys from the diabetic cilia (-) versus cilia (+) mice. Thus diabetes may be a significant risk factor for accelerated cyst formation and renal failure in PKD.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2457

**Novel *UMOD* Mutations Result in Decreased Ciliary Uromodulin Expression *In Vitro*** Matthias Tilmann Florian Wolf,[1,2] John A. Sayer,[3] Luca Rampoldi,[4] Bernd Hoppe,[1] Friedhelm Hildebrandt,[5] Frank Zaucke.[6] *[1]Department of Pediatrics, University Hospital Cologne, Cologne, Germany; [2]Department of Pediatrics, University of Texas, Southwestern Medical Center, Dallas, TX; [3]Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, United Kingdom; [4]Dulbecco Telethon Institute, DIBIT, San Raffaele Scientific Institute, Milan, Italy; [5]Departments of Pediatrics and Human Genetics, University of Michigan, Ann Arbor, MI; [6]Center for Biochemistry, Medical Faculty, University of Cologne, Cologne, Germany.*

Mutations in *Uromodulin* (*UMOD*) have been described in: 1. medullary cystic kidney disease type 2, 2. familial juvenile hyperuricemic nephropathy, and 3. glomerulocystic kidney disease. Previously, we have demonstrated Uromodulin expression in renal primary cilia. We were interested if *UMOD* mutations would result in a decreased ciliary Uromodulin expression in cell culture. We also compared in patients from the 3 disease groups whether presence or absence of *UMOD* mutations are associated with different clinical features.

We performed mutation analysis in *UMOD* for 33 kindreds and identified 13 (10 novel and 3 previously published) *UMOD* mutations. In IMCD3 cells we show that the mutant constructs C150S and T225K do no traffick to cilia in contrast to wildtype UMOD. Between 17 individuals from 13 kindreds with *UMOD* mutations and 20 individuals without *UMOD* mutations there was no significant difference in age of onset (25.3 vs. 19.1 years) or GFR (49.9 vs. 61 ml/min/1.73m$^2$). When comparing the group with *UMOD* mutations vs. the group without there was more frequently a positive family history (88% vs. 50%; p=0.01) and hyperuricemia (47% vs. 16.7%; p=0.04). Presenting symptoms in the patients with *UMOD* mutation were mostly hypertension (65%) and anemia (23.5%), whereas the non *UMOD* group presented initially with polyuria (29.1%).

We show that *UMOD* mutations may impair the UMOD ciliary expression. We identified 10 novel mutations. Patients with *UMOD* mutations have more frequently a positive family history and hyperuricemia and present initially more frequently with hypertension.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2458

**Expression of Mutant Uromodulin Leads to Progressive Tubulo-Interstitial Damage and Renal Function Defects in Transgenic Mice** Ilenia Bernascone,[1] Sylvie Janas,[2] Masami Ikehata,[3] Matteo Trudu,[1] Alessandro Corbelli,[3] Celine Schaeffer,[1] Antonio Amoroso,[4] Gian Marco Ghiggeri,[5] Francesco Scolari,[6] Maria Pia Rastaldi,[3] Olivier Devuyst,[2] Luca Rampoldi.[1] *[1]Dulbecco Telethon Institute c/o San Raffaele Scientific Institute, Milan, Italy; [2]Université Catholique de Louvain, Brussels, Belgium; [3]Fondazione IRCCS Ospedale Maggiore Policlinico, Milan, Italy; [4]University of Turin, Turin, Italy; [5]G. Gaslini Institute, Genoa, Italy; [6]Montichiari Hospital, Montichiari, Italy.*

Uromodulin-associated kidney diseases (UAKD) are autosomal dominant tubulo-interstitial disorders caused by mutations in UMOD, the gene encoding uromodulin. We recently reported on the generation and characterisation of the first UAKD mouse model. Transgenic mice expressing the C147W mutant uromodulin develop a tubulo-interstitial disease very similar to the human condition characterised by extensive fibrosis, inflammatory cell infiltration and tubular dilation. Interestingly, we observed specific cellular damage of the thick ascending limb of Henle's loop. These features are accompanied by urinary concentrating defect of renal origin and mild renal failure. As in UAKD patients, urinary excretion of uromodulin is markedly reduced and the protein accumulates in the ER of expressing cells.

All these features are clearly present in 24 weeks-old mice. To gain insight into the disease progression, we extended our analyses at earlier time points. Interestingly, a mild urine-concentrating defect, enhanced after furosemide treatment, could be seen at 9 weeks of age when the interstitial damage and infiltration are very moderate. ER enrichment of mutant protein is already present even in younger (6 weeks) pre-symptomatic animals and is likely the primary event in the disease pathogenesis. Notably, preliminary data on the molecular mechanisms of ER retention showed that mutant protein interacts with the ER-resident chaperone GRP78/BiP, a main player in the ER quality control process that has a pivotal role in the ER stress responses following accumulation of misfolded protein.

These data demonstrate a gain-of-toxic function of uromodulin mutations and provide insights into the pathogenesis of UAKD.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2459

**Albumin Induced Production of Proinflammatory Substances in Cystinotic Proximal Tubular Cells** Martijn J. Wilmer,[1,4] Leo A. H. Monnens,[3] Lambertus V. Heuvel,[1,4] Elena N. Levtchenko.[2,4] *[1]Laboratory of Pediatrics and Neurology, Radboud University Medical Center, Nijmegen, Netherlands; [2]Department of Pediatric Nephrology, University Hospitals Leuven, Leuven, Belgium; [3]Department of Physiology, Radboud University Medical Center, Nijmegen, Netherlands; [4]Laboratory of Pediatrics, KULeuven, Leuven, Belgium.*

**Objectives:** Lysosomal cystine accumulation is the hallmark of the autosomal recessive disorder cystinosis, caused by mutations in the *CTNS* gene. Patients with cystinosis usually develop renal Fanconi syndrome in the first year of life leading to progressive renal failure.

Whether tubular chemokine and cytokine production is involved in the pathogenesis of renal disease in cystinosis is investigated.

**Methods:** Conditionally immortalized proximal tubular cell lines (ciPTEC) of controls (n=4) and cystinotic patients (n=8) were developed by transfection with SV40T and hTERT. Production of IL-8, MCP-1 and TGF-1 were measured after incubation with bovine serum albumin (BSA; range 20-500g/ml) using ELISA and corrected for intracellular protein. Urinary levels of IL-8, MCP-1 and RANTES were measured and corrected for creatinine in cystinotic patients (n=11) and controls (n=6). Data are presented as pg/mol creatinine.

**Results:** In control and cystinotic ciPTEC, BSA dependent IL-8, MCP-1 and TGF-1 production was demonstrated. No differences between the two groups were observed at basal or stimulated conditions. IL-8 (control 1.5 versus cystinosis 7.5), MCP-1 (10.2 versus 107.9) and RANTES (0.7 versus 4.0) were increased in cystinotic urine ($p<0.05$).

**Conclusions:** Cystinotic ciPTEC are susceptible to albumin induced production of proinflammatory substances comparable to control cells. Because our previous studies in cystinosis patients showed, next to tubular proteinuria, glomerular protein loss, treatment targeting RAAS inhibition is indicated.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2460**

**A Novel Rat Model with >75% of Offspring Exhibiting Spontaneous Unilateral Renal Agenesis** Nicholas D. Kampa,[1] Leah C. Solberg Woods,[2] Kevin R. Regner,[1] Cary T. Stelloh,[1] Michael R. Garrett.[1] *[1]Department of Medicine- Nephrology, Medical College of Wisconsin, Milwaukee, WI; [2]Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI.*

Unilateral renal agenesis (URA) is a relatively common developmental defect that occurs in 1:500-1000 births. Children born with URA are often asymptomatic but may exhibit defects in the urinary tract or extra renal organs. The long-term prognosis of URA is poorly understood due to lack of long-term follow-up data. However, some patients with URA develop proteinuria, hypertension, and chronic kidney disease. Current animal models of URA utilize either invasive methods (i.e. nephrectomy or renal abalation) or genetic knockout of known developmental genes. There are no spontaneous models of URA that are feasible for use in large scale studies of this disorder. We recently screened NIH heterogeneous stock (HS) rats for variation in renal phenotypes and identified an animal exhibiting URA. Subsequent selection has established several breeding lines that consistently demonstrate URA ranging from 50-80%. In this model, URA most often affects the right kidney (80%) and is associated with other urogenital abnormalities including the absence of the ipsilateral adrenal, ureter, seminal vesicles or uterine horn and ovaries. URA rats exhibit significant renal hypertrophy (20-50% depending on age) with increased glomerular area (7092±627 $\mu m^2$), while having lower nephron numbers (18955±1126) compared to two-kidney littermates (5768±418 $\mu m^2$ and 24789±1126, respectively). Some male one-kidney animals do exhibit increased proteinuria (compared to normal two-kidney littermates) with concomitant histological injury characterized by glomerulosclerosis and tubulointerstitial damage later in life (week 32-40). In summary, this model exhibits a high incidence of spontaneous URA and demonstrates clinical features similar to the human disorder. The model will overcome the limitations of current URA models and will allow for long-term study of cardiovascular and renal implications of URA, provide insight into renal development, and serve as a unique genetic resource to better understand gene modifiers involved in renal agenesis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2461**

**Functional Loss of Cystin in the *cpk* Mouse Causes Necdin-Mediated *cmyc* Over-Expression: A Mechanism for Pathogenesis** Chaozhe Yang, Maoqing Wu, Binli Tao, Lisa M. Guay-Woodford. *Genetics, UAB, Birmingham, AL.*

Over-expression of *cmyc* has been proposed as a key modulator of renal cystogenesis in the *cpk* mouse model of ARPKD (JASN 1:1048, 1991). In previous studies, we have shown: 1) cystin, the cilia-associated protein disrupted in the *cpk* mouse, interacts with the anti-apoptotic protein, necdin; 2) the cystin C-terminal 25 AA harbors the necdin-binding site; 3) in luciferase reporter assays, necdin enhances *cmyc* P1 promoter (P1) activity by binding to the P1 GN box and full-length cystin, but not a C-25 deletion construct (cystin C-25), antagonizes this effect; 4) in ChIP assays, necdin, cystin, and cystin C-25 can be immunoprecipitated with the *cmyc* P1 GN box. In the current study, we sought to further characterize the mechanism through which cystin and necdin modulate *cmyc* expression. **Methods/Results**: To confirm previously published observations, we used quantitative immunoblotting to demonstrate that c-myc was highly expressed in kidneys from 14 day old *cpk* mice when compared to wild-type controls. We then generated a doubly transfected stable mIMCD3 cell line expressing cystin::GFP and necdin::RFP and observed that cystin co-localizes with necdin in the nucleus. Previous studies by our group demonstrated that cystin contains a functional nuclear localization signal (JASN 20:2570, 2009). In electrophoretic mobility shift assays (EMSA) using a biotin-labeled GN box and mIMCD3 cells transfected with pCMV-cystin-HA, pCMV-necdin-myc, pCMV-HA (empty vector) constructs, DNA-protein complexes were observed in the nuclear extracts of cystin- and necdin-transfected mIMCD3 cells, but not empty vector-transfected cells. **Conclusions**: Our data demonstrate: 1) both cystin and necdin interact with the *cmyc* P1 GN box; 2) cystin-DNA binding is independent of the cystin-necdin interaction; and 3) cystin antagonizes the stimulatory effect of necdin on the *cmyc* P1 promoter and down-regulates gene expression. We are testing whether this antagonistic action involves direct cystin-necdin binding. We propose that functional loss of cystin causes renal cystogenesis in this mouse ARPKD model by *cmyc* over-expression and dysregulation of *cmyc* downstream targets.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2462**

**Progressive Renal Failure in Infancy – A Manifestation of a New Mitochondrial Cytopathy** Ruth Belostotsky, Efrat Ben Shalom, Rachel Becker-Cohen, Sofia Feinstein, Choni Rinat, Yaacov Frishberg. *Pediatric Nephrology, Shaare Zedek Medical Center, Jerusalem, Israel.*

Mitochondrial cytopathy (MC) in childhood usually manifests as tubulopathy, the most common being Fanconi syndrome, or the nephrotic syndrome due to FSGS. Two infants born prematurely to an inbred kindred of Arab descent presented with a fatal multi-system disease manifesting as poor feeding, developmental delay, primary pulmonary hypertension and elevated serum lactate concentrations associated with metabolic alkalosis. Diabetes mellitus developed in one. Their renal manifestations included marked azotemia disproportionate to serum creatinine levels, persistent hyperuricemia, salt wasting, hypomagnesemia and progression to ESRD in the first year of life. Muscle biopsy obtained from one patient revealed extremely reduced activity of all respiratory complexes but complex II. The latter, which was relatively preserved, is the only complex encoded solely by the nuclear genome. Analysis of the consanguineous pedigree suggested that this would be an autosomal recessive disorder, associated with compromised energy production, and led to the assumption that this is likely a defect in nuclear DNA. Nuclear-encoded genes are central to a number of processes pertinent to the synthesis of mtDNA-encoded proteins (transcription, translation and replication machinery). Homozygosity mapping (Affymetrix 250K Array SNP) was implemented searching for homozygous identity-by-descent-regions, shared by both infants. KinSNP analysis of the data demonstrated 2 shared homozygous chromosomal fragments: on chromosome 19 - 5 Mbp, and on chromosome 4 - 2 Mbp. These loci contained 221 and 144 SNPs, respectively. A priority list of proteins located within these loci was constructed and analysis of candidate genes is underway. This is a new multi-organ MC with devastating renal involvement in infancy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2463**

**Targeting Cytostatic vs. Cytotoxic Effects on TSC Angiomyolipoma Cell Viability** Brian J. Siroky, Lu Lu, Anna R. Hellmann, John J. Bissler. *Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Tuberous sclerosis complex (TSC), an inherited tumor predisposition syndrome associated with mutations in *TSC1* or *TSC2*, affects approximately 1 in 6000 individuals. 80% of TSC patients develop renal angiomyolipomata, and renal involvement in TSC is a major contributor to patient morbidity and mortality. Recent work has shown that mammalian target of rapamycin complex 1 (mTORC1) inhibition with rapamycin caused angiomyolipoma shrinkage. However, angiomyolipomas trended toward baseline size when the drug was discontinued, indicating a greater cytostatic rather than cytotoxic effect. We sought to identify potential cytotoxic therapies. Endoplasmic reticulum (ER) stress can develop in TSC angiomyolipomas due to increased protein translation stemming from constitutive mTORC1 activity. We hypothesized that renal angiomyolipoma cells experience ER stress that can be leveraged to result in targeted cytotoxicity. We used a human angiomyolipoma cell line immortalized with HPV E6/7 and telomerase that was stably transfected with empty vector, or the *TSC2* gene (encoding tuberin). By western blot, we found that angiomyolipoma cells lacked tuberin and greatly over-expressed phospho-S6 ribosomal protein compared to tuberin-rescued cells. In cell viability assays, we found that everolimus (20 nM), an mTORC1 inhibitor, had no effect at 24 hours, but after 72 hours significantly suppressed proliferation of angiomyolipoma cells, while tuberin-rescued cells were not affected, supporting a cytostatic effect. Similar studies were performed with the proteasome inhibitor MG-132 (500 nM), which has been shown to induce ER stress. Western blot analyses showed induction of C/EBP-homologous protein (CHOP), a marker of ER stress, by MG-132 in both cell lines. Poly (ADP-ribose) polymerase (PARP) cleavage, which indicates apoptosis, was observed in MG-132 treated angiomyolipoma cells but not tuberin-rescued cells. By 8 hours of MG-132 treatment, viability was substantially reduced in angiomyolipoma cells, but not tuberin-rescued cells. These results suggest that human angiomyolipoma cells may be uniquely susceptible to therapies that exacerbate ER stress.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2464**

**IQCB1/NPHP5 Mutations Are Involved in Senior-Løken Syndrome with Very Late Onset of Renal Failure** Isabelle Perrault,[1] Remi Salomon,[2,3] Kallman Tory,[4] Vincent Moriniere,[2] Eduardo Silva,[5] Helene Dollfus,[6] Christian Hamel,[7] Albert Bensman,[8] Josseline Kaplan,[1] Corinne Antignac,[2] Jean-Michel Rozet,[1] Sophie Saunier.[2] *[1]Inserm U781, Necker Hospital, Paris, France; [2]Inserm U983, Necker Hospital, Paris, France; [3]Pediatric Nephrology Department, Necker Hospital, Paris, France; [4]Ist Department of Pediatrics, Semmelweis University, Budapest, Hungary; [5]Ophtalmology Department, University Hospital, Coimbra, Portugal; [6]Genetics Department, Faculté de médecine, Starsbourg, France; [7]Inserm U583, INM, Montpellier, France; [8]Nephrology Department, Trousseau Hospital, Paris, France.*

Senior-Løken syndrome (SLSN) is an autosomal recessive disorder with the main features of nephronophthisis (NPHP) leading to ESRD at a mean age of 12 years (12y), and retinal degeneration with variable visual impairment from mild to Leber congenital amaurosis (LCA). Mutations in five SLSN genes have been reported to account for SLSN including NPHP1, CEP290/NPHP6 and IQCB1/NPHP5. We aimed to evaluate the

occurrence of IQCB1 mutations in SLSN and to define the clinical course of renal disease in these patients. We considered 41 SLSN patients with no mutations in either NPHP1 or CEP290 and extended our study to 240 isolated LCA patients without obvious renal alterations. Probands' DNAs were screened for mutations in the 13 IQCB1 coding exons. A total of 42 IQCB1 disease alleles were identified in 15/41 SLSN families with LCA and 7/240 LCA families (26 patients; 14 different nonsense or splice mutations). Renal investigation revealed NPHP symptoms in 3/7 LCA families. The remaining LCA patients (range 5-12y, mean=10y, n=6) are still under investigation. A total of 20 patients from 18 families presented with NPHP symptoms of early to very late onset: 10 patients with ESRD from 7-55y (mean= 41y), 9 patients with chronic renal failure from 3-16y (mean=12y) and 1 patient (7y) with a urinary concentrating defect. Altogether, IQCB1 mutations lead to SLSN with LCA and very variable onset of renal failure even within families, ranging from early childhood to very late adulthood. Therefore, we recommend that renal function should be closely monitored in LCA patients with IQCB1 mutations, regardless of age.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2465

**Clinical Characterization of an Italian Group of Patients Suffering from Bardet-Biedl Syndrome** Miriam Zacchia,[1] Valentina Di Iorio,[3] Francesco Trepiccione,[1] Francesca Simonelli,[3] Elio Marciano,[2] Giovambattista Capasso.[1] *[1]Internal Medicine, SUN, Naples, Italy; [2]Neuroscience, Federico II, Naples, Italy; [3]Oftalmology, SUN, Naples, Italy.*

Bardet Biedl syndrome (BBS) is a rare genetic disorder characterized by multiple organ dysfunctions including the kidney. This study has been designed to elucidate renal and extra-renal phenotype in a group of Italian BBS patients.

22 patients with a clinical diagnosis of BBS have been enrolled; so far 10 patients aged 17-38 years have been screened. Study design: biochemical evaluation, ultrasound imaging, water deprivation test plus ddAVP (desmopessin 4 µg), electroretinal studies, audiometry and otoacustic emission (OAE) testing have been performed.

*eGlomerular filtration rate (eGFR) by CKD-EPI:* 8/10 patients showed normal eGFR (mean±SD, 111±28 ml/min/1.73 $m^2$); 2 adolescent patients were in ESRD.

*Tubular functions*: These studies were performed in patients with eGFR higher than 60 mL/min. No signs of proximal tubule dysfunction were found; plasma and urinary levels of electrolytes (Na, K, Ca, Mg) and acid-base status were normal (mean pH 7.42±0.03, $HCO_3^-$25.38±0.61 mEq/L, $pCO_2$ 39.85±2.50). The majority of patients (6/8) showed a defect in urinary concentration ability, with urine osmolality below 400 mOsm/Kg 12 hrs after water deprivation and lower than 500 mOsm/kg up to 2 hrs after ddAVP administration. Only one patient was frankly poliuric.

*Renal ultrasound imaging:* fetal lobulations were found in 8 patients, renal dysplasia, renal cysts and calyceal diverticula in 2, 6 and 3 patients, respectively.

*Extrarenal phenotype*: retinal degeneration and a story of obesity were found in all patients; 2 patients manifested severe hearing loss, while in 8 it was confined to high frequencies (4 to 8 kHz); polydactyly and cognitive impairment were found in 9 and 4 patients respectively; a patient with normal eGFR was hypertensive.

In conclusion, urinary concentration defect is the most common renal dysfunction, while CKD is rare and not age-dependent. All patients showed renal malformations. In addition to retinal degeneration, a high prevalence of hearing loss limited to high frequences has been revealed, a finding never described in literature.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2466

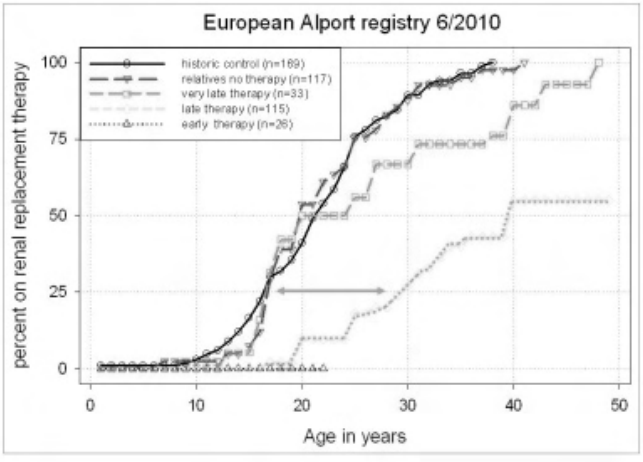
**Primary Endpoints of the European Alport Registry and Its Implications for Future Trials: Early Diagnosis and Therapy Delays Renal Failure** Oliver Gross, Gerhard A. Mueller. *Nephrology & Rheumatology, University Medicine Center Goettingen, Goettingen, Germany.*

Alport syndrome inevitably leads to end stage renal failure (ESRF) in adolescents and young adults. In Alport mice, ACE-inhibition delays renal failure. In humans, however, no efficient therapy can be offered - despite early diagnosis. For that reason, the European Alport registry evaluates the outcome of ACE-inhibition therapy in children and young adults.

552 individuals (237 carriers and 315 patients) have been included into the registry.

A total of 286 individuals (112 carriers and 174 patients) were treated with ACE-inhibition. No SAEs were reported. AEs that lead to discontinuation of therapy included symptomatic hypotension in 2.0% and hyperkalemia (less than 5.5mmol/l) in 1.7% of patients. All AEs were classified as minor and did not cause hospital admission. Treated patients were divided into: (1) early therapy (n=33, proteinuria less than 0.3g/day or hematuria only); (2) late therapy (n=115, proteinuria >= 0.3g/day); (3) very late therapy (n=26, Crea-Clearance<60 ml/min).

Untreated controls (black circles) reach ESRF at the same time point as untreated uncles or brothers (red triangles) with the same genetic defect as treated patients.




In contrast, very late therapy (green squares, p=0.014) and late therapy (yellow diamonds, p<0.0001) significantly delay ESRF. No patient on early therapy (blue triangles) did yet reach renal failure, therefore, effectiveness of early therapy can not (yet) be calculated.

For the first time in humans, our registry data show that Alport syndrome is a treatable disease and ESRF can be delayed by ACE-inhibition. Early start of therapy may be more effective than late therapy. Our data strongly support the need for early diagnosis and therapy in children with Alport syndrome. Hereby, the registry created the essential base for future prospective international trials.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2467

**Progression of Nephropathic Cystinosis in Adults: The Impact of Cysteamine Therapy** Albane Brodin-Sartorius,[1] Patrick Niaudet,[2] Corinne Antignac,[3] Christophe M. Legendre,[4] Philippe H. Lesavre,[1] Aude Servais.[1] *[1]Nephrology, Necker Hospital, Paris, France; [2]Paediatric Nephrology, Necker Hospital, Paris, France; [3]Genetics, Necker Hospital, Paris, France; [4]Transplantation, Necker Hospital, Paris, France.*

Nephropathic cystinosis is a multisystem autosomal recessive disease caused by cystine accumulation. The effect of long term oral cysteamine therapy has to be examined.

Eighty-six adult patients (mean age 26.7±7.1 years) diagnosed with nephropathic cystinosis between 1961 and 1995 were studied. Data were collected on diagnosis, genetics, socio-professional outcome and mortality; time of initiation and duration of cysteamine; and abnormalities of thyroid, pancreas and neuromuscular function.

Cysteamine was administered in 89% of patients, initiated at a mean age of 9.9±10.3 years. By last follow-up, 91% of the patients had end-stage renal disease (mean age 11.1±4.0 years), 72% had hypothyroidism (13.4±6.2 years), 56% diabetes (17.1±7.2 years), and 37% neuromuscular disorder (23.3±6.3 years). Twenty-four patients died. Life expectancy was significantly higher in cysteamine-treated patients versus untreated patients (mean 29.4±7.9 versus 21.6±4.6 years, respectively). Survival curves showed that cysteamine treatment from >5 years significantly delayed the onset of end-stage renal disease, hypothyroidism, diabetes and neuromuscular disorder. Initiating cysteamine before the age of 5 years also significantly delayed the occurrence of those complications.

Cysteamine treatment increases the life expectancy of cystinotic patients. This series indicated that cysteamine treatment for more than 5 years delays the onset of complications, particularly if it is initiated before the age of 5 years.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2468

**Serum Uric Acid Is the Predictor for Progression of Renal Dysfunction Since the Appearance of Overt Nephropathy in Type 2 Diabetes** Kentaro Tanaka,[#1,2,4] Shigeko Hara,[#2,3] Akifumi Kushiyama,[#2] Yoko Yoshida,[#2] Yoshifumi Ubara,[#3] Sonoo Mizuiri,[#4] Atsushi Aikawa,[#4] Shouji Kawazu.[#2] *[1]Depatment of Nephrology, Saiseikai Kanagawa-ken Hospital, Yokohama, Japan; [2]Divison of Diabetes and Metabolism, The Institute for Adult Diseases, Asahi Life Foundation, Tokyo, Japan; [3]Kidney Center and Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Tokyo, Japan; [4]Depatment of Nephrology, Toho University School of Medicine, Tokyo, Japan.*

**Purpose** In diabetic microalbuminuric stage, control of hypertension and hyperglycemia bring in reversible effect. Even though intensive therapy, some groups progress to the renal dysfunction since overt nephropathy. This study is retrospective cohort investigation to reveal the predictor for progression of renal dysfunction since the appearance of overt nephropathy in type 2 diabetic individuals.

**Methods** 290 patients (231men, 59women, age61.9yr, follow-up period5.9yr) were investigated at baseline(retinopathy with UACR ≥300mg/gCr and/or urinary dipstick≥+). We examined the progression of renal dysfunction by Cr doubling. Cr doubling and no

doubling group were compared in clinical findings. The risk factor of Cr doubling was assessed by Cox models.

**Results** 85 of 290 patients developed Cr doubling.In two groups,Cr doubling group showed significantly, early onset of overt nephropathy,higher smoking rates, higher levels of LDL-C, $HbA_{1C}$,serum uric acid(SUA).

In univariate analysis, risk factors of Cr doubling were women, diabetes duration,LDL-C,smoking,$HbA_{1C}$ and SUA. In multivariate analysis, smoking(1.76;1.08-2.91,p=0.02),HbA1c(1.26;1.10-1.44,p=0.0006) and SUA(1.36;1.14-1.62,p=0.0005) remained significant.The highest tertile(≥6.2mg/dl for men, ≥5.1 for women) of SUA levels showed higher rate of Cr doubling than the lower two tertiles (5.1-6.2, ≤5.1 for men, 4.1-5.1, ≤4.1 for women) by the Kaplan-Meier method(log-rank test, p=0.0007).

**Conclusions** Independent predictors for progression of renal dysfunction since the appearance of overt nephropathy in type 2 diabetes are smoking, $HbA_{1C}$ and SUA levels. SUA levels at the overt nephropathy is the strongest predictor for progression of renal dysfunction.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2469

### Entity of CKD Stage 4 over 75 Years Old Is Different from That of Less Than 75 Years of Age

Hiromichi Suzuki, Tsutomu Inoue, Yusuke Watanabe, Tsuneo Takenaka, Hirokazu Okada. *Nephrology, Saitama Medical University, Iruma gun, Saitama, Japan.*

**Background**: Although dependent on the age factor, estimated glomerular filtration rate (eGFR) is commonly used for evaluation of kidney function. Recently, a population based study demonstrated that the eGFR-based criteria for evaluation of CKD were not reliable in patients over 75 years of age. However, whether the assessment of CKD stage 4 patients older than 75 years is better suited for their management and prognosis has not been clearly established.

**Aim:** To compare the characteristics of patients with CKD stage 4 older than 75 years (elderly group) with those between 74 and 65 years (younger group).

**Subjects and Methods**: A single center, retrospective cohort study was performed which included 183 patients (female/male: 66/117**;** 79.2 ± 2.6 years old) with CKD stage 4 (eGFR 19.3 ± 1.7 ml/min/1.73 $m^2$) and 312 patients (female/male: 129/183**;** 70.8 ± 2.0years old) (eGFR 21.5 ± 1.9 ml/min/1.73 $m^2$) from 2004 to 2009. Electronic databases were used to examine the characteristics and mortalities.

**Results**: The ratios of females and males were significantly different between the two groups (p<0.01). The underlying diseases of the elderly group: 146 (79.8%) were due to hypertension, 22(12.0%) were diabetic and others. In the younger group, 118 (37.8%) were diabetic, 96 (30.7%) had glomerulonephritis and others. These were also significant differences (p<0.01). The causes of death were: 9 of 31 (29.0%) in the elderly and 24/47 (51.0%) in the younger group were due to cardiovascular diseases (p<0.01). Progression of eGFR were 0.5 ± 0.2 ml/min/1.73 $m^2$ /year in the elderly group and 1.4 ± 2 ml/min/1.73 $m^2$/year in the younger group (p<0.01). During 6 years, 27 (14.7%) in the elderly group and 140 (44.8%) in the younger group were introduced into dialysis therapy.

**Conclusions**: First, CKD stage 4 patients older than 75 years of age are a different entity from the same stage of the younger group. Second, application of eGFR in CKD stage 4 patients older than 75 years needs to be reconsidered, and other markers such as cystatin C should be explored

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2470

### Glomerular Structure Predicts Diabetic Nephropathy (DN) in Normoalbuminuric (NA) Type 1 Diabetic (T1D) Patients (pts)

Alicia Parks, Michael Mauer, Maria Luiza A. Caramori. *University of Minnesota.*

Over half of the new end-stage renal disease (ESRD) cases in the US are due to diabetes (D). Early identification of pts at increased DN risk would allow preventative strategies to be targeted at this population. We determined whether renal biopsy findings in NA T1D pts predict progression to a composite endpoint of overt proteinuria, ESRD, or cardiovascular (CV) death. Kidney research biopsies and function tests were performed on 106 NA pts [D duration ≥5 years (yrs)]. Albumin excretion rate (AER) was measured by immunoassay (3 samples). Retinopathy was assessed by fundoscopy. Mesangial fractional volume [Vv(Mes/glom)], glomerular basement membrane (GBM) width, and surface density of peripheral GBM per glomerulus [Sv(PGBM/glom)] were estimated by electron microscopic morphometry. *T*-test and Fisher's exact test were used to compare pts remaining NA (non-progressors; NP) to those reaching the endpoint (progressors; P). After 12.4±6.6 (mean±SD) yrs of follow-up, 61 pts were NP, 13 were P, 4 were microalbuminuric, 12 died of non-CV causes, and no follow-up was available in 16. Baseline characteristics, with the exception of hemoglobin $A_{1c}$ ($A_{1c}$) (8.4±1.6 *vs.* 9.4±2.1%; p=0.038) and AER (7.3±4.6 *vs.* 10.1±5.4 µg/min; p=0.033), were not different between pts studied and not studied, respectively. Sex (4M/9F *vs.* 26M/35F), D duration (16.4±8.2 *vs.* 20.1±10.1 yrs), AER (7.2±5.0 *vs.*7.5±4.8 µg/min), mean blood pressure (88.9±10.6 *vs.* 85.6±8.6 mmHg), hypertension (38 *vs.* 25%), retinopathy (55% *vs.* 31%), and duration of follow-up were not different between P (12.1±8.5) and NP (12.9±5.8 yrs), respectively. P were younger at D onset (10.9±6.0 *vs.* 15.3±9.4 yrs; p=0.04) and at kidney biopsy (27.3±8.2 *vs.* 35.4±9.5 yrs; p=0.006), had higher $A_{1c}$ (10.1±2.5 *vs.* 8.0±1.2%; p=0.016) and greater GBM width (584.9±118.3 *vs.* 452.3±79.0 nm; p<0.00001) than NP, respectively. No other variables were different between P and NP. GBM width, $A_{1c}$ and age at D onset were independent predictors of progression. This study found that, despite the absence of clinically apparent renal disease, NA T1D pts with younger onset of D, higher $A_{1c}$ and greater GBM width are at increased risk of progression.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2471

### Safety and Efficacy of Acthar Gel (ACTH) on Albuminuria and Progression of Diabetic Nephropathy in Patients with Nephrotic Range Proteinuria: A Randomized Prospective Study

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Tight blood glucose control and ACE/ARB therapy can reduce proteinuria in diabetic nephropathy. Diabetics with nephrotic range proteinuria despite ACE/ARB therapy are more likely to develop ESRD. Treatment of membranous glomerulonephritis with ACTH can reduce proteinuria through activation of endothelial and podocyte melanocortin-1 (MCP-1) receptors. To determine whether ACTH reduces proteinuria in diabetic nephropathy, we conducted a randomized, prospective trial of Acthar Gel (ACTH) in patients with nephrotic range proteinuria.

| Demographics | No. Pts | Age (yrs) | DM (yrs) | % ACE | %ARB | % Biopsy |
|---|---|---|---|---|---|---|
| No./% Pts | 18 | 53±3 | 15±2 | 46% | 60% | 53% |
| Table-1 | | | | | | |
| Proteinuria | Baseline | Month-1 | Month-3 | Month-6 | Complete | Partial |
| Total Pop. | 7.4±0.8 | 4.5±0.8 | 3.90.8 | 2.30.4 | 7.6% | 61.5% |
| 16 units | 7.6±1.4 | 4.4±1.1 | 2.4±0.6 | 2.3±0.6 | 14% | 57% |
| 32 units | 7.3±0.9 | 4.5±1.2 | 5.1±1.1 | 2.4±0.5 | 0.0% | 66.0% |

Methods: Patients with type I or II diabetes and > 3000 mg proteinuria/24 hrs on ACE alone or > 2000 mg/24 hrs on combination ACE/ARB or other protein lowering agent were enrolled. All patients had GFRs ≥ 20 mls/min by Cockcroft-Gault and HgBA1c ≤ 9.0%. Patients were randomized to SQ ACTHar Gel (16 or 32 units) daily for 6 months. Primary endpoint was the percent patients achieving complete (<300mg/24hr) or partial response (> 50% reduction) proteinuria. Secondary endpoints included changes in serum Cr and HgBA1c after 6 months therapy.

As shown in Table-1, mean proteinuria reduced from 7.8±0.8 to 2.3±0.4 gms/24 hrs after 6 months ACTH therapy (P=0.018). Despite advanced diabetic nephropathy (mean GFR-42.0 ml/min), serum Cr remained stable over six months (2.8±0.4 to 2.6±0.5 mg/dl). There was no difference in protein reduction between patients randomized to 16 or 32 units ACTHarGel. Two patients required reduction in ACTH dose secondary to hyperglycemia. We find that SQ ACTH significantly reduces proteinuria in advanced diabetic nephropathy and stabilizes renal function over 6 months. The use of ACTH and other MCR agonists may represent a novel pathway for treatment of diabetic nephropathy.

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## SA-PO2472

### Microalbuminuria and Silent Cerebral Infarction Independently Predict Renal Outcomes in Type 2 Diabetes

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Recently, we reported that silent cerebral infarction was an independent risk factor for renal failure in type 2 diabetes. This study indicates that the presence of vascular diseases is a predictor of the decline in renal function in patients with type 2 diabetes. On the other hand, albuminuria has been well established as a marker strongly predictive of renal and cardiovascular prognosis. However, there are few data regarding whether albuminuria and the presence of vascular diseases are separate and independent risk factors for cardiovascular and renal events among individuals with type 2 diabetes. In the present study, we studied the effects of microalbuminuria (MA) and SCI on the risk for renal events in 608 type 2 diabetic patients without overt proteinuria (513 with normoalbuminuria and 95 with microalbuminuria) by a post hoc analysis of our results (Uzu T, et al. JASN 2010). The patients underwent cerebral magnetic resonance imaging at baseline, and 177 of 608 patients had SCI. Over the average 7.5-year follow-up period, 58 patients reached the primary composite end point of end stage renal disease (ESRD) or death. In addition, 99 patients reached the secondary renal end point of any dialysis and doubling of the serum Cr concentration. Multivariate analysis using the Cox proportional hazards model found that both SCI and MA were independent prognostic factors for the primary composite outcome. In addition, SCI and MA were also found as independent prognostic factors for the secondary renal outcome. Patients with both MA and SCI at baseline had a 4.2-fold higher risk for primary events and a 6.4-fold higher risk for secondary renal events, compared with patients with neither of these risk factors. In conclusion, SCI and MA are independent risk factors for renal events among type 2 diabetic patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2473**

**Relation between Echocardiography and Coronary Artery Disease in Asymptomatic Type 2 Diabetic Patients with Elevated Urinary Albumin Excretion Rate** Henrik Reinhard,[1] Peter R. Hansen,[2] Niels Wiinberg,[3] Claus Leth Petersen,[3] Hans-Henrik Parving,[4] Peter Rossing,[1] Peter Karl Jacobsen.[1] *[1]Steno Diabetes Center; [2]Gentofte Hospital; [3]Frederiksberg Hospital; [4]Rigshospitalet.*

Left ventricular (LV) hypertrophy and systolic/diastolic abnormalities are common in type 2 diabetic patients with elevated urinary albumin excretion rate (UAER) but relation to coronary artery disease (CAD) is unclear. This study examined echocardiographic parameters, including LV mass index, LV systolic and diastolic function, and their relation to previously undiagnosed CAD in type 2 diabetic patients with UAER >30mg/24h.

We included 200 type 2 diabetic patients without prior CAD. Patients with P-NT-proBNP >45.2 ng/L and/or coronary calcium score (CCS) >400 were stratified as high risk patients for CAD (n=133), and all other patients as low risk (n=67). High risk patients were examined by myocardial perfusion imaging (MPI; n=109) and/or coronary angiography (CAG; n=86). LV systolic and/or diastolic dysfunctions were evaluated in all patients by conventional echocardiography and tissue Doppler**.** Moderate-severe LV hypertrophy was defined by LV mass index >131 g/m$^2$ in men and >108 g/m$^2$ in women.

Results: Patients received mulitifactorial intervention. LV mass index was 87 (21) g/m$^2$ and 4% of patients had moderate-severe LV hypertrophy. LV systolic function was impaired (<50%) in only 5% of patients. LV diastolic dysfunction (LVDD) was found in 54.5% of patients. In 70 high risk patients, significant CAD was demonstrated by MPI and/or CAG. Adjusted odd ratio (OR [95% CI]) for having significant CAD was 2.91 (1.41-6.00) in patients with LV mass index above the median. LVDD was not associated with LV mass index or significant CAD but was associated with poor metabolic control with adjusted OR 1.46 (1.14-1.87) per 1% increase of $HbA_{1c}$ (p=0.003).

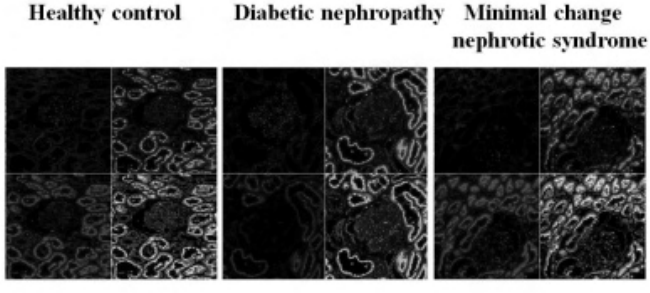
Conclusions: In our study of asymptomatic diabetic patients with elevated UAER that received multifactorial intervention, the prevalence of abnormal LV mass and function was surprisingly low. However, even within normal range, LV mass index was an independent predictor of significant CAD. LVDD was common but not associated with significant CAD

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2474**

**ACE/ACE2-Angiotensin (1-7)-MAS Receptor Expression in Diabetic Nephropathy** Sonoo Mizuiri,[1] Makoto Hamanoue,[2] Hiromichi Hemmi,[3] Michitsune Arita,[1] Toshiyuki Aoki,[1] Yasushi Ohashi,[1] Ken Sakai,[1] Minoru Shinozaki,[4] Kazutoshi Shibuya,[4] Atsushi Aikawa.[1] *[1]Nephrology, Toho University of School of Medicine, Tokyo, Japan; [2]Physiology, Toho University of School of Medicine, Tokyo, Japan; [3]Molecular Biology, Toho University of School of Medicine, Tokyo, Japan; [4]Surgical Pathology, Toho University of School of Medicine, Tokyo, Japan.*

ACE2-Ang (17)-Mas receptor (MAS) axis is possibly acting as a counter-regulatory system against the ACE-Ang II-AT1 Axis. The detail of the ACE2-Ang (1-7)-MAS axis was uncertain in human diabetic nephropathy. We studied renal ACE/ACE2-Ang (1-7)-MAS expression by double immunofluorescence staining using confocal microscopy and DAB staining. Percentage and intensity of the labeled area in the DAB staining were evaluated by a computerized imaging analysis. Subjects were 17 patients with diabetic nephropathy (DN), 11 patients with minimal change nephrotic syndrome (MCNS) and 17 healthy kidney donors (controls). Median value of serum creatinine was 1.1 mg/dl, 0.8 mg/dl and 0.8 mg/dl in DN, MCNS and controls. Median value of proteinuria was 3.7 g/day and 6.8 g/day in DN and MCNS. ACE2 was mainly stained in proximal tubules but also in glomeruli, and co-localized with ACE, Ang (1-7) and MAS. In diabetic kidneys, increased ACE and decreased ACE2 expression was observed compared with other specimens.




**Double immunofluorescence staining of ACE (green) and ACE2 (red) with nuclear staining with DAPI (blue). Merged images (right lower panels, yellow or green) show co-localization of ACE and ACE2.**

Tubular Ang (1-7) expression (density/pixel) was significantly (p<0.05) downregulated in DN [12.6. (5.7-18.7)] compared with controls [31.3 (25.9-36.4) ] and MCNS [19.0 (16.7-28.3)]. Tubular MAS expression was also downregulated in DN compared with controls (p<0.001) but similar to MCNS. There were no significant changes in glomerular Ang (1-7) and MAS expression across groups.

**Tubular ACE2-Ang (1-7)-MAS axis is downregulated in patients with diabetic nephropathy and this may exacerbate renal injury.**

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2475**

**Cardiovascular Risk Factors in Type 2 Diabetic Patients with and without Renal Impairment – The Swedish National Diabetes Register (NDR)** Henrik Hadimeri,[1] Hanri Afghahi,[1] Bjorn Eliasson,[2] Jan Cederholm,[3] Maria Svensson.[2] *[1]Dept of Nephrology, Kärnsjukhuset, Skövde, Sweden; [2]Inst of Medicine, University of Gothenburg, Gothenburg, Sweden; [3]Public Health and Caring Sciences,Family Medicin and Clinincal Epidemiology, Uppsala University, Uppsala, Sweden.*

**Aim of study:** To assess differences in cardiovascular risk factors in type 2 diabetic patients with and without renal impairment in a cross-sectional study using data from a nation-wide population-based diabetes register.

**Patients and method:** 62661 patients with T2D (18-80 years) with complete datasets on albumin excretion, renal function and clinical characteristics reported to the Swedish National Diabetes Register (NDR) in 2008. Albuminuria was defined as urinary albumin excretion rate > 20 µg/min and renal impairment as estimated glomerular filtration rate; eGFR< 60 ml/min/1.73 m2 according to MDRD. **Results**: 15% of all patients had renal impairment (n=9308). 58% of these patients were non-albuminuric. Patients with renal impairment were older (71.2±6.7 vs. 64.0 ±9.3 years), had a longer diabetes duration (11.1±7.7 vs. 7.8±6.4 years), more often women (50 vs. 40%), had lower total cholesterol (4.6±1.0 vs. 4.7±1.0 mmol/L), higher triglycerides (2.0±1.2 vs. 1.8±1.1 mmol/L), higher HbA1c (7.1±1.1 vs. 7.0± 1.1 % (DCCT)), higher BMI (30.2±5.3 vs. 29.7±5.2 kg/m$^2$), higher systolic blood pressure (138±18 vs. 137±16 mmHg) and a smaller proportion were smokers (10 vs. 15%) (all p-values <0.001) compared to patients without renal impairment. When patients with and without renal impairment were compared at GLM regression adjusting for all other variables similar relationships were found except for HbA1c and systolic blood pressure where adjusted values were lower in patient with renal impairment.

**Conclusions:** The majority of patients with type 2 diabetes and renal impairment have non-albuminuric renal disease. Several differences in cardiovascular risk factor pattern were found between type 2 diabetic patients with and without renal impairment. The cause-effect relationship and potential treatment effects could not be assessed in this cross-sectional study and thus prospective studies are warranted.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2476**

**Determinants of Diabetic Nephropathy (DN) in Patients (pts) with Type 1 Diabetes (T1DM)** Maria Luiza A. Caramori, Youngki Kim, York Marahrens, Michael Mauer. *University of Minnesota.*

Stochastic and environmental factors may cause persistent epigenetic changes that alter phenotype. Genes susceptible to such variations have been shown to derive their epigenetic properties from nearby transposable elements, which make up 45% of the human genome. We studied the relationships between Alu and LINE-1 transposable element concentrations and microarray (Affymetrix HG-U133A) gene expression levels in skin fibroblasts (SF) of T1DM pts with ("fast-track") and without ("slow-track") DN. The mRNA expression value of each gene in each of the 18 "fast-track" pts (proteinuric and very rapid development of glomerular lesions) was compared to the averaged normalized SF expression value of each gene in the 20 "slow-track" pts [normoalbuminuric and very slow development of lesions]. Genes that are up- or down-regulated (≥2.4-fold) between healthy controls are typically flanked by lower Alu and higher LINE-1 concentrations than genes with lesser differences, this defining the four components of the "typical Alu/LINE-1 pattern". Eleven "fast-track" pts had the typical Alu/LINE-1 pattern and 7 were atypical, 1 with downregulated genes displaying robustly lower rather than higher concentrations of LINE-1 sequence in their flanking regions. The other 6 deviated in one or more components of the typical pattern without weakened typical patterns in the others. $HbA_{1c}$ was higher ($P$=0.001) in the atypical (10.6±1.7%) *vs*. typical (8.9±1.0) Alu/LINE-1 pattern DN "fast-track" pts. These two "fast-track" groups also showed distinctly different Affymetrix microarray directional pathway gene expression differences when compared with the "slow-track" group. OxPhos ($P$<0.001), pentose phosphate ($P$=0.013) and citrate ($P$=0.046) pathways were up-regulated in the typical Alu/LINE-1 pattern "fast-track" group, while the differences in the atypical Alu/LINE-1 pattern "fast-" *vs*. "slow-track" T1DM pts were in ribosome ($P$<0.001), aminoacyl-tRNA ($P$<0.01) and ubiquitin mediated proteolysis ($P$<0.05) pathways, these overlapping with the ones we previously observed in T1DM pts *per se vs.* normal controls (unpublished data). These studies support the concept that "fast-track" T1DM DN pts are not a homogeneous group.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2477**

**Obstructive Sleep Apnea Contributes to Renal Dysfunction in Type 2 Diabetes Mellitus** Ashish Tikotekar,[1] Anthony Sica,[2] Jean Fleischman,[1] Chinmay Patel,[1] Harigopal Reddy,[1] Erik Perez,[1] Sharon Merdian,[1] Harly Greenberg.[2] *[1]Queens Hospital Center; [2]Long Island Jewish Medical Center.*

Introduction:

Obstructive sleep apnea (OSA) & type 2 diabetes mellitus (DMII) are frequent co-morbid conditions in patients with chronic kidney disease (CKD). While microvascular renal injury is related to DMII, the contribution of OSA to renal dysfunction is unclear.

We evaluated the association of metrics describing OSA severity with markers of renal function in patients with DMII.

Method:

221 patients, age >18 yrs with DMII & symptoms of OSA who underwent polysomnography were evaluated. Pregnancy, connective tissue disorders & CKD stage 4 or 5 were exclusionary criteria. Variables assessed included measures of OSA severity [apnea hypopnea index (AHI) & % sleep time with SaO2 <90% (T90] measures of renal function [serum creatinine (SrCr), creatinine clearance (CrCl), spot urine albumin (SPA)], systolic & diastolic blood pressure [SBP & DBP], hemoglobin A1c [HbA1c] & use of ACE I or ARB.

Results:

Subjects were 58±11 yrs old, 92 m & 129 f, BMI=38±11 kg/m2, SrCr=1.04 ±0.5mg/dl, CrCl 105.6±50.9ml/min & HbA1C=7.2±1.3%. Mean AHI was in the moderate range 28.0±28.6/hr of sleep with T90%=14.1±22.5%. The percent of subjects by quartile of AHI was: Normal: 0-5=19.5%; Mild: 5-14=23.7%; Moderate:15-29=22.8%; Severe:> 30=34%.

Median (IQR) SrCr & SPA were higher in subjects with AHI>15 than in those with AHI<15 (SrCr: 1.0 (0.8 – 1.2) vs. 0.9 (0.8 – 1.0), p<0.035; SPA: 20.8 (5.0 – 28.0) vs.10 (5.0 – 52.8) µg/mg, p<0.05). Kruskal–Wallis one-way analysis of variance demonstrated a significant effect of AHI group on SrCr, p=0.04.

A multiple linear regression using a combination of predictor variables provided by best subsets analysis showed that SrCr is predicted by a highly significant (p<0.0001) combination of: age (p=0.001); AHI (p=0.027); SBP (p=0.03); HbA1C% (p=0.005); & female gender(p=0.001); R2adjusted=21.4%. Use of an ACEI or ARB was not a significant contributor to the regression.

Conclusion:

OSA severity, as assessed by the AHI, significantly contributes to renal dysfunction in DMII. Future studies should assess the impact of OSA treatment on progression of renal dysfunction in DMII.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2478

**Association of Urinary MCP-1, Serum Aldosterone, Renal Function and Microalbuminuria in Recently Diagnosed Type 2 Diabetes Mellitus Patients** Carlos Kornhauser. *Ciencias Medicas, University of Guanajuato, Leon, Mexico.*

INTRODUCTION AND AIM: The exact mechanisms of diabetic renal disease and progression remain unclear and require further investigation. MCP-1 is a highly chemotactic citokine associated with kidney disease development and progression. Our main objective was to evaluate the association of urinary MCP-1 (uMCP-1), serum aldosterone, microalbuminuria and kidney disease in recently diagnosed type 2 diabetes mellitus (DM2) patients with and without metabolic control.

MATERIAL AND METHODS: Serum glucose, creatinine, uric acid, HbA1, FGF-23, and C reactive protein, were assessed, as well as serum and uMCP-1, EGF, aldosterone, and microalbuminuria in 100 DM2 patients diagnosed in the last 14 months. Glomerular filtration rate (GFR) was estimated with the MDRD formula.

RESULTS: 15 patients out of the controlled metabolic group were in stage 1 or 2 of chronic kidney disease (CKD), 18 patients in the uncontrolled metabolic group were in stage 1 or 2 of CKD.

Microalbuminuria showed a positive association with serum uric acid (r=0.31; p<0.05) and with uMCP-1, (r=0.20; p<0.05). uMCP-1 also shows an independent positive association with serum aldosterone (r=0.20; p<0.05).

Urinary EGF showed an independent positive association with urinary aldosterone (0.22, p<0.05).

Serum uric acid as well as microalbumiuria showed a negative association with GFR (r=-0.54, p<0.05; and -0.26, p<0.05) respectively.

CONCLUSIONS: The association between urinary aldosterone and EGF may be due to EGFR activation by aldosterone stimulation in mesangial cells. Urinary MCP-1 association with microalbuminuria and kidney disease in DM2 patients shows that it may be used as an early predictor of kidney disease in these patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2479

**Nephrinuria Is a Potential Biomarker for a Loss of Renal Function in Singaporean Chinese Patients with Type 2 Diabetes Mellitus Even in the Absence of Albuminuria** Daniel P. K. Ng,[1] Mervi Ristola,[2] Bee-Choo Tai,[1] Elaine Tan,[3] Helen Soh Sum Leong,[3] Kee Seng Chia,[1,4] Chia Siong Wong,[4] Wei-Yen Lim,[4] Harry B. Holthofer.[2] *[1]Epidemiology and Public Health, National University of Singapore, Singapore, Singapore; [2]The Haartman Institute, University of Helsinki, Helsinki, Finland; [3]Clinical Services, National Healthcare Group Polyclinics, Singapore, Singapore; [4]Centre for Molecular Epidemiology, National University of Singapore, Singapore, Singapore.*

The involvement of nephrin in controlling renal function is unclear with the literature only emphasizing its role in albuminuria. We therefore investigated the potential association between nephrinuria as evidenced by the appearance of urinary immuno-positive nephrin fragments, with renal function in 381 Chinese type 2 diabetic patients. Western blot analysis of the urine samples using an antibody against human nephrin revealed the presence of up to four distinct protein bands. Each fragment was associated with a decline in estimated glomerular filtration rate (eGFR; largest P=0.003). lnACR was not a significant independent predictor of eGFR and even with the deliberate retention of lnACR in the statistical model, the 25, 50 and 60 kDa fragments remained associated with eGFR reduction (P<0.05). The 25 kDa fragment was the strongest predictor of eGFR decline, being associated with eGFR reduction of 6.55 ml/min/1.73 $m^2$ (95%CI: 0.07-13.03). Stratifying the patients according to the presence of the 25 kDa fragment did not reveal any significant differences in clinical characteristics apart from eGFR. Aside from the 75 kDa fragment, nephrinuria was significantly associated with a loss of eGFR in patients with normoalbuminuria (ACR <=30mg/g). Particularly, the presence of the 25 kDa fragment was associated with a loss in eGFR of 17.29 (95%CI: 6.56-28.01) ml/min/1.73 $m^2$ (P = 0.002). Nephrinuria may therefore provide new clinical insights into renal biology in diabetes even in normoalbuminuric patients who have traditionally been perceived as having a low risk of chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2480

**Treatment with Synthetic ACTH in Patients with Idiopathic Membranous Nephropathy and High Risk for Renal Failure** Julia M. Hofstra,[1] Hans S. Brink,[2] Jos J. Van de Kerkhof,[3] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; [2]Internal Medicine, Medisch Spectrum Twente, Enschede, Netherlands; [3]Internal Medicine, Bernhoven Hospital, Veghel, Netherlands.*

BACKGROUND: Therapy in idiopathic membranous nephropathy remains debated. New therapeutic agents are warranted. Previous studies with synthetic ACTH, in which nephrotic patients with iMN at both low and high risk for renal failure were included, showed remission rates up to 85% and no significant side effects. We conducted a prospective study in patients with iMN and a high risk for renal failure (NCT00694863).

METHODS: Patients with a biopsy proven iMN, a nephrotic syndrome, normal renal function (eGFR > 60 ml/min) and a high risk for progression (based on elevated urinary β2m and IgG levels) were treated with intramuscular injections of synthetic ACTH during 9 months. Maximal treatment dose was 1 mg tetracosactide-hexacetate (Synacthen Depot®) twice a week.

RESULTS: Preliminary results of 14 patients (M/F 11/3, age 52 ±16 yrs, sCr 102 ±20 umol/l, sAlb 23 ±6 g/L, proteinuria 11.7 ±5.5 g/day) who have completed the study so far are presented here. Twelve patients completed treatment; in 1 patient treatment was changed because of progressive renal failure, in another patient ACTH was stopped because of side effects. During follow-up renal function was stable in all but 1 patient. Proteinuria decreased with median 84% (range 29-96%), with 7 patients (50%) reaching proteinuria <2 g/day. No patient reached a complete remission. At the end of follow up (median 13 months, range 10-21) 3 out of 4 patients with a remission and follow-up >3 months after end of treatment had experienced a relapse.

CONCLUSION: Treatment with synthetic ACTH induced significant reduction of proteinuria in high risk patients with iMN. However, sustained remission was induced in only a minority of patients. These preliminary data suggest that synthetical ACTH has limited value in the treatment of high risk patients with iMN.

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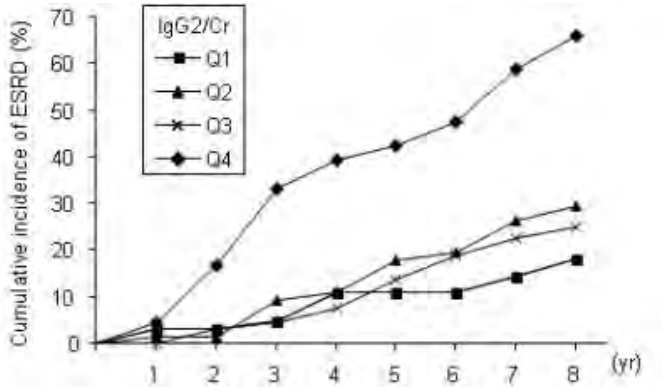
Disclosure of Financial Relationships: nothing to disclose

## SA-PO2481

**Urinary Excretion of Immunoglobulin Classes and Progression to End-Stage Renal Disease (ESRD) in Patients with Type 1 Diabetes (T1D) and Proteinuria (PT)** Tomohito Gohda, William Walker, Jan Skupien, James Warram, Andrzej S. Krolewski, Monika A. Niewczas. *Research Division, Joslin Diabetes Center, Boston, MA.*

Elevated urinary albumin excretion is a hallmark of diabetic nephropathy (DN). Some data suggest that albumin does not display a toxic effect on tubular cells. Therefore the aim of this study was to evaluate whether urinary excretion of proteins different in size and charge, classes of immunoglobulins, may predict ESRD in patients with T1D and PT. Urinary concentrations of total protein, albumin and immunoglobulins (IgG1-4, IgA, IgM), were measured at baseline using nephelometry and Luminex based-immunoassay, respectively. Total of 312 patients with T1D and PT at baseline were followed for median 5.8 yr. At entry median proteinuria was 1567 mg/g creatinine and median eGFR was 62 ml/min/1.73m². During follow-up 126 patients (40%) reached ESRD. Baseline concentrations of all immunoglobulins, albumin and total protein were significantly higher in patients who progressed to ESRD compared to those who still had functioning kidneys. In Cox analysis controlled for clinical covariates, including eGFR and AER, high concentrations of IgG2 (quartile 4 vs quartile 1-3) remained significant predictors of ESRD (hazard ratio, 95% CI, 2.3,1.5-3.8).




Spearman correlations among all proteins at baseline were statistically significant. Interestingly, all proteins also correlated strongly with urinary markers of inflammation (IL8, MCP1, IP10, IL6) and MMPs. In conclusion, increase in urinary excretion of immunoglobulins predicts progression to ESRD in subjects with T1D and proteinuria. The effect of IgG2 on this outcome was independent from AER and GFR. Our data suggest that impairment of filtration barrier as well as inflammatory processes, distinct for different proteins, might contribute to the progression of DN. This research was supported by JDRF.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2482

**Increased Platelet-Leukocyte Aggregation in Diabetic Patients with Chronic Kidney Disease (CKD) Stages 3-4** Tora C. Almquist,[1,2] Stefan H. Jacobson,[2] Per-Eric Lins,[3] Paul Hjemdahl.[1] *[1]Dept of Medicine, Clinical Pharmacology Unit, Karolinska University Hospital (Solna), Stockholm, Sweden; [2]Dept of Nephrology, Danderyd Hospital, Stockholm, Sweden; [3]Dept of Medicine, Diabetology Unit, Danderyd Hospital, Stockholm, Sweden.*

Diabetes mellitus (DM) is associated with hyperreactive platelets and increased platelet-leukocyte cross-talk, but the impact of concomitant renal dysfunction, i.e. diabetic nephropathy, has been much less studied. Platelets interact with coagulation, endothelial function and inflammation, and have a central role in the pathophysiology of atherosclerosis and thrombosis. Platelet- and leukocyte activation may contribute to the high risk of suffering atherosclerotic cardiovascular disease in patients with DM and chronic kidney disease (CKD).

Platelet- and leukocyte function, and platelet-leukocyte aggregation was evaluated in 18 DM patients with CKD stages 3-4 (estimated glomerular filtration rate (eGFR) 15-59 ml/min x1.73 m² (mean 41±14 ml/min) and in 21 DM patients with eGFR >75 ml/min x 1.73 m² (mean 87±11 ml/min), using whole blood flow cytometry.

Platelet-leukocyte aggregation (PLA) was significantly higher in patients with CKD 3-4 both in resting samples (4.9±2.2% vs. 3.4±1.7%; p=0.02) and in samples stimulated with a Collagen-Related Peptide (CRP) (53.4±5.1% vs. 46.4±11.0%; p=0.02). There was no significant difference in platelet activity between the two groups, measured as platelet P-selectin expression in unstimulated samples, or adenosine diphosphate (ADP), thrombin or CRP- induced platelet P-selectin expression or as basal or stimulated PAC-1 binding (activated fibrinogen receptor). Leukocyte activation, assessed as expression of leukocyte CD11b, tended to be higher on monocytes in unstimulated samples, in patients with CKD 3-4 (MFI 2.0 ±0.3 vs.1.8±0.4; p<0.1).

We conclude that patients with DM and CKD stages 3-4 have signs of increased platelet-leukocyte aggregation compared to DM patients with eGFR>75 ml/min. This may contribute to an increased cardiovascular risk.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2483

**Excess Urinary Excretion of Protein Glycation Adducts Predict Subsequent Early GFR Loss in Type 1 Diabetes** Bruce A. Perkins,[1] Naila Rabbani,[2] Andrew J. Weston,[2] Adaikalakoteswari Antonysunil,[2] Linda Hanna Ficociello,[3] Monika A. Niewczas,[3] James Warram,[3] Andrzej S. Krolewski,[3] Paul Thornalley.[2] *[1]University of Toronto; [2]University of Essex; [3]Joslin Diabetes Center.*

Although protein glycation is implicated in onset of microalbuminuria (MA) in type 1 diabetes, its role in the etiology of *'early GFR loss'* is unknown. Among 86 patients with normal GFR and new onset MA followed for 12 years in the First Joslin Kidney Study, we selected 22 cases of early GFR loss, defined by change in $GFR_{CYSTATIN\ C}$ exceeding -3.3 percent per year and 33 stable GFR controls. For comparison, we selected 30 contemporaneous controls with normoalbuminuria (NA) and stable GFR. Quantification of eight glycation free adducts was made by liquid chromatography-tandem mass spectrometry in baseline urine and plasma ultrafiltrate. The 55 subjects with new onset MA had similar characteristics and baseline GFR regardless of subsequent stable GFR or early GFR loss. The levels of most (7 of 8) adducts were incrementally the same or lower in the NA, MA-stable GFR, and MA-early GFR loss groups, respectively. However, urinary fractional excretion was highest in early GFR loss for the majority (5 of the 8) adducts.

Fractional Excretion (median Values) of Glycation Adducts

| | NA (n=30) | MA-Stable GFR (n=33) | MA-Early GFR Loss (n-22) | P Multi-Variate* |
|---|---|---|---|---|
| N-frutosyl-lysine | 1.00 | 1.00 | 2.04 | 0.04 |
| N-carboxymethyl-lysine | 0.30 | 0.32 | 0.52 | 0.01 |
| N-Carboxyethyl-lysine | 0.26 | 0.23 | 0.42 | 0.007 |
| Pentosidine | 0.087 | 0.72 | 1.09 | <0.001 |
| Glyoxal-Derived | 0.31 | 0.097 | 0.18 | NS |
| Methyldlyoxal-derived | 1.14 | 0.82 | 1.82 | NS |
| 3-deoxyglucosone-derived | 0.15 | 0.13 | 0.20 | NS |
| N-carboxymethylarginine | 0.043 | 0.18 | 0.32 | <0.0001 |

* adjusted for albumin excretion rate and A1c

Increased fractional excretion - not circulating levels - of glycation free adducts were independently associated with subsequent risk of early GFR loss. This "superexcretion" phenotype may be etiologically related to and serve as a biomarker for early GFR loss.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2484

**Proteinuria in Type 2 Diabetic Patients with Renal Impairment: The Changing Face of Diabetic Nephropathy** David K. Packham, Sara Ivory, Anne T. Reutens, Rory Wolfe, Richard D. Rohde, Hiddo Jan Lambers Heerspink, Jamie P. Dwyer, Robert C. Atkins, Julia Lewis. *Collaborative Study Group.*

Aim

To investigate whether traditional clinical paradigm of type 2 diabetic nephropathy (type 2 DN) has been influenced by widespread use of angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs) and to establish whether patients with lower initial PCR have a better short term renal prognosis.

Background

Type 2 DN patients who progress to end stage renal disease (ESRD) traditionally develop significant proteinuria prior to the development of renal impairment. The effect of the widespread use of ACEi or ARBs on this clinical paradigm has not been established.

Methods

2303 patients enrolled in the Sulodexide Overt Nephropathy Study (OVERT) were analysed. Incidence of renal impairment with and without significant proteinuria was established and comparison made between groups. Follow up data on randomized patients for those with urinary protein creatinine ratio (PCR) <1000mg/g at screening was compared with those with PCR ≥1000 mg/g.

Results

Twenty two percent had significant renal impairment with a PCR at screening of <500mg/g. Therapy with ACEi and/or ARBs at the time of screening was recorded in 94%, where prior medication data was available. Randomised patients who had screening PCR < 1000mg/g had an annualized incidence rate of ESRD 0.5%, less than one tenth of those with screening PCR > 1000mg/g (5.4%).

Conclusions

In patients with type 2 DN and moderately severe renal impairment, low levels of proteinuria are found in over one fifth of patients. This suggests an effect of the widespread usage of ACEi or ARBs on the traditional clinical paradigm of type 2 DN.

Patients with an initial screening PCR <1000mg/g appear to have a better long-term prognosis than those with PCR ≥1000mg/g.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2485

**Progression of Diabetic Kidney Disease in Type 1 and Type 2 Diabetic Patients** Nobue Tanaka, Tetsuya Babazono, Yasuhiko Iwamoto. *Diabetes Center, Tokyo Women's Medical University, Tokyo, Japan.*

The impact of type of diabetes (type 1 or type 2) on the progression of diabetic kidney disease is not well known. We therefore conducted this observational cohort study to evaluate the differences in the decline in glomerular filtration rate (GFR) and progression of albuminuria between type 1 and type 2 diabetic patients.

A total of 3,856 adult patients with diabetes were recruited into this study between 2003 and 2005, including 693 patients with type 1 (mean [± SD]; 38 ± 12 years) and 3,163 patients with type 2 diabetes (60 ± 12 years). Albuminuria was assessed based on albumin-to-creatinine ratio (ACR), and classified into normoalbuminuria (n=2,664), microalbuminuria (n=824), and macroalbuminuria (n=368). The rate of change in estimated GFR (eGFR) was determined by regression analysis with eGFR as a function of time, and was compared by means of analysis of covariance, adjusting for age, sex, blood pressure, duration of diabetes, eGFR at baseline, ACR, hemoglobin, HbA1c and total cholesterol. Cumulative incidence of transition from any stage to a more advanced stage of albuminuria was estimated using the Kaplan-Meier method.

eGFRs (mL/min/1.73 m²) at baseline were 88.0 ± 20.2 for type 1 and 74.8 ± 20.8 for type 2 diabetic patients (p<0.001). Patients were followed-up for 4.5 ± 0.8 years. The mean rates of change in eGFR (%/year), after adjustment for clinical parameters, were -0.97 ± 0.16 for type 1 and -1.09 ± 0.08 for type 2 in normoalbuminuric patients ($p$=0.53, ANCOVA), -2.56 ± 0.55 and -2.82 ± 0.16 in microalbuminuric patients ($p$=0.66), and -9.36 ± 1.78 and -9.94 ±0.50 in macroalbuminuric patients ($p$=0.76), respectively. In Kaplan-Meier method, the incidence of microalbuminuria was significantly higher in type 2 than type 1 diabetic patients (log-rank test p<0.001). There was no difference, however, in the progression to macroalbuminuria from microalbuminuria (log-rank; p=0.419).

In conclusion, this longitudinal study suggests that, while the decline in kidney function is similar in type 1 and type 2 diabetic patients in each stage of albuminuria, the incidence of microalbuminuria is likely to be higher in type 2 diabetic patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2486**

**Which Level of $HbA_{1c}$ Is Appropriate for Prevention of ESRD in Diabetes?**
Sewon Oh,[1] Ho Seok Koo,[2] Kook-Hwan Oh,[2] Ki Young Na,[1] Kwon Wook Joo,[2] Chun-Soo Lim,[2] Yon Su Kim,[2] Dong Wan Chae,[1] Curie Ahn,[2] Jin Suk Han,[2] Suhnggwon Kim,[2] Ho Jun Chin.[1] *[1]Department of Internal Medicine, Seoul National University Bundang Hospital, Kyeong-Kido, Democratic Peoples Republic of Korea; [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Democratic Peoples Republic of Korea.*

**Background** : Diabetes mellitus (DM) is a common cause of end stage renal disease (ESRD). The KDOQI guidelines recommend glycated hemoglobin ($HbA_{1c}$) should be lower than 7.0% to reduce the rate of decrease in glomerular filtration rate (GFR). However, it is controversial whether tighter glycemic control is associated with better clinical outcomes. We assessed the relationship between ESRD and the $HbA_{1c}$ and the association between all cause mortality and $HbA_{1c}$ in patients with DM.

**Methods:** A cohort of patients aged 25 years and older who had treated for DM were generated from Seoul National University Bundang Hospital (SNUBH) database from January 2004 to December 2004. We identified 4494 patients and classified patients into four groups by the first measured $HbA_{1c}$ in 2004 ($HbA_{1c}$ <6.5%, 6.5-7.5%, 7.6-8.5% and >8.5%). We obtained data from SNUBH electronic resources to search for medical history by International Classification of Disease (ICD)-10 code and medication history in 2004 by Anatomical Therapeutic Chemical Cassificatio System (ATC) code. Age, sex, cholesterol, blood pressure, GFR, medication history, cardiovascular disease, cancer and the duration of DM were identified as confounding factors, and cox survival models were adjusted for these factors.

**Results:** Ninety one patients developed ESRD during 63 months of mean follow up period. The observed event rates were 1.3%, 1.8%, 2.4% and 3.1% in $HbA_{1c}$ <6.5%, 6.5-7.5%, 7.6-8.5%, and >8.5% group, respectively. The cumulative renal survival benefit was observed in the lowest $HbA_{1c}$ group. Compared with the lowest $HbA_{1c}$ group with the lowest hazard, the adjusted hazard ratio of ESRD in $HbA_{1c}$ 6.5-7.5 % group was 2.93, $HbA_{1c}$ 7.6-8.5 % group was 4.44 and $HbA_{1c}$>8.5 % group was 4.51. All cause mortality was not associated with $HbA_{1c}$.

**Conclusions:** Lowered $HbA_{1c}$ value to 6.5% was associated with reduced development of ESRD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2487**

**Vitamin D Levels and Asymptomatic Coronary Artery Disease in Type 2 Diabetic Patients with Elevated Urinary Albumin Excretion Rate**
Christel Joergensen,[1] Henrik Reinhard,[1] Anne Schmedes,[2] Peter R. Hansen,[3] Niels Wiinberg,[4] Claus Leth Petersen,[4] Kaj Winther,[8] Hans-Henrik Parving,[5] Peter Karl Jacobsen,[7] Peter Rossing.[1] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]Dept. of Clinical Biochemistry, Lillebaelt Hospital, Vejle, Denmark; [3]Dept. of Cardiology, Gentofte University Hospital, Denmark; [4]Dept. of Clinical Physiology and Nuclear Medicine, Frederiksberg University Hospital, Denmark; [5]Dept. of Medical Endocrinology, Copenhagen University Hospital, Rigshospitalet, Denmark; [6]The Heart Centre, Copenhagen University Hospital, Rigshospitalet, Denmark; [7]Dept. of Clinical Biochemistry, Frederiksberg University Hospital, Denmark.*

***Aims*** Severe vitamin D deficiency has been shown to predict cardiovascular mortality in type 2 diabetic patients. We investigated the association between severe vitamin D deficiency and asymptomatic Coronary artery disease (CAD) as well as coronary calcium score (CCS) in type 2 diabetic patients with urinary albumin excretion rate (UAER) > 30mg/24h.

***Methods*** A cross sectional study including 200 type 2 diabetic patients without clinical signs of CAD. Severe vitamin D deficiency was defined as p-25OHD < 12.5nmol/l. Patients with p-NT-proBNP > 45.2ng/L and/or CCS > 400 were stratified as high risk patients of CAD (n=133). High risk patients were examined by myocardial perfusion imaging (MPI; n=109), and/or CT-angiography (CTA; n=20), and/or coronary angiography (CAG; n=86).

***Results*** Median (range) vitamin D level was 36.9 (3.8-118.6)nmol/l. The prevalence of severe vitamin D deficiency was 9.5% (19/200). In 70 (35%) patients, significant CAD was demonstrated by MPI and/or CAG.

Severe vitamin D deficiency was associated with asymptomatic CAD, odds ratio (OR) [95% CI] 2.2 [0.9-5.8]. After adjusting for additional risk factors, OR was 5.0 [1.3-19.5]. The prevalence of CCS > 400 was 34% (68/200). Severe vitamin D deficiency was associated with CCS>400, OR 4.3 [1.5-12.1]. The association persisted after adjusting for additional risk factors, OR 4.0 [1.2-13.1].

***Conclusion*** In high risk type 2 diabetic patients with elevated UAER, low levels of vitamin D are strongly and independently associated with asymptomatic CAD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2488**

**Urine Metabalomic Profile of Diabetic Nephropathy**
Anna V. Mathew,[1] Satish P. Ramachandrarao,[1] Joachim H. Ix,[2] Ronghui Xu,[3] Bruce A. Barshop,[4] Robert K. Naviaux,[4] Kumar Sharma.[1] *[1]Center for Renal Translational Medicine, University of California San Diego; [2]Department of Nephrology, University of California San Diego; [3]Department of Mathematics, University of California San Diego; [4]Mitochondrial and Metabolic Disease Center, University of Califonia San Diego, La Jolla, CA.*

We studied the urine metabolite profile of diabetic patients with kidney disease to help identify organic acid derangements that can serve as biomarkers.

**Methods:** Our study included 14 patients with diabetic kidney disease (eGFR 31.79±7.329)**(DN)**. This population was compared to a control of 23 healthy volunteers with no diabetes or kidney disease. The control group contained 6 (26%) and the DN group contained 5 (35%) females. 24 hour urine was collected from both groups. A composite quantitative urine organic acid estimation was done using the Agilent 5973 GC/MS. 76 different organic acids were looked at per sample and the results were standardized per mmol of creatinine. We compared the 2 groups for each of the 76 metabolites and found 11 significant metabolites using the unpaired t-test. We chose a cut off of P <0.00846 to have an estimated false discovery rate <0.05. Glomerular filtration rate (GFR), albumin creatinine ratio (ACR) of the DN group were then correlated to the 11 significant metabolites using linear regression analysis**.**

**Results:** We found that glycolic acid, 3OH isobutyric acid, 3OH isovaleric acid, aconitic acid, homovanillic acid, succinic acid, citric acid, uracil and glutaric acid were significantly decreased in the DN group. 5OH hexanoic acid was significantly increased in the DN group when compared to the controls. Of these metabolites, only citric acid ($r^2$=0.4755) negatively correlated and 3OH isovaleric acid levels ($r^2$=0.4755) positively correlated to ACR but not to GFR.

**Conclusion:** We found that the organic acid profile of urine from DN patients to be significantly decreased in metabolites related to the mitochondrial intermediary metabolism. This finding will be validated in a larger patient population and hopefully will lead to identification of biomarker profiles that provide more information than GFR in these patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2489**

**Greater Frequency of End Stage Renal Disease Than Cardiovascular Mortality in Proteinuric Type 2 Diabetic Nephropathy: Results from the DIAMETRIC Database**
David K. Packham, Tahira P. Alves, Jamie P. Dwyer, Robert C. Atkins, Dick De Zeeuw, Shahnaz Shahinfar, Julia Lewis, Hiddo Jan Lambers Heerspink. *Clinical Pharmacology, University Medical Centre, Groningen, Netherlands.*

Aim

To investigate comparative incidence of cardiovascular death (CVD) versus progression to end stage renal disease (ESRD) in a cohort of proteinuric type 2 diabetic patients with renal impairment.

Background

Previous studies have demonstrated that patients with chronic kidney disease (including diabetic nephropathy) are more likely to die from cardiovascular disease than ESRD. This study was conducted to determine whether ESRD is a more common primary outcome than CVD in patients with established proteinuric type 2 diabetic nephropathy (type 2 DN) and renal impairment.

Methods

Data on 3228 adult patients with type 2 DN from the IDNT and RENAAL trials were combined to establish the Diabetes Mellitus Treatment for Renal Insufficiency Consortium (DIAMETRIC) database. The incidence rate of ESRD was compared with CVD and all cause mortality (ACM) in the overall population and in subgroup analysis.

Results

Mean follow up was 2.8 years. A total of 19.5% of patients developed ESRD, roughly five times the incidence of CVD and three times the incidence of ACM. ESRD was more common than CVD in all subgroups analysed with the exception of subjects with low levels of albuminuria and well preserved levels of renal function at baseline.

Conclusions

Patients with type 2 DN, characterized by renal impairment and significant proteinuria, are more likely to reach ESRD than die. Given the rapidly increasing number of cases of type 2 diabetes worldwide, this has implications for predicting the future renal replacement therapy (RRT) requirements.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2490**

**Effect of Oral Cholecalciferol (Vitamin $D_3$) on Proteinuria and Cytokines in Type 2 Diabetic Patients with Chronic Kidney Disease (CKD)**
Min Jeong Kim,[1] Andrew H. Frankel,[1] Mandy Donaldson,[2] Sarah J. Darch,[2] Charles D. Pusey,[1] Frederick W. K. Tam.[1] *[1]Kidney and Transplant Institute, Hammersmith Hospital, Imperial College London; [2]Clinical Biochemistry, Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: Anti-inflammatory effects of active vitamin D have been demonstrated *in vitro* as well as *in vivo*. Growing evidence indicates that active vitamin D analogues may have anti-proteinuric and renoprotective effects in CKD patients. However, it remains

unclear, if treatment with inactive vitamin D can achieve similar effects. In a prospective study, the effect of cholecalciferol on proteinuria and urinary cytokines were investigated in type 2 diabetic patients.

Methods: Type 2 diabetic patients with CKD stage 2-4 attending the Hammersmith Hospital renal outpatient clinic were enrolled in a prospective study. Patients taking vitamin D analogues were not eligible. Serum 25(OH)D and $1,25(OH)_2D$, and urinary albumin-creatinine ratio (ACR), monocyte chemoattractant protein-1 (MCP-1) and transforming growth factor-β1 (TGF-β1) were measured. Patients with insufficient levels of 25(OH)D (< 80 nmol/L) were treated with cholecalciferol according to the hospital standard management guidelines and monitored at 2 and 4 months.

Results: From 67 enrolled patients, 54 patients with insufficient 25(OH)D were treated and 45 patients were monitored at 2-month follow-up. Serum 25(OH)D and $1,25(OH)_2D$ increased from 38.5 nmol/L (median; interquartile range 22-47.5) to 95 (85 – 123) and 20.2 pg/mL (13.6 – 32.5) to 48.9 (38.1 – 66.4) ($p < 0.0001$) respectively. ACR decreased from 13 mg/mmol (4.1 – 89.3) to 10.7 (2.7 – 53.8) ($p= 0.0042$). All patients except 3 were on RAS inhibition at the enrolment. Blood pressure and eGFR were stable. No significant changes of urinary MCP-1/creatinine ratio (10.6 ng/mmol (7.1 – 22.2) to 11.7 (6.9 – 19.7)) and TGF-β1/creatinine ratio (34.1 (16.9 – 56.2) to 17.9 (9.3 – 47.4)) were observed. No adverse effect was observed.

Conclusions: This is the first report showing a potential anti-proteinuric effect of cholecalciferol in type 2 diabetic patients. There was a trend of reduction in urinary TGFβ1. The ongoing follow-up data will clarify the results.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2491

**Impaired Renal Function Modifies the Risk of Severe Hypoglycemia among Users of Insulin but Not Glyburide: A Nested Case-Control Study** Matthew A. Weir,[1] Tara Gomes,[2] Muhammad Mamdani,[2,3,4] David N. Juurlink,[2,3] Daniel G. Hackam,[5,6] Arsh Jain,[1] Amit X. Garg.[1,2,6] *[1]Medicine, Division of Nephrology, University of Western Ontario, London, ON, Canada; [2]Institute for Clinical Evaluative Sciences, Toronto, ON, Canada; [3]Health Policy, Management and Evaluation, University of Toronto, Toronto, ON, Canada; [4]Applied Health Research Center, Li Ka Shing Knowledge Institute, Toronto, ON, Canada; [5]Clinical Pharmacology, University of Western Ontario, London, ON, Canada; [6]Epidemiology and Biostatistics, University of Western Ontario, London, ON, Canada.*

Background: Little evidence justifies the avoidance of glyburide in patients with impaired renal function. We aimed to determine if renal function modifies the risk of hypoglycemia among patients using glyburide.

Methods: We conducted a nested case-control study using administrative records and laboratory data from Ontario, Canada. We included outpatients 66 years of age and older with diabetes mellitus and prescriptions for glyburide, insulin or metformin. We ascertained hypoglycemic events using administrative records and we estimated glomerular filtration rates (eGFR) using serum creatinine concentrations.

Results: From a cohort of 19,620 patients, we identified 204 cases whose eGFR was ≥ 60 ml/min/1.73m2 (normal renal function) and 354 cases whose eGFR was < 60 ml/min/1.73m2 (impaired renal function). Compared to metformin, glyburide associated with a greater risk of hypoglycemia in patients with both normal (adjusted OR 9.0, 95% CI 4.9 to 16.4) and impaired renal function (adjusted OR 6.0, 95% CI 3.8 to 9.5). We observed a similar relationship when comparing insulin to metformin; the risk was greater in patients with normal renal function (adjusted OR 18.7, 95% CI 10.5 to 33.5) compared to those with impaired renal function (adjusted OR 7.9, 95% CI 5.0 to 12.4). Tests of interaction showed that among glyburide users renal function did not significantly modify the risk of hypoglycemia, but among insulin users, impaired renal function associated with a lower risk.

Conclusions: In this population-based study, impaired renal function did not augment the risk of hypoglycemia associated with glyburide use.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2492

**Gene Expression Profiles in Human Proximal Tubular Cells (PTC) Exposed to Urines from Patients with Type 1 Diabetes (T1D) and Early GFR Loss** Bozena Krolewski,[1] Krzysztof Wanic,[1,2] Monika A. Niewczas,[1,3,4] William Walker,[1] James Warram,[1] Andrzej S. Krolewski.[1,3] *[1]Research Division, Joslin Diabetes Center, Boston, MA; [2]Metabolic Research Unit, Department of Endocrinology, St. James's Hosital, Trinity College, Dublin, Ireland; [3]Department of Medicine, Harvard Medical School, Boston, MA; [4]Department of Immunology, Transplantology and Internal Diseases, Medical University of Warsaw, Poland.*

The goal of our study was to identify novel genes possibly involved in the development of early GFR loss in patients with T1D and microalbuminuria. We hypothesize that development of an early GFR loss may be initiated by toxic protein(s) and/or growth factors present in urines of these patients possibly inducing proximal tubular cell damage.

In our in vitro experiments, human immortalized PTC (HK-2) cells were exposed to pooled concentrated (2x), endotoxin-free urines collected from two groups of T1D patients with microalbuminuria who were followed for 4-8 years and during that follow-up had either early GFR loss or stable renal function. At baseline, when urine specimens were collected, all patients had normal GFR and similar elevated urinary albumin excretion.

Following 6 hrs exposure to the pooled urines, RNA was extracted from cells and genome-wide expression profiles were examined using Ilumina Sentrix Beadchip Array (Human-8V2) in two experiments and a pattern of expression of specific genes was confirmed in the third experiment by qPCR. In each experiment urines from different sets of patients were used. In the analysis, we used the fold change criterion <0.77or > 1.3. Interestingly in all three experiments, we found that urines obtained from patients who were at high risk of early GFR loss induced significant changes in expression of four distinctive inflammatory genes: *IL8, CSF2, MAPK6* (all up-regulated) and *LRRF1PI* (down-regulated) (False Discovery Rate < 0.0001).

All these genes (members of inflammatory pathways) are mechanistically closely related, most likely causing damage to PTC cells. These preliminary data could possibly help to identify and characterize novel therapeutic target(s) for intervention to prevent the early GFR loss in T1D patients.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2493

**Serum Fructosamine (SF), but Not Glycosylated Hemoglobin (A1c), Predicts Survival in Diabetic Hemodialysis (HD) Patients (pts)** Neal Mittman, Brinda Desiraju, Hitesh Kapupara, Jyotiprakas Chattopadhyay, Morrell M. Avram. *Avram Division of Nephrology, Long Island College Hospital, Brooklyn, NY.*

Diabetes is the most common cause of end-stage renal disease and an important risk factor for morbidity and mortality in dialysis patients. We and others have suggested that glycosylated hemoglobin (A1c) may not be a reliable measure of glycemic control in dialysis patients, and therefore have explored the use of serum fructosamine (SF) as an alternative marker. We recently reported that albumin-corrected fructosamine (AlbF), but not HbA1c, is associated with morbidity (hospitalizations and infections) in diabetic HD pts. The objective of this study was to investigate the prognostic importance of enrollment SF on survival. We enrolled 100 diabetic HD pts and followed them prospectively for up to 5 1/2 years. Demographics, clinical, biochemical and outcome data were recorded. The mean age was 63 years. Fifty percent were women and the majority were African-Americans (72%). SF values were corrected for the variations in the concentration of serum albumin (AlbF) (method of Lamb et al., 1993). Mean values for SF and AlbF were 373 µmol/l (range: 243-618 µmol/l) and 973 µmol/g (range: 607–1994 µmol/g), respectively. During the study period, 44 pts (44%) expired. Pts who died during the study had significantly higher AlbF (1025 vs. 911, p=0.033) compared to those who survived. Using Cox's multivariate regression analysis, adjusting for age, race, gender and months on dialysis at enrollment, AlbF was a significant independent predictor of mortality (Relative Risk=1.002, p=0.011) in these diabetic HD pts. In contrast, A1c did not predict mortality (p=0.15) in this population.

Predictors of Mortality in HD: Cox's Multivariate analysis

| Variable | Relative Risk | p |
|---|---|---|
| Age (years) | 1.033 | 0.066 |
| Gender (Male vs. Female) | 1.166 | 0.85 |
| Race (AA vs. others) | 0.62 | 0.16 |
| AlbF (µmol/g) | 1.001 | 0.031 |

AA= African American

In conclusion, AlbF, but not A1c, the most commonly utilized measure of glycemic control, predicts long-term survival as well as morbidity in diabetic HD pts.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2494

**Residual Proteinuria and eGFR Predict Progression of Renal Impairment within 2 Years in Type 2 Diabetic Patients with Established Proteinuria and Renal Impairment Who Have Received Irbesartan for a Minimum of 3 Months** David K. Packham, Sara Ivory, Rory Wolfe, Anne T. Reutens, Richard D. Rohde, Julia Lewis, Robert C. Atkins. *Collaborative Study Group, Chicago, IL.*

Aim

To investigate whether residual urinary proteinuria (UPr) and estimated glomerular filtration rate (egfr) predict incidence of significant progression of renal impairment in patients with established type 2 diabetic nephropathy (type 2 dn) already receiving full dose angiotensin receptor blocker (ARB) therapy.

Background

Both UPr and egfr are established predictors of progression in type 2 dn patients with established proteinuric renal impairment and ARBs have been shown to significantly improve their renal prognosis. The relative significance and interaction between UPr and egfr in patients already receiving a maximal dose of ARB before measurement of these predictors is yet to be established.

Methods

1,278 patients with type 2 diabetic nephropathy and moderate proteinuric renal impairment were analysed retrospectively, 413 from the Irbesartan Diabetic Nephropathy Trial and 885 from the Sulodexide Overt Nephropathy Study. Patients had received irbesartan 300mg daily, or a maximum tolerated dose, for at least 3 months. A significant renal event was defined as end stage renal failure, a doubling of baseline creatinine or a creatinine > 6 mg/l. Survival analysis was used to provide estimates of 24 month absolute risk for nine categories in a 3x3 matrix for levels of UPr and eGFR.

Results

UPR and egfr were strongly related to risk of significant progression of renal failure. The interaction between eGFR category and proteinuria category was not statistically significant.

eGFR PCR
<1000 1000-<2000 2000+
45 - <60 0.00% 3.1% 10.7%
30 - <45 6.7% 4.0% 21.4%
15 - <30 7.0% 12.9% 45.2%

Conclusions

Patients with type 2 dn and moderately severe proteinuric renal impairment already stabilized on ARB, have increased risk of renal events with increasing proteinuria and decreasing eGFR over the medium term. Awareness of the wide variation in incidence among subgroups is of value in clinical management and the design of clinical trials.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2495**

**Lack of Racial/Ethnic Differences in the Progression of Nephropathy in a Hypertensive Diabetic Population** Raymond Estacio,[1,2] Brenda Beaty,[2] Rebecca Hanratty,[1,2] Michel B. Chonchol.[2] *[1]General Internal Medicine, Denver Health, Denver, CO; [2]University of Colorado Denver, Denver, CO.*

Purpose: We examined the relationship of race and ethnicity, glycemic and blood pressure control on the occurrence or progression of diabetic nephropathy.

Methods: A retrospective cohort study of diabetic patients with hypertension in a safety-net community health system in Denver, Colorado. A total of 923 type 2 diabetic patients with concurrent measures of systolic blood pressure (SBP), hemoglobin A1c (HbA1c), and urinary albumin/creatinine ratio (ACR) were included. We evaluated the relationship of selected sociodemographic and clinical factors on the change in $\log_{10}$ (ACR). Mixed effects longitudinal models to partition between and within patient effects of SBP and HbA1c on log (ACR) over time were utilized

Results: The mean follow-up period was 47.3 ± 23.9 months. Analyses were adjusted for age, gender, race/ethnicity, insurance status, history of vascular disease, time between diabetes diagnosis and ACR measurement, and baseline estimated glomerular filtration rate. Higher baseline HbA1c and SBP were associated with higher baseline $\log_{10}$(ACR) values in the whole population ($p<0.001$ for both). No differences in the $\log_{10}$(ACR) were seen when comparing African Americans and Hispanics with non-Hispanic whites. Analyses with SBP and HbA1c as time-varying covariates and complete partitioning of between and within patient effects demonstrated that a decline in SBP and in HbA1c were independently associated with a decline in $\log_{10}$(ACR) ($p<0.0001$ for both), after adjusting for patients' overall SBP and HbA1c levels and level of albuminuria.

Conclusion: In a population of hypertensive diabetic patients, race and ethnicity was not associated with the progression of diabetic nephropathy. The current findings suggest that any improvement of BP and glycemic control no matter the overall level of control or albuminuria is independently associated with improvement of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2496**

**Glycated Albumin as an Indicator of Glycemic Control in Predialysis Diabetic Nephropathy Patients** Hyeong Cheon Park,[1] Sung Jin Moon,[1] Jung Eun Lee,[2] Sung Chang Bae,[1] Sung-Kyu Ha.[1] *[1]Internal Medicine, Gangnam Severance Hospital, Seoul, Korea; [2]Internal Medicine, Yongin Severance Hospital, Yongin, Korea.*

Glycated albumin (GA) has been proposed to better reflect glycemic control in diabetic hemodialysis patients. We assessed the usefulness of GA as a parameter of glycemic control in predialysis diabetic nephropathy patients.

A total of 499 diabetic patients (296 men, mean age 61.6 ± 12.5 years) were enrolled in this cross-sectional study. Parameters of glycemic control (mean glucose, HbA1C, and GA) were investigated along with other biochemical and clinical parameters.

The patient distribution according to CKD stages from 1 to 5 were 51, 116, 115, 35, and 182, respectively. When CKD 5 was compared to CKD 1, the mean glucose level was significantly higher (179±60 vs. 158±43 mg/dL, $p<0.02$) whereas HbA1c was much lower (7.3±1.3% vs. 8.4±1.8%, $p<0.001$) in CKD 5. The slope of the regression line between mean glucose and HbA1C was more shallow (CKD 1: beta=0.027 vs. CKD 5: beta= 0.007, $p<0.001$), and the GA/HbA1C ratio was significantly increased (CKD 1: 2.58 vs CKD 5: 3.84, $p<0.001$) in CKD 5 patients. However, no significant differences in the relationship between GA and mean glucose were observed between CKD 1 and 5. HbA1C in CKD 5 was negatively associated with weekly erythropoietin dose. In CKD 3 and 4 patients, the GA/HbA1C ratio also showed significant increase compared to CKD 1 (2.9±0.6 and 3.0±0.6, $p<0.001$) and the slopes of the regression lines between mean glucose and HbA1C in CKD 3 and 4 were significantly more shallow compared with CKD 1 (beta: CKD1=0.027, CKD2= 0.020, CKD3= 0.016, CKD4= 0.016, $p<0.05$). However, slopes of the regression lines between mean glucose and GA were comparable among the 4 groups. Multiple linear regression analysis showed that CKD stage 3, 4 status significantly impacted HbA1C, without significant effect on GA. In conclusion, our results suggest that HbA1C underestimate the status of glycemic control even in predialysis diabetic nephropathy patients as well as diabetic hemodialysis patient. The GA, however, may more accurately reflect glycemic control in these predialysis diabetic patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2497**

**Pentoxifylline for Renoprotection in Diabetic Nephropathy: The PREDIAN Study** Juan F. Navarro,[1,2] Carmen Mora,[2] Mercedes Muros,[3] Antonio Rivero,[1] Nieves Del Castillo,[1] Javier García.[1] *[1]Nephrology Service, University Hospital Nuestra Señora de Candelaria (HUNSC); [2]Research Unit, HUNSC; [3]Clinical Biochemistry, HUNSC, Santa Cruz de Tenerife, Spain.*

Diabetic nephropathy (DN) is the main cause of end-stage renal disease (ESRD). Despite renin-angiotensin system (RAS) blockade, a significant proportion of patients progress to ESRD. Pentoxifylline (PTF) posses antiproteinuric and anti-inflammatory effects, suggesting potential renoprotective efficacy. The aim of the Pentoxifylline for Renoprotection in Diabetic Nephropathy (PREDIAN) study is to test the efficacy of PTF addition to RAS blockade on the progression of DN. Here we report the study design and the baseline patient characteristics.

The PREDIAN study is 2-year, investigator-initiated, single-center, prospective, randomized, controlled, clinical trial funded by the Spanish Ministry of Science and Innovation. One-hundred and sixty nine type 2 diabetic patients with chronic kidney disease (CKD) stages 3 and 4 were randomized to a control group (N=87) or an active group (N=82). All patients received angiotensin-convering enzyme inhibitor (ACEI) or angiotensin II receptor blockers (ARB) at the maximal recommended dosage for more that 6 months. Patients in the active group were treated with PTF (1200 mg/day) on top of ACEI or ARB, whereas control subjects continued with RAS blockers. The primary outcome measure was the difference in estimated glomerular filtration rate (eGFR) between the groups at the end of the study. Baseline characteristics of the subjects are: 116 patients (68.6%) stage 3 and 53 (31.3%) stage 4 CKD, age 69±9 years, duration of diabetes 15±3 years, eGFR 37±12 ml/min/1.73m$^2$, albuminuria 1.39±1.16 g/day, blood pressure 142±8/86±8 mmHg. Serum concentrations of inflammatory cytokines (tumor necrosis factor-alpha (TNFa), interleukin-6 (IL-6) and interleukin-10 (IL-10)) and polymorphisms of the coding genes for these molecules (TNFa, –174 G/C; IL-6, –308 G/A; and IL-10, –1082 G/A), and the relationships among these variables with albuminuria and eGFR are studied. In conclusion, the PREDIAN study will provide evidence on the renoprotective benefit of PTF in addition to RAS blockade in DN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2498**

**Pulse Pressure Predicts All-Cause and Cardiovascular Mortality, Cardiovascular Events and Progression to End Stage Renal Failure in Type 1 Diabetic Patients** Simone Theilade,[1] Maria Lajer,[1] Anders Jorsal,[1] Lise Tarnow,[1] Hans-Henrik Parving,[2] Peter Rossing.[1] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]Dept. of Medical Endocrinology, Copenhagen University Hospital, Rigshospitalet, Denmark.*

***Background and Aim***

Patients with diabetes have an elevated risk of early death due to cardiovascular disease (CVD) and development of end stage renal disease (ESRD). The aim of this analysis is to evaluate whether office pulse pressure (PP) (as an estimate of arterial stiffness) predicts mortality, cardiovascular events and progression to ESRD in patients with type 1 diabetes.

***Materials and Methods***

A prospective observational follow-up study including 900 patients with type 1 diabetes (512 men; age 44±11 years (mean±SD) ; duration of diabetes 28±11 years). Of these patients, 458 had diabetic nephropathy (glomerular filtration rate (GFR) 76±34ml/min/1.73 m$^2$) and 442 had persistent normoalbuminuria. The patients were followed for 8(0-13) years (median(range)).

***Results***

During follow-up 178(20%) patients died, of which 109(12%) where CVD deaths and 75 patients (16% of patients with diabetic nephropathy) developed ESRD. Patients with elevated PP had significantly higher all-cause mortality (adjusted HR (per 10 mmHg increase) 1.2(1.1-1.4); $p<0.001$), CVD mortality (adjusted HR 1.3(1.2-1.5); $p<0.001$), non fatal CVD events (adjusted HR 1.2(1.0-1.3); $p=0.01$) and combined fatal and non-fatal CVD (adjusted HR 1.2(1.1-1.3); $p<0.001$), (adjusted for sex, age, duration of diabetes, smoking, diastolic blood pressure, $HbA_{1c}$, cholesterol, UAER, history of CVD and nephropathy status).

In patients with diabetic nephropathy elevated PP was associated with progression to ESRD (adjusted HR (per 10 mmHg increase) 1.2(1.0-1.4); $p=0.048$), (adjusted for sex, age, duration of diabetes, diastolic blood pressure, $HbA_{1c}$, cholesterol and UAER).

***Conclusion/interpretation***

Elevated office PP predicts all-cause and CVD mortality, CVD events and progression to ESRD in patients with type 1 diabetes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2499**

**Serum Levels of Cleaved and Intact Forms of Tumor Necrosis Factor Receptor 1 (TNFR1) and Early Progressive Renal Function Loss in Patients with Diabetes** William Walker, Monika A. Niewczas, Souphatta Sasorith, Andrzej S. Krolewski. *Joslin Diabetes Center, Boston, MA.*

In diabetes, progressive renal function decline can begin early when patients have microalbuminuria and normal renal function. Recently we have shown that the serum concentration of the soluble TNFR1 protein is an extremely good predictor of this early GFR loss, and subsequent development of ESRD. Also, it has been shown that sTNFR1

exists in two forms in serum: a cleaved 28-kDa form and an intact, full length 55-kDa form. Thus, the goal of this study was to determine which form of the protein is associated with early GFR loss in patients with Type 1 Diabetes.

We chose 79 individuals from the *Second Joslin Kidney Study on the Natural History of Microalbuminuria*. All subjects had microalbuminuria at baseline examination and were followed for 4-6 years to obtain serial measurements of serum cystatin C for tracing the trajectory of GFR loss. Changes over time were summarized as percent change in GFRcystatin per year using a mixed effects model. All patients had GFRcystatin >60 ml/min at entry. During follow-up, the annual percent change in GFRcystatin varied among patients from -32.3 to + 3.7 (median -2.53). Baseline specimens were analyzed using western blots to quantitatively determine the presence of both forms of the protein. These results, combined with ELISA measurements, allowed for calculation of the concentration of each form of the receptor. Simple linear regression analysis of the percent change in GFRcystatin in comparison with six important clinical characteristics (intact and cleaved TNFR1 concentration, HbA1C, AER, baseline GFR, and age) revealed that the only significant predictor of renal function loss is the intact TNFR1 concentration ($p = .0034$). This form of the receptor remains as the only significant contributor when including all six of the covariates in a multiple regression model. More specifically, as the intact TNFR1 concentration increases by quartile, the GFRcystatin decreases at an accelerated rate of 1.41% per year ($p = .02$). In conclusion, these results support the hypothesis that the differing forms of sTNFR1 have distinct effects on renal function loss in patients with T1D.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2500

**Epidemiology of Diabetic Nephropathy in African American Children with Type 2 Diabetes Mellitus** Josefina Sharma, Shirley Shwu-Shiow Chang. *Pediatrics Division of Nephrology, SUNY Downstate Medical Ctr, Brooklyn, NY.*

We conducted a retrospective longitudinal study on the natural course of diabetic nephropathy in African American Children with type 2 diabetes mellitus (T2DM) followed at SUNY Downstate and Kings County Hospital Center from 1999 to 2010. Inclusion criteria consist at least 2 spot urine albumin to Cr (UAC) ratios, at least 6 months of diabetes duration, and childhood onset T2DM. There were 31 subjects (68% F, 32% M; 84% AA, 10% Hispanics, 6% Asians), with diabetes duration 5.0 ± 5.7 years (mean ± 2SD), age of T2DM onset at 13 ± 5 years, initial urinary albumin excretion (UAE) status (74% were normoalbuminuric, 26% microalbuminuric). Clinical characteristics are as follows: BMI 33.3 (20.9-50.9) kg/m$^2$ [median (range)], mean BP 88 (80-107) mmHg, HbA1c 9.3 ± 6.5%, serum Cr 0.75 ± 0.29 mg/dL with eGFR by Schwartz 137 ± 39 mL/min/1.73m$^2$, spot UAC 5.6 (0.01-190.9) mg/gram, cholesterol 169 ± 73 mg/dL, triglycerides 113 ± 91 mg/dl, HDL 45 (34-88) mg/dL, LDL 100 ± 70 mg/dL. After follow-up of 3.6 ± 5.8 years, 87 % of all subjects with initial UAC in the normoalbuminuric range remained normoalbuminuric, 8.7 % developed microalbuminuria, and 4.3% progressed to macroalbuminuria. Of those subjects with initial UAC in the microalbuminuric range, 37.5% reverted back to normoalbuminuria, 50% remained microalbuminuric, and 12.5% progressed to macroalbuminuria. UAE is positively correlated with diabetes duration (r=0.526, p=0.02), and to LDL (r=0.408, p=0.025). Glycemic control is positively correlated to UAE (r=0.498, p=0.04), and cholesterol levels (r=0.435, p=0.016). With regards to UAE status at the end of the follow-up period, glycemic control and cholesterol level as well as LDL were different (p=0.05, 0.026, 0.04 respectively) among the normoalbuminuric, microalbuminuric, and macroalbuminuric groups. In this study, glycemic control is a predictor of UAE ($R^2$=0.246, p=0.003). Further prospective, larger, and longer studies are needed in elucidating the protective and/or progressive risk factors for diabetic nephropathy in minority children with T2DM.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2501

**Relationship of Visceral and Subcutaneous Adiposity with Renal Function in People with Type 2 Diabetes Mellitus** Biro Kim,[1] Yongkyun Kim,[2] Jihee Lim,[3] Minyoung Kim,[3] Cheolwhee Park,[3] Ho-Cheol Song,[2] Yongsoo Kim,[3] Euy-Jin Choi,[2] Yoonsik Chang,[1] Bumsoon Choi.[3] *[1]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea, Yeouido, St. Mary's Hospital, Republic of Korea; [2]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea, Bucheon, St. Mary's Hospital, Republic of Korea; [3]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea, Seoul, St.Mary's Hospital, Republic of Korea.*

**Background.** Obesity and diabetes mellitus (DM) are established risk factors for the development of chronic kidney disease. The visceral adiposity (VAT) and subcutaneous adiposity (SAT) may be associated with the differential metabolic risk. Our study was performed to determine whether VAT or SAT was associated with the deterioration of renal function in people with type 2 DM.

**Methods.** Nine hundred twenty-nine people with type 2 DM (488 women and 441 men) and who had undergone abdominal computed tomography assessment of the SAT and VAT areas were included. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease four-variable equation at the time of the assessment of the SAT and VAT areas.

**Results**. The mean visceral fat area was 118 ± 56 Cm$^2$ in women and 126 ± 65 Cm$^2$ in men. The mean subcutaneous fat area was 192 ± 79 Cm$^2$ in women and 124 ± 66 Cm$^2$ in men. The visceral fat area was independently associated with the eGFR after adjustment for age, gender, the duration of diabetes, the systolic blood pressure, the diastolic blood pressure, the serum triglyceride, the homeostasis model for insulin resistance score, uric acid and urinary albumin excretion (β-coefficient = -0.075. $p = 0.034$), while the body mass index, total fat area and subcutaneous fat area were not significantly associated with the eGFR.

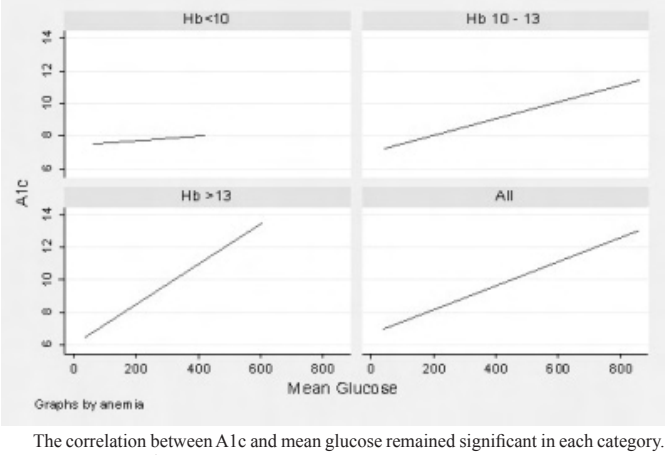
**Conclusion.** Our data suggest that VAT might be an additional prognostic factor for the deterioration of renal function in people with type 2 DM.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2502

**Impact of Anemia on Hemoglobin A1c and Average Glucose Correlations in Diabetic Chronic Kidney Disease** Salman Waheed,[1] Mark E. Williams.[2] *[1]Medicine, University of Massachusetts Medical School, Worcester, MA; [2]Renal Unit, Joslin Diabetes Center, Boston, MA.*

The optimal target for glycemic control in diabetic CKD has not been established, and the validity of hemoglobin A1c, its standard metric, is being called into question, due to both pathophysiologic and methodologic factors related to CKD. We evaluated the association of A1c with average glucose levels, and the effect of anemia on this association using a database of 3707 patients at a large diabetes referral center. The cohort consisted of patients with diabetic CKD (eGFR<60 and not on dialysis). Average A1c was positively correlated with average glucose levels with a Pearson correlation coefficient of 0.292 ($p<.0001$). We then categorized subjects into 3 groups: hemoglobin (Hb) <10, Hb10-13 and Hb>13, and correlated average A1c with average glucose levels in each category. We noted that there was no statistically significant difference among the three groups in terms of their mean eGFR (P=0.1) or age (P=0.4). However, the groups differed from one another in their mean glucose values (P<0.0001), A1c (P<0.001), calcium (<0.0001), vitamin D (P=0.04) and albumin/creatinine ratio (ACR) (0.001).




The correlation between A1c and mean glucose remained significant in each category. However, the level of correlation decreased at lower Hb values. In a regression model using A1c as dependent variable and mean glucose as the predictor variable, the correlation between A1c and mean glucose decreased with worsening anemia even after controlling for age, eGFR, calcium, vitamin D and ACR values.

Hemoglobin A1c may be less valid as a glycemia metric in CKD patients as anemia advances. Further evaluation with prospective data is needed to further support this finding.

Disclosure of Financial Relationships: nothing to disclose

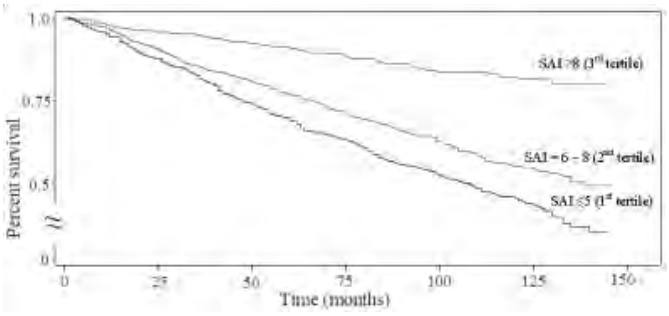
## SA-PO2503

**Social Adaptability Is Associated with Mortality in Patients with Diabetes** Alexander S. Goldfarb-Rumyantzev,[1] Preeti Rout,[1] Gurprataap Singh Sandhu,[1] Hongying Tang,[2] Anna Barenbaum,[3] Mark E. Williams.[1] *[1]Division of Nephrology, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA; [2]Transplant Institute, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA; [3]Tel Aviv University, Tel Aviv, Israel.*

Objective. A quantifiable assessment of socio-economic status and its bearing on clinical outcome in patients with diabetes is lacking. The social adaptability index (SAI) has previously been validated in the general population and in patients with chronic kidney disease. We hypothesize that SAI could be used in diabetes practice to identify disadvantaged population at risk for inferior outcome.

Research Design and Methods. The association of the SAI (calculated as linear combination of education status, employment, income, marital status and substance abuse) with patient survival was examined using a Cox model in NHANES-3 population.

Results. We identified 1,634 subjects with diabetes with mean age 61.9±15.3 years; 40.9% males; 38.5% White, 27.7% African American, and 31.3% Mexican American. The highest SAI was in Whites (6.9±2.5), followed by Mexican Americans (6.5±2.3), and then African Americans (6.1±2.6) (ANOVA, $p<0.001$). SAI was higher in subjects living in metropolitan areas (6.8±2.6) compared to the rural population (6.3±2.4) (T-test, $p<0.001$). Finally SAI was greater in males (7.1±2.4) than in females (6.1±2.4) (T-test, $p<0.001$). SAI had association with survival (HR 0.9, $p<0.001$) in the entire study population and in most of the subgroups (divided by race, sex, and urban/rural location). Similarly, SAI divided into tertiles (≤5, 6 to 8, >8) demonstrated a significant and "dose-dependent" association with survival.




Conclusions. Social adaptability index is associated with mortality in the diabetic population and is useful in identifying individuals who are at risk for inferior outcome.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2504**

**Sweet Pee: A Novel Mouse Model of SGLT2 Dysfunction for Studying Diabetes** Joseph Ly,[1] Tuncer Onay,[1] Colin Mckerlie,[4] Lucy Osborne,[5] Ann Flenniken,[1] Susan E. Quaggin.[1] *[1]Samuel Lunenfeld Research Institute, Toronto, ON, Canada; [2]Deparrtment of Medicine II, University Hospital Aachen, Aachen, Germany; [3]Hospital for Sick Children, Toronto, ON, Canada; [4]Toronto Center for Phenogenomics, Toronto, ON, Canada; [5]Department of Medicine, University of Toronto, Toronto, ON, Canada.*

The targeting of renal tubular SGLT2, a sodium glucose cotransporter, has become a new alternative to the management of Diabetes II. Dapagliflozin, among other SGLT inhibitors are being studied in clinical trials. Although early trial observed an increase in genitourinary infections, adverse effects have not been systematically studied. In this study, we describe a new mouse model of SGLT2 mutation, Sweet Pee, and report a significant mortality secondary to dehydration and urosepsis when these mice were treated with streptozotocin (STZ). We hypothesize that chronic tubular glucose exposure due to SGLT2 dysfunction will exert untoward effects on the health of these animals. Sweet Pee mutants were generated with ENU. We mapped the mutation to mouse chromosome 7 (LOD >4) within a critical region between 127-140 MB. Sequencing of Sweet Pee led to the discovery of a frameshift mutation in SLC5A2 (encodes SGLT2)from a single thymine insertion in exon 4 that resulted in a truncated neopepitide. Glucose tolerance test revealed a trend towards lower glucose levels. A total of 44 homozygous, 16 heterozygous and 7 wild type male mice were treated with low-dose STZ to induce diabetes and were followed for 16 weeks for survival. 26(60%) homozygotes, 13(81%) heterozygotes, and 7(100%) wild type demonstrated random serum glucose greater than 20 mmol/L. Of these, 8(31%) homozygotes, 5(38%) heterozygotes, and 7(100%) wild type survived. The sick mice exhibited severe dehydration and some developed urine infection. Histology of selected kidneys demonstrated dense white blood cells infiltration and parenchymal necrosis. We conclude that our diabetic Sweet Pee mutants had a significantly higher mortality compared to wild type. This data may suggest a heightened surveillance for urosepsis in patients treated with SGLT inhibitors.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2505**

**Is the MDRD Formula Valid during Renin Inhibition in Diabetic Kidney Disease?** Frederik I. Persson,[1] Hans-Henrik Parving,[2] Peter Rossing.[1] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]Medical Endocrinology, Rigshospitalet, Copenhagen, Denmark.*

It has been suggested that chronic RAS blockade with ACE inhibition in patients with diabetic kidney disease increases organic ion clearance e.g. creatinine. Consequently, it is possible that the benefit attributed to this therapy may be related to improved tubular secretion of creatinine, as this lowers serum creatinine. We therefore studied the effect of RAS blockade during renin inhibition on GFR (51Cr-EDTA plasma clearance) and estimated GFR. This was a pooled analysis from two studies of 43 patients with type 2 diabetes, hypertension and albuminuria, all having GFR measured after a 2 month placebo period (n=43) as well as after a 2 month treatment period with aliskiren 300 mg daily (n=43). We calculated eGFR using the MDRD formula and compared with the results of the GFR measurements.

After the placebo period mean GFR was 85 (27) and eGFR was 77 (27) ml/min/1.73m$^2$, mean difference 8.7 ml/min/1.73m$^2$ (95% CI 4.5, 12.9), p<0.001.

After 2 months treatment with 300 mg aliskiren once daily mean GFR was 81 (27) and eGFR was 73 (26) ml/min/1.73m$^2$, mean difference 7.0 ml/min/1.73m$^2$ (95% CI 3.2, 10.8), p=0.001 . There was no significant difference in decline in renal function from placebo to aliskiren evaluated by GFR: 4.7 (9) ml/min/1.73m$^2$or by eGFR: 3.0 (10) ml/min/1.73m$^2$, mean difference 1.7 ml/min/1.73m$^2$ (95% CI -1.0, 4.4)..

In conclusion the use of the MDRD formula is valid during renin inhibition in patients with type 2 diabetes, hypertension and albuminuria.

Disclosure of Financial Relationships: Ownership: NovoNordisk; Honoraria: Novartis.

**SA-PO2506**

**Quantitative Magentic Resonance Imaging of Diabetic Nephropathy** Christopher A. Flask,[1] John R. Sedor,[1,2] Lan Lu,[1] Katherine M. Dell.[1,2] *[1]Departments of Radiology, Biomedical Engineering, Medicine and Pediatrics, Case Western Reserve University, Cleveland, OH; [2]CWRU Center for the Study of Kidney Disease and Biology, MetroHealth System Campus, Case Western Reserve University, Cleveland, OH.*

Diabetic nephropathy (DN) is the leading cause of end-stage kidney disease in the US. However, there are currently no established non-invasive assessments that can reliably detect early-stage DN, the point at which targeted interventions are most likely to impact disease progression. Changes in estimated glomerular filtration rate (eGFR) may not be seen until significant damage has occurred; microalbuminuria may indicate early damage but regresses in a significant proportion of patients. The goal of this study was to examine changes in diffusion tensor imaging (DTI) parameters as a potential biomarker for DN in a well-characterized cohort of patients with diabetes and a range of eGFRs.

DTI images were obtained on 8 diabetic subjects with a range of kidney function (eGFR = 32-108 mL/min/1.73m$^2$) currently participating in the Renal Disease Progression Genes and Environmental Impact on Diabetic Nephropathy Study. eGFR was based on the CKD-EPI equation. Five normal subjects were scanned for comparison. Image maps of Fractional Anisotropy (FA) and Apparent Diffusion Coefficient (ADC) were computed, and 16 medullary and cortical regions of interest (ROIs) for left and right kidneys of each subject were selected for analysis.

Medullary FA strongly correlated with eGFR ($R^2$ = 0.78) for all subjects, while neither cortical nor medullary ADC values correlated with eGFR ($R^2$ = 0.19 and 0.16, respectively). Cortical FA values were also unchanged among all subjects. Mean FA values for all diabetic subjects were also significantly lower than controls (0.23 vs. 0.30, $p < 0.05$). Mean ADC and FA values for normal subjects were comparable to published reports.

These data suggest that medullary diffusion FA may be a sensitive assessment for DN progression. These data further suggest that quantitative MRI modalities may serve as biomarkers for early disease in other chronic kidney diseases.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2507**

**Noninvasive 7tesla MRI Determination of Intramyocellular Lipid Concentrations in Chronic Kidney Disease** Devasmita Choudhury, Manisha Shah, Maram Museitif, Lynne M. Roetzer. *Medicine, Dallas VA Medical Center/ UT Southwestern Medical Center, Dallas, TX.*

Insulin resistance (IR) is prevalent in chronic kidney disease (CKD). It is widely-accepted that, among patients with normal renal function, elevated intramyocellular lipids (IMCLs) correlate with fatty acid metabolites that cause IR. Although hypertriglyceridemia is common among patients with CKD, little is known about the impact of CKD on IMCL. Magnetic resonance spectroscopy offers noninvasive monitoring of IMCLs. At high fields (7T) detection of IMCL is improved compared to standard clinical systems. This study investigated the utility of noninvasive 7 Tesla MRS in IMCL accumulation in CKD(1-5, MDRD eGFR) patients with and without diabetes (DM) and compared intramyocellular lipid (IMCL) concentration (mmol/kg/ wet weight muscle) to aged matched healthy controls (HC). Calf muscle NMR spectroscopic data from of 14 male CKD and 8 male healthy subjects was analyzed to calculate [IMCL] based on the amplitude of the triglyceride methyl signal compared to creatine. Results: Of 14CKD patients (mean age 57±11, BMI 32±6), 6 patients, all with CKD 3-5 had high extramyocellular (EMCL) lipid signal that overwhelmed the IMCL signal. When this subgroup was compared to those with CKD 3-5, with detectable IMCL, 5/6 in high EMCL group were nonwhite compared to 3/8 in the IMCL detectable group. Further non-white IMCL detectable patients had less severe CKD stage. IMCL concentration of 8 CKD 1-5 (mean age 56±15, BMI 30±7) was 5.0 (2.1-6.6) mmol/kg wet weight, compared to 8 HC (52±10, BMI 29±4), with IMCL 3.7 (.005-10), p =n.s. In those with measurable IMCL, stage of ckd 1-2 vs 3-5, level of serum cholesterol, TGL, HBA1c or presence or absence of DM were not different as compared to HC. Early data indicates nearly 42% advanced CKD have marked increase in EMCL concentrations rendering IMCL measurements undectable even by high resolution 7tesla imaging, particularly in patients of nonwhite origin. Race may be important in intramuscular lipid distribution. Further exploration of IMCL to EMCL distribution using non-invasive 7Tesla MRI imaging may elucidate risks for early insulin resistance in non-white patient population.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2508**

**An Acute Fall in Glomerular Filtration Rate (GFR) during Treatment with an Angiotensin Receptor Blocker (ARB) Is Associated with Renoprotection: A Post-Hoc Analysis in RENAAL** Frank Holtkamp,[1] Hiddo Jan Lambers Heerspink,[1] Pieter de Graeff,[1] Mark E. Cooper,[2] Dick De Zeeuw.[1] *[1]Dep Clin Pharmacology, UMCG, Groningen, Netherlands; [2]Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia.*

**Introduction:** ARB treatment confers renoprotection. However, during therapy initiation, ARB treatment may induce an acute fall in GFR, which opposes renal protection. We hypothesize that this initial fall in GFR upon ARB treatment reflects a renal hemodynamic effect that is associated with long-term structural renal protection.

**Methods:** A post-hoc analysis in the RENAAL trial was conducted. The acute fall in estimated (e)GFR (MDRD formula) from baseline to month 3 and its relationship with long term eGFR slope (and hard renal endpoints) were determined.

**Results:** Patients assigned to losartan had a 0.73 ml/min greater acute fall in eGFR during the first 3 months (p=0.031) compared to patients assigned to placebo, but a 0.8 ml/min/year slower mean eGFR decline thereafter (-4.2 (95%CI -3.9, 4.6) versus -5.0 (-4.7, 5.4) ml/min/1.73m$^2$/year; p<0.001). A large inter-individual difference in acute eGFR change was noticed. When all subjects were divided in tertiles of initial eGFR change, subjects within the losartan group with a large fall in eGFR had a significant less steep slope in long-term eGFR compared to those with a moderate fall or rise in eGFR (table). In addition, a larger fall in acute eGFR upon ARB treatment was associated with a larger treatment effect on renal endpoints (table).

| Tertiles of acute eGFR change (ml/min/1.73m2) | Long term eGFR in losartan treated patients (ml/min/1.73m2/year) | Treatment effect vs placebo | | |
|---|---|---|---|---|
| mean (sd) | mean (95%CI) | HR | 95% CI | p value |
| -8.5 (4.0) | -3.65 (-4.20 to -3.10) | 0.71 | 0.54-0.93 | 0.014 |
| -2.1 (1.3) | -3.85 (-4.40 to -3.30) | 0.77 | 0.56-1.05 | 0.093 |
| +4.5 (2.9) | -4.43 (-4.97 to -3.90)* | 0.92 | 0.62-1.36 | 0.661 |

* p=0.045 vs tertile 1

**Conclusion:** An initial fall in eGFR during ARB treatment is independently associated with a slower rate of renal function loss during follow-up suggesting that an initial fall in eGFR can be interpreted as a marker of therapeutic effectiveness.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2509

**Early Renal Function Decline in Type 2 Diabetes** Meda E. Pavkov,[1] William Knowler,[2] Clinton C. Mason,[2] Kevin V. Lemley,[3] Bryan D. Myers,[4] Robert G. Nelson.[2] *[1]CDC, GA; [2]NIDDK, AZ; [3]USC, CA; [4]Stanford University, CA.*

Early renal function decline (ERFD), a decline in glomerular filtration rate (GFR) in excess of normal aging, is described in type 1 diabetic persons with normo- or microalbuminuria but not in type 2 diabetes. We measured GFR (iothalamate clearance) and urinary albumin/creatinine (ACR) at least 5 times during a median follow-up of 8.8 years (range=2.5-10.0 years) in 189 Pima Indians with type 2 diabetes. At baseline, 93 subjects had normal albuminuria (ACR <30 mg/g) and 96 had microalbuminuria (30≤ACR<300 mg/g). ERFD was defined by an average decline in GFR of ≥3.3%/year during this initial follow-up period, a threshold corresponding to the upper 2.5 percentile of the distribution of the decline in creatinine clearance in nondiabetic normotensive Caucasians in the Baltimore Longitudinal Study on Aging--the same definition that has been used in type 1 diabetes. Subsequently, subjects were followed for up to 17.5 years to the onset of end-stage renal disease, death, or December 31, 2009. At baseline, median GFR was 158 ml/min (range 49-296 ml/min), and the prevalence of ERFD was higher in subjects with baseline microalbuminuria (47%) than in those with normoalbuminuria (24%). The prevalence of ERFD was lowest at the end of the initial follow-up period in subjects who remained or regressed to normal ACR (13%), intermediate in those who remained or progressed to microalbuminuria (35%), and highest in those who progressed to macroalbuminuria (64%; ACR ≥300 mg/g). The cumulative incidence of end-stage renal disease 9 years after the initial follow-up period, when 75% of the observations were censored, is shown in the table according to albuminuria level at the end of the initial period.

Cumulative incidence of kidney failure

| | Remaining or regressing to normoalbuminuria | Remaining or progressing to microalbuminuria | Progressing to macroalbuminuria |
|---|---|---|---|
| ERFD - | 7.1% (1 event) | 0 | 14.9% (2 events) |
| ERFD + | 0 | 0 | 60.4% (13 events) |

In type 2 diabetes, progressive loss of GFR begins before the onset of macroalbuminuria, but a decline in excess of that expected by normal aging (ERFD) is strongly dependent on progression to macroalbuminuria.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2510

**Body Mass Index Is a Predictor of Angiotensin Receptor Blocker (ARB) Induced Albuminuria Reduction** Frank Holtkamp,[1] Hiddo Jan Lambers Heerspink,[1] Gozewijn Dirk Laverman,[1] Gerjan Navis,[1] Hans-Henrik Parving,[2] Julia Lewis,[3] Dick De Zeeuw.[1] *[1]University Medical Center, Groningen, Netherlands; [2]University Hospital, Copenhagen, Denmark; [3]Vanderbilt University School of Medicine, Nashville, TN.*

**Introduction** The effect of ARB-induced reduction in albuminuria varies between individuals. Sodium intake and concomitant diuretic use increase plasma RAAS activity and enhance the albuminuria response. Higher body mass index (BMI) has also been associated with increased tissue RAAS activity and an increased renal vascular response to RAAS blockade in healthy subjects and type 2 diabetes. We assessed whether a higher BMI was associated with a larger reduction in albuminuria during ARB therapy in patients with type 2 diabetes and nephropathy.

**Method** Available data for change in albuminuria in ARB and placebo treated patients in the combined RENAAL and IDNT trials were used (n=2661). Short term response in albuminuria was defined as change from baseline to month 6. Univariate and multivariate regression analyses was applied to determine whether baseline BMI predicted changes in 24h urinary albumin excretion, corrected for multiple response determinants including albuminuria, eGFR, blood pressure, diuretic use.

**Results** Albuminuria was reduced with 29.7% (95% range: -86.9% to +178.9%) during ARB therapy versus a decrease of 5.7% (95% range: -80.4 % to +280.4%) during placebo. A higher BMI was significantly associated with a larger albuminuria reduction (table). This was independent of other baseline parameters (multivariate (β=0.009 per kg/m$^2$; p=0.007). Interestingly, higher BMI was positively associated with the DD ACE-genotype polymorphism.

| BMI quartiles (kg/m2) | 25.8 < | > 25.8 and < 29.3 | > 29.3 and <33.6 | > 33.6 |
|---|---|---|---|---|
| Albuminuria response (%, month6 - baseline) (median, IQR) | -12.9 (-42, +30) | -16.5 (-48, +30) | -15.0 (-49, +27) | -21.8 (-53, +18)* |
| DD genotype (%) | 24.5 | 27.1 | 28.7 | 31.9* |

*p<0.05 vs lowest quartile

**Conclusion** A higher BMI was associated with a larger albuminuria response in patients with diabetes and nephropathy. These data suggest that a (genetically determined) intrinsic activity of the RAAS is an important determinant for ARB treatment response.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2511

**In Vivo Delivery of Megsin siRNA Plasmid Reveals Therapeutic Potential Against Diabetic Nephropathy by Down-Regulating P27$^{kip1}$ Level** Ying Li, Maodong Liu, Yanling Zhang, Yanqing Chi. *Nephrology, 3rd Hospital of Hebei Medical University, Shijiazhuang, Hebei, China.*

**Aims:** A mesangium-predominant gene,Megsin, has emerged as a participant in mesangial cell proliferation and/or mesangial matrix expansion. We hypothesize that Megsin may serve as a therapeutic target in the management of diabetic nephropathy and a series of in vivo or in vitro experiments were designed.**Methods:** We have constructed a Megsin siRNA plasmid and have examined the effect of Megsin inhibition on the progression of diabetic nephropathy in a mouse model. CD-1 mice underwent uninephrectomy and STZ treatment prior to receiving weekly tail-vein rein injections of the plasmid. Animals were sacrificed at week-1, week-2 and week-12. At each time point, urine and blood samples were collected, and kidney tissues were harvested. The level of serum glucose in every group's mice was measured in vena caudalis by the blood sugar analyzer. Furthermore, serum creatine ( Scr) and urine protein( UP) in 24 hours were estimated by the automatic biochemistry analyzer. In vitro Mouse mesangial cells (MCs) were transfected with pBAsi mU6 Neo megsin siRNA plasmid or pBAsi mU6 Neo plasmid using lipofectamine 2000 reagent . After 24 hours, cells were further cultured in DMEM containing high glucose (HG; 25mM) or normal glucose ( NG; 2.8mM). Cells in 6-well culture plates were collected for protein extraction and culture medium was collected for Collagen IV radioimmunoassay at hour-12, 24 and 48. The levels of megsin, MMP-2, TIMP-2 and P27$^{kip1}$ were measured by Immunohistochemistry or western blotting.**Results:** Inhibition of Megsin alleviated proteinuria and glomerular collagen IV accumulation 12 weeks after the STZ injection and inhibited renal cell proliferation. Furthermore, in vitro experiments showed that, after the transfection of Megsin siRNA plasmid, the mouse mesangial cellular abnormalities induced by high glucose, such as increased cell proliferation and collagen IV production were reversed. Significantly, the level of P27kip1 was down-regulated in transfected mesangial cells.**Conclusion:** the inhibition of Megsin might exert beneficial effects on the diabetic kidney partly through down-regulation of P27$^{kip1}$ level.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2512

**Dual Inhibition of Classical PKC-α and PKC-β Isoforms in a Pharmacological and Double Knock-Out Mouse Approach Leads to Protection Against Experimental Murine Diabetic Nephropathy** Jan Menne, Joon-Keun Park, Nelli Shushakova, Hermann G. Haller. *Nephrology and Hypertension, Medical School Hannover, Hannover, Germany.*

**INTRODUCTION AND AIMS:**

Both classical PKC isoforms, PKC-α and -β, have been implicated in the pathogenesis of diabetic microvascular diseases. We previously elucidated the specific role of these individual isoforms and revealed that activation of the PKC-β-isoform contributes to high-glucose-induced renal fibrosis whereas perlecan as well as nephrin and VEGF expression are regulated by a PKC-α-isoform-dependent signaling pathway leading to diabetic albuminuria.

**METHODS:**

We now tested the hypothesis if deletion of both classical PKC isoforms is able to completely abolish the development of diabetic nephropathy in the streptozotocin-induced diabetic mouse model. We therefore studied distinct pharmacological approaches while inhibiting both classical PKC isoforms and validated the phenotype of nondiabetic and streptozotocin-induced diabetic homozygeous PKC-α/β double knock-out mice (PKC-α/β$^{-/-}$) compared to appropriate 129/SV wild type mice.

**RESULTS:**

After 8 weeks of diabetes mellitus the high-glucose-induced renal and glomerular hypertrophy as well as the increased expression of extracellular matrix proteins such as collagen and fibronectin was abolished in the PKC-α/β$^{-/-}$ mice compared to WT controls. Furthermore, the high-glucose-induced expression of the profibrotic cytokine TGF-$\beta_1$ was significantly diminished in the PKC-α/β$^{-/-}$ mice in comparison to diabetic WT mice. The loss of the basal membrane proteoglycan perlecan and the podocyte protein nephrin is prevented in the diabetic state in the PKC-α/β$^{-/-}$ mice. Furthermore, we were able to demonstrate, that a PKC-α/β inhibitor had a similar effect.

**CONCLUSIONS:**

In summary, blockade of the two prominent PKC isoforms prevent early diabetic nephropathy while inhibiting prosclerotic glomerular and tubulo-interstitial changes as well as the development of albuminuria. These results demonstrate that downregulation of the dual PKC-isoform activation in the diabetic state *in vivo* is a suitable therapeutic target in the prevention of diabetic microvascular complications such as diabetic nephropathy.

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## SA-PO2513

**Reduction of Glomerulosclerosis and Prevention of Interstitial Inflammation in Diabetic CD-1 Mice by Pentosan Polysulfate and Pyridoxamine** Fabrizio Grosjean,[1] Vittoria Esposito,[1] Maya Ramdas,[1] Massimo Torreggiani,[2] Helen Vlassara,[1] Feng Zheng,[1] Gary E. Striker.[1] *[1]Geriatrics and Palliative Medicine, Mount Sinai School of Medicine, New York, NY; [2]Unit of Nephrology, IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy.*

The levels of both systemic and kidney inflammation markers are increased in diabetic nephropathy (DN). We showed that the levels of inflammation in diabetic patients directly correlate with those of advanced glycation endproducts (AGEs), that reducing AGEs lowers circulating and cellular markers of inflammation, and that AGE inhibitors reduce DN. Since inflammation is partly due to elevated AGEs, we studied a combination of two drugs, one that reduces inflammation (PPS) and one that reduces AGE levels (PYR) using STZ-induced diabetic CD1 mice, a model of rapidly progressive DN. After 4 weeks of hyperglycemia mice were randomized to EN (enalapril, 10 mg/kg/day), EN+PYR (PYR 200 mg/kg/day), EN+PPS (PPS 25 mg/kg/day), EN+PYR+PPS, or tap water. After 24 weeks, untreated diabetic mice (DB) had prominent glomerulosclerosis with thickened basement membranes, tubular atrophy, and diffuse interstitial inflammation. The percent glomerulosclerosis in DB mice (17.5±6) was reduced by EN (14.1±3), EN+PYR (13.7±3), and EN+PYR+PPS (10.5±3). While interstitial inflammation was also reduced in all treatment groups, it was nearly completely ablated in the PPS groups (DB+EN+PYR+PPS; 0.2±0.5 vs. DB: 2.3±0.7; $p<0.05$). All DB mice had an increased kidney weight/body weight ratio and hyperglycemia, which were not influenced by treatment. In conclusion, treatment of DN by the combined use of an inhibitor of inflammation (PPS) and an inhibitor of AGEs (PY) substantially reduces kidney lesions typical of DN in CD-1 mice. Since both drugs are FDA-approved and have few side-effects, this may represent an additive approach in the clinical management of DN.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2514

**The Effect of GW610742, PPAR-δ Agonist, on Diabetic Nephropathy and Albuminuria** Eun-Young Lee,[1] Geun Tae Kim,[1] Su-Ji Kim,[1] Sujin Seok,[1] Ran Choi,[2] Mi Young Lee,[2] Choon Hee Chung.[2] *[1]Internal Medicine, Soon Chun Hyang University, Cheonan, Korea; [2]Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea.*

Peroxisome proliferator–activated receptor (PPAR)-δ, a member of the ligand-activated nuclear receptor superfamily, plays an important role in lipid and glucose homeostasis and inflammation. However, the role of PPAR- δ in the pathogenesis of diabetic nephropathy has not been determined. We investigated the effect of PPAR-δ on kidney pathology and albuminuria in spontaneously developed diabetic rats. Otsuka-Long-Evans-Tokushima-Fatty rats, an animal model for type 2 diabetes, were randomized into a non-treated and a GW610742 (10 mg/kg, a highly specific ligand for PPAR-δ)-treated group. Long-Evans Tokushima Otsuka rats were used as a non-diabetic control. In diabetic rats, albuminuria, glomerular hypertrophy and glomerular sclerosis were more severe than in non-diabetic rats. GW610742 did not affect hyperglycemia, kidney weight and glomerular hypertrophy in diabetes. However, GW610742 did significantly ameliorate diabetic albuminuria. Diabetic glomerular basement membrane thickening was significantly prevented in GW610742-treated diabetic rats. Decreased density of slit pores between podocyte foot processes in diabetic rats was significantly recovered toward normal by GW610742. Nephrin protein was decreased in the diabetic rats but was significantly restored by GW610742. Taken together, PPAR-δ activation by GW610742 ameliorated albuminuria with corresponding change in podocyte-related structures, such as nephrin, glomerular basement membrane thickening and slit pore density. In conclusion, this is the first report that PPAR-δ activation by GW610742, PPAR-δ agonist, has a renoprotective effect via recovery of urinary filtration barrier integrity in diabetes.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2515

**Tubulointerstitial Injury Is Exacerbated in Streptozotocin-Induced Diabetic Matrix Metalloproteinase-2 Knockout Mice** Ryotaro Ando, Kei Fukami, Seiya Okuda. *Division of Nephrology Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan.*

Background and aims:Matrix metalloproteinnase2 (MMP-2) is one of the potent metalloproteinases which can degrade various types of extracellular matrix (ECM) . It has been suggested that diabetes-induced accumulation of ECM are, at least in part, by down-regulation of MMP-2. However there is no data whether MMP-2 activity and expression affect diabetes-induced renal damage in vivo. Therefore, we investigated the effects of MMP-2 deficiency on tubulointerstitial injury in diabetic animals.

Materials and methods: Diabetes was induced by streptozotocin (STZ)(50mg/kg) in male MMP-2 knockout mice (MMP-2 KO) and C57BL/6J mice (Ctrl). After 16 weeks,cortical MMP-2 expression and activity were measured by zymography and real-timePCR, respectively. Urinary albumin excretion (UAE) and N-acetyl β-D-glucosaminidase were measured by enzyme-linked immunosorbent assay. Alfa-smooth muscle actin (α-SMA) was evaluated by western blots and immnohistochemistry.Tubutointerstitial injury and fibrosis were evatuated by hematoxylin and eosin staining and Masson-trichrome staining.

Results: Plasma levels of glucose and HbA1c were increased by about 2-3-folds in 16-week diabetic mice compared with non-diabetic Ctrl mice (plasma glucose; 490.3±7.5mg/dl, HbA1c; 9.91±0.22 %). MMP-2 expression and activity in the total kidney cortex of diabetic mice were increased in zymography and RT-PCR analysis. Serum levels of BUN, creatinine (Cr) and UAE were significantly increased in MMP-2 KO DM mice compared with Ctrl DM mice (BUN; 23.2±1.7 vs 39.8±6.0 mg/dl,$p<0.01$, Cr; 0.07±0.01 vs 0.17±0.04 mg/dl, $p<0.01$, UAE; 0.08±0.01 vs 0.16±0.03 mg/mgCr, $p<0.05$). Further, tubulointerstitial injury and cortical α-SMA expression were enhanced in DM mice, which were further increased in MMP-2 KO DM mice.

Conclusions: We demonstrate for the first time that tubulointerstitial injury and fibrosis were exacerbated in diabetic MMP-2 knockout mice, even though MMP-2 levels were increased in diabetic mice. The present study suggests that the decrease in other matrix-degrading enzymes may be involved in the tubulointerstitial injury and fibrosis in diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2516

**Tissue Inhibitors of Metalloproteinases-1 (TIMP-1), Is a Urinary Marker for Proximal Tubular Cell Damage in Diabetic OVE26 Mice** Shirong Zheng,[1,2] Yun Huang,[1] Paul N. Epstein.[1] *[1]Juvenile Diabetes Research, Department of Pediatrics, University of Louisville, Louisville, KY; [2]Institute for Cellular Therapeutics, Department of Surgery, University of Louisville, Louisville, KY.*

Tissue inhibitor of metalloproteinases-1 (TIMP-1) is a major and specific inhibitor of matrix metalloproteinase, which regulate diverse processes including remodeling of extracellular matrix. TIMP-1 remains low in normal kidney and increases significantly in many kidney diseases with renal fibrosis. In this study, we investigated the expression of TIMP-1 in a mouse model of diabetic nephropathy. Diabetic OVE26 and control FVB mice were used and uninephrectomy and sham surgery was performed at two month of age to accelerate albuminuria and fibrosis. At four months of age, kidney TIMP-1 mRNA expression increased more than 4 fold in diabetic mice and almost 75 fold in uninephrectomized diabetic mice ($p<0.05$). Increased TIMP-1 gene expression correlated with the elevation of albuminuria and degree of fibrosis in diabetic mice. Immunohistochemistry revealed that most TIMP-1 was localized to tubular cells, especially tubules with atrophy or severe dilation. TIMP-1 staining was also evident on isolated cells in the interstitial space. TIMP-1 staining in tubular cells overlapped with staining for albumin, complement C3 and caspase 3. Double staining with megalin confined these proteins to proximal tubular cells. By Elisa analysis we detected TIMP-1 protein in the urine, which was positively correlated with urine albuminuria and renal fibrosis in diabetic mice. Since TIMP-1 expression in proximal tubular cells is strongly associated with albumin and complement accumulation, tubular cell atrophy and dilation and tubulointerstital fibrosis, these results indicate that urine TIMP-1 provides an indicator of proximal tubular cell damage and progression of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2517

**Long-Term Administration of Resveratrol Reduces Metabolic Disturbances and Renal Injury in db/db Mice** Ji Eun Lee,[1] Hyunwook Kim,[1] Young Youl Hyun,[2] Jinjoo Cha,[2] Kum Hyun Han,[3] Sang Youb Han,[3] Hyoung-Kyu Kim,[2] Young Sun Kang,[2] Dae R. Cha.[2] *[1]Department of Internal Medicine, Wonkwang University Sanbon Hospital, Gunpo-si, Gyeonggi-do, Korea; [2]Department of Internal Medicine, Korea University Ansan Hospital, Ansan-si, Gyeonggi-do, Korea; [3]Department of Internal Medicine, Inje University Ilsan Baek Hospital, Goyang-si, Gyeonggi-do, Korea.*

Resveratrol, a polyphenolic SIRT1 activator, has been found to possess beneficial properties against metabolic diseases such as obesity and type 2 diabetes. In this study, we investigated the effects of chronic daily administration of resveratrol on metabolic disturbances and renal injury in db/db mice.

Experimental animals at 8 weeks of age were given resveratrol (20 mg/kg/d) and vehicle for 3 months. After resveratrol treatment, there were no significant changes in the levels of fasting blood glucose, HbA1c and blood pressure. However, plasma insulin level, HOMA-IR, plasma lipid levels, and insulin resistance measured by insulin tolerace test were significantly improved. After 2 month-treatment, resveratrol-fed diabetic mice showed reduced urinary albumin compared with vehicle-fed diabetic mice. This effect was much more apparent after 3 month-resveratrol treatment. Resveratrol treatment decreased plasma isoprostane level and increased renal adiponectin receptor and AMPK mRNA expressions compared with vehicles. Furthermore, resveratrol treatment suppressed mesangial expansion and decreased the synthesis of pro-fibrotic molecules including TGF-β, PAI-1 and type IV collagen. In summary, long-term administration of resveratrol improves insulin resistance, dyslipidemia, and renal injury in db/db mice. Our results suggest that these effects may be associated with decreased oxidative stress and regulation of renal adiponectin receptor-AMPK pathway.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2518**

**Intermittent Administration of ONO-1301, a Sustained-Release Prostacyclin Analog, Ameliorates Renal Alterations in Obese Type 2 Diabetes Mice** Hiroko Yamasaki, Yohei Maeshima, Tatsuyo Nasu, Daisuke Saito, Katsuyuki Tanabe, Hitoshi Sugiyama, Hirofumi Makino. *Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan.*

Diabetic nephropathy is the most common pathological disorder predisposing end-stage renal disease, and novel therapeutic approaches are required. ONO-1301 is a novel sustained-release prostacyclin analog possessing thromboxane A2 synthase inhibitory activity, with potent therapeutic efficacies on experimental pulmonary hypertension, pulmonary fibrosis and myocardial ischemia. We recently observed the therapeutic efficacies of slow-release ONO-1301 (SR-ONO) in experimental type 1 diabetic nephropathy, an unilateral ureteral obstruction, and a rat progressive Thy-1 nephritis model. Here, we examined the therapeutic efficacy of intermittent administration of SR-ONO on renal alterations in the obese type 2 diabetes mouse. Db/db mice, a model of obese type 2 diabetes at 8 weeks of age exhibiting hyperglycemia, received subcutaneous injections of either SR-ONO (3mg/kg) or vehicle buffer every 3 weeks. Animals were sacrificed at 16 weeks of age. Cultured mouse mesangial cells (Mes13) were stimulated with high ambient glucose (HG; 25mM) in the presence of ONO-1301(1-100nM) for 24 hrs. Clinical parameters (albuminuria, creatinine clearance), kidney weight, glomerular volume and mesangial matrix index were examined, and immunohistochemistry and immunoblot was performed. SR-ONO treatment did not affect obesity or hyperglycemia, but significantly ameliorated albuminuria, glomerular hypertrophy, the increase of mesangial matrix index, glomerular accumulation of type IV collagen, F4/80+ monocyte/macrophage, TGF-beta1, alpha-smooth muscle actin (SMA) and MCP-1 in db/db mice compared with vehicle treatment. In Mes13 cells, ONO-1301 dose-dependently suppressed the increase of TGF-beta, type IV collagen and alpha-SMA induced by HG (immunoblot). Taken together, these results suggest the potential therapeutic efficacy of intermittent administration of SR-ONO on renal alterations in type 2 diabetes mediated via anti-fibrotic and anti-inflammatory effects, partially through its direct effects on mesangial cells.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2519**

**Amelioration of Microalbuminiuria in ROCK1 Knockout Mice with STZ-Induced Diabetic Kidney Disease** Li Zhou,[1] Fei Liu,[1] Hui Y. Lan,[2] Ping Fu.[1] *[1]Department of Medicine-Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China; [2]Department of Medicine and Therapeutic and Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Hong Kong, China.*

**Background.** It has been shown that blockade of Rho kinase with pharmacologic inhibitors ameliorates renal fibrosis and diabetic kidney disease (DKD). But the underlined mechanisms are still not clear. We hypothesize that Rho kinase is a key mediator regulating the expression of megalin/cubilin and tubular function of reabsorption for albumin in the early stage of DKD.

**Methods.** This study examined the role of Rho kinase in the early stage of STZ-induced DKD model of mice that do not express ROCK1 gene, a critical downstream mediator of Rho GTPase, in the aspect of effect on tubular function of reabsorption for albumin and the expression of megalin/cubilin, an endocytosis receptors complex. In vitro experiment on NRK52E, a normal rat tubular epithelial cell line with high glucose stimulating was introduced.

**Results.** Results showed that *microalbuminuria was attenuated in ROCK1 KO mice treated with STZ which was consistent to the attenuation of reduced megalin/cubilin expression in renal cortex at 8 week. In vitro* study with ROCK inhibitor Y-27632 further confirmed the protective role of ROCK inhibitor on the decrease of megalin expression and the reduced endocytosis of renal tubular epithelial cells induced by high glucose.

**Conclusions.** This study further confirmed the pathogenic role of ROCK1 in DKD and it indicated potential downstream pathway on an endocytosis receptors complex - *megalin/cubilin which was related to the pathogenesis of microalbuminuria in the early stage of DKD.*

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2520**

**Natural History of Albuminuria in Akita Diabetic Mice – Potential Role of USF1** Amber P. Sanchez, Young H. You, Shinichi Okada, Anne-Emilie Decleves, Kumar Sharma. *Department of Medicine, Division of Nephrology, University of California San Diego, La Jolla, CA.*

The clinical hallmark of diabetic nephropathy (DN) is mesangial matrix expansion, largely driven by TGF-β, and albuminuria. We have previously identified that the USF1 transcription factor mediates glucose-induced TGF-β1 stimulation in mesangial cells, however its role in regulating albuminuria is unknown. We investigated the natural history of albuminuria in male Akita mice (Ins2/Akita), a genetic model of type 1 diabetes, and determined the role of USF1 in the development of albuminuria by intercrossing USF1 knockout (KO) with Akita mice. Mice were evaluated at 6, 12, 20, and 28 weeks by 24h urine collection and compared to wild type control (WT) (n=6-8 each group). We observed a time-dependent fluctuation of albuminuria in Akita mice. As early as 6 weeks, Akita mice had significantly higher urinary albumin excretion (UAE) than WT (mean 267.8 vs 19.6μg/24h, P<0.0001). However, at 12 weeks albuminuria was reduced and not significantly different than WT (mean 52.5 vs 31μg/24h). Albuminuria peaked at 20 weeks (1113 vs 13.6 μg/24h, P<0.05) and returned to values similar to the 6 week time point by 28 weeks of age (275 vs 15.7μg/24h, P<0.001). Diabetic mice heterozygous for USF1 followed the same pattern of UAE as Akita mice. However, diabetic USF1 KO mice had no significant difference in UAE than WT mice at any of the time points (mean UAE <108μg/24h, all time points, P=NS). Kidney cortex was evaluated by quantitative real time PCR and immunostaining from mice sacrificed after 28 weeks. Akita mice had significantly higher TGF-β1 mRNA (p<0.05) whereas diabetic USF1 KO mice had no significant difference from WT. On immunostaining, Akita mice stained strongly for TGF-β in the glomeruli and tubules, whereas in diabetic USF1 KO mice TGF-β staining was substantially reduced. In conclusion, Akita mice exhibit a fluctuating pattern of albuminuria that is time-dependent. In mice lacking the USF1 transcription factor there is no significant increase in albuminuria or glomerular TGF-β staining in the setting of diabetes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2521**

**Nitric Oxide Negatively Regulates HB-EGF Expression in Vascular Endothelial Cells in eNOS Knockout Diabetic Mice** Fenghua Zeng, Suwan Wang, Xiaofeng Fan, Hongling Jiang, Huifang Cheng, Raymond C. Harris. *Medicine, Vanderbilt University, Nashville, TN.*

Diabetes mellitus (DM) is a heterogeneous disorder that initiates a diverse spectrum of effects on the vasculature and causes vasculopathy, such as macroangiopathy-related major cardiovascular events and end-stage renal disease caused by microangiopathy-related diabetic nephropathy (DN). A hallmark of diabetic vasculopathy is endothelial cell (EC) dysfunction, where the loss of nitric oxide (NO) and the consequent impairment of endothelium-dependent vasorelaxation play a major role. Studies have suggested that HB-EGF may serve as a mediator of EC dysfunction in diabetes. In the current studies, we investigated 1) whether HB-EGF expression is increased in the EC of the DN model; and 2) whether the loss of NO contributes to the augmentation of HB-EGF expression. As endothelial nitric oxide synthase (eNOS) is the main source of NO in EC, the well established eNOS knockout diabetic mouse model (*eNOSKO db/db*) was used. We found that HB-EGF was highly expressed in the glomeruli, EC of the small blood vessels and smooth muscle cells of the medium blood vessels in the DN kidney of *eNOSKO db/db* mice. HB-EGF expression levels in the kidney cortex were higher in both eNOSKO db/db and *eNOSKO* non-diabetic mice than either db/db or non-diabetic mice, with *eNOSKO db/db mice having* the highest HB-EGF level. HB-EGF expression correlated with the extent of renal injury, with increases noted by 12 weeks of age when DN begins to develop, and with further elevations paralleling the progressive glomerulopathy. Those results suggested that loss of NO may be a main contributor of HB-EGF expression. In a cultured glomerular endothelial cell line, administration of the eNOS inhibitors L-NAME or L-NIO increased HB-EGF expression in a dose- and time length-dependent manner. In conclusion, our results suggest that under normal conditions, NO may negatively regulate HB-EGF expression in the EC of the blood vessel, but that with endothelial dysfunction, decreased NO production may allow increased HB-EGF expression, which may be an important mediator of the vascular damage in the DN development and progression in *eNOSKO db/db* mice.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2522**

**Epidermal Growth Factor Receptor (EGFR) Inhibitor Ameliorates Apoptosis in Experimental Diabetic Glomeruli** Shin-Wook Kang, Jung Tak Park, Bo Young Nam, Tae-Hyun Yoo. *Brain Korea 21 Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.*

Kidney size is increased in diabetes due to glomerular and tubular hypertrophy. In addition to hypertrophy, apoptosis has been documented in the course of diabetic nephropathy (DN). Cell death by apoptosis is surmised to be involved in mesangial cell loss in the late stage of DN and podocyte loss, contributing to the development of albuminuria. In spite of numerous studies on hypertrophy and apoptosis in DN, little is known on the interrelationship between hypertrophy and apoptosis in diabetic glomeruli. To clarify the consequence of inhibiting hypertrophy on apoptosis in diabetic glomeruli, we examined the changes in glomerular expression of apoptosis-related molecules in experimental diabetic rats treated with a selective epidermal growth factor (EGF) receptor tyrosine kinase inhibitor, known to abrogate hypertrophy in diabetic glomeruli. Rats were injected either with diluent (n=16, C) or STZ IP (n=16, DM). After confirming diabetes, 8 rats from each group were treated with 100 mg/kg/day of PKI 166 (PKI), a selective EGF receptor inhibitor. Urinary albumin excretion were significantly higher in DM compared to C rats (p<0.05), and PKI treatment significantly reduced albuminuria in DM rats (p<0.05). The mean glomerular volume in DM was 60.6% larger than in C rats, and PKI treatment for 3 months significantly prevented glomerular hypertrophy in DM rats (p<0.05). The ratio of Bax/Bcl-2 protein expression and active caspase-3 protein expression were significantly increased in DM compared to C glomeruli (p<0.01, p<0.05, respectively), and these changes in DM glomeruli were significantly abrogated by the administration of PKI (p<0.05). PKI treatment also significantly inhibited the increase in TUNEL-positive apoptotic cells within DM glomeruli (p<0.05). Compared to C rats, the number of total glomerular cells was significantly decreased in DM, and this decrement in DM rats was significantly ameliorated by PKI treatment (p<0.05). PKI treatment attenuated apoptosis in DM glomeruli, suggesting that the inhibition of glomerular hypertrophy could inhibit glomerular cells apoptosis under DM conditions.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2523**

**Bradykinin Receptor 1 Deficiency Aggravates Albuminuria in Diabetic Mice** Nelly Blaes,[1] Lucie Libert,[1] Christianne Pecher,[1] Francoise Praddaude,[1&2] Acil Jaffar,[1&2] Julien Allard,[1] Ivan A. Tack,[1&2] Jean-Pierre Girolami.[1] *[1]U858, INSERM, Toulouse, France; [2]Physiology Medical School, University Paul Sabatier, Toulouse, France.*

Bradykinin receptor 1 (B1R) is induced during diabetes in several organs including the kidneys. We investigated the role of B1R during the onset of diabetic nephropathy using streptozotocin-induced diabetes in B1R knock out (B1KO) and wild-type (WT) mice. Early renal alterations, including albuminuria, cortical gene and protein expressions, and structural and ultrastructural changes, were monitored up to 4 weeks of diabetes. Diabetes, both incidence and hyperglycemia, was less severe in B1KO. However albuminuria was aggravated in B1KO. Electron micrographs from WT diabetic mice revealed irregularly thickened glomerular basement membrane, extensive podocyte foot effacement process and noticeable interstitial fibrosis. Surprisingly, podocytes lesions were not aggravated in diabetic B1KO. Glomerular hypertrophy and mesangial matrix expansion were unchanged in diabetic B1KO. Similar increase in cortical nephrin expression was observed during diabetes in both strains. Endothelial proteins (VE-cadherin , CD31, ESAM (Endothelial Specific Adhesion Molecule)) expression were higher in control B1KO. Diabetes induced similar alterations of these proteins in both strains. In contrast, diabetic B1KO showed a blunted increase in eNOS expression. The tight junction ZO1 protein was over expressed in control B1KO, but showed a lower increase during diabetes than diabetic WT. The data show that deletion of B1R results in diabetes-induced aggravated albuminuria. This early renal diabetic impact was associated with a marked alteration of cortical eNOS whereas podocytes lesions were similar in both strains. This suggests that B1R deficiency may aggravate diabetic nephropathy through at least impaired endothelial function. Therefore the physiopathological significance of B1R overexpression in diabetes has to be addressed.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2524**

**Resveratrol Improves Oxidative Stress and Protects Against Diabetic Nephropathy through Normalization of Mn-SOD Dysfunction** Munehiro Kitada,[1] Keizo Kanasaki,[1] Shinji Kume,[2] Daisuke Koya.[1] *[1]Diabetes & Endocrinology, Kanazawa Medical University, Kahoku-gun, Ishikawa, Japan; [2]Diabetes & Nephrology & Neurology, Shiga University of Medical Science, Otsu, Shiga, Japan.*

**Purpose:** Despite the beneficial effects of resveratrol (RSV) on renal diseases including type 1 diabetic nephropathy, its effects on type 2 diabetic nephropathy remains elusive. This study examined the renoprotective effect of RSV in db/db mice, a model of type 2 diabetes.

**Methods:** Db/db mice were treated with RSV (0.3% mixed in chow) for 8 weeks. We measured urinary albumin excretion (UAE), histological changes including mesangial expansion, fibronectin accumulation and macrophage infiltration, oxidative stress markers (urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) excretion, nitrotyrosine expression, 8-OHdG content and D-17 deletion in mitochondrial DNA), and manganese-superoxide dismutase (Mn-SOD) activity together with its tyrosine-nitrated modification in the kidney. Mitochondrial biogenesis was assessed by measuring mRNA expression levels of peroxisome proliferative activated receptor γ coactivator-1α (PGC-1 α), nuclear respiratory factors-1(NRF-1), mitochondrial DNA content and mitochondrial number counted under electron microscopy. Blood glucose, HbA1c and plasma lipid profiles were also measured.

**Results:** RSV significantly reduced UAE and attenuated renal histological changes in db/db mice. Oxidative stress and mitochondrial biogenesis were enhanced in db/db mice; however, Mn-SOD activity was reduced by its increased tyrosine-nitrated modification. RSV ameliorated systemic and renal mitochondrial oxidative stress, Mn-SOD activity, mitochondrial biogenesis, and partially improved blood glucose, HbA1c and abnormal lipid profiles in db/db mice.

**Conclusions:** RSV ameliorates renal injury and enhanced mitochondrial biogenesis with Mn-SOD dysfunction in the kidney of db/db mice, through improvement in oxidative stress via normalization of Mn-SOD function and glucose-lipid metabolism.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2525**

**Involvement of Fibrocytes in the Progression of Diabetic Nephropathy Via MCP-1/CCR2 Signaling** Akinori Hara,[1] Kiyoki Kitagawa,[1] Kengo Furuichi,[1] Takashi Wada.[2] *[1]Department of Disease Control and Homeostasis, Kanazawa University, Kanazawa, Ishikawa, Japan; [2]Department of Laboratory Medicine, Kanazawa University, Kanazawa, Ishikawa, Japan.*

**Purpose**: The migration and activation of a circulating mesenchymal progenitor cell, fibrocyte, by the action of the chemokine/chemokine receptor system has been implicated in the pathologic fibrogenesis including kidney fibrosis. In the present study, the involvement of fibrocytes via CC chemokine receptor 2 (CCR2), a cognate receptor of monocyte chemoattractant protein (MCP)-1/CCL2, was examined in the progression of diabetic nephropathy.

**Methods**: Human fibrocytes were isolated and incubated with high concentrations of D-glucose and/or MCP-1. Diabetic kidney injury was induced by the administration of streptozotocin in CCR2 knockout mice and mice treated with propagermanium (PG), a CCR2 inhibitor.

**Results**: *In vitro* examination revealed that stimulation of fibrocytes with high glucose increased the mRNA levels of transforming growth factor (TGF)-$\beta_1$ and pro $\alpha$1 chain of type I collagen (COL1A1) and MCP-1, and this effect was mediated in part by increased osmolality. In *in vivo* studies, fibrocytes, dual-positive for CD45 and type I collagen, infiltrated the diseased kidneys, especially the interstitium, and the number was reduced both in CCR2 knockout and PG-treated mice. Importantly, the blockade of CCR2 signaling reduced glomerulosclerosis and interstitial fibrosis. Concomitantly, transcripts of TGF-$\beta_1$, COL1A1, and MCP-1 in diseased kidney were decreased both in CCR2 knockout and PG-treated mice.

**Conclusion**: These findings suggest that fibrocytes infiltrate the kidney via MCP-1/CCR2 signaling and play a role in the progression of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2526**

**Simvastatin Alleviates Diabetes-Induced VEGF-Mediated Nephropathy Via the Modulation of Tight Junction Protein Occludin** Hui Peng,[1] Yan-Ru Chen,[1] Wenfang Chen,[2] Cheng Wang,[1] Farhad R. Danesh,[3] Tan-Qi Lou.[1] *[1]Division of Internal Medicine, Department of Nephrology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China; [2]Department of Pathology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China; [3]Department of Internal Medicine, Baylor College of Medicine, Houston, TX.*

Previous studies showed VEGF could increase glomerular endothelial cell permeability by disrupting tight junction protein occludin and ZO-1. We hypothesis occludin in glomerulis of diabetic nephropathy is disrupted, and simvastatin could ameliorate the disruption of occludin induced by VEGF in diabetic nephropathy. By using immunofluorescence, we assessed occludin expression in the renal biopsy sections of five diabetic nephropathy patients and three normal controls from renal carcinoma patients after nephrectomy. It showed the immunostaining of occludin was significantly decreased and discontinued in the glomerulis of diabetic nephropathy comparing normal renal control. Diabetic db/db mice received simvastatin (40 mg. kg. day p.o.) for 16 weeks, untreated db/db and db/m mice served as control. Occludin was detected by immuofluorescence and immunoblot, which was conducted with glomerulis extracted from mice kidneys. Urinary microalbumin concentrations were measured by ELISA. Simvastatin-treated mice exhibited significant reduction in albuminuria as well as significant recovery in occludin expression in the glomerulis compared with untreated db/db mice (n=3, $P<0.05$). We cultured glomerular endothelial cells, with or without simvastatin (1μM) pretreatment, in 50ng/ml VEGF. Simvastatin clearly attenuated the hyperpermeability promoted by VEGF when detected by trans-endothelial electrical resistance (TEER). Simvastatin also reversed VEGF induced occludin translocation from para-membrance to cytoplasm and reduced occludin tyrosine phosphorylation caused by VEGF. Based on these data, we propose that occludin is disrupted in glomerulis of diabetic nephropathy, which may be caused by elevated VEGF. It also suggests simvastatin may alleviates diabetes-induced VEGF-mediated endothelial via modulation of occludin.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2527**

**Paricalcitol, Vitamin D Analogue, Ameliorated Renal Injury through an Improvement of Lipid Metabolism and an Anti-Oxidative Effect in Diabetic Nephropathy** Young Sun Kang,[1] Young Youl Hyun,[1] Jinjoo Cha,[1] Ji Eun Lee,[2] Hyunwook Kim,[2] Kum Hyun Han,[3] Hyoung-Kyu Kim,[1] Dae R. Cha.[1] *[1]Nephrology, Korea University Ansan Hospital, Ansan, Republic of Korea; [2]Nephrology, Wonkwang University, Republic of Korea; [3]Nephrology, Inje University, Republic of Korea.*

Vitamin D deficiency is related to the cardiovascular and chronic kidney disease. Vitamin D improves albuminuria and the progression of renal injury. However, the mechanism in diabetic nephropathy remain unclear, independent of the classical action of vitamin D. We investigated the mechanism for the effect of vitamin D on type 2 diabetic nephropathy and lipid metabolism.Type 2 diabetic db/db mice were injected subcutaneously with 0.5ug/kg paricalcitol for 3 months. There were no differences in body weights, FPG, HbA1c, glucose/insulin tolerance test, serum creatinine level, systolic/diastolic blood pressure of the basal characteristic parameters. Organ weights such as kidney, heart, fat and liver were decreased in paricalcitol-treated diabetic mice. Paricalcitol improved cardiac LVMI and urinary albumin excretion. Paricalcitol-treated diabetic mice also showed improved plasma lipid profiles of total cholesterol, triglyceride and LDL.More interestingly, cholesterol and triglyceride contents in kidney tissue were dramatically decreased by paricalcitol, which showed the similar result in fat and liver. In addition, plasma and urinary 8-isoprostane levels were inhibited markedly by paricalcitol treatment. Tissue LPO in kidney, fat and liver was significantly improved. Paricalcitol suppressed the gene expressions and protein synthesis of TGF-b1, PAI-1 and type IV collagen. It also improved glomerulosclerosis, hepatic steatosis and adipose phenotypic change. These results were in accordance with *in vitro* experiment of cultured VDR siRNA mesangial cells that revealed the inhibitory changes in gene expression and protein synthesis of TGF-b1, PAI-1, type IV collagen and the enzymes related with lipid metabolism of FAS, HMG-CoA and SREBP1c. Collectively, paricalcitol might have the protective effect on diabetic nephropathy via an improvement of systemic and tissue lipid metabolism and an anti-oxidative effect.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2528**

**Hydrogen Sulfide Activates AMPK and Inhibits High Glucose Induction of Protein and Matrix Laminin Synthesis in Glomerular Endothelial Cells**
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Hydrogen sulfide (H2S) is a signaling gas that regulates vasculature; its role in diabetic nephropathy is not known. We hypothesized that H2S regulates high glucose effects on glomerular endothelial cells (GEndo). High glucose (30mM glucose), but not equimolar mannitol, significantly promoted global protein synthesis at 8 and 16 hrs and laminin (a1b1g1) synthesis at 1 hr; these events were inhibited by 250 uM NaHS, a H2S donor. Role of AMPK, an inhibitor of protein synthesis, was examined. NaHS stimulated activating Thr172 phosphorylation of AMPK peaking at 250 uM. High glucose reduced AMPK phosphorylation that was fully restored by NaHS at both 5 min and 8 hrs. H2S regulation of calcium-calmodulin kinase kinase beta (CamKKb) as an upstream kinase for AMPK was explored. STO-169 (10uM), an inhibitor of CamKKb, modestly but significantly inhibited NaHS stimulation of AMPK. Downstream of AMPK, high glucose induction of p70S6kinase (S6k) phosphorylation was inhibited by NaHS. High glucose induction of elongation phase events in mRNA translation that are controlled by S6k such as Ser366 phosphorylation of eEF2 kinase and Thr56 dephosphorylation of eEF2 were also inhibited by NaHS. Renal cortical content of cystathionine beta synthase (CBS), a H2S generating enzyme, was significantly reduced in OVE26 mice with type 1 diabetes and in db/db mice with type 2 diabetes compared to that in nondiabetic mice. These data suggest the following: 1. H2S stimulates AMPK phosphorylation in GEndos. 2. H2S inhibits high glucose induction of global and matrix laminin synthesis, probably by stimulating AMPK activity and inhibiting S6k and elongation phase of mRNA translation. 3. H2S induction of AMPK partly involves activation of CamKKbeta. 4. Reduction in CBS in renal cortex of diabetic mice suggests that H2S production in the kidney may be reduced in diabetes. Our data suggest that diabetes-induced reduction in H2S may contribute to renal hypertrophy and matrix accumulation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2529**

**The Post-Ischemia Inflammatory Syndrome of Diabetic Nephropathy**
Katherine J. Kelly,[1] Jesus H. Dominguez.[1,2] *[1]Medicine/Nephrology, Indiana University School of Medicine, Indianapolis, IN; [2]Medicine/Nephrology, Roudebush VAMC, Indianapolis, IN.*

Diabetes mellitus and its complications are a public health problem of epidemic proportions. Both diabetes and chronic kidney disease (CKD) increase the risk of acute kidney injury. In spontaneously obese-diabetic rats (ZS rats) we have demonstrated accelerated progression of nephropathy, with progressive increases in serum creatinine and proteinuria following a single episode of acute renal ischemia. Severe renal inflammation was closely linked to renal dysfunction in this novel model of "acute-on-chronic" renal failure. We termed this entity the post-ischemic inflammatory syndrome (PIIS). Currently, there is no specific therapy for this syndrome. We now demonstrate that renal function continues to deteriorate in rats over months after an acute injury and multiple inflammatory markers are significantly activated in the diabetic kidneys, including interleukin-6, cytokine induced neutrophil chemoattractant, macrophage migration inhibitory factor and transforming growth factor receptor. We further characterized the inflammatory phenotype in cultured tubular cells and found that tumor necrosis factor a and angiotensin II increased expression of both intercellular adhesion molecule-1 (ICAM-1) and the receptor for oxidized LDL, LOX-1, in NRK52E tubule cells. This proinflammatory response was abrogated by GW0742, a selective agonist of peroxisome proliferator activator receptor (PPAR) δ. Hence PPAR δ activation exerts powerful anti-inflammatory action on renal tubular cells. We speculate that PPARδ agonists could improve the postischemia inflammatory syndrome.

Disclosure of Financial Relationships: Ownership: Partial ownership of LLC providing microscopic imaging and consulting services.

**SA-PO2530**

**Osteopontin-to-Creatinine Ratio as a Biomarker of Type 2 Diabetic Nephropathy**
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Osteopontin (OPN) is a secreted, multi-functional, matrix glycoprotein present in low levels in kidneys, but is significantly upregulated in tissue injury. Increased OPN plays an important role in several animal models of chronic kidney disease, wound healing and coronary artery calcification and is a potential biomarker of coronary artery disease. We have demonstrated that OPN is critical in angiotensin II-induced tubulointerstitial fibrosis, and glomerulosclerosis of type 1 and type 2 diabetic nephropathy (DN). Here we show that increased urinary osteopontin-to-creatinine ratio (OCR) in the *db/db* mouse, a well characterized genetic model of human type 2 DN, correlates well with several structural parameters characteristic of human DN.

Non-diabetic *db/m* and diabetic *db/db* mice (n=5-7) were followed from the start of diabetes (age 8 weeks) to age 20 weeks. Plasma glucose and tail-cuff blood pressures were measured and animals were placed in metabolic cages for 24h urine and determination of OCR and albumin-to-creatinine ratio (ACR; by ELISA). At age 20 weeks, the right kidney was harvested for paraffin-embedded sections for Periodic Acid Schiff (PAS) staining and the left kidney was perfused-fixed and processed for light and electron microscopy.

As expected, plasma glucose significantly increased after 12 weeks of diabetes. In addition, there was a 1.6-fold, $p<0.05$ increase in % mesangial area, a 1.1-fold, $p<0.05$ increase in glomerular basement membrane thickening, 1.3-fold, $p<0.001$ increase in fractional volume of mesangium, and 1.6-fold, $p<0.005$ increase in glomerular volume. OCR and ACR were significantly increased between *db/m* and *db/db* mice, 15-fold and 24-fold, $p<0.05$ respectively. Urinary OCR in *db/db* mice showed a consistent increase up to 64.8-fold, $p<0.005$. However, ACR peaked at 16 weeks (6.4-fold, $p<0.05$) and then declined.

The data indicate that OCR is an acceptable indicator of the structural glomerular progression of DN and that OCR may be a better determinant of DN compared to ACR in the *db/db* mouse. The data also suggests that OCR may be a biomarker of DN in humans.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2531**

**Advanced Glycation End-Products (AGEs) Inhibit Focal Adhesion Kinase and Rac-1 Activition Via Suppression of Neuropilin-1 (NRP1) Expression**
Gunter B. Wolf. *KLinik für Innere Medizin III, University of Jena, Jena, Germany.*

Aim: We have recently demonstrated that glycated-BSA (AGE-BSA) inhibits podocytes migration in a NRP1 dependent manner. The purpose of this study was to analyze the contribution of AGEs and NRP1 in regulation of focal adhesion kinase (FAK) activity and small RhoA GTPases in differentiated podocytes.

Methods: Murine differentiated podocytes were treated with 5 mg/ml AGE-BSA or the same amount of a control-BSA(Co-BSA), for 24 h. After that the cells were left untreated or stimulated with 10-7M (Phorbol 12-myristate 13-acetate, PMA), a well-known FAK activator for 10 min and lysates were analyzed via western blot. NRP1 expression was down-regulated via SIRNA technology. Influence of NRP1 on FAK activation was also tested through forced overexpression of NRP1. The activity of Rac-1 and RhoA was analyzed in pull-down assays.

Results: Our data revealed that pFAK activation was significantly reduced in cells treated with AGE-BSA compared to Co-BSA. Similarly, podocytes depleted of NRP1 via transfection of Nrp1 SiRNA inhibited PMA dependent FAK activation, In contrast, forced overexpression of full length mouse NRP1 in podocytes induced stronger FAK Tyr 576 phosphorylation in PMA treated cells then the empty vector transfection. As migration is also related to the activation of the small RhoA GTPases we addressed the influence of AGE-BSA and NRP1. Our data demonstrate that treatment of podocytes with PMA induced an accumulation of the Rac-1 GTP, active state, in Co-BSA incubated cells, while AGE-BSA remarkably inhibited Rac-1 activation. Down-regulation of NRP1 suppressed PMA induced Rac-1 activity. In fact, there was not a detectable signal for Rac-1 in the western blots from the pull-down assays. Nevertheless, no inhibitory effect of the co siRNA was detected. Opposite, NRP1 overexpression increased the accumulation of Rac-1 GTP in PMA stimulated podocytes above the empty vector transfected cells treated with PMA. Conclusions: AGE-BSA treatment inhibits FAK activity in podocytes stimulated with PMA compared with Co-BSA. This effect was NRP1 dependent These mechanisms may contribute to diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2532**

**Advanced Glycation End Products (AGEs) Increase Human Mesangial Foam Cell Formation by Increasing SCAP Golgi Modification**
Xiong Z. Ruan,[3] Yang Yuan,[1,3] Lei Zhao,[2] John F. Moorhead,[3] Zachariah Varghese.[3] *[1]Endocrinology, Zhongda Hospital Southeast University, Nanjing, China; [2]Centre for Lipid Research, Chongqing Medical University, Chongqing, China; [3]Centre for Nephrology, University College London Medical School, London, United Kingdom.*

Diabetic nephropathy caused by advanced glycation end products (AGEs) is associated with lipid accumulation in glomeruli. This study was designed to investigate whether $N^{\varepsilon}$-(carboxymethyl) lysine (CML) (one of AGEs family) increase lipid accumulation in human mesangial cells (HMCs) via LDL receptor pathway by increasing SREBP cleavage-activating protein (SCAP) transcription and its posttranslational modification in Golgi.

HMCs were treated with CML. Intracellular cholesterol content was assessed by Oil Red O staining and cholesterol enzymatic assay. Expression of mRNA and protein of molecules controlling cholesterol homeostasis in the treated cells was examined by real-time quantitative PCR and western blotting, respectively. Golgi enzymes activity was determined using enzyme-based method. SCAP translocation was detected by confocal microscopy.

CML increased cholesterol accumulation in HMCs. Exposure to CML increased expression and abnormal translocation of SCAP from ER to Golgi even in the presence of a high concentration of LDL. The increased SCAP translocation carried more transcription factor SREBP2 to the Golgi for activation by cleavage which enhanced gene transcription of LDL receptor and HMGCoA reductase. Furthermore, CML enhanced SCAP glycolyzation by upregulating Golgi mannosidase activity. This prolonged the half-life and enhanced recycling of SCAP between the ER and the Golgi. The effects of CML were blocked by inhibitors of Golgi mannosidases.

*Conclusions:* AGEs (CML) increased lipid synthesis and uptake, thereby causing foam cell formation via increasing transcription and protein glycolyzation of SCAP by Golgi enzymes in HMCs. These data imply that Golgi enzymes inhibitors might have a potential renal protective role in prevention of mesangial foam cell formation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2533**

**Attenuation of Diabetic Nephropathy Via RAGE Deletion in Type 1 Diabetic Transgenic Mice Expressing Human Aldose Reductase** Anjali Ganda, Radha Ananthakrishnan, Rosa Rosario, Vivette D. D'Agati, Ravichandran Ramasamy, Ann Marie Schmidt. *Medicine, Surgery, Pathology, Columbia University College of Physicians & Surgeons, New York, NY.*

Substrate flux via aldose reductase (AR), the rate limiting enzyme in the polyol pathway, mediates the generation of pre-advanced glycation end-products (pre-AGEs) from glucose. AR-inhibitors suppress albuminuria, mesangial expansion, and glomerular basement membrane (GBM) thickening in animal models, and reduce microalbuminuria in human type 1 diabetics. AR gene polymorphism is present in patients with diabetic nephropathy. We tested the hypothesis that Receptor for Advanced Glycation End-Products (RAGE) gene deletion in Type 1 diabetic transgenic mice expressing human levels/activity of AR (TghAR+) will exert protection against indices of diabetic nephropathy (DN).

TghAR+ mice (C57BL/6) were bred into the RAGE-expressing(+) or RAGE-null background and rendered Type 1 diabetic with streptozotocin at age 6 wks. Mice were sacrificed at age 15 and 26 wks. Kidneys were stained with periodic-acid Schiff (PAS). A semi-quantitative mesangial sclerosis score was calculated by averaging findings in >100 glomeruli/mouse (scale 0-3+; 0=absent, 1=mild, 2=moderate, 3=severe). GBM thickness and foot process (FP) effacement were determined by ultrastructural analysis (≥ 8 glomeruli/mouse studied).

TghAR+RAGE+ mice sacrificed at 15 wks of age (n=2) had mean 0.5 mesangial sclerosis, GBM diameter 156.76±22.96 nm, and 5% FP effacement. TghAR+RAGE+ mice sacrificed at 26 wks of age (n=2) demonstrated progression of all indices of DN with mean 2.5 mesangial sclerosis, GBM diameter 268.22±117.25 nm, and 21% FP effacement. TghAR+RAGE-null mice sacrificed at age 26 wks (n=2) displayed sizable, although not complete, protection in all indices of DN with mean 1.5 mesangial sclerosis, GBM diameter 200.11±41.13 nm, and 12% FP effacement.

RAGE deletion is known to protect against indices of DN in animal models. One major previously unexplored mechanism may be its effect on inhibiting the downstream effects of toxic products generated by AR. Drugs targeting both AR and RAGE may exert additive/synergistic benefit in preventing progression of DN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2534**

**Kidney Handling of Glycated and Advanced-Glycated End Product Albumin Using Two-Photon Microscopy** Monika D. Gandhi, Mark C. Wagner, George Rhodes, Exing Wang, Ruben M. Sandoval, Silvia B. Campos-Bilderback, Bruce A. Molitoris. *Nephrology, Indiana University, Indianapolis, IN.*

Glycated and Advanced Glycation End Products (AGE) are different stages of glucosylated proteins commonly shown as strong markers of Diabetic complications. In particular, glucosylated proteins derived from the Glucose and Dicarbonyl α-oxaldehyde Methylglyoxal (MGO) incubations are closely associated with hyperglycemia and increased susceptibility to Diabetic Nephropathy. The, *Kidney* is the major site for glucosylated protein excretion with selective excretion of glucosylated albumin, known as the "editing" phenomenon - however, little is known about the *process of* editing.

Therefore, our study tests the hypothesis that glucosylated-modified albumin is filtered through the glomerular barrier at similar rates, but it is handled differently by the proximal tubule cells. This was evaluated by infusion of Texas Red conjugated, un-glycated, glucose-modified, and MGO-modified albumin into *Munich Wistar Fromter* rats. Two-photon microscopy was used to directly quantify *in vivo* albumin filtration and proximal tubule handling.

MGO-modified albumin showed a significant reduction in plasma fluorescence at 24 hrs compared to both un-glycated (P 0.006) and glucose-modified albumins (P 0.031) with percent plasma fluorescent at 24hrs/2hrs for Un-glycated of 19.41+/-3.33%, Glucose-modified of 20.17+/-5.91% and MGO-modified of 7.43+/-0.31%. We measured similar glomerular permeability (Glomerular Sieving Coefficient - GSC) between Glucosylated albumin (GSC for MGO-albumin of 0.0113+/-0.0081 and Glycated-albumin of 0.0099+/-0.0051) and un-glycated albumin (GSC of 0.0109+/-0.0047). These differences indicate that it is not the glomerular handling, but possible protein handling and trafficking by the renal proximal tubule of the AGE-modified proteins that is affected. However, the reversible mid-phase glucosylated proteins made by glucose-modification of the albumin is not handled differently as the end-phase Amadori products of AGEs. This is consistent with the pathogenic role of AGEs and early proximal tubule functional changes seen leading to hypertrophy and nephropathy in diabetes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2535**

**Advanced Glycation End-Products (AGEs) Differentially Regulate Morg1 Expression in Renal Cells** Gunter B. Wolf. *Internal Medicine II, University of Jena, Jena, Germany.*

Aim: The mitogen-activated protein kinase organizer 1 (Morg1) was recently identified as a novel protein that covalently binds to PHD3. The purpose of this study was to analyze the influence of AGEs on Morg1 expression in different kidney cell lines. Methods: MMC (mouse mesangial cells) and MTC (mouse tubular cells) . Differentiated murine podocytes were also studied. Cells were serum deprived for 24 h, followed by treatment with 5 mg/ml Co-BSA (not glycated BSA) or AGE-BSA (glycated - BSA) for 24 h. Morg1 expression was analyzed by real-time PCR and western blot. HIF-1alpha promoter activity was tested in a reporter-gene assay system. PHD3 activity was measured using hydroxylation-coupled decarboxylation of (1-14C)-2-oxoglutarate followed by liquid scintillation counting of the formed 14CO2. Results: All three kidney cell lines expressed Morg1, but podocytes somewhat less then MMC and MTC cells. Our data revealed that AGE-BSA increased Morg1 expression in MMCs and podocytes, whereas its expression in MTCs was significantly reduced. Western blot analysis confirmed the real time PCR findings. In addition we checked the HIF-1α and HIF-2α expression on a protein level but did not detect significant changes. Therefore, we analyzed HIF-1alpha expression using a gene–reporter assay, which is more sensitive method to test gene expression and regulation. We observed that HIF-1α promoter activity in MMC and MTC exposed to AGE-BSA was induced correspondingly 1.66±0.16 fold and 1.82±0.12 fold relative to Co-BSA treated cells. In contrast HIF-1α promoter activity demonstrated significant reduction in luciferase activity in differentiated podocytes incubated with AGE-BSA (0.7±0.1 fold vs. Co-BSA). We found that AGE-BSA treatment increased PHD3 activity in MMC and podocytes, but inhibited PHD3 activation in MTCs. Conclusion: The relationship between Morg1 expression, PHD3 activity and HiF1α activation is complex and cell specific. Although AGE-BSA-mediated suppression of PHD3 activity increased HiF1α expression, Morg1 appears to be concomitantly regulated with PHD3. Our finding may provide a novel link between AGEs and hypoxia in diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2536**

**Additive Renoprotective Effects of B2-Kinin Receptor Blocker and PPAR-γ Agonist in Uninephrectomized *db/db* Mice** Sydney C. W. Tang,[1] Loretta Y. Y. Chan,[1] Joseph C. K. Leung,[1] Hui Y. Lan,[2] Kar Neng Lai.[1] *[1]Department of Medicine, The University of Hong Kong, Hong Kong, Hong Kong; [2]Department of Medicine and Therapeutics, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, Hong Kong.*

We recently showed that the bradykinin B2 receptor (B2R) blocker icatibant and the perioxisome proliferator-activated receptor-γ agonist rosiglitazone (Ros) exerted anti-inflammatory effects in renal tubular cells exposed to a diabetic milieu. This study aims to explore whether these effects can be translated to an experimental model of type 2 diabetic nephropathy. *db/db* mice and their nondiabetic *db/m* littermates underwent sham operation or uninephrectomy (Unx) at 10 weeks and received vehicle, metformin, icatibant, Ros, or icatibant plus Ros for 8 weeks before sacrifice. Among the *db/db* group, mice that received icatibant or Ros had significantly lower serum creatinine and albuminuria, which was further reduced when icatibant and Ros were given in combination. These beneficial effects were not observed in the metformin group that achieved similar glycemic control as Ros-treated animals. Likewise, the severity of glomerulosclerosis, interstitial injury, cortical F4/80 and a-smooth muscle actin immunostaining, and CCL-2, ICAM-1, and TGF-β overexpression were all attenuated by icatibant and Ros, and these effects were enhanced when both agents were combined. At the signaling level, icatibant and Ros reduced ERK1/2 and STAT1 activation, respectively. Our results suggest a deleterious role of the kallikrein-kinin system in murine accelerated diabetic nephropathy, which can be ameliorated by the B2R blocker icatibant and enhanced by the addition of Ros. This may open a novel therapeutic approach for further evaluation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2537**

**Kidney Injury Molecule-1 (Kim-1) Mediates Tubular Epithelial Cell Injury in Diabetic Nephropathy** Takaharu Ichimura, Huiping Zhao, Suetonia Palmer, Shan Mou, Davide Pietro Cina, Heung-Myong Woo, Joseph V. Bonventre. *Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Diabetes mellitus is the leading cause of end-stage renal failure. Although diabetic nephropathy (DN) is characterized by glomerulopathy and albuminuria, disease progression best correlates with tubular degeneration and interstitial fibrosis. Kim-1, a scavenger receptor highly expressed by injured proximal tubule cells, is present in the urine of patients with DN.

To study the effects of Kim-1 on disease progression, we developed a murine model of DN combining streptozotocin injection, unilateral nephrectomy, and high fat diet. Mice developed albuminuria and progressive kidney injury; Kim-1 was expressed by proximal tubule cells. *In vitro* high glucose levels enhanced cultured epithelial cell Kim-1 expression, via a process that was reactive oxygen species dependent. *In vivo* and *in vitro*, Kim-1 augmented tubule cell uptake of advanced glycation end products (AGE) and oxidized lipids, two components of the proximal tubular fluid in patients with diabetes mellitus. In mouse DN kidneys *in vivo*, tubules that expressed Kim-1 had augmented AGE uptake, brush border loss, and were surrounded by F4/80 positive macrophages. In these kidneys, proximal tubules showed accelerated tubule cell senescence and increased interstitial myofibroblast expansion. Ingestion of oxidized lipids or AGE by primary tubule cells that express Kim-1 in culture markedly enhanced caspase 3 activity and apoptosis, reflecting tubule cell toxicity. Uptake of oxidized lipids by tubule cells also enhanced subsequent TNFα release by co-cultured macrophages.

In conclusion, Kim-1 expression in DN is induced by hyperglycemia and associated oxidant stress. Kim-1 enhances tubule cell uptake of AGE and oxidized lipids, and potentiates a pro-inflammatory tubular injury that ultimately contributes to tubule degeneration and fibrosis, and progressive kidney disease.

Disclosure of Financial Relationships: Patent: Kim-1 patents licensed by Partners Healthcare to Johnson and Johnson, BiogenIdec and Genzyme.

**SA-PO2538**

**Inhibition of Inflammation by Oral Pentosan Polysulfate Prevents Diabetic Nephropathy in Aging C57B6 Mice** Jin Wu, Fabrizio Grosjean, Helen Vlassara, Gary E. Striker, Feng Zheng. *Geriatrics and Palliative Medicine, Mount Sinai School of Medicine, New York, NY.*

Since inflammation plays a key role in the development and progression of diabetic nephropathy, we asked whether a drug that reduces inflammation would reduce diabetic nephropathy in mice. We previously found that PPS reduces interstitial inflammation and prevents glomerulosclerosis in 5/6th nephrectomy rats (JASN 12:2080-87, 2001). In addition, we recently found that, whereas young diabetic C57B6 mice develop only mild lesions, old diabetic C57Bl6 mice develop severe DN involving glomeruli, tubules, blood vessels and the tubulointerstitium (AmJPath 176:2163-76, 2010). Since PPS reduces interstitial inflammation, we examined old diabetic C57B6 mice to determine if PPS treatment affected DN. We examined fresh renal tissue and renal cells in culture to determine the mechanisms by which PPS affects proinflammatory responses in diabetes. C57B6 mice (18 months old) were treated with streptozotocin to induce diabetes in aging C57B6 mice. One month after the induction of stable diabetes, mice were randomized to receive either oral PPS (25 mg/kg/day) or tap water for 4 months. PPS treatment preserved renal function (Scr 0.109±.03 vs. 0.138±.02 mg/dl, $p<0.05$) and significantly reduced the progression of albuminuria (710 vs. 48 µg/mgCr, $p<0.01$) in aging diabetic mice, and decreased the severity of renal lesions, especially the tubulointerstitial inflammation (<1+ vs. 3+) and 70% reduction in apoptosis. The upregulation of TNFa and proinflammatory genes (MCP-1, RANTES, CXCl-1, MIP-2, and ICAM-1) in aging diabetic kidneys was reduced by >50% with PPS treatment. In cultured renal cells, PPS suppressed NF-kB and decreased the proinflammatory action of TNFa by >2-fold. PPS also decreased TNFa, high glucose, and advanced glycation end product-stimulated MCP-1 production. Finally, PPS decreased TNFa-induced increase in albumin permeability in podocytes in culture. Thus, 1) PPS treatment decreases inflammatory lesions of aging diabetic nephropathy and largely prevents disease progression, 2) PPS may be a useful adjunct in the management of DN in aging patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2539**

**Therapeutic Augmentation of Tubular Regeneration Following Acute Kidney Injury** Takuto Chiba,[2] Raymond C. Harris,[1] Neil A. Hukriede,[3] Mark P. De Caestecker.[1, 2] *[1]Nephrology Division, Vanderbilt University, Nashville, TN; [2]Cell and Developmental Biology, Vanderbilt University, Nashville, TN; [3]Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA.*

As part of a screen to identify compounds that promote expansion of renal progenitor cells, we identified a novel histone deacetylase (HDAC) inhibitor, methyl-4-(phenylthio) butanoate (M4PTB). M4PTB promotes expansion of renal progenitor cells in zebrafish embryos, and enhances renal tubular recovery in a zebrafish model of acute kidney injury (AKI). Other HDAC inhibitors such as phenylbutanoic acid and trichostatin A likewise promote expansion of the renal progenitor cells in zebrafish embryos. In comparison to M4PTB, these compounds induce generalized defects in embryonic patterning at equivalent effective doses indicating greater toxicity. To determine whether M4PTB augments renal tubular regeneration following AKI in mice, we first evaluated the effect of systemic administration of M4PTB on renal histone acetylation in mice. A single dose of m4PTB induced a marked increase in H3 K9 acetylation for 24 hours after treatment. Having established the effective dose of M4PTB, we developed a mouse model of AKI in which selective proximal tubular injury is induced by administration of diphtheria toxin (DT) to transgenic mice expressing the diphtheria toxin receptor under the control of gamma-GT promoter (γ-GT-hHB-EGF mice). M4PTB was administered subcutaneously 24 hours after DT injection in γ-GT-hHB-EGF mice and then daily thereafter. At four days post-injury there was a reduction in blood urea nitrogen (BUN) levels, and tubulointerstitial injury scores are decreased in M4PTB-treated vs. vehicle-treated mice after 10 days. Moreover, M4PTB treatment is associated with increased proximal tubular cell and decreased interstitial cell proliferation during recovery. These data indicate that treatment with M4PTB increases the rate of renal recovery, reduces post-injury tubulointerstitial fibrosis and suggest that these effects result from selective enhancement of renal tubular regeneration and inhibition of interstitial fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2540**

**Variability in PTEN Expression Regulates Glomerular Endothelial Cell Permeability** Zhaoyong Hu,[1] Wenjian Wang,[2] William E. Mitch.[1] *[1]Internal Medicine, Baylor College of Medicine, Houston, TX; [2]Internal Medicine, Guangdong General Hospital, Guangzhou, Guangdong, China.*

Growth factors have been implicated in modulating kidney functions because they can promote cell growth and mobility plus cytoskeletal rearrangement. Many growth factor-initiated signaling pathways include stimulation of phosphatidylinositol 3-kinase (PI3K) activity, producing the 2nd messanger, PIP3. PIP3 is also regulated by PTEN, a phsphatase converting PIP3 to the inactive product, PIP2. In db/db mice with diabetic nephropathy, PTEN expression was shown to be reduced in glomeruli leading to the conclusion that a decrease in PTEN causes measangial expansion as a prelude to diabetic nephropathy. In normal mouse kidney, we found that PTEN is expressed in glomerular endothelial cells,podocytes and tubular cells. In kidneys of db/db mice, we found PTEN expression decreassed in glomerular endothelial cells and podocytes. We then evaluated whether changes in PTEN expression could affect kidney function by measuring albumin permeability through a monolayer of glomerular endothelial cells. First, we knocked down PTEN in glomerular endothelial cells and found a 1.5-fold increase in albumin permeability compared to cells treated with a scrambled control siRNA. Second, we over expressed PTEN in glomerular endothelial cells: albumin permeability was unchanged from control, but permeability stimulated by VEGF or PDGF were blocked. Because changes in the actin cytoskeleton can regulate cell permeability in glomerular endothelial cells, we evaluated how varying PTEN expression would influence actin cytoskeleton rearrangement. PTEN over-expression inhibited the rearrangment of actin filaments induced by VEGF; Knokdown of PTEN increased F-actin formation, consistent with the increase in albumin permeability. We also studied the BpV inhibitor of PTEN, when we added BpV (Hopic) to glomerular endothelial cells, albumin permeability was increased.We concluded that changes in PTEN expression regulate the permeability of glomerular endothelial cells. Manipulation of PTEN could provide a target for preventing albuminuria associated with diabetic nephropathy and potenitally, other chronic kidney diseases.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2541**

**Mannose-Binding Lectin (MBL) and High-Sensitive C-Reactive Protein (hsCRP) Concentrations Predicts Cardiovascular Events and Kidney Function among Patients with Type 1 Diabetes and Diabetic Nephropathy** Maria Lajer,[1] Anders Jorsal,[1] Troels Krarup Hansen,[3] Lise Tarnow,[1] Steffen Thiel,[3] Hans-Henrik Parving,[2] Allan Flyvbjerg,[3] Peter Rossing.[1] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]Dep. of Medical Endocrinology, Rigshospitalet, Copenhagen University Hospital, Denmark; [3]Medical Department M and Medical Research Laboratories, Aarhus University Hospital, Denmark; [4]Medical Microbiology and Immunology, Aarhus University.*

Mannose-binding lectin (MBL) and high-sensitive C-reactive protein (hsCRP) are markers of complement activation and inflammation suggested to be involved in diabetic vascular damage.

This prospective observational follow-up study included 198 type 1 diabetic patients with overt diabetic nephropathy (121 men; age 40.9±9.5 years (mean±SD); $^{51}$Cr-EDTA-GFR 74±33ml/min/1.73m$^2$) and 175 longstanding type 1 diabetic patients with persistent normoalbuminuria (103 men; age 42.6±9.5 years; duration of diabetes 27.6±8.2 years). Patients were followed for 12.6(0.0-12.9) years (median (range)).

Among patients with diabetic nephropathy, 78 patients died and 84 suffered fatal or non-fatal cardiovascular event. Those with both serum MBL and hsCRP levels above the median had an increased risk of all-cause mortality and fatal or non-fatal cardiovascular disease, compared to patients with both levels below the median ($p<0.008$). After adjusting for covariates (sex, age, smoking, SysBP, $HbA_{1c}$, cholesterol, and GFR), hazard ratio (95% CI) were 1.9(1.0-3.9) and 2.0(1.0-3.8), respectively.

Regarding kidney function, the decline in GFR was faster among patients with higher MBL and hsCRP levels (-4.7±5.8) ml/min/year) vs. lower (-3.2±2.5) ml/min/year), ($p=0.042$). However, MBL and hsCRP levels did not predict development of end-stage renal disease (n=40), ($p=0.71$). Among the normoalbuminuric patients, MBL above vs. below the median did not predict either all-cause mortality or fatal and non-fatal cardiovascular disease 1.4(0.6-3.6) and 0.7(0.3-1.6), respectively.

In conclusion, combined higher levels of MBL and hsCRP are predictive of cardiovascular disease and loss of kidney function among type 1 diabetic patients with diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2542**

**Discovery of Novel Biomarkers for Diagnostics of Diabetic Nephropathy** Ulrika Lundin,[1] Angel Argiles,[2] Harald Mischak,[3] Klaus M. Weinberger.[1] *[1]BIOCRATES Life Sciences AG, Innsbruck, Austria; [2]RD Néphrologie, Montpellier, France; [3]Mosaiques Diagnostics & Therapeutics AG, Hannover, Germany.*

Diabetic nephropathy is one of the most severe complications from type II diabetes and often leads to end stage renal disease. The epidemiological increase of type II diabetes and the fact that conventional diagnostic markers like creatinine and albumin are insensitive and unable to detect renal damage at initial stages of pathogenesis emphasizes the need of novel and more sensitive markers.

In this study, samples from six cohorts, diabetics and non diabetics at different stages of nephropathy, were collected. Targeted metabolomics was used to quantitate about 460 metabolites in plasma and 270 in urine including the classes amino acids, biogenic amines, polyamines, acylcarnitines, phosphatidylcholines, reducing mono- and oligosaccharides, sphingomyelins, eicosanoids, bile acids and energy metabolism intermediates. The analytical methodology of choice was flow injection analysis (FIA) or HPLC-ESI-MS/MS in highly selective multiple reaction monitoring mode performed on an AB Sciex API 4000 QTRAP® instrument (AB Sciex). Additionally, 160 fatty acids were quantitated in plasma by GC-MS/MS.

The datasets were analyzed using principal components- and discriminant analysis and several advanced statistical tests to assess the most significant metabolites. By first looking at the different metabolic pathways and then grouping the significantly altered metabolites thereafter, we found compounds involved in dimethylarginine metabolism, in urea cycle / NO synthase, TCA cycle, certain low abundant acylcarnitines, and oxidative stress related compounds to be associated with progression of renal damage. These results both confirmed old findings, such as symmetric dimethylarginine (SDMA), already an established marker for renal damage, but also presented potential novel biomarkers like

the acylcarnitines glutarylcarnitine and pelargonylcarnitine, which both show an increase with progression of renal damage. With metabolomics, not only new markers can be found but by covering the main pathways of intermediary metabolism a deeper understanding of the cause of the disease can also be obtained.

Disclosure of Financial Relationships: Employer: BIOCRATES Life Sciences AG; Patent: BIOCRATES Life Sciences AG.

### SA-PO2543

**A Novel Model of Metabolic Syndrome and Insulin Resistance (IR) in Uremic Rodents and Effect of Inhibition of the Enzyme 11 beta Hydroxy-Steroid Dehydrogenase Type 1 (11HSD1)** Ananda Chapagain, Paul W. Caton, Julius Edward Kieswich, Nanda K. Nayuni, Steven Michael Harwood, Martin J. Raftery, Magdi Yaqoob. *Translational Medicine and Therapeutics, QMUL, London, United Kingdom.*

We have shown that IR in uremia can be partly explained by excessive gluconeogenesis due to increased hepatic glucocorticoid (GC)activity through 11HSD1 in 5/6 nephrectomized rats. Here, we present further data in a different animal model. Eight week old Wistar rats were subjected to a diet consisting of 0.75% adenine or standard chow for a total of 4 weeks.At 2 weeks carbenoxolone (CX; 50 mg/kg) was gavaged to half of the rats to give 4 groups (1) adenine-induced uremia (2) adenine-induced uremia plus CX (3) standard chow (4) standard chow plus CX.After 4 weeks,the rats were fasted, anaesthetized and plasma samples obtained. Rats were then subjected to glucose and insulin tolerance tests.The animals were sacrificed,and plasma and tissue samples obtained.To assess gluconeogenesis, phosphoenolpyruvate carboxykinase (PCK1) and Glucose-6 phosphatase (G6Pase) was assayed using western blot, along with protein levels of 11HSD1, PCK1 transcription factors p-CREB and FOXO1,PGC-1a (PPAR-gamma coactivator 1 alpha)and TORC2(PGC1a coactivator).Insulin and levels of inflammatory cytokines IL-1b and TNF-a,known up-regulators of HSD1,were measured using specific ELISA.Statistical comparisons were determined by ANOVA.Plasma urea (8.8 fold; $p<0.01$) and creatinine (6.2 fold; $p<0.01$) levels were raised in uremia,as well as higher fasting plasma Insulin levels(2.1 fold, 503±16 pg/ml, $p<0.05$), abnormal glucose and insulin tolerance tests, elevated circulating levels of IL-1b (1.9 fold, 66 ±12 pg/ml, $p<0.05$) and increased 11HSD1, p-CREB, TORC2, PGCa, PCK1 and G6Pase protein compared to sham animals.These changes reversed with CX treatment,with 11HSD1 and PCK1 protein reduced by 1.6± 0.4fold ($p<0.05$) and 1.4± 0.1 fold ($p<0.01$) respectively and similar reduction in associated proteins.

These results confirm that IR in uremia occurs through abnormally elevated GC-directed gluconeogenesis as a result of increased 11HSD1. Furthermore, we also demonstrate a novel and easily reproducible model of IR in uremia.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2544

**Mouse Model of Diabetic Cardiorenal Syndrome** Jae-Hyung Chang,[1] Seung-Yeol Paik,[2] Lan Mao,[1] Susan B. Gurley,[1] Phillip Ruiz,[3] Robert F. Spurney.[1] *[1]Duke University, Durham, NC; [2]Chung-ang University, Seoul, Korea; [3]University of Miami, Miami, FL.*

A significant subset of patients with diabetes mellitus develop cardiovascular disease and diabetic nephropathy (DN) which may result from a constellation of coexistent risk factors including hypertension, left ventricular hypertrophy (LVH), hyperglycemia and albuminuria. In developed countries, diabetic cardiorenal syndrome is a significant healthcare burden because the incidence of diabetes mellitus has reached epidemic proportions. Akita mice are a genetic model of type 1 diabetes caused by a mutation (*Ins2*$^{+/C96Y}$) that results in selective pancreatic β-cell failure as a result of proteotoxicity due to misfolding of insulin 2. The phenotypic effect of these abnormalities is, however, significantly influenced by genetic background. In the present studies, we determined if Akita mice on the FVB/NJ background have features of human diabetic cardiorenal syndrome including hypertension and durable hyperglycemia as well as histopathologic, functional and molecular characteristics of DN and LVH in humans. We found that male FVB/NJ Akita mice developed sustained hyperglycemia as well as polyuria by 4 weeks of age, which was associated with increased rates of podocyte apoptosis. These abnormalities were associated with a significant increase in systolic blood pressure in 10-week old Akita mice. At this time point, heart weight was significantly increased and was associated with echocardiographic evidence of LV systolic dysfunction as well as enhanced expression of molecular markers of cardiac hypertrophy including mRNA for β-myosin heavy chain as well as atrial natriuretic peptide. Pathological examination revealed interstitial fibrosis and inflammatory infiltrates in hearts of 10-week old Akita mice. By 20 weeks of age, Akita mice developed features of human DN including renal hypertrophy, a 10-fold increase in albuminuria, glomerular hypertrophy and a decrease in the number of podocytes. While additional studies are required to fully validate the FVB/NJ Akita model, these data suggest that Akita mice on the FVB/NJ background may be a useful platform for studying the mechanisms of diabetic cardiorenal injury in humans.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2545

**Self-Assemble Peptide Nanofibers Serve as an Acellular Develivery Platform for the Secretome from Human Embryonic Stem Cells: Implications for Diabetic Nephropathy** Yin Wang, Wenjian Wang, Farhad R. Danesh. *Medicine/Nephrology, Baylor College of Medicine, Houston, TX.*

Recent studies have suggested that paracrine/endocrine factors secreted from stem cells account for many of the beneficial effects of stem cells in the kidney recovery following acute kidney injury. However, the contribution of secretome from stem cells on chronic kidney diseases such as diabetic nephropathy (DN) is unknown. In this regards, human embryonic stem cells (hES) represent a powerful tool for cell therapy in the kidney due to their extensive profile of cytokines/chemokines and growth factors. In the current study, we developed a self-assembled peptide nanofiber preparation as an acellular platform for delivery of secreted paracine/endocrine factors released from human embryonic stem cells (hES). To prepare preconditioned nanofibers, nanofibers were exposed to secretome from hES for 24hrs using a two-compartment transwell system, in which nanofibers were placed in the upper compartment, and hES cells were seeded in the lower compartment. To assess the effect of preconditioned nanofibers on high glucose-induced endothelial cell permeability, preconditioned nanofibers were transferred to the lower compartment of a new transwell system, whereas kidney microvascular endothelial cells were seeded on the upper compartment. After 24 hours of coculture in the presence of high glucose, $^{125}$I-BSA was added into the upper compartment, and the permeability of $^{125}$I-BSA across confluent endothelial cell monolayer was determined. We observed that preconditioned nanofibers significantly decreased high glucose (25mM)-induced endothelial cell permeability ($4.7x10^{-2}$ul/min *vs*.$3.5x10^{-2}$ul/min, $P<0.05$). Preconditioned nanofibers also reversed high glucose-induced apoptosis in podocytes (25mM x 24hrs, 20% *vs*. 8%, $P<0.01$) through inhibition of caspase-3 activity. Our findings indicate that the use of preconditioned nanofibers can potentially address many concerns associated with the use of hES cells in vivo, and provide a novel scientific rationale for the use of preconditioned nanofibers in vivo in animal models of diabetes.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2546

**Renal Co-Regulation of Carnosine Synthase and Carnosinase in Developing Diabetic Nephropathy** Ana Zutinic, Antien Mooyaart, Jan A. Bruijn, Emile De Heer, Hans J. Baelde. *Department of Pathology, Leiden University Medical Centre, Leiden, Netherlands.*

L-carnosine is known for its antioxidant effects, degradation of advanced glycation end products, inhibition of ACE and reduction of TGF-β induced synthesis of extracellular matrix. These properties make it potentially protective against microvascular complications in a diabetic environment. Its rapid degradation by carnosinase (CNDP1) is therefore considered disadvantageous for diabetes patients. It has been shown that patients with more leucine repeats in CNDP1 show higher circulating carnosinase levels, and are more susceptible for diabetic nephropathy than patients with a low number of leucine repeats in CNDP1. Carnosine synthase (CARNS1) could compensate for this carnosine deficiency by increasing carnosine synthesis. We investigated whether carnosine synthase is expressed by the kidney and whether this is related to the transcription level of CNDP1 in diabetic nephropathy versus control samples.

Carnosine synthase mRNA expression level was measured in 41 kidney biopsies by Q-PCR. This included 28 diabetic samples and 13 healthy controls. Additionally, protein expression of carnosine synthase was determined by immunohistochemistry in the kidney using the antibodies described by Margolis et al, journal name, publication name.

CARNS1 mRNA expression was found both in microdissected glomeruli and tubulo-interstitial compartment (3.3 times higher in glomeruli than in the latter). There was no difference in mRNA levels in diabetic samples compared to controls. There was a strong correlation of the mRNA expression between CNDP1 and CARNS1 in diabetic samples as well as in healthy controls. Additionally, immunostaining of tubular epithelial cells was found with monoclonal antibodies against carnosine synthase, showing that CARNS1 is expressed in both tubuli and podocytes.

We have shown that both CARNS1 and CNDP1 are expressed in the kidney. The relation between CARNS1 and CNDP1 provides evidence for a simultaneous co-regulation of these two genes, and a coordinated regulation of carnosine metabolism in the kidney. The importance of this local carnosinase metabolism in diabetic nephropathy remains to be investigated.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2547

**Role of Previous Physical Training in the Renal Function and Autonomic System in Experimental Diabetes** Kleiton Silva,[1;2] Rafael Da Silva Luiz,[1] Nayda P. Abreu,[1] Maria Claudia Irigoyen,[2] Nestor Schor.[1] *[1]Department of Nephrology, Federal University of São Paulo, Brazil; [2]Heart of Institution, São Paulo of University, Brazil.*

The impact of previous physical training (PPT) in diabetes (DM) is largely unexplored. In this study the effect of PPT in the renal function and autonomic system in streptozotocin (STZ)-induced DM was evaluated. Wistar rats were induced to DM weighing 300 – 320g and were divided into 4 groups: control (C), diabetic (D), diabetic trained (DT), and pre-trained diabetic (4 weeks before STZ +10 post STZ, PTD). Physical capacity was evaluated by maximal speed test. Renal function was assessed by proteinuria, creatinine clearance and serum creatinine. Blood pressure was recorded to evaluate hemodynamic and autonomic parameters. We studied tachycardic (TR) and bradycardic (BR) responses.

The Systolic BP (SBP) was lower in group D (15%) and DT (12%) while no changing in PTD in comparison with C.

| Variable (final)/Groups | C | D | DT | PTD |
|---|---|---|---|---|
| Glycemia (mg/dl) | 89±2.8 | 509±23* | 372±28*# | 379±33*# |
| Physical capacity (km/h) | 1.3±0.09$ | 0.9±0.05$ | 1.6±0.1$ | 2.1±0.04$ |
| Proteinuria (mg/dl) | 21.82±1.2 | 100±14.2$ | 25.95±4 | 24.02± 8.5 |
| Creatinine clearance (ml/kg/min) | 1.4± 0.1 | 0.1± 0.01$ | 1.84± 0.3 | 2± 0.3 |
| Serum creatinine (mg/dl) | 0.3±0.04 | 1.2±0.05* | 0.9±0.02* | 0.6±0.04# |
| Tachycardic responses (bpm/mmHg) | 3.44±0.3 | 1.7±0.09* | 2.26±0.09* | 3.66±0.2#+ |
| Bradycardic responses (bpm/mmHg) | -2.24±0.1 | -1.2±0.2* | -1.52±0.2* | 2.49±0.2#+ |

*p<0.05 vs C; #p<0.05 vs D; +p<0.05 vs DT; $p<0.05 vs all groups.

The pulse interval (PIV) increased in group D, DT and PTD when compared to C group. The sympathetic (LF) as well as the parasympathetic (HF) cardiac autonomic modulation decreased in group D when compared to C. The DT and PTD groups presented similar values for the LF and HF components of PIV. These results suggest that PPT is effective to attenuate the renal and autonomic impaired function induced by experimental diabetes.

Suport: FAPESP, CAPES and CNPq.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2548

**Inhibition of Uncoupling Protein 2 by Genipin Ameliorates Diabetic Nephropathy** Wenjing Qiu, Ruoyun Tan, Junwei Yang. *Center of Kidney Disease, the 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Increasing evidence indicates that mitochondrial uncoupling protein 2 (UCP2) is associated with pathogenesis of diabetes. UCP2-mediated proton leak can be inhibited by genipin, which is extracted from the traditional Chinese herb gardenia fruit that has been used to relieve the symptoms of diabetes in clinical settings. However, the effect of genipin on diabetic nephropathy remains unknown. In this study, we examined the therapeutic potential of genipin on diabetic nephropathy induced by low–dose streptozotocin. One week after diabetes onset, half of diabetic mice were given genipin orally at a dose of 50mg/kg body weight once a day for 12 weeks. In vehicle treated diabetic mice, we identified positive correlation between the enhanced expression of UCP2 and the increased expression of a-smooth muscle actin (a-SMA), desmin, and fibronectin, as well as the decreased expression of slit diaphragm-associated protein nephrin, podocin, CD2-associated protein (CD2AP), and Wilms Tumor 1 (WT-1). However, genipin largely blunted UCP2 expression and the glomeruli injury in the diabetic mice. Our results were also confirmed with indirect immunohisto-chemical staining, immunofluorescence and electron microscope. *In vitro*, we cultured mouse podocyte cells in high glucose with and without genipin. We found that genipin effectively suppressed the expression of UCP2 at a dose of 50 μM. In high glucose environment, the expression of podocin and WT-1 was decreased, while a-SMA and desmin was increased in a time dependent manner. However, genipin almost completely suppressed these changes induced by high glucose. Furthermore, knock down of UCP2 with small interfering RNA yielded similar results as what we found with genipin. In conclusion, the expression of UCP2 was significantly upregulated in diabetic nephropathy, and genipin could mitigate the progression of diabetic nephropathy by effectively inhibiting UCP2.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2549

**Dual Bile Acid Receptors Agonist INT-767 Prevents Diabetic Nephropathy through Multiple Mechanisms** Xiaoxin X. Wang, Cydney Lynn Urbanek, Nathaniel L. Solis, Hannah Danielle Santamaria, Moshe Levi, Tao Jiang. *Medicine, University of Colorado Denver.*

Bile acids have recently emerged as versatile signaling molecules endowed with systemic endocrine functions. Bile acids are ligands for nuclear hormone receptor farnesoid X receptor (FXR) and G-protein-coupled receptors such as TGR5. To determine the functional consequences of bile-acid activated signaling in the pathogenesis of diabetic nephropathy, we induced type 1 diabetes in western diet fed DBA/2J mice using multiple low-dose streptozotocin injection and treated mice for 12-wk after diabetes with FXR/TGR5 dual agonist INT-767. The studies have shown the renal protective role of bile acid receptors activation on proteinuria, podocyte damage, accumulation of extracellular matrix proteins, and glomerulosclerosis through its coordinated effects on modulation of renal lipid metabolism, oxidative stress, inflammation, and profibrotic growth factors. Analysis of gene profiling further reveals that INT-767 acts on a) intrarenal renin-angiotensin-system and b) glucose transporters. INT-767 treatment dramatically decreased the renal expression of renin, renin receptor, angiotensinogen and angiotensin-converting enzyme. In addition, INT-767 treatment markedly decreased multiple renal glucose transporters such as GLUT1 and GLUT4. These results therefore indicate a new and important role for FXR and TGR5 in the kidney and provide new therapeutic avenues for the treatment of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2550

**Effect of Mutual Epididymal Fat Transplantation between db/db Mice and db/m Mice on Urinary Albumin Excretion Level** Hyunwook Kim,[1] Ji Eun Lee,[1] Young Youl Hyun,[2] Kum Hyun Han,[3] Young Sun Kang,[2] Dae R. Cha.[2] *[1]Department of Internal Medicine, Wonkwang University College of Medicine Sanbon Hospital, Gunpo, Kyunggi, Korea; [2]Department of Internal Medicine, Korea University College of Medicine Ansan Hospital, Ansan, Kyunggi, Korea; [3]Department of Internal Medicine, Inje University College of Medicine Ilsan Baik Hospital, Goyang, Kyunggi, Korea.*

The adipose tissue is now accepted as a major regulatory organ that plays a key role in diabetic milieu, but the metabolic effects associated with diabetic db/db mouse versus non-diabetic db/m mouse visceral fat on kidney have not been fully explored. Therefore, in this study, we performed the mutual transplantation of the same amount epididymal fat from diabetic db/db mice and non-diabetic db/m mice into the subcutaneous back of db/m mice and db/db mice, respectively, and investigated the effects on renal parameters as well as changes in insulin resistance and diabetic conditions compared with non-transplanted db/m and db/db mice, respectively. At 3 months after transplantation, although there were no significant differences in fasting blood glucose, HbA1c, adipokine levels, creatinine clearance, and blood pressure, db/db mouse epididymal fat-transplanted db/m mice showed significantly increased urinary albumin excretion (25.54 ± 8.86 μg/day vs. 3.21 ± 0.99 μg/day, $P = 0.014$) along with the trend towards aggravation of insulin resistance assessed by fasting plasma insulin and homeostasis model assessment of insulin resistance (HOMA-IR) as well as more weight gain compared with non-transplanted db/m mice. In case of db/m mouse epididymal fat-transplanted db/db mice, we also found no differences in adipokine level, creatinine clearance, and blood pressure. However, there was a definite trend towards decreased urinary albumin excretion (206.09 ± 83.37 μg/day vs. 697.99 ± 139.12 μg/day, $P = 0.066$) along with significant improvement of insulin resistance represented by insulin tolerance test, fasting blood sugar, blood cholesterol, and HbA1c levels compared with non-transplanted db/db mice. Collectively, this study suggests that adipose tissue plays a central role in albumin excretion level, likely by modulating insulin resistance status.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2551

**Effect of Gastric Bypass Surgery on Renal Glucose Handling** Havovi Chichger,[1] Joanne Marks,[1] Marco Bueter,[2] Carel W. Le Roux,[2] Edward S. Debnam,[1] Robert J. Unwin.[1] *[1]Neuroscience, Physiology & Pharmacology, University College London; [2]Investigative Medicine, Imperial College London.*

The epidemic of obesity that has developed in the last 20 years is thought to be largely diet-related, and it is strongly associated with a range of clinical disorders, including hypertension and type II diabetes. One method of treatment for severe obesity is gastric bypass surgery, Roux-en-Y (RYGB), which has been shown to rapidly correct hyperglycaemia in type II diabetes.

Hyperglycaemia in type I diabetic rats induces expression of the facilitative glucose transporter, GLUT2, at the proximal tubule (PT) brush border membrane (BBM); moreover, GLUT2 correlates positively with expression of PKC-βI (Goestemeyer, 2007). In models of insulin-resistance and obesity, such as Goto-Kakizaki type II diabetes, junk-food induced obesity, and a high-fat fed rat model (unpublished data), we have shown that GLUT2 expression also occurs at the PT BBM and correlates with PKC-βI expression. In the present study we have investigated the expression of GLUT2 and PKC-βI at the PT BBM in response to RYGB gastric bypass surgery in a rat model.

RYGB surgery was performed in male Wistar rats, with sham surgery as control (Bueter, 2010). After 16 weeks, kidneys were removed and the cortex dissected for BBM vesicle preparation and western blotting, or RNA extraction and real-time PCR.

Despite no diiferences in fasting plasma glucose levels between sham and RYGB animals (8.5 ± 0.2 mM vs 8.5 ± 0.8 mM), RYGB rats exhibited a significant decrease in renal GLUT2 mRNA (80% $p<0.001$) and PT BBM protein expression (55% $p<0.05$), with an accompanying decrease in PKC-βI protein expression (30.8% $p<0.05$).

This study shows that in RYGB animals there is a reduction in renal BBM GLUT2 expression and its upstream signal PKC-βI, which suggests an important relationship between intestinal glucose absorption, improved glucose homeostasis and renal glucose handling post-RYGB. It also highlights the potential value of this animal model in investigating altered renal transport mechanisms following gastric bypass surgery, and it confirms the close signalling relationship between renal PKC-βI activity and PT BBM GLUT2 expression *in vivo*.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2552**

**Insulin Resistance, Visceral Obesity, Fasting Hyperglycemia, and Dyslipidemia in Mice with Homozygous Mutation of the Gene Encoding Canonical Transient Receptor Potential 1 (TRPC1) Channel: A Mouse Model of Metabolic Syndrome**

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TRPC superfamily includes various channels involved in signal transduction & metabolism. Some members have been implicated in obesity, triglyceridemia, diabetes & hypertension, features of Metabolic Syndrome (MS). TRPC1 expression was reduced in diabetes. But the relationship was unclear despite being the 1st TRP channel cloned & identified in beta cells. We tested the hypothesis that TRPC1 knockout impairs glucose tolerance due to blunted insulin release or sensitivity. No abnormalities were found till 9th mon, when non-fasted plasma glucose in TRPC1 -/- males was 35% higher than age- & sex-matched +/+ mice (135 vs. 100 mg/dl, p<0.01). At 10 mon, they were 10% heavier & fasting glucose was 50% higher (109 vs. 72 mg/dl, p<0.01). By 1yr, they were 20% heavier. Hyperglycemia persisted. Glucose tolerance test with 2 mg/kg IP after 13 h fast revealed significant & sustained hyperglycemia in -/- mice, ~ 2 fold higher the 1st 3 h & 65% higher at 6th h. Plasma insulin was ~ 2.5 fold higher throughout. Fasting insulin (23 vs. 7 µunits/ml, p<0.01) & glucose (6.9 vs. 3.7 mM, p<0.01) were both elevated. Insulin resistance (IR) by homeostatic model assessment (HOMA) was greatly increased (7.9 vs. 1.1, p<0.01), with normal HOMA beta cell function (90 vs. 99 %). At 13 mon, HOMA IR (4.6 vs. 1.1, p<0.05) remained elevated, with unchanged beta cell function (84 vs. 105 %). In -/- mice, fasting plasma total cholesterol (153 vs. 118 mg/dl, p<0.01) was elevated by 39% & LDL cholesterol (94 vs. 59 mg/dl, p<0.01) by 60%. Liver echogenicity was elevated by 50% at 7 mon & by 75% at 1y, suggesting hepatic steatosis. Hypertension was absent. Our data support the hypothesis that TRPC1 deficiency produces insulin resistance, visceral obesity, hyperglycemia & dyslipidemia. TRPC1 null mice represent an excellent model of MS without hypertension.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2553**

**Dietary Energy and Carbohydrate Decrease on Non-Dialysis Treatment Days in Female Maintenance Hemodialysis (MHD) Patients**

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**Background:** Protein energy wasting (PEW) is commons in MHD patients and associated with adverse outcomes. Inadequate dietary intake may engender or aggravate PEW. Previous studies indicated lower energy intake on dialysis days when compared to non-dialysis days. **Methods**: We evaluated differences between dietary energy (DEI), protein (DPI), fat (DFI), and carbohydrate (DCI) intake in MHD patients on dialysis days (D) and non–dialysis treatment days (ND1=1 day after dialysis; ND2=2 days after dialysis) in 136 adults [59 female (F), 77 male (M)] enrolled in the "Nutrition and Inflammation Evaluation in Dialysis Patients" (NIED) Study using interview assisted 3-day food records. **Results:** In males, DEI, DPI, DFI, and DCI did not differ between treatment days. However, DEI tended to be lower on ND1 and ND2 compared with D in female patients [p= 0.08 and 0.11], as did DCI [p= 0.01 and 0.06]. The drop in DCI from D to ND1 and ND2 was greater in females vs. males [p=0.02, 0.07]. DPI was lower on all treatment days in females vs. males (p=0.04). DFI was not different between males and females.

Table: Dietary intake in 136 MHD patients (mean [95% CI])

| | DEI (kcal) | DEI (kcal) | DPI (g) | DPI (g) | DFI (g) | DFI (g) |
|---|---|---|---|---|---|---|
| Day | Woman | Man | Woman | Man | Woman | Man |
| D (dialysis day) | 1600 [1408,1792] | 1756 [1588,1923] | 61.2* [52.4,69.9] | 73.4 [65.8,81.0] | 59.9 [50.6,69.1] | 68.0 [60.0,76.1] |
| ND-1 | 1440* [1247,1632] | 1767 [1599,1934] | 57.5* [48.7,66.2] | 75.8 [68.2,83.5] | 57.9 [48.7,67.2] | 65.0 [56.9,73.1] ] 65.0 [56.9,73.1] |
| ND-2 | 1455* [1263,1647] | 1749 [1582,1917] | 59.1* [50.4,67.9] | 72.2 [64.6,79.8] | 55.3 [46.0,64.5] | 64.5 [56.4,72.6] |

*Female vs. Male p<0.05

**Conclusion:** Contrary to previous studies, the DEI tended to be lower on ND days in female MHD patients, which may be at least in part due to lower DCI, whereas in male MHD patients DEI and DPI were not different on D and ND days. Attention to different dietary status on different weekdays in MHD patients may correct PEW.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2554**

**Postprandial Metabolic Response to a Fat- and Carbohydrate-Rich Meal in Patients with Chronic Kidney Disease**

Tetsu Miyamoto, Abdul Rashid Tony Qureshi, Tae Yamamoto, Ayumu Nakashima, Bengt Lindholm, Peter Stenvinkel, Anders Alvestrand, Jonas Axelsson. *Divisions of Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden.*

**Background**: While chronic kidney disease (CKD) is associated with dysmetabolism including a marked insulin resistance, the postprandial response has not comprehensively been studied in CKD patients.

**Methods**: We conducted an interventional study comparing fasting and postprandial circulating biomarkers of glucose and lipid homeostasis, incretins, anabolic hormones, inflammation, oxidative stress and pulse wave analysis (PWA) in nine prevalent non-diabetic hemodialysis (HD) patients and ten matched controls assessed after a standardized meal consisting of 75 g of milk fat, 80 g of carbohydrates and 6 g of proteins/m$^2$.

**Results**: Glucose and triglyceride increased in a similar manner following the meal, while insulin, glucose-dependent insulinotropic polypeptide and especially C-peptide ($\Delta AUC_{\text{C-peptide 240min}}$ 1550 ±190 ng.min/L in patients vs 740 ± 80 ng.min/L in controls; P=0.001) increased more in HD-patients. HDL and LDL cholesterol decreased slightly with no significant difference between the groups. The elevated baseline growth hormone in patients dropped, resulting in comparable levels in both groups 240 min after the meal ($\Delta AUC_{GH240min}$ -1220 ± 490 ng.min/mL in patients vs. -410 ± 160 ng.min/mL in controls; p<0.05); however, there was no change in IGF-1 levels. No marked changes of IL-6 and TNF-α were observed in either group. A marked increase in the DNA oxidative product 8-hydoxydeoxyguanosine (8-OHdG) was observed in HD-patients ($\Delta 8\text{-}OHdG_{240min}$ 0.05 ± 0.02 ng/mL vs. 0.001 ± 0.008 ng/mL; p<0.05). Postprandial increase in heart rates as well as pulse pressures, and the drop in subendocardial viability ratio (SEVR) calculated by PWA, were smaller in HD-patients ($\Delta AUC_{SEVR180min}$ - 27.8 ± 5.2 %.hr in patients vs. – 66.0 ± 11.2 %.hr in controls; p<0.05)

**Conclusions**: The postprandial state in CKD is characterized by impaired insulin sensitivity with an increased incretin levels, along with GH/IGF-1 axis uncoupling, a marked rise in an oxidative stress marker and a blunted hemodynamic response.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2555**

**Association of the Malnutrition-Inflammation Score (MIS) with First 90-Day Mortality in Incident Hemodialysis (HD) Patients**

Deborah A. Benner,[1] Kamyar Kalantar-Zadeh,[2] Lilia R. Lukowsky,[2] Steven M. Wilson,[1] Kathy Lahr,[1] Karen M. Spach,[1] Mahesh Krishnan.[1] *[1]DaVita Inc., Denver, CO; [2]Division of Nephrology & Hypertension, Harbor-UCLA, Los Angeles, CA.*

Protein-energy wasting (PEW) is associated with increased mortality in HD patients but its association with first 90-day death in incident HD patients is not clear. The MIS, an evaluator-friendly composite score ranging from 0 (well-nourished) to 30 (severely malnourished), includes 10 components (each with a score between 0 to 3) across nutritional history (5 components), nutritional physical exam (2 components), BMI and 2 laboratory values (albumin and transferrin). We hypothesized that worsening nutritional status detected by MIS is associated with increased first 90-day mortality in incident HD patients.

Methods: During the first calendar quarter of 2009 the MIS was recorded by over 1100 dietitians in 9,441 incident (vintage <30 days) and 19,174 prevalent HD patients. MIS scores were divided into 4 worsening quartiles. Incident patient mortality was defined as death occurring within 90 days of the first treatment at the facility per 100 patient years. Crude and relative mortality across quartiles were calculated including after adjustment for age, gender, diabetes, race/ethnicity and vintage.

Results: Incident HD patients were 64.6±14.6 years old and included 43.9% women, 34.2% African Americans and 69.1% diabetics. Incident HD patients had worse nutritional status (MIS=7.8 ± 4.4) than prevalent patients (MIS= 6.4 ± 4.5, p<0.001). The standardized first 90 day mortality was the lowest in the quartile with the best nutritional status, whereas it was 8.6 times higher in the worse nutritional quartile (see Table):

| MIS | 0, 1, 2 and 3 | 4 and 5 | 6, 7 and 8 | 9 and higher |
|---|---|---|---|---|
| Incident Patient N | 7,151 | 5,840 | 6,919 | 8,708 |
| Deaths per 100 Patient Years | 4.9 | 9.1 | 15.3 | 41.6 |
| Relative Mortality | 1.0 (ref) | 1.9 (1.7-2.7) | 3.1 (2.4-3.9) | 8.6 (6.7-10.9) |

Conclusions: In incident HD patients, worse nutritional status detected by MIS is a strong predictor of short-term death. Improving nutritional status in incident HD patients may improve survival, which needs to be tested in controlled trials.

Disclosure of Financial Relationships: Employer: DaVita Inc.

**SA-PO2556**

**Protein-Energy Wasting Modifies the Association of Endogenous Ghrelin with Mortality in Hemodialysis Patients**

Juan J. Carrero, Ayumu Nakashima, Abdul Rashid Tony Qureshi, Bengt Lindholm, Olof Heimburger, Peter F. Barany, Peter Stenvinkel. *Divisions of Renal Medicine and Baxter Novum, CLINTEC, Karolinska Institutet, Sweden.*

**Introduction:** Ghrelin abnormalities may contribute to anorexia, inflammation and cardiovascular risk in hemodialysis patients, leading to worse outcome. Conflicting results of circulating ghrelin levels in patients with chronic kidney disease have been reported, and multiple confounding factors may contribute to these seemingly contradicting findings. One of such factors may be the individual's nutritional status. We hypothesized that the consequences of ghrelin in hemodialysis patients are context-sensitive and dependent on the presence of protein-energy wasting (PEW).

**Methods:** This is a cross-sectional study of hemodialysis patients (n = 217; 125 men; average +/- SD age: 66 +/- 14 y) followed for 31 (25$^{th}$-75$^{th}$ percentile 20-38) months where ghrelin, leptin, PEW (subjective global assessment) and CRP were assessed.

**Results:** Patients with low ghrelin (low third) were older, with higher BMI and leptin, presenting an increased mortality risk that persisted after adjustment for age, sex and dialysis vintage ((HR 1.55 95% CI [0.99-2.40]) but was lost after correction for comorbidities. Four categories were created according to ghrelin groups and the presence of PEW. Patients with PEW and low ghrelin values showed abnormally high levels of CRP and leptin (MANOVA interaction, p<0.05), while also presenting the highest all-cause (adjusted HR 3.34 [1.74-6.41]) and cardiovascular-related (adjusted HR 3.54 [1.40-8.91] mortality risk.

**Conclusions:** The consequences of low ghrelin concentration may depend on pre-existent PEW. Low ghrelin values in wasted hemodialysis patients were linked to a markedly increased (cardiovascular) mortality risk. As these patients exhibited a more anorectic phenotype (increased CRP and leptin), our results provide a clinical scenario where ghrelin therapies may be particularly useful.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2557

**Oral Nutritional Supplementation (ONS) as Part of Disease Management (DM) Improves Clinical Outcomes in the End Stage Renal Disease (ESRD) Population** Christine Cheu,[1] Sylvia Paz B. Ramirez,[1] Jeffrey Pearson,[1] Claudia Dahlerus,[1] Peter F. Sauer,[2] Robert E. Farrell,[2] Stephen D. McMurray,[2] Tania Chowdhury.[1] *[1]Arbor Research; [2]Fresenius Medical Care Health Plan.*

Serum albumin (ALB) is a predictor of morbidity and mortality, however, there have been limited changes in the nutritional management of ESRD patients (pts). As part of DM, Fresenius Medical Health Care Plan (FMCHP) provided ONS to enrollees at ALB<3.8 g/dL. This study evaluates the impact of FMCHP's ONS program on ALB, hospitalization, and mortality. FMCHP placed pts with a 3 mo mean ALB <3.8g/dL on ONS, revising to a 2 mo mean ALB<3.8g/dL within 6 mos of program initiation. ONS was discontinued when the 3 mo mean ALB exceeded 3.8 g/dL. The United States Dialysis Outcomes Practice Patterns Study (DOPPS) was the comparison group; only baseline ONS use was available. Logistic regression was used to evaluate associations between ONS and ALB. Cox models were used to calculate hospitalization and mortality percentages. Adjustments were age, sex, race, Hispanic ethnicity, ESRD vintage, comorbidity, new Medicare enrollee status. Among 1,377 pts, 712 (52%) received ONS. Pts were on continuous ONS use for a mean of 3.4 (±4.0) mos. Pts who received ONS the previous month were more likely to have an increase in ALB (OR=1.08, p=0.01). FMCHP pts on ONS had lower hospitalization percentages compared to non ONS pts. U.S. DOPPS pts on ONS had higher hospitalization percentages at one year compared to non-ONS users. ONS use in FMCHP was associated with a lower mortality percentage at one year regardless of achievement of target ALB. Similar protective effects of ONS on mortality were seen in U.S. DOPPS among pts with an ALB<3.8 g/dL. Findings show that FMCHP's ONS program as part of DM was associated with increased ALB, and reduced hospitalization and mortality.

| | FMCHP | | U.S. DOPPS | |
|---|---|---|---|---|
| **Hospitalization** | **No ONS (95% CI)** | **ONS (95% CI)** | **No ONS (95% CI)** | **ONS (95% CI)** |
| Achieved ALB >/= 3.8 g/dL | 68.4% (64.3, 72.0) | 18.1% (11.9, 23.9) | 33.6% (29.4, 37.5) | 39.7% (27.6, 49.7) |
| Always < 3.8 g/dL | 90.3% (83.4, 94.4) | 62.7% (35.8, 78.3) | 57.4% (48.6, 64.6) | 78.7% (62.3, 88.0) |
| **Mortality** | **No ONS (95% CI)** | **ONS (95% CI)** | **No ONS (95% CI)** | **ONS (95% CI)** |
| Achieved ALB >/= 3.8 g/dL | 10.0% (6.7, 13.2) | 5.5% (3.6, 7.4) | 9.2% (7.0, 11.4) | 12.9% (6.6, 18.0) |
| Always < 3.8 g/dL | 56.3% (32.9%, 71.5) | 26.0% (15.0, 35.6) | 24.9% (9.2, 17.7) | 19.2% (7.2, 29.6) |

Disclosure of Financial Relationships: nothing to disclose

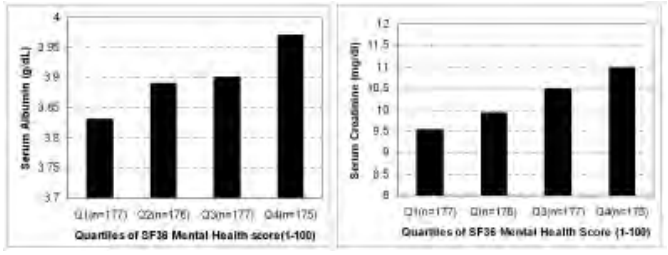
### SA-PO2558

**Association of Nutritional Status and Health-Related Quality of Life SF-36 Score in Maintenance Hemodialysis Patients** Usama Feroze,[1] Gangadarshni Chandramohan,[1] Ramanath B. Dukkipati,[1] Nazanin Noori,[1] Deborah A. Benner,[2] Csaba P. Kovesdy,[3] Joel D. Kopple,[1] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]DaVita, Lakewood, CO; [3]Salem VA, Salem, VA.*

**Background:** Maintenance hemodialysis (MHD) patients usually report an unfavorable health-related quality of life (HR-QOL). Many MHD patients also suffer from protein-energy wasting (PEW), as indicated by low serum albumin and/or creatinine levels. We hypothesized that better nutritional status is related to a better self-perceived HR-QOL

**Methods:** Out of 893 MHD patients who were followed from 2001 to 2007 in the NIED Study, SF-36 mental health score was obtained from 705 patients. Patients were ranked according to SF-36 mental health score and then divided into 4 quartiles(<37, 37-<51, 51-<69, and >=69).

**Results:** MHD patients across the above 4 SF36 mental health score quartiles were 55.2±13.2, 53.6±14.4, 52.51±15.1 and 52.7±16 years old (mean±SD) and included 56%, 49%, 45% and 38% women; 64%, 58%, 54% and 47% diabetics; and 37%, 26%, 33% and 31% African Americans, respectively. Both serum albumin and creatinine levels showed incrementally higher values for the better (higher) score groups across the SF-36 quartiles (see Figure).




**Conclusions:** Better nutritional status as reflected by higher serum albumin and creatinine concentrations are associated with better self-reported HR-QoL score in MHD patients. Interventional studies to improve nutritional statues and to examine its impact on improving outcomes including HR-QOL are indicated.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2559

**Comparison of Body Composition Determined by Multifrequency Bioelectrical Impedance and Dual Energy X-Ray Absorptiometry in Stable Peritoneal Dialysis Outpatients** Antje Fürstenberg,[1] Andrew Davenport.[2] *[1]Centre for Nephrology, Royal Free Hospital, London, United Kingdom; [2]Centre for Nephrology, University College London, Medical School, Royal Free Campus, London, United Kingdom.*

Introduction

Malnutrition is common in peritoneal dialysis patients and associated with poor outcome. Therefore, simple, reliable and easily available methods to determe nutritional status and to recognise short-term changes in body composition are desirable. The accuracy of multifrequency bioelectrical impedance analysis (MF-BIA) as a tool to assess body composition is unknown in this population.

Methods

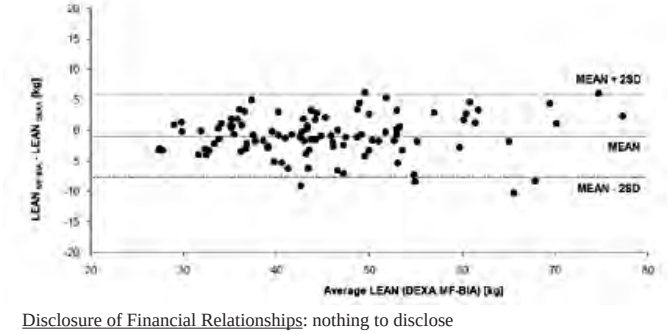
We compared whole body and segmental composition determined by MF-BIA with that obtained using dual energy x-ray absorptiometry (DEXA) in 104 outpatient adult peritoneal dialysis patients.

Results

Assessment of whole body composition showed that lean body mass (LBM) measured by the two techniques was highly correlated with good method agreement (Figure 1) using DEXA as the reference test (r=0.95, p<0.0001; BIAS -0.88kg; 95% CI -1.53 to 0.23kg). Similarly, high correlation and good method agreement were found for fat mass (FM) (r=0.93, p<0.0001; bias 0.69kg; 95% CI 0.03kg to 1.6kg). Segmental analysis of the lean body mass revealed strong correlations between lean body mass of trunk, left and right arms and legs (r= 0.90, 0.86 0.84, 0.89 and 0.90 respectively, p<0.0001). Bone mineral content (BMC) derived by MF-BIA overestimated that measured by DEXA (bias 0.740 kg; 95% CI 0.66 to 0.82kg).

Conclusion

MF-BIA appears to be a robust tool for measuring and monitoring body composition in peritoneal dialysis patients.




Disclosure of Financial Relationships: nothing to disclose

### SA-PO2560

**Long Term Changes of Body Composition in HD Patients** Peter Wabel,[1] Ulrich Moissl,[1] Sebastian Wieskotten,[1] Volker Wizemann.[2] *[1]Fresenius Medical Care D GmbH; [2]PHV - Dialysis Centre Giessen.*

*Background*

Body composition in haemodialysis (HD) patients is prone to long term changes. The aim of this work was to highlight changes in lean (LTM) and adipose tissue mass (ATM) in prevalent HD patients over a period of 7 years and to compare this data to reference ranges in age and gender matched healthy subjects.

*Subjects and Methods*

In 124 HD patients (72 male, 52 female, 16 male and 14 female diabetics) bioimpedance measurements (BCM – Body Composition Monitor, Fresenius Medical Care) were performed over 2-7 years. The BCM calculated LTM, Lean Tissue Index LTI (= LTM / Height²), ATM and Fat Tissue Index FTI (= ATM / Height²) according to the model of Chamney (AJCN 2007). For each patient LTI and FTI data over time was fitted by

a regression line and the mean slope of LTI and FTI was calculated. Additionally this information was compared with existing data of 2071 healthy subjects with gender- and age-dependent ranges of body composition (JASNsuppl2009).

*Results*

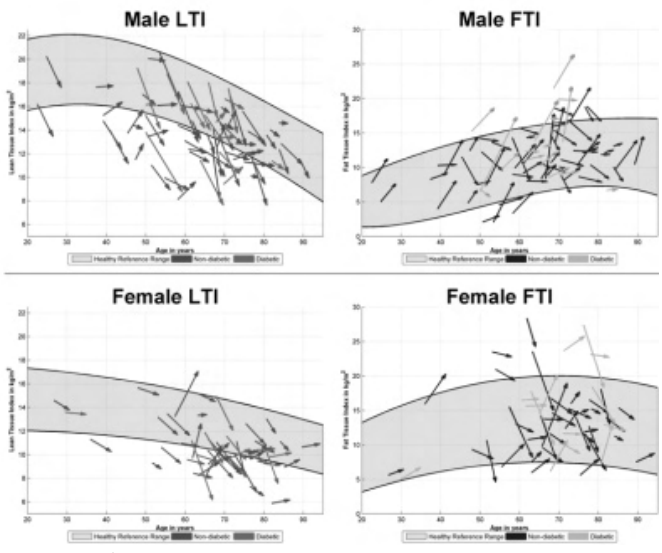
The differences between the slopes are tested by a Kruskal-Wallis test.

Mean changes of body composition in HD patients over 7 years

| Unit (kg/m²/year) | Male | Female |
|---|---|---|
| BMI | 0.037 | -0.284 # |
| BMI subgroup diabetics | 0.145 | -0.139 |
| subgroup diabetics | -0.363 # | -0.133 # |
| LTI subgroup diabetics | -0.493 # | -0.291 # |
| Adipose tissue (FTI) | -0.332 # | -0.251 #,* |
| FTI subgroup diabetics | 0.536 | 0.035 |

# significantly different to a mean change of 0 kg / m²/year ($p < 0,05$)

Male patients exhibited a significant loss of LTI and significant accumulation of FTI. Female patients showed a significant decrease in BMI, LTI and FTI. In contrast to male patients females decreased their FTI. There were no significant differences between diabetic and non-diabetic HD patients. The comparison of the individual patients with the reference population is shown in figure 1.




Disclosure of Financial Relationships: Employer: Fresenius Medical Care.

## SA-PO2561

**Ghrelin and Obestatin in Pediatric Patients with Chronic Renal Insufficiency and Following Renal Transplantation: Possible Insights into Appetite Dysregulation** Rainer Buescher, Anja K. Büscher, Peter F. Hoyer. *University of Duisburg-Essen, Pediatrics 2, Pediatric Nephrology, Essen, Germany.*

**Background:** Cachexia and growth retardation are common problems in pediatric patients with chronic renal insufficiency. Disturbances of appetite-regulating hormones are discussed to be causative factors. Acyl ghrelin is a potent orexigenic hormone, whereas desacyl ghrelin and obestatin, an alternative splicing product of the ghrelin gene, exert opposite functions. The understanding of the regulation of acyl ghrelin and its anorexigenic opponents and its role for the development of cachexia in renal insufficiency remains unclear.

**Methods:** We measured plasma total ghrelin, acylated ghrelin, obestatin, serum leptin and adiponectin in children with chronic kidney disease (CKD, n=29), children undergoing hemodialysis (HD) or peritoneal dialysis (PD, n=29), children following renal transplantation (RTx, n=91) and healthy controls (n=27), and analyzed the data in relation to the auxological parameters BMI and height.

**Results:** Patients with renal insufficiency showed lower BMI SDS and height SDS compared to healthy controls and children undergoing RTx. Total ghrelin was elevated in CKD and PD patients compared to controls or transplant recipients ($P<0.001$). As acyl ghrelin showed no difference between groups, the acyl ghrelin/total ghrelin ratio was reduced in uremic patients ($P<0.05$). Obestatin plasma levels were increased in uremic children compared to controls and RTx patients ($P<0.05$).

**Conclusions:** Uremia leads to an accumulation of the anorexigenic hormones desacyl ghrelin and obestatin, whereas orexigens like acyl ghrelin are not elevated. An imbalance between anorexigenic and orexigenic hormones may have an impact on cachexia development in pediatric patients with chronic renal insufficiency.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2562

**Novel Equations To Estimate Lean Body Mass in Maintenance Hemodialysis Patients** Nazanin Noori,[1] Csaba P. Kovesdy,[2] Rachelle Bross,[1] Antigone Oreopoulos,[3] Deborah A. Benner,[4] Rajnish Mehrotra,[1] Joel D. Kopple,[1] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA, Torrance, CA; [2]Nephrology, Salem VA, Salem, VA; [3]Univ. of Alberta, Edmonton, AB, Canada; [4]DaVita, Lakewood, CO.*

**Background:** Lean body mass (LBM) represents muscle mass, somatic protein storage and an important nutritional measure in maintenance hemodialysis (MHD) patients. We developed and tested equations to estimate LBM in MHD patients using simple measures. **Methods**: In 118 MHD patients, who underwent dual energy X-ray absorptiometry (DEXA) to assess their LBM. The validity of equations were tested against the near-infrared (NIR) measured LBM in 612 additional MHD patients. **Results:** DEXA measured LBM correlated with serum creatinine, mid-arm muscle circumference (MAMC), hand grip strength (HGS). MAMC in men and serum creatinine and HGS in women were the best surrogates of LBM. Three equations to estimate LBM were developed based on these 3 surrogates and gender, height, weight, ageand urea reduction ratio (for the serum creatinine).

Equations to estimate LBM in MHD patients

| | |
|---|---|
| Equation #1 | LBMSCr = 0.34*SCr (mg/dL) + 5.58*gender + 0.30*weight (kg) + 0.67*height (inch) - 0.23*URR - 5.75 |
| Equation #2 | LBMHGS = 9.09*HGS (unit) + 5.15*gender + 0.33*weight (kg) + 0.74*height (inch) - 29.06 |
| Equation #3 | LBMMAMC = 0.28*MAMC (cm) + 5.52*gender + 0.28*weight (kg) + 0.82*height (inch) - 35.30 |

The first and third equation estimates correlated well with NIR measured LBM ($R^2 \geq 0.88$) in the NIR cohort, although difference (Bland-Altman) plot showed that in higher LBM ranges they tended to underestimate it. **Conclusions:** Comparing to DEXA and NIR measured LBM, our developed equations using serum creatinine, MAMC, or HGS and demographic variables can relatively accurately estimate LBM in long-term MHD patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2563

**Characteristics of Body Composition and Nutritional Status in Patients Receiving Hemodialysis Therapy for More Than 30 Years** Shigeru Otsubo,[1] Miwa Ishihara,[1] Naoki Kimata,[2] Takashi Akiba,[2] Kosaku Nitta.[3] *[1]Department of Blood Purification, Sangenjaya Hospital, Tokyo, Japan; [2]Department of Blood Purification, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan; [3]Department of Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.*

***Backgroud***

The number of long-term survivors of chronic kidney disease (CKD) receiving hemodialysis has been increasing. On the other hand, patients with advanced CKD, especially those on long-term dialysis, often suffer from muscle wasting. In the present study, we investigated the body composition and nutritional status of extremely long-term hemodialysis patients.

***Methods***

Eighty outpatients receiving maintenance hemodialysis (including 18 for more than 30 years) were enrolled. The biochemical parameters were measured before the start of the hemodialysis session. The muscle mass was assessed by measuring the mid-upper arm muscle area (AMA). We classified the patients according to the duration of hemodialysis therapy (less than 10 years, 10 - 20 years, 20 - 30 years, or over 30 years) and compared the laboratory and anthropometric data between the groups. AMA and body mass index (BMI) were corrected using the following formula.

Corrected AMA (BMI) = measured AMA (BMI) / mean AMA (BMI) for same age and same sex, as obtained from the Japanese Anthropometric Reference Data 2001.

***Results***

No significant differences in age or the total protein, albumin, total cholesterol, triglyceride or CRP levels were observed among 4 groups. The corrected BMI was significantly lower in the more than 30 years group than in the less than 10 years group ($0.88 \pm 0.11$ versus $0.98 \pm 0.19$, $p=0.0417$). The corrected AMA was significantly lower in the more than 30 years group ($0.79 \pm 0.13$) than in the other groups ($P=0.0003$ compared with less than 10 years group ($1.01 \pm 0.22$), $P=0.0002$ compared with 10 - 20 years group ($1.04 \pm 0.17$), and $P=0.0046$ compared with 20 - 30 years group ($1.04 \pm 0.29$)).

***Conclusion***

In extremely long-term hemodialysis outpatients (for more than 30 years), the BMI and AMA were reduced, whereas nutritional markers such as the total protein, albumin, total cholesterol and triglyceride levels were relatively preserved.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2564**

**Relationship between Total Ghrelin and Inflammation in Hemodialysis Patients** Denise Mafra,[1] Najla Elias Farage,[2] Julie Lobo,[2] Milena Barcza Stockler-Pinto,[2] Viviane Oliveira Leal,[1] Luciana Catunda Brito,[4] Denis Fouque,[3] Maurilo Leite.[2] *[1]Clinical Nutrition, Federal University Fluminense, Niterói, Rio de Janeiro, Brazil; [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [3]University Claude Bernard Hopital Edouard Herriot, Lyon, France; [4]Federal University of Sergipe.*

**Introduction:** Ghrelin is a peptide involved in appetite and food intake. Previous studies show that plasma ghrelin is increased in hemodialysis (HD) patients, without particular impact on body composition, suggesting that there may be resistance to the ghrelin action in these patients. On the other hand, some studies have speculated that the high levels of ghrelin might be related to systemic inflammation in renal patients. The present study addressed this issue. **Materials and Methods:** Fifty HD patients from RenalCor Clinic at Rio de Janeiro, Brazil (19 women, 11 diabetics, mean age 54.3 ± 12.6 yr; BMI 24.4 ± 4.1 kg/m$^2$; % body fat 29.4 ± 7.4%), were studied. Blood samples were collected during fasting, before a regular HD session. Serum total ghrelin levels were measured using the enzyme immunometric assay (EIA) method and compared to 21 healthy subjects (12 women, 50.7 ± 15.7yr and BMI 25.6 ± 4.0 kg/m$^2$; % body fat 30.0 ± 5.7%). The body composition was evaluated by dual energy X-ray absortiometry (DEXA). Energy and protein intake were evaluated using a 2-day food record.

**Results:** Patients showed elevated plasma ghrelin levels when compared to healthy subjects (1.21 ± 1.0 ng/mL *vs* 0.42 ± 0.2; P<0.001). There was a positive correlation between ghrelin levels and TNF-a (r=0.25; P<0.01), IL-6 (r=0.42; P<0.05), and a negative correlation between TNF-a and protein intake (r=-0.28; P<0.05) and energy intake (r=-0.34; P<0.01). No correlation was observed with any aspect of body composition. **Conclusion:** Plasma ghrelin levels are elevated in HD patients and associated with the state of systemic inflammation. We suggest that inflammation may impact on plasma ghrelin bioactivity and metabolism in hemodialysis patients. *Supported by CNPq and FAPERJ.*

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2565**

**Comparisons on Control of Mineral Metabolism and Prescription Patterns by Dialysis Modality in ESRD Patients: Cross-Sectional Study of Clinical Research Center for End Stage Renal Disease in Korea** Young-Deuk Yoon,[1,2] Ja-Yong Park,[1,2] Eun-Joo Song,[1,2] Ji-Young Choi,[1,2] Se-Hee Yoon,[1,2] Sun-Hee Park,[1,2] Chan-Duck Kim,[1,2] Sung-Ho Kim,[1] Jun-Young Do,[1] Seong Eun Kim,[1] Sang Heon Song,[1] Yeong Hoon Kim,[1] Yon Su Kim,[1] Shin-Wook Kang,[1] Chul Woo Yang,[1] Nam Ho Kim,[1] Yong-Lim Kim.[1,2] *[1]Kyungpook National University School of Medicine; [2]Clinical Research Center for End Stage Renal Disease, Korea.*

To examine the abnormality of mineral metabolism and prescription pattern by dialysis modality in ESRD patients. Using the data from1,141 ESRD patients in CRC for ESRD in Korea between April 2009 and March 2010, we investigated albumin corrected-serum calcium, serum phosphate, serum parathyroid hormone(**iPTH**), prescription pattern of vit D-related drug (Calcitriol, Alfacalcidol, Paricalcitol) and phosphate-binders. Of 1141 patients (HD:PD 794:347, mean age of 56.2±13.6 years), men were 59.3%, mean dialysis duration was 43.8±52.8 months. There were no differences in dialysis duration, gender ratio, percentage of incident patients between HD and PD patients. Mean age was higher in HD group, whereas body mass index was higher in PD group (p=0.001). Patients with diabetes as a co-morbidity is higher in HD group (48.7% vs 40.3%, p=0.009). Many patients did not reach the recommended K/DOQI guideline in terms of serum phosphorus (16% of patients below lower target range, 30% of patients above upper target range), corrected calcium (27% below, 23% above), calcium-phosphorus product (20% above), and iPTH (47% below, 26% above). There were no differences in phosphorus, Calcium, Calcium×phosphorus product control between HD and PD patients, but iPTH is higher in PD group (323.8 vs 207.2 pg/ml, p<0.001). Only 10% of patients are satisfied with recommended target range. Higher prescription of vit D-related drug was noted in PD group (21.0 vs 14.2%, p=0.003), whereas higher prescription of phosphate binder in HD group (68.5 vs 59.9%, p=0.004). In cross-sectional analysis of dialysis patients, there are no significant differences in the control of mineral metabolism between HD and PD patients except iPTH. In addition, the prescription of vit D-related drug was higher in PD group.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2566**

**Inflammatory Marker (IL-6 -174 G/C) Gene Polymorphism in Malnourished Patients with End Stage Renal Disease** Raj K. Sharma, Richa Sharma, Anita Saxena, Suraksha Agarwal. *Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, UP, India.*

End Stage Renal Disease (ESRD) is an inflammed state and mediators of inflammation are activated. Retention of uremic toxins in the body, contact with foreign membrane (Dialysis) and kidney failure induces various immunological and metabolic imbalances in body. Patients develop inflammation, malnutrition, atherosclerosis, anemia etc. Strong associations between malnutrition, inflammation and atherosclerosis in the patient population suggest the presence of syndrome of Malnutrition, Inflammation, and Atherosclerosis (MIA), which when associated with various co-morbidities results in exceptionally high mortality rate. Therefore, based upon above hypothesis we studied the interleukin-6 (IL-6 -174G/C) gene polymorphism with malnutrition in maintenance hemodialysis patients. A total of 40 patients on MHD were enrolled in the study, 4mL venous blood was collected in EDTA vial. To assess the nutritional status, Subjective Global Assessment (SGA), body composition (BMI and waist circumference), bioelectrical impedance analysis (BIA) were used. Carotid atherosclerosis was investigated by ultrasonographically by carotid intimal-media thickness (cIMT). IL6 -174G/C gene polymorphism was done using standard PCR-RFLP method. Serum albumin for the patients was 3.6±0.67g% and was found to be below normal for 13 patients. Serum protein was found to be 6.33±0.93g% with severe hypoalbuminuria in 37% of patients, PTH was 158.2±200pg/ml, CRP levels were 4.02±12.9pg/ml and 12 patients (30%) had elevated CRP levels. 14, 5 and 20 patients had normal, mild and moderate SGA score respectively. Out of 40 patients 3 patients showed increased carotid intimal thickness (CIT) on carotid doppler more than normal limits. We observed significant increase in the genotype frequency of the IL-6-174 G/C (p=<0.05) in malnourished patients on MHD. The C allele of IL-6 -G174C were significantly different in ESRD when compared to controls (p=<0.05). Our results suggest that IL-6 -174G/C gene polymorphism may be a risk factor for patients of MIA for development of malnutrition in ESRD patient on MHD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2567**

**Dietary Intake in Incident Hemodialysis Patients: The Predictors of Arrhythmic and Cardiovascular Risk in End Stage Renal Disease (PACE) Study** Julia J. Scialla,[1] Cheryl A. Anderson,[1] Stephen M. Sozio,[1] Bobbie Henry,[1] Bernard G. Jaar,[1] Tariq Shafi,[1] Wen Hong Linda Kao,[1] Rulan S. Parekh.[2] *[1]Johns Hopkins University; [2]University of Toronto.*

Biomarkers of malnutrition are risk factors for death in ESRD patients, but directly measured dietary intakes have not been well described. We recruited 176 incident hemodialysis patients from the Baltimore area between 10/08 and 4/10. Diet was assessed by a registered dietician using a 24 hour dietary recall interview in 115 participants. From the 2005-2006 National Health and Nutrition Examination Survey (NHANES), a survey of the non-institutionalized US population, we created a matched comparison population among those with 24 hour dietary recall interviews performed and estimated GFR of ≥ 30 mL/min/1.73 m$^2$. We matched 4:1 on categories of age, sex and race to the PACE population. Intakes were compared between the populations using linear regression. Mean age of the PACE study population was 53y (range 20 to 90 y); 43% were female; 68% black; 47% had diabetes. Energy intake was lower (p<0.001) in PACE, but macronutrient composition was similar.

| Nutrient † | PACE (n=115) | Matched NHANES population (n=460) | p-value |
|---|---|---|---|
| Energy (kcal/d) | 1522 (1065, 2140) | 1996 (1433, 2693) | <0.001 |
| % kcal from protein | 16.5 (13.1, 20.7) | 15.3 (12.3, 18.0) | 0.08 |
| % kcal from fat | 32.9 (26.2, 40.2) | 33.6 (28.1, 39.8) | 0.8 |
| % kcal from saturated fat | 10.6 (8.1, 13.1) | 10.5 (8.3, 13.3) | 0.8 |
| % kcal from carbohydrate | 48.6 (41.1, 56.8) | 48.8 (39.8, 56.8) | 0.7 |
| Sodium* | 1763 (1348, 2139) | 1570 (1260, 1876) | <0.001 |
| Potassium* | 966 (755, 1252) | 1209 (915, 1511) | <0.001 |
| Phosphorus* | 519 (457, 633) | 577 (464, 692) | 0.04 |

†Median (IQR); *mg/1000 kcal

After adjustment for diabetes, energy (p<0.001), potassium (p<0.001) and phosphorus (p=0.002) intake remained lower and sodium intake higher (p=0.01) in PACE compared with the matched NHANES population. Our results suggest that decreased energy intake, as opposed to a selective decrease in protein intake, is present early in the course of ESRD. Greater attention to energy intake in dialysis patients is needed.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2568**

**Prevalence of Metabolic Syndrome in PD Patients and the Correlation with Vascular Calcification** Hye Won Kim, Ha Na Yang, Won-Yong Cho, Hyoung-Kyu Kim, Sang-Kyung Jo. *Department of Internal Medicine, Korea University Anam Hospital, Seoul, Republic of Korea.*

**Background**: Although metabolic syndrome is known to be closely related to increased cardiovascular morbidity and mortality in general population, its prognostic value in patients with peritoneal dialysis (PD) is largely unknown. This study was planned to evaluate the prevalence of metabolic syndrome and its correlation with other cardiovascular risk factors in stable PD patients.

**Methods**: One hundred eleven patients who have been treated by peritoneal dialysis for more than 1 year were enrolled. Metabolic syndrome is defined as the presence of three or more components using the modified National Cholesterol Education Program (Adult Treatment Panel III) guidelines. Patients were also divided as the number of corresponding components and the correlation between the number of components of metabolic syndrome with various parameters including simple vascular calcification score (SVCS), brachial-ankle pulse wave velocity (ba-PWV), plasma inflammatory cytokines and other clinical parameters was performed.

**Results**: The prevalence of metabolic syndrome was 81.7%. Two patients (1.8%) had none of the component, 13 patients (11.9%) had two components, 41 patients (37.6%) had three components, 39 patients (35.8%) had four components and 8 patients (7.3%) corresponded all of five criteria. Patients with metabolic syndrome were significantly older, had higher CRP, WBC count, percentage of neutrophil and SVCS. With multiple regression analysis, as the number of satisfied components increased, WBC counts, CRP and SVCS were increased after adjustment by age. However, there were no significant correlation between ba-PWV or plasma inflammatory cytokines and number of metabolic syndrome components.

**Conclusion**: PD patients showed higher prevalence of metabolic syndrome than general population. Our observation that there is positive correlation between the number of corresponding components of metabolic syndrome and CRP, WBC counts, medial vascular calcification might suggest that metabolic syndrome in PD patients might also be served as a strong predictor of cardiovascular morbidity and mortality.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2569

**Estimation of Average Plasma Glucose Levels by Glycated Albumin and Optimal Target Value of Glycated Albumin in Patients with Diabetic ESRD** Jwa-Kyung Kim, Jung Tak Park, Bo Young Nam, Hye-Young Kang, Shin-Wook Kang, Tae-Hyun Yoo. *Brain Korea 21 Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.*

Compared to glycated hemoglobin (HbA1c), serum glycated albumin (GA) level reflects recent glycemic control more accurately in DM ESRD patients. However, no definite relationship between serum GA and mean plasma glucose (MPG) level is determined and the optimal reference value of serum GA is not set up. A total of 117 ESRD patients, including 99 subjects with type 2 DM and 18 non-DM subjects were included in this study (76 on HD, 41 on PD). Patients were asked to perform four–point daily self-monitoring blood glucose (BG) at least 3 continuous days per week for 4 weeks. Mean BG level was determined using area-under-the-curve analysis and MPG was estimated by adding 11% of mean BG to mean BG level. Serum GA and other biochemical parameters were obtained at the end of one month and HbA1c level was checked at 8-10 weeks after BG monitoring. Approximately, 41 BG values were checked by each subject. MPG, GA and HbA1c levels were 163.2±44.2mg/dl, 19.6±6.9% and 6.45±1.4%, respectively. GA level of DM patients was 21.2±6.4% and 11.1±1.5% in non-DM patients, but there was no significant difference between dialysis modalities (HD 20.8±7.3%, PD 17.3±5.5%; p=0.13). GA showed a positive correlation with dialysis duration (r=0.42, p<0.001), but there was a significant negative correlation between GA and serum albumin level (r=-0.32, p<0.001). Among individual time points, postprandial glucose level showed higher correlation with GA than fasting glucose level (r=0.51, p=0.01 vs. r=0.20, p=0.06). Linear regression analysis between MPG and GA or HbA1c provided a close relationship of MPG (mg/dl)=5.95 X GA% + 47.52 (r= 0.74, p<0.001) and MPG (mg/dl)=27.7 X HbA1c% - 38.91 (r=0.60, p<0.001). Considering that the recommended average MPG level is 155 mg/dl, which is equivalent to 7% of HbA1c in the general population, the optimal target range of GA% is thought to be 17-18% in diabetic ESRD patients. Compared to HbA1c, GA levels had better correlation with MPG, and average MPG levels of the preceding 4 weeks could be estimated using the GA values.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2570

**Changes of Serum Adipokine Concentration after Start of Peritoneal Dialysis** Chul Ho Chung, Jung Hwan Park, Eun Jung Kim, Moo Yong Park, Soo Jeong Choi, Jin Kuk Kim, Seung D. Hwang. *Division of Nephrology, Soonchunhyang University Bucheon Hospital, Bucheon-si, Gyeonggi-do, Korea.*

**Background -** Fat tissue is complex organ that secretes several cytokines. In the PD patients, continous glucose absorption from dialysate lead to weight gain and fat mass increase. In this study, we prospectively investigated the effect of initiating PD on fat mass and the relation of adipokine changes.

**Methods -** Adipokines(leptin, adiponectin, TNF-α, IL-6), abdominal fat CT, several nutritional parameters, peritoneal equilibration teats(PET) were measured 7days, 6 months and 12 months after the initiation of PD.

**Results -** 36 patients were enrolled. At baseline, woman was higher than man in leptin(383.3±116.6 vs. 95.1±29.6, p=0.029) and adiponectin levels(330.9±40.9 vs.193.9±37.3, p=0.018). Body weight and total cholesterol increased continuously for 12 months. There was no significant change in visceral and subcutaneous fat mass. Subcutaneous fat was correlated positively with leptin(r=0.804, p<0.01), and negatively with adiponectin(r=-0.593, p<0.01). Adiponectin was negatively correlated with leptin(r=-0.556, p<0.01). Nutritional parameter and PET result was no significant changes. There was no significant changes in leptin, adiponectin and IL-6. TNF-α decreased at 6months, but increased at 12months(p=0.011).

Serial changes

| | Baseline | 6 months | 12 months | p-value |
|---|---|---|---|---|
| Weight(Kg) | 60.1±9.6 | 61.8±10.2 | 62.9±11.2 | **0.022** |
| Cholesterol(mg/dL) | 149.3±46.0 | 172.1±59.7 | 173.9±38.2 | **0.009** |
| Visceral fat mass(cm2) | 83.5±63.6 | 103.2±71.9 | 74.1±29.9 | 0.981 |
| Subcutaneous fat mass(cm2) | 95.8±58.5 | 125.9±64.7 | 112.4±51.0 | 0.172 |
| Leptin(ng/mL) | 247.2±66.9 | 383.5±103.3 | 418.8±147.2 | 0.159 |
| Adiponectin(ug/mL) | 266.2±29.8 | 256.1±29.2 | 203.5±23.0 | 0.138 |
| IL-6(pg/mL) | 11.3±1.7 | 7.7±3.2 | 10.4±2.3 | 0.948 |
| TNF-a(pg/mL) | 9.4±1.4 | 6.6±0.8 | 8.0±0.9 | **0.011** |

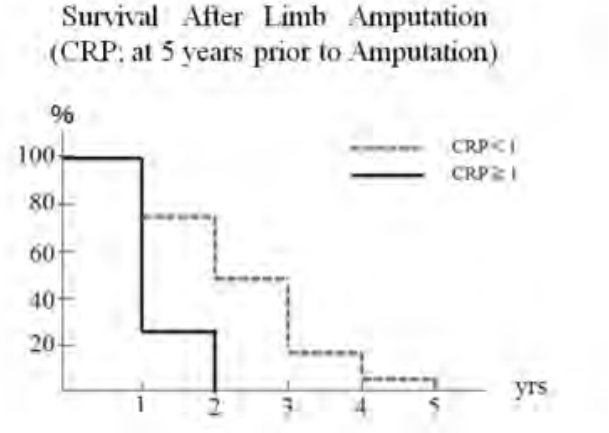
**Conclusion -** Baseline subcutaneous fat was correlated positively with leptin, and negatively with adiponectin. Body weight increased consistently for 12 months. There was no change in leptin, adiponectin and IL-6 levels, but TNF-α decreased at 6 months, and increased at 12 months.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2571

**Continuous Inflammation Is a Strong Indicator of Patient Survival Following Limb Amputation in Hemodialysis (HD) Patients** Masao Aoyagi,[1] Miwa Shirahama,[1] Satoshi Funakoshi,[1] Yoshiaki Lee,[1] Takashi Harada,[1] Mineaki Kitamura,[2] Akira Furusu,[2] Shigeru Kohno.[2] *[1]Sakuramachi Clinic, Nagasaki, Japan; [2]Nagasaki University School of Medicine, Nagasaki, Japan.*

Background: Limb-threatening ischemia due to arteriosclerosis obliterans is becoming more common in HD patients along with an increase in diabetic nephropathy in elderly ESRD. Purpose: The survival rate of HD patients who had undergone limb amputation was analyzed to determine common factors affecting patient survival. Methods: From 1999 to 2009, there were 36 HD patients (26 DM, 10 non-DM) who had undergone limb amputation at our facility. Various parameters including average Hb, serum albumin, and CRP, were determined 5 years prior to each amputation, when no symptoms had yet been documented in their limbs. Results: There was no significant change in average Hb, albumin during the period of 5 years. As shown in Figure 1, patients with a CRP level of < 1.0 mg/dL 5 years prior to amputation (11 DM / 6 non-DM) had a 3.1 +2.2 year survival rate following limb amputation.




On the other hand, no patients with a CRP level of > 1.0 mg/dL (6 DM / 3 non-DM) survived for more than 2 years. Interestingly the presence of DM or nutritional status was not observed to be a critical factor for patient survival. Conclusions: Our data suggest that continuous systemic inflammation may have a stronger impact than nutrition status for the prognosis of HD patients with potential arteriosclerosis obliterans.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2572

**Leptin: Adiponectin Ratio Is an Independent Predictor of Mortality in Non Diabetic Peritoneal Dialysis Patients** Jung Tak Park, Jwa-Kyung Kim, Bo Young Nam, Hye-Young Kang, Tae-Hyun Yoo, Shin-Wook Kang. *Brain Korea 21 Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.*

Background: Leptin/Adiponectin (L:A) ratio has been suggested as an atherosclerotic index in diabetic patients and as a useful parameter for insulin resistance in patients with and without diabetes. Although peritoneal dialysis patients are well characterized for alterations in adipocytokine metabolism, the corresponding effects of L:A ratio in this particular population have not been clearly defined. In this study, we prospectively investigated the effect of L:A ratio on outcome in non-diabetic peritoneal dialysis (PD) patients.

Methods: The study subjects included 131 stable non-diabetic PD patients who had been on PD for more than 3 months. Baseline serum leptin and adiponectin levels were measured. Mortality was evaluated during the follow-up period.

Resluts: During the follow-up period (mean follow-up duration: 49.2 ± 24.6 months), 17 patients died (12.9 %), 9 (52.9 %) as a result of cardiovascular disease. L:A ratio showed a significant positive correlation with body mass index ($r = 0.69$, $P < 0.001$), HOMA-IR ($r = 0.44$, $P < 0.001$), hsCRP($r = 0.31$, $P < 0.001$). However a significant negative correlation was found with lean body mass index ($r = -0.26$, $P = 0.003$). There was no significant correlation with normalized protein catabolic rate ($r = -0.29$, $P = 0.74$) or serum albumin levels ($r = 0.05$, $P = 0.61$). Cox proportional hazards analysis revealed that log L:A ratio [RR (95% CI): 1.67 (1.09-2.55)] (P = 0.02), log leptin levels [RR (95% CI): 2.02 (1.08-3.79)] (P = 0.03), and lower adiponectin levels [RR (95% CI): 0.91 (0.84-0.99)] (P = 0.03) were independent predictors for mortality. The area under the curve of the L:A ratio (0.73) in receiver operator characteristic analysis for mortality was greater than that of log leptin (0.68) or adiponectin (0.33) indicating that log L:A ratio better predicts death than leptin or adiponectin alone.

Conclusion: L:A ratio, which correlates with metabolic and inflammatory parameters, could be a better predictor for mortality than each single adipocytokine in non diabetic patients undergoing peritoneal dialysis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2573**

**Correlates of Plasma Gelsolin Levels in Prevalent Hemodialysis (HD) Patients** Laura Rosales,[1] Georges Ouellet,[1-2] Viktoriya Kuntsevich,[2] Boris Medvedovsky,[2] Mary Carter,[1] Peter Kotanko,[1-2] Nathan W. Levin,[1-2] Stephan Thijssen.[1-2] *[1]Renal Research Institute; [2]Beth Israel Medical Center, New York.*

Plasma gelsolin (pGSN) is an actin binding protein and muscle tissue is its major source. The association between pGSN levels and co-morbidities, anthropometric, demographic and routine laboratory parameters has not been studied

Methods:

pGSN levels were determined with the 2C4 pGSN ELISA kit (Critical Biologics, Cambridge, MA) in maintenance HD patients from 3 HD centers; demographics (age, HD vintage, race, gender, BMI), co-morbidities (diabetes (DM), atherosclerotic cardiovascular disease (CVD)), were recorded. Univariate associations with pGSN were assessed with Pearson (r) or Spearman (rho) correlations and groups comparison by t test. Multiple linear regression with backward parameter elimination determined independent predictors of pGSN.

Results

We studied 153 patients (mean age 61±15 years; median HD vintage 2.3 (range 0.2-20.0) years; 52% male; 42% Blacks, 52% DM). In univariate analysis, pGSN positively associated with pre-HD creatinine (r=0.38, P<0.001), BMI (rho=0.2, P=0.01), and pre-HD body weight (rho=0.2, P=0.01), and inversely correlated with age (r=-0.294, P<0.001). pGSN levels were lower in non-blacks vs. blacks (P=0.047), DM vs. non-DM (P=0.023), and patients with CVD vs. without CVD (P=0.053). After backward stepwise regression, BMI, pre-HD serum creatinine concentration and CVD status remained as independent predictors of pGSN level in the final model, which yielded an adjusted R2 of 0.16 and an overall significance of P<0.001. (Table 1).

Table 1. Parameter estimates and significance levels of pGSN

| Predictors | B | SE | P |
|---|---|---|---|
| BMI | 48.7 | 20.6 | 0.02 |
| Creatinine | 216 | 52 | <0.001 |
| Cardiovascular disease | -471 | 280 | 0.094 |

Conclusion:

Our study shows that serum creatinine, BMI, and atherosclerotic CVD status(borderline) are independent predictors of pGSN levels in chronic HD patients. The significant association between pGSN and surrogates of muscle mass is in line with muscle being its primary source. The relationship with CVD may be linked to microinflammation and warrants further investigation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2574**

**Are Circulating Zinc α2-Glycoprotein (ZAG) Levels Increased in Hemodialysis Patients?** Viviane Oliveira Leal,[1] Milena Barcza Stockler-Pinto,[2] Najla Elias Farage,[2] Julie Lobo,[2] Maurilo Leite,[2] Denis Fouque,[3] Denise Mafra.[1] *[1]Federal University Fluminense, Niteroi, Brazil; [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [3]University Claude Bernard Hopital Edouard Herriot, Lyon, France.*

Zinc α2-glycoprotein (ZAG), a soluble glycoprotein secreted from the liver and adipose tissue, acts a lipid-mobilizing factor involved in regulating lipid metabolism and adiposity. The role of ZAG is also connected with its ability to directly influence expression of uncoupling proteins which are implicated in the regulation of energy balance. In cancer cachexia, current data point to ZAG as a potential candidate for mediating lipid catabolism. Cachexia also occurs in chronic kidney disease. Nevertheless, to date, there is no information about ZAG levels in hemodialysis (HD) patients.

**Objective**: The purpose of this study was to describe plasma ZAG levels in HD patients.

**Methods**: Twenty-nine HD patients (18 men, 7 diabetics, aged 53.5 ± 12.1 yr, 36 (8-144) months on HD, Kt/V of 1.40 ± 0.25, body mass index (BMI) of 24.3 ± 4.4 kg/m$^2$) from RenalCor Clinic at Rio de Janeiro, Brazil, were studied. Blood samples were collected during fasting, before a regular HD session. ZAG was determined by ELISA (Biovendor®) and compared to 10 healthy subjects (6 men, 53.8 ± 14.2 yr old, BMI of 25.7 ± 2.3kg/m$^2$). The SPSS for Windows (version 11.0) was used as statistical program.

**Results**: Plasma ZAG levels were approximately 3-fold higher in HD patients (171.6 ± 41.6mg/L) when compared to healthy subjects (62.5 ± 21.7mg/L) (p<0.0001). ZAG levels were not correlated with age, time on HD, BMI and % body fat.

**Conclusions**: The circulating ZAG levels are increased in HD patients. Therefore, the role of ZAG on lipid metabolism and energy balance in this population deserves further investigation. *Supported by CNPq and FAPERJ*

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2575**

**Insulin-Resistance and End Stage Renal Disease: The Role of Retinol Binding Protein 4** Elisa Gabanti, Giovanni Piotti, Valeria Corradetti, Teresa Valsania, Camillo Carrara, Eleonora Pattonieri, Pasquale Esposito, Teresa Rampino, Antonio Dal Canton. *Unit of Nephrology Dilaysis and Tranplantation, IRCCS Fondazione Policlinico San Matteo and University of Pavia, Pavia, Italy.*

INTRODUCTION

Insulin resistance (IR) affects patients with end stage renal disease (ESRD). Studies on animals and humans demonstrated the adipose tissue of glucose transporter protein 4 (GLUT4) decrease results in serum RBP4 increase that in turn induces IR. Since its renal excrection, we investigated whether RBP4 plays a role in inducing IR in ESRD.

METHODS

We enrolled 16 controls (C) and 16 patients on hemodialysis (HD), 18-72, free from diabetes, matched for sex, age and BMI. We obtained history, blood pressure, BMI, biochemical exams and we measured serum RBP4 levels by ELISA. Moreover we collected subcutaneous fat samples, from 6 HD at the time of renal transplantation and 6 paired C undergone hernia correction, to evaluate rbp4 and glut4 expression on adipose tissue by RT-PCR.

RESULTS

Serum glucose was similar in C and HD. Serum RBP4 was four times higher in HD than C (HD: 176,8+/-63,2 mg/dl; C: 39,2+/-17,4 mg/dl; p=0,0001). As expected HD insulin levels and HOMA index were significantly higher than C (insulin, HD: 15,8+/-12,2 µIU/ml; C: 6,7+/-4,3 µIU/ml, p=0,005; HOMA index, HD: 3,2+/-2,6, C: 1,4+/-1,0, p=0,008). Serum RBP4 in HD was significantly correlated with fasting serum glucose (p=0,04), total serum cholesterol (p=0,04), triglyceride (p=0,0004) and serum protein (p=0,005).

Serum RBP4 levels were lower in HD patients with a residual diuresis greater than 5000 ml/24 hours (D) compared to anuric patients (A) (D: 141,3+/-12,1 mg/dl, A: 193,4+/-16,1 mg/dl, p=0,02).

HD glut4 mRNA expression on adipose tissue was lower than C; it was inversely correlated with age, BMI in HD and age, BMI and urea in C (p=0,05). HD rbp4 mRNA expression too was lower than C, it was significantly correlated with age and renal function in C and with age and insulin levels in HD (p=0,05).

CONCLUSIONS

Our results demonstrate IR may be mediated by reduced adipose glut4 expression and high serum RBP4 levels in ESRD. The decrease of RBP4 renal clearance may be responsible for high levels of serum RBP4 in ESRD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2576**

**The Short-Term Influence of Hepatitis C Virus Activity on Malnutrition-Inflammation Complex Syndrome in Maintenance Hemodialysis Patients** Hung-Bin Tsai.[1,2] *[1]Department of Internal Medicine, Buddhist Dalin Tzu Chi General Hospital, Chiayi, Taiwan; [2]School of Medicine, Tzu Chi University, Hualian, Taiwan.*

**Background.** Patients with maintenance hemodialysis (MHD) have a significantly higher prevalence of hepatitis C virus (HCV) infection and malnutrition-inflammation complex syndrome (MICS). This study was conducted to find clinical characteristics and influence of HCV infection on MICS using a nutritional-inflammatory scoring system known as the Malnutrition-inflammation Score (MIS) in Taiwanese MHD patients. **Methods.** This was a prospective, longitudinal study performed at a single hemodialysis center in Taiwan from September 2007 through March 2008. The study had 58 (37.9%) subjects in the active HCV group and 95 (62.1%) subjects in the non-HCV group, whose two or three weekly hemodialysis sessions were reviewed over a seven-month period. An additional 18 patients were included, and their inactive HCV status indicated previous HCV eradication spontaneously or after successful antiviral therapy. The malnutrition-inflammation score (MIS) was assessed using 10 components, combining the 7 components of the conventional Subjective Global Assessment (SGA) of Nutrition with 3 new elements (body mass index, serum albumin, and total iron binding capacity). **Results.** Hemodialysis duration and total MIS score increased with active HCV risk. The active HCV group had lower predialysis mean blood pressure, postdialysis weight, serum albumin, total cholesterol, and triglycerides levels but higher MIS scores compared to the non-HCV group. The MIS 5 score, indicative of major comorbid conditions, was significantly higher in the active HCV group than the non-HCV group throughout the trial. **Conclusion.** HCV infection incidence remains significant in MHD patients. The HCV-infected MHD patients have a greater MICS-associated metabolic and physiological disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2577**

**Differences in Muscle Action Potential in Patients with ESRD Compared to Age-Matched Sedentary Control Subjects** Cynthia Delgado,[1] Julie W. Doyle,[1] Kirsten L. Johansen.[1,2] *[1]Nephrology, UCSF, San Francisco, CA; [2]Nephrology, SFVAMC, San Francisco, Ca.*

Introduction: Compound motor action potentials (CMAPs) represent the summated electrical activity of individual nerve fibers activated by stimulation. CMAPs are known to be an accurate measure of activated muscle fibers, with reduction in amplitude suggestive of denervation injury. Evaluation of CMAPs has been applied in the clinical assessment of person with ALS in addition to other neuromuscular disorders. ESRD patients have

muscle weakness and sarcopenia compared to age matched controls. The purpose of the study was to determine whether differences in muscle function among patients with CKD are associated with EMG signs of denervation/reinnervation injury.

Methods: Eighty patients on hemodialysis > 3 months were included in the study if there was evidence of malnutrition or poor quality of life assessed by questionnaire. 20 age matched control subjects who were not on dialysis were studied for comparison. Participants underwent EMG action potential evaluation of the anterior tibialis muscle using surface electrodes. Cross-sectional area of the contractile tissue of the anterior tibialis muscle was measured by MRI.

Results: 48 patients had EMG and muscle CSA data available. The majority of subjects were male (64%) with mean age 55 (±13 yrs). 54% of participants were African American, 12% Caucasian and 25% Asian. ESRD patients had smaller muscle CMAP amplitude (P<0.005) than control subjects. In multivariate analysis, ESRD patients had lower CMAP amplitude than control subjects even after adjustment for muscle area, age and sex (coef -.0031, p<0.01).

Conclusion: ESRD patients have decreased CMAP potentials beyond what is expected based on their muscle atrophy. This is suggestive of axonal damage and loss, possibly as a result of denervation reinnervation injury or uremic toxins despite adequate dialysis by modern standards.

Disclosure of Financial Relationships: nothing to disclose

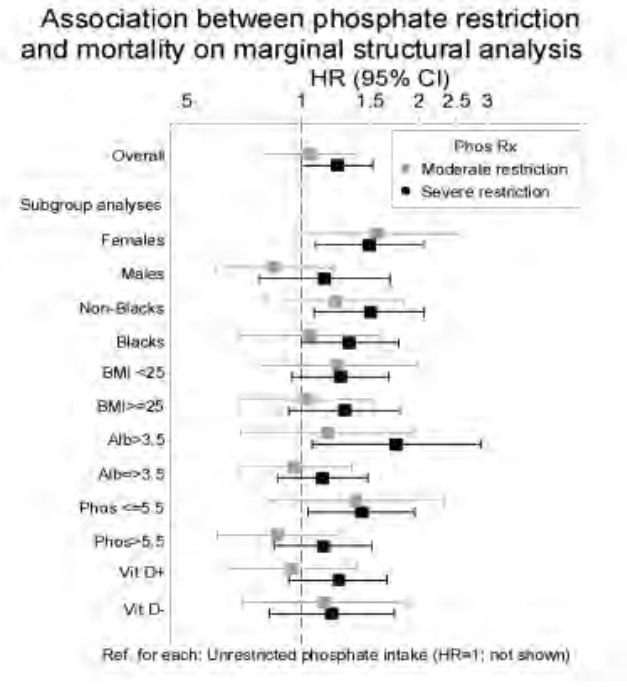
## SA-PO2578

**Prescribed Dietary Phosphate Restriction and Mortality in Hemodialysis (HD)** Katherine E. Lynch,[1] Rebecca Lynch,[2] Gary C. Curhan,[2] Steven M. Brunelli.[2] *[1]Beth Israel Deaconess Medical Center; [2]Brigham and Women's Hospital.*

**Purpose:** To evaluate the association between prescribed dietary phosphate restriction and mortality among HD patients.

**Methods:** We conducted a post-hoc analysis of data from the Hemodialysis Study. Dietary phosphate prescription (considered as *unrestricted*, *moderately restricted* (1-2 g/day), and *severely restricted* (≤1 g/day)) was assessed at baseline and yearly thereafter. Covariates (also updated annually) included pertinent demographics, comorbidities, procedural data, laboratories, and dietary protein/caloric intake. Marginal structural models were fit in order to estimate the adjusted association between dietary phosphate restriction and mortality in the setting of time-dependent confounding.

**Results:** The cohort consisted of 1780 subjects; mean age was 58+/-14 years; 57% were female; 62% were black; total at-risk time was 5025 pt-years; 838 deaths were observed. At baseline dietary phosphate was unrestricted, moderately restricted and severely restricted in 525(29.5%), 304(17.1%), and 951(53.4%), respectively. Compared to *unrestricted* dietary phosphate, *severe* restriction was associated with greater mortality: adjusted HR (95% CI) 1.24 (1.00-1.53). *Moderate* phosphate restriction bore an intermediate association with mortality, which did not achieve statistical significance: HR (95% CI) 1.05 (0.80-1.39). The association between *severe* phosphate restriction and mortality was accentuated among women, non-blacks, subjects with hypoalbuminemia and those without hyperphosphatemia (Figure).




**Conclusions:** This study suggests that prescribed dietary phosphate restriction is not associated with improved survival among prevalent hemodialysis patients, and severe restriction may be associated with greater mortality particularly in some subgroups. Further work is needed to confirm and generalize these findings.

Disclosure of Financial Relationships: nothing to disclose

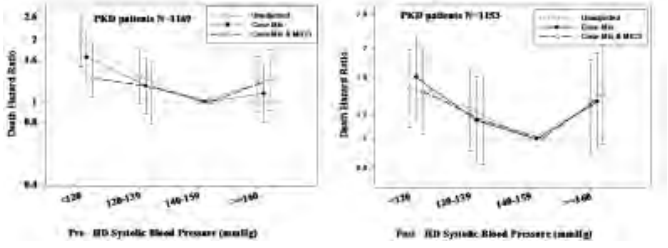
## SA-PO2579

**Blood Pressure and Survival in Long-Term Hemodialysis Patients with and without Polycystic Kidney Disease** Miklos Z. Molnar,[1] Lilia R. Lukowsky,[1] Ramanath B. Dukkipati,[1] Jennie Jing,[1] Allen R. Nissenson,[2] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]DaVita Inc, Lakewood, CO; [3]Division of Nephrology, Salem VA Medical Center, Salem, VA.*

**Background:** In maintenance dialysis patients, low blood pressure (BP) is associated with higher death rates when compared to normal to moderately high values. This "hypertension paradox" may be related to comorbid conditions. Dialysis patients with polycystic kidney disease (PKD) usually have a lower comorbidity burden and greater survival. We hypothesized that in PKD dialysis patients, a representative of a healthier dialysis patient population, high BP is associated with higher mortality.

**Methods:** Time-dependent survival models including after multivariate adjustment were examined to assess the association between pre- and post-hemodialysis BP and all-cause mortality in a 5-year cohort of 67,085 non-PKD and 1,579 PKD hemodialysis patients.

**Results:** In PKD patients low pre- and post-hemodialysis systolic BPs were associated with increased mortality, whereas high pre-hemodialysis diastolic BP was associated with greater survival. Fully adjusted death hazard ratios (and 95% confidence levels) for pre- and post-hemodialysis BP of <120 (reference: 140-<160 mmHg) were 1.30 (1.06-1.92) (Figure 1) and 1.45 (1.04-2.02) (Figure 2), respectively, and for pre-hemodialysis diastolic BP of >=80 (reference: 70-<80 mmHg) was 0.68 (0.49-0.93, all p-values <0.05). Similar associations were observed in non-PKD patients. In pooled analyses, within each commensurate BP stratum, PKD patients exhibited superior survival to non-PKD patients.



**Conclusions:** Among hemodialysis patients, those with PKD display a similar BP paradox as without PKD, even though within each BP category PKD patients maintain superior survival.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2580

**Zinc May Regulate Serum Leptin Concentrations in Hemodialysis Patients** Denise Mafra,[1] Luciana Nicolau Aranha,[1] Julie Lobo,[2] Milena Barcza Stockler-Pinto,[2] Viviane Oliveira Leal,[1] Najla Elias Farage,[2] Denis Fouque.[3] *[1]Nutrition, Federal University Fluminense, Niteroi, Rio de Janeiro, Brazil; [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [3]Nephrology, University Claude Bernard Hopital Edouard Herriot, Lyon, France.*

Anorexia is a common complication in hemodialysis (HD) and is associated with development of malnutrition and consequently with increased risk of mortality. Recent evidences suggests that zinc can be a mediator of leptin production indicating a possible relationship between zinc deficiency and leptin levels in the pathogenesis of anorexia in HD.

**Aim:** The purpose of this study was to evaluate the relationship between zinc levels and plasma leptin in hemodialysis patients.

**Methods:** Forty eight HD patients (mean age 54.4 ± 12.8 yr, 62.5% men) from RenalCor Clinic at Rio de Janeiro, Brazil, were studied and compared to 21 healthy individuals, aged 50.7 ± 15.7 yr. Blood samples were collected at fasting, before a regular HD session. Serum zinc concentration was measured by atomic absorption spectrometry and plasma leptin levels were determined using Multiplex kits (R&D System) on a Luminex. Anthropometry was used to evaluate the nutritional status.

**Results:** Leptin levels were significantly higher in patients than in healthy individuals (22.3 ± 48.5 *vs.* 7.6 ± 8.6 µg/mL; p = 0.04), whereas serum zinc levels were significantly lower (54.5 ±16.3 *vs.* 78.4 ±9.4 µg/dL; p= 0.0001). Plasma leptin was negatively correlated with plasma zinc (r=-0.34; p=0.006) and positively correlated with BMI (r=0.38; p=0.008) and % body fat (r=0.48; p=0.002). Plasma zinc was negatively associated with TNF-α (r=- 0.37; p=0.002).

**Conclusion:** In conclusion, this study showed that plasma zinc is possibly involved in leptin regulation. Further studies are required to investigate the regulatory pathways by which zinc and leptin regulate energy intake and body composition in hemodialysis patients. *Supported by Faperj, CNPq.*

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2581**

**Impact of the Route of Erythropoietin Administration on Hemoglobin Variability in Hemodialysis Patients** Youngki Lee, Ja-Ryong Koo, Seung Min Lee, Soo Jin Kim, Sung Gyun Kim, Ji Eun Oh, Jung-Woo Noh. *Department of Internal Medicine, Hallym Kidney Research Institute, Hallym University, Seoul, Republic of Korea.*

**Background:** Hemoglobin variability is a commonly occurring problem in hemodialysis patients receiving erythropoietin (EPO), and may portend poor outcomes. Drug-related factors such as difference in pharmacokinetics among EPO and different route of administration may affect hemoglobin variability. We reported that the risk of vascular access failure may be greater with subcutaneous (SC) compared to intravenous (IV) administration of EPO previously (Am J Kidney Dis 53:815-822, 2009). The present study was undertaken to analyze the impact of the different route of EPO administration on hemoglobin variability in hemodialysis patients.

**Methods**: 78 Korean hemodialysis patients were randomized to receive either IV (n=40) or SC (n=38) EPO therapy. EPO was administered during dialysis and the dose titrated to maintain hemoglobin level between 9 to 12 g/dL. We calculated the number of hemoglobin measurements out of the target range during all visits. Study duration was 4-77 months.

**Results:** The number of hemoglobin values out of the target range in patients receiving SC administration of EPO was higher than that of IV group (0.36±0.19 vs. 0.27±0.12/visit, P=0.03). There were no significant differences in the probability of the CVD-free rate between the groups. However, increased number of hemoglobin values out of the target range was associated with high probability of cardiovascular events regardless the route of EPO administration (p=0.018). And it was independently associated with increased CVD (HR: 2.85, 95% CI: 1.02, 7.94, P=0.04) after adjustment for age, previous CVD, dialysis vintage, and diabetes mellitus. There were no significant differences in either hemoglobin concentration or EPO doses between the two groups during the study period.

**Conclusions:** This study suggests that the risk of hemoglobin variability is greater with SC compared to IV administration of EPO in hemodialysis patients. Number of hemoglobin values out of the target range may be associated with increased risk of CVD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2582**

**Cost-Savings Associated with Reduction in Lower Limit Hemoglobin Target in EPO-Treated Hemodialysis Patients** Timothy V. Nguyen,[1] David S. Goldfarb.[2] *[1]Pharmacy Practice and Administration, Arnold & Marie Schwartz College of Pharmacy, Long Island University and The Mount Sinai Medical Center, New York, NY; [2]Medicine and Nephrology, NYU School of Medicine and NY Harbor VA Medical Center, New York, NY.*

**Purpose**: ESAs are widely used in dialysis patients. Patients treated with ESAs to Hb level >12 g/dL experience multiple complications including death. The FDA issued black box warnings and recommended not to treat to Hb level >12 g/dL. The NKF-KDOQI guidelines recommend target Hb levels in the range of 11-12 g/dL not to exceed 13 g/dL. We hypothesized that reducing target Hb in ESA-treated patients would prevent Hb levels rising above 12 g/dL and lead to significant cost savings.

**Methods**: In response to complications associated with ESA therapy (CHOIR, CREATE, TREAT), our target Hb levels were reduced to 9-11 g/dL from 11-12 g/dL. Thirty five chronic adult HD patients received EPO and IV iron from January to December 2009. Retrospective data analysis include: Hb levels, EPO dose, TSAT and ferritin levels. EPO was administered via subcutaneous injection weekly or twice weekly. IV iron ferric gluconate was administered to maintain TSAT >20% and ferritin >200 ng/mL.

**Results**: The mean monthly Hb levels changed from 11.2 g/dL in Jan to 10.6 g/dL in Dec. The percentage (%) of patients with mean monthly Hb(g/dL) >10, >12 and >13 were 82±6.5, 10±5.6 and 1.8±1.9 respectively. The mean weekly EPO doses decreased from 9,500 to 5,600 units. The reduction was approximately 40% per dose per patient, which translated to a 40% reduction in costs of treatment. The savings exceeded $60,000 for 35 patients. More than 80% of patients had TSAT>20% and ferritin level >200 ng/mL throughout the entire period.

**Conclusions**: Lowering target Hb level to 9-11 g/dL in HD patients treated with EPO achieved quality anemia management and resulted in significant cost savings. The literature suggests that this cost savings would be associated with minimal reduction in quality of life and no change in cardiovascular morbidity or mortality.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2583**

**The MIRACEL Study – Dosing and Dose Modifications Following the Direct Switch from Shorter-Acting ESA to Once Monthly C.E.R.A.** Danilo Fliser.[1] *[1]Klinik für Innere Medizin IV, Univeritätsklinikum des Saarlandes, Homburg/Saar, Germany; [2]Dialysezentrum Karlstrasse, Düsseldorf, Germany; [3]Medizinische Klinik III, Klinikum Fulda, Fulda, Germany.*

**INTRODUCTION AND AIMS:** The MIRACEL study was the first post-approval study with CERA to investigate hemoglobin (Hb) levels in CKD stage V pts receiving hemodialysis. We present additional in-depth data on dosing patterns and dose modification throughout the trial.

**METHODS:** In this prospective, single-arm national trial, 424 pts were converted from epoetin (Epo) α, β, δ, or Darbepoetin α (Dα) to CERA trt, of those 416 (98.1%) formed the ITT population. After a screening phase (2 months), pts were directly switched to once monthly CERA iv, regardless of their previous ESA, route of administration or dosing interval. Starting doses were 125µg or 200µg/months, depending on the pre-switch ESA-dose, followed by individual adaptation. After a titration phase (5 months), an evaluation phase (3 months) followed.

**RESULTS:** 311 (73.3%) pts started with 125µg/m, 106 (25.0%) pts initially received 200µg/months. During titration, dose was adjusted in 310 (73.1%) pts, and during evaluation in 171 (40.3%) pts. 260 (62.5%) pts needed only one dose modification during titration, to achieve target Hb levels. 120 (28.3%) pts required >1 dose modification during titration, while during evaluation, a second modification became necessary in only 41 (9.7%) pts. Overall, 1.19 ± 0.93 and 0.57 ± 0.68 dose modifications were needed in titration and evaluation phase, respectively. Mean CERA dose was 142 ± 48 µg/months throughout the study. When comparing the initial two pre-defined doses of 125 µg/months resp. 200 µg/months – with the last dose, the 125-µg-group was treated a mean of 129.6 ± 60.9 µg/months and the 200-µg-cohort arrived at a mean dose of 203.4 ± 58.5 µg/months.

**CONCLUSION:** Data from this large multi-center study emphasizes the clinical ease of switching hemodialysis pts from shorter acting ESAs at different dosing intervals to once monthly CERA. Starting from the recommended dosing, the majority of pts needed only one dose modification, with a robust starting-to-end-correlation of employed doses.

Disclosure of Financial Relationships: Consultancy: RocheResearch Funding: Daiichi-Sankyo, Ortho-Biotech; Honoraria: Amgen, Abbott, Boehringer-Ingelheim, Daiichi-Sankyo, FMC, Genzyme, Ortho-Biotech, Novartis, Roche, Shire; Scientific Advisor: Amgen, Daiichi-Sankyo, Roche.

**SA-PO2584**

**$^{14}$C-Peginesatide Clearance, Metabolism, and Excretion in Monkeys Following IV Administration** Kathryn W. Woodburn,[1] Yuu Moriya,[2] Yoshihiko Tagawa,[2] Christopher P. Holmes.[1] *[1]Affymax Inc, Palo Alto, CA; [2]Takeda Pharmaceutical Company, Ltd, Yodogawa-ku, Osaka, Japan.*

**Background:** Hematide™/peginesatide is a PEGylated, investigational, peptide-based erythropoiesis-stimulating agent that was designed and engineered to stimulate specifically the erythropoietin receptor dimer that governs erythropoiesis. This study was conducted to evaluate the pharmacokinetics and excretion of $^{14}$C-peginesatide in monkeys. **Methods:** Four male Cynomolgus monkeys were given a single IV dose of 5 mg/kg $^{14}$C-peginesatide. Blood samples for pharmacokinetic analysis were obtained at 0.25, 1, 6, 24, 48, 72, 96, 120, 144, 168, 240, and 336 h postdose. Urine and fecal samples were obtained at 24 h intervals up to 336 h. Samples were measured for radioactivity and plasma, urine, and fecal metabolite profiles were assessed by high performance liquid chromatography. **Results:** Radioactivity in the plasma exhibited a $t_{1/2\,\alpha}$ of 35.7 h and a $t_{1/2\,\beta}$ of 80.0 h. The mean $AUC_{0\text{-}336\,h}$ in the plasma was 10756 µg equiv.·h/mL. By 24 h, the mean recovery from the urine and feces was 12.4% and 0.2% of the dose, respectively. At 336 h, the recovery into the urine and feces was 59.7% and 7.0% of the dose, respectively. The sum of the excretion ratios was 66.7% of the dose. Peginesatide was identified as the predominant compound in the plasma, urine, and fecal samples. **Conclusions:** Elimination of peginesatide derived radioactivity from the plasma was prolonged after intravenous administration. Negligible metabolites of $^{14}$C-peginesatide were detected in plasma, urine, and feces.

Disclosure of Financial Relationships: Employer: Affymax Inc; Ownership: Affymax Inc.

**SA-PO2585**

**Clearance, Metabolism, and Excretion of $^{14}$C-Peginesatide in Rats Following IV Administration** Kathryn W. Woodburn,[1] Yuu Moriya,[2] Yoshihiko Tagawa,[2] Christopher P. Holmes.[1] *[1]Affymax Inc, Palo Alto, CA; [2]Takeda Pharmaceutical Company, Ltd, Yodogawa-ku, Osaka, Japan.*

**Background:** Hematide™/peginesatide is a PEGylated, investigational, peptide-based erythropoiesis-stimulating agent that was designed and engineered to stimulate specifically the erythropoietin receptor dimer that governs erythropoiesis. This study was conducted to characterize the clearance and major routes of elimination of peginesatide. **Methods:** Mass balance and pharmacokinetic evaluation was performed in male Sprague-Dawley rats following IV administration of 5 mg/kg $^{14}$C-peginesatide. Blood samples were collected at 0.25, 1, 6, 24, 48, 72, 120, 168, 240, and 336 h, the expired air was collected up to 24 hours, and urine and feces were collected at 24 h intervals up to 336 h postdose. Samples were measured for radioactivity and metabolite characterization was determined by HPLC. **Results:** Plasma half-life values of the radioactivity, $t_{1/2\,\alpha}$ and $t_{1/2\,\beta}$ were 27.8 and 109.7 hours, respectively with an $AUC_{0\text{-}336h}$ of 6198 µg equiv.•h/mL. No radioactivity was measured in expired air. At the end of the study (336 h), the radioactivity recovered in urine, feces, and carcass was 41.3%, 11.8%, and 49.9%, respectively. Metabolite profiling from collected samples demonstrated that $^{14}$C-peginesatide was the main radioactive component in plasma, feces, and urine. **Conclusions:** Clearance of $^{14}$C-peginesatide derived radioactivity was prolonged, with urinary excretion being a predominant elimination route. Negligible metabolites of $^{14}$C-peginesatide were detected in plasma, urine, and feces.

Disclosure of Financial Relationships: Employer: Affymax Inc; Ownership: Affymax Inc.

**SA-PO2586**

**Hematide™/Peginesatide Is Metabolically Stable and Does Not Induce or Inhibit Human Cytochrome P450 Enzymes** Sue Im,[1] Kei-Lai Fong,[2] Mitsuhiro Nishihara,[3] Jyunzo Takahashi,[3] Yoshihiko Tagawa,[3] Christopher P. Holmes,[1] Kathryn W. Woodburn.[1] *[1]Affymax Inc, Palo Alto, CA; [2]Accellient Partners LLC, Berkeley, CA; [3]Takeda Pharmaceutical Company Ltd, Osaka, Japan.*

**Background:** Peginesatide is a PEGylated, investigational, peptide-based erythropoiesis-stimulating agent that was designed and engineered to stimulate specifically the erythropoietin receptor dimer that governs erythropoiesis. Patients receiving peginesatide treatment for chronic kidney disease may be taking other medications concomitantly; therefore, drug-drug interactions resulting from the alteration of drug metabolizing enzymes may affect safety or efficacy responses. Peginesatide and its non-pegylated dimer and monomer were evaluated for their potential to induce or inhibit human cytochrome P450 enzymes (CYPs). In vitro metabolism of peginesatide was also assessed. **Methods**: To assess induction of CYP activities, cultured human hepatocytes were treated for 72 h with peginesatide (≤500 µg/mL), dimer or monomer (≤10 µg/mL). To assess inhibition, peginesatide, dimer or monomer (same concentrations) were incubated (37°C) with human liver microsomes or microsomes expressing human CYP in the presence of an NADPH generating system and marker substrates for CYP activities. In both evaluations, negative and positive controls were included and the percent change in enzyme activity determined. Separately, radiolabeled peginesatide was incubated (37°C) with liver and renal microsomes and S9 fractions from rats, monkeys and humans in the presence of an NADPH generating system. Metabolite formation was monitored by HPLC. **Results**: Peginesatide and its dimer and monomer did not cause either inhibition or induction of human CYP enzymes. In vitro, peginesatide was the main component following incubation with microsomes from all 3 species. **Conclusions**: Peginesatide, its dimer, or monomer did not cause inhibition or induction of human CYP enzymes. Peginesatide was metabolically stable in vitro. These in vitro results suggest that administration of peginesatide is not likely to result in CYP-mediated drug-drug interactions; however, the clinical significance of these results is unknown.

Disclosure of Financial Relationships: Employer: Affymax Inc.; Ownership: Affymax Inc.

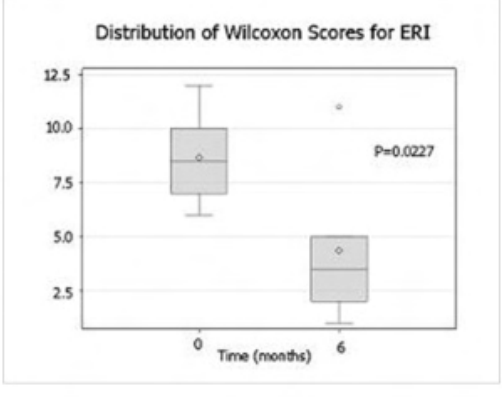
**SA-PO2587**

**N-Acetylcysteine (NAC) May Improve Erythropoietin Resistant Anemia (ERA) in Hemodialysis Patients** Nancy A. Finnigan,[1] Michael M. Chernick,[3] Robert L. Benz.[1,2] *[1]Nephrology, Lankenau Hospital, Wynnewood, PA; [2]Lankenau Institute for Medical Research, Wynnewood, PA; [3]Biostatistics, Lankenau Institute for Medical Research, Wynnewood, PA.*

PURPOSE: We studied the effect of N-acetylcysteine on hemodialysis patients with EPO resistance of undetermined etiology, presuming oxidative stress as the cause.

METHODS: All chronic hemodialysis patients from a single center were screened for eligibility. Inclusion criteria were age > 18, on hemodialysis > 3 months, and an ERI of >10 (Erythropoietin-Resistance Index, defined as weekly EPO dose / weight Kg / Hgb) for 2 months. Exclusion criteria included recent hospitalization, bleeding, infection, major surgery, active malignancy, iron deficiency, Kt/V<1.2, prior renal transplantation, and severe hyperparathyroidism. Subjects were administered oral N-acetylcysteine 600mg twice daily for 6 months. Hemoglobin was monitored monthly, and EPO dose adjusted per dialysis unit protocol.

RESULTS: Of 12 subjects enrolled, 2 discontinued due to GI side effects, 6 have completed the protocol, and 4 are actively receiving NAC. Of subjects completing the protocol, all had reduction of ERI from baseline with one exception, whose increase in ERI was attributed to a dental infection requiring dental extractions. Average ERI reduction was 19.9 to 9.3 (53%).




Wilcoxon Rank Sum Test on 6 subjects with complete data sets shows an ERI reduction from baseline (p=0.0227). Two month follow-up data is available on 5 patients, 4 of which are uninfected and show an ERI increase of 56% after NAC discontinuation.

CONCLUSIONS: The preliminary results of this small pilot study demonstrate an improvement in ERI with the administration of N-acetylcysteine to hemodialysis patients with EPO resistance presumed due to oxidative stress. Recruitment of subjects is ongoing to confirm these results.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2588**

**Erythropoietin Utilization in Hemodialysis Patients Receiving Paricalcitol vs. Doxercalciferol for Secondary Hyperparathyroidism** Samina Khan, Steven E. Marx, Beverly A. Johns, Utpaul Audhya. *Abbott, Abbott Park, IL.*

OBJECTIVE: To assess the impact of erythropoietin utilization among hemodialysis patients previously receiving erythropoietin who began paricalcitol or doxercalciferol.

METHODS: A retrospective cohort analysis from 2004 thru 2009 was conducted using database from a large dialysis organization of hemodialysis patients receiving erythropoietin. Patients were assigned to either paricalcitol or doxercalciferol treatment cohorts based on patients receiving a minimum of 10-doses of the respective drug. Inclusion criteria: erythropoietin use prior to index date, age >17year, minimum 90 days of data availability pre-index date, survival first 90 days after index date, & presence of consistent records. The index date was defined as the date of first dose of paricalcitol or doxercalciferol. A multivariate analysis of 14,043 patients adjusting for (1) age, gender, race, diabetes status, duration of dialysis, hemoglobin; (2) plus ferritin, and (3) plus study entry period was conducted. Additionally, 2,270 propensity matched patients using age, gender, race, and baseline hemoglobin were assessed as above. Sub-analyses were performed replacing ferritin with iron saturation & adding baseline iPTH to propensity match.

RESULTS: Multivariate analyses demonstrated statistically significant lower erythropoietin utilization for all three adjustments models comparing paricalcitol vs. doxercalciferol: (1) n=12,908 vs. 1,135, -0.05, p<0.05, (2) n=12,747 vs. 1,116, -0.05, p<0.05, and (3) n=12,747 vs. 1,116, -0.06, p<0.05. Similarly propensity matched analysis showed a trend towards lower erythropoietin use comparing paricalcitol vs. doxercalciferol for the first two, while statistically significant for the third adjustment analysis: (1) n=1,135 vs. 1,135, -0.05, p=0.10; (2) n=1,126 vs. 1,126, -0.05, p=0.09; (3) n=1,126 vs. 1,126, -0.08, p<0.05. Sub-analyses demonstrated similar results.

CONCLUSION: Hemodialysis patients with secondary hyperparathyroidism at this provider who received paricalcitol appear to have less erythropoietin dose requirements than those receiving doxercalciferol. Further prospective studies are required to confirm these results.

Disclosure of Financial Relationships: Employer: ABBOTT Laboratories; Ownership: I own Abbott stock.

**SA-PO2589**

**Prevalence of Atrial Fibrillation and Anticoagulation for Stroke Prophylaxis in Patients with Atrial Fibrillation: A Cross-Sectional Study of Patients on Hemodialysis** Juan Guzman,[1] Andrew Edward Gibson,[1] Amindeep S. Sandhu,[1] Trevor J. Wilkieson,[1] Nischal Ranganath,[1] Deborah Lynn Zimmerman,[2] Catherine M. Clase.[1] *[1]Department of Medicine - Division Nephrology, McMaster University, Hamilton, ON, Canada; [2]Department of Medicine - Division Nephrology, University of Ottawa, Ottawa, ON, Canada.*

**BACKGROUND:** Atrial fibrillation (AF) appears more frequent in haemodialysis (HD) patients. Previous studies have reported prevalences between 11 and 27% in previous studies.

**OBJECTIVE:** To determine the prevalence of AF and the prevalence of anticoagulation in those with AF in HD patients at St Joseph's Healthcare Hamilton (SJHH) dialysis unit.

**METHODOLOGY:** We performed a cross-sectional chart review of all 415 patients on HD attending to the SJHH. We collected data using an electronic-based survey linked to an electronic network database. We recorded demographic data, past medical history, anticoagulation data, risk of major bleeding (HEMORR2HAGES score), AF clinical information and AF embolic stroke risk score (CHADS2) in all the participants. The McMaster University research ethics board reviewed and approved the protocol. Data collection and analysis was completed from December 2009 to May 2010. Alpha level was set at 0.05. Data was analyzed with SPSS Version 18.0 (Chicago, IL, USA).

**RESULTS:** From 415 total study population, 32 (7%) patients were excluded. A total of 81 patients were identified as having either current AF or history of AF (prevalence: 22%; 95% CI 18 to 26%). Of the 81 patients, 28 (34.6%) were anticoagulated with Warfarin and 53 (65.4%) were not. There was no statistically significant difference in the mean total CHADS2 score [3.2 (1.4) vs. 3.4 (1.6)] or the mean total HEMORR2HAGES score [3.96 (1.6) vs. 4.6 (1.4)] between the Warfarin and no Warfarin groups respectively.

**CONCLUSION**: Warfarin use for stroke prevention in HD patients with AF is not prevalent and the variation in the use of Warfarin does not seem to be explained by risk of stroke or risk of bleeding. We hypothesize that the practice variation might be related to equipoise or uncertainty in the community, because of lack of direct evidence of benefit in this group of patients

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2590**

**Cardiovascular Risk Factors Correlate with Cardiac Anatomy in Pediatric Dialysis Patients** Douglas M. Silverstein. *Pediatric Nephrology, Children's National Medical Center, Washington, DC.*

Cardiovascular disease (CVD) is a major cause of mortality in children with end-stage renal disease (ESRD). We assessed the prevalence of 12 CVD risk factors in 28 pediatric ESRD patients: 12 on hemodialysis (HD) and 16 on peritoneal dialysis (PD), age 14.8±0.8 years, dialysis vintage 26.9±5.1 months. Pre-treatment systolic blood pressure index (SBPI) values were those recorded over 1 month. Values for homocysteine and 25 hydroxy vitamin D represent one value; all other lab values were those averaged over 3 months. 39% of patients had elevated left ventricular mass index (LVMI). 25 OH Vitamin D and hemoglobin levels, and BMI percentile were significantly more abnormal in patients with high LVMI. There were 4.1±0.3 CVD risk factors/patient. 50% of patients with normal LVMI had <4 CVD risk factors compared to 17% of those with high LVMI.

CVD RISK FACTORS: IMPACT ON LVMI

| RISK FACTOR | % OF PATIENTS WITH RISK FACTOR | NORMAL LVMI | HIGH LVMI | p VALUE |
|---|---|---|---|---|
| Pre-Treatment Systolic BP Index | 50% | 0.99±0.01 | 1.02±0.03 | 0.5 |
| Serum Albumin (g/dl) | 4% | 4.1±0.2 | 4.1±0.1 | 0.9 |
| Serum homocysteine (mmol/L) | 71% | 15.5±2.8 | 18.3±2.5 | 0.5 |
| HDL (mg/dl) | 18% | 117.7±22.3 | 115.5±28.1 | 0.9 |
| LDL (mg/dl) | 21% | 44.3±2.1 | 45.3±4.2 | 0.8 |
| 25 Hydroxy Vitamin D (ng/ml) | 100% | 13.7±1.7 | 8.5±1.4 | 0.03 |
| Serum phosphorous (mg/dl) | 50% | 5.3±0.3 | 5.7±0.4 | 0.3 |
| PTH (pg/ml) | 57% | 669.0±168.8 | 514.0±159.1 | 0.5 |
| Ca X Pi | 39% | 50.5±2.4 | 55.7±4.5 | 0.3 |
| Hemoglobin (g/dl) | 14% | 12.1±1.4 | 10.6±0.5 | 0.02 |
| CRP (mg/L) | 43% | 4.3±1.1 | 9.2±4.5 | 0.2 |
| BMI Percentile | 14% | 26.0±7.1 | 60.7±9.1 | 0.005 |
| Number of CVD Risk Factors | Not Applicable | 3.4±0.4 | 5.0±0.5 | 0.01 |

We conclude that accumulation of CVD risk factors predicts abnormal cardiac anatomy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2591**

**The Effect of Hemodialysis Modality on Insulin Resistance** Sung Jin Moon,[1] Jwa-Kyung Kim,[2] Jung Eun Lee,[3] Hyeong Cheon Park,[1] Tae-Hyun Yoo,[2] Shin-Wook Kang,[2] Ho Yung Lee,[2] Sung-Kyu Ha.[1] *[1]Internal Medicine, Gangnam Severance Hospital, Seoul, Korea; [2]Internal Medicine, Severance Hospital, Seoul, Korea; [3]Internal Medicine, Yongin Severance Hospital, Yongin, Korea.*

Introduction: Cardiovascular disease (CVD) is the leading cause of mortality in patients with end-stage renal disease (ESRD). Insulin resistance (IR) is associated with development of the CVD. We examined the factors containing hemodialysis modalities, associated with insulin resistance in hemodialysis patients.

Methods: In a cross-sectional study, 82 non-diabetic HD patients (47 men, mean age 59.2±14.4 years) were enrolled and divided into two groups by median homeostasis model assessment index (HOMA-IR). Other clinical and bio-chemical data associated with IR were collected.

Results: Higher HOMA-IR group had higher BMI (23.0 ± 3.1 vs. 20.9 ± 2.3 kg/m$^2$, p=0.001) and lower HDL cholesterol (42.4 ± 11.0 vs. 47.5 ± 10.4 mg/dL, p=0.038) levels. In addition, lower HOMA-IR group had more β-2 microglobulin reduction rate (39.1 ± 25.1 vs. 16.0 ± 22.8 %, p=0.001) and more proportion of hemodiafiltration (HDF) modality (p=0.002). HOMA-IR was significantly correlated with β-2 microglobulin reduction rate (r=-0.318, p=0.004). On multivariate logistic regression analysis adjusted by age, sex, BMI, hemodialysis modality, HDL cholesterol, and medication of statins and beta blockers, BMI and HDF modality were significant factors associated with HOMA-IR.

Conclusion: Lower HOMA-IR was significantly associated with HDF modality. It suggests that HDF therapy, enhancing removal of middle-molecular-weight substances by convection may reduce insulin resistance in hemodialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2592**

**Coronary Artery Calcification and Cardiovascular Events in Incident Patients on Haemodialysis** Trevor J. Wilkieson, Mohammed Omair Rahman, Maurice D. Voss, Alistair J. Ingram, Nischal Ranganath, Charlie H. Goldsmith, Cathy Z. Kotsamanes, Azim S. Gangji, Mark A. Crowther, Christian G. Rabbat, Catherine M. Clase. *Medicine, McMaster University, Hamilton, ON, Canada.*

**Background.** Coronary artery calcification (CAC) is prevalent in patients with end stage renal disease. CAC is progressive and is a risk factor for many adverse outcomes including cardiovascular events and death.

**Methods.** We conducted a prospective cohort study of consenting adult incident patients on haemodialysis (HD) at St Joseph's Healthcare Hamilton (SJHH), recruiting from October 2004 to October 2007. Patients were characterized in terms of age, sex, presence of diabetes, serum calcium, phosphorus, type of phosphate binder used, warfarin use, active vitamin D compounds, and baseline cardiovascular disease. CAC was measured by spiral CT and scored using the Agatston-Janowitz (AJ-130) method. Patients were followed until May 2008 for the primary outcome composite: all cause death, first myocardial infarction (MI) or stroke.

**Results.** The longitudinal cohort study recruited 164 patients at SJHH. 51 patients consented to spiral CT. There were 16 primary outcomes. The hazard ratio for CAC above the median was 2.5 (95% confidence interval [CI] 0.87, 7.3) (Figure 1). In the analysis adjusted for age, the hazard ratio for CAC above the median was 1.7 (95% CI 0.55, 5.4).

**Conclusion.** The primary limitation of this study is that few events occurred; which precluded a more comprehensive multivariate analysis. Accepting this limitation, in these data, CAC is associated with all cause death, first MI or stroke in incident patients on HD. However, the relationship is confounded by age.

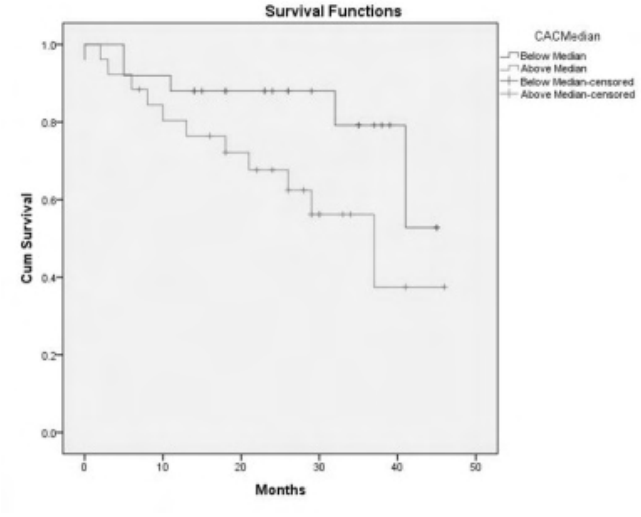



Figure 1. Relationship between CAC and all cause death, first MI or stroke; CAC divided at the median.

Disclosure of Financial Relationships: Employer: Astellas Pharma Canada Inc. Research Funding: Bodystat Ltd.

**SA-PO2593**

**Apo E2 Polymorphism Is Associated with Higher Levels of HDL2 Subfraction in Dialysis Patients** Fasika M. Tedla, Olusegun L. Amao, Moro O. Salifu, Clinton D. Brown. *Department of Medicine, Division of Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: Apo E gene polymorphisms have been associated with dyslipidemia in the general as well as dialysis population but the impact of these polymorphisms on lipoprotein subfractions has not been studied in dialysis patients.

Methods: We measured total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), apolipoprotein B (apo B), lipoprotein a (Lpa), C-reactive protein (CRP) levels; apo E genotype; and percentages of HDL2b and small dense LDL in 67 hemodialysis patients using assays available at an independent laboratory (Berkeley HeartLab, Alameda, CA). Patients were classified into three Apo E genotypes: E2 (e2/e2, e2/e3), E3 (e3/e3, e2/e4) or E4 (e4/e4, e3/e4). Genotypes were compared using analysis of variance or $\chi 2$ for continuous or categorical variables, respectively. Genotype frequencies were tested for Hardy-Weinberg equilibrium using exact probability test. Statistical significance was determined if $\alpha < 0.05$.

Results: The genotype frequencies were found to be in Hardy-Weinberg proportion. Apo E2, E3, and E4 genotypes represented 15%, 53.7% and 31.3%, of the study subjects respectively, and had no significant difference in gender, mean age (57.3±7., 57±5, 60.1±8.2 years), TC (139.2±20.1, 146±13.6, 144.3±17.4 mg/dL), LDL-C (67±13.8, 78±11.2, 78±14.6 mg/dL), HDL-C (55±10.8, 46±5, 52±20.5 mg/dL), TG (84±28.4, 107±23, 107±32.7 mg/dL), apo B (60±11.5, 73±9.2, 73±10.2 mg/dL), Lpa (54±29, 64±18, 72±24 mg/dL), CRP (13.3±14, 10±3.2, 10.5±6.5 mg/L), or small dense LDL % (13±3.8, 19±2.5, 19±5). The genotype groups, however, had significantly different HDL2b % (37.8±7.8, 24.5±7.5, 24.4±5.3, p<0.01). Significant inverse correlation was noted between HDL2b concentration and percentage of small dense LDL for Apo E2 (r=-0.74, p=0.02)and Apo E4 (r=0.68, p=0.01) polymorphisms, but not the wild genotype (Apo E3).

Conclusion: Dialysis patients with Apo E2 polymorphism have higher percentage of the cardio-protective HDL2b subfraction compared to dialysis patients with Apo E3 and Apo E4 polymorphisms.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2594**

**Effects of HFR Aequilibrium Therapy on Cardiovascular Stability: A European Multicentric Study** Francesco Locatelli,[1] Sergio Stefoni,[2] Thierry Petitclerc.[3] *[1]Manzoni, Lecco; [2]S. Orsola Malpighi, Bologna; [3]AURA, Paris.*

HFR Aequilibrium (HFR Aeq) is a dialysis technique based on the combination of HFR treatment with dialysate $Na^+$ and UF profiles elaborated automatically by a mathematical kinetic model and supported by the Natrium sensor measure of the patient's blood $Na^+$ concentration. The objective is to stabilize intradialytic BV taking into account the BW decrease and $Na^+$ balance of the session. Aim of the study was to evaluate the efficacy of HFR Aeq vs HFR.

An international study was carried out in 50 patients. After 1 month of wash out treatment, they were dialyzed with HFR or HFR Aeq (2 months)and then crossed. The study was carried out evaluating: intradialytic SBP, DBP and HR (time 0, every 30' and at 240'). Other intradialytic symptoms, operative and medical interventions were registered.

More than 900 sessions were performed for each period (Wash out, standard HFR, HFR Aeq). Intradialytic SBP and DBP significantly increased on HFR Aeq vs HFR respectively from 60' and 120' from start through session end. Intradialytic HR was more stable with HFR Aeq. Symptomatic hypotension episodes resulted significantly lower on HFR Aeq vs HFR (23±20% vs 31±28% of sessions respectively, $p<0.05$), as lower was the % of operative and medical interventions (17±20% of sessions with almost 1 intervention on HFR Aeq vs 22±24% on HFR; $p<0.01$). HFR Aeq resulted more effective on more unstable patients (35±15% of sessions with hypotension on HFR Aeq vs 60±28% on HFR; $p<0,01$). No clinical or biochemical signs of $Na^+$ - water overload appeared during the periods of treatment with HFR Aeq.

Disclosure of Financial Relationships: Consultancy: I'm a member of an advisory board of Abbott, Affimax,Amgen,Genzyme,Merck,Shire, Takeda, Roche, GlaxoSmithKline and member of a safety committee of Sandoz.

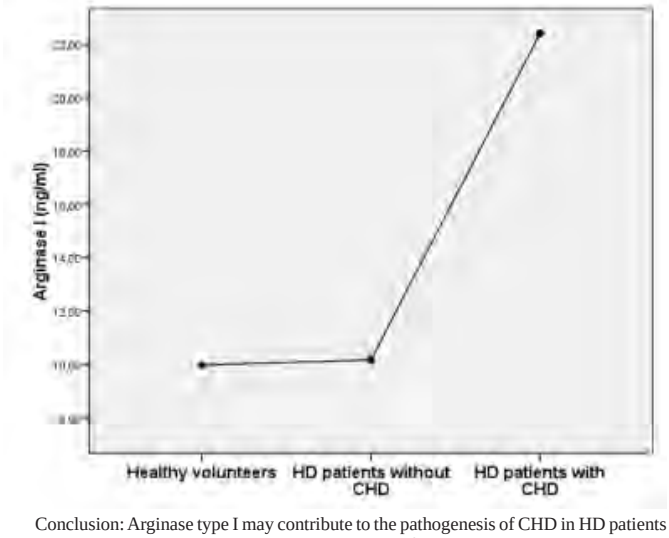
**SA-PO2595**

**Arginase Type I as a Marker of Coronary Heart Disease in Hemodialysis Patients** Theodoros Eleftheriadis, Vassilios Liakopoulos, Georgia Antoniadi, Ioannis Stefanidis. *Department of Nephrology, University of Thessaly, Larissa, Greece.*

Background: Cardiovascular disease, and mainly coronary heart disease (CHD), is the leading cause of death in hemodialysis (HD) patients. Non-traditional risk factors may play an important role in this population. Arginase is known to contribute directly in atherosclerosis progression and to counteract the beneficial effects of nitric oxide. HD could be considered as an inflammatory condition. Inflammation contributes to atherosclerosis progression and influences both arginase and nitric oxide synthase expression. In the present study serum arginase type I was evaluated as a marker of CHD in HD patients. The markers of inflammation nterleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were also assessed.

Patients and methods: 68 HD patients and 24 healthy volunteers were enrolled into the study. 20 HD patients suffered from CHD confirmed with coronary angiography, while the rest 48 HD patients were asymptomatic. The asymptomatic HD patients were followed up for a period of 24 months after the blood collection and none of them developed symptoms of CHD. Serum arginase type I, IL-6 and TNF-α were measured with ELISA.

Results: IL-6 and TNF-α levels were increased in HD patients, but did not differ between HD patients with or without CHD. On the contrary, arginase levels did not differ between healthy subjects and HD patients, but were twice higher in HD patients with CHD than in HD patients without CHD (22.41±15.47 ng/ml vs. 10.16±8.13 ng/ml).




Conclusion: Arginase type I may contribute to the pathogenesis of CHD in HD patients and its levels in the serum could be used as a marker of CHD in this population.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2596**

**Impact of Functional Genetic Polymorphisms of the Renin-Angiotensin-Aldosterone System on Coronary Artery Disease, Cardiovascular Complications and Mortality in Dialysis Patients** Sendogan Aker, Christos Bantis, Philip Reis, Peter J. Heering, Christina Schwandt, Lars C. Rump, Katrin Ivens. *Department of Nephrology, Heinrich-Heine University, Düsseldorf, Germany.*

We investigated the impact of angiotensin converting enzyme (ACE) I/D and aldosterone synthase gene C-344T polymorphisms on coronary artery disease (CAD) in chronic dialysis patients.

We studied n=462 patients on chronic dialysis with angiographically confirmed (n=217) or excluded (n=245) CAD followed up for 5.4±4.0 years. Cardiovascular complications and the need for intervention (coronary angioplasty or coronary artery bypass grafting) were monitored. The polymorphisms were determined by PCR. Serum aldosterone and ACE activity were determined by ELISA and RIA in 57 and 91 CKD patients respectively.

Aldosterone synthase C-344T and ACE-I/D polymorphisms influenced the serum aldosterone levels (CC/CT: 106.8±70.4, TT: 243.2±323 pg/ml, $p=0.013$) and ACE serum activity (DD/DT: 98.7±21, II: 65.6±18 U/L, $p<0.001$). Patients with CAD were comparable to those without CAD regarding age at coronary angiography, time on dialysis, LDL cholesterol and prevalence of arterial hypertension (ns). Among patients with CAD there were significantly more men (73.9 vs. 61.2%, $p<0.01$), diabetics (29.8 vs. 17.6%, $p<0.01$) and smokers (42.8 vs. 34.3%, $p<0.05$). Patients with CAD had also higher CRP (1.15±1.17 vs. 0.94±1.15mg/dl, $p<0.05$) and fibrinogen levels (453±134 vs. 417±109 mg/dl, $p<0.05$). There was no difference in the genotype distribution in patients with CAD (ACE I/D: DD=26.3%, DI=48.8%, II=24.9%; C-344T: CC=14.9%, CT=52.6%, TT=32.6%) and without CAD (ACE I/D: DD=25.4%, DI=51.2%, II=23.4%; C-344T: CC=20.4%, CT=47.8%, TT=31.8%, ns). Furthermore, no impact of the investigated polymorphisms was detected in the Kaplan-Meier analysis of intervention-free or myocardial-infarction-free survival as well in the general patient survival (ns).

The functional ACE-I/D and aldosterone synthase gene C-344T polymorphisms are not risk factors for the development of coronary artery disease in dialysis patients. Furthermore, they do not influence the incidence of myocardial infarction, need for intervention and mortality in this patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2597**

**Chronic Ambulatory Ultrafiltration Therapy for Heart Failure: Lack of Impact on Established Predictors of Mortality** Amir Kazory,[1] Matthias Goldstein,[2] Ardalan Enkeshafi,[3] Jerald Insel.[2] *[1]Division of Nephrology, Hypertension, University of Florida, Gainesville, FL; [2]Division of Cardiology, Good Samaritan Hospital, Baltimore, MD; [3]Department of Medicine, Western Maryland Health System, Cumberland, MD.*

**Background**: Although several studies have demonstrated the efficacy of ultrafiltration therapy in patients with heart failure (HF), there is currently no data on the impact of this therapy on long-term outcomes. Blood urea nitrogen (BUN) and serum sodium levels are the two "modifiable" laboratory parameters that have consistently been shown to predict outcomes in this population both at short- and long-term. We sought to explore the impact of chronic ultrafiltration on these established predictors of mortality.

**Methods:** All ambulatory patients with HF who were referred to the "aquapheresis clinic" for extracorporeal therapy were included. The clinic provided chronic ultrafiltration therapy to patients with refractory HF. Patients with less than 3 sessions of therapy were excluded. For each ultrafiltration session, relevant clinical and laboratory data at various time points (e.g. prior to initiation of therapy, one week, and four weeks) were recorded and compared.

**Results:** Between March 2006 and December 2008, 16 patients underwent a total of 279 sessions of ultrafiltration. The mean ejection fraction was 38% , with a baseline eGFR of 52.2 ml/min. The patients who completed a minimum of 4 weeks of ultrafiltration (9) had a mean baseline BUN of 38.5 mg/dl and serum sodium of 137.4 mEq/l; two of them were hyponatremic (serum sodium level ≤ 135 mEq/l) with a mean serum sodium concentration of 134 mEq/l. After 4 weeks of ultrafiltration therapy, BUN levels increased to 43.6 mg/dl and there was no significant change in the serum sodium level of the overall population (138.1 mEq/l) or the hyponatremic subgroup (135 mEq/l).

**Conclusion**: This study suggest that chronic ultrafiltration therapy does not portend a beneficial impact on certain predictors of mortality (BUN and serum sodium) in patients with HF. Whether this translates into a lack of effect on long-term outcomes needs to be addressed by future studies.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2598**

**Impact of Predialysis Care by Nephrologists on Selection of Dialysis Modality for Therapy and Prognosis of Stages 4 and 5 CKD Patients Older Than 75 Years of Age** Hiromichi Suzuki, Tsutomu Inoue, Yusuke Watanabe, Tsuneo Takenaka, Hirokazu Okada. *Nephrology, Saitama Medical University, Iruma gun, Saitama, Japan.*

**Background:** Overwhelming increases in numbers of the elderly population undergoing dialysis have become a serious problem in many countries with respect to medical as well as economic aspects. Early referral to nephrologists is recommended in patients with CKD. Moreover, it is well known that predialysis care is important for delaying introduction to renal replacement therapy (RRT). However, no conclusive data are available on whether predialysis care affects the selection of dialysis modality in the therapy and prognosis of elderly patients.

**Aim**: To examine the influence of predialysis care by nephrologists on the selection of dialysis modality and to evaluate the prognosis of elderly patients.

**Methods and Subjects**: A single center, retrospective cohort study was performed that included 210 patients (female/male**:** 52/158**;** 78 ± 9 years old) with stages 4 and 5 CKD (estimated GFR (eGFR) 8.7 ± 1.3 ml/min/1.73 $m^2$) referred to the kidney disease center from 2004 to 2009. Electronic databases were used to obtain information on selection of RRT and mortality.

**Results**: Only 60 (28%) patients were referred to the center 6 months prior to the introduction of RRT. Of the 60 patients, 42 selected continuous ambulatory peritoneal dialysis (CAPD), while 18 selected hemodialysis (HD) therapy. Of the other patients, 139

selected HD, 9 patients did CAPD and 2 refused RRT. More than 50% (32) of patients receiving predialysis care survived more than 29 months after introduction of RRT. In contrast, less than 10% (12) of late referral patients survived more than 30 months ($p<0.01$). There were significant differences in the levels of eGFR, albumin, hemoglobin, potassium, calcium, and phosphate between the two groups at the start of RRT, but no difference was seen for age.

**Conclusion**: Predialysis care by nephrologists is important for selection of RRT modality, and for improvement of prognosis after introduction of RRT in stages 4 and 5 CKD patients older than 75 years of age.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2599

**Beta Blockers for Prevention of Sudden Cardiac Death in Patients on Hemodialysis: A Propensity Score Analysis of the HEMO Study** Navdeep Tangri, Shani Shastri, Hocine Tighiouart, Gerald J. Beck, Alfred K. Cheung, Gary Eknoyan, Mark J. Sarnak. *Division of Nephrology, Tufts Medical Center, Boston, MA.*

Hemodialysis (HD) patients are at high risk for sudden cardiac death (SCD). Although beta blockers have been proven beneficial for prevention of SCD in the general population, little evidence exists for their efficacy in HD. The purpose of this analysis was to evaluate the potential benefit of beta blockers in a post-hoc analysis of the HEMO study.

The HEMO study was a randomized trial that enrolled 1,846 HD patients. Medications were ascertained from the dialysis chart, and SCD was a secondary outcome of interest. We used Cox regression models, as well as propensity score methods to study the effect of beta blockers on SCD.

1,747 patients were included in this analysis, and 521 were on beta blockers at baseline. Mean age was 58 years with 57 % females. 39% had ischemic heart disease (IHD) and 12% had severe heart failure (HF). 181 patients died from SCD during a mean follow-up of 31 months. Beta blocker use was not associated with a lower risk of SCD in univariate, multivariable or propensity-based analysis(Table). There was a significant interaction ($p=0.03$) between beta blocker use and IHD (HR 0.65(0.42,1.01) for patients with IHD and 1.61(0.92,2.80) for those without IHD), but not with HF ($p>0.05$). There was no association between beta blockers and other cardiac or all-cause mortality.

In HD patients without preexisting IHD, beta blocker use is not associated with a lower risk of SCD. These findings suggest that clinical equipoise may exist for a randomized controlled trial of beta blockers in HD patients without IHD.

Table: Effect of beta blockers on sudden cardiac death using different statistical methods

| Method | HR (95 % CI) |
|---|---|
| Survival Analysis | |
| Univariate | 0.89 (0.64 – 1.24) |
| Multivariable* | 0.87 (0.62 – 1.22) |
| Propensity Score (PS) Analysis | |
| PS Matching | 0.86 (0.58 – 1.28) |
| PS Stratification | 0.92 (0.43 – 1.95) |
| PS Adjustment | 0.87 (0.62 – 1.22) |

*Adjusted for age, sex, race, dialysis access, albumin, BMI, presence of diabetes, ischemic heart disease, HF, arrhythmia and cerebrovascular disease at baseline

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2600

**Increasing PD Reduces Medicaid Spending** Eric Berger, Kelly Yori, Renee Jg Arnold, John E. Moran. *DaVita Inc., Denver, CO.*

To meet 2011 budget shortfalls, states are enacting Medicaid budget cuts, including proposals to reduce dialysis reimbursement. In dual-eligible (Medicaid/Medicare) dialysis patients, Medicaid covers the 1st 3 months of tx until Medicare becomes effective. If a dual-eligible patient initiates training for PD, Medicare coverage becomes effective immediately. We examined the potential Medicaid savings associated with increasing PD among incident dialysis patients. This is a cost minimization model, taking a California Medicaid (MediCal) perspective, with a 1-year time horizon. We assumed 10% of ESRD incident patients who start dialysis without previous nephrologic care ("crash") and 25% of non-"crash" ESRD incident patients will choose PD.

| Assumptions | N* | Target PD Population | Model PD | Annual Savings |
|---|---|---|---|---|
| Incident Patients | | | | |
| Dual eligible | 1358 | | 217 | $6.1M |
| -"Crash" | 815 | 10% | 82 | |
| -Non-"Crash" | 543 | 25% | 135 | |
| Non-Medicare | 1280 | | 205 | $3.6M |
| -"Crash" | 768 | 10% | 77 | |
| -Non-"Crash" | 572 | 25% | 128 | |
| | Medicare Costs 12-month * | Medicare Costs 1st 3 months | MediCal Costs 1st 3 months | MediCal Savings 12-month |
| Per Dual-Eligible Patient | | | | |
| HD | $73,000 | $35,300 | $31,808 | N/A |
| PD | $53,500 | $25,900 | $5,174 | $28,101 |
| Difference | $19,500 | | $26,634 | |
| -with 10% Medicare discount | $17,600 | | | |

* 2009 MediCal actual ** 2008 USRDS

The model assumes an increase in incident PD from 5.8% (actual) to 7% in the dual-eligible patients and from 7.6% (actual) to 10.3% for MediCal-only patients. The model calculated the 3-month savings associated with changing coverage from MediCal to Medicare in dual-eligibles, and the annual savings for lower PD reimbursement (relative to HD) for non-Medicare eligible patients.The cumulative savings of treating 217 additional dually-eligible incident patients with PD vs HD was $6.1M over 1 year and treating 205 more non-Medicare eligible incident patients with PD was $3.6M yielding California an annual savings of $9.7M.Increasing the number of incident patients treated with PD instead of HD could save MediCal $5 to $10 million per year, likely the latter. This is a viable alternative for state officials seeking options to decrease Medicaid spending without compromising patient outcomes.

Disclosure of Financial Relationships: Employer: DaVita Inc.

## SA-PO2601

**Predictors of Mismatch between Chosen and Actual Dialysis Modality** Scott E. Liebman,[1] Steven P. Lamontagne,[1] Nancy Caswell.[2] *[1]Medicine, University of Rochester, Rochester, NY; [2]National Kidney Foundation of Upstate New York, Rochester, NY.*

**Background:** Although dialysis modality education (DME) is associated with higher rates of peritoneal dialysis (PD) use, some patients start hemodialysis (HD) despite an initially selecting PD as their modality of choice. This study tests the hypothesis that patients choosing and subsequently starting PD are educated earlier than those who choose PD, but start HD.

**Methods**: We performed a retrospective analysis of all patients who 1) Received DME at the University of Rochester between January 2004 and September 2009, 2) chose PD, and 3) subsequently started dialysis. Patients with contraindications to PD and those receiving education within two weeks of starting dialysis were excluded.

**Results:** Of the 112 patients receiving DME and choosing PD, 52 started dialysis with PD, 60 with HD. The primary hypothesis was negative. There was no difference in time between education and dialysis start between the two initial modalities ($41.4 \pm 42.9$ weeks for those starting PD vs. $46 \pm 49.9$ weeks for those starting HD, $p= 0.5$). Those starting PD had fewer comorbidities (2.1 vs.2.7) and were more likely to be under 75 years of age (94% vs. 78%) and working (24% vs. 6%) compared to those starting HD. In addition to the factors above, glomerular disease as ESRD cause was predictive of being on PD at day 90 of dialysis (26% of PD patients vs. 10% of HD patients), irrespective of initial modality.

Patients starting on HD were significantly more likely to have an unplanned dialysis start compared to those starting PD (57% vs. 2%).

Of the 54 patients who started HD and completed 90 days of dialysis, only six had transferred to PD by day 90.

Glomerular filtration rate (GFR) at DME, race, gender, ethnicity, BMI, education level, smoking , marital or insurance status and time of or GFR at initial nephrology encounter were not associated with initial modality.

**Conclusion:** Our data suggest that many people who initially select PD do not start with this modality, and few variables predict this discrepancy. Once on HD, most patients do not change modality. Further investigation using a qualitative approach may help explain the mismatch.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2602

**Predialysis Education and Care for Peritoneal Dialysis Catheters: A Need for Improvement** Leslie P. Wong,[1,2] Kalani T. Yamamoto,[2] Hien Pham.[2] *[1]Northwest Kidney Centers, Seattle, WA; [2]Nephrology, University of Washington Medical Center, Seattle, WA.*

**Background:** Peritoneal dialysis (PD) catheter-related complications are an important cause of morbidity and technique failure. Although guidelines outlining predialysis PD catheter- and exit-site care have been published by the International Society for Peritoneal Dialysis (ISPD), it is not known if these recommendations are followed in clinical practice. We are conducting a population-based study at a large U.S. PD program to examine this question.

**Methods:** We have enrolled 90 patients to date. Subjects answered a questionnaire about the care and instructions they received before and after PD catheter placement. Questions were derived directly from ISPD guidelines. Patient characteristics were recorded

**Results:** Fifty seven percent of participants were female. Mean age was 56 (±16) years. Most subjects were White (52%) and 69% had attended college or graduate school. The majority (99%) of patients had their PD catheters placed by surgeons. Before surgery, 46% of patients were checked for a hernia. Only 60% were asked if they could visualize their planned exit site. Instructions to shower with soap (52%) or perform bowel cleansing (20%) were not always given before surgery. Following PD catheter placement, most patients were told to call their surgeon (47%) or PD nurse (32%) for problems, not their nephrologist (5%). Only 44% of patients were instructed to leave their catheter dressing undisturbed after surgery. Thirty two percent of patients were not counseled about signs of PD catheter infection and 23% were not instructed on avoiding constipation. Many (42%) patients reported having a suture at their exit site. Only 58% of patients had their catheter examined by their nephrologist prior to starting PD.

**Conclusions:** Our preliminary results suggest that recommended predialysis PD catheter- and exit-site practices are not followed consistently. Improved education and implementation of current guidelines is needed.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2603**

**Superior Survival Rate in Long-Duration PD Patients with Peritoneal Dialysis Fluids (PDFs) with Neutral pH and Low Glucose Degradation Product (GDP)** Hoon Young Choi, Yong Kyu Lee, Beom Seok Kim, Hyeong Cheon Park, Ho Yung Lee. *Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.*

Long-term peritoneal dialysis has shown that peritoneal membrane performance and anatomy deteriorates with time of exposure to PDFs.

We carried out the retrospective cohort study comparing the long-term clinical impact of peritoneal dialysis (PD) treatment with either conventional PDFs or low GDP PDFs. On Dec. 31th 2009, the database included 1,257 individual PD patients' records. According to TR (treatment received) classification, PD patients were grouped either conventional PDFs (C-PDF group) or low GDP PDFs (L-GDP PDF group) in Korea single center. We investigated the demographic data, patient and technical survivals.

A total of 1,257 patients were included in this study (TR classification; C-PDF group= 573, L-GDP PDF group= 684). Age, sex, DM distribution were not significantly different between the two groups. L-GDP PDF group has longer PD duration than in C-PDF group (65.0 ± 56.4* vs. 58.1 ± 51.3 months, p<0.05).

The technical survival by Kaplan-Meir analysis was not different between the two groups. (Log rank; p=0.071). Patient-survival rate were significant higher in L-GDP PDF group than in C-PDF group (log rank; p=0.000).

Our data showed that the long-duration PD patients with low GDP PDFs treatment showed better patient-survival in Korea single center.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2604**

**Impact of Icodextrin on Residual Renal Function during 3 Years in Incident CAPD Patients** Jun-Young Do, Sun Young Jung, Kyu-Hyang Cho, Jong-Won Park, Kyung-Woo Yoon. *Internal Medicine, Yeungnam University Hospital, Daegu, Korea.*

Objectives: This study was designed to verify the impact of icodextrin solution on preservation of residual renal function in incident CAPD patients.

Methods: Among 301 incident CAPD patients during Apr. 2001 to Dec. 2006, 99 incident CAPD patients completed 36months protocol, prospectively. Residual renal function (RRF: mean of CCr and Curea), daily UFV, dialysate(D)-CA125, adequacy, 4.25% modified PET and clinical indices were measured at 1st, 6th,12th, 18th, 24th, 30th and 36th months. There used one solution among 4 PD solutions (2 low GDP: Physioneal and Balance, 2 high GDP:Dianeal and stay safe) through the 36 months and patients who changed PD solution were excluded. Icodextrin solution was used in 32 patients for volume control or improvement of quality of life. Sex, age, percent of diabetes were not significantly different between icodextrin and non-icodextrin groups. Pearson chi square analysis and independent T test were used to analyze the data.

Results: There were no significant differences of RRF, D/P4Cr and D/P1Na between icodextrin and non-icodextrin groups at the 1 month (5.08±6.68 vs. 4.03±2.27 ml/min, 0.70±0.16 vs. 0.69±0.09 and 0.87±0.16 vs. 0.88±0.11, p>0.05, respectively). At the 36 month, icodextrin group showed higher RRF (2.98±5.09 vs. 1.60±1.67 ml/mim, p<0.05) and higher D/P1Na (0.90±0.03 vs. 0.88±0.03, p<0.01) than non-icodextrin group but daily ultrafiltration volume was not significantly different.

In conclusion, Icodextrin group showed beneficial effect on preservation of RRF but not on preservation of aquaporin function.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2605**

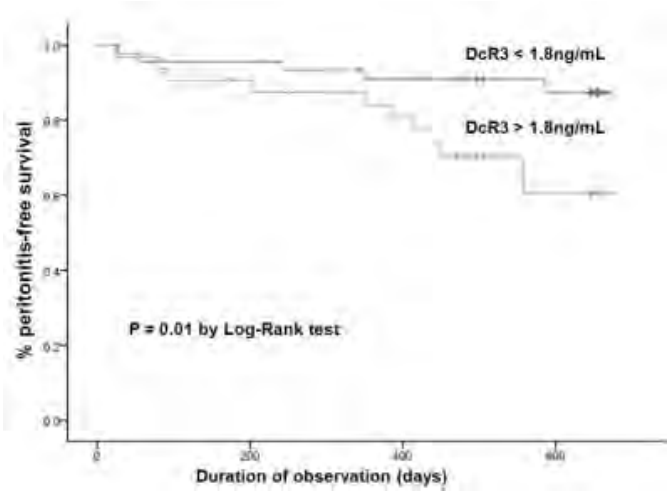
**Risk Factors of Peritoneal Dialysis (PD)-Related Peritonitis: A Novel Maker of Serum Decoy Receptor 3 (DcR3)** Yi-Sheng Lin,[1,2] Szu-Chun Hung,[3] Der-Cherng Tarng.[1,4] *[1]Department and Institute of Physiology, National Yang-Ming University; [2]Division of Nephrology, Taipei City Hospital; [3]Division of Nephrology, Buddhist Tzu Chi General Hospital; [4]Division of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan.*

**Aim**

Peritonitis is a major complication in PD, which is associated with technique failure and mortality. Till now, the risk factors of PD-related peritonitis are inconsistent. DcR3 is a member of tumor necrosis factor receptor family with immunomodulatory effects. Elevated serum DcR3 was found in several chronic inflammation status such as malignancy and could be served as a predictor for survival. However, the role of DcR3 in predicting PD-related peritonitis remains unknown.

**Methods and Results**

Seventy-seven patients (38 male and 39 female; mean age 58 ± 13 years) undergoing PD (vintage 24.5 months) were recruited for this observational study. Serum DcR3 and C-reactive protein (hs-CRP) levels were measured at the beginning of the study. Forty-six (60 %) patients were diabetic. The mean follow-up duration was 499 ± 17 days. The peritonitis incidence rate was 0.17 episodes/patient-year. The baseline serum DcR3 level was 1.94 ± 1.23 ng/mL. We observed that age (>55 years), gender, diabetes mellitus, baseline serum albumin (>3.5 g/dL), and hs-CRP (>0.5 mg/dL) were not associated with PD-related peritonitis (all $p$> 0.05). Only patients with serum DcR3 level >1.8 ng/mL had higher peritonitis rate as compared to those with serum DcR3 level <1.8 ng/mL ($p$< 0.05). Kaplan-Meier survival analysis showed PD patients with serum DcR3 level >1.8 ng/mL had higher risk for peritonitis ($p$=0.01).




**Conclusion**

Serum DcR3 level is a potential predictor of peritonitis in chronic PD patients. DcR3 has been shown a surrogate of malnutrition-inflammatory complex in our previous studies. Its role in modulating immune competency in PD patients is highly speculative.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2606**

**Impact of Peritonitis on Ex-Vivo Phenotype of Human Peritoneal Mesothelial Cells (HPMCs) during 3 Years in Incident CAPD Patients** Jun-Young Do, Sun Young Jung, Kyu-Hyang Cho, Jong-Won Park, Kyung-Woo Yoon. *Internal Medicine, Yeungnam University Hospital, Daegu, Korea.*

Objectives: This study was designed to verify the impact of peritonitis on ex vivo phenotype of HPMC and the associating factors with higher epithelial to mesenchymal transition (EMT) in CAPD patients.

Methods: Among 301 incident CAPD patients during Apr. 2001 to Dec. 2006, 99 incident CAPD patients completed 36months protocol, prospectively. Effluent cell cultures, dialysate(D)-CA125, adequacy, 4.25% modified PET and clinical indices were measured at 1st, 6th,12th, 18th, 24th, 30th and 36th months. One solution among 4 PD solutions (2 low GDP: Physioneal and Balance, 2 high GDP:Dianeal and stay safe) was used through the 36 months and patients who changed PD solution were excluded. D-CA125 and HPMCs from overnight effluent were completely isolated and cultured on the T25 flask at 1st and every 6 months. Cell score was blindly measured by the same person at near confluence (score 1: cobble-stone shaped HPMCs, score 2: mixed, score 3: fibroblastoid cell). Incidence of peritonitis and levels of D-CA125 during 36 months were compared between Score 1(n=32) and score 3(n=22) groups at the 36 months. Pearson chi square analysis and independent T test were used to analyze the data.

Results: There were no significant differences of D/P 4Cr, D/P 1hr Na, ultrafiltration volume during PET, sex, age, diabetes between cell score 1 and score 3 groups. Cell score 1 (less EMT) group at the 36th month showed significantly higher D-CA125 level than cell score 3 group (32.16±29.77 vs. 15.65±9.51, 37.62±31.91 vs. 13.74±6.02, 36.48±24.84 vs. 13.87±8.29, 35.19±17.65 vs. 11.98±6.84, 36.00±25.69 vs. 14.30±11.21 and 34.02±26.57 vs. 11.89±7.43, p=0.009, p=0.000, p=0.029, p=0.000, p=0.029 and p=0.000 at 6th , 12th , 18th , 24th , 30th and 36th months). Incidence of peritonitis was not significantly different between cell score 1 and score 3 groups during 36 months (0.32±0.41/year vs. 0.38±0.45/year, p=n.s.).

In conclusion, Dialysate CA125 showed significant association with ex vivo phenotype of HPMC but usual incidence of peritonitis didn't show significant impact on EMT during 3 years.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2607**

**Dialysis Vintage and Number of Previous Episodes Are Risk Factors of Peritonitis – A Single Center Study** Mira Varagunam, Ananda Chapagain, Magdi Yaqoob, Stanley Fan. *Department of Translational Medicine and Therapeutics, William Harvey Research Institute, London, United Kingdom.*

Despite the technical improvements in PD (peritoneal dialysis ) systems peritonitis remains a major problem leading to catheter loss, technique failure , sepsis and mortality. Understanding risk factors of peritonitis which are modifiable as well as those factors which are not modifiable is an important aspect of clinical management.We investigated the risk factors associated with peritonitis in patients who initiated PD between 2003 and 2007 in our renal unit. Our primary outcome was to quantify the risk of peritonitis with time on dialysis using random effects Poisson regression with Lexis expansion which allows the rates to be determined in specific time periods after initiating dialysis . Secondary outcome was to identify other putative risk factors associated with peritonitis . Out of the 367 patients 208 (57%) had peritonitis episodes. 159 (43.3%) patients had 0 episodes, 138(37.6%) had 1 episode, 49 (13.4%) had 2 episodes 19 (5.2%) had 3 episodes and 2 (0.5%) had 4 episodes. The median(min, max) follow up time was 1.5(0.01,5.7) years. Table 1 shows the rate ratio comparing different time periods after inception with the base line of 0-2 years.

| Time bands | Absolute rate (episodes/ person year) | *RR(95% CI) | p value |
|---|---|---|---|
| 0-2 years (Reference) | 0.45 | 1 | |
| 2-4 years | 0.83 | 1.85 (1.29,2.67) | 0.001 |
| 4-6 years | 1.85 | 4.11 (1.72,9.8) | 0.001 |

* rate ratio

Age at start of dialysis, sex, diabetes as cause of end stage renal disease, race, season, starting modality (APD vs PD) ,adjusted for dialysis vintage did not show a significant association with peritonitis rate . An increase in episode of peritonitis (after adjusting for dialysis vintage) was associated with peritonitis rate in a linear manner, with an increase in peritonitis episode associated with a rate ratio (95%CI) 2.1 (1.77,2.52) p<0.001.

Both time on dialysis and number of previous episodes are significantly associated with peritonits rate, and this may have to be taken into account when making a clinical descion to switch to haemodialysis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2608**

**Anuric Patients and Peritoneal Patients** Ricardo Vizinho, Patricia Quadros Branco, Maria Augusta Cabrita Silva Gaspar, Jose Diogo Barata. *Nephrology, Hospital Santa Cruz, Centro Hospitalar Lisboa Ocidental, Carnaxide, Lisboa, Portugal.*

Introduction: Anuric patients do not have the advantage of renal funtion preservation associated to Peritoneal Dialysis (PD) technique and so they do not benefit form its favorable impact on quality of life and survival.

Objective: To compare outcomes of baseline anuric Vs non-anuric PD patients, based on the experience of our center.

Methods: Retrospective analysis of data regarding 122 PD patients followed in our center, between 2002 and 2009, 48 of them under active treatment at the moment of data collection. Statistical analysis was done using SPSS version 16.0 and a p value < 0,5 was considered significant.

Results: The studied population had an average age of 59 years, 58% females, an average Body Mass Index (BMI) of 24.5, a mean Glomerular Filtration Rate (GFR) of 4.69ml/min, with 24% of anuric patients at baseline and 19% diabetic patients. Seventeen patients were on PD because they had no other option of dialysis (vascular access failure for hemodialysis).

There was no statistical significant difference between anuric and non-anuric patients regarding daily fluid-removal, type of transporters, kt/v, number of peritonitis or of in-stays for any cause, or time on DP.

When evaluating global mortality, adjusting for age, anury, peritonitis, in-stays and fluid-removal, diabetes was a positive predictor factor of mortality (HR=15, p=0.04).

In multivariate analysis, when adjusted for age, diabetes and time on DP and dialysis efficacy, anury was the only predictor of change to other dialysis modality (RR=2.6, p=0,035).

Conclusions: Based on our experience, although anury predicts transfer to hemodialysis, we found no difference regarding other major outcomes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2609**

**How Renal Transplantation Induces Bias in Peritoneal Dialysis Survival Analysis** Jean-Baptiste Beuscart,[1,2] Dominique C. Pagniez,[2] Luc Frimat,[3] David Evans,[4] Celia Lessore,[2] Christian Verger,[4] Alain Duhamel.[1] *[1]Biostatisctics, EA2694, UDSL, Lille, France; [2]Nephrology, CHU Lille, France; [3]Nephrology, EA 4003, Nancy-Université, France; [4]RDPLF, Pontoise, France.*

Background: Survival analyses on dialysis usually do not exclude patients for whom renal transplantation (RT) is planned. Only these patients can access to RT, which may occur before death during PD is observed. This competition between death and RT can lead to biased estimations in the total population.

Patients and methods: (i) Single center cohort of 293 incident PD patients (1992-2007) (ii) Analysis verified on a cohort of 6650 PD patients from the Registre de Dialyse Peritoneale de Langue Francaise (RDPLF). We estimated cumulative incidences for death and RT, according to whether RT was planned or not. We explored risk factors for death and RT using the Cox proportional hazard model.

Results: (i) RT was planned for 114 (39%) patients, none of whom died. At 6 years, their estimated probability of RT was 69%. They were younger and had fewer comorbidities.

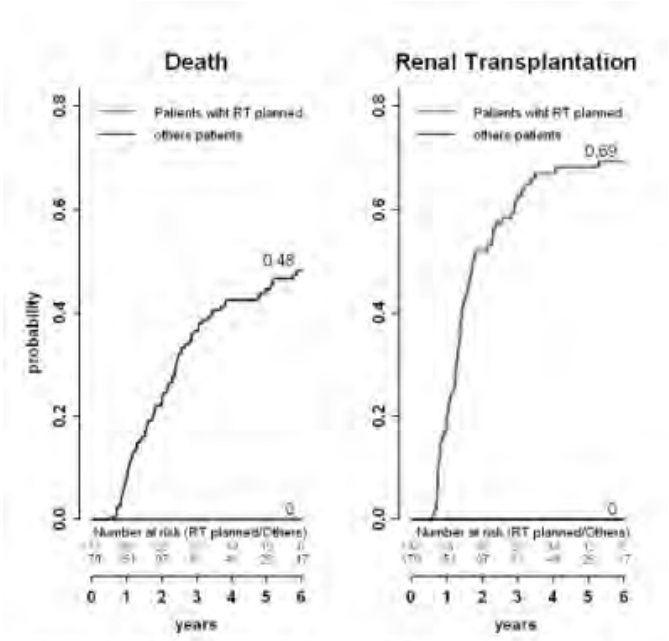



**Figure 1:** Cumulative incidences of death and renal transplantation (RT) during PD, according to whether RT was planned or not.

In the total population, older age, diabetes, low albumin, low residual renal function, and high CRP were significant risk factors for death and significant protective factors for RT. Therefore, the death hazard ratio could not be estimated independently of RT. (ii) The RDPLF data confirmed these results. RT was planned for 2205 (33%) patients. Their probability of death was estimated at 5% at four years, against 57% for the 4422 (67%) other patients. Age and diabetes were inversely related to death and RT.

Conclusion: Patients for whom RT is planned are much more likely to undergo renal transplantation than to die on PD. This competition between death and RT means that relative risks for death cannot be estimated independently of RT in the total population. PD survival analyzes should be stratified on inscription on RT waiting list.

Disclosure of Financial Relationships: Research Funding: Baxter Healthcare Corp. (Deerfield, IL).

**SA-PO2610**

**Characteristics of the CKD Patients Preferentially Directed to Pre-Emptive Renal Transplantation (Pre-RT) in the PREPARE Study** Bertrand Dussol,[1] Malik Touam,[2] Eric Daugas,[3] Paul Stroumza,[4] Yvon Berland,[1] Patrick Henri,[5] Georges J. Mourad.[6] *[1]Nephrology, Aix Marseille University, Marseille, France; [2]AURA, Cedh, Paris, France; [3]Nephrology, Hopital Bichat, Paris, France; [4]Diaverium, Clinique du Parc, Marseille, France; [5]Nephrology, Caen CHU, Caen, France; [6]Nephrology, Montpellier Medical School, Montpellier, France.*

The characteristics of the CKD patients preferentially directed by the nephrologists to pre-RT are not known. The aim of the PREPARE study was to describe the characteristics of stage 3 and 4 CKD patients, and the nephrologist's intention concerning type of ESRD treatment in case of evolution to stage 5. 1382 patients seen by their nephrologists during the first week of November 2009 were included in the study: a pre-RT was considered for 105 (7.5%), dialysis followed by RT (D-RT) for 317 (23%) and dialysis only (D) for 863 (62.5%). We compared the characteristics of pre-RT, D-RT, and D groups.

Table 1

| | pre-RT | D-RT | D | p |
|---|---|---|---|---|
| Age (years) | 50±13 | 50±13 | 74±10* | < 0.001 |
| Sex (male/female) % | 63/37 | 61/39 | 61/39 | ns |
| Nephrologic follow up (years) | 7.5±8 | 6.4±8 | 4.5±6 | ns |
| eGFR (ml/min) | 33±13 | 27±15* | 29±13* | <0.01 |
| HTA (%) | 80 | 88* | 87* | <0.05 |
| Diabetes mellitus (%) | 19 | 31* | 42* | <0.0001 |
| Other cardiovascular risk factors (%) | 12 | 13 | 19* | <0.05 |
| Cardiovascular complications (%) | 11 | 23* | 47* | <0.001 |
| Charlson score | 3.3±1 | 4.1±2* | 6.5±2* | <0.0001 |

*significantly different of pre-RT

This study showed that nephrologists in France envisaged pre-RT in a very small percentage of stage 3 and 4 CKD patients.This result explained that only 2.3% of the renal transplantations performed in France in 2008 were pre-emptive (Agence de la Biomedecine registry). Pre-RT patients were significantly younger, had higher eGFR, and had less cardiovascular risk factors and complications. French nephrologists are probably reluctant to give access to renal transplantation waiting list to some old and/or diabetic and/or patients with comorbidities, while it is known that pre-RT provides the best results and that duration of dialysis negatively impacts results of RT.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2611**

**Quality of Life (QoL) in the Oldest Dialysis Patients – Important Differences' between Hemodialysis (HD) and Peritoneal Dialysis (PD)?** Inger Laegreid,[1,2] Knut Aasarod,[1,2] Marit Jordhoy.[3] *[1]Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway; [2]Department of Nephrology, St. Olavs University Hospital, Trondheim, Norway; [3]Regional Center of Excellence in Palliative Care, Oslo University Hospital, Oslo, Norway.*

The elderly dialysis population is increasing, little is known about their QoL, and very few choose PD. **Aims**: was to describe and compare the QoL of the older Norwegian HD and PD population, and to explore whether the starting point of PD and HD differ(early vs late start).

**Method:** All patients > 75 years (n = 320) who according to the Norwegian Renal Registry (NRR) were in dialyses by 01.01.2009 and alive by Sept 2009 were asked to participate and mailed the QoL questionnaire SF 36. Medical data were collected from the NNR.

**Results**. In total, 230 pts (72%) responded, 152(66%) men and 78 (34%) women, 189 pts (82%) were in HD, 41 pts (18%) in PD. Mean age HD: 80.5 years, PD: 80.0 years. At start of dialysis, there was no significant difference in the distribution of comorbidities (diabetes mellitus type II, coronary and cerebrovascular diseases and malignancies), estimated glomerular filtration rate (eGFR, ml/min) (HD: 8.7; PD: 8.1) or the proportion of early start (eGFR > 10 ml/min), 27% in both groups. Preplanned start was more frequent among PD pts (86% vs73%) and duration of treatment was shorter (27 vs 38 months).

PD pts had lower blood pressure (mean 131/72 mm/Hg vs 143/71, annual report NNR 2008). Se-Albumin g/l (HD: 38.1, PD: 36.2) and BMI (kg/m²) (HD: 24.0, PD: 25.9) were comparable.

Table.1 SF 36 scores

| mean | HD | PD |
|---|---|---|
| Physical function | 42.0 | 38.3* |
| Role physical | 19.1 | 11.4* |
| Bodily pain | 56.7 | 60.6* |
| General health | 46.2 | 44.7* |
| Vitality | 40.2 | 35.4* |
| Social function | 61.4 | 69.7* |
| Role emotional | 43.5 | 39.0* |
| Mental health | 73.9 | 72.2* |

*p > 0.05 (Mann-Whitney U test)

Overall, the QoL scores were low, and there was no statistical or clinically significant difference between the two groups. In conclusion there are little differences between HD and PD pts in terms of QoL scores and basic characteristic. The findings may encourage a higher PD proportion in the elderly dialysis population.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2612**

**The Comparison of Incidence of Tuberculosis in Patients on Hemodialysis and Peritoneal Dialysis** Rumeyza Kazancioglu, Meltem Gursu, Savas Ozturk, Zeki Aydin, Sami Uzun, Serhat Karadag, Emel Tatli. *Nephrology, Haseki Training and Research Hospital, Istanbul, Turkey.*

**INTRODUCTION:** The increased incidence of tuberculosis in dialysis population has been shown by many studies. Tuberculosis has different clinical presentation, laboratory findings and drug side effects in this population. We aimed to compare tuberculosis incidence in hemodialysis(HD) and peritoneal dialysis(PD) patients.

**METHODS:** Patients who were on HD or PD treatment in our city for more than 3 months were included. Among the screened population; those who had tuberculosis during the dialysis period have been selected and divided into 2 groups according to dialysis modality: HD group and PD group. Their demographic and clinical data were recorded. Data were analyzed using SPSS ver 13.0.

**RESULTS:** 935 HD patients from 7 centers and 322 PD patients from 5 centers were screened. Thirty one patient in the HD group (3.35%); and 4 patients in the PD group (%1.24) were found to have diagnosis of tuberculosis during their dialysis treatment. The incidence of tuberculosis was statistically significantly higher in HD group [risk ratio:1.63; confidence interval: 95% (1.23-2.16)]. Patients in HD group were older (52.3±13.5 versus 26.7±5.9 years, p=0.001). Male/female ratio in HD and PD groups were 18/13 and 3/1, respectively. The time periods between the start of dialysis and the diagnosis of tuberculosis in HD and PD groups were 21.7±25.7 and 10.2±8.1 months, respectively (p=0.67). Extra pulmonary involvement in the groups was similar (48% in HD and 50% in PD group). One patient died due to tuberculosis in each group. But 12 patients in HD group and 1 patient in PD group were continuing their treatment at the time of analysis.

**DISCUSSION:** Tuberculosis was more frequent in HD group in the population we screened. This may be related with the fact that patients who prefer PD in our country are younger, have higher socioeconomic level and less co-morbidities in our country. Another factor may be related with less change in immunity against tuberculosis in PD population. These findings favor PD in terms of risk of tuberculosis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2613**

**Evaluation of the Utility of beta2-Microglobulin Adsorbent Column (Lixelle) for Dialyasis-Related Amyloidosis** Yuichiro Yamamoto,[1] Gen Yasuda,[1] Nobuhito Hirawa,[1] Satoshi Umemura.[2] *[1]Kidney Internal Medicine, Yokohama City University Medical Center, Yokohama, Kanagawa, Japan; [2]Kidney / Hypertensive Internal Medicine, Yokohama City University Hospital, Yokohama, Kanagawa, Japan.*

***Background.*** Dialysis-related amyloidosis (DRA) is one of major complication often seen in long-term dialysis patients,and is a factor decrease quality of life(QOL). Beta2-microglobulin (β2-m) is considered a major pathogenic factor in DRA . Lixelle column has been developed to absorb circulating β2-m ,and that various type of capacity column has been developed . Using minimum capacity of β2-m absorbing column :Lixelle Type S-15, we evaluated the utility and safety of column for patients of DRA. ***Methods.*** Seventeen hemodialysis patients with DRA were treated with Lixelle column for 1 year. Lixelle S-15 were used every haemodialysis . During the study period, pinch strength, visual analog scale , and activities of daily living were evaluated every three months,and collected blood sample every six months. ***Results.*** After 1-year treatment with Lixelle column, the β2-m level decreased 29.3 ± 9.6mg/L to 24.7 ± 5.1mg/L ($P < 0.05$),and the high sensitive c-reactive protein (hs-CRP) level decreased from 2996 ± 4380ng/ml to 1292 ± 1774ng/ml . After 1 year of Lixelle column use, the pinch strength increased from 5.9 ± 3.0 pounds to 7.2 ± 3.2 pounds ($P < 0.05$),and the visual analog scale and the activities of daily living score was also significantly improved. ***Conclusion.*** Long term use of Lixelle S-15 is effective for improvement of quality of life on dialysis patients with DRA , and is possible to improve the prognosis of them by suppressing chronic inflammation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2614**

**Impact of Changes in Predictors of Mortality during the First Year of Peritoneal Dialysis on Long-Term Patient Survival** Sung Hee Chung,[1,2] Jin Seok Jeon,[1] Soon Hyo Kwon,[1] Bengt Lindholm,[2] Dong-Cheol Han.[1] *[1]Hyonam Kidney Laboratory, Soon Chung Hyang University, Seoul, Korea; [2]Baxter Novum and Renal Medicine, Karolinska Institutet, Stockholm, Sweden.*

**Background**: We hypothesized that factors influencing patient survival and thus the predictive power of risk factors may change during the course of treatment with peritoneal dialysis (PD).

**Aim:** To evaluate impact of changes in predictors of mortality during the first year of PD on long-term patient survival after the first year, 148 PD patients (85 males, mean age 55±13 years) who underwent two assessments of nutritional status, residual renal function (RRF), dialysis adequacy, and peritoneal transport characteristics at a mean of 0.3 months and 11.5 months were included. After the second assessment patients were followed for 37 ± 25 months (range 7 - 115 months).

**Results**: At start of PD, patients who died during the follow-up period (Group D, n=43) were older and had higher prevalence of cardiovascular disease (CVD) and protein-energy wasting (PEW), higher fasting blood glucose (FBS), and lower serum creatinine (SCr) compared to patients who survived. At reassessment, Group D had higher prevalence of PEW, higher FBS, and lower serum albumin (SAlb), normalized protein equivalent of total nitrogen appearance, and RRF. During the first year of PD, SAlb and RRF decreased significantly and SCr increased significantly in Group D. On Cox proportional hazards multivariate analysis, independent predictors of mortality were based on initial assessment age (RR =1.06), CVD (RR =2.68), PEW (RR=2.16), and FBS (RR=1.04) and based on 1-year assessment age (RR =1.06), CVD (RR =2.93), SAlb (RR=0.26), and RRF (RR=0.79). Initial FBS correlated with initial SAlb (rho=-0.21), 1-year SAlb (rho=-0.16), and change in dialysate to plasma creatinine concentration ratio (rho=0.19).

**Conclusions.** This study demonstrates that in a selected group of PD patients who had been on PD more than 7 months, the predictive power of risk factors of mortality is changed during the initial treatment period, underlying the importance of repeating the analysis of risk factors during the course of treatment on PD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2615**

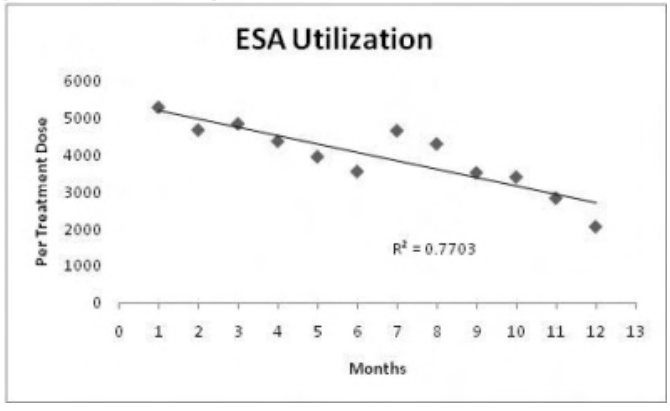
**A Nocturnal In-Center Hemodialysis Pilot Program – Logistic Issues and Improved Clinical Outcomes** Sheila Doss, Brigitte Schiller, Donna Lemus. *Satellite Healthcare, Mountain View, CA.*

A pilot in-center nocturnal hemodialysis (HD) program to evaluate the logistics and feasibility for alternative extended hour therapy was started in Jan 2009. A total of 21 patients were enrolled, providing 247 patient months of experience. Two patients died (1 withdrawal, 1 cardiac) and one transferred to home HD. Thrice weekly 8 hour overnight HD was offered to patients on conventional HD. Recruitment was easily achieved via meetings explaining to interested patients and families the purpose of the pilot. Reasons to switch to nocturnal were not doing well on current therapy (12/21), physician initiated (2/21), excess fluid gains (1/21), benefits of longer dialysis (4/21), employment (1/21), and 1 transfer from another nocturnal program.

The major operational issue was recruiting and retaining the RN staff for nocturnal schedule.

Clinical outcome measures improved during 12 months of nocturnal HD with 97% albumin ≥ 3.5 g/dL compared to 86% in the previous 12 months. 62% of phosphate values ≤ 5.5 mg/dL were reached on nocturnal compared to 46% prior on conventional HD. Mean

std Kt/V during the 12 months prior to starting nocturnal was 2.5 ± 0.4 and increased to 2.8 ± 0.3 on nocturnal HD. While maintaining Hb levels (pre-nocturnal 11.8 ± 1.2; nocturnal 12.3 ± 1.5g/dL) ESA utilization decreased over time reaching a 62% lower epogen dose per treatment by month 12 (p = 0.0002).




Quality of life assessment by KDQOL-SF36 at six months compared to baseline (n = 14) showed improvements in energy/fatigue (p = 0.04), effect of kidney disease (p = 0.05) and patient satisfaction with delivery of ESRD care (n = 0.009).

Patients on in-center nocturnal HD experience significant improvement in QOL. Challenges in implementation of nocturnal programs as part of routine ESRD care are outweighed by improved outcomes including decreased drug utilization.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2616

**Revalidation of the Kidney Disease Quality of Life Questionnaire (KDQOL)** Tracy Jack Mayne,[1] Duane V. Dunn,[1] Gilbert Marlowe,[1] Dorian R. Schatell.[2] *[1]DaVita Inc., Denver, CO; [2]MEI, Madison, WI.*

**Background** The Medicare Conditions of Coverage mandate the annual administration of the Kidney Disease Quality of Life questionnaire (KDQOL). The KDQOL was validated on 165 patients in 1997 and requires revalidation in a larger, more contemporary sample.

**Method** 19,337 KDQOLs were administered to dialysis patients between 1/08 and 12/09. We ran a confirmatory factor analyses and internal reliability on each subscale (SF-12; Kidney Disease Burden; Kidney Disease Symptoms; and Kidney Disease Effects).

**Results** *Burden* items produced a single factor explaining 69% of the variance, with good internal consistency, but floor & ceiling effects. *Symptoms* items produced two factors, but the second factor included only itchy/dry skin items, and the drop of in Eigen values occurred after the first factor. The parsimonious interpretation is a single factor explaining 27% of the variance, with good internal consistency, but ceiling effects. *Effect* items produced a single factor explaining 48% of the variance, with good internal consistency, but ceiling effects. A forced 2-factor solution of the SF-12 produced the expected *Physical* and *Mental Component* factors, explaining 28% and 25% of the variance respectively, with no floor/ceiling effects. However, internal consistency was low, and Cronbach's alfa improved appreciably with the removal of items.

| | SF-12 | | Kidney Disease | | |
|---|---|---|---|---|---|
| | Factor 1 Physical | Factor 2 Mental | Factor 1 Burden | Factor 1 Symptoms | Factor 1 Effect |
| Number of items | 12 | 12 | 4 | 12 | 8 |
| Eigen value | 4.93 | 1.41 | 2.77 | 4.11 | 3.81 |
| Variance explained | 28% | 25% | 69% | 27% | 48% |
| Cronbach's alpha | 0.60 | 0.57 | 0.85 | 0.82 | 0.84 |
| Item-total correlation range | -0.21 to 0.59 | -0.21 to 0.55 | 0.57 to 0.74 | 0.30 to 0.60 | 0.44 to 0.67 |
| Floor/Ceiling effects | No | No | F&C | C | C |

**Conclusion** The KDQOL, now mandated for annual collection by CMS, will become the *de facto* measure for tracking dialysis patient QOL in the US. Pre/post bundle comparisons are inevitable. The KDQOL does not adequately assess patients with very high dialysis Symptoms, Burdens & Effects, and thus may not detect if these measures worsen over time.

Disclosure of Financial Relationships: Employer: DaVita, Inc.; Honoraria: Sanofi-Aventis.

## SA-PO2617

**Subgroup Norms for the Kidney Disease Quality of Life Questionnaire in US Dialysis Patients** Duane V. Dunn,[1] Gilbert Marlowe,[1] Dorian R. Schatell,[2] Tracy Jack Mayne.[1] *[1]DaVita Inc., Denver, CO; [2]MEI, Madison, WI.*

**Background** CMS mandates annual administration of the KDQOL to US dialysis patients. Measuring norms before & after implementation of the new bundled payment system will allow us to assess its impact on patients.

**Method** 19,337 dialysis patients completed KDQOLs from 1/08 to 12/09. ANOVAs tested differences between groups by Gender; Race/Ethnicity; Age; Dialysis Vintage.

**Results**

| | SF-12 M±SD | | Kidney Disease M±SD | | |
|---|---|---|---|---|---|
| | *Physical* | *Mental* | *Burden* | *Symptoms* | *Effect* |
| GENDER | | | | | |
| Male (9085) | 36.8±10.4* | 49.5±10.9 | 47.5±28.9 | 76.2±16.1* | 67.4±22.7 |
| Female (9079) | 35.6±10.4 | 48.9±11.0 | 48.2±29.3 | 74.8±16.5 | 67.9±22.4 |
| RACE/ETHNICITY | | | | | |
| White (7867) | 35.5±10.4* | 49.3±10.9* | 47.5±28.6* | 75.9±15.9 | 67.5±22.1* |
| Black (6255) | 37.0±10.4 | 49.2±10.9 | 49.9±29.5 | 75.5±16.4 | 68.5±22.8 |
| Hispanic(1840) | 36.4±10.2 | 47.2±11.2 | 43.7±29.0 | 74.3±17.2 | 65.3±23.2 |
| Asian/Pacific Islander (253) | 36.9±10.7 | 48.7±10.3 | 40.2±26.7 | 75.5±18.6 | 65.6±23.1 |
| Native American (102) | 37.3±10.9 | 49.2±9.9 | 48.4±28.5 | 76.5±16.4 | 68.1±23.8 |
| Other/Unknown (3030) | 36.3±10.3 | 48.9±11.1 | 47.5±29.6 | 75.4±16.2 | 67.2±22.7 |
| AGE | | | | | |
| <18 (32) | 35.7±11.9* | 50.0±11.8* | 52.4±29.7 | 79.5±16.3* | 67.4±22.7* |
| 18-29 (240) | 42.1±10.0 | 47.1±11.4 | 46.9±28.3 | 76.4±17.4 | 66.2±23.8 |
| 30-39 (736) | 39.4±10.4 | 48.3±10.8 | 48.2±29.2 | 75.7±16.3 | 66.6±23.5 |
| 40-49 (1794) | 37.4±10.4 | 47.8±11.2 | 46.2±28.5 | 74.5±16.9 | 65.2±23.3 |
| 50-59 (3051) | 36.3±10.5 | 48.6±11.0 | 48.1±29.5 | 74.5±17.1 | 65.9±23.0 |
| 60-69 (3612) | 35.6±10.3 | 49.1±10.9 | 48.6±29.1 | 75.9±16.0 | 68.0±22.5 |
| 70-79 (3742) | 35.6±10.3 | 49.4±11.0 | 47.5±29.2 | 76.2±15.8 | 68.6±22.3 |
| 80+ (5918) | 36.1±10.3 | 49.6±10.9 | 47.9±29.1 | 75.7±16.1 | 68.4±22.1 |
| Missing (212) | 35.6±10.4 | 47.7±11.2 | 45.7±28.2 | 73.5±15.6 | 63.9±23.5 |
| VINTAGE | | | | | |
| ≤6 mo (622) | 35.6±10.2 | 48.8±11.1 | 48.0±28.9 | 75.8±16.2 | 68.5±21.8 |
| 7-12 mo (8393) | 35.4±10.1 | 48.4±11.6 | 45.5±28.9 | 75.9±15.8 | 66.8±22.7 |
| 2-5 years (3690) | 36.3±10.7 | 48.8±10.9 | 47.0±29.1 | 75.2±16.5 | 67.0±23.0 |
| 6-10 years (1423) | 36.2±10.6 | 49.4±10.7 | 49.6±29.1 | 75.9±16.2 | 68.0±22.5 |
| 11-20 years (744) | 36.3±10.4 | 49.1±11.0 | 47.9±29.1 | 75.6±16.3 | 67.7±22.6 |
| >20 years (1604) | 37.3±10.5 | 48.9±10.4 | 49.9±28.6 | 75.9±16.1 | 68.0±21.7 |
| Missing (2861) | 36.3±10.2 | 49.0±11.0 | 47.9±29.3 | 75.2±16.2 | 67.1±22.6 |

**Conclusion** There were statistically significant, but few clinically meaningful differences across groups. These norms establish the pre-bundling baseline on dialysis patient QOL.

Disclosure of Financial Relationships: Employer: DaVita, Inc.

## SA-PO2618

**Clinical Guidelines and Quality of Life in Hemodialysis Patients: A Wake-Up Call** Albert H. Mazairac,[1] G. Ardine De Wit,[2] E. Lars Penne,[1,3] Neelke C. Van Der Weerd,[1,3] Claire H. Den Hoedt,[1,4] Muriel Grooteman,[3] Marinus A. Van Den Dorpel,[4] Pieter M. Ter Wee,[3] Michiel Bots,[2] Peter J. Blankestijn.[1] *[1]UMCU, Utrecht, Netherlands; [2]JULIUS, Utrecht, Netherlands; [3]VUmc, Amsterdam, Netherlands; [4]Maasstad Hospital, Rotterdam.*

**Background:** The quality of life (QOL) of hemodialysis patients is poor. The development of clinical practice guidelines in dialysis care is based on various outcomes. However, it is unclear if achievement of treatment goals is related to an improved QOL.

**Aim:** To assess the relation between recommended treatment goals and QOL in hemodialysis patients.

**Methods:** We analyzed the baseline data of 697 hemodialysis patients (mean age 64 ±14 years [±SD], 63% male) of the ongoing CONvective TRAnsport Study (CONTRAST). Five clinical target levels as recommended by the Kidney Disease Quality Outcomes Initiative (KDOQI) were evaluated: spKtV (≥1.2), hemoglobin (11 to 13 g/dL), vascular access (AV fistula), phosphate (2.3 to 4.5 mg/dL) and blood pressure (<130/80 mm Hg). QOL was measured with the KDQOL-SF, a questionnaire that provides a physical (PCS) and mental (MCS) composite score as well as 12 kidney disease-specific QOL domains. We applied linear or logistic regression models and adjusted for confounders that caused ≥5% change to the crude relation.

**Results:** After adjustments for confounding, none of the target levels achieved related with the PCS or MCS. With regard to disease-specific QOL: patients with optimal phosphate levels reported a preferable effect of their kidney disease on daily life (OR: 1.50; 95% CI 1.07 - 2.10; $p<0.05$) and an enhanced quality of social interaction (OR: 1.53; 1.09 - 2.14; $p<0.05$). The domain burden of kidney disease was improved when hemoglobin target levels were met (OR: 1.36; 1.00 - 1.86, $p<0.05$). Patient satisfaction was better if blood pressure was as recommended (OR: 1.64; 1.08 - 2.47; $p<0.05$).

**Conclusion:** Overall, the achievement of the assessed clinical guidelines, which are generally based on level B/C evidence, only show a limited relation with the quality of life of hemodialysis patients. An effort should be made to provide evidence for further incorporation of perceived health status in guidelines.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2619

**Factors Correlated with Time Taken To Recover from a Hemodialysis (HD) Session** Usama T. Hussein, Kwabena T. Awuah, Radu R. Raducu, Fredric O. Finkelstein. *Nephrology Department, Saint Raphael Hospital, New Haven, CT.*

**INTRODUCTION**: Patients with end stage renal disease (ESRD) on HD have impairment of health related quality of life (HRQOL). This impairment is associated with various symptoms after HD treatments that impair patients' ability to resume normal activities after each session. Patients on short daily (SD) or long nocturnal dialysis (ND) have a shorter time to recovery (TTR) than those maintained on conventional hemodialysis (CHD). This shortened TTR is associated with improved HRQOL measures. The present study was undertaken to assess the TTR and clinical factors related to it in a large cohort of HD patients.

**METHODS:** All patients on CHD and ND in our 4 HD units were invited to participate. Patients were asked how long it took them to recover from their previous session for 3 consecutive treatments; data on age, gender, duration on renal replacement therapy(RRT), co-morbidities, BP during hemodialysis, time spent on HD and amount of ultra filtration(UF) were assessed from corresponding records. The mean value of the three sessions was used for analysis. 12 patients on ND and 263 on CHD participated.

**RESULTS:** The mean age was 66.7(+/- 15.69) for CHD group and 42.1(+/- 14.42) for the ND group. Mean number of co-morbidities for the two groups were 1.4(+/- 0.88) for CHD and 0.67(+/- 0.65) for ND. The mean duration on RRT was 40.2(+/- 37.78) and 49.67(+/- 35.28) months for CHD and ND respectively.A multivariate regression analysis including age, gender, number of co-morbidities, duration on RRT, occurrence of hypotension during dialysis, amount of UF and time spent on HD per session revealed that none of these covariates was significantly associated with TTR from HD. Importantly, there was no correlation between TTR and UF volume There was a difference in the TTR between ND (100 +/-162minutes) and CHD group (246+/-450minutes) p=0.0464.

**CONCLUSION:** In this cohort of HD patients, there was no correlation between TTR after HD session and studied covariates . This information is important as we assess the impact of new HD treatments on TTR and HRQOL. The difference in TTR between ND and CHD groups noted by earlier studies was confirmed.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2620

**Associations of Fluid and Dietary Restrictions with Mental Components of Quality of Life and Sociodemographic Characteristics in Patients on Maintenance Hemodialysis** Antonio Alberto Lopes,[1,2] Luciana Ferreira Silva,[1] Lidiane Dias Ribeiro,[1] Lívia Santana Oliveira,[1] Gildete Barreto Lopes.[1] *[1]Faculdade de Medicina da Universidade Federal da Bahia (UFBA); [2]Núcleo de Epidemiologia Clínica do Hospital Universitário Professor Edgard Santos, UFBA, Salvador, BA, Brazil.*

Purpose. We investigated whether the degree that maintenance hemodialysis (MHD) patients reported being bothered by fluid or dietary restriction was associated with mental components of health-related quality of life (HRQOL) and sociodemographic factors.

Methods. Cross-section of data of 1107 patients from PROHEMO Study developed in Salvador, Brazil. Patients were asked about the extent that they were bothered by fluid and dietary restrictions. SF-36 was used to assess HRQOL components: general health, vitality, social functioning, emotional well-being and role emotional. Logistic regression was used to identify characteristics associated with the bother of fluid or dietary restrictions. The assessed characteristics were age, gender, education, race and living status. Linear regression was used for assessing associations of HRQOL with being bothered by fluid and by dietary restrictions. The models were adjusted for sociodemographics, vintage, dialysis by catheter, albumin, creatinine, hemoglobin, Kt/V, diabetes, heart failure, cerebrovascular and peripheral vascular disease.

Results. Significantly (P<0.05) higher adjusted odds of being moderately to extremely bothered by fluid restriction were observed for women (adjusted odds ratio(AOR)=1.40) and those not living with family(AOR=1.72). Compared to patients aged ≥60 yr, those aged 18-39 yr showed higher odds of being bothered by fluid(AOR=2.18) and dietary restrictions(AOR=1.91). Patients moderately to extremely bothered by fluid or dietary restriction had significantly(P<0.001) lower adjusted scores for all assessed mental HRQOL scales, with differences >7 points.

Conclusion. The results are consistent with an important role of the burden of fluid and dietary restrictions in reducing the mental HRQOL of MHD patients. The burden related to these restrictions is apparently greater for younger patients, women and those not living with family.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2621

**The Influence of Sleep Quality, Depression and the Bother of Dry Skin on the Association between Pruritus Severity and the Burden of Chronic Hemodialysis Treatment** Gildete Barreto Lopes,[1] Gabriel Schnitman,[1] Lidiane Dias Ribeiro,[1] Dandara Reis Silva,[1] Antonio Alberto Lopes.[1,2] *[1]Núcleo de Epidemiologia Clínica do Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia (UFBA); [2]Faculdade de Medicina, UFBA, Salvador, BA, Brazil.*

Purpose: The main objective of the study was to assess whether severe pruritus is associated with greater perceived burden of kidney disease in maintenance hemodialysis patients without and with adjustments for sleep quality, depression symptoms and the bother of dry skin.

Methods: Cross section of baseline data of 980 patients enrolled in the Prospective Study of the Prognosis of Chronic Hemodialysis Patients (PROHEMO), a prospective study developed at dialysis units in Salvador, Brazil. The Kidney Disease Quality of Life Short Form was used to determine scores (range: 0 to 100) of the burden of kidney disease and sleep quality with higher scores indicating less burden of kidney disease and better sleep quality, respectively. The Center for Epidemiological Studies Depression Index was used to determine scores of depression symptoms (range: 0 to 60) with higher scores indicating higher depression symptoms.

Results: Mean age of the patients was 48.8±14.2 years; 58.7% were represented by men. The prevalence of severe pruritus (very much or extreme) was 19.4%. Lower score of the burden of kidney disease was observed for patients with severe pruritus than for those with no or mild pruritus (difference=11.44, P<0.001). Severe pruritus was also significantly associated with lower sleep score, higher odds of reporting being bothered by dry skin and higher score of depression symptoms. The difference in the burden of kidney disease by degree of pruritus was virtually unchanged after adjustments for demographics and comorbidities. By contrast, the differences was almost totally eliminated after adjustment for sleep score, depression score and the bother of dry skin (difference=0.89, P=0.719).

Conclusions: The results indicate that the higher burden of chronic hemodialysis treatment in patients with severe pruritus is almost entirely explained by poorer sleep quality, higher level of depression symptoms and the bother of dry skin.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2622

**Prevalence and Correlates of Depression Symptoms in Haemodialysis Patients: A Multinational Cross-Sectional Study** Valeria Maria Saglimbene,[1] Giorgia De Berardis,[1] Fabio Pellegrini,[1] David W. Johnson,[2] Jonathan C. Craig,[3] Jorgen B. A. Hegbrant,[4] Giovanni F. M. Strippoli.[1,3,4] *[1]Mario Negri Sud Consortium; [2]University of Queensland; [3]University of Sydney; [4]Diaverum Medical Scientific Office.*

Depression is common but underrecognized in people on hemodialysis. Existing studies found variable prevalence and conflicting data on correlates/predictors. In this multinational cross-sectional study, we enrolled people receiving hemodialysis in 27 outpatient clinics selected randomly from a collaborative dialysis network. Depressive symptoms were evaluated with the Center for Epidemiological Studies Depression (CES-D) Scale. Depression was considered a CES-D score>=18. Multivariable logistic regression was used to determine correlates of depression adjusting for clinical and sociodemographic characteristics.

Of 2920 haemodialysis patients in the participating clinics, 2180 (75%) completed the CES-D questionnaire. Overall, 1092 (50%) respondents reported depressive symptoms. Depressive symptoms were correlated with female gender (AOR 1.55, 95% CI 1.28 to 1.88), alcohol abuse (AOR 2.82, 95% CI 1.32 to 6.03), previous cardiovascular events (AOR 1.32, 95% CI 1.07 to 1.64) and sexual dysfunction (AOR 2.11, 95% CI 1.36 to 2.12). Education >5 years and daily physical activity were protective factors for depression (AOR 0.38, 95% CI 0.24 to 0.62 and AOR 0.58, 95% CI 0.44 to 0.76 respectively). Managing physicians had diagnosed depression in only 109 (10%) patients compared with 1092 (50%) actually reporting CES-D scores ≥18. Only 89 (8%) received treatment for their depressive symptoms.

In conclusion, we found depressive symptoms in 50% of the study population. Potentially modifiable risk factors include physical activity, identification and treatment of sexual dysfunction. We contend that assessment of depressive symptoms should be incorporated in clinical practice and intervention studies for this condition should be designed and conducted.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2623

**Musculoskeletal Disease Severity and Outcome in the HEMO Study** Heather L. Bujnicki, Jennifer M. Harness, Thomas I. Phelps, Phillip R. Rozak, Jane S. Tschang, Raymond J. Vergona, Mark L. Unruh. *University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Background: Both hemodialysis and musculoskeletal (MSC) disorders significantly affect quality of life. Chronic hemodialysis is associated with decreased bone density and muscle mass which may predispose to more MSC. The relationship between severity of MSC disease and outcomes among patients on dialysis is uncertain.

Methods: We undertook a secondary data analysis of the HEMO study, a randomized clinical trial in which 1846 patients underwent thrice-weekly dialysis. The data collected from this study was analyzed and correlations between MSC scores and demographic, socioeconomic, clinical, and laboratory variables were identified. MSC scores in the HEMO study were separated into four classes; no musculoskeletal or connective disease diagnosis (0), disease diagnosis with mild distress (1), moderate disease progression requiring medication (2), and severe, incapacitating, uncontrolled pain (3). For data analysis, moderate (2) and severe (3) classes were combined.

Results: Of the 1,846 study participants, 1,037 (56%) had a musculoskeletal score of 0 (none), and 430 (23%) had a score of 1 (mild), while 379 (21%) had a score of 2(moderate-severe). Age (OR = 1.02, 95% CI = 1.01-1.03), increasing duration of dialysis (OR = 1.13, 95% CI = 1.10-1.15), and diabetes (OR = 1.44, 95% CI = 1.18-1.76) were independently associated with increasing severity of musculoskeletal scores after accounting for key demographic and clinical covariates. Increasing severity of musculoskeletal scores were found to be associated with several quality of life measures including physical function ($p < 0.0001$), bodily pain ($p < 0.0001$), and physical component score ($p < 0.0001$). Increasing severity of MSC scores were not found to be significantly associated with survival.

Conclusion: Although musculoskeletal disease severity was not significantly associated with survival, increased severity of disease was associated with key quality of life measures. Further investigations could focus on the association between musculoskeletal functioning such as ambulation and falls on quality of life and survival.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2624**

**Self-Reported Physical Functioning in Maintenance Hemodialysis Patients Is Unrelated to Cognitive Performance** Brian T. Agganis,[1] Daniel E. Weiner,[1] Lena M. Giang,[1] Hocine Tighiouart,[2] Tammy Scott,[3] Mark J. Sarnak.[1] *[1]Nephrology, Tufts Medical Center, Boston, MA; [2]Biostatistics Research Center, Tufts Medical Center, Boston, MA; [3]Psychiatry, Tufts Medical Center, Boston, MA.*

Prior research highlights physical activity as a possible moderator of cognitive decline in the general population. As part of the ongoing Cognition and Dialysis Study, we evaluated the association between physical functioning and cognitive performance in maintenance hemodialysis patients.

The Short Form Health Survey (SF-36) is a self-reported health questionnaire commonly used to evaluate disease burden in individuals and across populations. The SF-36's Physical Component Score (PCS) provides a global measure of physical functioning derived from questions related to general health, bodily pain, daily activities, and physical performance. Study participants with SF-36 questionnaires administered as part of their routine care within 3 months of cognitive testing were included in this analysis. Linear regression was used to evaluate the association between PCS and cognitive test performance in unadjusted and adjusted analyses.

195 participants met the inclusion criteria. Mean age was 65 years, 49% were female, 22% African American, 52% had diabetes, and 35% had coronary disease. The mean (SD) PCS score was 37 (10). In univariate analysis, individuals with higher PCS performed better on Digit Symbol Coding although this finding was not consistent across other measures of executive function and did not remain significant after multivariable analysis (Table). PCS was unrelated to cognitive performance in all other tests of cognitive function.

We found no significant or consistent relationship between self-reported physical functioning and cognitive performance in hemodialysis patients.

| Cognitive Test | Function Tested | Unadjusted Estimate per 1 SD increase in PCS , p-value | Adjusted Estimate per 1 SD increase in PCS, p-value |
|---|---|---|---|
| Mini Mental State Exam | Screen | 0.11, 0.398 | 0.16, 0.413 |
| Verbal Intelligence | Intelligence | -1.67, 0.053 | -1.08, 0.181 |
| WMS-III Short Delay | Primary Cortical (Memory) | 0.19, 0.397 | 0.13, 0.523 |
| WMS-III Delayed Recall | | 0.17, 0.407 | 0.04, 0.841 |
| WMS-III Retention | | 2.89, 0.165 | 1.25, 0.384 |
| WAIS-III Recognition | | 0.32, 0.156 | 0.21, 0.314 |
| WAIS-III Block Design | Primarily Subcortical (Executive function and processing speed) | 0.35, 0.642 | 0.35, 0.611 |
| WAIS-III Digit Symbol Coding | | 4.15, 0.001 | 2.02, 0.056 |
| Trails A | | -3.27, 0.239 | 0.95, 0.731 |
| Trails B* | | -14.10, 0.073 | -0.85, 0.900 |

All tests represent scores/tasks completed except Trails A and B which are time required to complete a task. Positive values correspond with better test performance for all tasks except Trails A and B. Multivariable adjustments included age, sex, race, and education. *Trails B analyses were performed using Tobit regression to account for failure to complete the task within 5 minutes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2625**

**Association of Frailty with Muscle Size among Dialysis Patients and Sedentary Control Subjects** Cynthia Delgado,[1] Julie W. Doyle,[1] Kirsten L. Johansen.[1,2] *[1]Nephrology, UCSF, San Francisco, CA; [2]Nephrology, SFVAMC, San Francisco, CA.*

Frailty is a physiological syndrome characterized by decreased reserve and diminished resistance to stressors. Frailty has been independently linked to decreased survival in several large cohort studies, including studies of patients with chronic kidney disease (CKD). The prevalence of frailty in the CKD population is quite high – estimated to be 21% based on NHANES data. The purpose of the study was to determine the extent to which frailty is linked to measured muscle size and function in patients with ESRD and controls using baseline data from patients enrolled in an interventional study.

Methods: Eighty patients undergoing hemodialysis for > 3 months were included in the study if there was evidence of malnutrition or poor quality of life assessed by questionnaire. 20 age matched control subjects were not on dialysis. Electromyography action potentials of the anterior tibialis muscle were recorded using a surface electrode. Cross-sectional area of the contractile tissue of the muscle was measured using MRI . Frailty was defined based on questionnaire measures of physical functioning, exhaustion, and physical activity as has been previously described. These included SF-36 physical function score < 75 (2 points), SF-36 Vitality score < 55 (1 point), and the lowest quartile of weekly kcal energy expenditure on leisure activity calculated from the Physical Activity Scale for the Elderly (PASE) questionnaire. A frailty score of ≥ 3 was defined as frail.

Results: 48 subjects had EMG and muscle CSA data available for analysis. The majority of subjects were male (64%) with mean age 55 (± 13 yrs). 54% of participants were African American, 12% were Caucasian and 25% Asian. 14% of control subjects met frailty criteria compared to 61% of those on dialysis (p<0.0001). In multivariate analysis, frailty was associated with decreased muscle area even after adjustment for age and sex (coef -0.64, p<0.03)

Conclusion: Frailty as defined by the Woods criteria is associated with muscle atrophy, providing important validation of the syndrome as reflective of sarcopenia.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2626**

**Blood Pressure and Cognitive Function in Dialysis Patients** Lena M. Giang, Daniel E. Weiner, Hocine Tighiouart, Brian T. Agganis, Tammy Scott, Mark J. Sarnak. *Nephrology, Tufts Medical Center, Boston, MA.*

There are few data on the relationship of blood pressure and cognitive function in chronic dialysis patients.

The Cognition and Dialysis Study is an ongoing investigation of cognitive function and its risk factors in 5 Boston area DCI units. In this analysis we evaluated the relationship of different domains of cognitive function to systolic and diastolic blood pressure as well as pulse pressure in univariate and multivariable linear regression models adjusted for age, sex, race and education.

Among 273 participants with complete data, mean age was 63 years; 47% were female, 22% African American and 48% had diabetes. Mean (SD) of pulse pressure, systolic blood pressure and diastolic blood pressure were 69 (14) and 143 (21), and 73 (12) mm Hg, respectively. In univariate analyses, the performance on cognitive tests assessing memory, executive function and processing speeds was worse among participants with higher pulse pressure and higher diastolic blood pressure (data not shown).

| Cognitive Test | Function Tested | Unadjusted Estimate Per 1 SD increase in Pulse Pressure, p-value | Adjusted Estimate Per 1 SD increase in Pulse Pressure, p-value |
|---|---|---|---|
| Mini Mental State Exam | Screen | -0.30, 0.089 | -0.11, 0.518 |
| Verbal Intelligence | Intelligence | 0.36, 0.621 | -0.34, 0.624 |
| WMS-III Short Delay | Primary Cortical (Memory) | -0.46, 0.009 | -0.13, 0.295 |
| WMS-III Delay Recall | | -0.23, 0.058 | -0.07, 0.687 |
| WMS-III Retention | | -1.35, 0.425 | 0.57, 0.744 |
| WMS-III Recognition | | -0.43, 0.018 | -0.19, 0.279 |
| WAIS-III Block Design | Primarily Subcortical (Executive function and processing speed) | -1.38, 0.035 | -0.04, 0.941 |
| WAIS-III Symbol Coding | | -3.16, 0.004 | -0.58, 0.521 |
| Trails A | | 5.14, 0.029 | 1.08, 0.639 |
| Trails B* | | 15.73, 0.025 | 0.62, 0.229 |

All tests represent scores/tasks completed except Trails A and B which are time required to complete a task. Positive estimates are consistent with improved performance except for Trials A and B.
*Trails B analyses were performed using Tobit regression to account for failure to complete the task within 5 minutes.

These relationships however were not significant after multivariable adjustment. There was no association between cognitive function and systolic blood pressure in either univariate or multivariable analysis.

We found no association between blood pressure and cognitive function in this cross sectional analysis. These results need to be reproduced in longitudinal studies.

Disclosure of Financial Relationships: nothing to disclose

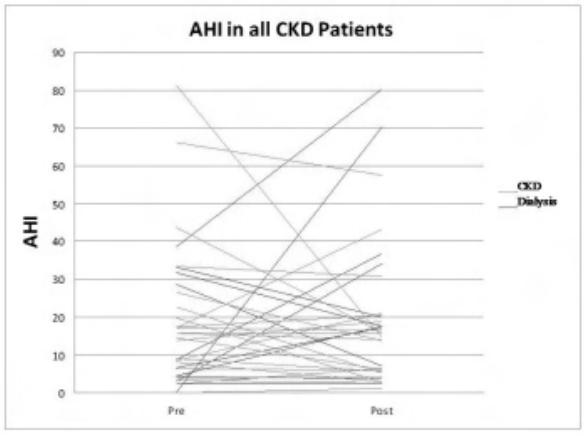
**SA-PO2627**

**Influence of Dialysis on Severity of Sleep Apnea in Advance Chronic Kidney Disease** Dionne C. Okafor, Sheena Ann Dohar, Mark L. Unruh. *University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Objective: While sleep apnea has been strongly linked to ESRD, very little is known regarding the prevalence of sleep apnea in advanced CKD. It is also unknown if the initiation of dialysis would change the severity of sleep apnea. Therefore, we initiatived a prospective study to describe longitudinal changes in sleep apnea severity among patients with advanced CKD and to assess whether sleep apnea improves with the initiation of dialysis.

Methods: We recruited 61 patients. Of these patients, 32 participants completed two unattended home polysyomnographies (PSG). The first PSG study completed at time of enrollment into the study, the next performed either 3 months following the start of dialysis or 1 year after initiating the study (post).

Results: The study sample was an average of 53 years old, predominantly men (66.6%), and white (71.8%), with a mean BMI of 30.51 kg/m2. The overall average eGFR was 21.51; CKD patients had an eGFR of 23.84 vs. 17.49 in the dialysis group. There was no signficant increase in Apnea-hypopnea index (AHI) from pre to post (18.56 vs. 19.30; P=0.86). However, those who remained off dialysis at follow-up had a decrease in AHI (20.566 vs. 14.78) while those who started dialysis demonstrated an increase in AHI (14.76 vs. 27.94) (P=0.03). The vast majority of apneic events were obstructive in both groups. There was no significant difference in nocturnal hypoxemia as measured by the amount of time with oxygen saturation less than 90% while sleeping (pre 6.03 vs. post 6.67; p=0.16)




Conclusions: Sleep apnea is prevalent in the CKD population and significantly worsens with the initiation of dialysis. However, there still needs to be more exploration into this field to understand the factors connecting CKD to obstructive sleep apnea.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2628**

**Significance of Circadian Chronotype on Sleep in HD Patients** Tushar Sharma,[1] Daniel Hahn,[1] Kelly Glazer Baron,[2] James J. Paparello,[1] Vincent L. Yang,[1] Shyam J. Uttamchandani,[1] William A. Schlueter,[1] Lisa Wolfe,[1] Shubha Ahya.[1] *[1]Medicine, Northwestern University, Chicago, IL; [2]Neurology, Northwestern University, Chicago, IL.*

PURPOSE: 1-evaluate the prevalence of sleep disturbances in an outpatient HD population. 2-identify a relationship between circadian chronotype (morning type vs evening type) and dialysis adherence.

METHODS: 72 patients from 6 dialysis shifts (12 per shift) completed 8 surveys to assess for circadian preference (Horne Ostberg), sleep quality (PSQI), daytime sleepiness (ESS), restless legs syndrome, depression, sleep apnea syndrome (OSA STOP), barriers to care, and affect of sleep on daytime functioning (modified FOSQ). Adherence was measured by a quantitative and qualitative assessment of missed dialysis treatment sessions in each dialysis shift over the past 1 year.

RESULTS: RLS symptoms were noted in 47.2%. The mean score on the PSQI was 7.68 and 61% of the patients had a score of >5, indicating poor sleep quality. Excessive daytime sleepiness was found in 45% of the patients. Patients were identified as morning type (43%) or neither type (54%). Only 2 patients were evening type. Neither types had shorter sleep duration (p=0.013), longer sleep latency (p=0.000), greater daytime dysfunction (p=0.006), overall higher scores on the PSQI (p=0.002), and more barriers to treatment (p=0.013). There was a trend for higher rates of nonadherence with neither types vs morning types (p=0.091). When biochemical parameters were analyzed, only PTH was found to be higher in the neither types vs morning types (672 vs 297, p=0.048). Morning types were older (63 vs 55, p=0.03) and had less years on dialysis (3.1 vs 5.6 years, p=0.01). When regression analysis was performed, Horne Ostberg scores were significant predictors of barriers to care (p=0.001) and sleep quality (p=0.002). Dialysis nonadherence correlated with transportation barriers (r= .29 p.024), barriers total(r=.29 p=.023), more time on HD (r=-.27 p=.024) and PTH (r=-.28, p=.023).

CONCLUSION: Morning types have better sleep quality,overall functioning and are on dialysis for a shorter time. Dialysis vintage and transportation may be reasons for dialysis nonadherence.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2629**

**Difficulties in Sexual Arousal, Enjoyment and Perceived Sex Life among New Dialysis Patients: A National Study** Austin G. Stack,[1] Catherine A. Wall,[2] Kieran Hannan.[3] *[1]Regional Kidney Centre, Letterkenny General Hospital, Letterkenny, Donegal, Ireland; [2]Department of Nephrology, Adelaide and Meath Hospitals, Dublin, Ireland; [3]Department of Nephrology, Cavan General Hospital, Cavan, Ireland.*

Sexual dysfunction is thought to be common in patients with advanced chronic kidney disease. The aim of this study was to describe and measure the impact of factors associated with sexual arousal, enjoyment and overall sex life in a national cohort.

Methods: Data were obtained on a subset of patients (n=2,480; 62 %) from the Dialysis Morbidity and Mortality Study Wave 2, an incident dialysis cohort study. A validated quality of life instrument (KDQOL-SF) was used to assess patient's attitudes to kidney disease at the start of dialysis and its impact on quality of life. Multivariable logistic regression was used to determine associations (Odds Ratio (OR)) of demographic, clinical and laboratory factors with difficulty in sexual arousement and sexual enjoyment.

Results: Overall, 39% of new dialysis patients reported moderate to severe impact on their sex life; 44% reported moderate to severe difficulties in becomming sexually aroused and enjoying sex. The likelihood of reporting moderate-severe difficulties in relaxing & enjoying sex were significantly greater for younger patients (Age18-50, OR=1.60; Age 50-70, OR=1.62; Age > 70, OR=1.00), males (OR=2.71), married couples (OR=2.59), those reporting negative affect (OR=2.40), receiving prescribed antidepressants (OR=1.27), and treated with PD (OR=1.21). Conversely, the likelihood of reporting difficulties were significantly lower for patients who exhibited positive affect (OR=0.53), exercised regularly (OR=0.64), were in full or part-time employment (OR=0.80 and 0.79), and who were dialyzing with a permanent vascular access (OR=0.79). Surprisingly, none of the co-existing cardiovascular or non-cardiovascular comorbidities recorded at dialysis initiation were significant.

Conclusions: Difficulties in relaxing and enjoying sex are common among new dialysis patients and negatively impact on sex life. Further studies should address in-depth the barriers to sexual enjoyment in this patient population in order to improve overall quality of life.

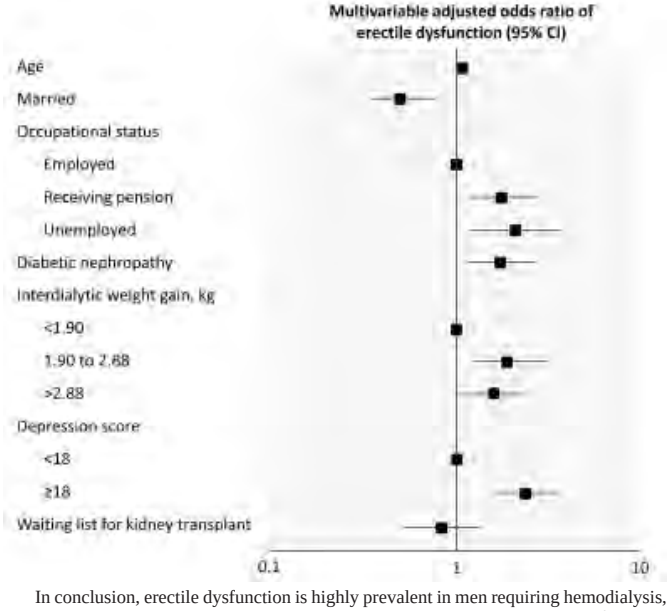
Disclosure of Financial Relationships: nothing to disclose

**SA-PO2630**

**The Prevalence and Correlates of Erectile Dysfunction in 946 Men Receiving Hemodialysis: A Multi-Center Cross-Sectional Study** Mariacristina Vecchio,[1] Suetonia Palmer,[2] Giorgia De Berardis,[1] Fabio Pellegrini,[1] David W. Johnson,[3] Jonathan C. Craig,[4] Giovanni F. M. Strippoli.[1,4,5] *[1]Consorzio Mario Negri Sud; [2]University of Otago Christchurch; [3]University of Queensland; [4]University of Sydney; [5]Diaverum Medical Scientific Office.*

Existing studies suggest erectile dysfunction is common in men receiving hemodialysis but have limited by study size and methods problems. In this multinational cross-sectional study, we enrolled men receiving hemodialysis in 27 outpatient clinics selected randomly from a collaborative dialysis network. Erectile function was assessed anonymously by the International Index of Erectile Function (IIEF) questionnaire. Depressive symptoms were evaluated with the Center for Epidemiological Studies Depression (CES-D) Scale. Multivariable logistic regression was used to determine correlates of erectile dysfunction adjusting for clinical and sociodemographic characteristics.

Overall, 946 (59%) of 1611 eligible men responded. Of these, 781 (83%) men reported erectile dysfunction and 445 (57%) reported severe erectile dysfunction. Only 33 (4%) men with erectile dysfunction reported receiving specific treatment. Depression (CES-D score ≥18) was the strongest correlate of erectile dysfunction whereas being married reduced the risk. Erectile dysfunction was also independently correlated with age, unemployment, diabetic nephropathy and interdialytic weight gain (**Figure**). Diabetic nephropathy nearly trebled the risk of severe erectile dysfunction (adjusted odds ratio (AOR) 2.54, CI 1.61-4.02)).




In conclusion, erectile dysfunction is highly prevalent in men requiring hemodialysis, and is rarely treated. Depression and unemployment are important correlates of erectile dysfunction in this population.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2631**

**The Seasonal Variations of Uremic Pruritus Prevalence in Patients with Hemodialysis** Jwa-Kyung Kim,[1] Sung Jin Moon,[2] Hyeong Cheon Park,[2] Sung-Kyu Ha,[2] Tae-Hyun Yoo,[1] Shin-Wook Kang,[1] Ho Yung Lee,[1] Sang Choel Lee,[3] Soo Young Yoon,[3] Jung Eun Lee,[4] Hyung-Jong Kim,[5] Dongho Yang.[5] *[1]Severance Hospital; [2]Gangnam Severance Hospital; [3]Kwandong University; [4]Yongin Severance Hospital; [5]Bundang CHA Hospital.*

Uremic pruritus is one of the most common and potentially disabling symptoms in patients with ESRD. Although uremia is regarded as the main cause of pruritus, the precise diagnostic methods of the condition and its treatment remains unknown. Moreover, the seasonal variation of uremic pruritus is not evaluated in patient with hemodialysis. A self-

administered questionnaire was constructed for the evaluation of uremic pruritus. Patients receiving maintenance hemodialysis therapy in 5 centers completed this survey with an interval of 6 months (at February 2009, August 2009). To assess the intensity of pruritus, each subject was asked to check a Visual Analogue Scale (VAS) and the score was graded from 0 to 5; 0 (none), 1,2,3,4 and 5 (very severe). The number of uremic patients who agreed to the questionnaire-based survey during the winter (February 2009) and summer (August 2009) was 297 and 336, respectively and 230 responders completed both of the questionnaires. The mean age of the 230 patients was 59.0±12.5 years and mean dialysis duration was 35±8.5 months. The prevalence rate of uremic pruritus in winter was 65.6% (n=150) and 48.3% (n=110) in summer. Although the prevalence rate showed decreasing tendency in summer, however, there was no significant seasonal variation in uremic pruritus (p=0.13). Whereas, the prevalence rate ranged from 16.7 to 83.3% among the five centers and this inter-hospital difference was statistically significant (p=0.03). Among the 80 patients who did not have pruritus in the winter, 27.6% of the patients (n=22) developed new itching sensation during the summer. Conversely, in the 34.4% of the patients who experienced pruritus in the winter (n=51), the pruritic symptom was disappeared during the summer season. This data suggest that there's no seasonal variation of uremic pruritus in maintenance hemodialyis patients. Approximately 30% of patients showed seasonal difference in pruritic symptom.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2632

**Levels of Indican and Restless Legs Syndrome in Hemodialysis Patients: The Retained Organic Solutes and Clinical Outcomes in Hemodialyis (ROSCO) Study** Laura C. Plantinga,[1] Michal L. Melamed,[2] Thomas H. Hostetter,[2] Timothy W. Meyer,[3] Tariq Shafi,[4] Nancy E. Fink,[4] Neil R. Powe.[1] *[1]University of California, San Francisco, CA; [2]Albert Einstein College of Medicine, Bronx, NY; [3]Stanford University, Palo Alto, CA; [4]Johns Hopkins University, Baltimore, MD.*

Restless legs syndrome (RLS) is a common symptom among hemodialysis (HD) patients. Indoxyl sulfate (indican), which accumulates in ESRD patients and is not removed entirely by HD, can influence central nervous system function and has been proposed to contribute to RLS. We examined the association of self-reported RLS in a U.S. cohort of 459 incident HD patients (enrolled 1995-1998) with indican levels measured by high-performance liquid chromatography in frozen plasma samples collected ~6 months after dialysis initiation. RLS was defined as patient report of being very to extremely bothered by RLS at baseline (BL) and/or 1 year. Logistic and GEE regression were used to calculate odds ratios for RLS at BL and longitudinally. At baseline, 14.4% of those with indican levels less than the median (1.6 mg/dl) and 17.8% of those with levels above the median (P=0.316) reported having RLS. With adjustment for age, sex, race, body mass index, and creatinine, those with indican levels above the median were more likely to report RLS [OR=1.53 (1.07-2.21)]. Stratified analyses revealed no significant interaction by diabetes, gastrointestinal (GI) disease, or albumin or creatinine level, although the positive associations seen in subgroups with diabetes and with no GI disease were statistically significant. Results were similar but less significant among the 358 patients with information on RLS at 1 year; change in RLS from BL to 1 year was associated with indican level [OR=1.35 (0.84-2.15)] but not statistically significantly. Severe RLS symptoms may be associated with high levels of indican, an organic solute that is retained in HD.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2633

**The Prevalence of Uremic Neuropathy in a Contemporary Hemodialysis Cohort** Meg J. Jardine,[1 2] Arun V. Krishnan,[3] Martin P. Gallagher,[1 2] Paul Snelling,[1] Ria Arnold,[3] Carmel M. Hawley,[4] Bruce A. Pussell,[5] Vlado Perkovic,[2] Michael G. Suranyi,[1] Matthew C. Kiernan,[3] Josette M. Eris.[1] *[1]Sydney South West Area Health Service; [2]The George Institute for International Health; [3]Institute of Neurological Sciences, University of New South Wales; [4]Princess Alexandra Hospital; [5]Prince of Wales Hospital.*

**Aim:** To establish the prevalence of neuropathy in a contemporary hemodialysis population enrolled in the FINESSE trial.

**Introduction:** People with End Stage Kidney Disease (ESKD) experience greater weakness, less muscle mass, diminished physical activity and lower physical quality of life scores than controls. They are susceptible to neuropathies, including uremic and diabetic neuropathies, causing symptoms of paresthesia, pain, weakness and muscle wasting. The FINESSE trial (Filtration In the Neuropathy of End Stage kidney disease Symptom Evolution) is a randomized controlled trial evaluating the effect of hemodiafiltration compared with standard hemodialysis.

**Methods:** The presence of neuropathy was assessed in a cohort of stable maintenance hemodialysis patients. A standardized neuropathy instrument, the Total Neuropathy Score combining symptoms, signs and nerve conduction components, was measured prior to randomization.

**Results:** Baseline assessment was completed by 58 participants. Comorbidities included diabetes (38%), ischaemic heart disease (27%), and any vascular disease (35%). Overall evidence of neuropathy was found in 81% of participants, including symptomatic neuropathy in 35%. Neuropathy was present in 95% of the participants with diabetes, and in 71% of participants without diabetes.

**Conclusions:** UN remains highly prevalent among contemporary hemodialysis patients. The neuropathy burden may increase with the greater prevalence of diabetes and longer durations spent on dialysis associated with lengthening transplant waiting periods. FINESSE will provide important information on UN and will contribute substantially to high level evidence on the benefits and harms of HDF therapy. (Trial Registration: ACTRN12609000615280)

Disclosure of Financial Relationships: Employer: The George Institute for International Health

Sydney South West Area Health ServiceResearch Funding: My employer has received an unrestricted grant from CSL Biotherapies.

### SA-PO2634

**Pain Is Underrecognized and Undertreated in Hemodialysis Patients** Monica C. Beaulieu,[1,2] Marianna S. Leung,[1] Lee Er,[2] Fong Hue Huynh,[1] Clifford Chan Yan,[1] Ronald Werb,[1] Beverly Jung,[1] Mercedeh Kiaii.[1] *[1]Division of Nephrology, UBC, Vancouver, BC, Canada; [2]BC Provincial Renal Agency, Vancouver, BC, Canada.*

Effective pain management improves patient's quality of life and is an integral component of patient care. Pain is commonly experienced by hemodialysis (HD) patients but is often underrecognized and undertreated.

The aim of this study was to (1) describe the prevalence, severity, and management of pain in HD patients (2) determine if there are factors that differentiate those with and without pain.

We performed a prospective cohort study, surveying patients at an in-centre urban dialysis unit. All patients in the HD unit were approached to participate. Patients who reported pain were interviewed using Short-Form Brief Pain Inventory (SF-BPI) and Short Form McGill Pain Questionnaire (SF-MPQ)

161/233 patients participated in the study (63% male, 40% caucasian, 29% asain). 34% (57/161) of patients reported pain. Patients with pain had slightly longer HD duration (40.6 vs 27.2 mos; p=0.14) and more cardiovascular disease (57% vs 41%; p=0.02).

Of the 54 patients with pain on the SF-BPI, 46.3% rated their pain at it's worse as severe (pain score ≥ 7). The most common descriptors of pain were aching and tiring-exhausting.

30% of patients had no treatment presecribed for their pain. Of the 70% (38/54) who were treated, 82% received pharmacologic treatment, 18% non-pharmacologic. Of treated patients, 33% reported that their pain was not relieved effectively.

In conclusion, in a cohort of HD patients, 34% reported pain. Of patients with pain, 30% are untreated and a large number of treated patients still report pain. Practitioners caring for HD patients should have a systematic program for pain assessment and management in their units.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2635

**Regimen Complexity, Burden of Therapy and Prescription Adherence in Dialysis** Luca Neri,[1, 2] Maurizio Gallieni,[4] Alma Martini,[4] Lisa Allegra Rocca Rey,[5] Diego Brancaccio.[3] *[1]Dipartimento di Medicine del Lavoro e Biostatistica, Universita' di Milano, Milano, Italy; [2]Center for Outcomes Research, Saint Louis University, St. Louis, MO; [3]Fondazione Italiana del Rene [Italian Kidney Foundation], Milano, Italy; [4]U.O. Nefrologia, Ospedale San Carlo Borromeo, Milano, Italy; [5]U.O. Nefrologia, Multimedica Holding, Castellanza - Varese, Italy.*

**Background and Aims.** Poor prescription adherence is common in dialysis patients and may cause sub-optimal outcomes and increased health care costs. We evaluated the association between regimen complexity, perceived burden of therapy and prescription adherence in hemodiaysis (HD).

**Methods.** We enrolled 1238 HD patients in 54 Italian centers. We collected data on patients' socio-demographic characteristics, perceived burden of therapy (BOT), quality of life, health care satisfaction, social support and prescription adherence with a self-administered questionnaire. Data on medication regimen, comorbidities, hospitalizations, transplant listing status were provided by the nursing staff. We estimated the adjusted association of regimen complexity, BOT and prescription adherence with logistic regression.

**Results.** Sample characteristics are described in table 1. Mean daily burden was 9.7 tablets and 48% of patients were adherent to medication prescriptions. Every tablet added to the medication regimen was associated to a 10% decrease in adherence likelihood after adjustment for possible confounders. Perceived burden of therapy moderated the association between tablet count and self-reported adherence (Interaction term, OR: 1.04, p<0.01; figure 1).

**Conclusion.** Poor adherence was very common in our sample. Reducing tablet burden might help patients be adherent. However our results suggest that modulating regimen complexity might be ineffective if patients' negative attitudes toward medications are not addressed concurrently.

| Characteristics | N (%) or mean (SD) | Characteristics | N (%) or mean (SD) |
|---|---|---|---|
| SRH | 5.9 (1.8) | Dialysis Vintage | |
| IIRS | 35.5 (14.3) | <1 years | 259 (20.7) |
| BOT | 2.3 (0.5) | 1-5 years | 541 (43.8) |
| Women | 449 (36.3) | >5 years | 446 (35.6) |
| Mean Age | 64.4 (16.8) | Hypertension | 736 (69.6) |
| > High School | 367 (29.9) | Hypotension | 128 (10.3) |
| E/P ratio | 147 (34.3) | CVD | 638 (51.5) |
| Disabled | 1052 (85.0) | Diabetes | 262 (21.2) |

Table 1. Subjects characteristic of the MgilorDialds sample. Legend: BOT, perceived Burden of Oral Therapy; E/P ratio: ratio between employed patients and working age individuals (legal retirement age is 60 years in Italy); CVD: cardiovascular disease. Hypotension refers to history of recurrent episodes of post-dialysis hypotension.

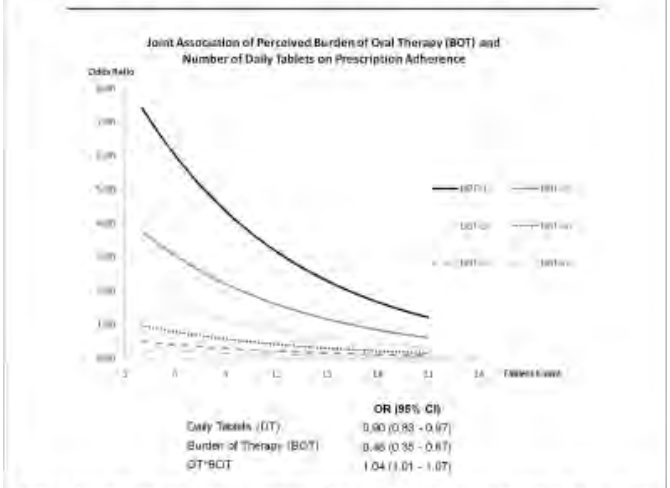



**Figure 1. Association of oral regimen characteristics and self reported adherence.** Multivariable logistic regression analysis evaluating correlates of regimen adherence among hemodialysis patients. Burden of therapy refers to self reported interference of oral therapy in daily life (6 items; ratings occurred along a 5-point Likert scale). Odds ratios refer to the marginal conditional likelihood associated to 1 unit increase in the independent variable. Analysis also adjusted for number of hospitalizations in the 12 months prior to enrollment, number of comorbidities, disability status, dialysis vintage, age, gender, employment status, education, support from health care providers, illness intrusiveness rating scale, sleep quality scale, transplant listing status, region of residence, living arrangement (living alone/nursing home vs. living with family members)

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2636**

**Hemodialysis Patients May Overestimate Their Ability To Manage Complex Medical Regimens** James B. Post, Kel G. Morin, Ann M. Spungen. *Research & Development, James J. Peters VAMC, Bronx, NY.*

Despite a high prevalence of cerebrovascular disease in hemodialysis (HD) patients, they are expected to adhere to complex treatment regimens in order to avoid adverse complications. Very little is known about the HD patient's perceived functional ability and whether or not it correlates with actual functional performance. The purpose of our study was to compare subjective and observed measures of independent activities of daily living (IADLs) in HD patients with no clinical evidence of dementia to appropriate controls matched for age, BMI, diabetes, hypertension, and hyperlipidemia. In 27 HD and 16 controls, the MMSE, Lawton IADL test (subjective), and Hopkins Medication Schedule (HMS) test (observed) were performed to compare the relationship between subjective and observed measures of IADLs in each group. Lawton-HMS differential scores were calculated by subtracting HMS scores from Lawton scores. Group comparisons were made using t-tests and chi-squared tests as appropriate. There were no significant differences between HD and controls in age 61±10 vs. 65±11 y, years of education, ethnicity, BMI, hypertension, diabetes, hyperlipidemia, or MMSE (29±1 vs. 29±1). HD patients scored significantly lower on IADL assessments vs. controls: Lawton 15±2 vs. 16±1 (p=.02), HMS 9±3 vs. 11±1 (p=.002). HD patients scored higher than controls, trending toward significance, on the Lawton-HMS differential score 6±3 vs. 4±1 (p=.07), suggesting that there may be a larger discrepancy between perceived and actual functional abilities in patients on HD. HD patients with no clinical or objective evidence of dementia demonstrate that they may overestimate their ability to adhere to and understand the complex medical regimens required of HD patients.

Disclosure of Financial Relationships: nothing to disclose

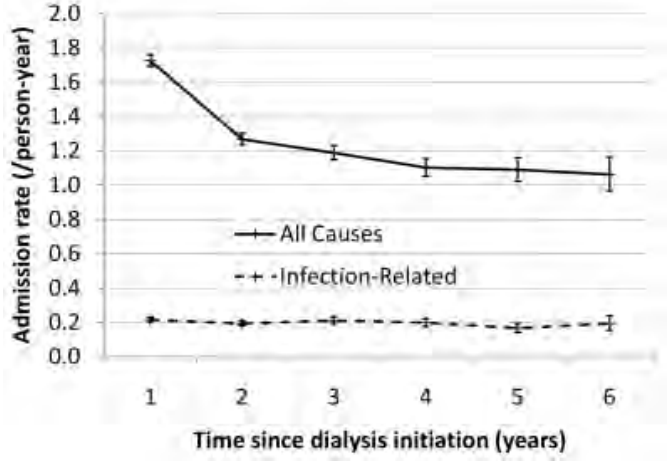
**SA-PO2637**

**Overall and Infection-Related Hospitalization Rate Trends in Dialysis Accounting for Dialysis Vintage** Jean-Philippe Lafrance,[1] Elham Rahme,[2] Sameena Z. Iqbal,[2] Michel Vallee.[1] *[1]Centre de recherche Hopital Maisonneuve-Rosemont, QC, Canada; [2]McGill University, QC, Canada.*

Chronic dialysis is associated with a high burden of morbidity requiring frequent hospital admissions. Trends of all cause and infection-related admissions have not been evaluated in newly dialyzed patients. We aimed at describing population-based incidence rate trends of all cause and infection-related hospitalizations in dialysis patients.

Using administrative databases from the universal health care system in Quebec, Canada (Regie de l'assurance maladie du Quebec), we built a retrospective cohort of all adults initiating chronic dialysis between 2001 and 2007. Patients with a prior history of kidney transplant or with <90d of follow-up were excluded. Hospitalizations during which a dialysis was initiated were not counted. Only patients in their 1st year of dialysis were included in the estimation of incidence rates per calendar years.

6567 patients (mean age=64.4±14.5 y, and 40% females) were included in the cohort. The mean follow-up was 2.38±1.7 y. In total 21,846 hospitalizations occurred and 86% of patients had ≥1 admission. All cause and infection-related hospitalizations remained stable between 2001 and 2006, (respectively: 1.81 vs.1.66 /p-y, P=0.11; and 0.23 to 0.21, P=0.55). Rates of all cause hospitalizations were higher in the 1st year following dialysis initiation (1.73 vs. 1.06, P<0.001), possibly explained by a survivor effect. However, infection-related rates remained stable over the follow-up period (0.22 to 0.19, P=0.09).




Overall hospitalization rates declined with dialysis vintage, which should be accounted for when estimating trends over calendar years in prevalent dialysis populations (if proportions of incident patients vary with time). Since the infection-related rates did not decline, the proportion of admissions due to infections increased with time on dialysis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2638**

**P-Cresol Sulfate Level and Infection-Related Hospitalizations in Hemodialysis Patients: The Retained Organic Solutes and Clinical Outcomes in Hemodialyis (ROSCO) Study** Neil R. Powe,[1] Laura C. Plantinga,[1] Thomas H. Hostetter,[2] Michal L. Melamed,[2] Bernard G. Jaar,[3] Tariq Shafi,[3] Nancy E. Fink,[3] Timothy W. Meyer.[4] *[1]University of California, San Francisco, CA; [2]Albert Einstein College of Medicine, Bronx, NY; [3]Johns Hopkins University, Baltimore, MD; [4]Stanford University, Palo Alto, CA.*

P-cresol sulfate (PCS), an organic solute formed by the action of colonic bacteria on the amino acids tyrosine and phenylalanine, accumulates in uremic patients and is poorly removed by conventional dialysis. Recent studies have identified relations between increased levels of PCS and adverse clinical outcomes in hemodialysis (HD) patients and between PCS and decreased leukocyte transendothelial migration *in vitro*. We examined the association of PCS with infection-related hospitalizations (IH) in a U.S. cohort of 518 incident HD patients enrolled in 1995-1998 and followed for an average of 3.4 years. PCS levels were measured by high-performance liquid chromatography in frozen plasma samples collected ~6 months after dialysis initiation. We linked USRDS Medicare billing records to ascertain IH over follow-up. We used Poisson regression to calculate incidence rate ratios (IRRs) for IH. The incidence of IHs per 1000 patient-years was 180.7 in the lowest tertile, 277.9 in the middle tertile and 256.2 in the highest tertile of PCS level. After adjustment for age, sex, race, body mass index, comorbid disease, albumin and creatinine, compared to patients with PCS levels in the lowest tertile, those with levels in the middle and highest tertiles had a 40-60% higher risk of IH [IRR=1.42 (1.09-1.86) and 1.60 (1.21-2.12), respectively. Stratified analyses suggested an interaction by presence of GI disease, with the relation only being present in those without any GI disease. Higher levels of PCS, an organic solute that is retained in patients undergoing HD, are independently associated with infection-related hospitalizations. Methods for removal of PCS and the effect on outcomes should be investigated further.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2639**

**Prevalence of Nephrogenic Systemic Fibrosis in Dialysis Patients: The Pro-FINEST Study** Sabine Amet,[1] Vincent Launay-Vacher,[1] Benedicte Stengel,[2] Anne Castot,[3] Camille Frances,[4] Nicolas Grenier,[5] Jean-Yves Gauvrit,[6] Genevieve M. Reinhardt,[7] Olivier Clement,[8] Nicolas Janus,[1] Carmen Kreft-Jais,[3] Gabriel Choukroun,[9] Maurice Laville,[10] Gilbert Deray.[1] *[1]Service ICAR, Nephrology, Pitie-Salpetriere Hospital, Paris, France; [2]Paul Brousse Hospital, Villejuif, France; [3]Afssaps, Saint-Denis, France; [4]Tenon Hospital, Paris, France; [5]Pellegrin Hospital, Bordeaux, France; [6]Pontchaillou Hospital, Rennes, France; [7]Haguenau Hospital, Haguenau, France; [8]HEGP Hospital, Paris, France; [9]South Hospital, Amiens, France; [10]Edouard Herriot Hospital, Lyon, France.*

NSF is a cutaneous and systemic disorder characterized by widespread tissue fibrosis. It has been suggested that gadolinium-based contrast agents (GBCA) may be responsible for NSF, especially in dialysis patients. The Pro-FINEST study is a national prospective study endorsed by the French Drug Agency (Afssaps), and the French Societies of Nephrology, Dermatology, and Radiology. It aims at determining the prevalence of NSF after a Magnetic Resonance Imaging (MRI) examination, +/- GBCA, in dialysis patients.

The study is based on a 3-section patient form. Section 1: demographics and dialysis; Section 2: MRI examination; Section 3: any dermatological event (DE). Further investigations are planned in case of DE. When a NSF diagnosis is confirmed, an ancillary study is to be performed, with random selection of 4 patients (same gender, dialysis technique, centre, and without any DE after MRI with the same GBCA, if injected).

Since 01/2009, 297 patients have been included (247 centres): mean age 64.1 years, 60.3% men 55.5% of the patients received GBCA; 87.7% Gadoterate. 5 patients reported DE although no NSF (1 hemodialysis-related prurit, 1 localized skin reaction to a trinitrine patch, 1 thoracic zona, 1 hardening feet skin linked to vascular lesions and no clear dermatological diagnosis in the fifth case but no evidence of NSF.

So far thus, no case of NSF has been reported in 195 dialysis patients among whom the majority received a GBCA. Most patients received a macrocyclic gadolinium chelate for which no case of NSF has been observed worldwide (Gadoterate).

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2640**

**Characteristics of and Outcomes for Patients Recovering from ESRD** Michael Heung, Flannery Campbell, John Kalbfleisch, T. H. Shearon, V. B. Ashby, Rajiv Saran. *Univ of Michigan.*

By definition, ESRD certification implies an "irreversible and permanent" state, yet many patients recover each year. The ability to identify such patients and to understand their prognosis following recovery is of major clinical significance.

ESRD recovery events were identified by a specific code in the Centers for Medicare and Medicaid Services (CMS) ESRD database (2002-2008). Multivariate logistic regression identified patient characteristics associated with renal recovery and Cox regression was used to assess survival up to 5 years following the recovery event compared to non-recovering ESRD and transplant patients.

During the study period, 37,482 patients recovered from ESRD among 744,143 patients starting ESRD. The incidence of recovery in the first year after starting dialysis rose throughout the study period, from 3.9% in 2002 to 5.6% in 2007 ($p<0.001$). Recovery occurred within 3 months in 56% of patients, and within 1 year for 90%. African-Americans were less likely to recover than Caucasians (OR 0.53, $p<0.05$). Patients with ATN and scleroderma as the primary cause of ESRD were more likely to recover than patients with a primary diagnosis of DM (OR 15.4 and 4.7 respectively, $p<0.05$ for both). Most (73%) patients remained recovered at 1 year, and 25% were still dialysis-independent after 5 years. Compared to patients recovering renal function, patients remaining on dialysis had worse long-term mortality (HR 2.15, $p<0.001$) while transplanted patients had a lower mortality (HR 0.30, $p<0.001$).

An increasing percentage of patients recover from ESRD each year. We identified characteristics that may be predictive of renal recovery, and these factors may aid in the counseling of patients starting dialysis. Interestingly, even causes that are considered irreversible (such as DM) showed evidence of recovery, highlighting superimposed acute kidney injury as a likely unrecognized factor in CKD progression. A majority of recovering patients remained dialysis-independent at 1 year following recovery, but these patients need to be followed closely as ~10% return to dialysis each year. Recovery of renal function is associated with a significantly better survival than remaining on dialysis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2641**

**A Randomized Controlled Trial To Compare the Safety and Efficacy of Oral Paricalcitol with Calcitriol in Dialysis Patients with Secondary Hyperparathyroidism** L. M. Ong, N. Punitha, H. K. Goh, A. B. Manocha, Ghazali T. V. Ahmad, M. Sukeri, B. L. Goh, S. Shahnaz, M. R. Seman, V. Indralingam. *MOH, Malaysia.*

There have been no studies comparing oral paricalcitol (OP) with oral calcitriol (OC) in treatment of secondary hyperparathyroidism (SH) in dialysis patients (DP).

**Aim**

To compare the efficacy and safety of OP with OC in DP with SH.

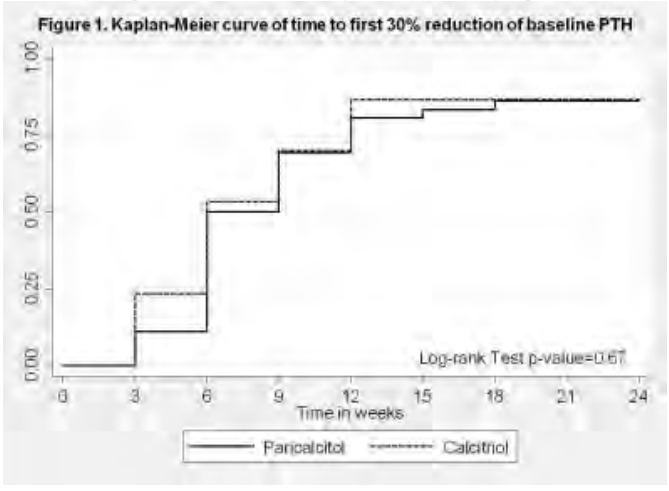
**Method**

We conducted a multicentre, open-labeled, parallel group RCT over 24 weeks. The initial thrice weekly dose of OP (PTH/120µg) and OC (PTH/360µg) was titrated 3-weekly to serum intact parathyroid hormone (PTH), calcium (Ca) & calcium-phosphate product (CaP). The primary end-point was time to ≥30% reduction from baseline PTH.

**Results**

36 patients were randomized to OP and 30 to OC. Baseline characteristics were similar between groups.

Primary end-point (PEP) did not differ significantly between groups.




86.1% (OP) vs 86.7% (OC) achieved PEP by 24 weeks. The mean time (weeks) to first event was 7.7 (OP) vs 6.9 (OC) p=0.4. The mean time (weeks) to maximal reduction in PTH (MRP) was 16.1 (OP) vs 15.5 (OC) p=0.7.The mean % of MRP from baseline was 68.7% (OP) vs 72.4% (OC) p=0.3. There were no significant differences in bone mineral parameters except for phosphate (P) & CaP at week 12.

Table 1. Mean change from baseline

| | Week | OP | OC |
|---|---|---|---|
| PTH (%) | 12 | -42.1 | -52.7 |
| | 24 | -44.2 | -40.7 |
| Ca (mg/dl) | 12 | 0.69 | 0.87 |
| | 24 | 0.78 | 0.77 |
| P (mg/dl) | 12 | -0.38 | 0.46* |
| | 24 | -0.04 | 0.8 |
| CaP (mg2/dl2) | 12 | -0.22 | 9.22* |
| | 24 | 3.94 | 11.9 |
| ALP (U/l) | 12 | -36.1 | -50.1 |
| | 24 | -51.1 | -89.5 |

*$p<0.05$

Drug compliance was comparable. 23 episodes of hypercalcemia were seen in 6 patients (17%) in OP & 16 episodes in 6 patients (20%) in OC group p=0.7. Other adverse events were similar in both groups.

**Conclusion**

OP has similar efficacy and safety compared with OC in dialysis patients with SH. There was no difference in the incidence of hypercalcemia.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2642**

**Exposure to Ionizing Radiation in End Stage Kidney Disease (ESKD)** Sinead Kinsella,[1] Joe P. Coyle,[2] Siobhan McCarthy,[3] Sebastian R. McWilliams,[3] Michael Clarkson,[1] Michael M. Maher,[2] Joseph A. Eustace.[1] *[1]Renal Medicine, Cork University Hospital, Cork, Ireland; [2]Radiology, Cork University Hospital, Cork, Ireland; [3]University College Cork, Cork, Ireland.*

Introduction: Exposure to ionizing radiation is associated with an increased risk of malignancy, as is ESKD.

Methods: We conducted a retrospective study of 394 patients (244 on maintenance haemodialysis (HD) and 150 with a functioning renal transplant) to quantify and compare ionizing radiation exposure from medical procedures in these populations. Patient demographics and comorbidities were obtained from medical record review. The number and type of radiological procedures were obtained from a computerised radiology database. Cumulative effective radiation dose (CED) expressed in milliSieverts (mSv) was calculated using published reference effective doses for diagnostic imaging studies.

Results: Patients were followed for a median (Intraquartile Range [IQR]) of 4 (1.7 to 6) years, data from 1513 patient years was included. Mean (sd) age at study entry was 52.7 (16.8) years. During the study period a total of 7311 radiological procedures were performed exposing the study population to an estimated total of 10548mSv of ionizing radiation. Computed tomography accounted for 61% of total CED while accounting for only 9% of the total number of radiological procedures performed. The median (IQR) CED per patient was 9.2 (1 to 33) mSv and was significantly higher in HD patients compared to the transplant group (15.1 Vs 2.9 mSv, $p<0.001$). On univariate logistic regression analysis HD patients had a significantly increased Odds Ratio (OR) (95% CI) of exposure to >50mSv of ionizing radiation compared to the transplant group (OR 3.5 (1.8-6.8)). Following simultaneous adjustment for age, duration of follow-up, cause of ESKD, and recorded comorbidities, HD remained significantly and independently associated with radiation exposure >50mSv.(OR 9.5, (95% CI 3.8-22.6)).

Conclusion: Patients with ESKD undergo repeated and frequent radiological procedures exposing them to substantial cumulative doses of radiation. HD patients in particular are at increased risk of exposure to high doses of ionizing radiation from medical procedures.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2643

**Risk Factors and Prevalence of Calciphylaxis in Japanese Hemodialysis Population; a Case-Control Study by Japanese Calciphylaxis Study Group** Matsuhiko Hayashi, Yoshihiko Kanno, Tadashi Yoshida. *Apheresis and Dialysis Center, University of Keio, School of Medicine, Shinjuku-ku, Tokyo, Japan.*

Calciphylaxis, also called calcific uremic arteriolopathy, is a rare and often fatal complication of end-stage renal disease, characterized by cutaneous ischemia and ulcer with medial calcification and intimal proliferation of small arteries. Previous studies reported its incidence ranging from 1 to 5 % of the patients on chronic hemodialysis. To reveal the characteristics of calciphylaxis in Japanese dialysis population, we conducted a nationwide surveillance and a case-control study. Firstly, we sent a questionnaire to 3760 hemodialysis centers in Japan, asking whether calciphylaxis cases were encountered in the past decade, and then the detailed clinical data with skin biopsy specimens were collected. Furthermore, two control dialysis patients matched to age and duration of hemodialysis were identified from the participated centers for each calciphylaxis case. The results of surveillance showed that there were 151 cases in the past 10 years and suggested that prevalence rate is lower than 2/10000 hemodialysis patients per year. Sixty-seven detailed clinical data were collected and 28 cases were diagnosed as definite cases by clinical characteristics and skin biopsy findings. In comparison with 56 matched controls, a logistic regression model identified that warfarin administration (odds ratio [OR] 10.8, 95% confidence interval [CI] 2.90 to 40.5, p<0.001), each 1 g/dl decrease in serum albumin (OR 19.7, 95% CI 4.36 to 89.5, p<0.05), and each 1 mg/dl increase in adjusted serum calcium level (OR 3.21, 95% CI 1.63 to 6.30, p<0.05) at the time of diagnosis were associated with calciphylaxis, while association of female gender, vitamin D analogue, serum phosphate level, adjusted calcium-phosphate products, and serum alkaline-phosphatase were not significant. We conclude that prevalence of calciphylaxis is much lower in Japan than in previous reports, suggesting that incidence of calciphylaxis may be different by races and geographic factors, and that warfarin therapy, lower serum albumin, and higher serum calcium level are risk factors for the development of calciphylaxis.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2644

**Employment in Inner City Hemodialysis Patients: A Study of the Interplay of Socio-Demographic and Health Related Factors** Adeyinka A. Adegoroye, Ikechukwu O. Nwobu, Ayoola Adekile, Moro O. Salifu. *Depatment of Medicine, SUNY Downstate Medical Center, Brooklyn, NY.*

Introduction:

Employment status of hemodialysis (HD) patients is an index of a rehabilitated patient with a chronic illness. Many factors including co-morbidities, physical deconditioning, emotional/psychological problems, quality of life, educational status, occupational history and US legal resident status may impact the employment status of HD patients.

Methods:

Cross sectional study of patients aged 50 and below in two outpatient dialysis centers. Data was collected using a self-administered questionnaire which included the Beck depression inventory-ll (BD-II), a depression screening tool and the Short form 36 health survey (SF-36), a quality of life evaluation tool.

Results:

65 HD patients participated in the study, of whom 37 (57%) were males. 60 (92.3%) were African or African American, 4 (6.2%) were Hispanic and 1 (1.5%) Asian American. 35 (53.8%) were employed. There was no difference between the employed and unemployed groups in Age, Gender, US residency status (Permanent resident/US citizen, Undocumented alien), Education level (Primary, High school and College), Pre-Hemodialysis employment status, duration of Hemodialysis therapy, Hemoglobin, Serum Albumin, Urea reduction ratio (URR) and BD-II. The two groups however significantly differed in the SF-36 score (72±16 vs. 62±20, p=0.037) and Diabetes Mellitus (p= 0.032). Using logistic regression analysis, only the SF-36 score predicted employment in this population. We did not identify any predictors of the SF 36 score.

Conclusion:

Employed HD patients tend to have a higher SF-36 score than unemployed HD patients. These results need verification in prospective studies.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2645

**Pulmonary Hypertension in Hemodialysis Patients: Insights from Hemodynamic Measurements** Chirag B. Patel,[1] James Gossage,[2] John White.[1] *[1]Medicine, Nephrology, Medical College of Georgia, Augusta, GA; [2]Medicine, Pulmonary Critical Care, Medical College of Georgia, Augusta, GA; [3]Augusta, GA.*

Pulmonary hypertension (PH) is common in hemodialysis (HD) patients and is associated with adverse outcomes. Potential etiologies include high flow states from AV grafts or fistulae, the high prevalence of sleep apnea, and chronic volume overload. Previous studies estimated pulmonary artery systolic pressure (ePASP) by 2D echocardiography (echo), the reliability of which has not been adequately assessed in HD. The purpose of this study is to determine the accuracy of echo in this population for diagnosing PH as compared to right heart catheterization (RHC) and to determine the role of volume status.

We conducted a retrospective review of 17 HD pts referred for suspected PH. Demographic data, dialysis access, lab, echo data, and RHC data were collected. ePASP >= 35 mmHg was used to diagnose PH by echo. Mean pulmonary artery pressure (mPAP) >= 25 mmHg was considered diagnostic by RHC. Pulmonary vascular resistance (PVR) > 200 dynes/sec/cm-5 and pulmonary capillary wedge pressure (PCWP) >= 15 mmHg were considered elevated. In patients with elevated PCWP, the trans-pulmonary gradient (TPG) was calculated. TPG < 15 suggests heart failure as cause of PH while TPG > 15 suggests mixed disease. The relation between mPAP, PCWP, and other important clinical factors were assessed.

87% (n = 15) pts were confirmed to have PH by RHC. PCWP was elevated in 13 pts. TPG was < 15 in 2 pts and > 15 in 11 pts. In patients with elevated PCWP, PVR was elevated in 54% (n = 7). mPAP was strongly related to PVR (0.719) but moderately related to PCWP (0.521) and PASP (0.483). Despite only 7 measurements, there was a strong relation between BNP and RAP (0.935), PASP (0.868), and mPAP (0.74).

In conclusion, in this study 2D echo reasonably predicts PH in HD patients. The majority had elevated PCWPs, but also TPGs and PVRs suggesting mixed processes. The relation between PCWP and mPAP is relatively weak confirming the need for further investigation of the chronic effects of fluctuating volume status and high flow dialysis shunts on the pulmonary vasculature.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2646

**Hepatitis B Virus (HBV) e Seromarkers in Prevalence Hemodialysis (HD) Patients** Alicja E. Grzegorzewska,[1] Jerzy Uzar.[2] *[1]Department of Nephrology, University of Medical Sciences, Poznan; [2]B.Braun Avitum, Legnica, Poland.*

HBV e antigen (HBeAg) serves a marker of active HBV replication. Antibodies to HBeAg (HBeAb) appear once HBeAg has been cleared and persist for 5-6 years. Total antibodies to core HBV antigen (HBcAb) appear before HBeAg disappearance and last for the entire life, possibly with HBV minichromosome in liver cells of both HBV surface antigen (HBsAg) carriers and non-carriers. Our aim was an evaluation of epidemiological status of HD population by examination of HBsAg negative patients for presence of HBV e seromarkers in relation to results of total HBcAb testing. Among prevalence HD patients, known as HBsAg negative according to at least every 6-month HBsAg monitoring, positive HBcAb/negative HBV DNA (n = 89) and negative HBcAb (n = 112) patients were blindly selected. Interval between seroconversion and detection of HBcAb positivity remained unknown. HBeAg and HBeAb were determined in HBcAb positive group; results of HBeAb testing were related to demographic, clinical and laboratory data. HBeAg and HBcAb (for control) were simultaneously determined in HBcAb negative group. Early non-HBsAg carrier stage (HBeAb positive) was shown in 46 (51.7%), whereas late non-HBsAg carrier stage (HBeAb negative) – in 43 (48.3%) of all HBcAb positive patients. As compared to HBeAb negative patients, HBeAb positive subjects were younger (53.6 ± 15.6 vs 64.0 ± 13.5 years), had shorter interval between first detection of HBcAb and current HBeAb determination (16.5 ± 15.5 months vs 22.1 ± 19.3 months) and showed lower prevalence of positive antibodies to hepatitis C virus (HCVAb) (28.3% vs 41.9%). None HBcAb positive patient was HBeAg positive. HBcAb negativity was confirmed in all 112 patients. In this group, one patient (0.89%) was HBeAg positive, what indicated an early stage of HBV infection (confirmed by positive HBsAg testing immediately performed). Testing of HD patients for HBV e seromarkers shows that prevalence of HBeAb positivity in HBcAb positive non-HBsAg carriers is present in approximetwly 50% of cases and is related to younger age and lower prevalence of HCVAb positivity. Testing of HBsAg negative patients for HBeAg can occasionally detect HBV replication.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2647

**Increased Local Expression of Connective Tissue Growth Factor (CTGF) Is an Earlier Event Than Peritoneal Thickening in Peritoneal Dialysis Patients** Alferso C. Abrahams,[1] Amelie Dendooven,[2] Tri Q. Nguyen,[2] Roel Goldschmeding.[2] *[1]Nephrology, UMC, Utrecht, Netherlands; [2]Pathology, UMC, Utrecht, Netherlands.*

**Introduction**

Chronic treatment with peritoneal dialysis (PD) is complicated by peritoneal fibrosis and thickening, which leads to loss of ultrafiltration capacity. It has been suggested that transforming growth factor-β1 (TGF-β1) plays a central role in this process. CTGF is a downstream mediator of profibrotic TGF-β1 activity and plays a key role in the pathogenesis of multiple fibrotic disorders. We hypothesized that increased CTGF precedes PD-associated peritoneal thickening.

**Methods and Results**

A biopsy of the parietal peritoneum was obtained during kidney transplantation procedure in 17 PD patients and 17 non-PD patients, i.e. ESRD patients treated by either hemodialysis or during a pre-emptive kidney transplantation. Mean PD duration in the PD group was 31±18 months, and none of the PD-patients had signs of ultrafiltration failure. The thickness of the submesothelial compact zone was independently and blindly assessed by two examiners on H&E-stained formalin-fixed tissue using image analysis software. TGF-β1 and CTGF mRNA expression were assessed by quantitative real-time PCR.

Although average peritoneal thickness was not different between the PD patients and non-PD patients (111±86 μm vs. 101±42 μm), peritoneal expression of CTGF mRNA was significantly increased in PD patients compared to non-PD patients (fold change 2.4±0.8 vs. 1.0±0.6, p < 0.05). There was no correlation between peritoneal CTGF mRNA expression and peritoneal thickness. Gene expression of TGF-β1 in peritoneum was not significantly

different (fold change 1.8±0.9 in PD patients vs. 1.0±0.8 in non-PD patients, p = 0.19), but there was a correlation between TGF-β1 and CTGF ($r^2 = 0.21$, $p < 0.006$).

**Conclusions**

In PD patients, increased peritoneal CTGF expression appears to precede peritoneal thickening and loss of PD efficiency. This temporal relation suggests that CTGF might play an important role in fibrotic events leading to peritoneal thickening and ultrafiltration failure. Almost 80% of the peritoneal CTGF expression is explained by other factors than TGF-β1.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2648

**Survival of Neonates, Infants and Toddlers Commencing Renal Replacement Therapy** R. Todd Alexander,[1] Bethany J. Foster,[2] Marcello Tonelli,[1] Andrea Soo,[3] Alberto Nettel-Aguirre,[3] Brenda Hemmelgarn,[3] Susan M. Samuel.[3] *[1]University of Alberta; [2]McGill University; [3]University of Calgary.*

BACKGROUND: It is known that children younger than 4 years old experience increased morbidity and mortality with ESRD, however, there is a paucity of detailed long-term follow-up data.

METHODS: We utilized a population-based retrospective cohort design employing data from a national organ failure registry and administrative data from Canada's universal health care system. We included 87 children (ages 0-2) who initiated renal replacement therapy (RRT) during the period 1992 to 2007 and followed them until death or date of last contact (median follow-up 4.67 years [interquartile range 1.37-9.83]). We assessed overall survival and impact of age at onset of RRT on survival.

RESULTS: Patients were mostly male (69.0%) and the etiology of ESRD was due to renal malformations in 54%. Peritoneal dialysis was the most common initial renal replacement modality (83.9%) and 57 (65.5%) children received a renal transplant. During 490 person-years of follow-up, there were 23 (26.4%) deaths; 22 of these occurred in patients who never received a transplant. Average age at renal transplantation was 2.82 years (SD: 1.14). Average weight at transplantation was 12.78 kg (SD: 3.07). Overall 5 and 10 year unadjusted survival from initiation of RRT were 74.9% (95% CI: 66.1% - 84.8%) and 72.9% (95% CI: 63.7% - 83.4%). In unadjusted analysis, children who initiated RRT between 0-3 months had decreased survival compared to those initiating RRT between 1-2 years (Fig. 1, log rank p = 0.04).

CONCLUSION: Children initiating RRT before 3 months of age have significantly increased mortality compared with those initiating RRT at an older age. Among those children receiving a transplant the overall survival was good.

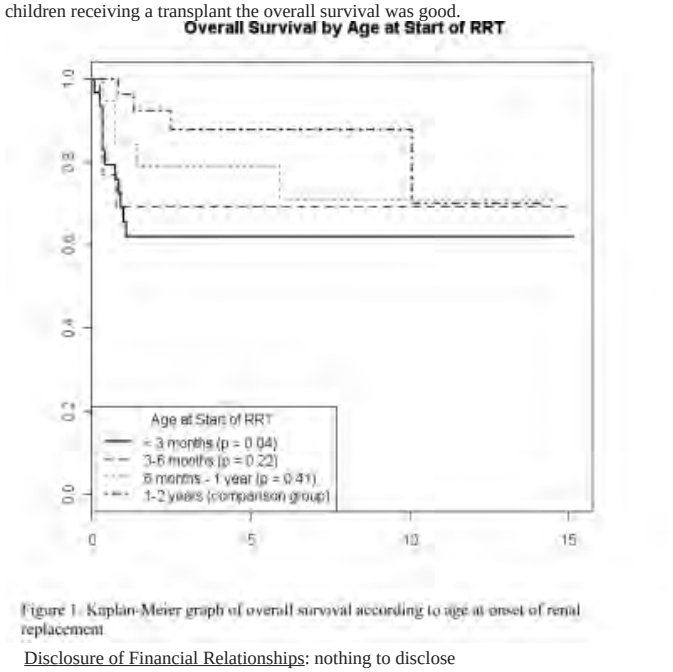



Figure 1. Kaplan-Meier graph of overall survival according to age at onset of renal replacement

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2649

**Death, Dialysis and Renal Transplantation among Aboriginal Children with Kidney Failure in Canada** Susan M. Samuel,[1] Marcello Tonelli,[2] Bethany J. Foster,[3] Alberto Nettel-Aguirre,[1] R. Todd Alexander,[2] Andrea Soo,[1] Brenda Hemmelgarn.[1] *[1]University of Calgary, Calgary, AB, Canada; [2]University of Alberta, Edmonton, AB, Canada; [3]McGill University, Montreal, QC, Canada.*

**Background:** Ethnicity is a risk factor for adverse outcomes among patients with end-stage renal disease. We evaluated differences in overall survival, time spent on dialysis and kidney transplantation rates among Aboriginal and Caucasian children in Canada. **Methods:** We used a population-based retrospective cohort design utilizing data from a national organ failure registry and administrative data of a universal healthcare system. Patients (ages 0-18) who were Aboriginal or Caucasian starting renal replacement therapy (RRT) in Canada between years 1992 and 2007 were followed until death, last contact or study end (Dec 31, 2007). **Results:** Among 843 paediatric patients who initiated RRT during study period, 104(12.3%) were Aboriginal and 521(62.5%) were Caucasian. During study period, 65(12.5%) Caucasians and 15(14.4%) Aboriginals died. Overall 5 and 10 yr survival from start of RRT for Aboriginal patients were 91.3%(95% CI: 85.6% - 97.3%) and 84.8%(95% CI: 76.4% - 94.3%), and among Caucasians were 92.2%(95% CI: 89.8% - 94.7%) and 85.8%(95% CI: 82.2% - 89.6%) respectively. There was no evidence of a difference in overall survival between the two groups (log rank p=0.29) Aboriginal children spent longer on dialysis before first kidney transplant: 1.75 years(IQR 0.69-2.81); compared to Caucasians: 0.75 year(IQR 0.08-1.75); p < 0.001. Unadjusted kidney transplantation rates per 100 person years were 10.8(95% CI 8.5-13.6) for Aboriginals and 12.3(95% CI 11.2-13.4) for Caucasians (Rate ratio: 1.14(95% CI: 0.89-1.45), p = 0.30). Among Aboriginals, 67.1% of first kidney transplants were from deceased donors and in Caucasians, 42.9% first kidney transplants were from deceased donors(p <0.001).**Conclusions:** There was no evidence of a difference in unadjusted survival between Canadian Aboriginal and Caucasian children starting RRT. Aboriginal children spent longer on dialysis prior to receiving a transplant. Evaluation is needed to examine barriers to receiving a kidney transplant among Aboriginal children.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2650

**Benfotiamine Protects Human Peritoneal Mesothelial Cells Against AGE Mediated Damage in PD** Sandra Muller-Krebs, Lars Kihm, Laura Mertes, Martin G. Zeier, Vedat Schwenger. *Nephrology, University of Heidelberg, Heidelberg, Germany.*

Introduction

High glucose and advanced glycation end-products (AGE) were suggested as contributing factors for local peritoneal damage. Previous studies demonstrated the ability of the lipid-soluble thiamine derivative benfotiamine (BF) to decrease high glucose induced tissue damage by an activation of transketolase (TK).

Methods

Human peritoneal mesothelial cells (HMPC) were isolated from omenta and incubated with low (1.5%) and high (3.9%) glucose containing PD fluids (PDF) either ± BF substitution for 48 h.

Expression of TK was assessed using immunofluorescence (IF). AGE mediated damage was analyzed by IF and western blot regarding the expression of the receptor for AGE (RAGE), epithelial to mesenchymal transition (EMT), and the cytoskeletal organization. For quantitation a semiquantitative score was used. The inflammatory status was analyzed by ELISA technique. Data are shown as mean ± SD.

Results

After incubation with PDF, HPMC revealed a decreased level of TK, higher expression of RAGE and vimentin as a marker of EMT. Moreover there was a higher percentage of HPMC exhibiting a reorganized actin cytoskeleton.

When cells were incubated with BF and PDF, TK expression was higher in the low glucose group in comparison to low glucose PDF without BF incubation (PDF1.5: 1.30±0.15 vs. PDF1.5+BF: 1.61±0.22, p<0.05).

RAGE expression was significantly lower when incubated with BF (PDF1.5: 1.65±0.11 vs. PDF1.5+BF: 1.37±0.14, p<0.001; PDF3.9: 1.71±0.16 vs. PDF3.9+BF: 1.49±0.14, p<0.001), EMT, shown by vimentin expression, was reduced (PDF1.5: 1.78±0.37 vs. PDF1.5+BF: 1.60±0.40, p<0.05; PDF3.9: 2.15±0.57 vs. PDF3.9+BF: 1.69±0.64, p<0.05). The actin cytoskeleton was reorganized in less % of when incubated with BF (PDF1.5: 26.3±13.1 vs. PDF1.5+BF: 17.5±7.54, p<0.01; PDF 3.9: 28.5±9.75 vs. PDF3.9+BF: 24.8±11.8). Interleukin-6 release was reduced by incubation with BF (PDF3.9 [ng/ml]: 6.14±2.91 vs. PDF3.9+BF [ng/ml]: 3.57±2.19).

Conclusions

Our findings suggest that BF mediates activation of TK and thereby provides specific protective effects to HMPC that affect RAGE, EMT, cytoskeletal organization, and inflammation during AGE mediated damage in PD.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2651

**Protective Effects of Non Glucose-Based Peritoneal Dialysis Fluid (PDF) Against PD-Induced Hypoxic Peritoneum** Wasin Manuprasert,[1] Sirigul Kanchanabuch,[1] Somchai Eiam-Ong,[2] Talerngsak Kanjanabuch.[1,2] *[1]Kidney&Metabolic Disorder Research Center, Chulalongkorn University, Thailand; [2]Medicine, Chulalongkorn University, Thailand.*

Long-term exposure of the peritoneal membrane to unphysiologic PDF results in an injury of peritoneum. The roles of polyglucose against PDF-induced hypoxic peritoneum were investigated in both *in vitro* & *in vivo*.

**Methods:** 20 Male Sprague-Dawley rats, divided into 3 groups were subjected to twice daily injections with 1) 0.9%NSS, 2) 3.86%G PDF, 3) 7.5%polyglucose solution. After 4- & 12-weeks injections, morphologies of peritoneal membrane, HIF-1α, and VEGF were investigated. To confirm hypoxic condition, pimodazol (hypoxyprobe) was immediately injected to animals prior to sacrifice and was assessed. In *in-vitro* study, omental-derived primary human mesothelial cells (HPMC) were exposure for 15 hours to either of 1.36%G PDF, 7.5%polyglucose solution, or 0.1%FCS in normoxic condition (5%CO2, 95%Air) and hypoxic condition (1%O2, 5%CO2, 94%Air). The mesothelium injury & death were assessed by morphological changes, %LDH release, and TUNEL staining.

**Results:** Rat submesothelial thickness & fibrosis in the omental & parietal peritoneum were increased significantly and corresponded to up-regulation of HIF-1α and VEGF expressions. Both changes were significantly ameliorated compared to injection with

7.5%polyglucose. HPMC cultured with glucose-based PDF for up to 15 hours showed time-dependent morphological changes, which was accompanied by cell death and loss of mesothelial markers, in both normoxic and hypoxic conditions. Using polyglucose significantly ameliorated HPMC injury, loss of mesothelial markers, and apoptosis in response to PDF in both conditions. Beneficial effects of non glucose-based PDF on peritoneal hypoxia were further confirmed using hypoxyprobe. PDF activated VEGF in a dose-dependent manner with attenuation by polyglucose which was consistent both *in-vivo* and *in-vitro* studies.

**Conclusions:** PDF-induced peritoneal hypoxia may act in parallel or synergistically with bio-incompatible properties of PDF to develop long-term peritoneal damages. Using of non glucose-based PDF can prevent PD-induced hypoxic peritoneum.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2652

**Nebivolol (NV) Prevents Peritoneal Membrane (PM) Failure Induced by Peritoneal Dialysis (PD) Fluids** Anna Rita Aguirre,[1] Guadalupe González-Mateo,[3] Hugo Abensur,[1] Patricia Albar-Vizcaino,[2] Mª Luisa Perez-Lozano,[2] Rafael Selgas,[3] Manuel Lopez-Cabrera,[2] Abelardo I. Aguilera.[2] *[1]Nephrology Department, University of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Servicio de Nefrología, Hospital Universitario de la Princesa, Madrid, Spain; [3]Servicio de Nefrología, Hospital Universitario La Paz, Madrid, Spain.*

In PD patients, β-blockers have been considered deleterious to PM by their association with loss of ultrafiltration (UF) capacity due to splanchnic vasoconstriction and fibrosis. NV is a new β-blocker with novel characteristics mediated by nitric oxide release. Herein, we analyzed the effects of NV on human peritoneal mesothelial cells (HPMC), on a mice PD model and on 16 PD patients receiving oral NV after suffering an acute myocardial infarction.

***In vitro,*** HPMC were co-treated with NV and TGF-β, to induce epithelial-to-mesenchymal transition (EMT). NV decreased fibronectin and pre-collagen-I production, increased tPA but did not affect the EMT, VEGF, neither vessel-formation (matrix-gel).

***In vivo***, 21 C57BL/6 mice were divided in 3 groups. Control group (CG) carried a PD catheter without PD fluid infusion. Study group (SG) received 2 ml of intraperitoneal dextrose (D) 4.24%/ day and oral NV during 30 days. Positive control (PC) group received 2 mL/ day of intraperitoneal D for 30 days. SG showed lower PM thickness, lower counts of submesothelial vessels (Tie1+), less mesothelial cells suffering EMT (cytokeratin + and α-SMA +) and less AGEs than PC group. The lymphatic vessel counts were similar in all groups. In PD effluent, SG also showed a tendency of decreased IL-6 production. Functionally, NV protected the UF capacity and small solute peritoneal transport (SPT).

***The PD patients*** who received NV showed a lower frequency of high SPT rate than a CG matched by age, gender and PD events during a 2 year follow-up.

***Conclusion:*** NV showed an anti- fibrotic, anti- EMT and pro- fibrinolytic effect on PM exposed to bio - incompatible PD fluids, functionally protecting from UF failure.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2653

**Exposure of Peritoneum to Polymer Catheters for 1-7 Days Results in Acute Inflammation in Rats** Michael F. Flessner,[1] Xiaorong Li,[1] Elise Peery Gomez-Sanchez,[1] Zhi He.[2] *[1]Medicine, University of Mississippi Medical Center, Jackson, MS; [2]Pathology, University of Mississippi Medical Center, Jackson, MS.*

To test the hypothesis that measurable changes in the peritoneum occur rapidly after catheter exposure, polyethylene rings were placed in the peritoneal cavity of 18 Sprague-Dawley rats (E); controls (C, n=18) underwent sham laparotomy without catheters. After 1, 3, 7 days, peritoneal lavage was carried out, the rings recovered and cells adhering to the catheters separated using ultrasound; the cells, catheter, and abdominal swab were cultured for 96 hours to insure sterility. Data from 4 E rats and 3 C rats were discarded due to bacterial contamination. Abdominal wall tissues were collected after sacrifice and processed for CD31, Trichrome, and cytokine immunohistochemistry (IHC). Initial cell densities on the catheter material (#cells/cm$^2$, mean±SE) were: day 1, 976±298; day 3, 18371±2291; day 7, 27313±3343. CD31 staining and peritoneal thickness were significantly greater than controls by day 1; overall means respectively were: E 29.4±3.6 vs C 3.8±3.1 vessels/mm peritoneum; E 46.4±4.6 vs C 25.1±4.1 microns. IHC for VEGF, TGF, and αSMA of E animals was significantly elevated at day 1 and remained elevated through day 7. Real time RT-PCR was done on lavage cells for TNFα, TGFβ, IL-1, CD68, and mineralocorticoid receptor (MR). As expected, inflammation increased after the injury of surgery and was reflected by an increase in inflammatory markers in both groups over the 7 days. However cells recovered from the peritoneal lavage of E rats expressed significantly ($p<0.05$) less mRNA for CD68 and TNFα than C rats. MR expression in peritoneal lavage cells in E rats was more than 10-fold greater than in C rats. Our hypothesis was upheld: innate immune responses likely begin immediately with the introduction into the cavity of the foreign body resulting in measurable structural change in the peritoneum within 24 hours. Sequestration of macrophages may explain the decrease in CD68 and TNFα expressing cells in the E groups. MR, which is also associated with inflammatory events in cardiovascular and renal damage, appears to be involved in the process.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2654

**Chronic HCV Infection Is Associated with Low Serum C-Reactive Protein (CRP) in Hemodialysis (HD)** Philip Goldwasser,[1] Maria Ajaimy.[2] *[1]VA NY Harbor Healthcare System, Brooklyn, NY; [2]SUNY Downstate Medical Ctr, Bkln, NY.*

CRP level is a sensitive indicator of inflammation and mortality risk. CRP has been reported to be lower in HCV patients (pts), but only a borderline effect of HCV on CRP has been reported in HD (Nascimento et al, 2005). In a prevalent cohort of 69 HD pts, we examined the relationship of high sensitivity-CRP to HCV, adjusting for markers of inflammation derived from routine monthly bloodwork, and other potential confounders. After excluding 16 pts who, in the prior month, were diagnosed with an inflammatory condition or hospitalized, 53 pts remained. Samples with CRP below the detectable limit (<0.01 mg/dL) were assigned a value of 0.01 mg/dL (n=4). HCV was present in 26% of the cohort (14/53). HCV pts tended to be younger than non-HCV pts and more often HIV+ (both $p<0.01$), but the groups were similar in diabetes, race, serum albumin, ferritin, white count (WBC), and HD access. CRP ranged from 0.01 to 9.22 mg/dL (median = 0.50 mg/dL). Median CRP was lower in pts with HCV (0.09 mg/dL *vs*. 0.55 mg/dL; $p<0.01$ by Mann-Whitney U-test). When CRP was divided into quartiles, the frequency of HCV was very high (62%) in the lowest quartile (≤0.12 mg/dL), but lower (15%, 7%, and 23%, respectively) in the other quartiles ($p<0.01$ for quartile × HCV status). The 4 pts with undetectable CRP all had HCV, and their mean albumin was 4.1±0.4[sd], suggesting adequate hepatic synthetic status. Log (CRP) correlated with albumin ($r = -0.39$, $p<0.005$), log (ferritin) ($r=0.36$, $P=0.01$), and WBC ($r=0.31$, $P<0.03$). In a multivariate model, log (CRP) continued to be associated with HCV ($P<0.04$), adjusting for albumin ($p<0.06$), WBC ($p<0.08$), log (ferritin) ($P<0.21$), and age ($p=0.3$). When HIV+ pts were excluded, HCV remained the strongest predictor ($p<0.05$), adjusted for the same covariates. **In summary**, among prevalent HD pts, HCV was associated with a lower CRP in both univariate and multivariate analyses. A high proportion of HCV pts had very low CRP. Possible explanations include reduced synthesis, increased clearance, assay interference, and survivor bias. This association may affect the clinical and epidemiologic utility of CRP.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2655

**Hepatitis C Virus Infected Chronic Hemodialysis Patients Have Increased Cardiovascular Risk** Nageswara Reddy Pamidi. *Nephrology, Kasturba Medical College, Manipal, Karnataka, India.*

**BACKGROUND:** Cardiovascular disease (CVD)is the leading cause of mortality in hemodialysis subjects.This study is done to assess effect of HCV infection on oxidative stress markers and its correlation to arterial stiffness in HD.

**METHODS:** A prospective study done with IEC approval and informed consent. Serum levels of malondialdehyde as thiobarbituric acid reactive substance (TBARS), protein carbonyl content (oxidant stress), protein sulfohydryl groups as antioxidant marker are measured and were correlated with Carotid-Femoral pulse wave velocity (cfPWV) and Aortic augmentation(Aix) done by SphygmoCor.®

**RESULTS:** Demographics comparable among all groups ($p>0.05$). As the duration of HD is significantly higher in HCV positive subjects they were divided into 2 sub groups to nullify the effect of HD duration. HD is associated with raise in serum TBARS and protein carbonyl content and a decrease in protein sulfhydryl levels. HCV infection augments the adverse effects of HD on serum markers of oxidative stress as shown in Table1.

Table 1

| PARAMETER | CONTROL(a) | HCV NEGATIVE(b) | HCV POSITIVE < 2 YRS(c) | HCV POSITIVE ≥ 2 YRS(d) |
|---|---|---|---|---|
| Serum TBARS (nmol/L) | 3.54±0.32 | 8.82±2.21 | 12.59±3.68* | 11.01±2.75* |
| Protein carbonyl (nmol/L) | 0.66±0.21 | 1.23±0.38 | 1.77±0.89* | 2.00±0.76* |
| Protein sulfhydryl (µmol/mL) | 588.3±29.03 | 302.3± 99.54 | 234.8±77.9* | 233.1±77.8* |
| Serum NO (µmol/L) | 33.67±5.78 | 8.18±4.12 | 9.13±3.51 | 10.29±7.84 |
| cf PWV (m/s) | 7.4±1.19 | 11.0±1.45 | 11.91±0.76* | 13.0±1.35* |
| AIx (%) | 8.7±0.63 | 28.7±4.64 | 31.0±6.41 | 37.5±5.27* |

* p=<0.001 betqween (b) & (d)

The increased oxidative stress in HCV positive patients is probably not due to increased duration on HD as there is no significant difference between these among group **c** compared with those of group **d**. The markers of arterial stiffness viz. PWV and AiX are higher in HCV positive compared to those without HCV again after nullifying the effect of duration on HD.

**CONCLUSION:** As there is increased oxidative stress and arterial stiffness in HCV infected hemodialysis patients they can have increased cardiovascular mortality and morbidity. Long term follow up studies are recommended.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2656

**Effect of Gadolinium Contrast on Iron Metabolism Pathways and Inhibition by Iron Chelator** Sundararaman Swaminathan,[1] Chhanda X. Bose,[1] Sudhir V. Shah,[1] Kim M. Hiatt.[2] *[1]Nephrology, University of Arkansas for Medical Sciences, Little Rock, AR; [2]Dermatopathology, University of Arkansas for Medical Sciences, Little Rock, AR.*

**Background**: Nephrogenic Systemic Fibrosis (NSF) is characterized by fibrosis and infiltration of fibrocytic cells. We have recently demonstrated iron mobilization and tissue accumulation of iron in NSF. Cellular pathways that participate in iron dysmetabolism of NSF are unknown. CD163- Heme Oxygenase (HO-1) – Ferroportin pathway is well known to be a pivotal regulator of iron metabolism in the body.

**Methods:** We first evaluated the *in vitro* effects of Omniscan on cultured human peripheral blood mononuclear cells (PBMC). Unsorted PBMC were cultured with various concentrations of Omniscan (0.1 to 2.5 mmol/Kg BW) in DMEM medium. Between Day 5-8, adherent cells were isolated and evaluated with flow cytometry and immunofluorescence for phenotype and protein expression confirmed with western blot. We performed additional *in vitro* studies with oral iron chelator- deferiprone to evaluate its effect on Omniscan-induced changes. We also performed immunohistochemistry on NSF biopsy specimen for a panel of cellular markers.

**Results:** Omniscan treatment induced increased number of adherent spindle cells which morphology similar to fibrocytes. Omniscan-treated cells strongly expressed markers of Iron metabolism such as CD163, HO-1, H-Ferritin and *Iron export protein*- Ferroportin and inhibited Hepcidin. Iron chelator significantly decreased the development of $CD163^+$ adherent spindle cells. Spindle cells in NSF skin biopsies showed strong expression of CD163 and Ferroportin.

**Conclusion:** Omniscan induces development of $CD163^+$ spindle cells *in vitro* and $CD163^+$/ Ferroportin-$1^+$ cells accumulate in human NSF lesions. Strong activation of CD163-HO-1-H-Ferritin-Ferroportin pathway and inhibition of Hepcidin *in vitro* by Omniscan taken along with inhibition of $CD163^+$ spindle cell development by deferiprone suggests a potent role of CD163 scavenger pathway and iron export in NSF pathogenesis.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2657

**Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Hemodialysis (HD) Patients Correlates with Iron Status, Increases by Intravenous Iron Administration (IVIR) and May Modulate Its' Complications** David Tovbin,[1] Shani Kesari,[3] David Sola-Del Valle,[6] Jonathan M. Barasch,[6] Amos Douvdevani,[2] Moshe Zlotnik,[1] Amir Abd Elkadir,[4] Shimon Storch.[5] *[1]Nephrology, Soroka Medical Center; [2]Nephrology Laboratory, Soroka Medical Center; [3]Joyce and Irving Goldman Medical School; [4]Biomedical Engineering, Ben-Gurion University of the Negev; [5]Nephrology, Bnai-Zion Medical Center, Haifa, Israel; [6]Nephrology, Columbia Medical Center, New York, NY.*

Neutrophil gelatinase-associated lipocalin (NGAL) binds the bacterial iron carrier siderophore protecting against infection, and increases in infection, inflammation and oxidative stress. These disturbances are present in hemodialysis (HD) patients, augmented by intravenous iron (IVIR) and associated with impaired iron homeostasis, anemia and atherosclerosis. We hypothesized that NGAL in HD patients is affected by iron status and IVIR and modulates their complications. Thus, we assessed the relation of serum NGAL levels and their changes by IVIR to iron, inflammation and oxidative stress related parameters. In a prospective study, 20 chronic HD patients in 2 hospitals received 100 mg /week iron-sucrose in midweek HD for 4 weeks after 4 weeks without IVIR. Serum NGAL levels and iron, inflammation and oxidative stress (AOPP-advanced oxidation protein products) related parameters were evaluated at mid week sessions in HD without IVIR and with the 1st and 4th IVIR. Comparing the 1st center 14 patients to the 2nd center 6 patients, pre-1st IVIR serum ferritin (116 ± 235 ng/ml Vs 417 ± 574 ng/ml, p<0.01) and iron (45 ± 24 Vs 71 ± 37 µg/100 ml, p<0.05) levels were lower, and were associated with lower NGAL levels (69 ± 46 Vs 227 ± 141 ng/ml, p<0.05). In 1st center, IVIR increased serum NGAL to pre-4th IVIR levels of 188 ± 47 ng/ml (P<0.001)), parallel to increase in ferritin (to 236 ± 290 ng/ml, p<0.0001). Pre-1st IVIR NGAL levels were negatively associated with 1st IVIR % CRP change (r=-0.45, p<0.05) and AOPP change (r=-0.54, p<0.054, NS). Conclusions: Serum NGAL in HD patients correlates with iron status and in depleted patients increases with IVIR. NGAL may modulate IVIR related complications.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2658

**The Effect of Different Types and Doses of Iron on Inflammaton and Oxidative Stress in Hemodialysis Patients** Rumeyza Kazancioglu,[1] Savas Ozturk,[1] Meltem Gursu,[1] Cagdas Kaya,[2] A. Baki Kumbasar,[2] Abdulkadir Ergen,[2] Zeki Aydin,[1] Sami Uzun,[1] Serhat Karadag.[1] *[1]Nephrology, Haseki Training and Research Hospital, Istanbul, Turkey; [2]Internal Medicine, Haseki Training and Research Hospital, Istanbul, Turkey.*

**INTRODUCTION:** There are many studies about inflammatory and atherogenic effects of intravenous iron(IVI). Moreover, monthly total dose iron application has not been compared with standard intermittent therapies. Herein, different doses and types of IVI were examined for late effects on inflammatory-oxidative stress indices.

**METHODS:** Stable chronic hemodialysis(HD) patients with hemoglobin levels more than 8gr/dl were included if they did not have history of recent bleeding, transfusion, oral or parenteral iron use within the last 15 days and active infection. Patients were grouped as those not receiving IVI(Group 1, n=29, 100mg/week), those having intermittent iron sucrose(Group 2, n=25, 100mg/week), those receiving intermittent iron dextran(Group 3, n=24, 100mg/week) and those having monthly total dose iron dextran(Group 4, n=23, 400mg/week). Samples for MDA(malone dialdehyde), AOPP(advanced oxidation protein product), CRP(C-reactive protein) and TNF-α were taken at days-0, 2, 7 and 30 within the first 10 minutes of dialysis. Statistical analysis was carried on by SPSS for Windows with p values less than 0.05 being accepted as significant.

**RESULTS:** 101 patients with a male/female ratio of 48/53 were included. The mean age of the patients was 47.6±13.9 years. Groups were similar in terms of age, gender, hemoglobin, iron indices and total monthly amount of IVI. When all patients were considered together; MDA levels at days-7 and 30; AOPP levels at days-0 and 30, CRP levels at day-30 and TNF-α levels at day-7 were significantly higher than other days. But, there were not any significant differences within and between groups in multi-and bilateral group analysis. IVI injections were well tolerated in all groups without significant side effects.

**CONCLUSION:** Although IVI has potential oxidant effects; the different types and doses of IVI treatments are well tolerated without negative effects on lipid and protein oxidation and inflammatory indices in chronic HD patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2659

**Effects of Intravenous (IV) Iron Sucrose (IS) Preparations on Oxidative/ Nitrosative Stress and Inflammatory Markers in Rat Brain** Jorge E. Toblli, G. Cao, J. Giani, M. Munoz, L. Oliveri, F. Dominici. *Hospital Aleman, University of Buenos Aires.*

**Purpose:**IV iron is effective in treating iron deficiency, but iron may be associated with oxidative stress and inflammation. Clinical experience with the original IS Venofer® is based on >240 million doses used up to 2010, but subtle differences in IS similar (ISS) products affect iron release. This study explores potential differences on oxidative and nitrosative stress and inflammatory response in the brain of normal rats. **Methods:**5 groups of Sprague-Dawley rats were studied: G1(IS); G2(ISS Portugal); G3(ISS Colombia); G4(ISS Argentina), G5(controls). G1,G2,G3&G4 received IV iron weekly (40mg iron/kg), and G5 normal saline solution, on days 0, 7, 14, 21 and 28. TBARS and GSH/GSSH ratio were evaluated in brain homogenates. Nitrotyrosine, IL-6, TNF-alpha, heat shock protein-70 (HSP70) and caspase-3 were detected by immunohistochemistry.

Results after 5 weekly injections of 40mg iron/kg

| Mean±SD | GI (n=7) | G2 (n=7) | G3 (n=7) | G4 (n=7) | G5 (n=7) |
|---|---|---|---|---|---|
| Hemoglobin(g/dl) | 16.4±0.4 | 16.1±0.4 | 16.1±0.3 | 16.2±0.4 | 15.9±0.3 |
| Serum iron (µ/dl) | 371±18** | 431±14 | 426±11 | 455±22 | 317±15* |
| TSAT (%) | 68.5±5.7** | 85.0±3.5 | 82.0±6.4 | 83.1±7.1 | 44.4±4.9* |
| TBARS (nmol MDA/g protein) | 217.8±18.8** | 309.1±20.6 | 322.7±21.1 | 315.4±25.0 | 191.2±21.5** |
| GSH/GSSG | 6.0±1.2** | 3.5±1.2 | 3.8±1.3 | 3.9±0.7 | 6.7±1.1** |
| Nitrotyrosine (+staining/area) | 2.1±1.4** | 6.7±2.1 | 6.3±1.4 | 6.1±2.1 | 1.0±0.7** |
| TNF-alpha (+cells/area) | 3.6±0.8** | 10.9±3.5 | 9.3±1.4 | 9.0±2.1 | 2.0±0.7** |
| IL-6 (+cells/area) | 4.2±1.8** | 9.8±2.5 | 9.4±2.7 | 10.1±2.8 | 1.4±0.6** |
| HSP70 (+cells/area) | 5.5±2.3** | 11.2±2.9 | 13.0±3.5 | 11.5±1.9 | 2.7±1.2** |
| Caspase-3 (+cells/area) | 0.7±0.4** | 4.1±1.0 | 3.5±0.9 | 3.8±0.6 | 0.4±0.5** |

*p<0.01 vs all groups **p<0.01 vs G2, G3 & G4

**Conclusions:**The tested ISSs cause substantially more oxidative and nitrosative stress, increased inflammatory response and apoptosis in the brain of normal rats than the original IS. It is possible that, in contrast to the originator IS, significant amounts of weakly-bound iron are liberated from these ISS due to lower stability of the iron complex.

Disclosure of Financial Relationships: Research Funding: Vifor (International) AG, a company of Vifor Pharma group.

## SA-PO2660

**Comparison of Iron Sucrose and Iron Sucrose Similar Substances on Oxidative Stress and Inflammatory Parameters in the Rat** Jorge E. Toblli, G. Cao, L. Oliveri, M. Angerosa. *Hospital Aleman, University of Buenos Aires.*

**Purpose:** Subtle differences in the structure of intravenous (IV) iron carbohydrate complexes like iron sucrose (IS) may affect iron release, which can result in oxidative stress and inflammation. This abstract summarizes 5 nonclinical studies evaluating IS similar (ISS) preparations vs the original IV IS (Venofer®) using identical methodologies. **Methods:** 11 groups of Sprague-Dawley rats (n=8) received IV iron preparations weekly (40mg/kg body weight on days 0, 7, 14, 21 & 28): $ISS_1$ Feriv, Spain; $ISS_2$ Hematin, Colombia; $ISS_3$ Generis, Portugal; $ISS_4$ Energavit, Argentina; $ISS_5$ Fe-lib, Taiwan; $ISS_6$ Ferplex SS, Pakistan; $ISS_7$ Encifer, India; $ISS_8$ Fesin, Japan; $ISS_9$ Anerrum, South-Korea; IS Venofer; controls (C) received normal saline. At the end of the 4-week studies, oxidative stress parameters (TBARS) were evaluated in liver homogenates. TNF-a was evaluated by immunohistochemistry. **Results:** IS and ISS groups differed in terms of iron markers, markers of oxidative stress and inflammation, and functional parameters (p<0.01).

| Mean± SD | Serum iron (µg/dL) | TSAT (%) | TNF-α† (%/area) | ALT (UI/L) | Proteinuria (mg/day) | TBARS††(nmol MDA/g prot) |
|---|---|---|---|---|---|---|
| $ISS_1$ | 415±11* | 83±4* | 7.9±0.8* | 410±45* | 26.5±5.5* | 83±11* |
| $ISS_2$ | 404±21* | 88±3* | 7.4±1.1* | 225±22* | 13.7±2.7* | 80±11* |
| $ISS_3$ | 423±18* | 83±4* | 8.8±0.9* | 335±78* | 27.7±4.2* | 103±19* |
| $ISS_4$ | 439±29* | 84±4* | 2.6±0.4* | 175±37* | 21.7±4.9* | 150±23* |
| $ISS_5$ | 488±39.9* | 87±4* | 4.3±0.4* | 340±58* | 29.8±7.9* | 179±17* |
| $ISS_6$ | 478±45* | 84±4* | 4.3±0.3* | 404±44* | 35.5±7.0* | 196±21* |
| $ISS_7$ | 454±41* | 89±6* | 4.1±0.4* | 409±66* | 39.4±8.2* | 168±19* |
| $ISS_8$ | 472±60* | 89±6* | 6.2±2.5* | 501±77* | 49.2±6.0* | 128±12* |
| $ISS_{9l}$ | 499±45* | 89±6* | 6.1±2.9* | 453±76* | 38.9±7.9* | 125±8* |
| IS | 364±18** | 68±4** | 1.3±0.3** | 59±11 | 4.7±1.2 | 74±8 |
| C | 300±9 | 44±4 | 0.4±0.1 | 49±12 | 3.2±2.2 | 70±9 |

†heart; ††liver *p<0.01 vs IS and control; **p<0.01 vs control

**Conclusions:** (a) ISS may have potentially harmful clinical effects due to subtle structural modifications, possibly arising from variations in manufacturing procedures and (b) additional equivalence criteria may be required to assess complex molecule copies such as iron sucrose

Disclosure of Financial Relationships: Research Funding: Vifor (International) AG, a company of Vifor Pharma group.

**SA-PO2661**

**Effects of α-Lipoic Acid on Oxidative Stress in ESRD Patients Receiving IV Iron** Arif Showkat,[1] Joanna Q. Hudson.[1,2] *[1]Medicine, University of Tennessee, Memphis, TN; [2]Clinical Pharmacy, University of Tennessee, Memphis, TN.*

Oxidative stress is associated with increased risk of cardiovascular disease in ESRD patients (pts). IV iron has been shown to increase oxidative stress in this population. The purpose of the study was to evaluate changes in oxidative stress markers following administration of IV sodium ferric gluconate (SFG) to hemodialysis pts with and without prior administration of the antioxidant α-lipoic acid.

ESRD pts who met inclusion criteria were enrolled in this open-label, crossover study. During a control (C) and intervention (I) visit 125 mg IV SFG was administered over 10 minutes. During the I visit 600 mg of α-lipoic acid was given orally 30 minutes prior to IV SFG. Pts received both treatments in random order. Blood samples were collected at 0, 15, 30, 60, 90, 120, 150, and 180 minutes post-infusion for measurement of malondialdehyde (MDA), F2-isoprostane (FIP), lipid hydroperoxide (LHP), and iron indices. The change in oxidative stress markers over time and differences in the percent change at each time point between C and I were evaluated.

Ten African-American ESRD pts were enrolled; 5 males; mean age 45±9 yrs; mean Hb13±1 g/dL; ferritin 571±241 ng/mL; TSat 27±4%. MDA, FIP, and LHP increased significantly for both C and I visits after administration of IV SFG with a greater increase observed in the I group.

Percentage Change of Oxidative Markers Over Time

| Time (min) | 30 | 60 | 90 | 120 | 180 |
|---|---|---|---|---|---|
| MDA-C | 12.6±8.9 | 21.7±26.5 | 19.4±30.5 | 22.3±38.7 | 19.6±39 |
| MDA-I | 25±22 | 72.1±96.3* | 66.7±70.6* | 60.6±60** | 65.9±69.3** |
| FIP-C | 20.7±8.9 | 13.8±20.7 | 19.5±17.5 | 7.7±21.1 | 10.8±15.9 |
| FIP-I | 30.3±18.7 | 56.8±32.4** | 37.6±28.7 | 38.9±40.4** | 19.9±34 |
| LHP-C | 28.2±18.3 | 23.5±13.2 | 16.2±10 | 9.4±10.4 | 5.1±6.1 |
| LHP-I | 36.1±15.8 | 37.4±18** | 28.7±15.3** | 23.1±13.2** | 14.8±8.4** |

Values expressed as mean ± SD. MDA & LHP in μmol/L, FIP in pg/mL. *P≤0.01, **0.01>P ≤0.05 compared to C group.

Administration of IV SFG is associated with an acute rise in oxidative stress . In contrast to previous studies, administration of an antioxidant was associated with a greater increase in oxidative stress.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2662**

**Prolyl Hydroxylase Inhibitors Reduce Hepcidin Levels and Affect Multiple Iron-Modulating Proteins** Richard A. Brigandi,[1] Mangatt Biju,[2] Jennifer L. Ariazi,[2] Sanjay Kumar,[1] Kevin James Duffy,[2] Connie L. Erickson-Miller.[1] *[1]GlaxoSmithKline, King of Prussia, PA; [2]GlaxoSmithKline, Collegeville, PA.*

Hepcidin and other iron-modulating proteins are responsible for the regulated release and transport of iron to the marrow to optimize erythropoiesis. The role of prolyl hydroxylase inhibitors (PHI) on these proteins was investigated using several small molecule α-ketoglutarate mimetic compounds. Hep3B cells were treated for 6 h with 25 ng/mL BMP-6 to stimulate an up-regulation of hepcidin. Following 16 h treatment with 3-50 uM PHI, quantitative RT-PCR (qRT-PCR) was performed to assess mRNA levels. PHI compounds stabilized HIF-1 and HIF-2 protein expression in Hep3B cells and induced EPO production. Results from qRT-PCR demonstrated that BMP-6 enhanced the level of hepcidin mRNA 12-15-fold, while the amount of hemajuvelin mRNA increased very slightly. However, following PHI treatment there was a consistent 3-fold decrease in hepcidin and 3-6-fold decrease in hemajuvelin mRNA. TMPRSS6 mRNA was not induced by BMP-6, but increased ~2-fold in response to PHI. These data suggest that PHI compounds can regulate an expanded set of iron modulating proteins rather than just the BMP-induced hepcidin. This is most likely a direct effect of the PHI as evidenced by data in which treatment with EPO (10-1000 mIU/mL) did not reduce the level of hepcidin mRNA in BMP-6 stimulated Hep3B cells. To determine the effects of PHI on iron-regulation in vivo, rats were treated with a single oral dose of vehicle or PHI (30 mg/kg) and livers collected at 0, 2, 4, 6, 8, 10, 12, 14, 16 and 24 h. The levels of mRNA were determined by qRT-PCR. There was a statistically significant increase in mRNA expression of EPO as early as 2 h and of ferroportin over 5 timepoints. There was a 15-fold decrease in hemojuvelin and hepcidin mRNA in the liver. Thus, inhibition of prolyl hydroxylases by small molecule compounds affects multiple iron-modulating genes in both in vitro and in vivo systems. The changes in this set of iron utilization proteins suggest that the PHI compounds can directly modulate the release and transport of iron to ensure adequate availability for more efficient erythropoiesis.

Disclosure of Financial Relationships: Employer: GlaxoSmithKline; Ownership: GlaxoSmithKline.

**SA-PO2663**

**The Prolyl Hydroxylase Inhibitor, GSK1278863A, Induced EPO *In Vitro* and Efficient Erythropoiesis Leading to Increased Hemoglobin *In Vivo*** Richard A. Brigandi,[1] Jennifer L. Ariazi,[2] Kevin James Duffy,[2] Lusong Luo,[2] David F. Adams,[1] Connie L. Erickson-Miller.[1] *[1]GlaxoSmithKline, King of Prussia, PA; [2]GlaxoSmithKline, Collegeville, PA.*

GSK1278863A is a highly selective orally bioavailable small molecule prolyl hydroxylase inhibitor that demonstrated *in vitro* and *in vivo* responses predictive of erythroid responses in humans. In biochemical assays, GSK1278863A inhibited EGLN 1(PHD2) and EGLN3(PHD3) with an IC50 of 22 and 5.5 nM, respectively. Treatment of the liver carcinoma Hep3B cell line for 6 hours with 25 and 50 μM GSK1278863A resulted in the accumulation of both HIF1α and HIF2α protein. Erythropoietin (EPO) mRNA was induced 7-fold relative to vehicle at 16 hrs following treatment of Hep3B cells and a 4-fold increase in EPO protein was detected in Hep3B conditioned medium at 24 hrs. These *in vitro* responses were predictive of the production of EPO and elevation of hemoglobin observed in mice, rats and dogs. A single 60 mg/kg oral dose of GSK1278863A in normal mice increased EPO mRNA by 450- and 60-fold in the liver and kidney, respectively at 6-8 hours post treatment. An 8-fold increase in plasma EPO protein levels was also observed at 8 hrs. Daily oral GSK1278863A gavage of normal mice for 8 days resulted in significant changes in all red blood cell parameters. Increases in hemoglobin of up to 2.8 g/dL relative to vehicle were observed after dosing with 3-30 mg/kg GSK1278863A. Increases in hematocrit and RBC were also observed. Daily oral GSK1278863A gavage in normal rats resulted in statistically significant 9-22% increases in hemoglobin, hematocrit and RBCs at 3 and 10 mg/kg/day following 14 and 21 days treatment. Progressive increases in red blood cell parameters, preceded by reticulocytosis, were also observed over the course of a one month study in dogs receiving daily oral doses of 3 mg/kg/day. Thus, stabilization of HIFa subunits and the production of EPO mRNA and protein by GSK1278863A predicted the *in vivo* responses in mice, rats and dogs of increased plasma EPO protein, and increased hemoglobin and other red blood cell parameters. Increased plasma EPO was also observed in healthy subjects treated with a single dose of GSK1278863A.

Disclosure of Financial Relationships: Employer: GlaxoSmithKline; Ownership: GlaxoSmithKline.

**SA-PO2664**

**Ezetimibe Improves Dyslipidemia and Oxidative Stress Conditions in Hemodialysis Patients** Yoshifumi Hamasaki, Kent Doi, Eisei Noiri, Toshiro Fujita. *Nephrology and Endocrinology / Hemodialysis and Apheresis, The University of Tokyo, Tokyo, Japan.*

[Background] Dyslipidemia and oxidative stress are the traditional and non-traditional cardiovascular risk factors for hemodialysis (HD) patients. Ezetimibe (EZT) is a new cholesterol-lowering agent that inhibits cholesterol absorption at small intestine. Little is known whether administration of EZT to HD patients is safe and effective to improve dyslipidemia and oxidative stress.

[Method] Twenty hyperlipidemic ESRD patients receiving maintenance HD were treated by EZT (10mg/day) for 6 months. Lipid profile and oxidative stress conditions were compared with control HD patients who had untreated dyslipidemia. Oxidative stress condition was evaluated by Reactive Oxygen Metabolites test (d-ROMs test) and Biological Anti-oxidant Potential test (BAP test) that have been developed to quantify oxidative stress and anti-oxidant capacity in blood easily.

[Results] After administration of EZT for 6 months, three patients were discontinued EZT. LDL cholesterol level and non-HDL cholesterol level were significantly reduced in the EZT group (n=17) compared with the control group (n=16) [-25.7% versus 4.0%, $p<0.05$ and -24.7% versus 2.4%, $p<0.05$, respectively]. MDA-LDL level was also reduced in the EZT group (-36.6% versus -13.8%, $p<0.05$). EZT significantly reduced value of d-ROMs test in the patients with low inflammation [$\log_{10}$(hs-CRP) <3.5] and high HDL cholesterol level (HDL-C >45 mg/dl) at 0 month compared with the control patients of the same profile [-10.0% in the EZT subgroup (n=11) versus 13.2% in the control subgroup (n=9), $p<0.05$]. In the patients with low serum albumin (<4.0 g/dl) at 0 month, value of BAP test (anti-oxidative stress) were maintained at 6 month in the EZT group [the change rate is 0.6% in the EZT subgroup (n=5) versus -12.9% in the control subgroup(n=9), $p<0.05$].

[Conclusion] EZT in HD patients improve lipid profile effectively and safely. EZT showed potential beneficial effect to reduce oxidative stress in HD patients with low inflammation and high HDL cholesterol, and to maintain anti-oxidant capacity in patients with low serum albumin level.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2665**

**Effect of Brazilian Nut Supplementation on Selenium and Glutathione Peroxidase in Hemodialysis Patients: A 12-Month Follow-Up** Milena Barcza Stockler-Pinto,[1] Denise Mafra,[2] Najla Elias Farage,[1] Viviane Oliveira Leal,[2] Julie Lobo,[1] Carolina Souza Sulis,[2] Gilson Teles Boaventura,[2] Olaf Malm,[1] Denis Fouque,[4] Silvia Maria Franciscato Cozzolino.[3] *[1]Federal University of Rio de Janeiro, Brazil; [2]Federal Fluminense University, Brazil; [3]University of São Paulo, Brazil; [4]University Claude Bernard Hopital Edouard, France.*

In hemodialysis (HD) patients large amounts of reactive oxygen species (ROS) are produced and, at higher concentrations, ROS are thought to be involved in the pathogenesis of cardiovascular disease. Selenium (Se) may exert an anti-atherogenic influence by reducing oxidative stress. The richest known food source of selenium is the Brazil nut (*Bertholletia excelsa*) found in the Amazon region. **Objective**: To evaluate the effect of Brazil nut supplementation on plasma Se and glutathione peroxidase (GSH-Px) activity in HD patients and verify if the effects could be sustained after 12 months.

**Methods**: 81 HD patients (52.0 ± 15.2 yr, dialysis vintage 82.3 ± 91.4 mo, BMI of 24.9 ± 4.4 kg/m$^2$) were studied. 21 patients were followed 12 months without supplementation. All patients received 1 nut, average of 58.1 μg Se/g, a day for three months. Se concentration in nuts and in plasma was determined by atomic absorption spectrophotometry with hydride generation and the levels of GSH-Px were measured by using commercial kits.**Results**: Plasma Se (17.3 ± 19.9 μg/L) was below normal (60 - 120μg/L) before nut supplementation. After 3 months of supplementation, plasma Se increased to 106.8 ± 50.3 μg/L ($p< 0.0001$), and after 12 months it decreased to 31.9 ± 14.8 μg/L ($p< 0.0001$). The activity of GSH-Px also increased after supplementation from 46.6 ± 14.9 to 55.9 ± 23.6 U/gHb ($p<0.0001$). Before supplementation, 11% of patients had GSH-Px activity below normal range (27.5

– 73.6 U/gHb) and after supplementation, all patients showed GSH-Px activity within the normal range.**Conclusion**: HD patients were Se deficient and consumption of only one brazilian nut per day (5 g) during 3 months was effective to increase Se and GSH-Px parameters. Selenium levels 12 months after the supplementation period were not as low as pre-supplementation levels but yet significantly lower.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2666**

**Impact of Intradialytic Low-to Moderate Intensity Exercise Training on Cellular Inflammation** Christof Ulrich,[1] Henning Theissinger,[1] Roman Fiedler,[1] Sylvia Hanika,[1] Axel Schlitt,[2] Eric Seibert,[1] Matthias Girndt.[1] *[1]Internal Medicine II, Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany; [2]Internal Medicine III, Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany.*

Exercise training strengthens the performance of haemodialysis patients and appears to lower their high cardiovascular risk burden. We speculated that improvement of cardiovascular outcome by exercise training may partially be attributed to a reduction of cellular inflammation to which the inflammatory CD16+ monocyte subset is linked.

21 pts were enrolled in a low-to-moderate intradialytic exercise program, 14 of whom (49.8±8.0 years) were willing to take part in cycling exercises (EX) thrice a week. 7 pts served as controls (CO: 54.0±10.5 years). Serum parameters were analysed at baseline (t0), 4 (t4), 8 (t8) and 12 (t12) weeks. Monocyte subsets, CD14++CD16- (Mo1), CD14++CD16+ (Mo2), CD14(+)CD16+ (Mo3) were determined by flow cytometry at t0, t8 and t12. Analysis was performed using Repeated Measures ANOVA.

The mean cycling time exceeded 30 min (t4:38.8±9.4; t8:41.3±13.9; t12:41.1±15.8). The average cycling power capacity increased over time (t4:18.7±6.9; t8:21.0±10.5; t12:27.0±13.0 (watt), $p<0.01$ (t4 vs. t12). CRP was not different in both groups. Phosphate and potassium serum levels did not change; calcium levels significantly increased in EX (t0:2.2±0.2; t8:2.3±0.2; t12:2.4±0.1, $p<0.001$, t0 vs. t12]. Mean blood pressure slightly decreased in EX (t0:90.8±15.3; t4:89.9±12.5; t8:86.5±11.0; t12:87.4±13.0, $p=0.06$), and remained unchanged in CO (t0:96.2±11.6; t4:96.7±10.3; t8:94.1±8.8; t12:94.8±8.3, $p=0.33$). Although Mo3 values tended to be lower in CO (%Mo3; t0:17.1±7.4; t8:12.0±8.3; t12:12.7±9.2; $p=0.24$), the frequency of inflammatory Mo3 only significantly declined over time in EX (%Mo3; t0:23.1±11.2; t8:16.0±4.6; t12:12.5±6.9; $p<0.001$, t0 vs. t12).

Our data indicate a trend in blood pressure lowering and therefore improvement of the cardiovascular risk profile by exercise training. We observe a trend for reduction of cellular inflammation, however, a potential decrease of CD16+ monocytes remains to be confirmed after longer exercise training.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2667**

**Ethylene-Vinyl Alcohol Copolymer Dialyzer Membrane Reduces Protein Oxidation in Hemodialysis Patients** Yoshihiro Matsumoto,[1] Hiromachi Kumagai.[2] *[1]Nephrology, Shizuoka City Hospital, Shizuoka, Japan; [2]University of Shizuoka, Shizuoka, Japan.*

*Background:* Oxidative stress has been implicated in the cardiovascular complications that affect hemodialysis (HD) patients. Ethylene-vinyl alcohol copolymer (EVAL) dialyzer membrane induces less production of reactive oxygen species compared to conventional dialyzers. We evaluated the impact of EVAL membrane on plasma protein oxidation in HD patients.

*Methods:* HD patients treated with cellulose triacetate (CTA) dialyzers were selected. In the first study performed in a 2-month crossover design alternating between CTA and EVAL, nonmercaptalbumin (HNA) and advanced oxidation protein products (AOPP) levels were measured in the predialysis blood from 10 subjects. In the second study, predialysis plasma myeloperoxidase (MPO) levels were measured before and after a 2-week EVAL treatment on 12 patients.

*Results:* Plasma AOPP levels were significantly reduced after a 2-month EVAL treatment and increased again after CTA treatment, although the HNA proportions were not affected significantly by the change in dialyzer membranes. The following study, a 2-week EVAL treatment, showed the decrease in MPO levels immediately before HD.

*Discussion:* The frequent use of EVAL dialyzers has been shown to reduce protein oxidation, possibly through the suppression of circulating phagocytes. This novel biocompatible dialyzer is expected to protect cardiovascular mortality in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2668**

**Increasing Continuous Renal Replacement Therapy Intensity in Septic Patients with Acute Kidney Injury: A Single-Center Randonmized Clinical Trial** Ping Zhang, Lina Shao, Jing Yuan, Jianghua Chen. *The Kidney Disease Center, The First Affiliated Hospital, Medical College, Zhejiang University, Hangzhou, Zhejiang, China.*

Acute renal injury (AKI) is an important complication in septic patients and is an independent predictor of mortality. However the optimal renal replacement therapy intensity and timing in septic patients with AKI is still unclear. We randomly assigned septic patients with AKI to continuous replacement therapy (CRRT) with high volume hemofiltration (50ml per kilogram per hour ,HVHF group) or extra high volume hemofiltration (85ml per kilogram per hour , extra high volume group, EHVHF group).*The primary study outcome was death from any cause within 28 day, 60 days and 90days after randomization.* Of the 280 patients enrolled patients, 141 was assigned to the EHVHF group and 139 to the HVHF group. Data were available for 230 patients, 114 patients in EHVHF group and 116 in HVHF group. The two groups had similar baseline characteristics and received the study treatment for average of 10.9 and 10.8 days respectively ($p=0.95$). At 28 days after randomization, 59 patients (51.75%) died in the EHVHF group and 57 (49.13%) in the HVHF group. At 9o days after randomization, 64 patients died in the EHVHF group (56.14%) and in the HVHF group (55.17%) respectively The probability of death in 90days was also similar between the two groups by Kaplan –Merier method ($p=0.942$). No differences were found of renal outcome in survivors to 90d after randomization between the two groups ($p=0.374$). Inotropic support of norepinephrine, time in hospital more than 7 days, Blood platelet count less than 8*10E9/L, APACHE II score >25 and total bilirubin >170 umol/L before randomization were the independent risk in 90d survival after renal replacement for sepsis and AKI patients. In septic patients with AKI, increasing intensity of CRRT didn't improve the survival at 28 days and 90 days.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2669**

**Evolution of Endothelial Injury on Dialysis and after Kidney Transplantation** Keren Mandelzweig,[1] Claudio Rigatto,[2] Lisa M. Miller,[2] Manish M. Sood,[2] Paul Komenda,[2] David N. Rush,[2] Joe A. Bueti.[2] *[1]Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada; [2]Section of Nephrology, Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada.*

Cardiovascular disease progresses on dialysis and improves after renal transplantation. Endothelial injury (EI) may play a role, but changes in EI over time on dialysis and post-transplantation are poorly characterized. We measured soluble Vascular Cell Adhesion Molecule-1 (VCAM), a validated measure of EI, in a retrospective cohort of kidney transplant patients between Jan. 1, 2000 and Dec. 31, 2005 (n=186). Patients with a minimum of 2 serum samples drawn at least 6 months apart in both the pre and post transplant periods were analyzed (n=172). Data abstraction was by chart review. VCAM was measured by ELISA. Mixed linear modeling was used to analyze changes in VCAM as a function of time and transplantation, adjusted for age, gender and several time dependent variables (co-morbidities, dialysis modality, medications and heparin induced antibodies (HIA)).

VCAM levels increased progressively on dialysis (49 ng/mL per year, $p<0.0001$). Transplantation was associated with a large decrease in VCAM (-664 ng/mL, $p<0.0001$ within one month). VCAM levels continued to decline in the 24 months post transplantation (143 ng/mL per year, $p<.0001$). Adjustment for all confounders did not alter these relationships. Medications and HIA levels were not associated with VCAM. Age and heart failure were associated with higher VCAM levels; however, these variables did not predict change in VCAM slope before or after transplant (i.e. all variable*time interactions=NS). VCAM pre transplant was a predictor of death ($p=.04$) and the composite outcome of death and vascular events ($p=.03$). In conclusion, EI worsens over time on dialysis and significantly improves post renal transplantation. These longitudinal repeated measures data confirm less rigorous cross-sectional observations, and may partly explain the elevated CVD rate in dialysis and the salutary effect of transplantation. We speculate that components of dialysis not measured in our analysis drive EI in ESRD.

Disclosure of Financial Relationships: nothing to disclose

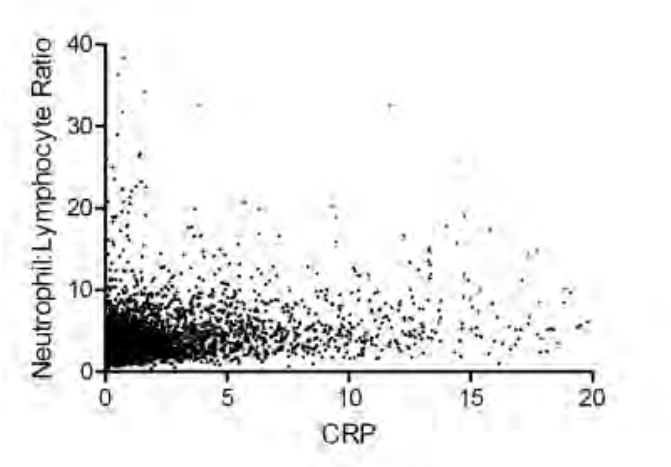
**SA-PO2670**

**Neutrophil to Lymphocyte Ratio, White Blood Cell Count, and Ferritin Are Not Validated Surrogates for Inflammation in End-Stage Renal Disease Patient** Mahesh Krishnan, Carey Colson, Steven M. Wilson, Robert Provenzano. *DaVita Inc., Denver, CO.*

Background: Despite the fact that patients on dialysis have significant amounts of inflammation, the gold standard test, C reactive protein (CRP) is not routinely monitored. As a result, epidemiologic studies have attempted to use surrogates such as Neutrophil to Lymphocye ratio (NLR), white blood cell count (WBC) and ferritin. To date, these surrogates have not been validated but continue to be widely used in analyses. Using a large US dialysis provider's database, we sought to complete this validation.

Methods: CRP values from 4,565 dialysis patient samples collected between 2007 and 2009 were correlated with same day NLR , absolute WBC count and ferritin. Correlation coefficient was determined by Pearson's for each value

Results: CRP values did not correlate with NL ratio ($r2 = 0.07$; see Figure), WBC ($r2 = 0.10$) or ferritin ($r2 = 0.02$).




Conclusions: NLR, WBC and ferritin were not validated as surrogates for inflammation (through CRP values) in a large number of ESRD patient samples. These values should not be used to adjust for inflammation in prospective or retrospective studies in dialysis patients. Further work should be done to find better surrogates for inflammation in ESRD patients until CRP or similar testing is more routinely used.

Disclosure of Financial Relationships: Employer: DaVita Inc; Other Relationship: Previous Employer Amgen Inc. (until July 2009).

**SA-PO2671**

**Pentraxin 3 Is a Useful Marker of the Microinflammatory Response during a Single Hemodialysis Session** Tae Yamamoto,[1] Marcelo M. Nascimento,[1,3] Shirley Yumi Hayashi,[1,2,3] Abdul Rashid Tony Qureshi,[1] Jacek Waniewski,[1] Björn Anderstam,[1] Astrid Seeberger,[1] Britta Lind,[2] Bengt Lindholm.[1] *[1]Renal Medicine and Baxter Novum, Karolinska Institute, Stockholm, Sweden; [2]Medical Engineering, Royal Institute of Technology, Stockholm, Sweden; [3]PUCP, Curitiba, Brazil.*

The acute impact of the hemodialysis (HD) procedure on cardiovascular disease (CVD) risk markers such as circulating cytokines and proteins involved in vascular calcification is not fully known. Therefore we studied changes in circulating CVD risk markers during a single HD session, and examined putative associations with carotid intima-media thickness (IMT).

We enrolled 44 clinically stable patients (median age 56 years; 64% males) who were treated by conventional HD with cellulose acetate membrane, three times a week (3.5-4 hours per session) and measured circulating markers of *inflammation* (CRP, IL-6, TNF-alpha and PTX3), *vascular calcification* (FGF23, OPG and fetuin-A) and *oxidative stress* (8-OHdG) before and after a single session of HD. Protein concentrations were corrected for ultrafiltration during HD. IMT was measured by ultrasonography.

Whereas small molecules decline during HD (e.g., 8-OHdG from 0.26 to 0.16 ng/ml; $p<0.001$), the pattern of changes of larger not dialyzable substances differs markedly: Markers related to *vascular calcification* did not change (fetuin-A) or decreased (OPG and FGF23), and among *inflammation markers*, hsCRP was unchanged and TNF-alpha decreased. In contrast, the median IL-6 increased by 16 % from 4.9 to 5.6 pg/ml ($p<0.05$) and the median PTX 3 increased by 45 % from 4.5 to 7.1 ng/ml ($p<0.001$). Changes in PTX3 and IL-6 were observed in patients with normal IMT, while in patients with abnormal IMT who had already high values of PTX3 and IL-6, the concentrations did not change significantly during HD. Among the measured risk markers, OPG, IL-6 and hsCRP levels were associated with IMT.

We conclude that a single HD session resulted in increased levels of IL-6 and PTX3 whereas CRP did not change. IL-6 and especially PTX3 could be valid markers to monitor the intradialytic inflammatory response during HD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2672**

**Decrease in Frataxin (FRX) Is Linked to Oxidative Stress in Patients on Maintenance Hemodialysis (MHD)** Yukiko Hasuike, Hiroshi Nonoguchi, Yoshinaga Otaki, Takanori Nagai, Masayoshi Nanami, Takahiro Kuragano, Takeshi Nakanishi. *Internal Medicine, Division of Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.*

**Introduction**. The mitochondrial (Mito) protein, FRX, regulates iron metabolism in Mito and attenuates iron-mediated oxidative damage. FRX is reduced in neuro- and cardio-degenerative disease, Friedreich's ataxia. We have already reported the dysregulation of iron metabolism in polymorphonuclear leukocytes (PMNLs), from MHD patients. To clarify whether FRX is affected and linked to oxidative stress in MHD, the association of FRX and several parameters related to uremic status were investigated. **Methods**. 17 MHD patients and 15 healthy controls were recruited in the study. Relative quantitative polymerase chain reaction was used to measure frataxin messenger RNA (mRNA) in PMNLs and FRX content was semiquantified by means of Western blot analysis. Serum albumin (Alb) and several parameters of iron metabolism, inflammation, (interleukin-6 [IL-6], tumor necrosis factor-α [TNF-α], high sensitivity-C reactive protein [hCRP]), malondialdehyde (MDA) as a marker of oxidative stress and dose of erythropoietin were determined. **Results**. FRX mRNA levels in PMNLs from MHD patients were significantly lower compared with those in healthy subjects (54.5%). Western blot analysis confirmed that content of the corresponding protein paralleled that of the mRNAs (65.0%). Serum MDA levels as well as markers of inflammation were significantly elevated in MHD. FRX mRNA levels were significantly associated with serum iron, TNF-α, IL-6, hCRP, MDA and Alb. In stepwise multiple regression analyses, MDA was only selected as an independent predictor of FRX in PMNLs ($R^2=0.661$, $p<0.0001$). **Discussion**. Decrease in FRX content could cause the acceleration of oxidative stress in MHD patients, which might be related to the dysregulation of iron metabolism in Mito.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2673**

**Growth Arrest Specific Gene 6 (Gas6) Levels Are Elevated in Chronic Hemodialysis Patients** Iris J. Lee,[1] Gautam S. Choure,[1] Brijesh S. Bodiwala,[1] Brendan A. Hilliard,[2] Jesse M. Goldman,[1] Jean Lee,[1] Philip L. Cohen.[2] *[1]Section of Nephrology, Temple University, Philadelphia, PA; [2]Section of Rheumatology, Temple University, Philadelphia, PA.*

Purpose: Gas6 is a vitamin-K dependent protein with diverse biologic functions. Animal and in vitro studies demonstrate a role for Gas6 in mediating endothelial and vascular smooth muscle cell function, atherosclerosis and vascular calcification. Gas6 also has multiple functions in leukocyte biology and inflammation. Little is still known about the role of Gas6 in humans, and no data exists on Gas6 levels in hemodialysis patients. Given the increased prevalence of vascular disease and chronic inflammation in the ESRD population, we investigated whether Gas6 plasma levels are altered in patients on chronic hemodialysis.

Methods: Blood samples were obtained from 23 normal subjects and 53 chronic hemodialysis patients from a university affiliated dialysis center. Patients with known malignancy, coagulation disorders, or on warfarin therapy were excluded. Pre-dialysis blood was collected and Gas6 quantified in plasma by ELISA.

Results: Plasma samples from 52 chronic dialysis patients were compared to 23 healthy normal subjects. The mean Gas6 level in normal subjects was 36.23ng/ml +/- 18.33 and the mean Gas6 level in dialysis patients was on average 3 fold higher 102.8 +/- 35.19, ($p<.0001$). The dose of intravenous iron was directly associated with higher Gas6 levels. ($r=.33$; $p=.0138$) Conversely, albumin levels were inversely associated with Gas6 levels. ($r=-.34$; $p=.0121$) In a multivariate analysis, albumin and iron dose were found to be independently associated with Gas6. There was no significant correlation found between Gas6 levels and hemoglobin, Epogen™ dose, ferritin, PTH, Ca, Phos, Kt/V, presence of DM, HTN and CAD.

Conclusions: This is the first report of Gas6 levels in hemodialysis patients. Gas6 may be increased in dialysis patients secondary to chronic inflammation. Low albumin and higher IV iron administration was associated with higher Gas6 levels, suggesting a possible connection between inflammation, oxidative stress mediated by iron and development of vascular disease in dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2674**

**Serum Calprotectin Increases with Time in Haemodialysis Patients: An Important New Factor in the Modulation of Response to Inflammatory Stress** Laura E. A. Harrison,[1] Ruth J. Pepper,[2] James O. Burton,[1] Alan D. Salama,[2] Chris W. McIntyre.[3] *[1]Department of Renal Medicine, Royal Derby Hospital, United Kingdom; [2]Renal Section, Imperial College London, United Kingdom; [3]School of Graduate Entry Medicine and Health, University of Nottingham, United Kingdom.*

Haemodialysis (HD) patients suffer a complex of metabolic stress, systemic inflammation and dialysis induced end-organ ischaemia resulting in elevated cardiovascular mortality. Interactions within this pathophysiology are still poorly characterised. Calprotectin (MRP8/14), a protein expressed in neutrophils, is implicated in modulation of the inflammatory response. Previous study has identified elevated calprotectin levels in prevalent HD patients. We aimed to study prospective changes in calprotectin levels and the associated cardiovascular risk 'phenotype' in HD patients.

50 established HD patients were studied at baseline and 12 months later. Dialysis details and blood samples were taken pre-HD. Serum calprotectin (sCAL) was quantified with ELISA and endotoxin (ET) (resulting from presumed gut translocation and indicative of non-infective pro-inflammatory stress) by LAL assay.

At study inclusion, pre-HD sCAL level was 2961[IQR 2383-6206]ng/ml, with 34% of patients having undetectable levels. Only 28% had sCAL levels >3500ng/ml (clinically relevant cutoff cf healthy controls). There were no significant differences in sCAL levels between patients with or without diabetes or cardiovascular comorbidities and no significant correlations with systemic inflammation as measured by serum hsCRP or IL6.

A year later, median sCAL levels had significantly increased to 6874 [IQR 3693-11097] ng/ml ($p<0.05$), no patients had undetectable levels, and the proportion of patients with levels >3500ng/ml increased from 28% to 78%. Serum ET from presumed gut translocation (in the absence of clinically apparent infection) demonstrated a degree of significant correlation with sCAL in survivors.

Calprotectin levels increase with time in HD patients and appear in part to be associated with non-infective dialysis induced inflammatory stress. The role of regulation of pro-inflammatory neutrophils and macrophages in these processes awaits further elucidation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2675**

**Osteopontin Levels Are Elevated and Related to Bone, Vascular and Inflammatory Markers in Pediatric Dialysis Patients** Douglas M. Silverstein,[1] Poyyapakkam Srivaths,[2] Stuart Goldstein.[3] *[1]Pediatric Nephrology, Children's National Medical Center, Washington, DC; [2]Pediatric Nephrology, Baylor College of Medicine, Houston, TX; [3]Pediatric Nephrology, Cincinnati Children's Hospital Medical Center, Cncinnati, OH.*

Osteopontin (OPN) is an extracellular matrix cell adhesion protein that is expressed the bone, kidney and vasculature. OPN plays a role in bone turnover, immunity, and vascular modeling. We assessed serum OPN levels in 16 children (16.6±3.3 years) receiving maintenance hemodialysis (HD). The mean OPN level was 1099.0±331.8 (range 116.5-4603) ng/ml. Only two patients exhibited OPN levels <200 ng/ml while seven had levels >500 ng/ml. OPN levels were unrelated to either spKt/V (r= -0.01; p=0.9) or dialysis vintage (r=0.15, p=0.5). Serum intact parathyroid hormone (iPTH) and vascular cell adhesion molecule-1 (sVCAM-1) levels were significantly higher in patients with OPN levels >420 ng/ml. OPN levels were positively associated with markers of bone turnover (iPTH: r=0.77; p=0.0005), vascular disease (sVCAM-1: r= 0.65; p=0.006 and sE-Selectin: r= 0.76; p=0.0006) and inflammation (TNF-alpha: r= 0.52; p=0.04).

| PARAMETERS | CORRELATION (r) | p VALUE |
|---|---|---|
| Intact PTH | 0.77 | 0.0005 |
| Serum Calcium | 0.25 | 0.3 |
| Serum Phosphorous | 0.39 | 0.1 |
| sVCAM-1 | 0.65 | 0.006 |
| sE-Selectin | 0.76 | 0.0006 |
| sICAM-1 | -0.12 | 0.7 |
| TNF-alpha | 0.52 | 0.04 |

We conclude that serum OPN levels are elevated in pediatric ESRD patients and correlate with markers of bone turnover, vascular disease and inflammation. OPN therefore seems to be another important link in the bone-vascular-inflammation disease axis in ESRD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2676**

**Serum Zinc Concentrations Is Negatively Correlated with Electronegative LDL in Hemodialysis Patients** Julie Lobo,[1] Milena Barcza Stockler-Pinto,[1] Luciana Nicolau Aranha,[2] Najla Elias Farage,[1] Viviane Oliveira Leal,[2] Dulcinéia Saes Parra Abdalla,[3] João Paulo Torres,[1] Denis Fouque,[4] Denise Mafra.[2] *[1]Federal Univ of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Federal Univ Fluminense, Niterói, Brazil; [3]Univ of São Paulo, São Paulo, Brazil; [4]University Claude Bernard Hopital Edouard Herriot, France.*

Oxidative modification of low-density lipoprotein (LDL) plays a key role in the pathogenesis of atherosclerosis. The generation of minimally oxidized LDL, also called electronegative LDL [LDL(-)], is present in plasma at higher concentrations in diseases with high cardiovascular risk, such as chronic kidney disease (CKD). Zinc is an essential micronutrient that can function as an antiinflammatory and antioxidative agent, and as such, it may have atheroprotective properties. However, patients with CKD often exhibit zinc deficiency.Our aim was to investigate the relationship between LDL(-) levels and plasma zinc levels in hemodialysis (HD) patients. **Methods:** The levels of LDL(-), zinc and Tumos necrosis factor-alpha (TNF-α) were measured in 50 patients undergoing HD (31M/19F) from RenalCor Clinic, Rio de Janeiro, Brazil [age 54.3 ± 12.6yr, on dialysis for the mean period of 49.7 ± 46.5 months and Body mass index (BMI) 24.4 ± 4.1 Kg/m2)] and compared to 21 healthy subjects (HS) (9M/12F) (age 50.7 ± 15.7yr, BMI *25.5 ± 4* Kg/m$^2$). LDL(-) was measured by ELISA, zinc by atomic absorption spectrophotometry and TNF-α by a multiplex assay kit. **Results:** HD patients presented higher LDL(-) levels (0.18 ± 0.12 U/L) when compared to HS (0.11 ± 0.92 U/L) (p=0.02) and TNF-α was also higher in patients (5.9 ± 2.1 pg/ml) than in HS (2.49 ± 1.1 pg/ml) (p=0.0001). While zinc levels were significantly lower in HD patients (54.5 ± 16.3 µg/dL) than in HS (78.4 ± 9.4 µg/dL) (p=0.0001), 80% of the patients had zinc levels below normal values. We found a negative correlation between LDL(-) and zinc levels in the patients (r=0.43; p=0.0001). **Conclusion:** In the present study, HD patients showed significantly increased levels of LDL(-) and TNF-α, and decreased levels of zinc than HS. Zinc deficiency can contribute to the development of atherosclerotic cardiovascular disease in these patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2677**

**Vascular Endothelial Growth Factor Increase Differentially Regulates Cytokines in End Stage Renal Disease** Vinod K. Bansal,[1] Debra Hoppensteadt,[2] Evangelos Litinas,[2] Indermohan Thethi,[2] Jawed Fareed.[2] *[1]Department of Nephrology, Loyola University Medical Center, Maywood, IL; [2]Department of Pathology, Loyola University Medical Center, Maywood, IL.*

Vascular endothelial growth factor (VEGF) mediates nitric oxide dependent angiogenic effects contribute to the inflammatory responses. VEGF is upregulated in ESRD and contributes to the cardiovascular dysfunction in ESRD. VEGF is also upregulated by erythropoietin which is commonly used in the management of ESRD patients. With the availability of BioChip Array Technology , a multiparametric screening of inflammatory mediators/cytokines can be measured and the relevance of VEGF can be established. Utilizing the Randox Biochip technology, we profiled the plasma levels from 53 ESRD patients to determine their relevance to VEGF levels. Pre-dialysis samples from 53 male and female ESRD patients on maintenance hemodialysis were analyzed using a cytokine biochip for VEGF, IL2, IL4, IL6, IL8, IL10, IFNG1, TNF, IL1, IL1, MCP-1 and EGF were analyzed. In addition, NO and asymmetric dimethyl arginine (ADMA) levels were also measured. A group of 50 normal healthy males and females constituted the control group. The circulating levels of VEGF and mediators were compared with the normals. In addition, VEGF levels were compared with the other mediators. In comparison to the normals, ESRD patients exhibited decreased levels of IL4, IL8, TNF and EGF which ranged from 20-210%. Increased levels of IL2, IL6, IL10, IFNG1, IL1 and MCP-1 were noted (20-350%). NO levels were also increased, however ADMA remained unchanged. VEGF levels showed wide variation, however on a cumulative basis a 30-210% increase in VEGF was noted. Except for the MCP-1, the correlation of VEGF with other inflammatory markers was relatively poor (r2=<0.25). These results show that ESRD patients can be profiled for various inflammatory mediators, VEGF levels were markedly increased, however only MCP-1 levels correlated well with the increase in VEGF levels. Additional studies are warranted to clarify the regulatory role of VEGF on inflammatory cytokines in ESRD.αβ®ααα

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2678**

**Vascular Endothelial Growth Factor Receptor 2 (VEGF-R2) Is Responsible for the Downstream Signaling Effects Attributed to the beta-Common Receptor (βC-R)** Jennafer Hochmuth, Laura Sautina, Mark S. Segal. *Department of Medicine, University of Florida, Gainesville, FL.*

We have previously demonstrated that the heterodimeric erythropoietin (EPO) receptor and the beta-common receptor (βC-R) are required for EPO-mediated endothelial progenitor cell migration via a nitric oxide (NO) dependent mechanism. Investigation into the mechanism revealed a novel interaction between the βC-R and VEGF-R2 and demonstrated that the stimulation of NO is not dependent on VEGF but is instead dependent on VEGF-R2. Examination of the literature demonstrates that many of the downstream activities of the βC-R and VEGF-R2, such as differentiation, proliferation, and inflammation, are identical. We tested the hypothesis that all of the downstream activities putatively mediated by the βC-R require and are mediated via VEGF-R2. To test this hypothesis we knocked-down expression of the βC-R or VEGF-R2 in HCAECs and studied the effect on tubulogenesis, migration, NO production, proliferation, and inflammation in response to EPO or VEGF stimulation.

We have found a significant increase in inflammatory cytokines in endothelial cells stimulated with high EPO doses (50 mU/mL), however we hypothesize that this is due to EPO-R's interaction with VEGF-R2. Since the km of the homodimeric EPO receptor is 10-fold less than the heterodimeric EPO-R/βC-R, 5 mU/mL versus 50 mU/mL respectively, we examined the downstream effects of low (10 mU/mL) and high (75 mU/mL) EPO doses.

An increasing number of clinical trials have shown that when higher doses of erythropoietin are used in chronic kidney disease patients there is an increase in the number of cardiovascular complications. In addition, high doses of EPO given to breast cancer patients lead to an increased mortality. Interaction and cross-stimulation of EPO-R, βC-R, and VEGF-R2 could explain these adverse outcomes. We believe that understanding each receptor's role will be helpful in correcting and ultimately avoiding adverse side effects and complications attributed to EPO therapy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2679**

**Association of the Metabolic Syndrome with Periodontal Disease in Hemodialysis Patients** Li-Ping Chen,[1] Chih-Kang Chiang,[2,3] Kuan-Yu Hung.[3] *[1]Department of Dentistry, Chang Gung Memorial Hospital, Taipei, Taiwan; [2]Department of Integrated Diagnostics & Therapeutics, National Taiwan University Hospital, Taipei, Taiwan; [3]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan.*

Metabolic syndrome and periodontitis both have an increasing prevalence worldwide; however, limited information is available on their association.

Objective: The objective of the study was to assess the association between periodontitis and the metabolic syndrome in a hemodialysis outcome cohort.

Design, Setting, and Participants: Data analysis from the Manutrition and Inflammation Cachexia Syndrome Survey on 253 hemodialysis patients who received periodontal examination was studied.

Main Outcome Measures: Association of diagnosis and extent of microbial dental plaque burdern and periodontitis (gingival bleeding and probing pocket depths by Plaque Index, Gingivial Inedx and Periodontal Disease Index) with the metabolic syndrome and its individual component conditions (central obesity, hypertriglyceridemia, low high-density lipoprotein-cholesterol, hypertension, and insulin resistance) were measured. Adjustment for age, sex, social economical status, general conditions, and smoking were considered.

Results: The prevalence of the metabolic syndrome was 46.2%, and 65.1% among individuals with no-mild and moderate-severe periodontitis, respectively. After adjusting for confounders, hemodialysis patients suffering from moderate to severe periodontitis were 2.03 times (95% CI 1.20–3.448) more likely to have the metabolic syndrome than unaffected and mild periodontitis individuals. Diagnosis of metabolic syndrome increased by 1.56 times (95% CI 1.12–2.16) in increasing severity of gingival inflammation and 1.72 times (95% CI 1.13–12.61) in increasing microbial dental plaque on tooth surface.

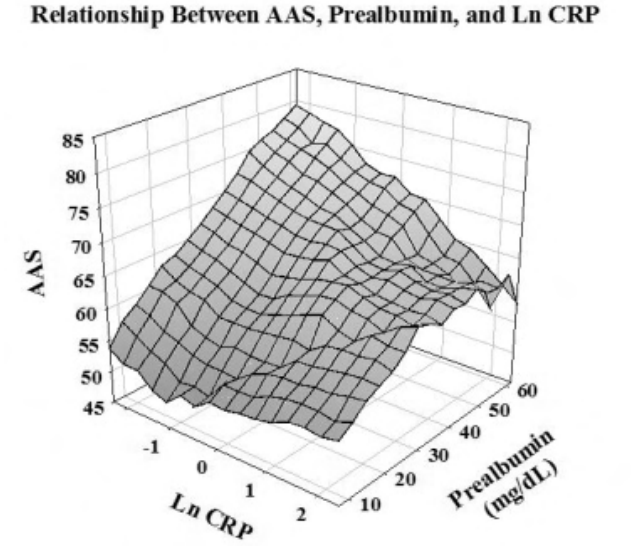
Conclusions: Moderate to severe periodontitis is associated with metabolic syndrome in hemodialysis patients. Further studies are required to test whether improvements in oral health lead to reductions in cardiometabolic traits and the risk of metabolic syndrome.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2680**

**Association of Self Reported Physical Activity with Laboratory Markers of Nutrition and Inflammation: The Comprehensive Dialysis Study** Shuchi Anand,[1] George A. Kaysen,[2] Kirsten L. Johansen,[3] Barbara A. Grimes,[3] Manjula Kurella Tamura,[1] Glenn M. Chertow.[1] *[1]Nephrology, Stanford University School of Medicine, Palo Alto, CA; [2]Nephrology, University of California, Davis, Davis, CA; [3]Nephrology, University of California, San Francisco, San Francisco, CA.*

Persons on dialysis maintain extremely low levels of physical activity. Prior studies have demonstrated a direct correlation between nutrition and physical activity but provide conflicting data on the link between inflammation and physical activity. Using a cohort of patients new to dialysis from the Comprehensive Dialysis Study, we examined the correlations among laboratory markers of nutrition and inflammation and physical activity. Baseline laboratory measures--albumin, prealbumin, C reactive protein (CRP) and alpha-1-glycoprotein (AAG)--were collected in a subset of participants who also provided estimates of physical activity using the Human Activity Profile. We present univariate correlation and multivariable analyses adjusting for age, sex, race/ethnicity, diabetes and center. The mean age (n=201) was 61 years. The adjusted activity scores (AAS) and maximal activity scores (MAS) were below the 10th percentile in comparison to healthy 60 year-old volunteers. Both activity scores were directly correlated with albumin (r2=0.3) and prealbumin (r2=0.3), and inversely correlated with CRP (r2=-0.2) and AAG (r2=-0.1). In multivariable analyses activity scores showed a significant direct correlation with prealbumin, and an inverse correlation with CRP.




The magnitude of correlation was modest; prealbumin higher by 0.3 mg/dL or CRP lower by 40% corresponded to a 1 point higher activity score. Our analyses suggest that dialysis patients with evidence of malnutrition or inflammation are likely to report lower levels of physical activity.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2681**

**Association of Dietary Omega-6 to Omega-3 Ratio and Inflammation in Maintenance Hemodialysis Patients** Nazanin Noori,[1] Sameer B. Murali,[1] John J. Sim,[4] Rachelle Bross,[1] Deborah A. Benner,[2] Allen R. Nissenson,[2] Joel D. Kopple,[1] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, Harbor-UCLA Medical Center, Torrance, CA; [2]DaVita, Lakewood, CO; [3]Salem VA, Salem, VA; [4]Nephrology, Kaiser Permanente, Los Angeles, CA.*

**Background :** Chronic inflammation, as shown by higher levels of C-reactive protein (CRP) is present in maintenance hemodialysis (MHD) patients with protein-energy wasting (PEW). It is not known whether diet has a bearing on inflammation. Many MHD patients eat fast food, which is known to have an unfavorably high ratio of omega-6 to omega-3 poly-unsaturated fatty acids (PUFA) and which may activate pro-inflammatory cascades. **Methods:** Using 3-day dietary record supplemented with dietary interview we examined the correlation of serum CRP changes during the first year and the estimated dietary omega-6 to omega-3 PUFA ratio at the start of the cohort of 145 MHD patients. **Results:** Higher omega-3 intake per se was associated with a non-significant trend towards decreased serum CRP. However, a higher omega-6 to omega-3 PUFA ratio was associated with a significant increase in serum CRP over 12 months, after adjustment for case-mix (age and gender), diet (energy, saturated fatty acids, trans fat, cholesterol and fiber intakes), body mass index, and history of hypertension (HTN) (r=0.20, p=0.03) (see Table).

| | Correlation (r) | p-value |
|---|---|---|
| Age and gender adjusted | 0.16 | 0.09 |
| Case-mix + diet adjusted | 0.19 | 0.04 |
| Previous + BMI + HTN adjusted | 0.20 | 0.03 |

Cubic spline analysis confirmed that higher omega-6 to omega-3 PUFA ratio was associated with likelihood of increase in CRP by at least 5 mg/L. **Conclusions:** Higher omega-6 to omega-3 PUFA intake ratios are associated with worsening inflammation in MHD patients, even after adjustments for other dietary components. These findings support the conclusion that dietary modulation of inflammation is possible in dialysis patients, a hypothesis which requires a randomized controlled trial to confirm.

Disclosure of Financial Relationships: nothing to disclose

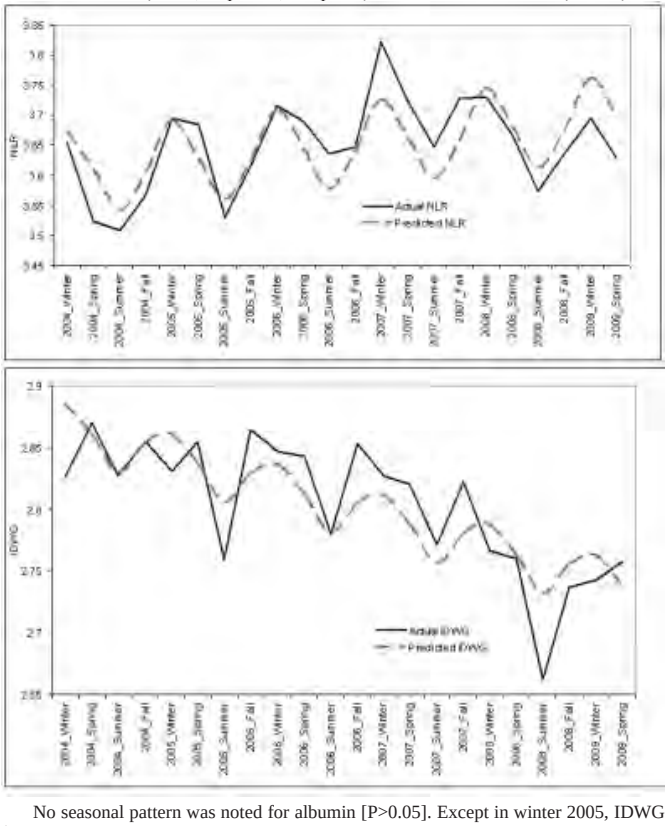
**SA-PO2682**

**Seasonal Variability in Inflammation and Interdialytic Weight Gain in Chronic Hemodialysis Patients** Len A. Usvyat,[1] Jeroen Kooman,[2] Frank Van der Sande,[2] Georges Ouellet,[1] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Renal Research Institute, NY, NY; [2]Maastricht University Medical Centre, Maastricht, Netherlands.*

In the hemodialysis (HD) population, blood pressure and body temperature follows circannual rhythm. However, it is unknown whether inflammatory parameters and intradialytic weight gains (IDWG) also follow a seasonal pattern.

We reviewed records of HD patients (pts) treated in RRI clinics between Apr 1, 2004 and Mar 31, 2009. Only pts with at least one treatment in each of the four seasons were included. Seasons were defined on a calendar basis: winter as Dec through Feb, spring as Mar through May, summer as June through Aug, fall as Sep through Nov. Neutrophil-to-lymphocyte ratio (NLR), albumin, and IDWG were recorded as averages per pt per month and per season. Cosinor analysis was conducted to test for seasonality in NLR, albumin, and IDWG.

In total 10,303 patients were studied (55% male, 49% black, 41% white, 49% diabetic, avg age [stdev]: 60.5 [15.5] years). Cosinor analysis over a five year period demonstrated a seasonal component of NLR with highest NLR in winter and nadir in summer. Coefficients of seasonal factors (mesor, amplitude, acrophase) were different from zero (P<0.05).




No seasonal pattern was noted for albumin [P>0.05]. Except in winter 2005, IDWG followed a cyclical pattern with highest IDWG in winter and lowest in summer [cyclical coefficients p-values<0.10]. IDWG declined over the 5-year period on average by 0.024 kg per year, suggesting improved fluid control in RRI pts.

This study in chronic HD patients demonstrates a significant influence of seasons on NLR with highest inflammation in the winter and lowest in the summer. Albumin did not change over seasons potentially suggesting more food intake in the winter months; this interpretation is corroborated by analysis of IDWG.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2683**

**Hemodialysis Patients with Low and Moderate Level of Inflammation Show a Comparable Immune Response to Influenza Vaccine** Jaromir Eiselt,[1] Terezie Sedlackova,[2] Lukas Kielberger,[1] Jaroslav Racek,[2] Lada Malanova,[3] Petr Pazdiora.[4] *[1]Internal Dept. 1, Charles Univ., Plzen, Czech Republic; [2]Dept. of Biochemistry, Charles Univ., Czech Republic; [3]Dialysis Unit, B.Braun Avitum, Czech Republic; [4]Dept. of Epidemiology, Charles Univ., Czech Republic.*

Hemodialysis patients (HD) suffer from a defective host defense and show a reduced response to vaccines. The immune response to vaccinations in HD can be negatively influenced by inflammatory state.

Our multi-center study was designed to compare the antibody response to the influenza vaccine (Influvac 2009/10) in 35 controls without renal disease and with normal C-reactive protein (CRP) ≤3 mg/L (Controls), 64 HD with CRP ≤3 mg/L (HD LowCRP) and 58 HD with CRP ≥4 and <30 mg/L (HD HighCRP). All participants were in stable condition and had no clinical signs of infection. In addition to CRP, we examined markers of inflammation interleukin-6 (Il-6) and hepcidin. Immune reactivity was evaluated according to an anti-hemaglutinine titer (anti-HA) at baseline and 4 weeks after the vaccination. Seroconversion was defined as at least a 4fold rise in anti-HA. Results are summarized in the table.

Inflammation and response to influenza vaccine

| | Controls (n=35) | HD LowCRP (n=64) | HD HighCRP (n=58) |
|---|---|---|---|
| Interleukin-6 (pg/mL) | 0.89 (0.70-1.60)[a] | 4.08 (2.59-5.34)[b] | 7.09 (4.43-9.38) |
| Hepcidin (ng/mL) | 10 (8-19)[a] | 21 (10-34) | 23 (10-38) |
| A H1N1 seroconversion (%) | 74[c] | 36 | 52 |
| A H3N2 seroconversion (%) | 57 | 39 | 47 |
| B seroconversion (%) | 66[c] | 27[d] | 47 |

Data is median (interquartile range) or % of positive results; Mann-Whitney, Fisher and $\chi^2$ tests; [a]p<0.01 vs. HD LowCRP and HD HighCRP, [b]p<0.001 vs. HD HighCRP, [c]p<0.001 vs. HD LowCRP, [d]p<0.05 vs. HD HighCRP

Normal CRP in HD patient does not exclude substantial elevation of other markers of inflammation, as documented by high Il-6 and hepcidin in HD LowCRP group. The entire HD population displayed diminished immune reactivity to vaccine strains A H1N1 and B when compared to the controls. However, patients with low level of inflammation and those with elevation of markers of inflammation showed comparable immune response to influenza vaccine and both groups should be vaccinated.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2684**

**Catheter Use Associates with Reduced Inflammation and Erythropoetin Resistance in Maintenance Hemodialysis** Albert J. Power, Seema Singh, Kakit Chan, Peter Hill, David Taube, Neill D. Duncan. *Imperial College Kidney & Transplant Institute, West London Renal & Transplant Centre, Hammersmith Hospital, London, United Kingdom.*

Erythropoetin resistance [EPO-R] in hemodialysis associates with greater systemic inflammation. Central venous catheter [CVC] use without superadded infection has been associated with greater inflammation and EPO-R and recent data suggest higher EPO dosing may be harmful. We studied the effect of vascular access type on inflammation and EPO-R.

This retrospective cohort study examined 1163 patients established [>90d] on hemodialysis [HD] Sept 2009-Mar 2010 [7077 observations spanning 8077 patient months, AVF n=213, mean age 63.8±14.4yrs, spKt/V 1.92±0.36, Hb 12.2±1.2g/dL, EPO dose 0.63±0.51mcg/kg/wk]. Dialysis adequacy was targeted monthly to spKt/V≥1.6, weekly IV darbepoetin targeted to hemoglobin [Hb] 10.5-12.5g/dL. A measure of EPO-R, erythropoesis resistance index [ERI] was calculated: weekly dose/postdialysis weight/Hb. Data in the presence of active infection was excluded.

Median CRP was 0.7mg/dL [IQR 0.3-1.5mg/dL] and levels did not differ by vascular access type [p=0.3]. Higher CRP associated with peripheral vascular disease on univariate & multivariate analysis [p<0.001] but not with age, diabetes and other comorbidities, spKt/V or HD vintage.

Older age, female gender, lower CRP [p<0.001] & CVC use [p=0.01] associated with lower ERI on univariate as well as multivariate regression [all p<0.01]. ERI was not significantly associated with spKt/V, ethnicity, HD vintage and major comorbidities.

This large representative study demonstrates that age, gender and low levels of systemic inflammation influence EPO-R. CVC do not associate with higher inflammation and appear associated with lower EPO-R which may relate to low access-related infection rates. This important but apparently counterintuitive finding warrants further prospective study.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2685**

**Activation of Caspase-3 in the Skeletal Muscle during Hemodialysis** Parham Eftekhari, Dominic S. Raj. *Renal Disease and Hypertension, George Washington University, Washington, DC.*

Background: Muscle atrophy in end-stage renal disease (ESRD) may be due to the activation of apoptotic and proteolytic pathways.

Objective: We hypothesized that activation of caspase-3 in the skeletal muscle mediates apoptosis and proteolysis during hemodialysis (HD).

Materials and Methods: Eight ESRD patients were studied before (pre-HD) and during HD and the findings were compared with those from six healthy volunteers. Protein kinetics was determined by primed constant infusion of L-(ring 13C6) Phenylalanine.

Results: Caspase-3 activity in the skeletal muscle was higher in ESRD patients pre-HD than in controls (24966.0±4023.9 vs. 15293.3±2120.0 units, p<0.01) and increased further during HD (end-HD) (37666.6±4208.3 units) (p<0.001). 14 kDa actin fragments generated by caspase-3 mediated cleavage of actomyosin was higher in the skeletal muscle pre-HD (68%) and during HD (164%) compared to controls. The abundance of ubiquitinized carboxy-terminal actin fragment was also significantly increased during HD. Skeletal muscle biopsies obtained at the end of HD exhibited augmented apoptosis, which was higher than that observed in pre-HD and control samples (p<0.001). IL-6 content in the soluble fraction of the muscle skeletal muscle was increased significantly during HD. Protein kinetic studies showed that catabolism was higher in ESRD patients during HD compared to pre-HD and control subjects. Muscle protein catabolism was positively associated with caspase-3 activity and skeletal muscle IL-6 content.

Conclusion: Muscle atrophy in ESRD may be due to IL-6 induced activation of caspase-3 resulting in apoptosis as well as muscle proteolysis during HD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2686**

**Oxidized LDL Promotes Tregs Apoptosis through GSK-3b Activity in Uremic Patients** Pascal Meier. *Nephrology, CHCVs, Sion, Switzerland.*

Several lines of evidence support the idea that oxidative stress may play a major role in uremic T cell dysfunction. Patients with end-stage kidney disease (ESKD) have increased plasma levels of oxidized low-density lipoproteins (oxLDL). We have demonstrated that oxLDL induce regulatory $CD4^+/FOXP3^+$ T cells (Tregs) cell cycle arrest and apoptosis affecting their suppression capacity and finally promote a constant micro-inflammatory state in patients with ESKD. The present study used human Tregs to test the hypothesis that a physiologically relevant level of oxLDL influences Tregs apoptosis through the reduced expression of the canonical Wnt signaling and the increase expression of glycogen synthase kinase 3 beta (GSK-3b) both responsible for the increase phosphorylation of b-catenin, which affects Bcl-$X_L$ expression. The immunoprofile of peripheral Tregs was examined with protein quantification assays, flow cytometry and quantitative RT-PCR. Tregs from five healthy subjects were cultured in 10% uremic serum or in presence of increasing concentrations (50 to 200 mg/mL) of either native LDL or oxLDL for 72 hours. Uremic serum and oxLDL (200 mg/mL) but not native LDL led to the reduced Wnt signaling and the high expression of phosphorylated GSK-3b in cultured Tregs. The same results were obtained with Tregs from five uremic patients. The down-regulation of the Wnt cascade resulted in cytoplasmic and nuclear phosphorylated b-catenin accumulation. Consecutively, the nuclear expression of Bcl-$X_L$ was reduced and Tregs apoptosis was confirmed (DNA fragmentation). Tregs suppressive capacity was significantly reduced when cultured in 10% uremic serum or with 200 mg/mL oxLDL. In contrast, *in vitro* assays done in the presence of the GSK-3b inhibitor lithium led to dramatically prolonged Tregs survival and improved suppressive capacity *via* nuclear factor of activated T cells (NFAT) that remained in the nucleus. Together, these data demonstrate, in uremic patients, that oxLDL promotes Tregs apoptosis *via* GSK-3b activity, which negatively regulates Tregs physiological response and may contribute to immune dysfunction in patients with ESKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2687**

**Altered Function of IL-17 Producing $FOXP3^+$ Regulatory T Cells in Patients with End-Stage Kidney Disease** Pascal Meier. *Nephrology, CHCVs, Sion, Switzerland.*

The IL-17-producing $CD4^+$ T helper cell (Th17) differentiation is induced by a combination of IL-6 and TGF-β and is augmented by induction of IL-21. Signaling induced by these cytokines results in expression of the orphan nuclear receptor RORγt, transcription factors that are required for induction of IL-17 expression. Furthermore, human Th17 memory cells selectively express high levels of CCR6. We report that human peripheral blood from chronic HD patients (n = 10), chronic PD patients (n = 10) and patients with chronic kidney disease (CKD) (n = 10) show a significantly lower number of $CD4^+FOXP3^+$ T cells compared with healthy subjects with normal kidney function (Controls, n = 10) ($p < 0.01$). In HD patients, these cells express significantly less CCR6 and lose the capacity to produce IL-17 upon activation (PMA/iono for 24 h), ($p < 0.02$). $CD4^+FOXP3^+$ T cells from HD patients do not coexpress FOXP3 and RORγt transcription factors. Whereas, the $CD4^+FOXP3^+CCR6^+$ IL-17-producing cells strongly inhibit the proliferation of $CD4^+$ responder T cells in CKD patients and Controls, these cells do not present any regulation in chronic HD and PD patients. $CD4^+FOXP3^+CCR6^-$ regulatory T cells from Controls differentiate into IL-17 producer cells (Th17) upon T-cell receptor (TCR) stimulation in the presence of IL-1β, IL-2, IL-21, IL-23, and human serum. However, this differentiation is excessively marked when cells from Controls are cultured in presence of 10% uremic serum (HD patients). The same results are found with cells from HD patients. This suggests first that in human, the $IL\text{-}17^+FOXP3^+$ Treg cells are found in the periphery. Second, IL-17-producing Treg cells may play critical roles in controlling inflammation. Third, HD patients present a low number of IL-17-producing Tregs, which show an altered function *ex vivo* and *in vitro* with a parallel high number of Th17 with a normal high function. Although the biological significance of T cells that display the function of Tregs and the opposing function of Th17 remains unclear. This may be one mechanistic explanation of the T cell immune dysfunction in uremic patients, and may have important implications in clinics.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2688**

**Uremic HDL Disrupts Macrophage Lipid Homeostasis and Potentiates Inflammation** Suguru Yamamoto,[1] Patricia G. Yancey,[2] T. Alp Ikizler,[3] W. Gray Jerome,[4] Valentina Kon.[1] *[1]Vanderbilt University, Pediatrics, Nashville, TN; [2]Vanderbilt University, Medicine, Nashville, TN; [3]Vanderbilt University, Nephrology, Nashville, TN; [4]Vanderbilt University, Pathology, Nashville, TN.*

**Background** Although chronic renal disease (CKD) increases atherosclerotic cardiovascular disease (CVD) the underlying mechanisms are unknown, not explained by traditional risks, and resist conventional lipid lowering therapies. Since macrophage foam cell is the hallmark of atherogenic lesions, we examined if normal lipid homeostasis and inflammatory functions of macrophages are perturbed by lipoproteins from CKD patients on dialysis.

**Methods** Cell cholesterol content, efflux and inflammatory response were assessed in human macrophage-like THP-1 cells and peritoneal macrophages of apolipoprotein E-deficient mice (apoE-/-). Cells were exposed to high density lipoprotein (HDL) fraction isolated from hemodialysis patients (n=10) and matched normal controls (n=10). Sequential density ultracentrifugation was used to isolate HDL fractions. Gas liquid chromatography measured cellular lipid content which determined cholesterol efflux. mRNA expression assessed inflammatory markers.

**Results** Compared with controls, HDL fraction of uremics was less effective in facilitating cholesterol efflux from cholesterol-loaded THP-1 cells (5.3±1.8 vs 16.4±4.8%) or apoE-/- cells (18.6±3.8 vs 22.5±3.3%). Further, HDL of uremics heightened expression of inflammatory cytokines in THP-1 cells (relative mRNA expression for TNF-a: 4.1±0.6 vs 2.0±0.4, IL-6: 13.0±0.8 vs 2.0±0.8, IL-1b: 7.7±0.8 vs 2.0±0.1, $p<0.05$) and apoE-/- cells (relative mRNA expression for TNF-a: 13.0±1.9 vs 5.1±1.4, IL-6: 32.3±6.0 vs 8.4±3.7, and IL-1b: 33.3±4.0 vs 12.6±5.8, $p<0.05$).

**Conclusion** Thus, compared with individuals with intact kidney function, HDL of dialysis patients is revealed to have impaired cholesterol acceptor functions that reduce cholesterol efflux together with heightened inflammatory modulators. These findings predict increased foam cell formation and CVD that may explain dissociation of CVD with plasma lipid levels and resistance to standard lipid lowering therapies in patients with advanced CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2689**

**Soluble Epoxide Hydrolase Inhibitor (sEHI) Attenuates Release of Monocyte Chemotactic Protein-1 (MCP-1) from Human Monocytes** William G. Sanders,[1] Christi M. Terry,[2] Yuxia He,[2] Bruce D. Hammock,[3] Alfred K. Cheung.[2,4] *[1]Pharmaceutics, University of Utah, Salt Lake City, UT; [2]Medicine, University of Utah, Salt Lake City, UT; [3]Entymology, UC-Davis, Davis, CA; [4]Medical Service, VAMC, Salt Lake City, UT.*

In synthetic hemodialysis arteriovenous (AV) grafts, monocytes and T-cells appear early at the vein-graft anastomosis following placement and may participate in stenosis formation. Cellular epoxyeicosatrienoic acids (EETs) possess anti-inflammatory properties via down-regulation of NF-kB, a transcription factor important in cytokine expression. Soluble epoxide hydrolase (sEH) catabolizes EETs, thus sEH inhibitors (sEHI) may be effective as anti-inflammatory agents. Previously we showed increased sEH protein and activity in our pig model of AV graft stenosis. Here we investigated the expression of sEH in cultured monocytes and T cells and the effect of sEHI on cytokine release from these cells to determine if they could be targets for the anti-inflammatory actions of sEHI.

sEH expression in cultured human blood monocytes and T-cells was assessed by immunocytochemistry (ICC) and immunoblotting. The effect of pretreatment with a specific pharmacological sEHI (5 mM), with or without EETs (0.1 mM), on release of the proinflammatory cytokines monocyte chemotactic protein (MCP-1), macrophage inflammatory protein-1a (MIP-1a) and IL-6, from these cells exposed to LPS (10 ng/ml) for 24h was tested by ELISA. MCP-1 expression in explanted pig AV graft tissue was examined by immunohistochemistry (IHC).

Cultured monocytes and T-cells expressed sEH protein. sEHI inhibited MCP-1 release by ~50% ($p<0.05$), but not MIP-1α or IL-6, from monocytes and no further inhibition was observed with addition of EETs. No inhibitory effect of sEHI was observed on the release of any cytokine from T-cells. Thus the inhibitory effects of sEHI appear to be cytokine- and cell-specific. At 3 weeks post-placement, MCP-1 expression in the graft anastomosis was elevated compared to control vein. As monocytes are a primary source of MCP-1, which is vital in leukocyte recruitment and activation, sEHI may decrease inflammatory response and therefore stenosis in AV grafts.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2690**

**Increased Generation of Superoxide Anion (SA) and Decreased SA Scavenging in a Rat Arteriovenous Fistula (AVF) Model** Mykola V. Tsapenko, Anthony J. Croatt, Livius V. d'Uscio, Melissa C. Hernandez, Allan W. Ackerman, Joseph P. Grande, Zvonimir S. Katusic, Karl A. Nath. *Mayo Clinic, Rochester, MN.*

We have recently described a rat femoral AVF model (Am J Pathol 176:2530, 2010) characterized by neointimal hyperplasia and thrombogenesis at 4 weeks, and upregulation of vasculopathic genes and eNOS at 1 week. Since increased SA production can contribute to neointimal hyperplasia, the current study examined SA generation in the venous segment of this AVF model at 1 week. SA production, measured by HPLC, increased significantly in the venous limb of the AVF as compared to the control vein (3.41±0.71 vs. 9.59±2.04 μmol/mg protein, P<0.02). To determine the basis for such generation, we examined potential mechanisms including content of tetrahydrobiopterin (BH4, reduced form, the co-factor for eNOS) and 7,8-dihydrobiopterin (BH2, oxidized form) in the AVF: BH4 and BH2 were both significantly increased in the AVF, BH2 disproportionately so, thereby leading to a lower BH4/BH2 ratio in the venous segment of the AVF as compared with control veins (56±12 vs. 16±3, P<0.005). Such reduction in the BH4/BH2 ratio can cause uncoupling of eNOS, and thus the generation of SA rather than NO by eNOS. Other sources of SA, including NADPH oxidase (NOX1, NOX2, NOX4, subunits p47phox and p67phox), and COX (COX1 and COX2) isoforms, determined by Western analysis, were not significantly different between the groups. We also examined superoxide dismutase activity (SOD), the main SA scavenger. In the venous limb of the AVF as compared to control veins, activities of total SOD (17.2±1.4 vs. 11.5±0.9 U/mg protein, P<0.005) and Cu/Zn-SOD (14.6±1.3 vs. 9.7±0.9 U/mg protein, P<0.01) were both significantly diminished. In summary, SA is generated in increased amounts in the venous limb of the AVF because of a reduced BH4/BH2 ratio and an attendant uncoupling of eNOS, and because of reduced SA scavenging due to diminished SOD activity. We suggest that such increased generation of SA may contribute to venous neointimal hyperplasia that occurs in this AVF model.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2691**

**Abstract Withdrawn**

**SA-PO2692**

**Investigation of the Association between TCF7L2 and Kidney Function in the Multi-Ethnic Study of Atherosclerosis (MESA)** Jamison W. Chang,[1] Michele Sale,[2] Stephen Rich,[2] Carmen A. Peralta,[3] Nehal N. Mehta.[4] *[1]Division of Nephrology and CIIR, University of Virginia, Charlottesville, VA; [2]Center for Public Genomics, University of Virginia, Charlottesville, VA; [3]Divsion of Nephrology, UCSF, San Francisco, CA; [4]Cardiology, University of Penn, Phildelphia, PA.*

The transcription factor 7-like 2 gene (TCF7L2), coding for a high-mobility box-containing transcription factor is an important effector in the canonical Wnt pathway. To date, variants of TCF7L2 show the strongest associations with type 2 diabetes among the common type 2 diabetes genes. In 2008, Kottgen et al demonstrated an association between TCF7L2 variants and progression of chronic kidney disease and eGFR both in diabetics and non-diabetics. These results suggested that variants in TCF7L2 may confer a risk of kidney disease independent of their influence on type 2 diabetes risk.

To further explore the relationship between variants in TCF7L2 and kidney disease, we examined the effects of 64 SNPs in TCF7L2 on kidney function in a large, diverse cohort, the Multi-Ethnic Study of Atherosclerosis (MESA) candidate gene study (n=2800). Using multivariable linear regression and adjusting for age, gender, race, and center of ascertainment, two SNPs in TCF7L2, rs11196199 and rs11196203 were found to be associated with mean eGFR in cross-sectional analysis of MESA (p value= 0.0004 and 0.0003 respectively). These SNPs were not associated with type 2 diabetes (P>0.9). Finally, adding in diabetic status and serum glucose to the model above, rs11196199 and rs11196203 remained significantly associated with eGFR (p value 0.0004 and 0.0003). None of the 64 variants in TCF7L2 were associated with urine albuminuria or progression of CKD in longitudinal analysis.

Taken together, the results suggest that variants in TCF7L2 may influence kidney function independently of their effect on type 2 diabetes risk. Planned analyses will determine whether these results replicate in the remainder of the MESA cohort (n=3500). The mechanism by which these variants influence kidney function is unclear at this time but will be studied further if this association is replicated.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2693**

**Accounting for Risk Variants in *MYH9* Reveals *FRMD3* Association with Diabetic ESRD in African Americans** Barry I. Freedman,[1] Carl D. Langefeld,[2] Lingyi Lu,[2] Jasmin Divers,[2] Mary E. Comeau,[2] Jeffrey B. Kopp,[4] Cheryl Winkler,[5] George W. Nelson,[5] Nicholette D. Allred,[3] Pamela J. Hicks,[3] Meredith A. Bostrom,[3] Jessica N. Cooke,[3] Caitrin W. Mcdonough,[3] Donald W. Bowden.[3] *[1]Internal Medicine, Wake Forest Univ Sch Med; [2]Biostatistical Sciences/Center for Public Health Genomics, Wake Forest Univ Sch Med; [3]Biochemistry/Center for Diabetes Research, Wake Forest Univ Sch Med, Winston-Salem, NC; [4]Kidney DIsease Section, NIDDK, Bethesda, MD; [5]Laboratory of Genomic Diversity, SAIC-Frederick, National Cancer Institute, Frederick, MD.*

Variants in *MYH9* are strongly associated with end-stage renal disease (ESRD) in African Americans (AA) and may mask identification of variants in other genes. The Affymetrix SNP Array 6.0 was genotyped in 966 AA with type 2 diabetes-(T2D) associated ESRD and 1032 non-diabetic, non-nephropathy (NDNN) controls. A T2D-ESRD case-only logistic regression analysis tested for gene-gene interactions with the *MYH9* E1 haplotype; and *MYH9* E1-stratified analyses tested for additional gene associations. Seven *FRMD3* SNPs exhibited significant interaction with E1 in an initial T2D-ESRD case-only analysis (*e.g.*, rs942280 p-value $1.48 \times 10^{-4}$ additive; OR=0.67, 95% CI 0.54-0.82). Analyses were repeated in another 640 AA with T2D-ESRD, 683 NDNN controls and 513 AA with T2D lacking nephropathy. *FRMD3-MYH9* interactions were observed in the replication analysis with 640 T2D-ESRD cases (rs942280 $p=1.37 \times 10^{-3}$) and combined 1,592 T2D-ESRD cases

(p=9.28 x $10^{-7}$ additive; OR=0.67). In non-*MYH9* E1 homozygotes, this *FRMD3* SNP was associated with T2D-ESRD (OR=1.28; heterozygosity p-value vs. E1 homozygotes 4.3 x $10^{-4}$) and 5 *FRMD3* SNPS were significantly associated with ESRD when contrasting T2D-ESRD cases with T2D non-nephropathy controls. Significant *FRMD3* associations with T2D-ESRD were detectable only after accounting for the effect of *MYH9*. *MYH9-FRMD3* statistical interactions likely result from *MYH9* identifying diabetic cases with non-diabetic ESRD. These analyses support the role of *FRMD3* in T2D-ESRD and suggest that identifying diabetic nephropathy genes in AA benefits from accounting for variation in *MYH9*.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2694**

**A Genome Wide Association Study for Diabetic Nephropathy Genes in African Americans** Caitrin W. Mcdonough,[1] Nicholette D. Allred,[2] Pamela J. Hicks,[2] Meredith A. Bostrom,[2] Lingyi Lu,[3] Maggie Ng,[4] Jasmin Divers,[3] Carl D. Langefeld,[3] Barry I. Freedman,[5] Donald W. Bowden.[2,5] *[1]Program in Molecular Medicine and Translational Science, Wake Forest University School of Medicine; [2]Biochemistry, Wake Forest University School of Medicine; [3]Biostatistical Sciences, Wake Forest University School of Medicine; [4]Pediatrics, Wake Forest University School of Medicine; [5]Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.*

African Americans (AA) have increased risk of developing ESRD compared to Caucasian Americans and many studies reveal that a genetic component contributes. We performed a genome-wide association study (GWAS) in 965 AA type 2 diabetic (T2D)-ESRD cases and 1,029 AA non-T2D non-nephropathy controls on the Affy6.0 platform. The top 754 SNPs were genotyped in a replication sample of 709 AA T2D-ESRD cases and 690 AA non-T2D non-nephropathy controls. To discriminate between T2D, T2D-ESRD, and/or all-cause ESRD, SNPs that replicated were then genotyped in 1,216 AA non-T2D ESRD cases and 1,246 AA T2D non- nephropathy controls. Analyses were adjusted for admixture. We detected 25 SNPs associated with T2D-ESRD in 19 regions ($P_{ADD} \leq$ 2E-04 in the combined analysis (GWAS+Replication), associated in T2D-ESRD vs T2D comparison, and with no/nominal association with T2D vs non T2D controls). Although genome wide significance for T2D-ESRD was not observed with any SNP, several genes, including *RPS12*, *LIMK2*, and *SFI1* are strong nephropathy candidates. *RPS12*, ribosomal protein S12, SNPs rs9493454 and rs7769051 showed combined P-values of 1.7x$10^{-5}$ and 2.2x$10^{-6}$, and odds ratios (OR) and 95% confidence intervals (95% CI) of 1.24(1.13-1.37) and 1.28(1.16-1.42), respectively. Two SNPs, rs2106294 and rs4820043 in *LIMK2*, LIM domain kinase 2, had combined P-values of 4.1x$10^{-6}$ and 5.1x$10^{-6}$, and OR (95% CI) of 0.57(0.45-0.72) and 0.57(0.45-0.73), respectively. A combined analysis of all 2,890 ESRD cases (1,674 T2D-ESRD; 1,216 non-T2D ESRD) revealed SNPs in *LIMK2* and *SFI1* with P-values 3.3x$10^{-8}$ to 6.5x$10^{-11}$ suggesting that they may contribute to all-cause ESRD. These results suggest that multiple loci underlie nephropathy susceptibility in AA with T2D.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2695**

**Association of Adrenomedullin 2 Gene I/D Polymorphism with Cerebral and Renal Damage in a Japanese General Population: The Ohasama Study** Kazuhito Totsune,[1,2] Takuo Hirose,[1] Hirohito Metoki,[1,3] Kei Asayama,[1] Masahiro Kikuya,[1] Takayoshi Ohkubo,[1,4] Kazuhiro Takahashi,[5] Yutaka Imai.[1] *[1]Planning for Drug Development and Clinical Evaluation, Tohoku Univ Grad Sch of Pharm Sci and Med, Sendai, Japan; [2]Faculty of Synthetic Welfare, Tohoku Fukushi Univ, Sendai; [3]Obstetrics and Gynecology, Tohoku Univ Grad Sch of Med, Sendai; [4]Department of Health Science, Shiga Univ of Med Sci, Otsu; [5]Endocrinology and Applied Med Sci, Tohoku Univ Grad Sch of Med, Sendai, Japan.*

Adrenomedullin 2 (AM2), also called intermedin, is a possible reno-protective peptide with various effects on the cardiovascular system and the kidney function. An exonic insertion/deletion (I/D) polymorphism (rs3840963) likely influences generation of AM2-53, due to its location within the N-terminal sequence. We previously reported the association of this polymorphism with blood pressure in a Japanese population (Renal Week 2008). In the present study, we examined the association of the polymorphism with renal function and the risk of silent cerebrovascular lesions (lacunar infarction and white matter hyperintensity). We recorded the estimated glomerular filtration rate (eGFR) and proteinurea of 1073 individuals over 40 years of age. Silent cerebrovascular lesions were recorded in 794 individuals over 55 years of age. Chronic kidney disease was diagnosed when an individual had proteinuria and/or decreased eGFR less than 60 ml/min/1.73$m^2$. DD carriers, compared to II and ID carriers, displayed significantly lower eGFR (75.4 vs. 82.6 and 82.9 mL/min/1.73$m^2$, respectively, P=0.04). DD carriers also had a significantly higher odds ratio (OR) for prevalence of chronic kidney disease (OR: 2.7, P=0.003), presence of lacunar infarction (OR:2.4, P=0.01), and white matter hyperintensity (OR:2.7, P=0.003), compared with II carriers. AM2 I/D polymorphism had no significant association with any other clinical manifestation tested, such as cardiac infarction and symptomatic strokes. Thus, the AM2 I/D polymorphism is associated with renal dysfunction and asymptomatic cerebrovascular diseases in the Japanese general population. These results suggest that AM2 has a certain role against the renal and cerebrovascular damages.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2696**

**Sequencing of α-Actinin-4 To Identify Variants Associated with Non-Diabetic End-Stage Renal Disease (ESRD) in African Americans** Meredith A. Bostrom,[1,5] Pamela J. Hicks,[1,5] Lingyi Lu,[2] Maggie Ng,[4,5] Carl D. Langefeld,[2] Barry I. Freedman,[3] Donald W. Bowden.[1,5] *[1]Biochemistry, Wake Forest University School of Medicine; [2]Biostatistical Sciences, Wake Forest University School of Medicine; [3]Internal Medicine, Wake Forest University School of Medicine; [4]Pediatrics, Wake Forest University School of Medicine; [5]Centers for Human Genomics and Diabetes Research, Winston-Salem, NC.*

African Americans (AA) disproportionately develop non-diabetic (non-DM) forms of ESRD and studies reveal a genetic component. *ACTN4* (α-actinin-4) is an actin binding protein expressed in podocytes; rare mutations have been implicated in familial focal segmental glomerulosclerosis. Nineteen exons and 2800 bases of the promoter of *ACTN4* were sequenced in 96 AA non-DM ESRD cases and 96 non-nephropathy controls. Sixty-seven SNPs were identified (49 were novel), including 33 intronic, 21 promoter, 12 exonic, and 1 3' of the gene. Sixty-one SNPs were genotyped in 278 AA non-DM ESRD cases and 327 AA non-nephropathy controls. One promoter SNP, rs10404257, was associated with non-DM ESRD (p=0.004, odds ratio (OR)=0.60, confidence interval (CI) 0.43-0.85; dominant model). Forty SNPs had minor allele frequencies less than 5%. These SNPs were collapsed into a single marker, designated by the presence (n=251) or absence (n=404) of any rare allele. There was no association with having a rare allele at any of these SNPs in non-DM ESRD. SNPs were tested for interaction with *MYH9*, previously associated with non-DM ESRD in AA, and no interaction was detected with single SNPs or collapsed rare alleles. 21 of the most associated SNPs were genotyped in an independent set of 525 AA non-DM ESRD cases and 267 controls. Although rs10404257 was not associated in the replication sample (p=0.768, OR=1.05, CI=0.75-1.48; dominant), it remained modestly associated when all samples were combined (p=0.033, OR=0.78, CI 0.62-0.98; dominant). We have sequenced the promoter and coding regions in the *ACTN4* gene and detected limited evidence of association with non-DM ESRD in AA. This negative finding for nephropathy suggests that common to moderately uncommon *ACTN4* variants impacting gene function may affect survival.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2697**

**Association of (Pro)renin Receptor Gene Polymorphism with Cardiac Hypertrophy, Asymptomatic Cerebrovascular Lesions and Chronic Kidney Disease in Japanese: The Ohasama Study** Kazuhito Totsune,[1,2] Takuo Hirose,[1] Hirohito Metoki,[1,3] Kei Asayama,[1] Masahiro Kikuya,[1] Takayoshi Ohkubo,[1,4] Kazuhiro Takahashi,[5] Yutaka Imai.[1] *[1]Planning for Drug Development and Clinical Evaluation, Tohoku Univ Grad Sch of Pharm Sci and Med, Sendai; [2]Faculty of Synthetic Welfare, Tohoku Fukushi Univ, Sendai; [3]Obstetrics and Gynecology, Tohoku Univ Grad Sch of Med, Sendai; [4]Department of Health Science, Shiga Univ of Med Sci, Otsu; [5]Endocrinology and Applied Med Sci, Tohoku Univ Grad Sch of Med, Sendai, Japan.*

(Pro)rennin receptor (PRR) is a new member of renin-angiotensin system. We previously reported the association of intervening sequence (IVS)5+169C>T (rs5918007) polymorphism of PRR with blood pressure in Japanese men (Am J Hypertens, 2009). In the present study, we investigated the association of PRR gene polymorphisms with left ventricular hypertrophy (LVH), silent cerebrovascular lesions (lacunar infarction and white matter hyperintensity (WMH)) and chronic kidney disease (CKD). For association study, we selected three polymorphisms: -782A>G (rs2968915), IVS5+169C>T, and +1513A>G (rs6609080). A total of 779 subjects were recruited. We defined LVH by ECG records and lacunar infarction and/or WMH by MRI. Since PRR gene is located on the X chromosome, men and women were analyzed separately. In women, the prevalence of LVH and lacunar infarction was significantly higher in subjects with the +1513GG genotype than in those with the AA or AG genotype (*P*=0.003 and *P*=0.01, respectively), but not WMH (*P*=0.07) nor CKD. Multiple logistic regression analysis adjusted for confounding factors demonstrated that +1513A>G polymorphism was significantly and independently associated with the risk of LVH (trend *P*=0.007) and lacunar infarction (trend *P*=0.04). Plasma renin activity (PRA) levels in women with the GG genotype were significantly lower than those in women with the AA or AG genotypes (*P*=0.01). In men, no significant association was observed with LVH, lacunar infarction, WMH and CKD. Thus, PRR gene polymorphism +1513A>G is associated with LVH and lacunar infarction in Japanese women. These results suggest that PRR has a role in organ damage in humans.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2698**

**Investigation of Genetic Mutations That Influence Telomerase Length for Association with End Stage Renal Disease** A. J. McKnight, Alexander P. Maxwell. *Nephrology Research Group, Queen's University of Belfast, Belfast, Northern Ireland, United Kingdom.*

Renal dysfunction is associated with reduced telomere length. Genetic mutations influencing telomerase (an enzyme that maintains telomere length) are an important disease mechanism that may have crucial implications for patients with end stage renal disease (ESRD).

A recent genome-wide association study (Nat Genet 42(3):197, 2010) reported two robustly replicated SNPs that were strongly associated with telomere length; rs12696304, *P*=$10^{-14}$, rs16847897, *P*=$10^{-12}$, $r^2$=0.49, SNPs across the 87 kb interval were also significantly

associated with reduced telomere length. These SNPs are located in a prioritised genomic region for renal disease (3q26) and are near the telomerase RNA component (TERC) gene.

To investigate the influence of these SNPs on ESRD, we conducted a case-control study in a White, European population. Individuals recruited to the case group had received a kidney transplant (n=574) and were compared to individuals in the control group that comprised kidney donors (n=517). SNPs were genotyped using commercial TaqMan kits with a genotype success rate greater than 99%. Genotype and allele frequencies were compared between cases and controls using SPSS. Statistically significant association was not observed in our collection, despite 1,091 individuals providing approximately 80% power to identify a risk allele ($p<0.05$) with an odds ratio of 1.3 for a minor allele frequency of 30%.

These SNPs are not major risk factors for ESRD within our population, however we suggest that studies evaluating age-related telomere length for renal phenotypes should also consider the influence of genetic contributions on observed telomere length.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2699

**Exome Sequencing of Type 1 Diabetes (T1D) Survivors and T1D End-Stage Renal Disease (ESRD) Cases Identifies Coding SNPs Implicated in the Pathogenesis of Diabetic Nephropathy** Marcus G. Pezzolesi,[1] Hillary A. Keenan,[1] Gabriel David Poznik,[1] Stephen Rich,[3] James Warram,[1] George L. King,[1] Andrzej S. Krolewski,[1] Jonathon Dunn,[1] Josyf Mychaleckyj.[3] *[1]Research Division, Joslin Diabetes Center, Boston, MA; [2]Center for Public Health Genomics, University of Virginia School of Medicine, Charlottesville, VA.*

Objective

Diabetic nephropathy (DN) affects 30% of all patients with T1D. Only 5% of patients with T1D survive without this complication for 50 or more years following the onset of disease. To identify genetic variants that contribute to protection or risk of DN in T1D, we performed exome sequencing in individuals who either remained free of renal complications despite a long duration of T1D or developed ERSD after a short duration of disease.

Research Design

Targeted resequencing of approximately 180,000 exons for 18,673 protein-coding genes was performed on 3 long-duration 'survivors' with normoalbuminuria and 3 short-duration 'ESRD cases'.

Results

We identified 6,193 previously reported nsSNPs, including 6,144 missense and 49 nonsense variants, per exome. These nsSNPs map to 6,004 distinct genes. Six-hundred eighteen genes contained nsSNPs that were exclusively observed in survivors. Ten genes contained nsSNPs present only among all 3 survivors. Similarly, we identified 537 genes that contained nsSNPs exclusively observed in ESRD cases, including 15 that contained nsSNPs present in all case subjects. We also identified 4,669 novel coding SNPs not annotated in dbSNP or present in data available from the 1000 Genomes Project. Of these, novel coding SNPs mapping to 4 genes were present only among survivors, while 6 genes contained novel coding SNPs found only in ESRD cases.

Conclusions

Exome sequencing using an extreme-trait study design identified multiple genes containing coding SNPs exclusive to either T1D survivors or ESRD cases. While further characterization of these SNPs in a larger collection of survivors and ESRD cases is necessary to determine their contribution to disease, our data demonstrate the utility of this approach in identifying potentially important functional changes associated with this complication.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2700

**Meta-Analysis of Genome-Wide Association Studies for Nephropathy in Type 1 Diabetes** A. J. McKnight,[1] Rany M. Salem,[2] Niina Sandholm,[3] Eoin P. Brennan,[4] On Behalf of the GENIE Consortium.[5] *[1]Queen's University of Belfast, Belfast, United Kingdom; [2]Broad Institute, Cambridge; [3]Folkhalsan Research Center, Helsinki, Finland; [4]University College Dublin, Dublin, Ireland; [5]GENIE Consortium, International.*

We have performed novel, independent genome-wide association studies (GWAS) and meta-analyses to advance understanding of the genetic contribution to diabetic nephropathy.

As part of the GENIE (GEnetics of Nephropathy, an International Effort) consortium we have assembled a large collection of individuals with type 1 diabetes (T1D, n=6,462). Recruited individuals include cases with overt diabetic nephropathy compared to normoalbuminuric controls that had T1D for at least 15 years with no evidence of renal disease. The All Ireland-Warren 3-UK GoKinD collection (823 cases, 903 controls) was genotyped on the Illumina Omni1-quad while FinnDiane (1,411 cases, 1,708 controls) was genotyped using the Illumina 610-quad. Publicly available data from US GoKinD (782 cases, 835 controls), genotyped on the Affymetrix 5.0 array, was obtained from dbGAP. Following standardised quality control, genotypes were imputed using MACH with the HapMap2 CEU panel. Association was investigated for approximately 2.4 million SNPs using PLINK with principal components of ancestry, age at recruitment, duration of diabetes, gender and study site as covariates; meta-analysis was performed using METAL.

A total of 15 SNPs achieved a p value of less than $10^{-6}$ in the combined analysis for diabetic nephropathy. Several of these SNPs mapped to 3q13, 4q34 and 20q11 with multiple SNPs mapping to a biologically plausible gene at 2q34. Further analyses have been conducted for secondary phenotypes and genetic outcomes are being examined in relation to our microarray and RNA Seq data. The best evidence for association was observed for ESRD where several SNPs in close vicinity achieved genome-wide significance at 2q11.2-q12. The top SNPs are undergoing replication in additional, independent collections.

By combining genetic and expression data we provide further novel insights into the pathogenesis of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2701

**Genetic Variants Identified by GWAS Suggest Intracellular Calcium, Vascular Remodeling, and Cardiac Repolarization Pathways Impact Dialytic Survival in African Americans with Diabetes** Mariana Murea,[1] Lingyi Lu,[2] Pamela J. Hicks,[3] Donald W. Bowden,[1,3] Jasmin Divers,[2] Carl D. Langefeld,[2] Barry I. Freedman.[1] *[1]Internal Medicine/Nephrology, Wake Forest University School of Medicine; [2]Department of Biostatistical Sciences, Wake Forest University School of Medicine; [3]Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC.*

Relative to Caucasians, African Americans (AA) have a nearly 40% lower risk of death on dialysis. As genetic factors may play a role, we performed a genome-wide association study (GWAS) in 610 AA patients with end-stage renal disease (ESRD) attributed to type 2 diabetes using the Affymetrix 6.0 platform (868,155 single nucleotide polymorphisms; SNPs). Time to all-cause mortality was assessed by a Cox proportional hazard model with adjustment for ancestry, age at ESRD, gender, BMI, and incident year. Cases were censored at kidney transplant or if alive at the final observation (n= 160); 430 deaths were observed after mean follow-up of 5.4 years. Primary inference is the additive genetic model unless significant departure from additivity ($P<0.05$), then minimum of dominant, additive or recessive model was reported. Five SNPs were associated with time to death at $p<1x10-7$: rs2681019 near KLHL29 (Hazard Ratio [HR]=2.58, PREC=8x10-8), rs815815 on CALM2 (HR=1.51, PADD=6x10-7), rs926392 (HR=2.37, PREC= 4x10-7) and rs926391 (HR=2.30, PREC= 7x10-7) near DHX35, and rs11128347 on PDZRN3 (HR=0.57, PADD=6x10-7). Ten SNPs in 8 other genes were associated at $P<1x10-6$: GAS2, LOC729980, PTPRM, ABCA4, PCSK2, NRG1 with high HR; and SVIP, SH3BP4 with low HR for time to death. Several intergenic SNPs located near ADAMTS3, ADAMTS5, HAS2, SLC24S4, IRX3, and IRX5 were also associated at $P<1x10-6$. Genetic variation was predominantly located within or in close proximity to genes playing critical roles in intracellular calcium trafficking, extracellular matrix turnover and mineralization, vascular remodeling under mechanical stretch, and cardiac ventricular repolarization. These results warrant additional investigation and replication to detect genes that contribute to survival on dialysis.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2702

**Genetic Associations for Diabetic Nephropathy: A Meta-Analysis** Antien Mooyaart,[1] Lisette Valk,[1] Jan A. Bruijn,[1] Emile De Heer,[1] Olaf Dekkers,[2] Hans J. Baelde.[1] *[1]Pathology, LUMC, Leiden, Netherlands; [2]Clinical Epidemiology, LUMC, Leiden, Netherlands.*

*Background* Many genetic association studies for diabetic nephropathy have been performed. The aim of our meta-analysis study was to assess the pooled effect of genetic variants reproducibly associated with diabetic nephropathy.

*Methods* PubMed, EMBASE, and Web of Science were searched for genes associated with diabetic nephropathy. All genetic variants associated with diabetic nephropathy, which are reproduced in at least two independent studies were selected. The association between these variants and diabetic nephropathy (macroalbuminuria/ proteinuria or ESRD) was calculated at the allele level and expressed as odds ratios. Pre-specified subgroup analyses were performed, stratifying type 1/type 2 diabetes, proteinuria/ESRD, different ethnic groups. The main measure of effect was the pooled odds ratio in a random effects model.

*Results* Our initial literature search yielded 3455 citations of which 610 were genetic association studies investigating diabetic nephropathy. A number of 33 reproduced genetic variants was found, of which 21 remained significantly associated with diabetic nephropathy in a random-effects meta-analysis. These variants were in or near the following genes: *ACE, ALR2* (2 variants), *APOC1, APOe, ELMO1, EPO, HSPG2, IL-1B, VEGF, FRMD3* (2 variants), *CARS* (2 variants), *UNC13B, CPVL/CHN2, GREM1*, four variants were not near genes. The odds ratios of these genetic variants ranged between 0.48 and 1.70. Additional variants were found from the subgroup analyses: *CCR5* (subgroup: Asians), *CNDP1* (subgroup: type 2 diabetes).

*Conclusion* In conclusion, this meta-analysis shows that several genetic variants are associated with the risk of diabetic nephropathy. The road ahead is to investigate the role of these variants in the pathogenesis of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2703**

**Polymorphisms in *MYH9* Are Associated with Diabetic Nephropathy in European Americans** Jessica N. Cooke,[1,2] Meredith A. Bostrom,[1,2] Pamela J. Hicks,[1,2] Maggie Ng,[1,2,3] Mary E. Comeau,[4] Jasmin Divers,[4] Carl D. Langefeld,[4] Barry I. Freedman,[5] Donald W. Bowden.[1,2,5] *[1]Biochemistry, Wake Forest University School of Medicine; [2]Center for Genomics and Personalized Medicine Research and Center for Diabetes Research, Wake Forest University School of Medicine; [3]Pediatrics--Medical Genetics, Wake Forest University School of Medicine; [4]Public Health Sciences, Wake Forest University School of Medicine; [5]Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.*

Diabetes is the leading cause of end-stage renal disease (ESRD) in the U.S., accounting for 44% of incident cases each year. Polymorphisms in the non-muscle myosin IIA gene (*MYH9*) are strongly associated with focal segmental glomerulosclerosis (FSGS) and non-diabetic ESRD in African Americans (AA) and with FSGS in European Americans (EA), less strongly with type 2 diabetic (T2D)-ESRD in AA. We tested for association of single nucleotide polymorphisms (SNPs) in *MYH9* with T2D-ESRD in EA. Fifteen *MYH9* SNPs were genotyped in 1948 EA; 540 cases with T2D-ESRD and 1408 non-nephropathy controls (461 with T2D and 947 without diabetes). Comparing T2D-ESRD cases with the 461 T2D non-nephropathy controls, single SNP associations were detected with *MYH9* SNPs rs4821480, rs2032487 and rs4281481, comprising part of the major E1 risk haplotype (p-values 0.014-0.041 recessive, odds ratio [OR] 4.31-8.66). Comparing T2D-ESRD cases to all 1408 non-nephropathy controls confirmed association with these three SNPs as well as the fourth SNP in the E1 haplotype (rs3752462; p-values 0.004-0.026, OR 1.42-3.77). Haplotype analysis contrasting T2D-ESRD cases to all 1408 non-nephropathy controls suggested that the partial *MYH9* E1 risk haplotype was associated with nephropathy risk (GCC haplotype two-sided p-value 0.039 additive [one-sided p-value 0.020], OR 1.54: haplotype frequency 7.1% in T2D-ESRD cases vs. 4.8% in non-nephropathy controls). These analyses indicate that *MYH9* E1 haplotype SNPs are associated with T2D-ESRD susceptibility in European Americans and that three of the four E1 risk haplotype SNPs that are associated with FSGS in African Americans appear to confer risk.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2704**

**Genome-Wide Association Study (GWAS) Identifies Five New Susceptibility Loci for IgA Nephropathy (IgAN)** Ali G. Gharavi,[1] Krzysztof Kiryluk,[1] Murim Choi,[2] Yifu Li,[1] Jingyuan Xie,[5] Ping Hou,[4] Simone Sanna-Cherchi,[1] Jan Novak,[3] Robert J. Wyatt,[9] Bruce A. Julian,[3] Silvana Savoldi,[7] Landino Allegri,[12] Gian Marco Ghiggeri,[10] Riccardo Magistroni,[8] Maurizio Salvadori,[11] Giuliano Boscutti,[6] Antonio Amoroso,[7] Francesco Scolari,[6] Nan Chen,[5] Hong Zhang,[4] Richard P. Lifton.[2] *[1]Columbia Univ.; [2]Yale Univ.; [3]Univ. of Alabama; [4]Peking Univ.; [5]Shanghai Ruijin Hospital; [6]Univ. of Brescia; [7]Univ. of Torino; [8]Univ. of Modena; [9]Univ. of Tennessee; [10]Gaslini Hospital; [11]Univ. of Florence; [12]Univ. of Parma.*

Objective: IgAN is a common cause of kidney failure. We performed a GWAS to identify common genetic variants underlying susceptibility to IgAN.

Methods: We performed a GWAS and replication in 5966 subjects. We genotyped 1194 cases and 902 controls from China on Illumina 610K SNP chips. Potentially significant SNPs were studied in 2 follow-up cohorts: Chinese (712 cases and 748 controls) and Caucasian (1,238 cases and 1,172 controls). SNPs with $p \leq 5 \times 10\text{-}8$ across all cohorts were considered significant. We formulated a genetic risk score model based on the discovered loci, and we applied it to diverse populations across 4 continents.

Findings: Our top association signal resides in the MHC-II on Chr. 6 and conveys a strong protective effect against IgAN (odds ratio = 0.59, $p = 3.1 \times 10\text{-}27$). This association is strongest with the DQB1*602-DQA1*102-DRB1*1501 haplotype, which has previously been shown to confer risk to or protection from other immune disorders. In addition, we discovered four other independent loci with genome-wide significance. Disease risk varies 10-fold between the highest and lowest risk score groups and these loci explain 7% of the variation in disease risk. Interestingly, mean risk scores are significantly different among populations, and are highest in Chinese, intermediate in Europeans, and lowest in Africans, which parallels disease frequency among these groups.

Conclusions: We have discovered five new disease susceptibility loci for IgAN which provide new insight into the biology and geographic variability in the prevalence of IgAN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2705**

**Distinguishing Familial IgA Nephropathy from COL4A Associated Nephropathy** Yifu Li,[1] Simone Sanna-Cherchi,[1] Hussein H. Karnib,[2] Gianluca Caridi,[3] Krzysztof Kiryluk,[1] Gian Marco Ghiggeri,[3] Ali G. Gharavi.[1] *[1]Medicine, Div. of Nephrology, Columbia University, New York, NY; [2]Medicine, American University of Beirut, Beirut, Lebanon; [3]Pediatric Nephrology, G. Gaslini, Genoa, Italy.*

**Introduction.** Familial IgA Nephropathy (IgAN), thin basement membrane nephropathy (TBMN) and Alport syndrome (AS) are primary glomerular disorders which all present with micro- or macroscopic hematuria, proteinuria and variable progression to ESRD. Distinguishing between these entities can be complicated if there is concomitant IgA deposition on kidney biopsy. This distinction is further complicated because a locus for familial IgA nephropathy was mapped to the COL4A3/A4 locus on Chr. 2q36.

**Methods.** We evaluated the possibility of familial IgA nephropathy in 4 multigenerational kindreds ascertained via an index case with renal failure, mesangial sclerosis and IgA deposition on kidney biopsy; each kindred featured multiple individuals with dominant transmission of hematuria/proteinuria with/without chronic renal failure, and absence of hearing or ocular defects. We performed linkage analysis and mutational screening of all exons of the COL4A3, COL4A4 and COL4A5 genes.

**Results.** Linkage analysis demonstrated linkage to the COL4A3/A4 or the COL4A5 loci in three families (LOD = 2.1 and 2.2 at 2q35-37, and LOD = 3.6 at Xq22-25). Mutational screening revealed a single segregating mutation in three families (COL4A3 G291E, COL4A4 G852A, and COL4A5 G174C). These mutations affected conserved glycine residues, segregated in all affected individuals and were not present in at least 200 ethnically-matched controls, thus establishing the diagnosis of COL4A-associated diseases. In the fourth family, we found no linkage to, nor mutations in the collagen loci, suggesting it was a true case of familial IgAN.

**Conclusions.** In familial hematuric disease, linkage analysis and mutational screening of COL4A genes can help distinguish between familial IgAN and COL4A-associated diseases, resolving confounders such as the presence of renal failure and coincidental mesangial IgA deposition.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2706**

**Genome-Wide Linkage Scan for Familial IgA Nephropathy among Southeast Asian Chinese: Identification of a Novel Susceptibility Locus on Chromosome 8p22-23** Yuxin Niu,[1] Sonia Davila,[2] M. F. Lam,[3] Sydney C. W. Tang,[3] Kar Neng Lai,[3] Stephen I.-Hong Hsu.[1] *[1]Division of Nephrology, Hypertension and Renal Transplantation, College of Medicine, University of Florida, Gainesville, FL; [2]Department of Population Genetics, Genome Institute of Singapore, Singapore; [3]Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong.*

IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide with a particularly high incidence reported by Asian/Southeast Asian biopsy registries. Over the past decade, three independent genome-wide scans for linkage to familial IgAN among Caucasians have identified two susceptibility loci on chromosomes 6q22-23 (*IGAN1*) and 2q36, as well as a locus suggestive for linkage on 4q26-31. We now report the results of a genome-wide scan based on a large 4-generation Singaporean Chinese family (F66) as well as 23 smaller Chinese IgAN kindreds from Hong Kong (HK23). Linkage analysis of F66 was first performed at 10 cM resolution using microsatellite markers. By parametric analysis (assuming autosomal dominant inheritance, allele frequency of 0.001, phenocopy rate of 0.01 and penetrance of 75%), a region of suggestive linkage with a maximum multipoint LOD score of 2.23 was identified on chromosome 8p23. By non-parametric (NPL) analysis, a significant linkage to 8p23 (maximum multipoint LOD score 3.89, *p*-value 0.004) was confirmed. Genotyping of 4 additional microsatellite markers in this region in F66 and HK23 yielded a maximum heterogeneous LOD (HLOD) score of 3.26 ($\alpha$=0.5) with *D8S552* located near the boundary of 8p22 and 8p23. The LOD-1 support interval is flanked by markers that define a physical distance of ~4.5 Mb. Affected-only analysis yielded a maximum HLOD score of approximately 2.37 with the marker *D8S552*. We are genotyping 11 additional markers to conduct fine resolution mapping of the 8p22-23 locus, expected to increase the HLOD score and narrow the critical region. Our results indicate that a novel locus on chromosome 8p22-23 contributes a large genetic effect in 50% of IgAN kindreds in our study, representative of large populations of Southeast Asian Chinese among whom familial IgAN is not uncommon.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2707**

**Genetic Evidence for Involvement of Adaptive Immunity in the Development of IgA Nephropathy: Several Alleles of MHC Class II Are Protective in a Caucasian Population** Mai Tuyet Vuong,[1,3,4] Sigrid Lundberg,[2] Iva Gunnarsson,[1] Lars Wramner,[7] Emeli Lundstrom,[1] Anders Fernstrom,[6] Lars Alfredsson,[5] Stefan H. Jacobson,[3] Leonid Padyukov.[1] *[1]Rheumatology Unit, Department of Medicine, Karolinska Institutet, Stockholm, Sweden; [2]Nephrology Unit, Department of Medicine, Karolinska University Hospital, Stockholm, Sweden; [3]Department of Nephrology, Danderyd Hospital, Karolinska Institutet, Stockholm, Sweden; [4]Department of Internal Medicine, Hanoi Medical University, Hanoi, Viet Nam; [5]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; [6]Department of Nephrology, Linköping University Hospital, Linköping, Sweden; [7]Transplantation Center, Sahlgrenska University Hospital, Göteborg, Sweden.*

Background: There is evidence suggesting that IgA nephropathy (IgAN) is an immunological disease and genetic findings contributing to disease development have been demonstrated. The role of Human Leukocyte Antigen (HLA) class II DR beta 1 (DRB1) has previously not been well studied. The aim of our study was to investigate the association of HLA-DRB1 alleles with IgAN in a Swedish Caucasian cohort.

Patients and Methods: The 1782 individuals included in our study consisted of 213 patients with biopsy proven IgAN, all of self-reported Caucasian ancestry. As a control cohort, 1569 healthy subjects from the same population in Sweden were included. HLA-DRB1 low resolution genotyping was performed and odds ratios adjusted for age and gender were calculated to assess the risk.

Results: In a simple allelic model the HLA-DRB1 *01, *04, *10 and *14 alleles represented an increased risk for IgAN with a cumulative odds ratio reaching 1.85 (95%CI 1.3-2.6). In opposite, the HLA-DRB1*03, *07, and *15 indicated a protective effect for IgAN, with cumulative OR=0.49 (95%CI 0.35-0.69). When the influence of risk alleles was adjusted for protective alleles and vice versa, only the protection from HLA-DRB1 remained significant.

Conclusion: The variants of HLA-DRB1 were associated with IgAN of which the HLA-DRB1 *03, and *15 revealed strong protective effect for IgAN. Our data suggest that involvement of adaptive immunity may be of importance in the development of the disease.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2708

### Polymorphisms in the Nonmuscle Myosin Heavy Chain 9 Gene (MYH9) Are Associated with the Progression of IgA Nephropathy in Chinese

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**Background** IgA nephropathy (IgAN) is the leading cause of end-stage renal disease (ESRD) in China considering different composition of ESRD causes in different ethnicities. Recent genome-wide association study(GWAS)indicated MYH9 gene was significantly associated with nondiabetic ESRD in African Americans, and also influenced kidney function in Europeans. Thus, in the present study, we aim to clarify whether MYH9 confers a shared mechanism among different causes of ESRD, and to seek a possibly further insight into our understanding of IgAN by applying GWAS data from ESRD to IgAN.

**Methods** 1116 Chinese, including 527 patients with renal biopsy-proved IgAN and 589 healthy controls, were enrolled in the present study. 3 SNPs (rs3752462, rs4821480, rs11089788) reported to be associated with ESRD with the most significance were genotyped by TaqMan assay and for further case control study.

**Results** None of the three SNPs was associated with the susceptibility to IgAN or clinical characters(systolic and diastolic blood pressure, serum creatinine, eGFR and urinary protein excretion)at the time of renal biopsy. However, eGFR decline rate was associated with rs11089788 in dominant model (p=0.028). Cox regression showed that rs11089788 (hazard ratio, 4.16; 95% confidence interval,1.58 to 11.00; $P=4.0\times10^{-3}$) was an independent predictive factor for renal survival besides time average proteinuria and serum creatinine.

**Conclusion** Based on a large Chinese IgAN cohort, we found an association between rs11089788 and prognosis of IgAN, mounting evidence of MYH9 as an important gene in IgAN to ESRD.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2709

### Genetic Variants in *Arhgef11* Linked to Proteinuria, Renal Hemodynamic Parameters and GFR in the Dahl Salt-Sensitive Rat

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The Dahl salt-sensitive (S) rat is a widely studied model of salt-sensitive hypertension which develops significant renal injury. Previously, a genetic analysis using the S and spontaneous hypertensive rat (SHR) identified a locus on chromosome 2 that strongly influences the development of renal injury. Temporal study (weeks 8-20) of blood pressure (BP) and renal parameters were compared between the S and a small congenic strain (which narrowed the locus to <1Mb). No significant difference in BP was detected from week 8-20 between strains (week 20, 202±6.4 and 194±2.1 mmHg, respectively). The congenic strain did exhibit significantly reduced proteinuria and improved renal function over the entire period of study. At week 20, proteinuria was 40% lower in the congenic and creatinine clearance was significantly higher (0.60±0.08 ml/min/gkw) compared to the S (0.40±0.02). Renal hemodynamic parameters were evaluated including: RBF, GFR and glomerular permeability ($P_{alb}$) at week 12. RBF was significantly higher in the congenic (4.4±0.33 ml/min/gkw) compared to S (3.0±0.19) as was FITC-inulin measured GFR (1033±101 and 564±63 µl/min/gkw, respectively). No significant difference was observed in $P_{alb}$ or nephron number between strains. Lastly, the congenic lived significantly longer compared to the S (median survival = 345 vs. 176 days). Using genetic and genomic methods we identified a small number of likely genetic variants linked to renal injury. The current evidence [promoter and coding sequence variants, gene expression differences, and a strong biological role] suggests that *Arhgef11* is a strong candidate underlying the locus. ARHGEF11 protein levels (in the S rat) are associated with up-regulation of the Rho/ROCK signaling pathway whereas this pathway is down-regulated in the congenic. In summary, this work provides evidence that renal injury and decline in renal function exhibited in the S rat may be linked to Rho/ROCK signaling and influenced by allelic variants in *Arhgef11*.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2710

### Survival and Renal Damage during Development Salt-Sensitive Hypertension Can Be Regulated by Genes in Rat Chromosome 2

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The existence of genes associated with salt-sensitive hypertension on rat chromosome 2 has been confirmed previously using different congenic strains. It is plausible that the chromosome 2 congenic can attenuate renal damage primarily through an altered fibrotic response. However, long-term studies of survival and renal sclerosis had not been conducted. We used a chromosome 2 substitution strain (SS-2(BN)) in which one chromosome was transferred from the Brown Norway (BN) rat onto the Dahl salt-sensitive (SS) genetic background. At the weaning SS (n=8) and SS-2(BN) (n=8) males had been placed on 1% salt diet for seven months. By the end of this period 75% of SS and none of SS-2(BN) rat died. Heart weight and histological evaluation did not reveal any differences between rat strains. Kidney weight was significantly increased in SS compared to SS-2(BN) strain (1.98±0.11 vs. 1.68±0.02 g). Daily urine volume was elevated in SS compared to SS-2(BN) strain (36.9±9.0 vs. 10.9±1.7 ml/day). These effects were accompanied by sever glomerulosclerosis, protein deposition and tubular degradation. Expression of several proteins, involved into pro-fibrotic signaling was analyzed in kidney cortex and medulla using Western blotting. BCAR3 expression in cortex was increased and C3G expression in medulla was decreased in SS-2(BN) compared to SS strain. Immunohistochemistry evaluation of C3G localization uncovered strong positive signal in glomerular and abnormal tubular cells. In conclusion, these observations indicate that BN rat chromosome 2 harbor genes that contribute to survival and renal damage during development of salt-sensitive hypertension.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2711

### Polymorphisms in the Protein C Pathway Increase Mortality in Dialysis Patients

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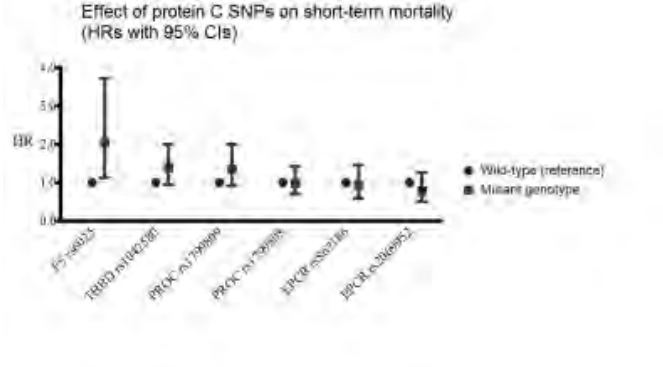
**Objectives:** The protein C pathway plays an important role in the protection of endothelial barriers and in inflammatory and anticoagulant processes, which are fundamental in patients treated with dialysis. The aim of this study was to investigate the mortality risk of known SNPs in the protein C pathway.

**Methods:** 1070 incident dialysis patients were genotyped for rs6025 (Factor V Leiden), rs1042580 (THromBomoDulin), rs1799808 and rs1799809 (PROtein C), and rs867186 and rs2069952 (Endothelial Protein C Receptor). Hazard ratios (HRs) with 95% confidence intervals (95% CIs) were calculated for 1.5-year (short-term) and 5-year (long-term) all-cause and cause-specific mortality risk for homozygotes and heterozygotes combined for the minor allele (mutant genotype) as compared to homozygotes for the major allele (wild-type).

**Results:** Factor V Leiden was associated with an increased short-term all-cause mortality risk (HR 2.05; 95% CI 1.13-3.72) (Figure 1) and cardiovascular mortality risk (HR 2.34; 95% CI 1.00-5.47). *PROC* rs1799809 was associated with an increased short-term mortality risk due to other causes than infections and cardiovascular diseases (HR 1.95; 95% CI 1.05-3.60), especially due to treatment cessation. Furthermore, these two SNPs combined with *THBD* rs1042580 showed an increased short-term term all-cause mortality risk (HR 3.32; 95% CI 1.09-10.16). None of the SNPs was associated with long-term mortality risk conditional on survival of 1.5 years.

**Conclusion:** Our data study suggests that Factor V Leiden, *PROC* rs1799809, and *THBD* rs1042580 contributes to an increased short-term mortality risk in dialysis patients.




Disclosure of Financial Relationships: nothing to disclose

**SA-PO2712**

**Variation in the VDR Gene and Neuroantibody Markers in Dialysis Patients** Darius Mason,[1] Magdalene M. Assimon,[1] Michael H. Akroush,[2] Jeffrey R. Bishop,[2] Hassan El-Fawal.[1] *[1]Albany College of Pharmacy & Health Sciences, Albany, NY; [2]University of Illinois at Chicago, Chicago, NY.*

Cognitive deficits associated with reduced survival are prevalent in hemodialysis (HD) patients. Vitamin D receptor (VDR) SNPs have been linked to neuroprotection and neurodegeneration (ND). Chemokines and autoantibodies (Ab) to myelin basic protein (MBP), glial fibrillary acidic protein (GFAP), and neurofilament (NF) triplet proteins have been reported in ND studies. These Ab arise secondary to nervous system (NS) damage providing a means to assess NS injury in HD patients. Characterization of Ab biomarkers of neuropathy in HD patients and their association with VDR SNPs was studied.

Biomarkers in HD subjects receiving ergocalciferol (ergo) were compared to non-users. Subjects were genotyped for VDR BsmI [rs154410]. IL-6, TNF-α, and IgG titers against NF (NF-68, NF-160, and NF-200), GFAP and MBP were measured by immunoassay. BsmI genotypes and ND biomarkers relationships were assessed.

Subjects (age 63.3±16.1 years, 66% male) genotype frequencies (Bsm1;CC/CT/TT: 41.4%/49.5%/9.1%) did not deviate from Hardy-Weinberg equilibrium(p=0.34). Ergo users with the C allele had lower anti-NF160 and anti-MBP titers compared to non-users ($p<0.05$).

| | Ergo: n=40 | No Ergo: n=71 |
|---|---|---|
| Log IL-6 (pg/mL) | 0.60±0.40 | 0.52±0.43 |
| TNF-α (pg/mL) | 11.56±5.6± | 12.97±7.0 |
| anti-NF68 | 1.71±0.34 | 1.69±0.27 |
| anti-NF160 | 1.48±0.31* | 1.63±0.31 |
| anti-NF200 | 1.66±0.33 | 1.71±0.26 |
| anti-GFAP | 1.69±0.34 | 1.78±0.41 |
| anti-MBP | 1.63±0.25* | 1.76±0.28 |

* $p<0.05$; Values: mean±SD; Ab values=absorbance at 405nm

IgG against NS proteins in HD patients suggests neuronal and glial insult. Anti-NF presence, particularly anti-NF160 in patients with the C allele, is consistent with studies showing reduced expression of these proteins in vitamin D deficiency. Loss of these structural proteins, indicated by serum IgG, contributes to ND. Vitamin D deficiency may also contribute to demyelination, indicated by anti-MBP, which was ameliorated by ergo therapy. This preliminary study suggests Ab detection may be useful in monitoring ND changes and the efficacy of ergo in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2713**

**KCNMB1 Variants Contribute to Glonmerular Filtration Rate and Progression of Chronic Kidney Disease** Jia Han, Yuqing Chen, Yu Liang, Ying Liu, Hong Zhang, Haiyan Wang. *Renal Division, Peking University First Hospital, Beijing, China.*

Background: Glomerular filtration rate (GFR) is a heritable trait, suggesting roles for genes in its regulation and decline. Mesangial and vascular smooth muscle cells control GFR by contraction dependent on voltage-gated Ca2+ influx. After membrane depolarization in these excitable cells, activation of large-conductance heteromeric K+ ("BK") channels repolarizes the plasma membrane, terminating Ca2+ influx in negative-feedback fashion. The regulatory beta1-subunit (KCNMB1) of BK, expressed in smooth muscle and mesangial cells, regulates the negative-feedback mechanism and determines contraction as well as basal tone. KCNMB1 variants were reported to be associated with hypertension as well as other disease. Here we asked whether the KCNMB1 variant influences GFR, in the basal state or during progressive renal decline.

Methods: We explored 3 KCNMB1 variants of rs11739136 (Glu65Lys), rs2301149 (Val110Leu), rs827778 (5'UTR) effects on GFR in three populations. A community-based population of Chinese Han (CH); individuals with IgA nephropathy with more than 24 months follow-up (IGAF) and end stage renal disease (ESRD) accepted dialysis. eGFR from serum creatinine, was calculated by CKD-EPI.

Results: The Glu65Lys variant predicted eGFR in CH population, and 65Lys carriers exhibited higher GFR (by ~1%, p=0.020, n=1176). In IGAF population with progressive renal disease as a result of IgA nephropathy, Val110Leu was associated with GFR decline. 110Leu carriers displayed faster chronic GFR decline slope (by ~1.7%, p=0.020, n=256), and 110L were more significantly frequent in ESRD persons (Chi-square=23.48, $p<0.001$, ESRD n=136 Vs control n=730).

Conclusions: Common KCNMB1 variants influence GFR. The results suggest that KCNMB1 is one of the candidate genes that influence GFR and affect the progression of chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2714**

**Association of Common Variants in Mendelian Disorder Genes with Renal Function: The CKDGen Consortium** Afshin Parsa,[1] Christian Fuchsberger,[2] Anna Kottgen,[6] Carsten A. Böger,[5] Conall M. O'Seaghdha,[8] Tennille S. Leak,[4] David Siscovick,[3] Wen Hong Linda Kao,[7] Caroline S. Fox.[8] *[1]University of Maryland; [2]University of Michigan; [3]University of Washington; [4]University of Pittsburgh; [5]Regensburg University, Germany; [6]Freiburg University, Germany; [7]Johns Hopkins University; [8]NHLBI's Framingham Heart Study, on Behalf of the CKDgen Consortium.*

INTRODUCTION: Results from population-based genome-wide association studies (GWAS) have uncovered new associations between common genetic variants and a variety of traits. Many of these associations map to genes previously linked to rare Mendelian disorders. Thus, we hypothesized that common variations in genes that harbor rare mutations causing Mendelian diseases with a renal phenotype would be associated with renal function in the general population.

METHODS: Using the keyword "kidney", we queried ~4000 listings within the OMIM public database. From this list, we curated 227 disease entries with described renal phenotypes and one or more identified putative autosomal gene. Our final candidate gene list included 283 unique entries. The renal phenotypes associated with these diseases are quite diverse and include renal dysplasia, tubular dysfunction, cysts and vesico-ureteric reflux. We then looked for association between common variants (minor allele frequency>5%) in these genes with eGFRcrea in 67,093 Caucasian participants of the CKDGen Consortium.

RESULTS: We identified a total of 6 novel associated candidate genes at $p < 10\text{-}5$ and identified another 11 moderately associated candidates ($p<10\text{-}4$). These findings include variants within the LRP2, NSD1, TSC1 and GSS genes. Replication efforts for our candidate loci are underway.

CONCLUSION: By exploring an extensive list of Mendelian genes known to be associated with renal pathology, we have identified numerous potentially novel common genetic variants that are associated with renal function in the general population. While preliminary, these findings suggest that database mining of common complex diseases may be feasible using a targeted Mendelian candidate gene approach.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2715**

**High Resolution Mapping of a CAKUT Locus on Mouse Chromosome 2** Francesca Lugani,[1,2] Zongyu Zheng,[1] Ali G. Gharavi.[1] *[1]Department of Medicine, Division of Nephrology, Columbia University, New York, NY; [2]Laboratory on Pathophysiology of Uremia, G. Gaslini Insitute, Genoa, Italy.*

**Objective**: Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) are the most common causes of end stage renal disease in children. We studied a spontaneous mouse mutant model of CAKUT, the Danforth's short tail mouse (*Sd*) . The heterozygous *Sd* mutants display a short tail with 100% penetrance and CAKUT (especially agenesis/hypodysplasia) with 80% penetrance. The homozygous mutants die shortly after birth due to severe multiorgan malformations, including bilateral renal agenesis, anorectal malformation, intestinal dysganglionosis and major defect of the axial cytoskeleton.

**Methods**: The *Sd* locus had previously been localized to Chr 2A3. To refine this locus, we produced 147 F2 segregants between Rsv/LeJ $^{Sd/+}$ mice and C57BL/6J mice and genotyped 10 informative markers (5 microsatellites and 5 SNPs) across the Sd locus. Multipoint lod scores were calculated using the R/QTL package, utilizing the discrete trait analytic model.

**Results**: Analysis of linkage yielded a peak lod score of 39 ($p=6x10^{-41}$) at rs33491214. Analysis of critical recombinants refined the Sd locus to a 1.3 cM region delimited by rs27129240 and rs29504224, which contains 15 transcriptional units. Of these positional candidates, 4 are known to be expressed in the developing urinary tract. Critically none of the positional candidates have been implicated in human malformation syndromes.

**Conclusions:** Identification of the *Sd* mutation and downstream pathways will provide significant insight into pathways underlying urogenital development and may clarify pathogenesis of human CAKUT and related malformation syndromes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2716**

**Familial Focal Segmental Glomerulosclerosis Is Linked to a Locus on Chromosome 6q16~22** Nan Chen, Jun Ma, Jingyuan Xie, Zhaohui Wang, Weiming Wang, Hong Ren, Wen Zhang. *Department of Nephrology, Ruijin Hospital, Shanghai JiaoTong University, School of Medicine, Shanghai, China.*

**Objective** Recently, mutations in several genes including ACTN4, TRPC6, PLCE1 and INF2 were found to cause familial focal segmental glomerulosclerosis( FSGS) by linkage analysis . But most of the pathogenic genes were identified in Caucasian pedigrees. So we performed linkage analysis to map the disease gene in a large Chinese FSGS family.

**Methods :** A Chinese FSGS family was investigated. 78 members in this family were screened. Peripheral blood sample were taken and genomic DNA was extracted. Polymerase chain reaction(PCR) and sequence-direct were used to exclude already known genes. Affymetrix 10k SNPs chip were selected as markers for the whole genome-wide scan in some family members. Merlin and SimWalk2 software were taken for linkage analysis.

**Results** The family comprised four-generation, 103 members which is the largest FSGS kindred reported in China. It was a late-onset, autosomal dominant inherited FSGS family with eleven affected members. There was no mutation found in the NPHS2,

ACTN4 and TRPC6 gene. Linkage analysis of single nucleotide polymorphism (SNP) data identified consistently positive log of the odds (LOD) scores across chromosome 6q (maximal LOD score of 3.795 at rs1431213). Recombination narrowed the conserved haplotype to 5 Mb at 6q16~22(flanking markers rs2001102 and rs722366), which were not reported previously.

**Conclusions** The family is the largest late-onset, autosomal dominant inherited Chinese FSGS kindred. Pathogenic gene for this family may linked to a new locus on chromosome 6q16~22.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2717

**The Uridine Diphosphate Glucuronosyltransferase (UGT) 2B7 C802T Single Nucleotide Polymorphism (SNP) and Small Vessel Vasculitis** Melanie S. Joy,[1] Yichun Hu,[2] Susan L. Hogan,[2] Philip C. Smith,[3] Ronald J. Falk.[2] *[1]Schools of Medicine and Pharmacy, University of North Carolina, Chapel Hill, NC; [2]School of Medicine, Kidney Center, University of North Carolina, Chapel Hill, NC; [3]School of Pharmacy, University of North Carolina, Chapel Hill, NC.*

The uridine diphosphate glucuronosyltransferases (UGTs) are Phase II metabolism enzymes that conjugate glucuronic acid to functional groups including carboxylic acids (e.g. mycophenolic acid), alcohols, amines, and thiols. Numerous xenobiotics associated with drug-induced vasculitis are also substrates for UGTs. The *C802T* variant allele in *UGT2B7* is associated with decreased metabolic activity. The current study evaluated the susceptibility of small vessel vasculitis (SVV), lupus nephritis, and rheumatoid arthritis in patients who carried the *UGT2B7 C802T* allelic variant.

Genotyping for the *UGT2B7 C802T* single nucleotide polymorphism was conducted on DNA from disease and control populations. Allelic and genotype frequencies were reported by disease cohort and Hardy Weinberg Equilibrium was assessed. The odds of disease were calculated by the Logistic Model and controlled for race.

A total of 320 DNA samples were genotyped; 170 SVV, 53 lupus nephritis, 26 rheumatoid arthritis, and 71 healthy controls. The SVV patient demographics showed: 84F/86M and 98% Caucasian. The odds ratio between *UGT2B7* genotype groups, using healthy controls as a reference group are shown in the Table. The analyses controlled for race.

| Subject Group | OR (95% CI) | p value |
|---|---|---|
| Small Vessel Vasculitis | 3.46 (1.883, 6.345) | <0.0001 |
| Lupus Nephritis | 1.43 (0.620, 3.320) | 0.3998 |
| Rheumatoid Arthritis | 0.89 (0.348, 2.265) | 0.8025 |

The association between SVV and a polymorphism in the gene of a phase II metabolizing enzyme is intriguing given numerous publications that suggest exposure to various xenobiotics may be one etiology of SVV. A review of implicated drugs demonstrate that propylthiouracil, isotretinoin, diuretics, and NSAIDs are substrates for UGT2B7. These data could have implications for SVV risk secondary to potential environmental toxins that are also UGT2B7 substrates.

Disclosure of Financial Relationships: Consultancy: Lilly, Amylin, Novo Nordisk (spouse); Ownership: Katharos, Inc.Research Funding: DeCode (Spouse); Scientific Advisor: CeQur (Spouse).

## SA-PO2718

**Denys-Drash Syndrome Associated with a Novel Exon 6 *WT1* Gene Mutation in a 18-Year-Old Girl** Pietro Claudio Dattolo,[1] Stefano Michelassi,[1] Giuseppe Ferro,[1] Alma Mehmetaj,[1] Paraskevas Iatropoulos,[2] Francesco Pizzarelli.[1] *[1]Nephrology and Dialysis Unit, S. M. Annunziata Hospital, Florence, Italy; [2]Genetic Department, Mario Negri Institute, Bergamo, Italy.*

Wilms tumor suppressor gene WT1 maps on chromosome 11p13 and is involved in several syndromes characterized by genital and kidney abnormalities. Classically, WT1 mutations occurring in exons 8 or 9 have been associated with Denys-Drash syndrome (DDS), characterized by early-onset and progressive nephropathy with progression to uraemia within the first four years of age, pseudoermaphroditism and Wilms tumor. Intron 9 splice donor site mutations have been associated with Frasier syndrome (FS), characterized by pseudoermaphroditism, slow progressive nephropathy and gonadoblastoma. The full-blown clinical picture only occurs in XY subjects as XX patients usually show normal genital development.

We describe a 18-year-old female patient with a past history of uninephrectomy in childhood for Wilms tumor and presenting to our unit because of end-stage renal disease secondary to chronic proteinuric nephropathy discovered two years before. The girl had a normal genital development except for evidence of right ovarian hypertrophy on ultrasound investigation. Genetic analysis showed XX karyotype and a WT1 exon 6 non-synonimous mutation due to aminoacid substitution (c.1012A>T) at nucleotide position 338 (R338X). Such a mutation has never been reported in literature and it was found neither in her parents nor in 178 chromosome controls. This case reinforces the hypothesis that WT1 mutations can be more numerous than the ones just reported in literature and cause a spectrum of different nephropathy-associated syndromes, with DDS and FS representing only the most known ones. WT1-related nephropathies may be less rare than we commonly believe and they should be kept in mind in every not immunologically mediated primary proteinuric nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2719

**Genetic Polymorphism of Proinflammatory Cytokines and Albuminuria in Japanese General Population: The Takahata Study** Ami Ikeda, Tsuneo Konta, Kazuko Suzuki, Yusuke Mashima, Kazunobu Ichikawa, Satoshi Takasaki, Isao Kubota. *Department of Cardiology, Pulmonology, and Nephrology, Yamagata University School of Medicine, Yamagata, Japan.*

**Background:** A cluster of proinflammatory cytokines play an important role in the development of various renal diseases and the expression of these cytokines is genetically modified. To examine the relation between genetic polymorphism of proinflammatory cytokines and albuminuria, we conducted a cross-sectional study in general population.

**Methods:** We genotyped the SNPs of six proinflammatory cytokines including IL-1beta, IL-6, IL-8 TNF-alpha, CCL1 and MCP-1 in 2927 Japanese subjects. Urine albumin-creatinine ratio (UACR) was obtained from morning spot urine.

**Results:** Albuminuria (UACR $\geq$ 30 mg/g) was observed in 19.2% of total subjects and was significantly related with the A/A+A/G genotypes of rs2069852 in IL-6 ($P$ = 0.010) and the A/A genotype of rs2282691 in CCL1 ($P$ = 0.002), as compared with the remaining genotypes. The multivariate analysis adjusted with traditional risk factors showed that these genotypes independently predicted albuminuria (Odds ratio [OR] 1.782, 95% confidence interval [CI] 1.171-2.712, $P$ = 0.007 for the A/A+A/G genotypes of rs2069852 in IL-6, and OR 1.432, 95%CI 1.128-1.770, $P$ = 0.003 for the A/A genotype of rs2282691 in CCL1, respectively). Along with the increase in the number of risk genotypes, the prevalence of albuminuria and UACR levels were increased.

**Conclusion:** This study revealed that the genotypes of IL-6 and CCL1 were related with albuminuria and the combination of these genotypes showed an additive effect on the prevalence and severity of albuminuria. This indicates that genetic background of inflammatory response might affect the development of renal injury in Japanese general population.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2720

**Transcriptional Complexity of the PKD1 Gene** Robert L. Bacallao,[1] Almira Kurbegovic,[2] Jeanette N. Mcclintick,[3] Marie Trudel.[2] *[1]Medicine, Richard Roudebush VAMC and Indiana University, Indianapolis, IN; [2]Molecular Genetics and Development, Institut de Recherches Cliniques de Montreal,Universite de Montreal, Montreal, QC, Canada; [3]Biochemistry and Molecular Biology, Indiana University, Indianapolis, IN.*

The PKD1 gene is comprised of 45 introns and exons encoding a 450 kDa protein. Mutations in this gene are associated with autosomal dominant polycystic kidney disease, the most common monogenetic cause of renal failure. While the gene locus is known to produce a 14 kb mRNA, recent evidence in the ACEView database suggests that the PKD1 locus may produce up to 14 different transcripts. To evaluate the degree of transcriptional complexity, we isolated RNA from mouse embryonic fibroblasts derived from 3 mouse lines; a PKD1 transgenic mouse ($PKD1^{++/++}$), a wild type ($PKD1^{+/+}$) mouse and a PKD1 knockout mouse ($PKD1^{-/-}$). The $PKD1^{-/-}$ mouse was generated by a knockout of exon 3 in the pKD1 locus. Transcript expression was analyzed using the GeneChip® Mouse Exon 1.0 ST Array (Affymetrix, Santa Clara, CA). The Mouse Exon 1.0 ST array has at least four probes per exon. Intra-sample variability in the RNA was less than 5 %. Comparing the intensity values from the exon probes between the $PKD1^{++/++}$ RNA and $PKD1^{+/+}$ RNA revealed that virtually all the PKD1 exons were expressed at two times the level observed in the $PKD1^{+/+}$ mouse save exons 18 and 30. Exons 18 and 30 were expressed six fold more in the $PKD1^{++/++}$ mouse. Strikingly evaluation of the RNA from the PKD1-/- mouse line showed significant signals from exons 1,5, 6,8,10,11, 14, 15, 18, 22-23, 25-27, 29, 34, 36, 38, 39-43. Also one exon 45 probe also showed significant level of expression. This data suggests that multiple transcripts arise from the PKD1 locus and their expression pattern has not been fully elucidated.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2721

**PC1 Regulates ER $Ca^{2+}$ Release through Activation of the PI3K/Akt Pathway To Modulate PC2-$IP_3$R-STIM1 Interaction** Netty Santoso, William B. Guggino. *Physiology, Johns Hopkins School of Medicine, Baltimore, MD.*

Mutations of Polycystin-1 (PC1) and Polycystin-2 (PC2) are the main cause for development of the genetic disease Polycystic Kidney disease (ADPKD). However, the molecular mechanism underlying how these mutations cause ADPKD is still unclear. PC2, a member of the TRP channel family, has been suggested to function as a $Ca^{2+}$ channel in the Endoplasmic reticulum (ER) and on the cell surface. Meanwhile, PC1 was shown to interact with PC2 to facilitate the trafficking and function of PC2 as a $Ca^{2+}$ channel on the cell surface. Although PC1 is thought not to have channel properties, misregulation of intracellular $Ca^{2+}$ homeostasis has been implicated in both PC1 and PC2 mutations. Here, we try to investigate whether PC1 is involved in the regulation of $Ca^{2+}$ homeostasis. We utilized a MDCK cell line that has stable expression of full-length PC1 to analyze intracellular $Ca^{2+}$ release from the ER. Expression of full-length PC1 in these cells caused inhibition of the $Ca^{2+}$ release compared to the control cells. We then correlated the $Ca^{2+}$ release inhibition with the reduction of endogenous PC2-$IP_3$R interaction in the PC1-expressing cells. Normally, PC2 interacts with the $IP_3$R, and this interaction is known to enhance $Ca^{2+}$ release. We also found that endogenous STIM1 interacted with the $IP_3$R, and this interaction was enhanced with PC1 expression. This interaction appeared competitive to the PC2-$IP_3$R interaction to inhibit ER $Ca^{2+}$ release. Finally, we show that the PI3K/Akt signaling pathway was involved in regulating the STIM1-$IP_3$R-PC2 interaction to modulate ER $Ca^{2+}$ release. Previously, it

was shown that PC1 expression in MDCK cells induces activation of the PI3K/Akt signaling pathway to suppress cell apoptosis. Here, we confirm the increased activation of the PI3K/Akt pathway in the PC1 cells, and we found that activation of the pathway inhibited the PC2-$IP_3R$ interaction, and ER $Ca^{2+}$ release, while increasing the STIM1-$IP_3R$ interaction. We propose that PC1 expression suppresses cell apoptosis through activation of the PI3K/Akt pathway by reducing the intracellular $Ca^{2+}$ release.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2722**

**Mechanisms of Plasticity of CD133+ Renal Progenitor Cells** Benedetta Bussolati, Cristina Grange, Aldo Moggio, Federica Collino, Giulia Aghemo, Giovanni Camussi. *Internal Medicine, University of Torino, Torino, Italy.*

The tubular compartment of the nephron displays a high regenerative ability. $CD133^+$ renal cells have been isolated in the tubular compartment and display characteristics of progenitor cells. However, the possible contribution of this resident population to repair is unknown. The purpose of this study was to evaluate whether hypoxia modulates stemness of $CD133^+$ progenitor cells. Methods: We isolated and cultured in normal and hypoxic ($1\%O_2$) conditions CD133+ renal progenitors. Expression of stem transcription factors was evaluated by Western Blot analysis and quantitative RT-PCR. Activation of Oct4 promoter was evaluated after cell lentiviral infection with an Oct4 GFP-reporter system. In vivo differentiation was evaluated after cell injection within Matrigel under the renal capsule of immuno-compromised mice.Results: CD133+ cells showed an expression of Oct4A, Oct4B and Oct4B1. Klf4 and c-Myc mRNA and protein were expressed, at low level, in renal CD133+ progenitors, whereas Sox2 expression was inconsistent. Hypoxia by increasing HIF-1alpha up-regulated Oct-4 isoforms in CD133+ cells via regulation of the Oct4 promoter. In parallel, hypoxia increased proliferation, clonogenicity and CD133 expression in the renal progenitors. When injected in vivo, CD133+ hypoxic progenitors showed an increase in their differentiative ability. In particular, the injected cells differentiated in structures resembling the different segments of the nephron, and expressed aminpeptidase, Tamm-horsfall protein and aquaporin-2 and 3 markers. In conclusion, these results suggest that hypoxia may direct $CD133^+$ progenitors toward a more stem phenotype, via Oct4A activation, inducing an increased proliferation, clonogenicity and differentiative potency. The plasticity of renal CD133+ cells could be implied in renal regeneration after hypoxic conditions.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2723**

**Phenotypic Dispersion in a Rodent Model of Hypercalciuria Is Linked to Sex and to Genes for Spliceosome Formation, RNA Manipulation, Metal Ion Binding and Energy Metabolism** Guy M. L. Perry,[1] Keith Nehrke,[2] David A. Bushinsky,[2] Steven J. Scheinman.[1] *[1]Medicine, SUNY Upstate Medical University, Syracuse, NY; [2]Medicine, University of Rochester, Rochester, NY.*

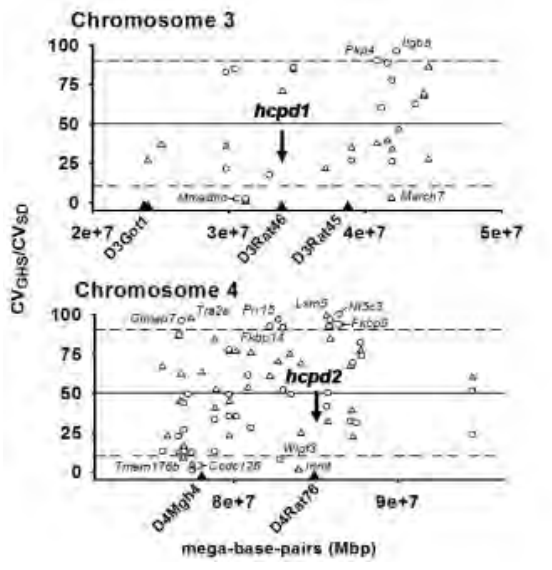
Empirical evidence from several animal models indicates that residual phenotypic variance is under genetic control and may be affected by sex; urinary physiology is also notable for high residual variability. We mapped two quantitative trait loci (QTL) for phenotypic dispersion (*PD*) in urinary calcium (*hcpd1, hcpd2*) in F2 intercrosses of Genetic Hypercalciuric Stone-forming (GHS) rats - Wistar-Kyoto (WKY) controls. In female F2s, *hcpd1* was significantly associated with PD ($P_{Bon}$<0.05) and *hcpd2* and two other microsatellite markers were suggestively ($P_{FDR}$<0.05) associated with *PD.*

Table1: QTL for PD in F2 GHS-WKY rats

| Locus (QTL) | n | $\sigma^2(CV)_{WKY}$ | $\sigma^2(CV)_{Het}$ | $\sigma^2(CV)_{GHS}$ | *P* |
|---|---|---|---|---|---|
| *D3Rat46* (*hcpd1*) | 125 | 3.76 (66.4) | 1.23 (45.8) | 1.58 (53.7) | 0.0002 |
| *D4Rat76* (*hcpd2*) | 126 | 0.972 (43.8) | 2.15 (62.2) | 2.41 (49.4) | 0.0024 |
| *D9Mgh2* | 105 | 1.15 (42.4) | 2.72 (68.5) | 2.61 (52.9) | 0.0009 |
| *D12Rat25* | 101 | 3.49 (64.5) | 1.49 (55.0) | 0.983 (45.6) | 0.0007 |

No locus was associated with PD in males (*P*>0.2). Relative variability in gene expression in GHS rats was compared to that in unselected Sprague-Dawley controls using coefficient of variance (CV) ratios; 31% of genes with extreme expression ratios nearby (10 MB) *PD* QTLs had methylation, RNA transcription or spliceosome functions, 20% were involved with energy metabolism and 19% with metal ions.

Figure 1: Expression CV ratios for genes linked to *hcpd1* and *hcpd2*




Sexual patterns in urinary PD were also detected in congenic rat lines. Our results indicate the existence of genes causing residual variance in urinary calcium excretion, expressed primarily in females and via RNA-modifying genes, a finding of enormous potential importance to nephrological genetics.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2724**

**Sleep Disordered Breathing Does Not Improve with Alternate Nightly Nocturnal Home Hemodialysis** Carolyn L. Van Eps,[1] Brett Duce,[2] Carmel M. Hawley,[1] Craig Hukins.[2] *[1]Department of Nephrology, Princess Alexandra Hospital, Brisbane, Queensland, Australia; [2]Department of Respiratory Medicine, Princess Alexandra Hospital, Brisbane, Queensland, Australia.*

Sleep disordered breathing (SDB) is common in ESKD and linked to adverse cardiovascular and quality of life outcomes. Daily dialysis regimens have been shown to improve SBD. We hypothesize that alternate nightly nocturnal home hemodialysis (NHD) may improve SDB.

47 patients converted from conventional home hemodialysis to NHD (6-9hours for 3.5-5sessions weekly). 32 patients(Age 55±11years, 88% Male, BMI 28.8±7.2kg/$m^2$, Diabetes 25%) at baseline and 25 patients at 3 months consented to sleep assessment using polysomnography with esophageal pressure assessment; ventilatory response to hypoxia and hypercapnia using rebreathing techniques; Epworth Sleepiness Score (ESS).

97% of patients had SDB which was predominantly obstructive(>98% of events). Most patients had moderately severe (Total Apnea Hypopnea Index(AHI) 95% CI 17.8-29.8/sleep hour),and 21% severe obstructive sleep apnea (OSA). There was no significant sleep hypoxia. 56% of patients had >15 leg movements/hour. There were no significant changes in sleep architecture, AHI, Arousal Index or ESS (6.9±3.1 vs7.0±4.1, p=0.20). Non-REM sleep mean partial pressure of oxygen improved but other measures of oxygenation did not. Benzodiazepine use increased(6-28%). There was a decrease in the intercept of the carbon dioxide-minute ventilation relationship but no change in its slope. The slope of the minute ventilation-oxygen saturation relationship during hypoxic challenge did not change. Interdialytic weight gains trended to increase.

Prevalence of OSA, periodic leg movements and the relationship between ventilatory drive and arterial carbon dioxide concentration did not improve with conversion to NHD. Central apneic events were unusual suggesting that control of uremic toxins and acidemia is of minimal importance in SDB in ESKD. Our failure to show improvement in OSA may result from persistently high interdialytic fluid gains with pharyngeal narrowing and obstruction when excess fluid redistributes as patients are recumbent. Increased benzodiazepine use may contribute.

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**SA-PO2725**

**Gentamicin Pharmacokinetics during Short-Daily Hemodialysis** Brian S. Decker,[1] Sharon M. Moe,[1] Kevin Sowinski.[1,2] *[1]Department of Medicine, Division of Nephrology, Indiana University School of Medicine, Indianapolis, IN; [2]Department of Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, IN.*

**Introduction**

Short-daily hemodialysis (SDHD) is emerging as an alternative to conventional, thrice-weekly hemodialysis due to its putative physiological benefits. However, there is a paucity of data regarding the appropriate dosing of medications in patients receiving SDHD.

**Purpose**

The purpose of this study was to investigate the effect of SDHD on the pharmacokinetics (PK) and intradialytic removal of gentamicin.

**Methods**

Six non-infected anuric adults treated with SDHD were studied and received four dialysis sessions over 4 days. Following the completion of a subject's first SDHD, gentamicin IV 2 mg/kg was administered. Blood samples were obtained over the ensuing 3 days during each subsequent inter and intradialytic period. During that time, subjects received three additional SDHD sessions. Gentamicin concentrations were determined by EMIT. Candidate pharmacokinetic models were fit to the concentration-time data using ADAPT 5 and PK parameters were determined by standard methods. Data are presented as median (range).

**Results**

5 men and 1 women [Age: 53.5 (28-59 yrs); Weight: 91 (59-110 Kg)] were studied. All subjects received SDHD with a new CT dialyzer (Exeltra 150, Baxter Healthcare, Inc.). SDHD dialysis operating characteristics were: dialysis time: 2.5 (2-2.5 hours) and Qb: 500 (450-500) mL/min. Pharmacokinetic parameter estimates and other relevant data are shown in Table 1. A two compartment open model best fit the pharmacokinetic data.

Gentamicin Pharmacokinetic Data

| CLs (mL/min) | CLdial (mL/min) | Vc (L) | Vss (L) |
|---|---|---|---|
| 3.1 (2.7-11.1) | 128 (77.9-252) | 13.1 (3.85-32.9) | 24.6 (14.5-46.0) |

**Conclusions**

The pharmacokinetic data for gentamicin in our SDHD patients is similar to the pharmacokinetic data for gentamicin administered to thrice-weekly HD patients. This suggests that the dosing recommendations for gentamicin in thrice-weekly HD can be used for patients receiving SDHD.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2726

**Continuos Ultrafiltration Can Improve Blood Pressure Control and the Efficiency of Hemodialysis** James P. Jones,[1] Edward F. Leonard,[2] Antony A. Farias,[1] Nathan W. Levin,[3] Maria E. Tarallo,[3] Stanley Cortell.[1] *[1]Medicine, St. Luke's Roosevelt Hospital Center, New York, NY; [2]Chemical and Biomedical Engineering, Columbia University, New York, NY; [3]Renal Research Institute, New York, NY.*

The high prevalence of hypertension (80%) is a major factor in the high mortality rate in ESRD patients. Fluid removal is essential for volume and blood pressure control. We have designed an ambulatory, ultrafiltration (UF) device based on microfluidic flow paths and ultrathin silicon-based nanopore filters. The device has a very small blood contact area ~50 $cm^2$ and an active transport volume of less than 0.5 ml. It operates at an ultrafiltration rate of 1 ml/min (equivalent to 10 kg/wk), and if worn continuously would maintain a nearly invariant, dry weight. As a simulation of the device, we have tested the hypothesis that hemodialysis patients maintained near their dry weight would have better controlled blood pressure and the efficiency of improved urea clearance. We studied 12 stable hemodialysis patients who received UF 4 days/week (M,W,Th,S) and twice weekly dialysis with UF (Tu,F). The pre-dialysis blood pressures and Kt/V of the 12 patients were compared in each patient with 4 weeks of standard 3 times per week hemodialysis and UF. MAP decreased from 110 to 95 ($P < 0.001$). Dialysis time decreased from 10.74 to 8.24 hours per week. UF per dialysis treatment decreased from 3.25 L to 1.82 L. Weekly Kt/V decreased from 4.19 to 3.75. Weekly Kt/V per minute showed an improvement of 16.65 %. This study supports the premise that an ambulatory UF device providing continuous UF would not only improve blood pressure control, but could also support a new regimen of continuous daily UF and in-center dialysis in two rather than three sessions/week. However this would require long term studies.

| | Pre Trial | | | | | | Daily Phase | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | Sys. | Dia. | MAP | HD Time (hours/wk) | weekly kT/V | HD UF Volume (L/wk) | Sys. | Dia. | MAP | HD Time (hours/wk) | weekly kT/V | HD UF Volume (L/wk) |
| 1 | 121 | 66 | 84 | 9.68 | 4.17 | 5.53 | 119 | 69 | 84 | 9.00 | 3.96 | 3.95 |
| 2 | 158 | 99 | 119 | 9.63 | 4.10 | 8.43 | 149 | 92 | 111 | 8.85 | 4.14 | 3.05 |
| 3 | 155 | 96 | 116 | 9.00 | 4.29 | 6.94 | 132 | 81 | 98 | 8.00 | 4.90 | 4.03 |
| 4 | 154 | 88 | 110 | 11.70 | 4.82 | 15.45 | 144 | 79 | 101 | 8.15 | 4.15 | 3.75 |
| 5 | 150 | 74 | 100 | 9.46 | 3.82 | 9.10 | 139 | 71 | 94 | 8.05 | 5.21 | 3.63 |
| 6 | 151 | 78 | 102 | 12.00 | 4.81 | 10.88 | 135 | 72 | 93 | 9.00 | 4.31 | 5.32 |
| 7 | 191 | 103 | 133 | 11.24 | 4.55 | 7.52 | 135 | 73 | 94 | 7.59 | 3.56 | 2.18 |
| 8 | 173 | 91 | 119 | 11.83 | 4.32 | 11.13 | 142 | 70 | 95 | 9.86 | 3.67 | 2.50 |
| 9 | 168 | 79 | 108 | 10.64 | 4.08 | 12.63 | 140 | 64 | 89 | 7.31 | 3.04 | 4.57 |
| 10 | 149 | 92 | 111 | 10.08 | 3.74 | 7.53 | 130 | 77 | 91 | 7.10 | 3.40 | 3.45 |
| 11 | 157 | 79 | 105 | 12.00 | 3.72 | 12.39 | 140 | 68 | 93 | 8.92 | 3.64 | 5.18 |
| 12 | 164 | 88 | 113 | 11.73 | 3.84 | 9.35 | 134 | 77 | 90 | 8.00 | 3.92 | 2.87 |
| Average | 150 | 86 | 110 | 10.74 | 4.19 | 9.74 | 136 | 74 | 95 | 8.24 | 3.75 | 3.64 |

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2727

**Nocturnal Hemodialysis in Pregnancy; the Mounting Toronto Experience** Tom Cornelis,[1] Johannes Keunen,[2] Moumita Barua,[1] Andreas Pierratos,[3] Marc B. Goldstein,[4] Christopher T. Chan,[1,5] Michelle A. Hladunewich.[1,2,5] *[1]Division of Nephrology, University Health Network, Toronto, ON, Canada; [2]Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON, Canada; [3]Division of Nephrology, Humber River Regional Hospital, Toronto, ON, Canada; [4]Division of Nephrology, St Michael's Hospital, Toronto, ON, Canada; [5]Equal Contribution.*

*Background*: Nocturnal hemodialysis (NHD) may improve fertility and lower pregnancy complications in women with chronic kidney disease (CKD). Here we expand our experience in NHD during pregnancy.

*Methods*: Cohort study of pregnant CKD patients on NHD from 2001 through 2010 in Toronto. Primary objective was to describe maternal-fetal outcomes and biochemical indices through pregnancy. Obstetrical follow-up included nuchal translucency ultrasound (US) (11-14 wk), quad test (16 wk), placental US (22 wk), and serial biophysical profile (BPP) scores and biometry US (from 26 wk).

*Results*: 11 patients (median age 34 y) had 14 pregnancies. At time of conception, 8 patients were on NHD (11 pregnancies), 1 patient was on conventional HD and 2 were not on dialysis. In NHD patients, the dialysis dose was increased to a weekly minimum of 42 hours and in non-NHD patients, NHD was started after conception. Median predialysis urea and blood pressure were maintained within physiological levels. Serial US revealed the estimated fetal weight averaged along the 50$^{th}$ percentile. BPP scores and placenta studies were by in large normal. Only 1 patient had transient polyhydramnios. Median gestational age and birth weight were 36 wk 5 days and 2020 g respectively. Complications were more common in patients not yet on NHD at time of conception and included placental insufficiency (n=1), low birth weight (below 10$^{th}$ percentile) (n=3), as well as preterm delivery (before 34 weeks) (n=4) secondary to cervical incompetence in 3 patients and leading to neonatal death in 1 patient.

*Conclusions*: NHD in pregnancy is feasible and may reduce maternal-fetal complications in ESRD patients. Pregnancy counselling in ESRD patients should include education on NHD as the best available dialysis modality. Strict combined obstetrical-renal follow-up is crucial.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2728

**A Single Centre Study Comparing Vascular Access Related Infection Rates in Nocturnal Versus Conventional Home Haemodialysis Patients Using Rope Ladder Needling Technique** Amy A. Crosthwaite, Rosemary Masterson. *Department of Nephrology, Royal Melbourne Hospital, Melbourne Health, Melbourne, Victoria, Australia.*

Background:

Extended hours nocturnal home haemodialysis (NHD) confers many advantages but there is concern about increased infection related morbidity and mortality in this cohort. In addition, it is unclear whether vascular access needling technique (buttonhole vs rope ladder) confers additional infection risk. We compared septic access event rates between cohorts of conventional (CHD) and nocturnal home haemodialysis patients to ascertain whether extended hours dialysis was associated with increased infection risk.

Methods:

A retrospective observational single centre cohort study comparing rates of infection and associated complications between NHD (n=78) and CHD (n=30) home dialysis patients between 2005 and 2010. All patients were followed for at least 12 months after commencing home dialysis.

Results:

Between May 2005- May 2010 261.60 (NHD 154.06, CHD 107.61) patient years of home haemodialysis were analysed. The average patient age was 47±19 years and 80% were male. Rope ladder needling technique was universally used. 19.5% of all patients experienced at least 1 septic access event. 67% events occured in the NHD patient cohort and 9 patients had ≥1 event. There was no significant difference in the rate of events between the 2 cohorts (NHD=0.14 events/patient year, CHD = 0.1 events/patient year, p=ns). Similar proportions of each group developed infections (NHD 27%, CHD 28%). The event rates in AVF and AVG were 0.08 and 0.03/patient year respectively. MSSA was the commonest pathogen identified. Complications of infected access included blood stream (NHD 66%, CHD 54.5%) and metastatic infection (NHD 19%, CHD 18%). There was no infection related deaths. Five patients discontinued home dialysis due to septic access events.

Conclusion:

This data confirms the significant morbidity associated with septic access events in the home dialysis population. In this cohort, the increased risk of infection was not associated with longer dialysis hours. Compared with other observational data. rope ladder needling technique appears to be associated with a lower rate of access infection.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2729**

**Impact of Urgent In-Hospital Chronic Kidney Disease Education among Patients with Unplanned Dialysis Start** Jean-Philippe Rioux, Harpaul Cheema, Diane M. Watson, Joanne M. Bargman, Christopher T. Chan. *Department of Medicine, Division of Nephrology, University Health Network, Toronto, ON, Canada.*

**Background:** The impact of urgent in-hospital educational interventions on the adoption of home dialysis (peritoneal dialysis (PD) and home hemodialysis (HHD)) after unplanned renal replacement therapy (RRT) start is unknown.

**Methods:** Clinical demographics of consecutive patients initiating RRT acutely from January 2005 to December 2009 were abstracted using our institutional electronic records. All patients received multimedia chronic kidney disease and modality education by the same consulting nurse practioner prior to discharge from the hospital. Clinical characteristics of patients choosing home dialysis or staying on in-center hemodialysis were compared.

**Results:** Between 2005 and 2009, 203 patients started hemodialysis acutely at our center. Seventy one patients adopted home dialysis (49 patients to PD and 22 to HHD) and 132 remained on in-center HD. Patients adopting home dialysis were similar to in-center HD patients in term of age and gender but had a different distribution of co-morbidities and etiology of end-stage renal disease. Patients known by a nephrologist before initiating RRT represent 39% of acute starters.

**Conclusion:** Home dialysis is feasible after unplanned renal replacement therapy start. Educational interventions are beneficial and should be promoted among inpatients initiating dialysis acutely. Prospective evaluation of clinical outcomes of this vulnerable population warrants further investigation.

Characteristics of patients adopting home or in-center dialysis

| | Home dialysis | In-center HD | p-value |
|---|---|---|---|
| | (n=71) | (n=132) | |
| | | | |
| Age, yrs | 55 ± 18 | 59 ± 16 | 0.09 |
| Gender, male, % | 49 | 52 | 0.2 |
| Etiology of ESRD | | | |
| Glomerulonephritis, % | 14 | 6 | 0.07 |
| Failed transplant, % | 24 | 12 | 0.045 |
| Ischemic nephropathy, % | 9 | 21 | 0.03 |
| Comorbidities | | | |
| Diabetes, % | 41 | 47 | 0.5 |
| Hypertension, % | 85 | 73 | 0.08 |
| PVD, % | 7 | 18 | 0.03 |
| CVD, % | 21 | 9 | 0.03 |
| CAD, % | 18 | 9 | 0.07 |

PVD, peripheral vascular disease; CVD, cerebro-vascular disease; CAD, coronary artery disease

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2730**

**Nocturnal Hemodialysis Is Associated with Improved Angiogenicity of Endothelial Progenitor Cells Compared with Conventional Hemodialysis** Darren A. Yuen,[1] Michael Kuliszewski,[1] Christine Liao,[1] Dimitry Rudenko,[1] Suzanne Advani,[1] Kerri Thai,[1] Howard Leong-Poi,[1] Christopher T. Chan.[2] *[1]St. Michael's Hospital, Toronto, Canada; [2]Toronto General Hospital, Toronto, Canada.*

**Background**: CKD is associated with an increased risk of peripheral vascular disease (PVD). Endothelial progenitor cells (EPCs) play important roles in maintaining vascular health. While EPCs from healthy individuals exert angiogenic effects in PVD models, uremia impairs EPC function *in vitro*. Nocturnal hemodialysis (NHD) increases uremic clearance and improves vascular function when compared with conventional hemodialysis (CHD). Whether NHD affects *in vivo* EPC angiogenic activity in the setting of ischemic limb disease is unknown. **Methods**: Peripheral blood-derived EPCs from healthy controls (n = 5) and age- and gender-matched CHD (12 hrs/wk, n = 10) and NHD (30 - 50 hrs/wk, n = 8) patients were cultured. 5 x $10^5$ EPCs or saline were injected at 5 sites in the ischemic hindlimb muscle of athymic nude rats 1 day post-left common iliac artery ligation. Hindlimb perfusion was assessed on day 28 via contrast bubble perfusion. Capillary density was assessed via lectin staining and expression of the angiogenic cytokines VEGF and SDF-1α via qPCR. **Results**: NHD was associated with increased clearance (CHD vs NHD PRU: 70 ± 6 vs 80 ± 3%, $p < 0.05$). While CHD EPC injection had no effect vs. saline, NHD EPC injection significantly improved ischemic hindlimb perfusion and capillary density to levels similar to that achieved with healthy control EPCs (Table 1). Levels of cytokines activated early in the angiogenic response (VEGF, SDF-1α) were not different 28 days post-ligation across all 4 treatment groups. **Conclusions**: NHD is associated with a significantly improved ability of EPCs to restore perfusion in a model of PVD, providing a possible explanation for the improved vascular outcomes seen with NHD.

| | Saline | Healthy Control | CHD | NHD | p value |
|---|---|---|---|---|---|
| Hindlimb perfusion ratio (ischemic:nonischemic limb) | 0.46 ± 0.05 | 0.80 ± 0.11 | 0.61 ± 0.06 | 0.85 ± 0.04 | < 0.05 |
| Ischemic hindlimb capillary density (capillaries/hpf) | 49 ± 6 | 75 ± 11 | 46 ± 7 | 86 ± 5 | < 0.05 |

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2731**

**Every-Other Night Home Hemodialysis with NxStage System One: A Crossover Pilot Study** Brigitte Schiller, Sheila Doss, John E. Moran. *Satellite Healthcare, Mountain View, CA.*

The standard practice for home hemodialysis (HD) with the NxStage System One is for the patient to dialyze 5-6 times weekly. We hypothesized that equally good outcomes could be achieved with higher doses of dialysis every second day, thereby potentially reducing burn-out with daily therapy.

We performed a crossover pilot study in prevalent patients following an A-B-$A^+$ design, with Phase A 4 weeks on current therapy, Phase B 8 weeks of high volume every-other night therapy (EON), and Phase $A^+$ 4 weeks of original therapy. Clinically stable patients undergoing weekly 5-6 times short daily home HD (SDHD) or nocturnal home HD (NHHD) with the NxStage System One took part. SDHD averaged 20 ± 3 hours with 151 ± 32 L dialysate, NHHD 47 ± 3 hours with 151 ± 23 L. Therapy for EON was prescribed with 50 or 60 L over a minimum of eight hours resulting in a mean of 31 ± 3 hours dialysis per week using 200 ± 17 L dialysate. Thus far 13 patients aged 52 ± 11 years have been enrolled, including 11 males, 5 diabetics; 7 have completed the study with 3 patients stopping prior to completion, and 3 still in study.

Results (mean ± SD) are shown from 27 patient months on EON, including follow-up.

| | Phase A | Phase B | Phase A+ | Follow-up |
|---|---|---|---|---|
| std Kt/V | 2.7 ± 1 | 3.4 ± 0.2 | 2.8 ± 0.8 | 3.0 ± 0.4 |
| Hgb | 10.6 ± 0.2 | 11.2 ± 0.7 | 11.2 ± 0.5 | 11.2 ± 0.4 |
| PO4 | 5.4 ± 1.5 | 5 ± 1.6 | 4.8 ± 1.3 | 4.6 ± 0.6 |
| Albumin | 4 ± 0.2 | 4.2 ± 0.2 | 4.2 ± 0.1 | 4.1 ± 0.1 |
| nPNA | 0.9 ± 0.02 | 0.9 ± 0.02 | 0.8 ± 0.02 | 0.9 ± 0.01 |

No change in antihypertensives or phosphate binders was observed. ESA usage was not evaluable due to conversion from a long-acting to a short-acting ESA during the study. All 7 completed study patients continued on EON. Only 1/13 patient stopped due to medical issues, 2/13 were unable to adhere to study timeline due to surgery.

These preliminary data suggest that EON with increased dialysate volume and longer times provides an alternative to daily HD in this stable patient group. EON is an attractive regimen, aligning solute and fluid removal with a less burdensome dialysis schedule. Long-term follow up is in progress.

Disclosure of Financial Relationships: Scientific Advisor: Affymax, Inc.; Other Relationship: Spouse employee at DaVita, Inc.

**SA-PO2732**

**Simulating Hemodialysis Modalities Using an Individualized Bayesian Urea Kinetic Modeling (IBKM)** Liping Zhang,[1] Adriana Hung,[2] Marc Pfister.[1] *[1]Dept. of Laboratory Medicine, University of California San Francisco, San Francisco, CA; [2]Dept. of Medicine, Vanderbilt University, Nashville, TN.*

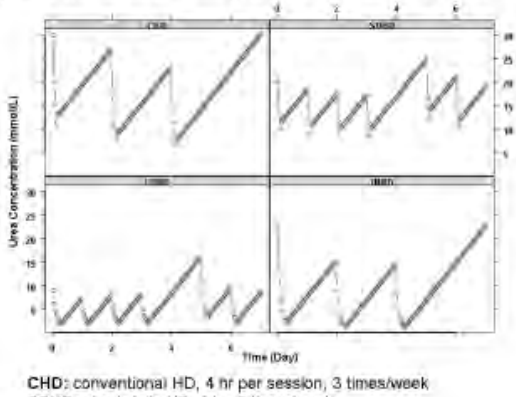
**Background:** Non-conventional hemodialysis (NCHD) modalities such as short daily HD are promising and increasingly used in practice as alternatives to "conventional HD (CHD)" of 3 treatments per week. Lacking of good standards to measure and methods to forecast their performance, the prescription of NCHD is often done empirically.

**Objective:** To simulate urea kinetic profiles under different HD modalities using individualized Bayesian urea kinetic model (IBKM) and to assess IBKM as a potential tool for forecasting NCHD prescription.

**Methods:** Based on a Bayesian framework, IBKM is a double-pool kinetic model developed with adult data on CHD to forecast and guide individual HD prescription. IBKM is applied to simulate urea kinetic weekly profiles in a single patient (i) at steady state under CHD and 3 NCHD modalities, and (ii) when HD prescription is switched from CHD to a NCHD modality.

**Results:** For a study patient with body weight of 75 kg, the IBKM-simulated urea kinetic profiles are shown in Figure 1.

Figure 1. IBKM-simulated urea kinetic profiles at steady state for various HD modalities




**CHD:** conventional HD, 4 hr per session, 3 times/week
**SDHD:** short-daily HD, 1 hr, 6 times/week
**LDHD:** long daily HD; 6 hr, 6 times/week
**INHD:** in-center nocturnal HD; 6 hr, 3 times/week

Average urea concentrations and area under concentration curves (AUC) above a hypothetical threshold of 15 mmol/L are calculated to illustrate how IBKM may be used to forecast and quantify HD performance.

**Conclusion:** IBKM is able to simulate individual urea kinetic profiles for various HD modalities, can be applied to other solutes, and is a promising tool for forecasting and guiding NCHD prescription.

Disclosure of Financial Relationships: Employer: Employee of Bristol-Myers Squibb Company; Ownership: Stockholder of Bristol-Myers Squibb Company.

### SA-PO2733

**Next-Generation Access for Peritoneal Dialysis without the Catheter Exit Site** Yudo Tanno,[1] Hiroyasu Yamamoto,[1] Keitaro Yokoyama,[1] Masami Uechi,[2] Tatsuo Hosoya.[1] *[1]Division of Kidney and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan; [2]Laboratory of Veterinary Internal Medicine, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, Kanagawa, Japan.*

Peritoneal dialysis (PD) is currently performed with a portion of the catheter exposed and consequently may cause catheter-related complications (catheter breakage, catheter exit-site infections, tunnel infection, peritonitis due to touch contamination, etc.). On the other hand, patients may refuse to undergo PD especially for cosmetic reasons as a part of the catheter is seen protruding from the abdominal wall. To address these issues, we have developed "Fully Embedded Subcutaneous Access for Peritoneal Dialysis" (FESTA for PD). While the PD catheter is placed into the peritoneal cavity, percutaneous access is gained via vascular graft (GRASIL®, Terumo, Japan), which has good elasticity and a high self-sealing capability, on the opposite side. The percutaneous access device is then embedded subcutaneously so that peritoneal access can be gained by subcutaneous puncture for changing the replacement/dialysate fluid bags.

The FESTA for PD was tested for durability and subcutaneous fluid leakage while the volume of dialysate instilled and drained was measured in pigs. Consequently, the estimated volume of subcutaneous fluid leakage per puncture in FESTA for PD was 2 g or less while it had an estimated life of 10 years. The volume of dialysate instilled and drained was almost equal to that in the conventional PD technique. In a 10-day study of FESTA for PD in beagle dogs, neither puncture-site infection nor peritonitis was observed.

These findings suggest that FESTA for PD, which is next generation peritoneal access system without the catheter exit site, will make a positive impact on the renal replacement therapy.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2734

**Impact of Catheter Style on Pediatric Peritoneal Dialysis Complications** Susan E. Ingraham, Hiren P. Patel. *Pediatric Nephrology, Nationwide Children's Hospital, Columbus, OH.*

Infection and catheter malfunction are common complications of chronic peritoneal dialysis (PD). Some studies have suggested that infection rate may be reduced by a caudally-oriented exit site, which is facilitated by use of a swan-neck catheter with a preformed bend in the extraperitoneal segment. In 2007, our pediatric renal dialysis unit began routine use of swan-neck catheters instead of straight catheters for chronic PD patients. We performed a retrospective chart review of all chronic PD patients treated in the Renal Dialysis Unit at Nationwide Children's Hospital from January 2004 through October 2009. We compared outcomes of swan-neck catheters to straight catheters with laterally-directed exit sites. Specific practice-based questions were: 1. Has use of swan-neck PD catheters reduced the incidence of catheter-related infections?; 2. Has use of swan-neck PD catheters altered the incidence of catheter malfunction?; 3. What factors are associated with increased risk of PD catheter malfunction?

A total of 31 straight and 37 swan-neck catheters were used during the study period, with 363 and 364 patient-months of use respectively. The overall incidence of catheter-related infection did not differ significantly, but rate of catheter removal due to infection decreased with use of swan-neck catheters (7/31 vs 1/37, p=0.014). Rate of catheter removal due to malfunction was higher with swan-neck catheters compared to straight catheters (14/37 vs 3/31, p=0.007). Patients with Prune Belly Syndrome demonstrated the highest rate of PD catheter malfunction, with a trend toward more frequent malfunction with swan-neck catheters (p=0.06). There was also a trend toward an increased rate of catheter malfunction with swan-neck catheters (56%) compared to straight catheters (11%) when placed by surgeons who insert PD catheters infrequently.

In conclusion, reduction in PD catheter-related infections by use of the swan-neck catheter style may be partially offset by an increased incidence of catheter malfunction. Placement by an unpracticed surgeon or diagnosis of Prune Belly Syndrome may lead to higher malfunction rates with swan-neck catheters compared to straight catheters in the pediatric population.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2735

**Infection, Technique Failure and Mortality in First Nations Peoples on Peritoneal Dialysis** Manish M. Sood, Paul Komenda, Mauro Verrelli, Martina Reslerova, Claudio Rigatto. *Nephrology, University of Manitoba, Winnipeg, MB, Canada.*

Background: The Canadian First Nations population suffers from high rates of end stage renal disease and the need for dialytic therapies. The objective of this study was to examine mortality, technique failure and peritonitis in First Nations patients on peritoneal dialysis and to explore whether differences may be accounted for by location of residence.

Methods: All adult peritoneal dialysis patients (N=727) from 1997-2007 residing in Manitoba, Canada were included. Data was extracted from a local administrative database, Canadian Organ Replacement Registry (CORR) and Peritonitis Organism Exit sites Tunnel infections (POET) databases. Cox and logistic regression models were used to determine the relationship between outcomes and First Nations status. Kaplan Meyer analyses were performed to examine the relationship between urban First Nations (<50 km from city) and rural First Nations (>50 km) status and outcomes.

Results: 161 First Nations and 566 Non-First nations were included in the analyses. Adjusted mortality (HR 1.476, CI 1.073-2.030), and adjusted time to peritonitis (HR 1.785, CI 1.352-2.357) were significantly higher in First Nations compared to Non-First Nations. There was no statistically significant difference in mortality, technique failure or peritonitis between urban or rural residing First Nations.

Conclusions: First Nations peoples on peritoneal dialysis suffer from higher mortality and faster time to peritonitis independent of co-morbidities and demographic characteristics. This effect is not influenced by place of residence, whether rural or urban. Efforts to improve the care of First Nations peoples on peritoneal dialysis should be targeted.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2736

**Peritonitis and Exit Site Infections in First Nations Patients on Peritoneal Dialysis** Manish M. Sood, Paul Komenda, Lisa M. Miller, Mauro Verrelli, Martina Reslerova, Claudio Rigatto. *Nephrology, University of Manitoba, Winnipeg, MB, Canada.*

Background: First Nations (FN) patients on peritoneal dialysis experience poor outcomes. Whether discrepancies exist regarding the microbiology, rate of infections and outcomes between FN and Non-FN peoples remains unknown.

Methods: All adult peritoneal dialysis patients (N=727) from 1997-2007 residing in Manitoba, Canada were included. Parametric and nonparametric tests were used as necessary. Negative binomial regression was used to determine the relationship of rates of exit site infections (ESI) and peritonitis between FN and Non-FN peoples.

Results: 161 FN and 566 Non-FN were included in the analyses. The unadjusted relative rates of peritonitis and ESI in FN were 132.7/100 pt yrs and 86.0/100-pt yrs compared to 87.8 and 78.2/100-pt yrs in non-FN populations. FN were more likely to have culture negative peritonitis (36.5 vs. 20.8%, p<0.0001) and *staphylococcus* ESI (54.1 vs. 32.9%, p<0.0001). The crude and adjusted rates of peritonitis were higher in FN for total episodes, culture negative and gram negative peritonitis. Catheter removal due to peritonitis was similar in both groups (42.9 FN vs. 38.1% non-FN, p=0.261).

Conclusions: FN patients experience higher rates of peritonitis and similar rates of ESI compared to non-FN. Interventions to improve outcomes and prevent infections should specifically be targeted to the First Nations population.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2737

**Peritoneal Dialysis in an Ageing Population: A 10-Year Single Centre Experience** Andrew Smyth, Evonne McCann, Lynn Redahan, Barbara Lambert, George J. Mellotte, Catherine A. Wall. *Department of Nephrology, Adelaide & Meath Hospital, Tallaght, Dublin, Ireland.*

*Background*

Chronic kidney disease (CKD) is becoming increasingly prevalent and there are increasing numbers of older patients with advanced CKD. Peritoneal dialysis (PD) is a potential treatment modality. Studies report older age to be a contra-indication for PD but others report co-morbidities to be more important. This study aims to assess PD outcomes in an elderly population in the largest PD centre in the Republic of Ireland over a ten-year period.

*Methods*

We retrospectively identified all adult patients, over the age of 50 years, who commenced PD for the first time between 1 January 1998 and 31 December 2008 at our institution. Primary outcome was survival with secondary outcomes of technique failure, peritonitis-free survival and transplantation.

*Results*

A total of 148 patients with a mean age of 63.17 years were included, who were stratified into three groups, 50-59 years (n=61), 60-69 years (n=46) and over 70 years (n=41). Twenty-two patients were on assisted peritoneal dialysis. Mean Charlson Co-Morbidity Index (CCMI) was higher in older patients (p<0.001), but modified Charlson Co-Morbidity Index (m-CCMI) was similar in all groups (p=0.737). Assisted PD was not associated with an increase in early complications (p=0.097) or technique failure (p=0.933) but death rates were higher (p=0.002). There were no significant differences by age group in early complication rates (p=0.222), number of hospitalizations (p=0.580), number of inpatient days (p=0.077), inpatient days during last year of life (p=0.135), peritonitis-free survival (p=0.794) and survival (p=0.905). CCMI was associated with mortality (p=0.002) as was

the m-CCMI (p=0.018) Renal transplantation occurred predominantly in younger patients (p=0.001) with a lower CCMI (p<0.001) who performed PD independently (p=0.004).

*Conclusion*

This study shows PD to be a safe option for renal replacement therapy in elderly patients with no differences in survival, technique survival or complication rates. Co-morbidities appear to play a stronger role in predicting survival than age alone. Assisted PD is a viable option in those unable to undergo PD independently.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2738**

**A Low-Site Tenckhoff Catheter Implantation Operation with Improved Peritoneal Dialysis Catheter Tip Migration** Jia Liu, Changying Xing. *Dept of Nephrology, First Affiliated Hospital of Nanjing Medical Univeristy, Nanjing, Jiangsu, China.*

Objective To report a low-site catheter implantation method with better catheter survival. Methods 180 ESRD patients starting PD from 2001 to 2009 in our center were screened. The operation procedure with the low-site catheter implantation method was different from the conventional method in three key steps. First, 3.5~4.5cm above the pubic symphysis was used as the body marker in this method other than 2 to 3cm under navel. Second, 1~2cm catheter tip was cut off with scissors so that the length could suit the shorter distance from the incision to the pouch of Douglas. Third, the catheter went through the rectus abdominis on the uppermost point of the incision, which meant the catheter went upward for about 2cm after it was fixed in the peritoneum incision. Results During the follow-up period, 3 patients (1.66%) experienced catheter tip migration. 4 patients (2.22%) experienced mechanical catheter obstruction caused by greater omentum. The incidence of the catheter tip migration and omentum obstruction is lower than that reported with conventional method by others. Conclusions Low-site catheter implantation method is an easy, safe, economic and effective technique for PD surgical procedure with lower morbidity of catheter tip migration and greater omentum obstruction.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2739**

**Peritoneal Dialysis as First Treatment Option in Late Referral (LR) Chronic Kidney Disease (CKD) Patients** Ewa Suchowierska,[1] Michal Mysliwiec,[1] Edyta Golembiewska,[2] Kazimierz Ciechanowski,[2] Jozef Penar,[3] Marian Klinger,[3] Jacek Lange,[4] Danuta Deckert.[4] *[1]Nephrology, Transplantation, Medical Univ., Bialystok, Poland; [2]Nephrology, Transplantology and Internal Medicine, Pomeranian Medical Univ., Szczecin, Poland; [3]Nephrology and Transplantation, Medical Univ., Wroclaw, Poland; [4]Baxter HealthCare.*

Introduction

One third of the patients are referred late to nephrology or dialysis units and are typically started on hemodialysis. It is known that unplanned hemodialysis initiation with central venous catheters (CVC) is independently associated with increased mortality and greater hospitalization rates, particularly vascular access–related and infections related hospitalizations. It is possible that starting with peritoneal dialysis as a first option may improve clinical outcomes. Education on dialysis treatment options in LR CKD patients may encourage patients to choose PD as first RRT option but is a challenge in this difficult patient group.

Results: From January to May, 2010 nine LR patients have participated in the educational program. At the start of the education program, 6 patients were requiring urgent HD via a CVC, and 2/6 chose PD, catheters were inserted and PD commenced after healing with no complications observed. Three patients were referred late but urgent HD was not required and after education they commenced PD first with rapid catheter insertion and PD starting before healing of the exit site (1-7 days after insertion). Within the first 30 days of PD, 2/3 of these patients had transient problems (peritonitis, scrotal hernia) but remained on PD but 1/3 had intractable fluid overload related to high peritoneal permeability and was transferred to HD. Patient reported outcomes (anxiety, depression and social isolation) were low in all 9 late referred patients commencing PD.

Summary This pilot study suggests that a specific educational program in LR CKD patients can allow patients to choose PD as a first long term option. Systems for rapid catheter insertion and starting of PD are possible but there is a risk of early complications in these patients. Further data gathering is essential to examine long term clinical outcomes in late referred patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2740**

**Symptom Burden in Older Dialysis Patients: Data from the BOLDE Study** Lina Johansson,[1] Ken Farrington,[2] Hugh Gallagher,[3] Edwina A. Brown.[1] *[1]Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom; [2]Lister Hospital, Stevenage, United Kingdom; [3]St Helier Hospital, Carshalton, United Kingdom.*

Data from BOLDE (Broadening Options for Long-Term Dialysis in the Elderly) shows that symptom burden is an independent predictor for quality of life, as determined by depression (Hospital Anxiety and Depression Scale), illness intrusion and physical and mental component scores on SF-12. Little is known, however, about the symptomatology of dialysis patients ≥65 years old.

A one-off questionnaire with 16 symptoms (graded none, mild, moderate or severe) was completed 70 pairs of patients on HD and PD matched for age [73.3±5.3 years], time on dialysis [30.9±27.3 months], gender [70% male], ethnicity [93% White European] and Index of Deprivation. The 6 most prevalent symptoms were: lack of energy (85%), pain in joints (73.6%), cold hands and feet (72.9%), cramps in legs (72.1%), weakness of limbs (68.6%) and sleep problems (68.6%).

Logistic regression modelling for each symptom identified which variables (age, length of time on dialysis, gender, dialysis modality, comorbidity score, depression score and nutritional status by Subjective Global Assessment) were associated with symptom severity (none/mild compared to moderate/severe). Increasing comorbidities and depression scores, female gender, malnutrition and being on HD were associated with greater symptom severity. The analyses also identified which individual symptoms were significantly associated with each of the 7 variables; an example is shown below.

Symptoms where greater severity is associated with dialysis modality

| Symptom | Odds ratio HD compared to PD | P value |
|---|---|---|
| Headaches | 5.952 | 0.039 |
| Dry mouth | 4.001 | 0.001 |
| Cramps in legs (comparable comorbidity scores) | 2.179 | 0.017 |
| Taste changes (comparable depression scores) | 1.447 | 0.035 |

In conclusion, older dialysis patients have a high symptom burden. Some symptoms were found to be associated with modality HD. Identifying the symptoms associated with specific variables will improve symptom understanding and management and therefore lead to improvements in quality of life.

Disclosure of Financial Relationships: Honoraria: Speakers fees from Baxter Healthcare.

**SA-PO2741**

**Use of Population (pop) Pharmacokinetic (PK) Modeling and Monte Carlo Simulation (MCS) To Determine Optimal Daptomycin (D) Dosing in Patients on Continuous Ambulatory Peritoneal Dialysis (CAPD)** Katie E. Cardone,[1,2] Thomas P. Lodise,[1,3] Nimish Patel,[1] Harold J. Manley,[4] Christopher D. Hoy,[5] Shari A. Meola,[5] George L. Drusano,[3] Darren W. Grabe.[1,2] *[1]Dept of Pharmacy Practice, Albany College of Pharmacy and Health Sciences, Albany, NY; [2]Albany Nephrology Pharmacy Group (ANephRx), ACPHS, Albany, NY; [3]Ordway Research Inst, Albany, NY; [4]Village Health Disease Management, Glenmont, NY; [5]Hortense & Louis Rubin Dialysis Center, Clifton Park, NY.*

Background: There is a dearth of data on optimal D dosing for pts on PD. Pop PK modeling & MCS were used to identify a D PD dosing scheme that yielded an exposure profile comparable to that obtained from a MCS using the D pop PK model derived from pts in the D vs. vancomycin S. aureus bacteremia infective endocarditis (SAB-IE) study (non-dialysis referent exposure).

Methods: Inclusion criteria: 18 yrs; non-infected; on PD (1 month); Hb 11mg/dL; non-pregnant; no peritonitis within 4 wks. Pts received a standardized CAPD prescription for 1 wk prior to study. Pts (n=8) received D 6mg/kg IV over 30 min. Dialysate and blood were collected over 12hrs & assayed with LC/MS/MS. Plasma conc-time profiles were modeled as a 3-compartment model with zero-order infusion & 1st order elimination & transfer (BigNPAG). A MCS (ADAPT II) using the D PD pop PK model simulated Cmax, Cmin48h, AUC0-24h & AUC0-48h for 6 mg/kg of D as a single dose. Exposure values were compared to those generated from a MCS using the D pop PK model derived from the SAB-IE study. For the SAB-IE MCS, D was given 6 mg/kg Q24H for 2 doses.

Results: Mean(SD) age:59(15) yrs, weight: 89(17) kg. Mean(SD) PK parameters: Vc:6.3 (1.2) L, CL:0.31 (0.17) L/h, k12:1.12 (1.20) h-1, k21:2.55 (2.97) h-1, k13:0.0077 (0.0025) h-1, k31:0.12 (0.11) h-1, Vpd:2.64 (1.27) L.

Conc-time profiles from MCS for PD & SAB-IE PK models (6 mg/kg)

| | CAPD | SAB-IE |
|---|---|---|
| Cmax | 55.4±23 | 44.9±31 |
| Cmin48h | 14±9 | 8.8±8 |
| AUC0-24h | 904±371 | 416±251 |
| AUC0-48h | 1358±585 | 927±542 |

Conclusions: Administration of D 6 mg/kg Q48H during CAPD yielded isometric exposure profiles relative to daptomycin 6 mg/IV Q24H from the SAB-IE (non-dialysis referent exposure) model.

Disclosure of Financial Relationships: Research Funding: Merck & Co.

**SA-PO2742**

**Increased Concentration of beta-1,3-D-Glucan in Dialysate from Peritoneal Dialysis Patients with Fungal Peritonitis** Masatoshi Kuratsune,[1] Kensuke Sasaki,[1] Akira Hirabayashi,[1] Yasuhiko Fukuda,[2] Noriaki Yorioka.[3] *[1]Nephrology, JA Hiroshima General Hospital, Hatsukaichi, Hiroshima, Japan; [2]Surgery, JA Hiroshima General Hospital, Hatsukaichi, Hiroshima, Japan; [3]Nephrology, Hiroshima University Graduate School of Medical Science, Hiroshima, Hiroshima, Japan.*

Background and aim : Since fungal peritonitis is a rare but very serious complication in peritoneal dialysis (PD) patients, its earliest diagnosis is desirable. The diagnosis usually requires identification of fungus by gram stain and positive culture. In this study, we measured the concentration of beta-1,3-D-glucan (BD), which is a component of the fungal cell wall, in dialysate and serum from patients with peritonitis.

Methods and patients : Between July 2007 and June 2010, a total of 54 incidences of peritonitis occurred in 28 PD patients at our hospital and BD was measured in 53 incidences. Peritonitis was defined according to the guideline by ISPD. At the immediate hospital visit with peritonitis symptoms, BD was measured by Beta-glucan Test Wako (Wako Pure Chemical Industries, Tokyo) in the dialysate (pBD) and serum (sBD) as well

as smear and culture sampling of dialysate. This test requires only 100 minutes to measure BD concentration and costs about 40 US dollars. Intergroup comparison was made by Mann-Whitney's U test.

Results : The causative organisms were identified by positive culture in 3 cases as fungus (Candida) (Group A) and 36 as bacteria (Gram negative 5, Gram positive 31)(Group B). As the other 14 were both culture and smear negative cases, we excluded them from further analysis. The average concentrations of pBD in Group A and B were 116.8±117.8 and 2.8±0.4 ng/mL, respectively ($p<0.001$). The average concentrations of sBD in Group A and B were 36.6±29.7 and 5.3±4.0 ng/mL, respectively ($p=0.32$). The calculated ratios of sBD to pBD in Group A and B were 0.33±0.26 and 1.93±1.36, respectively ($p<0.001$).

Conclusion : These results indicate that the measurement of BD in both dialysate and serum could be a quick method to differentiate fungal from bacterial peritonitis at early stage of peritonitis in PD patients.

Disclosure of Financial Relationships: Employer: JA HIROSHIMA GENERAL HOSPITAL.

**SA-PO2743**

**Connexin Hemichannels Mediate Low Calcium-Elicited Activation of cAMP Signaling Pathway and Elevation of Renin Production in Juxtaglomerular Cells** Tao Huang, Shotaro Nakajima, Masanori Kitamura, Jian Yao. *Department of Molecular Signaling, University of Yamanashi, Chuo, Yamanashi, Japan.*

Gap junctions, formed by the specific proteins called connexin (Cx), play an important role in the regulation of renin secretion in juxtaglomerular cells. Mice lacking Cx40 or Cx45 develop renin-dependent hypertension. Besides forming intercellular channels, connexins also form nonjunctional hemichannels. Once activated, hemichannels permit the rapid exchange of ions ($Ca^{2+}$, $Na^{+}$) and small molecules ($PGE_2$, ATP, $IP_3$, etc.) between the cytoplasm and the extracellular space. Given that hemichannels are activated by lowering extracellular calcium, a well-known stimulator of renin production, we speculated that hemichannels might be involved in the regulation of renin. Using a renin-secreting cell line As4.1 and primarily cultured mouse juxtaglomerular cells, we tested this hypothesis. Results: 1) As4.1 cells were characterized to express Cx43 and Cx45, and have the functional gap junctional intercellular communication, as evaluated by immunofluorescent staining, Western blot analysis, and diffusion of lucifer yellow (LY) after single cell injection. 2) Lowering extracellular calcium activated connexin hemichannels in As4.1 cells, as evidenced by the influx of LY and efflux of ATP. Hemichannel opening was associated with activation of cAMP signaling pathway, as revealed by increased levels of phosphorylated VASP (a PKA substrate) and CREB, as well as elevation of renin protein levels, as detected by Western blot analysis and renin activity assay. 3) Inhibition of hemichannels with heptanol or downregulation of Cx proteins with the specific siRNA for Cx40, Cx43 and Cx45 almost completely abolished the effects of calcium depletion on cAMP signals and renin production. 4) The similar results were achieved in primarily cultured mouse juxtaglomerular cells. Taken togethehr, our study indicates that Cx hemichannels mediates low calcium-elicited activation of cAMP signaling pathway and elevation of renin production in juxtaglomerular cells. Cx hemichannels could be a presently unrecognized mechansim involved in the control of renin synthesis and secretion.

Disclosure of Financial Relationships: nothing to disclose

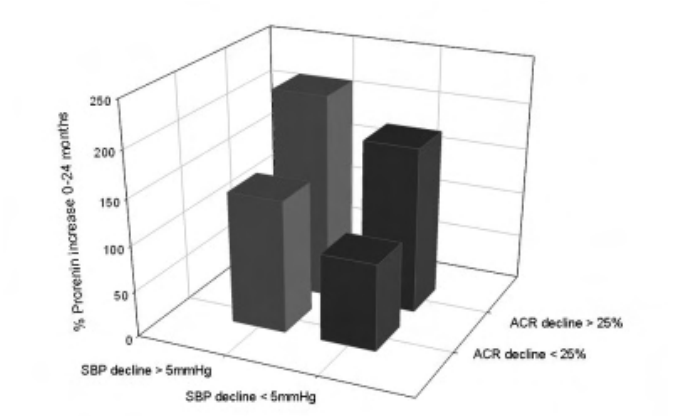
**SA-PO2744**

**Prorenin Is a Determinant of Angiotensin Receptor Blockade Response in Microalbuminuric Patients with Diabetes** Merel E. Hellemons,[1] Hiddo Jan Lambers Heerspink,[1] Stephan J. L. Bakker,[1] Hans-Henrik Parving,[2] Dick De Zeeuw,[1] Peter Rossing.[3] *[1]University Medical Center of Groningen, The Netherlands; [2]Rigshospitalet, Denmark; [3]Steno Medical Center, Denmark.*

The response in albumin-creatinine ratio (ACR) and systolic blood pressure (SBP) to Angiotensin Receptor Blockade (ARB) varies between individuals. Renin Angiotensin System (RAS) activity is an established determinant of the response to ARB-treatment. To date, however, no relation has been observed between ARB-induced changes in traditional RAS activity indicators (prorenin, renin and angiotensin II (ATII)) and individual ACR or BP response. We aimed to determine whether changes in these parameters correlated to the response in ACR and SBP during ARB-treatment in microalbuminuric patients with diabetes.

Data from the IRMA-2 trial comparing Irbesartan (150 and 300mg) versus placebo was used. Prorenin, renin and ATII concentrations were measured in a random sample (n=125 out of 590). Multivariate linear regression analysis was used.

Low baseline prorenin, but not renin or ATII, independently predicted larger reduction in ACR ($\beta$= 0.30; p=0.02). A dose-dependent (placebo-150-300mg) increase in concentrations of prorenin (88-110-204%), renin (71-189-412%), and ATII (43-131-215%) was observed during treatment. The rise in prorenin, but not of renin or AngII, was independently associated with each log unit reduction in ACR ($\beta$= -0.36; P=0.01) and each mmHg reduction in SBP ($\beta$= -0.23; P=0.04). Interestingly, the largest increases in prorenin were observed in patients with both ACR and SBP reduction and the lowest increase in patients with neither reduction in ACR nor SBP, whereas a reduction in either parameter was associated with an intermediate increase in prorenin (figure 1). The degree of increase in prorenin reflects the therapeutic efficacy in lowering ACR and SBP in irbesartan treatment.




Disclosure of Financial Relationships: nothing to disclose

**SA-PO2745**

**Ang-(1-7) Ameliorates p38-Related Renal Vasoreactivity in ApolipoproteinE Deficient Mice through Reduction of Reactive Oxygen Species** Sebastian A. Potthoff,[1] Tilman Clasen,[1] Michael Fähling,[2] Henning Hoch,[1] Eva Koenigshausen,[1] Magdalena Woznowski,[1] Lorenz Sellin,[1] Ivo Quack,[1] Oliver Vonend,[1] Andreas Patzak,[2] Lars C. Rump,[1] Johannes Stegbauer.[1] *[1]University of Duesseldorf, Germany; [2]Medical University of Berlin, Germany.*

Increased vascular reactivity to angiotensin (Ang)II in atherosclerotic vessels may contribute to the development of hypertension. Recent studies suggest that the p38-kinase is involved in regulating vascular reactivity. p38-kinase is activated by reactive oxygen species (ROS) which contributes to sustained smooth-muscle contraction by MLC(20) phosphorylation.

In apoE deficient (apoE-/-) and wild type (WT) mice we investigate if increased renal vasoreactivity to AngII is caused by ROS-related p38 activation and if Ang-(1-7) treatment influences renal vasoreactivity.

12 week old apoE-/- and WT mice on western diet were treated via minipumps either with saline or Ang-(1-7) (82µg/kg/hr) for 6 weeks. Vascular reactivity was tested in the isolated perfused kidney.

AngII induced renal pressor response was significantly increased in apoE-/- compared to WT mice. Ang-(1-7) treatment attenuates pressor response to AngII in apoE-/- mice. Accordingly, MLC(20) phosphorylation is increased in apoE-/- mice and reduced after Ang-(1-7) treatment. p38 inhibition (SB203580-5µmol/L) attenuates pressor response to AngII in apoE-/- but not in WT mice. Protein analysis of cortex and pre-glomerular vessel showed a two-fold increase in phosphor-p38 in apoE-/- compared to WT mice. Ang-(1-7) treatment decreased renal phosphor-p38 levels to WT baseline. In apoE-/- mice, expression of the NADPH-oxidase-subunit p47phox is 1.5-fold increased and restored by Ang-(1-7) treatment compared to WT mice. Concordantly, isoprostane-8 excretion in 24h-urine samples was significantly reduced in Ang-(1-7) treated apoE-/- mice.

Therefore, ROS-dependent p38-activation contributes to the increased renal vasoreactivity in apoE-/- mice. Ang-(1-7) treatment ameliorates vascular function at least partially through decreasing NADPH-oxidase overexpression and p38-activation. These data provide new insights in vascular function in atherosclerosis and beneficial mechanisms of Ang-(1-7).

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2746**

**Combined Treatment with Irbesartan and Vitamin D Retards Renal Injury in Salt-Loaded Uni-Nephrectomized Stroke-Prone Spontaneously Hypertensive Rats** Tsuneo Takenaka,[1] Tsutomu Inoue,[1] Hirokazu Okada,[1] Yoichi Ohno,[1] Takashi Miyazaki,[1] Akira Nishiyama,[2] Naohito Ishii,[3] Hiromichi Suzuki.[1] *[1]Saitama Medical University, Iruma, Saitama, Japan; [2]Kagawa University, Kagawa, Japan; [3]Kitasato University, Kanagawa, Japan.*

Vitamin D diminishes renin expression. We previously reported that vitamin D increased renal expression of klotho in rats with normal kidney function. In the present study, effects of vitamin D on renal injury was assessed in 4 groups of rats (n=6-8 for each group); uni-nephrectomized stroke-prone spontaneously hypertensive rats fed high salt (6%) diet as a control (C), those treated with irbesartan (100 mg/kg/day, I), rats treated with calcitriol (30 ng/kg/day, V), and rats treated with both irbesartan and calcitriol (I+V). Six weeks later, right kidney was harvested for analysis. Systolic blood pressure (SBP) in C was higher than I and I+V groups. While renal angiotensin II (AngII) concentration was lower in I and I+V groups than C, plasma AngII levels of I and V groups are higher and lower than C, respectively. In addition, urine albumin/creatinine ratio (Alb/Cr) was lower in I and I+V group than C, and 8-epi-prostaglandin F2$\alpha$ (PGF2$\alpha$) excretion was reduced in V and I+V groups. Immunoblot analysis revealed that compared to C, renal klotho expression was preserved in V and I+V group. Creatinine clearance (Ccr) was elevated in I+V group than C. The present data indicate that irbesartan effectively decreases blood pressure with reductions

in renal AngII and albuminuria. Our findings demonstrate that vitamin D enhances klotho expression, suppressing oxidative stress and albuminuria without substantial changes in renal AngII. The current results may provide evidence that renal renin-angiotensin system is regulated independently of circulating one.

| variables | unit | C | I | V | I+V |
|---|---|---|---|---|---|
| SBP | mmHg | 215±6 | 162±4* | 210±5 | 157±4* |
| Alb/Cr | mg/gCr | 310±98 | 29±9* | 102±35* | 11±3* |
| Ccr | ml/min | 1.6±0.1 | 1.8±0.2 | 1.7±0.2 | 2.3±0.3* |
| Renal AngII | fmol/g | 150±19 | 104±15* | 137±17 | 103±12* |
| Plasma AngII | pg/ml | 88±13 | 138±17* | 35±6* | 71±11 |
| 8-epi-PGF2α | ng/day | 15±2 | 12±2 | 8±1* | 7±1* |
| Klotho/β-actin | % | 50±11 | 74±16 | 96±20* | 102±25* |

* indicates p<0.05 vs C

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2747**

**Loss of Collectrin Results in Paradoxical Response to L-NAME** Sylvia Cechova,[1] Qing Zeng,[1] Rosa Chan,[1] Sandra M. Malakauskas,[2] Thu H. Le.[1] *[1]Medicine, University of Virginia, Charlottesville, VA; [2]Medicine, Salem Veterans Administration, Salem, VA.*

Collectrin is a protein with 40% homology to ACE2 but lacks any catalytic domain. It was first discovered in a search for genes that are upregulated during the hypertrophic phase after sub-total nephrectomy. We reported that collectrin-deficient mice (KO) display severe urinary amino acid wasting due to impaired trafficking of amino acid transporters in proximal tubular plasma membrane. On a mixed background, compared to wild-type mice (WT), KO mice have normal blood pressures (BP) at baseline, but have significantly elevated BPs after uni-nephrectomy (UNx). Here, to determine the mechanism(s) causing worse hypertension in the KO mice UNx, we compared renal plasma membrane expression of various salt transporters pre and post UNx, using Western blot analysis. Before UNx, there was no difference in expression in NHE3, NKCC, Na-K-ATPase, aENaC and NaPi2 channels between WT and KO mice. After UNx, there was a significant reduction in expression of aENaC and NaPi2 channels in KO mice (30% and 50%, respectively, by densitometry, $p < 0.006$). The reduced expressions of aENaC and NaPi2 channels are likely a compensatory response to elevated BPs. We posited that collectrin KO mice might have alteration in nitric oxide (NO) synthetic pathway that could play a role in elevated BP after UNx. We administered L-NAME in drinking water (20 mg/kg/day) for two weeks to adult mice at 3 months of age, without nephrectomy, and measured BP response using tail cuff manometer. Compared to baseline BPs, WT mice (n = 12) exhibited an average increase in systolic BP by + 14.7 ± 2.1 mm Hg after L-NAME (range + 4 to + 31 mm Hg). Surprisingly, collectrin KO mice (n = 18) exhibited no response to L-NAME, with average change in systolic BP of –2.1 ± 3.5 mm Hg (range – 27 to +16 mm Hg). The difference in change in BP in response to L-NAME was statistically significant, $p = 0.0004$. Our data suggest that collectrin KO mice have abnormalities in the NO synthetic pathway that may be exaggerated after loss of nephron mass. We speculate that arginine amino acid transport in endothelial cells is impaired in collectrin KO mice.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2748**

**Oxidative Stress Is Increased in ACE2 Deficiency and Restored Partially by Recombinant ACE2 Infusion** Jan A. Wysocki,[1] Karla Evora,[1] Minghao Ye,[1] Mirza S. Khan,[1] Susan B. Gurley,[2] Daniel Batlle.[1] *[1]Division of Nephrology & Hypertension, Northwestern University Feinberg School of Medicine, Chicago, IL; [2]Duke University Medical Center, Durham, NC.*

We have recently shown that human rACE2 effectively metabolizes circulating Angiotensin II and prevents Angiotensin II dependent hypertension in mice. Here, we examine kidney oxidative stress and the effect of rACE2 in male mice with genetic ACE2 deficiency and wild-type controls.

In ACE2 KO male mice, kidney H2O2 was increased as compared to wild type at 14 weeks of age (WT: 37.9 ± 1.4 μM/mg; ACE2 KO: 50.9 ± 3.1 μM/mg, $p < 0.001$). Likewise, at 40 weeks of age, H2O2 was also increased (KO: 58.1 ± 3.3; WT: 32.1 ± 3.0 μM/mg, $p < 0.001$). These changes in H2O2 were associated with corresponding increases in kidney NAD(P)H oxidase activity in both age groups.

The levels of kidney Angiotensin II were significantly higher in male ACE2 KO as compared to the wild-type (KO: 23.0 ± 4.8; WT: 10.7 ± 2.3 fmol/mg, $p < 0.05$), probably reflecting reduced Ang II degradation in the ACE2 KO. After three days of rACE2 administration, the ACE2 KO experienced a significant reduction in H2O2 (KO: 50.9 ± 3.1 μM/mg, KO/rACE2: 34.2 ± 8.3 μM/mg, $p < 0.05$). In ACE2 KO infused with Ang II, there was also a significant reduction in H2O2 when rACE2 was administered concurrently (ACE2 KO/AngII: 53.5 ± 1.8, ACE2 KO/AngII/rACE2: 42.7 ± 4.8 μM/mg, $p < 0.05$), but this level was still greater than that of the wild-type (WT: 32.1 ± 3.0 μM/mg, $p = 0.075$).

In conclusion, ACE2 deficiency leads to increased H2O2 production at the kidney level in association with increased kidney Ang II levels. The administration of human rACE2 attenuates H2O2 levels likely by increasing Ang II degradation, and thereby suggests a novel approach to reduce oxidative stress within the kidney.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2749**

**Chronic Antagonism of Mineralocorticoid Recepter Prevents Hypertension Progression and End Organ Damage in a Rodent Model of Salt-Sensitive Hypertension** Xiaoyan Zhou,[1] Martin Crook,[1] Wanda Sharif-Rodriguez,[1] Yonghua Zhu,[1] Zadok Ruben,[2] Gail M. Forrest,[1] Daphne Szeto,[1] Huawei Zhao,[1] Michael J. Forrest.[1] *[1]Cardiovascular Diseases, Merck & Co., Rahway, NJ; [2]Patoximed Consultants, Westfield, NJ.*

Excessive or inappropriate mineralocorticoid receptor (MR) activation not only enhances sodium and water retention and elevates blood pressure (BP), but may also promote deleterious effects on target organs via both hemodynamic and non-hemodynamic mechanisms. The aim of the present study is to investigate the effects of chronic MR antagonism with eplerenone on the development and progression of hypertension and target organ function in a rodent model of salt-sensitive hypertension, namely Dahl Salt Sensitive (Dahl SS) rats. Adult Dahl SS rats were administered eplerenone (100 $mg \cdot kg^{-1} \cdot d^{-1}$ for 8 weeks) mixed either in low salt (0.3% NaCl) or high salt (4% NaCl) rodent chow. BP was recorded daily by radiotelemetry and renal function was assessed weekly. Histopathological analyses of kidneys and hearts were performed at the end of the study. Low salt fed Dahl SS rats developed a slow progression of hypertension (systolic BP increased by 18±3 mmHg over 8 weeks), which was completely blocked by eplerenone. High salt fed Dahl SS rats developed severe hypertension, cardiac and renal hypertrophy, massive proteniura, glomerulosclerosis, perivascular fibrosis, tubular dilation, and renal interstitial injury. Eplerenone significantly attenuated the progressive rise in systolic BP (204±3 vs.179±3 mmHg, $p<0.05$), reduced proteinuria (605.5±29.6 vs. 479.7±26.1 mg/24h, $p<0.05$), improved injury scores of glomeruli (2.2±0.3 vs. 1.0±0.2, $p<0.05$), tubules (2.9±0.2 vs. 2.4±0.1, $p<0.05$), renal interstitium (2.2±0.1 vs.1.7±0.1, $p<0.05$), and vasculature (2.3±0.3 vs. 0.9±0.3, $p<0.05$). Cardiac collagen content score was not significantly changed by eplerernone (2.0±0.2 vs.1.8±0.2, $p>0.05$). These results demonstrate that MR antagonism provides target organ protection and attenuates the development of elevated BP in a model of salt-sensitive hypertension. Importantly, these data further support the promise of MR antagonists for the treatment of hypertension.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2750**

**Amelioration of AngiotensinII (AII)-Induced Salt-Sensitive Hypertension (SSHT) in Liver-Type Fatty Acid-Binding Protein (L-FABP) Transgenic Mice (Tg)** Ken Osaki,[1] Yusuke Suzuki,[1] Takeshi Sugaya,[2] Satoshi Horikoshi,[1] Yasuhiko Tomino.[1] *[1]Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan; [2]CMIC Co Ltd., Tokyo, Japan.*

Background: Recent findings indicated that inappropriate activation of the intrarenal renin-angiotensin system (RAS) induces reactive oxygen species production and tubulointerstitial inflammation, which may contribute to SSHT. It is well known that AII infusion induces enhancement of intrarenal RAS activation and subsequent experimental SSHT in mice.

On the other hand, L-FABP is expressed in proximal tubules in humans, but not rodents. L-FABP binds long-chain fatty acid oxidation products, and thus may play an endogenous antioxidative role in the human kidney.

Aim: The objective of the present study is to examine the antioxidative effect of L-FABP on AII-induced SSHT with Tg in which human L-FABP is overexpressed in proximal tubules.

Methods: Female Tg and their wild-type mice (WT) were subjected to the AII-infusion at doses of 500 ng/kg/day for 4 weeks, and then a high-sodium diet (HSD: 6% NaCl) was started from 5 to10 week.

Results: During AII infusion, a progressive increase of mean blood pressure (MBP) peaking at the week 4 (132.0±12.9 mmHg) was observed in WT, while a progressive increase of MBP was detected until week 2 in Tg. After AII infusion, both strains showed normal MBP (WT: 81.0±5.1, Tg: 85.6±7.1 mmHg) at week 5. MBP in WT gradually increased after week 6, although that SSHT was not observed in Tg until week 10. Renal renin mRNA expression at week 10 in Tg was significantly lower than that in WT ($P<0.05$). In addition, the protein level of angiotensinogen in Tg was also lower than that in WT. Urinary 8-OHdG concentration ($P<0.05$), renal 4-HNE protein expression and numbers of tubulointerstitial T lymphocytes were also attenuated in Tg.

Conclusion: The present findings showed that Tg with AII-induced SSHT were protected by attenuation of intrarenal RAS activation, oxidative stress and tubulointerstitial inflammation. Therefore, it appears that antioxidative action of L-FABP in proximal tubules may be important in this protection.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2751**

**ARB Protects Podocytes of HIV-1 Nephropathy Independently of Podocyte AT1** Akihiro Shimizu,[1] Taiji Matsusaka,[2] Jianyong Zhong,[3] Yoichi Miyazaki,[1] Tatsuo Hosoya,[1] Iekuni Ichikawa.[4] *[1]The Jikei University School of Medicine; [2]Tokai University; [3]Huashan Hospital; [4]Vanderbilt University School of Medicine.*

It is now well established that angiotensin (Ang) II via the Ang II type 1 receptor (AT1) plays important roles in the progression of glomerulosclerosis. It has been shown that podocytes express functional AT1 and transgenic rats overexpessing AT1 on podocytes develop glomerulosclerosis. In the present study, we investigated the mechanism of AII

action on podocytes in a mouse model of HIV-1 nephropathy. HIV-1 mice with sclerosis-prone FVB/N genetic background were treated with an ARB, losartan (30 mg/kg BW/day, s.c.) (n=7), or left untreated (n=7). At 4 weeks of age, HIV-1 mice with ARB showed significantly attenuated urinary protein/creatinine ratio (23.2+-9.8 vs. 55.3+-6.0 mg/mg) and glomerulosclerosis index (0.41+-0.21 vs. 1.34+- 0.27, on 0 to 4 scale) compared with control HIV mice.

We, then, studied the effect of podocyte-specific deletion of AT1 in HIV-1 mice on nephropathy-resistant C57BL/6 genetic background. At 8 months of age, mice carrying both HIV-1 gene and podocyte-selectively nullified AT1 gene (AT1KO/HIV-1) (n=13) and control/HIV-1(n=15) mice were statistically indistinguishable with respect to urinary albumin/creatinine ratio (2.5+-1.0 vs. 9.1+-4.8 mg/mg), glomerulosclerosis (0.61+-0.11 vs. 0.67+-0.17) and downregulation of nephrin (6.65+-0.28 vs. 6.71+-0.36 on 0 to 8 scale). These indicate that the injurious effect on podocytes and pro-sclerogenic effect of Ang II in HIV nephropathy are channeled through its receptors on cells other than podocytes, which include efferent arteriolar smooth muscle cells.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2752

**Absence of AT1A Angiotensin Receptors from Vascular Smooth Muscle Cells (VSMCs) Does Not Attenuate Vascular Remodeling in Hypertension** Matthew A. Sparks,[1] Eric W. Raasch,[1] Susan B. Gurley,[1] Johannes Stegbauer,[1] Anuradha Vivekanandan-Giri,[2] Subramaniam Pennathur,[2] Thu H. Le,[3] Thomas M. Coffman.[1] *[1]Division of Nephrology, Duke University; [2]Division of Nephrology, University of Michigan; [3]Division of Nephrology, University of Virginia.*

Vascular injury and remodeling are common pathological sequelae of hypertension. Previous studies have suggested that angiotensin II (ang II) acting through the AT1 receptor promotes vascular pathology in hypertension. To study the contribution of direct actions of AT1 receptors in this process, we generated mice with cell-specific deletion of the major murine AT1 receptor isoform (AT1A) from VSMCs using Cre/Loxp technology. To this end, we crossed a transgenic mouse line expressing Cre recombinase in smooth muscle (SM22α-Cre ) with a mouse line bearing a conditional allele of the Agtr1a gene, encoding the AT1A receptor. In SM22α-Cre+Agtr1a flox/flox (SMKO) mice, there was virtually complete elimination of AT1A receptor expression from VSMCs in the aorta, but not from small resistance vessels such as pre-glomerular arterioles. Thus, acute vasoconstrictor responses to angiotensin II were preserved in SMKOs. To induce hypertensive vascular remodeling, mice were continuously infused with ang II for 4 weeks. During infusion of ang II, mean arterial pressure measured by radiotelemetry increased significantly and to a similar extent in SMKOs (157±1 mm Hg) and controls (154±2 mm Hg). In the control mice, there was evidence of vascular oxidative stress indicated by enhanced hydrogen peroxide generation and nitrated tyrosine residues in segments of aorta; this was significantly attenuated in the SMKOs by 44% ($p<0.005$) and 74 % ($p<0.005$), respectively. With ang II infusion, the area of the aortic media increased by ~45% in both groups ($p<0.005$ vs. baseline) and despite the marked differences in free radical generation, the extent of medial expansion following the ang II infusion was not significantly different between the groups (50.5± 2.4 μ m in controls vs. 47± 4.6 μm in SMKOs). Thus, vascular AT1A receptors promote oxidative stress in the aortic wall but are not required for remodeling in angiotensin II-dependent hypertension.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2753

**Angiotensin II Infusion in Pregnant Rats as a Model of Preeclampsia** Steven Wagner,[1] Iasmina Craici,[1] Joseph P. Grande,[2] Karl A. Nath,[1] Vesna D. Garovic.[1] *[1]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Department of Pathology, Mayo Clinic, Rochester, MN.*

Preeclampsia is a pregnancy specific disorder characterized by hypertension and proteinuria manifested after 20 weeks gestation. The renin-angiotensin system is implicated in the pathophysiology of preeclampsia. Previous studies have shown that angiotensin II infusion in non-pregnant rats produces hypertension and proteinuria. We hypothesize that angiotensin II (Ang II) infusion in pregnant rats may provide a model of preeclampsia.

Pregnant Sprague-Dawley rats (n=15) were infused with either Ang II at 0.96 mg/kg/day (n=7) or normal saline (n=8) delivered via osmotic minipumps implanted at gestational day 5 (n=9) or day 9 (n=6). All Ang II-treated animals displayed hypertension one day after pump implantation (SBP 163 mmHg vs 125 mmHg in controls, $p=0.0045$). Systolic hypertension became more pronounced 5 days after implantation (181 mmHg vs 115 mmHg in controls, $p<0.0001$). At gestational day 17, 24-hour urinary protein was 3.1 mg in angiotensin II treated animals compared to 0.75 mg in saline controls ($p=0.049$). Average fetal mass was significantly decreased in Ang II-treated rats (3.64 vs. 4.16 grams, $p=0.032$), as was maternal weight (283 vs. 318 grams, $p=0.0004$).

Renal histology revealed arteriolar fibrinoid necrosis in three Ang II-treated rats; endotheliosis was not observed. Placental infarction was noted in one Ang II-treated rat, and decidual edema was consistently noted.

At sacrifice on gestational day 19, plasma levels of uric acid (0.67 vs 0.45 mg/dL, $p=0.007$) and BUN (25 vs 19 mg/dL, $p=0.008$) were increased in Ang II-treated rats. After correction for body weight, plasma creatinine was elevated in Ang II-treated rats (1.12 mg/dL/kg vs. 0.946 mg/dL/kg, $p=0.02$).

Our data demonstrate that Ang II infusion induces in pregnant rats features of preeclampsia, including systemic hypertension, proteinuria, and decreased GFR. We suggest that such chronic administration of Ang II in pregnant rats provides a model for preeclampsia.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2754

**The SKCa Ca-Activated K Channel Is a Target of Aldosterone in the Endothelium** Frederic Jaisser,[1] Violaine Charhbili,[1] Smail Messaoudi,[1] Aurelie Nguyen,[1] Ana-Maria Gomez.[2] *[1]INSERM U872 Team1, Paris, France; [2]INSERM U-637, Montpellier, France.*

To specifically address the role of the mineralocorticoid receptor (MR) in the endothelium in vivo, we have previously generated a mouse model with conditional overexpression of the MR in endothelial cells only (MR-EC mice). MR-EC mice presented with an altered myoendothelial crosstalk (Nguyen et al., FASEB J, 2010) leading to altered vasoreactivity. In the present study, we tested whether MR activation modulates the expression and activity of Ca-activated K channels (SKCa and IKCa) also known as Endothelium Derived Hyperpolarizing Factors involved in myoendothelial crosstalk. SKCa expression was induced by 10-8 M aldosterone in human and mouse endothelial cells, an effect prevented by 10-6 M spironolactone, a pharmacological MR antagonist. The expression of SKCa was also increased in the aorta of MR-EC mice. Pharmacological inhibition of the endothelial Ca2+-activated K+ channels with apamin and Tram-34 revealed an impaired relaxation response in MR-EC mice, indicating that the activity of endothelial Ca2+-activated K+ channels is increased upon long-term overexpression of the MR in the endothelium. Agonist-evoked Ca homeostasis was analyzed by confocal imaging in endothelial cells of intact aorta pre-incubated ex vivo with Fluo4-AM upon stimulation by Acetylcholine (Ach) in the presence or not of apamin and Tram34. Intracellular Ca transients were strongly increased upon Ach stimulation in the endothelium of aorta from aldosterone-treated wild-type mice or MR-EC mice as compared to control mice. This increased response was prevented upon inhibition of the endothelial Ca2+-activated K+ channels with apamin and Tram-34. Our results demonstrate for the first time that the SKCa Ca-activated K channel is a target of aldosterone in the endothelium and is involved in the altered vasoactive response and agonist-evoked Ca homeostasis modulated by endothelial MR activation

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2755

**Role of Hydrogen Sulfide and KCNQ Channels in Periadventitial Vasoregulation** Maik Gollasch,[1] Johanna Schleifenbaum,[1] Olga Zavaritskaya,[2] Rudolf Schubert,[2] Friedrich C. Luft,[3] Carolin Koehn,[4] Nadezda Voblova.[5] *[1]Nephrology/ECRC, Charité, Berlin, Germany; [2]Physiology, University Heidelberg, Germany; [3]Charite, Franz Volhard Clinic; [4]ECRC Berlin Buch, Charité Berlin, Germany; [5]Rostock University, Rostock.*

**Background:** Perivascular adipose tissue secretes an adipocyte-derived relaxing factor (ADRF) that opens voltage-dependent $K^+$ ($K_v$) channels in peripheral arteries. We studied the role of KCNQ-type $K_v$ channels and tested the hypothesis that hydrogen sulfide ($H_2S$) could be an ADRF in periadventitial vasoregulation. **Methods:** We performed isometric contraction studies on systemic arteries of rats and mice. **Results:** In mesenteric arteries and aortas without perivascular adipose tissue, the KCNQ channel openers retigabine, VRX0530727, VRX0621238, and VRX0621688 produced concentration-dependent vasorelaxation; VRX0621688 was the most potent vasodilator. The KCNQ inhibitor XE991 (30 μM) blocked the effects of both the drugs and ADRF. Inhibitors of cystathionine gamma lyase (CSE) ß-cyano-L-alanine (BCA, 5 mM) and 4-propargyl glycine (PPG, 10 mM) also blocked the relaxations. Cystathionine gamma lyase is expressed in perivascular adipose tissue and endogenously generates $H_2S$. The $H_2S$ donor NaHS produced concentration-dependent vasorelaxation, which was also blocked by XE991. The vasodilatory capacities of retigabine, VRX0530727, VRX0621238, and VRX0621688 were preserved following inhibition of $H_2S$ generation in perivascular fat. **Conclusions:** We suggest that KCNQ channel opening is a powerful mechanism to produce vasorelaxation of visceral arteries in rats and mice. Furthermore, KCNQ channels play a major role in the paracrine control of vascular tone by perivascular adipose tissue, which is at least in part mediated or modulated by $H_2S$. KCNQ channel opening is novel approach to improve impaired periadventitial vasoregulation, increased peripheral resistance and associated hypertension.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2756

**Cardiovascular Dysfunctions in Mice with Homozygous Mutation of the Gene Encoding Canonical Transient Receptor Potential 1 (TRPC1) Channel: Evidence for Hypoplastic Heart and Arterial Stiffness** Kai Lau,[1 & 4] Leonidas Tsiokas,[2] Becky Pennington,[1] Bonnie Eby,[1] Joel Abramowitz,[3] Lutz Birnbaumer,[3] Pedro Lozano.[1 & 4] *[1]Medicine, University of Oklahoma, Oklahoma City, OK; [2]Cell Biology, University of Oklahoma, Oklahoma City, OK; [3]Intramural Research, NIEHS, Research Triangle Park, NC; [4]Medicine, VA Hospital, Oklahoma City, OK.*

Previously we demonstrated the critical role of TRPC1 gene in the hypertrophic response to thoracic aorta constriction. But the impact of diploid deficiency of TRPC1 on the development & function of the circulatory system in the native state has not been defined. To test the hypothesis that TRPC1 null mice develop cardiovascular phenotypes in the absence of imposed stimuli, we measured left ventricular (LV) functions by echocardiographic studies and systemic arterial blood pressure (BP) by tail cuffs method using the computerized system of Visitech in 7-month-old TRPC1 -/- male mice and age- and sex matched wild type controls.Compared to TRPC +/+ controls, LV end-diastolic (ED) diameter (3.02 vs. 3.78 mm, $p<0.001$) and end-systolic (ES) diameter (1.38 vs. 2.09 mm, $p<0.001$) were both reduced, resulting in markedly reduced ED LV volume (29 vs. 56 μl, $p<0.001$), ES LV

volume (3 vs. 11 µl, p<0.01), and 42 % decrease in stroke volume (26 vs. 45 µl, p<0.001). Stroke index (SI) was thus reduced by 44 % in the null mice [0.8 vs. 1.4 µl/g body weight (BW), p<0.001] and cardiac output (CO) (14 vs. 21 ml/min, p<0.01) was reduced by 33 %. Systolic BP (113 vs. 121 torr, p<0.01), diastolic BP (77 vs.86 torr, p<0.01) and mean arterial (MA) BP (89 vs. 98 torr, p<0.001) were all reduced in the null mice. Systemic arterial resistance (SAR), calculated as MABP/CO, was markedly elevated (7.2 vs. 5 torr/ml/min, p<0.05). Pulse pressure was similar (36 vs. 33 torr). Arterial stiffness, calculated as pulse pressure / stroke index, was increased to 2 fold of wild type mice (51 vs. 25 torr/µl/min/g BW, p<0.001). Conversely, arterial compliance, the reciprocal of stiffness (22 vs. 43 µl/min/g BW/torr, p<0.001) was reduced by 49%. These data support our hypothesis of cardiovascular phenotypes in TRPC1 null mice.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2757

**Molecular Mechanisms of Ouabain-Induced Hypertension in Rats** Syed J. Khundmiri,[1] Sarah A. Salyer,[1] Nina W. Lesousky,[1] Eleanor D. Lederer.[1,2] *[1]Medicine/Kidney Disease Program, University of Louisville, Louisville, KY; [2]Medicine, Robley Rex VAMC, Louisville, KY.*

Increased levels of endogenous cardiac glycosides have been implicated in hypertension in patients with chronic kidney disease.We have recently demonstrated that low molar concentrations of ouabain increase phosphorylation, expression, and association of the α subunit of Na-K ATPase (NaK) with NHE-1 in rat kidney basolateral membranes and in human kidney proximal tubule cells. The ouabain-stimulated increase in NaK expression was accompanied by an increase in blood pressure in Sprague Dawley rats. We hypothesize that ouabain raises blood pressure through an NHE-1 dependent increase in renal Na-K activity. To address this hypothesis we treated Sprague Dawley rats with vehicle or ouabain (1mg/kg body weight/day) for 9 days in the presence or absence of an antibody to cardiac glycosides (Digibind) or an NHE-1 inhibitor (Zoniporide). Blood pressure was measured in anesthetized animals carotid artery catheter. NaK activity and expression was measured in basolateral membranes prepared from the above rats. Ouabain, but not vehicle, increased mean arterial pressure from 87.4±5.32 to 102.93±3.26 (p<0.01). Treatment with zoniporide prevented the increase in MAP (92.33±3.72, p=ns). Zoniporide alone did not alter the MAP (95.23±1.36, p=ns). Treatment with Digibind significantly decreased MAP given alone or in the presence of ouabain (66.17±9.64, p<.01 and 70.63±7.87, p<.01, respectively). Both zoniporide and digibind prevented ouabain induced Na-K activity and expression in kidney basolateral membranes. To confirm the NHE-1 dependence of ouabain induced increase in NaK, we compared ouabain-stimulated rubidium (Rb) uptake in proximal tubules isolated from wild type and NHE-1 knock-out mice. Ouabain increased Rb uptake in PT from WT mice (2.13±0.28 vs 4.87±0.73) but not in PT isolated from NHE-1-/- mice (2.21±0.32 vs 1.76±0.059). These data suggest that ouabain increases blood pressure through NHE-1 dependent activation of renal NaK.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2758

**Involvement of the Signaling Na/K-ATPase in Blood Pressure Regulation** Yiliang Chen,[1] Jiang Tian,[1] Changxuan Liu,[1] Joseph I. Shapiro,[2] Zi-Jian Xie.[1] *[1]Department of Physiology and Pharmacology, University of Toledo, Toledo, OH; [2]Department of Medicine, University of Toledo, Toledo, OH.*

Purpose of study:

To reveal the physiological role of the signaling function of the Na/K-ATPase

Major methods used:

Endosomal fractionation isolation; 22Na+ transcellular transport assay; mice blood pressure measurement by tail-cuff system.

Results:

The Na/K-ATPase (NKA), conventionally known as an ion transporter, also functions as a signal transducer. However, the physiological role of this newly discovered signaling function is not well understood. In this report, we demonstrate that the signaling NKA coordinates membrane trafficking and expression of NKA and sodium hydrogen exchanger isoform 3 (NHE3) in the renal epithelial cells. Activation of NKA signaling leads to endocytosis of both NKA and NHE3, which causes a reduction of transcellular Na+ transport in LLC-PK1 cells. Expression of the N-terminus of NKA α1 subunit (NT), which behaves as a dominant negative mutant, specifically blocks the NKA signaling and prevents ouabain-induced inhibition of transcellular Na+ transport. These *in vitro* experiments suggest that the signaling NKA be involved in renal sodium handling and therefore, may play a role in blood pressure regulation. To study the signaling NKA *in vivo*, we generated three lines of NT-YFP transgenic mice (NT mice). Then we fed the mice with either normal salt diet (0.5% NaCl) or high salt diet (4.0%) and measured blood pressure. As expected, the high salt diet produced hypertension (from 110 mmHg to 160 mmHg) in NT mice, but not in littermates. Moreover, the extent of blood pressure increase is correlated with NT-YFP renal expression level. Finally, renal functional curves show that expression of the NT produced salt-sensitive hypertension in NT mice.

Conclusion:

The signaling function of the NKA is involved in blood pressure regulation.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2759

**Sympathetic-Activity Induced Salt-Sensitive Hypertension Involved of WNKs** Shengyu Mu,[1] Tatsuo Shimosawa,[2] Fumiko Kawakami Mori,[1] Toshiro Fujita.[1] *[1]Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan; [2]Clinical Laboratory, University of Tokyo, Tokyo, Japan.*

(Background) Recent studies have showed that WNK kinases play important roles in the regulation of sodium transport in the distal nephron, therefore regulate the blood pressure. In this experiment we show the regulation of WNKs in sympathetic nerve activity-induced salt-sensitive hypertension.(Method) We used norepinephrine(NE) treated C57/B6 mice and DOCA-HS rats to show that sympathetic activation could decrease WNK4 expression, therefore increase sodium re-absorption and results in salt-sensitive hypertension. Then we used beta-1/2 knockout mice and mice distal nephrone cells (mDCTs) to show the mechanism of WNK4 regulation under high sympathetic activity. WNKs mRNA expression were measured by realtime-RT-PCR. The expressions of ion channels in the distal nephron were detected by western blot and immunostaining. Animal direct blood pressure was measured by catheter.(Result) After two weeks treatment either by NE or DOCA-salt, WNK4 expression was significantly lower than control groups (-50% in NE injected and -30% in DOCA-salt). Also sodium channels expression and activity were up-regulated in high sympathetic activity groups. NE treatment of beta1 but not 2 KO mice reduced WNK4 and salt sensitive hypertension. PKA inhibitor reversed effect of isoproterenol in reducing WNK4 expression.(Conclusion) In this study we found that sympathetic-activity inhibits WNK4, therefore increase the sodium re-absorption and finally induced to salt-sensitive hypertension. We suggest that regulation of WNK4 could be a new target for salt sensitive-hypertension.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2760

**Cyclosporine-Induced FHHt-Like Syndrome Is Associated with a Rise in WNK4; a Study in Rats and mDCT Cells** Haim Mayan,[1,2] Semyon Melnikov,[4] Shinichi Uchida,[3] Eliezer J. Holtzman,[2,5] Zvi Farfel.[1,2,4] *[1]Department of Medicine E, Sheba Medical Center, Israel; [2]Sackler School of Medicine, Tel Aviv University, Israel; [3]Tokyo Medical and Dental University, Tokyo, Japan; [4]Laboratory of Biochemical Pharmacology, Sheba Medical Center, Israel; [5]Laboratory of Molecular Nephrology, Sheba Medical Center, Israel.*

**Background**: Cyclosporine A is used for treatment of transplanted patients and for immune mediated diseases. Cyclosporine is known to cause a combination of metabolic side effects including hypertension, hyperkalemia , hypercalciuria and hypomagnesemia. These side effects except for hypomagnesemia are the cardinal features of familial hyperkalemia and hypertension (FHHt), also called pseudohypoaldosteronism type II (PHA II). FHHt is caused by mutations in the kinases WNK1 and WNK4 resulting in an increase in renal Na-Cl Cotransporter (NCC) apical distribution and function. Therefore we studied whether cyclosporine's metabolic side effects are mediated by WNK4 and NCC.

**Methods**: Sprague-Dawley (SD) rats were treated by cyclosporine 25 mg/Kg subcutaneously for 14 days. Blood pressure, blood chemistry values and kidney WNK4 protein were determined. In addition, mDCT cells were exposed to cyclosporine, and their WNK4 mRNA and protein content and their Na-Cl cotransporter (NCC) protein content were determined.

**Results**: The rats developed an FHHt-like syndrome including hypertension, hyperkalemia and salt sensitive hypercalciuria. These rats developed a significant increase in their kidney WNK4 protein content (0.13±0.01 vs 0.67±0.16 WNK4/GAPDH in controls, p=0.0183). In mDCT cells cyclosporine caused a rise in WNK4 mRNA levels and also a three fold rise in WNK4 protein content. This rise was followed by a rise in NCC protein content.**Conclusions**: Cyclosporine treatment causes an increase in WNK4 abundance in rats and in mDCT cells. This may be in part, the mechanism of cyclosporine- induced hypertension, hyperkalemia and hypercalciuria.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2761

**Apical NKCC2 Is Activated in Hypertensive Rats Contributing to Maintenance of Salt-Sensitive Hypertension** Monica Carmosino,[1] Patrizia Ferrari,[2] Lucia Torielli,[2] Mara Ferrandi,[2] Federica Rizzo,[1] Francesca Romano,[1] Giuseppe Bianchi,[3] Maria Svelto,[1] Giovanna Valenti.[1] *[1]General and Environmental Physiology, University of Bari, Bari, Italy; [2]Prassis Research Institute, Sigma Tau, Milan, Italy; [3]Università Vita Salute, S. Raffaele Hospital, Milan, Italy.*

The Milan hypertensive strain of rats (MHS) develops hypertension as consequence of the increased expression and activity of the tubular Na-K-ATPase. Regarding the other tubular sodium transporters no change in NKCC2 expression in the TAL was observed in this strain of rats (Capasso et al. 2008), despite NKCC2-dependent salt transport constitutes one of the major pathways for transepithelial salt reabsorption in the kidney. Since the activity of the renal-specific NKCC2 is regulated by changes in phosphorylation state, in this work we have analyzed the phosphorylation state of NKCC2 in Milan rats to verify whether the cotransporter activation is involved in the maintenance of hypertension in MHS rats.

Western blotting analysis using a specific antibody against the regulatory phospho-threonines in the NKCC2 N-terminus (R5 antibody) revealed that the renal expression of the phosphorylated-NKCC2 (p-NKCC2) increased by about 50% in hypertensive MHS rats compared to age-matched MNS control rats. Immunofluorescence analysis using the same antibody also showed that compared to age-matched controls, both the fluorescence

intensity of single tubules and the number of stained tubules significantly increased in MHS rats compared to MNS rats. On the other hand, western blotting analysis using T4 antibody, against both unphosphorylated and phosphorylated NKCC2, confirmed that the total expression of NKCC2 is unchanged in both strains of rats. Moreover, a six weeks oral administration of 20 mg/kg/day of furosemide prevented the insurgence of hypertension in treated MHS rats. In conclusion, we demonstrated that an increase in the activity of NKCC2 along the TAL might significantly contribute to sodium retention resulting in increase in systemic blood pressure in hypertensive MHS rats, placing NKCC2 as a key element in the pathogenesis of salt-sensitive hypertension.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2762

**Beneficial Effects of 4%KCl-1%NaCl and 4%KCl-2%NaCl vs. 1%KCl-2%NaCl Diets in SHR Rats** Alicia A. McDonough, Mien T. X. Nguyen, Donna Lee, Anne Riquier-Brison. *Cell and Neurobiology, Keck School of Medicine of USC, Los Angeles, CA.*

To assess the effects of dietary KCl on blood pressure and renal function, Spontaneously Hypertensive Rats (SHR) were fed one of 3 diets from 6–12 wks of age: 1)1%KCl-2%NaCl diet typical of average American diets (1K2Na), 2)4%KCl-1%NaCl diet recommended by Institute of Medicine, AHA (4K1Na), 3)4% KCl-2%NaCl, a K supplemented American diet (4K2Na). Table 1 summarizes physiological parameters.

| Measurement | 1K2Na | 4K1Na | 4K2Na |
|---|---|---|---|
| Plasma Na (mM) | 139±2 | 140±2 | 140±3 |
| Plasma K (mM) | 3.8±0.2 | 3.9±0.2 | 3.6±0.4 |
| MAP (mmHg) | 192±23 | 181±21 | 172±15 |
| Aldosterone (pg/ml) | 463±70 | 844±62 | 615±55 |
| U V (ml/24 h) | 8±4 | 12±4 | 25±10 |
| U Na (mM/24 h/bw) | 9±4 | 6±1 | 16±4 |
| U K (mM/24 h/bw) | 2.4±1.0 | 14±5 | 22±6 |
| Muscle Na,K-ATPase α2 | 1.0±0.1 | 1.25±0.1 | 1.25±.05 |

n=4, means±SD

The diets did not change plasma [Na] or [K]. Compared to 1K2Na, 4K2Na lowered MAP, increased UV, UNa and UK. Aldosterone was elevated with 4%KCl diets but lower in 4K2Na than in 4K1Na. Renal Na transporters, analyzed by microscopy and density gradient fractionation revealed: proximal NHE3 was retracted out of the microvilli but DCT NCC distribution was unaltered. Skeletal muscle sodium pump abundance was elevated by both 4%KCl diets. This set of results suggests that doubling SHR chow [KCl]: 1) provokes diuretic and natriuretic responses, perhaps emanating with lower PT NHE3 activity, that could drive flow sensitive K secretion in the distal nephron, 2) reduces MAP, more with 4K2Na, perhaps secondary to greater diuresis and lower [aldosterone], 3) increases muscle Na,K-ATPase expression, perhaps to buffer increased dietary K load, 4) has beneficial effects even with 2% NaCl, suggesting that further efforts should be directed toward increasing dietary K.

Disclosure of Financial Relationships: Employer: USC Keck School of Medicine; Honoraria: APS, AM Physiol Society; Patent: Chemicon Royalties for Sodium Pump Antibodies; Scientific Advisor: AM Physiol Society.

## SA-PO2763

**γ-Adducin Functions as a Novel Regulator of the Thiazide-Sensitive NaCl-Cotransporter** Henrik Dimke,[1] Pedro San Cristobal,[1] Mark J. J. De Graaf,[1] Jacob (Jaap) Deinum,[2] Jacques W. M. Lenders,[3] Joost G. Hoenderop,[1] Rene J. Bindels.[1] *[1]Physiology, Radboud University, Nijmegen Medical Center, Nijmegen, Netherlands; [2]Department of Internal Medicine, Section of Vascular Medicine, Radboud University, Nijmegen Medical Center, Nijmegen, Netherlands; [3]Department of Medicine, Carl Gustav Carus University Medical Center, Dresden, Germany.*

Hypertension is projected to affect more than 1 billion individuals worldwide. The National Vital Statistics Report lists heart disease as the primary cause of death, affecting more than 20 million people in the United States alone. Primary hypertension remains a major risk factor for the development of cardiovascular and chronic kidney diseases. The thiazide-sensitive NaCl cotransporter (NCC) plays a key role in renal salt reabsorption and thereby the maintenance of systemic blood pressure. This study was designed to further elucidate the molecular mechanisms governing the regulation of NCC. Pull down experiments coupled to mass spectrometry identified γ-adducin as a novel interactor of the transporter. γ-Adducin co-localized with NCC to the distal convoluted tubule. $^{22}Na^+$ uptake experiments in the Xenopus laevis oocyte showed that γ-adducin stimulated NCC activity in a dose-dependent manner. The stimulatory effect of γ-adducin occurs upstream from the With-No-Lysine Kinase 4. The binding site of γ-adducin mapped to the N-terminus of NCC encompassing three previously reported phosphorylation sites. Furthermore, competition with the N-terminal domain of NCC abolished the stimulatory effect of γ-adducin on the transporter. γ-Adducin was unable to increase NCC activity when these phosphorylation sites were made constitutively inactive or active. In addition, γ-adducin bound only to the dephosphorylated N-terminal of NCC. Taken together, our observations suggest that γ-adducin functions as a dynamic regulator of NCC, likely by amending the phosphorylation state of the transporter and consequently its activity. These observations suggest a novel mechanism by which γ-adducin influences renal NaCl transport and hence blood pressure maintenance.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2764

**Functional Analysis of Novel Thiazide-Sensitive NaCl-Cotransporter Sequence Variants in a New Cohort of Patients with Gitelman Syndrome** Pedro San Cristobal,[1] Bob Glaudemans,[1] Joost G. Hoenderop,[1] Rene J. Bindels,[1] Nine V. Knoers.[2] *[1]Physiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; [2]Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.*

Gitelman syndrome (GS) is an autosomal recessive disorder characterized by low blood pressure, hyponatremia, hypokalemia and metabolic alkalosis in conjunction with significant hypomagnesemia and hypocalciuria. Periods of muscle weakness and tetany are commonly observed in patients with GS. The underlying cause for the GS phenotype is genetic aberrations in the solute carrier family 12, member 3 (SLC12A3) gene that encodes the thiazide-sensitive NaCl cotransporter (NCC). NCC localizes to the luminal membrane of the distal convoluted tubule (DCT), where it facilitates the cotransport of $Na^+$ and $Cl^-$ from the pro-urine into the cell. Here, we analyzed DNA samples of 263 patients with clinical phenotype compatible with GS by direct sequencing of all 26 exons of the SLC12A3 gene. In total 114 different mutations were identified, 50 of which have not been reported in literature before. These novel variants include 7 deletions, 30 missense-, 9 splice site- and 4 nonsense mutations. 7 missense mutations were selected (Glu121Asp, Thr392Ile, Asn442Ser, Ser475Cys, Tyr489His, Pro751Leu, Gln1030Arg) to investigate their effect on NCC activity and plasma membrane localization using the Xenopus laevis oocyte expression system, by $^{22}Na^+$ tracer uptake and confocal microscopy. Our results demonstrated that the Thr392Ile mutant was retained in the endoplasmic reticulum (ER) (class 2 mutation), while the Asn442Ser and Gln1030Arg NCC mutants showed decreased plasma membrane localization and consequently function, likely due to impaired trafficking (class 3 mutation). Even though the NaCl uptake was hampered for NCC mutants Glu121Asp, Pro751Leu, Ser475Cys and Tyr489His, the transporters reached the plasma membrane (class 4 mutation), suggesting an effect on NCC regulation or ion affinity. The present study identifies and characterizes new mutations in NCC, thereby providing insight into the molecular mechanisms underlying GS.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2765

**Antagonists of Prostaglandin $E_2$ Receptors for the Treatment of Hypertension** Jason Duane Downey,[1] Richard M. Breyer.[2] *[1]Pharmacology, Vanderbilt University, Nashville, TN; [2]Medicine, Vanderbilt University, Nashville, TN.*

In contrast to blockade of all prostanoid production with NSAIDs, pharmacological blockade of $PGE_2$ pressor receptors EP1 and EP3 may decrease systemic blood pressure and protect from hypertensive renal damage. To test this hypothesis, EP1 and EP3 small molecule antagonists were synthesized and their pharmacology, pharmacokinetics, and physiologic effects on mean arterial pressure (MAP) were assessed. Two lead compounds, DG-041 and JD-200, were synthesized as subtype-selective antagonists of the pressor EP receptors. DG-041 binds the mEP3γ receptor with a $K_i$ of 449 ± 130.0 pM and potently blocks mEP3γ signaling with an $IC_{50}$ of 420.0 pM in an LVIP2.0zc cell-based CRE reporter assay. JD-200 blocked signaling through mEP1 with an $IC_{50}$ of 18.8 ± 11.5 nM in a fluorescent calcium flux cell-based assay. In vivo mouse pharmacokinetic experiments demonstrated that DG-041 is orally bioavailable with an elimination half-life of 1.2 h. JD-200 had an elimination half-life of 28 min. Preliminary studies demonstrate that while acute infusion of the EP1/EP3 agonist sulprostone caused a transient increase in MAP (+33.3 mmHg) in the EP1-/- mouse, pretreatment with DG-041 blocked the pressor effect of acute sulprostone infusion (+4.60 mmHg); the vasopressor effect of acute infusion of an unrelated pressor, α-adrenergic agonist phenylephrine, was not different (vehicle +42.0 mmHg, DG-041 +39.0 mmHg). Taken together these results suggest that these EP1 and EP3 receptor antagonists will be suitable for use in mouse models of hypertension.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2766

**EP1 Receptors Exacerbate Mortality in a Mouse Model of Hypertension** Christina E. Swan,[1] Kelli L. Boyd,[2] Roy Zent,[1] Richard M. Breyer.[1] *[1]Medicine, Vanderbilt University, Nashville, TN; [2]Pathology, Vanderbilt University, Nashville, TN.*

The role of the E-Prostanoid 1 (EP1) Prostaglandin E2 receptor in hypertensive end-organ damage was evaluated. Mice with a disrupted EP1 allele (EP1-/-; n = 25) or wild type controls (EP1+/+; n= 47) were subjected to a recently described model of hypertension and renal damage. Mice were uninephrectomized, and after two weeks recovery, a 50 mg deoxycorticosterone acetate pellet was implanted and 1 % NaCl was supplied in the drinking water. After a further week, angiotensin II (Ang II) was administered via Alzet minipumps (1.5 ng/min/g) implanted s.c. Blood pressure was measured by tail cuff plethysmography and proteinuria was assessed in spot urine collections on a weekly basis throughout the study. At the end of the study, mice were euthanized and histopathology was performed to assess renal damage. EP1+/+ mice in this model suffered from previously unreported high rates of mortality (56%), while EP1-/- mice had significantly lower mortality (23% P = 0.018). Systolic blood pressure was elevated in both groups post Ang II administration (EP1+/+ 179.8 ± 8.9 mmHg , EP1-/- 200.3 ± 8.0 mmHg P = 0.648). No differences were observed in levels of proteinuria between genotypes as determined by albumin to creatinine ratio (ACR) P = 0.216. Histopathological analysis revealed moderate hypertensive renal damage including glomerulosclerosis and mesangial expansion; but no differences were observed between the two groups. Taken together these data suggest that this deoxycorticosterone

acetate/Ang II model induces robust hypertension and modest renal damage in both EP1+/+ mice and EP1-/- mice. While the EP1 -/- mice are protected in this model, the lower mortality observed does not appear to be associated with either decrease in blood pressure or protection from renal damage.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2767**

**Changes in Extracellular Matriz (ECM) Composition Regulate COX-2 Expression in Human Mesangial Cells (HMC) Via FAK/PI3K/AKT/CREB Signaling Pathway** Laura Calleros,[1] Matilde Alique,[1] Alicia Luengo,[1] Mercedes Griera,[1] Diego Rodriguez-Puyol,[1, 2] Manuel Rodriguez-Puyol.[1] *[1]Department of Physiology, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain; [2]Nephrology Section and Research Unit, Hospital Universitario "Príncipe de Asturias", IRSIN, Alcalá de Henares, Madrid, Spain.*

Renal fibrosis is the common final outcome of many clinical conditions, both inflammatory and non-inflammatory, that lead to chronic renal failure. It is characterized by a progressive substitution of cellular elements by extracellular matrix (ECM) proteins, as well as the loss of the normal equilibrium between the synthesis and degradation of ECM. In normal conditions, collagen IV seems to be the most relevant glomerular collagen but in pathological conditions, such as hypertension or diabetes, characterized by an augmented profibrotic cytokines at the local level, an increased ECM protein synthesis with a progressive accumulation of interstitial collagen I takes place at glomerular level. ECM proteins, through the interaction with transmembrane proteins, may induce significant changes in cell phenotype. Interestingly, evidence obtained in the past decade indicates that progression to advanced renal fibrosis requires the participation of inflammatory events such as COX-2 up-regulation. Moreover it is known that AKT may act through different transcription factors in the regulation of the COX-2 promoter. Present results show that progressive accumulation of collagen I in the extracellular medium induces a significant increase of COX-2 expression in human mesangial cells and an enhancement in PGE2 production. This COX-2 overexpression is due to increased COX-2 mRNA levels. The analysis of the mechanism implicated in COX-2 up-regulation by collagen I showed FAK activation. Furthermore, we observed that the activation of the PI3K/AKT pathway by collagen I, and collagen I-induced COX-2 over expression was abolished by PI3K and AKT inhibitors, or abrogation of AKT expression. Additionally, we showed that the CRE transcription factor is implicated. In summary, our results provide evidence that collagen type I increases COX-2 expression via the FAK/PI3K/AKT/CREB signaling pathway.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2768**

**Pro-Fibrotic Effects of Aldosterone on Normal Heart and Aorta: Processes Attenuated by Endogenous Glucocorticoids and Their 11-Dehydro Metabolites** Andrew S. Brem,[1] David Morris,[2] Rujun Gong.[1] *[1]Division of Kidney Diseases and Hypertension; [2]Department of Pathology, Rhode Island Hospital, Brown Medical School, Providence, RI.*

Aldosterone (ALDO) is known to be a pro-fibrotic factor in both cardiovascular and renal tissues. The effects of ALDO on fibrosis have largely been studied in animal models of pre-existing hypertension and/or systemic disease. This study tests the hypothesis that prolonged ALDO exposure is able to directly induce fibrotic changes normal mice in the absence of hypertension or systemic disease. Experiments were conducted with intact or adrenalectomized (ADX) mice. Mice were divided into 8 groups (n=4/group) and treated for 1 week: CONTROLS; mice treated with ALDO (8 µg/kg/day via mini-pump); ADX-CONTROLS; ADX plus corticosterone (CORT 800 µg/kg/day); ADX-ALDO; ADX-ALDO plus the mineralocorticoid receptor (MR) antagonist RU-318 (800 µg/kg/day); ADX -ALDO plus CORT (800 µg/kg/day); and ADX-ALDO plus 11-dehydro-CORT (800 µg/kg/day). Four variables were measured: aortic smooth muscle to collagen ratio, aortic intimal thickness (µm), heart/body weight ratio (mg/gm), and left ventricular collagen volume (%). Mice in all 8 groups exhibited normal blood pressures at 1 week prior to sacrifice. CORT alone had no effect on any of the variables examined. However, ALDO exposure was associated with fibrotic changes in intact mice but the changes were significantly more pronounced in the ADX-ALDO mice. The fibrotic changes were distributed in a peri-vascular pattern, closely associated with micro-vessels. The ALDO induced effects were attenuated in the ADX-ALDO-RU-318 mice and in the ADX-ALDO-11-deydro-CORT treated mice. ADX-ALDO-CORT mice also showed a decrease in fibrotic changes but the findings were not as reproducible. Thus, extended low-level ALDO exposure induces fibrotic changes in heart and aorta from normal mice in the absence of hypertension or systemic disease. MR antagonists and the 11-dehydro-metabolite of vascular 11β-HSD (dehydrogenase) attenuate these fibrogenic effects. Vascular 11β-HSD isoforms may provide locally synthesized agents, which directly limit ALDO induced fibrotic activity in normal heart and aorta.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2769**

**Age-Dependent Impairment of Renal Vascular Function in Mature Ciliary Neurotrophic Factor Deficient Mice** Sebastian A. Potthoff, Yuriko Mori, Henning Hoch, Eva Koenigshausen, Magdalena Woznowski, Lorenz Sellin, Oliver Vonend, Johannes Stegbauer, Lars C. Rump, Ivo Quack. *Deparment of Nephrology, Heinrich-Heine University, Duesseldorf, Germany.*

Ciliary neurotrophic factor (CNTF), an interleukin-6-like cytokine with distinct impact on neuronal cell survival, mediates anti-inflammatory effects and reduces apoptosis by activating the JAK2-STAT3-signaling cascade and MAP kinases. CNTF is highly expressed in vessels and the kidney. To investigate a potential role of CNTF on renal vascular function, vasoreactivity to Ang II was tested in isolated perfused kidneys of CNTF-KO and WT mice. Furthermore, we investigated whether Ang II induced pressor responses were dependent on JAK2-STAT3 signalling pathway or MAP-kinase signal transduction. Therefore, dose-response-curves to Ang II were performend in the presence or absence of AG490 (JAK2-Inhibitor, 5µM), SB203580 (p38-inhibition, 5µM) and PD98059 (MEK1-inhibition, 5µm).

CNTF-KO vs. WT-mice at the age of 14 +/- 3 weeks (n=8) showed no significant difference in Ang II pressor response. However, at 31 +/- 7 weeks (n=12), CNTF-KO mice showed a significant decrease in Ang II pressor response with a minimum of 59.8% of WT-response at EC50. Neither in young nor old mice, acute inhibition of p38, MEK1 and JAK2 significantly altered Ang II dependent pressor responses.

Interestingly, pressor response to potassium chloride (1M) showed a trend to be reduced in old CNTF-KO vs. WT mice (KO vs. WT: 124+/-53 vs. 161+/-59 mmHg; p=0.16), whereas young CNTF-KO vs. WT mice mice had an equal pressor response to KCl (KO vs. WT: 152+/-24 mmHg vs. 159+/-33 mmHg; p=0.51).

In our study, we demonstrate that neither JAK2-STAT3 signaling pathway nor the MAP-kinases signal transduction have any impact on renal pressor response to Ang II in non-pathological conditions. However, CNTF deficiency causes an age-dependent impairment of renal vasoreactivity to Ang II. Thus, our data suggest that CNTF is nessecary to preserve maximal renal vascular function. Whether this observed age-dependant difference in pressor response is limited only to Ang II induced vasoconstriction or to general impairment of vasoreactivity is subject of ongoing research.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2770**

**SIRT1 Activation Protects the Endothelial Dysfunction To Promote Atherogenesis through LKB1/ AMP-Activated Protein Kinase Activation** Hideyuki Negoro.[1] *[1]Medicine, Harvard Medical School, Boston, MA; [2]Japanese Red Cross Medical Center Hospital, Tokyo, Japan.*

SIRT1 is a conserved NAD(+)-dependent deacetylase and possesses beneficial effects against aging-related diseases, but little information is available on a putative role of SIRT1 in endothelial and vascular homeostasis. Endothelial senescence causes endothelial dysfunction to promote atherosclerotic change and contribute to age-related vascular diseases. In the present study, we evaluate the protective effects of SIRT1 on the endothelial dysfunction and elucidate the underlying mechanisms. We established an in vitro senescence model by prolonged culture of primary endothelial cells isolated from bovine aorta. The freshly isolated young endothelial cells gradually underwent senescence during 1 month of repetitive passages. It was observed that protein expressions of SIRT1 were decreased time dependently in the senescent endothelial cells. In contrast, the protein levels of LKB1, a serine/threonine kinase, the phosphorylation of its downstream target AMP-activated protein kinase (AMPK) and plasminogen activator inhibitor (PAI)-1generation were dramatically increased in the senescent cells. On the other hand, resveratrol activated SIRT1 in the endothelial cells. SIRT1 activation with resveratrol inhibited the increase of LKB1, AMPK. At the same time, SIRT1 activation with resveratrol reduced PAI-1 generation in the endothelial cells significantly. We knocked down SIRT1 and the protein levels of LKB1, phosphorylated AMPK and PAI-1 elevated in the knocked down cells. The protein levels of LKB1, phosphorylated AMPK and PAI-1generation did not changed in the SIRT1 knocked down cells even if they were stimulated with resveratrol.

These findings indicate that activation of SIRT1 provides beneficial effects against the endothelial dysfunction to promote atherogenesis through LKB1/ AMPK pathways.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2771**

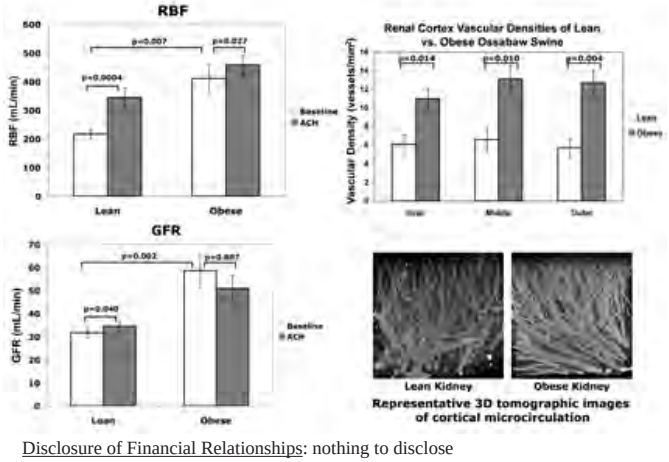
**Renal Hyperfiltration in Swine Obesity Is Associated with Increased Intra-Renal Microvascular Density** John R. Woollard, Michael James Korsmo, Kyra L. Jordan, Hui Tang, James Krier, Xiang-Yang Zhu, Lilach O. Lerman. *Nephrology & Hypertension, Mayo Clinic, Rochester, MN.*

**Objective:** Obesity is an important predictor of chronic kidney disease, and also associated with an early increase in glomerular filtration rate (GFR) and renal blood flow (RBF), which might contribute to progression of renal injury, but the mechanisms remain unclear. This study tested the hypothesis that obesity-induced hyperfiltration is associated with remodeling and dysfunction of the renal microvasculature. **METHODS:** Ossabaw littermate pigs (a model prone to obesity induced by an atherogenic diet and sedentary life style) were fed either a standard (lean, n=7) or atherogenic (obese, n=7) diet for 10 weeks. Visceral fat volume and renal hemodynamics and function were then studied in vivo using multi-detector CT before/after infusion of acetylcholine (Ach). Intra-renal microvascular density was then studied in vitro using micro CT.

**Results:** Obese were ~50% heavier than lean pigs (p=0.004) due to elevated visceral fat volume, and had increased serum cholesterol and oxidized-LDL (675±142 vs. 382±75

ng/mL, p<0.05) levels, while blood pressure was not different. Basal RBF and GFR were elevated in obese. RBF increased in response to Ach in both groups, but response was attenuated in obese compared to lean pigs (+17±6 vs +59±14%, p=0.007), as was GFR response to Ach (-10±5 vs +11±5%, p=0.004). Fractional vascular volume and spatial density of microvessels (20-200μm in diameter) were higher in the cortex of obese pigs.

**Conclusion:** Obesity leads to proliferation of dysfunctional intra-renal microvessels, and in turn an increase in RBF and GFR, likely secondary to inflammation and oxidative stress. The associated hyperfiltration and endothelial dysfunction may partly account for progression of renal injury in obesity.




Disclosure of Financial Relationships: nothing to disclose

## SA-PO2772

**Endothelial-to-Mesenchymal Transition (Endo-MT) in Human Vascular Endothelial Cells as a Novel Mechanism of Uric Acid-Induced Endothelial Dysfunction** Yang Hee Jang, Eun Sun Ryu, Mina Yu, Dong-Ryeol Ryu, Seung-Jung Kim, Kyu Bok Choi, Duk-Hee Kang. *Nephrology, Ewha Womans University, School of Medicine, Seoul, Republic of Korea.*

Recent data from experimental and clinical studies suggested a causative role of uric acid in the development and/or aggravation of cardiovascular, renal and metabolic diseases. Endothelial dysfunction, which is characterized by a decrease in nitric oxide (NO) production, an induction of oxidative stress and cell senescence, is regarded as one of the key mechanisms of uric acid-induced vascular and metabolic diseases. Endothelial-to-Mesenchymal transition (endo-MT) is an early and reversible process of endothelial dysfunction, and is known to play a role in cardiac fibrosis and interstitial fibrosis of diabetic nephropathy. We investigated whether uric acid per se induced endo-MT in cultured human vascular endothelial cells in order to define the early therapeutic target of uric acid-induced vascular, renal and metabolic disease. HUVECs were isolated from umbical cord, and stimulated with uric acid (3, 6, 9, 12 mg/dl). Endo-MT was evaluated by a comparison of the expression of the endothelial markers, CD31 or VE-cadherin and the mesenchymal marker, alpha smooth muscle actin (α-SMA) by western blot analysis and immunocytochemistry. Uric acid (3-12 mg/dl) induced the morphologic changes of HUVEC from 96 hours of stimulation with a loss of cell contact and elongation. From 48 hours of stimulation before the development of morphologic changes, uric acid down-regulated the expression of CD31 and VE-cadherin and up-regulated α-SMA expression in a dose-dependent and time-dependent manner, which were significantly attenuate by pre-treatment of organic anion transporter inhibitor, probenecid (1 mM). Fluorescence microscopy of uric acid-stimulated HUVEC also demonstrated that uric acid altered the expression of CD31 and α-SMA, Interestingly, uric acid-induced endo-MT in HUVEC was associated with a senescence of endothelial cells and a decrease in NO production. These findings suggested uric acid per se induced a phenotypic transition of endothelial cells, endo-MT, which could be one of the mechanisms of uric acid-induced endothelial dysfunction.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2773

**Indoxyl Sulfate, a Uremic Toxin, Promotes Cell Senescence in Aorta of Hypertensive Rats** Toshimitsu Niwa,[1] Ayinuer Adijiang,[1] Yuusuke Higuchi,[2] Fuyuhiko Nishijima,[2] Hidehisa Shimizu.[1] *[1]Department of Advanced Medicine for Uremia, Nagoya University School of Medicine, Nagoya, Japan; [2]Biomedical Research Laboratories, Kureha Co., Tokyo, Japan.*

We demonstrated that administration of indoxyl sulfate, a uremic toxin, promotes aortic calcification in hypertensive rats. This study aimed to clarify if indoxyl sulfate could contribute to cell senescence in the aorta of hypertensive rats.

The rat groups consisted of 1) Dahl salt-resistant normotensive rats (DN), 2) Dahl salt-resistant normotensive indoxyl sulfate-administered rats (DN+IS), 3) Dahl salt-sensitive hypertensive rats (DH), and 4) Dahl salt-sensitive hypertensive indoxyl sulfate-administered rats (DH+IS). After 32 weeks, their arcuate aortas were excised for histological and immunohistochemical analysis. Cell senescence was evaluated by immunohistochemistry of senescence-associated beta-galactosidase (SA-beta-gal), and senescence-related proteins such as $p16^{INK4a}$, $p21^{WAF1/CIP1}$, p53 and retinoblastoma protein (Rb).

Both DH and DH+IS rats showed significantly increased systolic blood pressure as compared with DN and DN+IS rats, respectively. Serum indoxyl sulfate levels were significantly increased in DN+IS and DH+IS rats as compared with DN and DH rats, respectively. DH+IS rats showed significantly increased serum creatinine and decreased creatinine clearance as compared with DH rats. In aorta, DH rats showed significantly increased aortic calcification and wall thickness, and increased expression of SA-beta-gal, $p16^{INK4a}$, $p21^{WAF1/CIP1}$, p53 and Rb in the calcification area of arcuate aorta as compared with DN rats. More notably, DH+IS rats showed significantly increased aortic calcification and wall thickness, and significantly increased expression of SA-beta-gal, $p16^{INK4a}$, $p21^{WAF1/CIP1}$, p53 and Rb in the cells embedded in the calcification area as compared with DH rats.

In conclusion, indoxyl sulfate promotes cell senescence with aortic calcification and expression of senescence-related proteins in hypertensive rats.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2774

**Mechanical Stretch Prevents Calcification and Regulates Calcium-Sensing Receptor Expression, Function in Long-Term Cultures of Human Aortic Smooth Muscle Cells** Daniel Zehnder,[1] Guerman Molostvov,[1] Rosemary Bland.[2] *[1]Clinical Siences Research Institute, University of Warwick, Coventry, United Kingdom; [2]BioMedical Research Institute, University of Warwick, Coventry, United Kingdom.*

Vascular smooth muscle cells (VSMC) play a crucial role in the development of arterial calcification, which is responsible for premature cardiovascular mortality in chronic kidney disease patients. This study investigates the role of calcium-sensing receptor (CaSR) and mechanical strain on VSMC phenotype and calcification.

Human aortic SMC (HAoSMC) were cultured under static or cyclic strain conditions using Flexcell apparatus for up to 2 weeks. HAoSMC phenotype and calcification were quantified by Western blot, alizarin red staining, alkaline phosphatase (ALP) and osteocalcin (OC) activity assays. For statistical analysis one-way ANOVA followed by Tukey's test was used.

Culture under cyclic strain resulted in up-regulation of α-actin expression by days 7 and 10 (23%, p<0.05) and a 45% increase in CaSR expression (p<0.05) compared to static cultures.

To assess the role of CaSR, cells were treated with CaSR agonists: 2 and 5mM $Ca^{2+}$ or 50μM $Gd^{3+}$ alone or in combination. Treatment of static HAoSMC with $Ca^{2+}$, $Gd^{3+}$ or both for 7 days induced a marked down-regulation (p<0.01) of CaSR expression and up-regulation (p<0.01) of HAoSMC calcification. ALP and OC levels were markedly increased in cells treated with both CaSR agonists (p<0.05). In HAoSMC cultured under cyclic strain, $Ca^{2+}$ and $Gd^{3+}$-induced down-regulation of CaSR expression and increased calcification were significantly attenuated (p<0.05 to p<0.001). In addition, cyclic strain induced a significant down-regulation of ALP and OC production in control and CaSR agonist-treated cells (p<0.01).

CaSR knockdown with a CaSR siRNA resulted in a further increase in calcification in $Ca^{2+}$ and $Gd^{3+}$-treated cells when compared to untransfected cells (p<0.05). In addition, there was a pronounced up-regulation of ALP and OC production in control and agonist-treated CaSR knockdown cells (p<0.01).

In conclusion, our findings indicate that a functional CaSR may serve to prevent a shift towards calcifying SMC phenotype and, therefore, protect against calcification.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2775

**Gene Therapy with Human Hepatocyte Growth Factor Prevents the Development of Hypertension and Suppresses Vascular MCP-1 and IL-6 mRNA Expression in Fructose-Fed Rats** Freddy J. Romero,[1] Maribel Ch. Chavez,[2] Jose L. Arcaya,[2] Jose D. Herrera,[2] Richard J. Johnson,[3] Bernardo Rodriguez-Iturbe.[1] *[1]IVIC-Zulia, Maracaibo, Venezuela; [2]Universidad del Zulia, Maracaibo, Venezuela; [3]University of Colorado, Denver.*

**Background**: Although previous studies have shown that increased serum concentration of hepatocyte growth factor (HGF) are present in association with the metabolic syndrome, the relationship of HGF abundance with hypertension and vascular inflammation remain largely unknown. The purpose of this study was to investigate the effect of gene therapy with human HGF on the development of these features in the fructose-induced model of metabolic syndrome. **Methods**: Six-weeks-old male Sprague Dawley rats were randomly divided into four groups (n=6 each):1)control group fed normal chow and water; 2)FFR group fed normal chow and given water containing 10% (w/v) fructose during 6 weeks; 3)FFR-pCMV-HGF group, fructose-fed rats that received hydrodynamic injection of HGF gene via naked plasmid vector (1 mg/Kg) and 4)FFR-pcDNA3.1 group, fructose-fed rats that received empty vector. Systolic blood pressure (SBP) and fasted plasma parameters were measured every week. The aorta was extracted to evaluate pro-inflammatory cytokines mRNA expression by real time PCR.

**Results**: As predicted, 6 weeks of fructose consumption in the drinking water resulted in mildly elevated SBP(mmHg) (control= 116 ± 2.01 vs. FFR= 140 ± 3.02) hypertriglyceridemia (mM) (FFR= 0.71 ± 0.04 vs. Control= 0.33 ± 0.05) and vascular inflammation compared with the controls rats. HGF gene therapy reduced SBP in FFR-pCMV-HGF vs. FFR-pcDNA3.1 (121.0 ± 2.76 vs. 139 ± 3.0, p<0.01) without modifying triglyceride blood levels. In addition, HGF therapy suppressed aorta mRNA expression of MCP-1 (0.98 ± 0.29 vs. 2.99 ± 0.96, p<0.05) and IL-6 (1.28 ± 0.42 vs. 3.53 ± 0.48, p<0.05) for FFR-pCMV-HGF vs. FFR-pcDNA3.1.

**Conclusions**: These results indicate that gene therapy with human HGF reduce vascular inflammatory cytokines and lowers blood pressure in fructose-induced metabolic syndrome without modifying hyperlipidemia. Elevated HGF levels in the metabolic syndrome may be an adaptive response to suppress vascular inflammation

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2776

**Bone Marrow-Derived Fibroblast Precursors Mediate Angiotensin II Induced Cardiac Fibrosis – Role of CCR2** Jing Xu, Song-Chang Lin, George E. Taffet, Mark L. Entman, William E. Mitch, Yanlin Wang. *Department of Medicine, Baylor College of Medicine, Houston, TX.*

Cardiovascular disease is a major cause of morbidity and mortality in patients with chronic kidney disease. Angiotensin II (Ang II) plays an important role of the development of cardiac hypertrophy and fibrosis, but the underlying cellular and molecular mechanisms are not completely understood. Recent studies have shown that bone marrow-derived fibroblast precursors are involved in the pathogenesis of tissue fibrosis. Since bone marrow-derived fibroblast precursors express chemokine receptor - CCR2, we tested the hypothesis that CCR2 can regulate the recruitment of bone marrow-derived fibroblast precursors into the heart, mediating Ang II-induced cardiac fibrosis. Wild-type (WT) and CCR2-deficient (CCR2-KO) mice underwent unilateral nephrectomy and were treated with angiotensin II (Ang II) via subcutaneous osmotic minipumps at 1500 ng/kg/min. Ang II treatment resulted in elevation of blood pressure and cardiac hypertrophy that was not significantly different between WT and CCR2-KO mice. Ang II treatment of WT mice caused cardiac fibrosis as measured by picrosirius red stain. There also was accumulation of bone marrow-derived fibroblast precursors expressing hematopoietic markers-CD34 and CD45, mesenchymal marker-collagen type I, and myofibroblast marker-α-smooth muscle actin (α-SMA) as determined by flow cytometry. Ang II-induced cardiac fibrosis and recruitment of bone marrow-derived fibroblast precursors in the heart were attenuated in CCR2-KO mice. Furthermore, Ang II treatment of WT mice increased collagen type I, fibronectin, and α-SMA expression in the heart as revealed by real time RT-PCR, immunohistochemistry, and Western blot analyses. These changes were abrogated in the CCR2-KO mice. Functional studies with cardiac ECHO showed that the reduction of cardiac fibrosis led to an impairment of cardiac systolic function and subsequent left ventricular dilatation in Ang II–treated CCR2-KO mice. Our results suggest that CCR2 play a pivotal role in the development of Ang II–induced cardiac fibrosis through regulation of bone marrow-derived fibroblast precursors.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2777

**CCR5 Deficiency Does Not Reduce Renal Endorgan Damage in Hypertensive Mice** Christian Krebs, Christoph Fraune, Jan-Eric Turner, Ulf Panzer, Rolf A. Stahl, Ulrich O. Wenzel. *III. Med. Klinik - Nephrology, University Hospital Hamburg-Eppendorf, Hamburg, Germany.*

The chemokine-receptor CCR5 has been linked to pathology in hypertension; i.e. CCR5 antagonists influence hypertension and CCR5 is expressed on infiltrating T cells in hypertensive mice.

By combining DOCA salt and Ang II we have recently established a model of hypertension in C57Bl/6 mice with massive renal endorgan damage (Kidney Int 73:643-50, 2008). To evaluate the role of CCR5 in hypertension we compared wildtype (WT) and CCR5 deficient mice in this model.

DOCA + Ang II induced hypertension in WT and CCR5 $^{-/-}$ mice and blood pressure and renal function did not differ between hypertensive WT and CCR5 $^{-/-}$. DOCA + Ang II induced massive albuminuria and glomerular injury as assessed by scoring and renal PAI-1 expression but no difference was found. In addition, no difference was found for renal inflammation as measured by infiltrating cells (T cells and macrophages) and CCL-2 expression. The renal expression of CCR5 ligands CCL4 and CCL5 were increased in hypertensive mice with no difference between the two groups. CCL3 was increased in CCR5 $^{-/-}$ as compared to hypertensive WT (rel. exp. 1.8 vs. 2.8; p<0.05).

In conclusion, CCR5 deficiency does not influence hypertensive renal endorgan damage. Since infiltrating T cells have a high expression of CCR5, these cells are either not pathogenic or CCR5 positive T cells immigrate via a different chemokine receptor. Beneficial effects of CCR5 antagonists in hypertension are most likely due to unspecific effect of the antagonists. Other chemokine receptors in concert with CCR5 must be important for hypertensive injury.

| | number | systolic blood pressure (mmHg) | albumine-creatinine-ratio | glomerulo-sclerosis index | BUN (mg/dl) | CCL-2 (rel. exp.) | PAI-1 (rel. exp.) |
|---|---|---|---|---|---|---|---|
| control | 6 | 97.92±2.24 | 0.10±0.01 | 0.18±0.04 | 28.67±2.43 | 1.00±0.02 | 1.09±0.20 |
| wildtyp DOCA + Ang II | 11 | 135.60±4.16* | 159.40±52.69* | 1.51±0.21* | 38,50±2.31* | 10.27±1.81* | 6.50±1.21* |
| CCR5 DOCA + Ang II | 9 | 134.10±4.13* | 133.30±26.64* | 1.17±0.38* | 35.70 ±1.57* | 19.89±5.69* | 10.63±3.64 |

* p<0.05 vs. control

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2778

**Mizoribine Ameliorates Hypertension and Renal Injury Caused by Aldosterone-Salt Treatment** Toshiki Doi,[1] Kouichirou Kawaoka,[1] Noriaki Yorioka.[2] *[1]Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan; [2]Department of Advanced Nephrology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan.*

Purpose. We investigated whether mizoribine, an effective immunosuppressant, could ameliorate hypertension and renal injury in a rat model of aldosterone-salt treatment.

Methods. Six-week-old male Sprague-Dawley rats underwent right nephrectomy under anesthesia. After 10 days for recovery from surgery, the rats were given 1% NaCl as drinking water. The animals were divided into 3 groups (n=7 each): vehicle infusion, 0.75 μg/hour aldosterone infusion, or aldosterone infusion and 3 mg/kg/day of oral mizoribine. Systolic blood pressure was measured by the tail cuff method every other week. Two days before killing, 24-hour urine was collected for measurement of protein. Six weeks after the start of treatment, all of the rats were sacrificed. Renal sections were assessed for glomerulosclerosis, interstitial fibrosis, and T lymphocyte and macrophage infiltration. Expression of interferon-gamma (INF-gamma), tumor necrosis factor-alpha (TNF-alpha), transforming growth factor-beta1 (TGF-beta1), monocyte chemotactic protein-1 (MCP-1), and endothelin-1 (ET-1) was measured by real-time PCR.

Results. Rats with aldosterone infusion exhibited hypertension and renal injury characterized by proteinuria, glomerulosclerosis, interstitial fibrosis, and interstitial infiltration of T lymphocytes and macrophages. Renal cortical mRNA expression of INF-gamma, TNF-alpha, TGF-beta1, MCP-1, and ET-1 were increased by aldosterone infusion. Treatment with mizoribine improved both hypertension and renal injury. Expression of INF-gamma, TNF-alpha, TGF-beta1, MCP-1, and ET-1 showed a decrease with mizoribine treatment.

Conclusion. Infiltration of immune cells was associated with hypertension and renal injury in rats infused with aldosterone.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2779

**The Glucocorticoid Receptor in the Vascular Endothelium Is a Critical Mediator of Nitric Oxide Release in LPS-Induced Sepsis** Julie Goodwin,[1] Yan Feng,[1] William C. Sessa.[2] *[1]Pediatrics, Yale University School of Medicine, New Haven, CT; [2]Pharmacology, Yale University School of Medicine, New Haven, CT; [3].*

Introduction: Glucocorticoids are commonly used as therapy for septic shock, yet there is continued debate as to whether they provide benefit and which of their many effects are relevant. Study of the glucocorticoid receptor in the vascular endothelium may provide insight into the mechanism of action of glucocorticoids in this setting.

Methods: Using an in vitro model of tissue-specific glucocorticoid receptor (GR) knockout in the endothelium through targeted siRNA in HUVECs we exposed control and knockout (KO) cells to LPS treatment with and without dexamethasone pre-treatment. Nitric oxide (NO) levels in the conditioned media were assessed by chemiluminescent NO analyzer. In vivo, mice with tissue-specific GR KO in the endothelium were treated with LPS with and without dexamethasone and iNOS expression was assessed in aortic lysates by Western blotting.

Results: Knockdown of GR in HUVECs by siRNA was >95% efficient. At baseline, loss of GR in HUVEC resulted in a 16% increase in total NO levels (p<.001). After treatment with LPS, KO cells showed an increase of 33% in NO levels compared to WT cells (p<.001). When pretreated with dexamethasone, KO cells had 70% higher NO levels compared to control cells that were similarly treated (p<.001). KO animals treated with 12.5 mg/kg LPS had both increased and earlier temporal expression of iNOS compared to control animals. When control animals were pre-treated with dexamethasone 2 mg/kg 2 hours prior to LPS, iNOS expression was significantly decreased as well as delayed. Similar treatment in KO animals resulted in no such improvement.

Conclusions: These studies suggest that:

(1) loss of GR in HUVECs increases basal expression of NO as well as NO expression after LPS alone and with dexamethasone pre-treatment

(2) loss of GR in the endothelium in vivo results in increased iNOS expression in response to LPS

(3) the glucocorticoid receptor in the vascular endothelium is likely a critical mediator of NO release in sepsis

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2780

**High-Fat Diet-Induced Renal Lipotoxicity in Spontaneously Hypertensive Rat Via PPARα-PI3K/Akt/FoxO3a-Oxidative Stress** Hyun Wha Chung, Ji Hee Lim, Min Young Kim, Seok Joon Shin, Bumsoon Choi, Cheol Whee Park. *Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea.*

Phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT)/forkhead box-containing protein O (FoxO) signaling regulates cell metabolism, cell cycle arrest, oxidative stress, and apoptosis. Therefore, we investigated which PI3K/AKT-FoxOs pathways would be involved in high-fat diet-induced intra-renal lipotoxity in Spontaneously Hypertensive Rat (SHR).

SHR and Wistar-Kyoto rat (WKY) at 8 weeks age were treated with either a normal diet (SHR-NF or WKY-NF group) or a high-fat diet (SHR-HF or WKY-HF group) with or

without fenofibrate and tempol for 12 weeks. After the end of 12 week, a high-fat diet in SHR gained more body weight and systolic blood pressure, elevated plasma insulin and triglycerides levels, and induced glucose intolerance. The high-fat diet resulted in increases in intra-renal free fatty acid and triglycerides accumulation related to a decrease in PPAR-α expression in the kidney as well as albuminuria, the expansion of mesangial areas, and inflammation in the kidney. The high-fat diet also significantly down-regulated PI3K-Ser473 pAKT-Ser253 pFoxO3a signaling, resulted in a decrease in MnSOD and an increase in Bim expression leading to the oxidative stress and apoptosis respectively. On the contrary, no such appreciable differences were observed between WKY and WKY-HF. Administration of fenofibrate or tempol in the high-fat diet-induced SHR reversed the elevation of the systolic blood pressure, insulin resistance, and intra-renal lipotoxicity via the induction of intra-renal PPARa and the activation of PI3K-Ser473 pAKT-Ser253 pFoxO3a signaling, which led to decreases in oxidative stress and apoptosis.

Our results suggest that PPAR-α agonists or antioxidants can ameliorate high-fat diet-induced renal lipotoxicity and hypertension by the up-regulation of the PI3K-pAkt-pFoxO3a signaling.

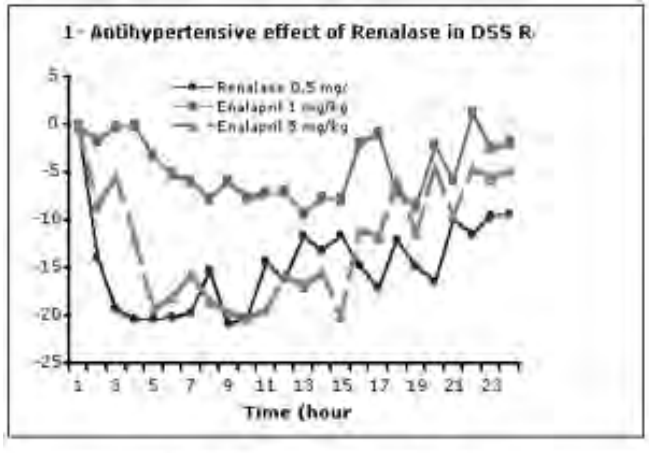
Disclosure of Financial Relationships: nothing to disclose

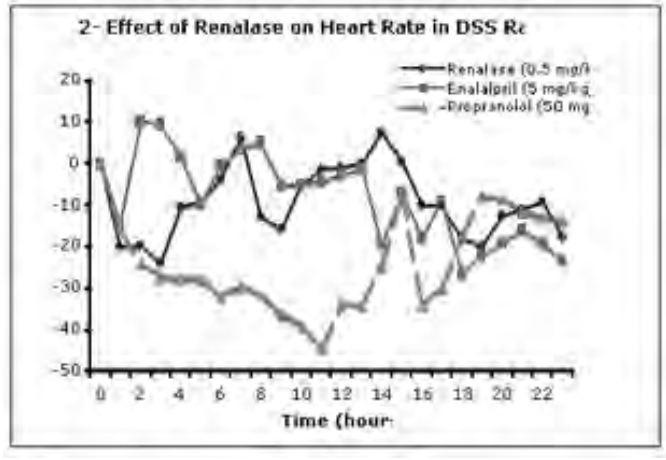
### SA-PO2781

**Antihypertensive Effect of Recombinant Renalase in Dahl Salt Sensitive (DSS) Rats** Gary V. Desir,[1,2] Lieqi Tang,[1,2] Peili Wang,[1,2] Guoyong Li,[1,2] Heino Velazquez.[1,2] *[1]Medicine, Yale School Med, New Haven, CT; [2]Medicine, VACHS, West Haven, CT.*

Renalase is a FAD containing protein that is expressed in kidney, heart, skeletal muscle, and small intestine, and is secreted into blood. Its levels are decreased in CKD, and vitro data indicate it specifically degrades catecholamines. Renalase deficiency in a mouse knockout model causes hypertension in the absence of changes in cardiac and renal function, and plasma aldosterone, suggesting that renalase directly modulates blood pressure. It is not known if recombinant renalase administration modulates ambulatory blood pressure.

Therefore, the effect of recombinant renalase on ambulatory blood pressure was assessed in DSS rats. Radio transmitters were surgically implanted into DSS rats (200 to 300 g body wt) maintained on a low salt diet, and blood pressure values were recorded continuously for the duration of the experiment. The animals were allowed to recover for 1 week until the diurnal variation in blood pressure returned to normal, and then switched to a high salt diet for the remainder of the study period. Test compounds were administered once the animals developed salt dependent hypertension. A single dose of recombinant renalase (0.5 mg/kg subcu) significantly decreased systolic and diastolic pressure for up to 12 hours (n=9, p<0.001).




Renalase lowered blood pressure significantly more than enalapril at a dose of 1 mg/kg (n=9, p<0.002), and comparably to enalapril (5 mg/kg) (n=9), (Fig 1) and propranolol (50 mg/kg) (n=9) (Not shown). Heart rate was reduced to a significantly greater degree by propranolol (n=9, p<0.001), than by either renalase or enalapril (Fig 2).

These data indicate that recombinant renalase has a potent and prolonged anti hypertensive effect in a rat model of salt sensitive hypertension.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2782

**The Effect of Changes in Salt Intake on the Circadian Rhythm of Blood Pressure and Heart Rate in Diabetic Akita Mice** Yan Qin,[1,3] Yuning George Huang,[1] Josephine P. Briggs,[2] Jurgen B. Schnermann.[1] *[1]NIDDK, National Institutes of Health, Bethesda, MD; [2]NCCAM, National Institutes of Health, Bethesda, MD; [3]Nephrology Division, Peking Union Medical College Hospital, Beijing, China.*

Mice heterozygous for an Ins2 mutation (Akita mice) develop severe insulin-dependent diabetes mellitus and represent a useful mouse model to study diabetic nephropathy. We determined long-term blood pressure and heart rate levels, their circadian variability, and their regulation by changes in salt intake. Circadian cardiovascular characteristics of mean blood pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), pulse pressure (PP) and locomotor activity were measured in conscious and unrestrained 12-week old Ins2+/- mice (C57BL/6J background) and age-matched C57BL/6J mice by the telemetry. After baseline studies, the mice were placed on diets with either a high (8.0%) or low (0.04%) NaCl content in a random order.

Akita mice demonstrated robust circadian rhythms of MAP, SBP, DBP, PP, HR and locomotor activity without significant differences in the mesor, amplitude, and acrophase of the rhythms compared with C57BL/6J mice respectively. However, 24-h mean HR was significant lower in Akita mice compared with C57BL/6J mice (527 ± 10 vs 581± 14 bpm, p=0.008), especially in the dark period. Both Akita and C57BL/6J mice adapted to the low NaCl diet without significant changes of the rhythm and the mean values of MAP, DBP, PP, and locomotor activity although blood pressure tended to be lower in Akita reaching significance for SBP. On the high NaCl diet, Akita mice showed a striking further reduction in HR in both light and dark phases (489 ± 12 vs 571 ± 10 bpm, p=0.001), and the appearance of a second heart rate and blood pressure minimum at 2 AM. We conclude that circadian patterns of blood pressure, heart rate and locomotor activity are not dramatically altered in 12-week old Akita mice with established diabetes mellitus. However, reduced heart rates in response to a high salt diet and reduced blood pressures during low NaCl diet suggest an impairment of autonomic regulation and baroreflex function in type 1 diabetes.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2783

**Dissecting the Genetics of Kidney Disease Using *Rf*-3 and *Rf*-4 Congenic Models** Caitlin C. O'Meara, Jozef Lazar, Howard J. Jacob. *Physiology, Medical College of Wisconsin, Milwaukee, WI.*

It is well known that genetic components play a role in the onset and progression of chronic kidney disease (CKD). As CKD is a complex disease, there are multiple genes, as well as gene-gene interactions that contribute to the development of the disease. These genes can cause changes in glomerular capillary pressure, glomerular barrier permeability, or impaired tubular function, all of which can lead to kidney damage. The focus of the present study was to identify the role of two separate renal failure quantitative trait loci (QTL), *Rf*-3 and *Rf*-4, in the development of kidney disease in the FHH rat, and to identify the gene(s) causing the phenotype in these regions using positional cloning and next generation sequencing approaches.

Five renal failure QTL (*Rf*-1 to *Rf*-5) have been identified in a cross between the CKD susceptible FHH, and the CKD resistant ACI rat strains. *Rf*-1 has been shown to synergistically interact with *Rf*-3 and *Rf*-4, so to examine the contribution of the *Rf*-3 and *Rf*-4 QTLs to the development of CKD, we have generated single (*Rf*-1), double (*Rf*-1+4), and triple (*Rf*-1+3+4) congenic strains by introgressing the FHH genome onto the resistant ACI genetic background. Using these models, we have been able to determine that gene(s) in the *Rf*-4 region cause increased glomerular permeability to albumin leading to modest proteinuria at 13 weeks of age. We found *Rf*-3 also results in glomerular permeability to albumin, and these animals present with tubular protein casting as well as mild increases in blood pressure and severe proteinuria at just 9 weeks of age.

From these congenic lines, we have subsequently generated minimal congenic lines targeting the *Rf*-3 and *Rf*-4 regions in order to narrow the number of positional candidate genes. Generating these minimal congenic lines has allowed us to narrow both the *Rf*-3 and *Rf*-4 candidate region to just a few megabases (Mb), and a handful of known and predicted genes. Next generation sequencing technology has allowed us to elucidate gene polymorphisms within the minimal congenic regions that could potentially cause the observed CKD phenotype.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2784**

**Sex Differences in Cardiac Pro-Hypertrophic Signaling in Cardiorenal Interaction Depend on Estrogen Receptor-beta and Determine Adaptive and Maladaptive Phenotypes** Bjoern Hegner,[1] Dennis B. Guergen,[1] Rusan Catar,[1] Lyubov Chaykovska,[1] Angelika Kusch,[1] Friedrich C. Luft,[2] Duska Dragun.[1] *[1]Nephrology and Intensive Care Medicine; [2]ECRC, Charité University Hospital, Berlin, Germany.*

In renal diseases, pre-menopausal women are protected from cardiac pathologies which has been attributed to less severe hypertension. In the deoxycorticosterone acetate (DOCA)/salt model for cardiorenal interaction we found worse myocardial hypertrophy (MH) in normotensive male mice dependent on calcineurin signaling. We hypothesized the contribution of estrogenreceptor beta (ERβ) to observed sex differences.

Blood pressure independent MH was induced in wild type (WT) and ERβ-/- uninephrectomized female and male mice by 6-weeks release DOCA-pellet, 1% NaCl and 250 mg/L hydralazine in drinking water. Telemetric recordings excluded blood pressure differences in all groups. In ERβ-/- animals, increases in heart weight/body weight ratios (HW/BW) upon DOCA/salt were opposite to WT littermates (male: WT 16%, ERβ-/- 10%; female: WT 7%, ERβ-/- 23%). Echocardiography revealed decompensated dilative MH in female ERβ-/- mice whereas male WT mice had concentric MH. This was reflected by different levels of a-type and b-type natriuretic peptide (ANP, BNP) transcripts. Elevation of TGFβ and collagen I characteristic for maladaptive MH was observed in DOCA treated WT males and ERβ-/- females. Signal transduction analyses demonstrated an inversion of protective Akt phosphorylation in ERβ-/- mice with lowest levels in male WT and female ERβ-/- mice. In addition, in ERβ-/- mice Akt activation was not followed by phosphorylation of the downstream target p70S6K which may be crucial for an adaptive response. Expression of Calcineurin Aβ and its positive regulator MCIP-1 was greatly reduced in DOCA treated ERβ-/- compared to WT males and increased in DOCA treated female ERβ-/- mice indicating divergent effects on maladaptive calcineurin signalling in ERβ-/- animals.

ERβ influences MH in the cardiorenal syndrome by modulating protective Akt and maladaptive calcineurin signalling. Our findings may provide a rationale for sex-specific therapeutic strategies to prevent MH in chronic renal diseases.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2785**

**Serum Protein and Nephrin Expression Changes in Living Mouse Glomeruli under the Acute Hypertensive Condition** Zilong Li, Juan Wang, Fengxia Yu, Jun Wang, Hua Zhou, Lining Wang. *Department of Nephrology, First Affiliated Hospital of China Medical University, Shenyang, Liaoning Province, China.*

**ntroduction:** To visualize topographical serum proteins and nephrin changes in living mouse glomerular capillary loops under various hemodynamic conditions by novel "in vivo cryotechnique"[1, 2].

**Materials and Methods: "In vivo cryotechnique" group:** The "in vivo cryotechnique" was performed on left kidneys of anesthetized C57BL/6 mice, as reported before[3]. **Control group:** the kidney tissues were fixed with the conventional immersion and perfusion methods. Their serial sections were stained and observed by light, confocal laser scanning microscopy[4] and immunoelectron microscopy. The animals were evaluated for renal nephrin mRNA and protein expression.

**Results:** By the "in vivo cryotechnique", the distribution of serum proteins: albumin and immunoglobulinG light chain kappa (KL) was disorder, which immunolocalized in apical areas of the foot processes and urinary space, not slit-diaphragm, and the immunoreactivity of them markedly increased, but the nephrin expression seriously decreased under the acute hypertensive condition. The abnormal distribution of serum proteins and nephrin was also found in control group under the normotensive condition, similar to that under the acute hypertensive condition by "in vivo cryotechnique".

**Conclusion:** The redistribution of serum proteins and nephrin was the important factor about proteinuria under the acute hypertensive condition. The "in vivo cryotechnique" should be a reliable tool to observed serum or renal proteins in situ and capture transient images of functioning glomeruli in living mice.

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Disclosure of Financial Relationships: nothing to disclose

**SA-PO2786**

**Peripheral Pulse Pressure as a Surrogate Marker of Central Arterial Stiffness in Patients with Predialysis CKD and Post Kidney Transplantation** Nina Ashraf Kashani, Jennifer R. Joslin, Nihil Chitalia, Debasish Banerjee. *Renal, St Georges Hospital, London, United Kingdom.*

**Introduction**

Measurement of central arterial stiffness, namely pulse wave velocity (PWV), has been proposed to be a better predictor of adverse outcomes than brachial blood pressure (BP). The central BP is proposed to be better related to measures of central artery stiffness- PWV and augmentation index (AI), than brachial BP in patients with essential hypertension. Such relationships have not been examined in predialysis (CKD) and post kidney transplantation.

**Methods and Results**

This study investigated the relationship of brachial Pulse Pressure (PP) with carotid-femoral PWV, radial AI, and central BP in predialysis CKD, post kidney transplantation and normotensive controls; using the SphygmoCor Pulse Wave Velocity system, *AtCor Medical Inc*. Informed consent was obtained from all participants. Data were anonymised and analysed using SPSS V17.

74 CKD patients (42 predialysis CKD, 32 transplant) and 18 healthy normotensive controls participated in the study; mean age = 55 ± 17 yrs. The PWV in the controls lower ($6.9 \pm 1.2$ m/sec$^2$ vs $8.6 \pm 2.3$ m/sec$^2$; $p<0.001$) compared to patients with CKD. However the controls were younger and had lower brachial systolic BP. The central SBP and PP were lower in controls but the AI was not different. Predialysis and transplant patients had no difference in age, eGFR, brachial BPs, central BPs, PWV or AI.

In CKD patients (predialysis and post transplant) brachial PP correlated well with central PP ($r=0.96$ ,$p= <0.001$) The PWV was related to both brachial and central PP ($r=0.35$, $p=0.002$ and $r=0.30$, $p=0.01$). Similarly AI correlated well both brachial and peripheral PP ($r=0.45$, $p<0.001$ and $r=0.63$, $p<0.001$). In controls the PWV and AI did not correlate with brachial PP.

**Conclusion**

In patients with predialysis CKD and post kidney transplantation the measurements of central arterial stiffness correlates well with brachial PP. Such correlations do not exist in normotensive controls. We propose that peripheral PP could be used as a surrogate for measurements of central arterial stiffness for follow up trials in patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2787**

**Effect of Tamsulosin on Bladder Microcirculation in a Rat Ischemia-Reperfusion Model, Evaluated by Pencil Lens Charge-Coupled Device Microscopy System** Hideki Mizuno, Tokunori Yamamoto, Naoto Sassa, Norihisa Matsukawa, Masashi Katoh, Yasushi Yoshino, Kazuo Mizutani, Ryohei Hattori, Momokazu Gotoh. *Urology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.*

**Aim:** To investigate the effect of tamsulosin hydrochloride on bladder microcirculation in a rat ischemia-reperfusion model using a pencil lens charge-coupled device microscopy system (PLCMS).

**Methods:** Changes in blood flow through a submucosal capillary of the rat bladder were measured during bladder filling using the PLCMS. One week after starting infusion of either physiological saline or tamsulosin, blood flow in the bladder was halted by bladder overdistension via an infusion of physiological saline. The bladder was then emptied to be reperfused with blood. Changes in blood flow through a submucosal capillary of the bladder during ischemia and reperfusion were measured using a PLCMS, and the data obtained for the control group and tamsulosin group were compared.

**Results:** As the bladder was distended, the velocity of red blood cell flow in a submucosal capillary of the bladder slowed, and stopped altogether when the bladder became overdistended. In the control group, capillary blood flow improved over time after release from overdistension, but failed to return to the baseline level, demonstrating that reperfusion injury to bladder micro-circulation was caused by bladder overdistension and emptying. In the tamsulosin group, capillary blood flow rapidly returned to baseline after release from overdistension.

**Conclusions:** Using a PLCMS, bladder micro-circulation was able to be visualized and quantitatively assessed by measuring the velocity of blood flow in a submucosal capillary of the bladder. Findings from the present study suggest that tamsulosin hydrochloride exerts a protective effect on blood flow in ischemia-reperfusion injury of the bladder.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2788**

**NLRP3 Inflammasome Activation in the Kidney: Implications for Glomerular Diseases** Julia Lichtnekert, Khader Valli Rupanagudi, Mi Ryu, Onkar Kulkarni, Hans J. Anders. *Medizinische Poliklinik, LMU, Munich, Germany.*

Inflammasomes are cytoplasmic multiprotein complexes that mediate the maturation of the proinflammatory cytokines interleukin-1β (IL-1β), IL-18 and IL-33. The NLRP3 inflammasome is the most extensively studied inflammasome and has large number of stimuli, such as various inflammatory crystals, bacterial toxins, ATP and several endogenous molecules. A few studies reported the role of caspase 1 and IL-1β in kidney injury and fibrosis. Here we studied the role of NLRP3 inflammasome in immune complex glomerulonephritis. In mouse models of lupus nephritis and nephrotoxic serum nephritis we detected elevated levels of IL-1β, NLRP3 and caspase 1 in the kidney. These data support the role of NLRP3 inflammasome in renal inflammatory diseases. The kidney is composed of several intrinsic renal and immune cells, however the source of IL-1β production in glomerulonephritis is obscure. Whether renal cells contain the NLRP3 inflammasome machinery is also still unknown. Here we investigated the functional role of NLRP3 inflammasome in intrinsic renal cells such as mesangial cells, podocytes, glomerular endothelial cells, tubular epithelial cells and tubular fibroblasts. We found that various NLRP3 stimuli did not lead to caspase 1 activation and production of IL-1β in these cells. However, in renal dendritic cells (CD11c+) these stimuli induced caspase 1 activation and production of IL-1β, emphasizing the important role of renal dendritic cells in innate immune response in the kidney. Together, these findings provide functional data for NLRP3 inflammasome in renal cells and draw attention to the presumable role of NLRP3 inflammasome activation in renal inflammatory diseases.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2789**

**Antigens in Glomerular Mesangium Recruit Activated T Cells and Induce Glomerulonephritis** Yogesh M. Scindia. *CIIR-Div of Nephrology, Dept of Medicine, University of Virginia, Charlottesville, VA.*

Purpose: We have previously described anti-a8 integrin immunoliposomes (a8ILs) for targeted delivery specifically to the glomerular mesangium. Here, we have used the a8ILs to establish an antigen specific model of glomerulonephritis (GN). This model will be used to investigate the pathogenic role of antigen-specific T cells in GN.

Methods: A rabbit polyclonal antibody to a8 integrin was conjugated to the surface of liposomes to generate a8ILs. The a8ILs were loaded with ovalbumin (ova-IL) and injected i.v. into C57BL/6 mice. This was followed by injection of activated ovalbumin-reactive CD4+ T cells obtained from OT2 mice. OT2 mice express a transgenic T cell receptor specific to ovalbumin. The recipient mice were sacrificed 3, 8 and 14 days later. Trafficking of OT2 T cells into the renal lymph nodes and kidney was studied by flow cytometry. Kidneys were evaluated for pathology, and infiltrating cells studied by immunostaining.

Results: Ovalbumin was rapidly deposited in the mesangium after ova-IL injection. Activated CD4+OT2 T cells accumulated preferentially in the renal lymph nodes on day 3 after cell transfer. By day 8, OT2 cells were detected in the kidney progressively increasing to day 14. Significantly, this was associated with an increase of non-OT2 CD4+ T (endogenous) cells. Infiltration of T cells, macrophages and dendritic cells was seen in and around glomeruli.

Conclusions: We have established an antigen-specific model of GN using ovalbumin as a surrogate mesangial antigen. Our study shows that CD4+ T cells reactive to mesangial antigens traffic to the kidney and induce intra- and peri-glomerular inflammation. These antigen-specific T cells recruit macrophages, dendritic cells and other T cells to induce GN. In lupus nephritis, immune complex deposition leading to macrophage infiltration into the renal interstitium has been identified as the critical indicator of progressive renal inflammation. Our studies suggest that this macrophage recruitment may be driven by glomerular antigen-specific T cell infiltration

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2790**

**Activin Receptor-Like Kinase Inhibitor, SB431542, Attenuates Experimental Mesangial Proliferative Glomerulonephritis** Yu-Hsiang Chou, Yung-Ming Chen, Shuei-Liong Lin, Wen-Chih Chiang, Tun-Jun Tsai. *National Taiwan University Hospital, Renal Division, Department of Internal Medicine, Taipei, Taiwan.*

Transforming growth factor-b (TGF-b) isoforms are considered the most important regulators in renal fibrogenesis. Here in this study, we investigated an inhibitor of type I activin receptor-like kinase (ALK) receptors , SB431542, for its therapeutic efficacy in rat mesangial cells (MC) in culture and anti-Thy1 glomerulonephritis *in vivo*. Our results showed that SB431542 dose-dependently inhibited TGF-b1-induced Smad2 activation, and target gene expression *in vitro*. It did not exert any effect on platelet-derived growth factor-stimulated mitogenesis or TGF-b1-induced extracellular signal-regulated kinase activation. In our anti-Thy1 model where glomerular mRNAs for TGF-b1, TGF-b3 and activin A were maximally upregulated on day 3, SB431542 (20 mg/kg/day) given intraperitoneally reduced the mRNA induction of these genes. Further, treatment with SB431542 (20 mg/kg/day) for 5 days reduced glomerular Smad2 activation in association with reduction of proteinuria, glomerular sclerosis, activated MC and macrophages, and glomerular mRNA and protein expression of PAI-1 and collagen I(a1). SB431542 did not affect the expression of Smad7, nor activate the NF-kB pathway in the nephritc animals. In sum, our data indicate that downregulation of Smad2 activation by selective inhibition of TGF-b/activin signaling attenuates anti-Thy1 disease and may have a therapeutic value in acute phase or relapses of mesangial proliferative glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2791**

**Tamm-Horsfall Protein Acts as a Cofactor for Factor I-Mediated Complement C3b Degradation** Diana Rhodes. *Division of Anatomy, Pacific Northwest University of Health Sciences, Yakima, WA.*

Tamm-Horsfall protein (THP), an abundant urinary protein of renal origin, previously was shown to bind C1q of the classical complement pathway and to inhibit activation of this pathway. THP also binds complement factor H (CFH) of the alternate complement pathway. The initial purpose of the present study was to determine if THP's binding to CFH altered the normal ability of CFH to act as a cofactor in complement factor I (CFI)-mediated complement C3b degradation, C3b being a key molecule at the cross-roads of all three complement pathways. *In vitro* C3b degradation assays were performed with purified C3b, CFI, CFH, and THP. Degradation of the alpha chain of C3b into its two fragments was monitored using silver-stained 8% SDS-PAGE gels. Control experiments in these assays suggested that THP, without added CFH, could act as a cofactor in C3b degradation. Having detected this unexpected result, the study purpose shifted to exploring further the potential cofactor activity of THP. The cofactor activity of THP did not appear to be due to contamination of the THP with CFH since CFH was not detected in THP samples using Western blots evaluated by a sensitive infrared imaging system. Furthermore, while cofactor activity was significantly decreased in CFH that was heated to 56°C or 100°C prior to the assays, cofactor activity in THP samples was unaffected by this heating. The ability of THP to act as a cofactor in CFI-mediated degradation of C3b appears to be an inherent property of THP since 10 different THP samples, from a wide range of individuals, all possessed similar cofactor activities. This activity most likely resides in the protein portion of THP since the cofactor activity was present in THP from which most of the carbohydrate moieties had been removed. In conclusion, THP appears to participate directly in complement regulation by its ability to act as a cofactor for CFI-mediated C3b degradation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2792**

**Characterization of the Glomerular Endothelial Binding Sites for Full-Length Complement Factor H (FH) and for Domains FH19-20 and FH5-7** Angelique Rops,[1] Markus J. Lehtinen,[2] Mohamed R. Daha,[3] Marinka Bakker,[1] Karita Haapasalo,[2] Richard J. Smith,[4] Jo H. M. Berden,[1] T. Sakari Jokiranta,[2] Johan Van der Vlag.[1] *[1]Dept. of Nephrology, Radboud University Nijmegen Medical Centre, Netherlands; [2]Dept. of Bacteriology and Immunology, University of Helsinki, Finland; [3]Dept. of Nephrology, Leiden University Medical Centre, Netherlands; [4]Dept. of Internal Medicine and Otolaryngology, University of Iowa.*

Complement factor H (FH) is the major regulator of the alternative complement pathway and binds via its domains FH19-20 to endothelial cells. Abnormalities in FH are associated with the renal diseases membranoproliferative glomerulonephritis type II (Dense Deposit Disease) and atypical hemolytic uremic syndrome. Furthermore, the Y402H polymorphism within domain 7 of FH is associated with age-related macular degeneration and DDD. Domains 7, 8-15 and 19-20 in FH can bind glycosaminoglycans/heparin. FH displays anti-inflammatory activities and endothelial heparan sulfate (HS) plays a crucial role during inflammation. Here, we evaluated the role of glomerular endothelial HS in binding of full-length FH and FH domains.

Binding of full-length FH and the domains FH19-20, FH5-7(402)Y, FH5-7(402)H to (activated) mouse glomerular endothelial cells (mGEnC-1) was evaluated by enzyme-linked immunosorbent assay (ELISA) and flow cytometry. To determine the involvement of HS in this binding, HS on mGEnC-1 was removed by treating with heparinase I/II/III, or competition ELISAs were performed with heparin(oids) or HS.

Full-length FH and the FH domains exhibited a dose-dependent binding to mGEnC-1. Activation of mGEnC-1 with TNF-$\alpha$ resulted in a significant increased binding of FH and FH domains. Heparin and HS inhibited dose-dependently the binding of FH, FH19-20 and FH5-7(402)Y/H to mGEnC-1, whereas N-, 2-O-, and 6-O-desulfated heparin showed differential inhibitory effects. Pre-incubation of mGEnC-1 with heparinase I/II/III inhibited the binding of FH and FH5-7(402)Y/H 2-fold, while the binding of FH19-20 was inhibited only 1.2-fold.

Conclusion: Binding of FH and domains FH19-20 and FH5-7 to (activated) glomerular endothelial cells is differentially mediated by HS.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2793**

**Protective Role of Mast Cells in Autoimmune Anti-Myeloperoxidase Induced Glomerulonephritis** Poh-Yi Gan, Shaun Andrew Summers, A. Richard Kitching, Stephen R. Holdsworth. *Centre for Inflammatory Diseases, Department of Medicine, Monash Medical Centre, Monash University, Melbourne, Victoria, Australia.*

Mast cells (MC) are traditionally viewed as barrier defense sentinels and first responders required in innate immunity, especially in the context of asthma and allergy. It is now clear that MC are able to produce and release a variety of inflammatory mediators that are important in the activation of both effector and regulatory cells. However little is known about the role of MC in immune tolerance and autoimmunity. To study the role of MC in nephritogenic autoimmunity, we compared the outcomes of an experimental model of myeloperoxidase (MPO)ANCA associated and segmental necrotizing GN between MC deficient Kitwsh/Kitwsh (Wsh) and WT mice.

MC deficient and WT mice were immunized with murine MPO in Complete Freund's adjuvant and GN was triggered by glomerular neutrophil recruitment and MPO deposition initiated by low dose anti-GBM antibody. Four days later, Wsh mice had developed enhanced T cell autoimmunity to MPO. Lymphocytes isolated from the lymph node (LN) draining the MPO immunization site in Wsh mice showed enhanced MPO recall proliferation (9773±2342counts/min vs 4767±386counts/min, p=0.02) and IFN-$\gamma$ production (31.7±6.0 vs 6.9±2.1ng/ml p=0.05). Dermal MPO induced DTH was also enhanced in Wsh mice (0.2±0.1 vs 0.4±0.1$\Delta$mm, p=0.04). MC accumulation in WT draining LNs was quantified after 6 days (non-immunized 5.0±1.0 vs immunized 24.6±4.8c/LN). By immunostaining prominent interaction between MC and T regs was apparent. In Wsh mice the number of LN CD4+FoxP3+ T regs was reduced (17.1 0.3% vs 15.9 0.4%, p=0.02). Increased MPO autoimmunity in Wsh mice was associated with increased severity of GN demonstrated by proteinuria (7.41 1.9 vs 2.5 0.6mg/24hr, p=0.01) and increased glomerular fibrinoid necrosis (27 vs 16%, p=0.02). Glomerular leukocyte effectors were also increased; T cells (0.6 0.1 vs 0.2 0.1c/gcs, p=0.01) and macrophages (1.7 0.2 vs 0.8 0.2c/gcs, p=0.01).

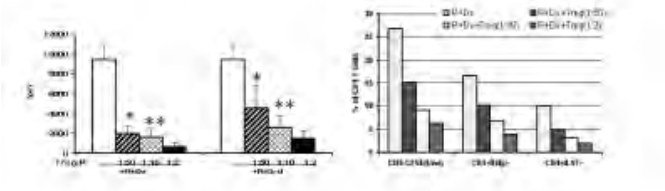
This study demonstrates that MC play a protective role in the development of autoimmunity to MPO and suggests that MC interact with T regs to facilitate peripheral tolerance.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2794**

**MTOR Inhibitors Generated Human Tregs Have Suppressive Activity, but Their Generation Is Impaired by Inflammatory Cytokines** Lorenzo G. Gallon,[1] Anton I. Skaro,[2] Giovanna La Monica,[1] Joseph Ross Leventhal,[1] Luting Xu.[1] *[1]Medicine/Nephrology, Northwestern University Medical School, Chicago; [2]Transplant, Northwestern University Medical School.*

Regulatory T cells (Tregs) play a major role in facilitating regulatory immune-mechanisms for the development of a pro-tolerant state towards the transplant. MTOR inhibitors (Sirolimus, SRL) can facilitate the expansion of Tregs. Little is known, in human, about the functionality of SRL-induced Tregs on allospecific T-cell subset responses. Methods: Alloreactive CD4+ T cells were first generated in MLR culture with responding CD4+CD45RA+ naive T cells and allogeneic CD14+ monocytes. Alloreactive CD4+ T cells, from the primary MLR, were enriched and re-stimulated by co-culturing with autologous CD14+ monocytes in the presence of antigenic stimulation of anti-CD3 and SRL to increase the frequency of Treg cells. SRL-induced Tregs were then tested for their capacity to suppress T cell responses by adding Tregs at increasing doses (1:50, 1:10, 1:2) in a MLR. Thymidine incorporation and CFSE dilution technique were used as a read out methodologies. Results: FOXP3 expression (Treg) was markedly increased when SRL was added to the culture. When inflammatory cytokines (IL-6, IL-1 β) were added with SRL to the cell culture, there was a marked decrease of the generation of SRL-induced Tregs (data not shown). SRL-derived Tregs showed alloantigen specific suppression of alloreactive CD4+CD25- T cells and of subpopulation of Th1 and Th17 measured, respectively, by thymidine incorporation and CFSE labeling.



SRL-derived CD4+CD25high Tregs showed alloantigen-specific suppression of proliferation of allo-reactive CD4+CD25- T cells and the subpopulations of Th1 and Th17

Conclusions: MTOR inhibitor can expands human Tregs and these cells have suppressive capacity on Th1 and Th17 alloresponses. Inflammatory conditions can block the generation SRL-induced Tregs. These findings have important clinical implications for patients receiving MTOR inibitors as part of their main immunosuppressive regimen.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2795**

**The Class II Phosphatidylinositol 3 Kinase C2 Beta Is Required for the Activation of the K+ Channel KCa3.1 and CD4 T Cells** Shekhar Srivastava,[1] Lie Di,[1] Olga Zhdanova,[1] Jon Backer,[3] Heike Wulff,[2] Edward Y. Skolnik.[1] *[1]Skirball Institute, Department of Internal Medicine, New York University Langone Medical Center, New York, NY; [2]Department of Pharmacology, University of California, Davis, Davis, CA; [3]Department of Molecular Pharmacology, Albert Einstein College of Medicine, New York, NY.*

The Ca2+-activated K+ channel KCa3.1 is required for Ca2+ influx and the subsequent activation of T cells. We previously showed that Nucleoside Diphosphate Kinase Beta (NDPK-B), a mammalian histidine kinase, directly phosphorylates and activates KCa3.1 and is required for the activation of human CD4 T lymphocytes. We now show that the class II phosphatidylinositol 3 kinase-C2beta (PI3K-C2β) is activated by the T cell receptor (TCR) and functions upstream of NDPK-B to activate KCa3.1 channel activity. Decreased expression of PI3K-C2β by siRNA in human CD4 T cells resulted in inhibition of KCa3.1 channel activity. The inhibition was due to decreased PI(3)P because dialyzing PI3K-C2β siRNA treated T cells with PI(3)P rescued KCa3.1 channel activity. Moreover, overexpression of PI3K-C2β in KCa3.1-transfected Jurkat T cells led to increased TCR-stimulated activation of KCa3.1 and Ca2+ influx, whereas silencing of PI3K-C2β inhibited both responses. Using total internal reflection fluorescence microscopy (TIRF) and planar lipid bilayers, we found that PI3K-C2β co-localized with Zap70 and the TCR in peripheral microclusters in the immunological synapse. This is the first demonstration that a class II PI3K plays a critical role in T cell activation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2796**

**The K+ Channels KCa3.1 and Kv1.3 as Therapeutic Targets To Treat Autoimmune Diseases** Lie Di,[1] Shekhar Srivastava,[1] Olga Zhdanova,[1] Heike Wulff,[2] Edward Y. Skolnik.[1] *[1]Internal Medicine, Skirball Institute, New York University Langone Medical Center, New York, NY; [2]Department of Pharmacology, University of California, Davis, Davis, CA.*

The calcium activated K+ channel KCa3.1, and the voltage activated K+ channel, Kv1.3 play critical roles in T lymphocyte Ca2+ signaling by helping to maintain a negative membrane potential which provides an electrochemical gradient to drive Ca2+ influx. To assess the role of KCa3.1 channels in lymphocyte activation in vivo, we studied T cell function in KCa3.1-/- mice. CD4 T helper (Th0) cells isolated from KCa3.1-/- mice lacked KCa3.1 channel activity, which resulted in decreased TCR-stimulated Ca2+ influx and interleukin 2 (IL-2) production. While loss of KCa3.1 did not interfere with CD4 T cell differentiation, both Ca2+ influx and cytokine production were markedly impaired in KCa3.1-/- Th1 and Th2 CD4 T cells, while T-regulatory and Th17 function were normal. In contrast, Th17 activation was dependent upon Kv1.3 for activation. We found that KCa3.1-/- mice were protected from developing severe colitis in two mouse models of inflammatory bowel disease (IBD) that are dependent upon CD4 Th1 cells, which were induced by (i) the adoptive transfer of mouse naïve CD4 T cells into rag2-/- recipients and (ii) trinitobencene sulfonic acid (TNBS). Thus, these findings suggest that KCa3.1 may be a novel therapeutic target to treat autoimmune diseases mediated by Th1 cells, while Kv1.3 may provide a novel therapeutic target to treat Th17-mediated autoimmune disease. Pharmacologic inhibitors of KCa3.1 and Kv1.3 have already been shown to be safe in humans. Thus, extending these observations to various forms of experimental glomerulonephritis may identify new opportunities to treat kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2797**

**Attenuation of Experimental Crescentic Glomerulonephritis in IL-17 Deficient and STAT3β-Deficient Mice** Seung Hee Yang,[1] Un Sil Jeon,[1, 2] Jung Pyo Lee,[3] Ran-Hui Cha,[1, 3] Yon Su Kim.[1, 3] *[1]Seoul National University Kidney Research Institute, Seoul, Republic of Korea; [2]Internal Medicine, Korea University Guro Hospital, Seoul, Republic of Korea; [3]Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.*

T cells play a major role in the pathogenesis of crescent glomerulonephritis (GN) which is the most severe form of GN and progresses to end-stage renal disease unless treated adequately. Th17 cells have been reported to contribute to renal injury in crescentic GN. In the present study, we examined whether modulation of Th17 response could reduce the renal injury in experimental cresentic GN. IL-17 deficient and STAT3β deficient mice were used because IL-17 is the major cytokine of Th17 cells and STAT3 is the essential transcriptional factor of Th17 cell differentiation.

Experimental crescentic GN was induced by injection of anti-glomerular basement membrane (GBM) antibodies in Balb/c, C57BL/6, IL-17 deficient Balb/c, and STAT3β deficient C57BL/6 mice. After 7 days of disease induction, elevation of BUN and urine protein/creatinine ratio reduced significantly in IL-17 deficient (BUN 55 ± 22.7 vs 154 ± 17.5mg/dl, $p<0.01$; U prot/cr 114 ± 10. 6 vs 143 ± 16.6 mg/mg, $p<0.05$) and STAT3β deficient mice (BUN 116 ± 25.0 vs 156 ± 8.0 mg/dl, $p<0.05$; U prot/cr 53 ± 6.8 vs 99 ± 6.8, $p<0.05$) compared to those of wild type mice. In addition, glomerular injury and crescent formation by anti-GBM antibody reduced in IL-17 deficient and STAT3β deficient mice compared to those of wild type mice. Renal mRNA expression of IL-1β, TGF-β, MCP-1, IL-12p19 and STAT3 increased in wild type mice, but these changes were attenuated in IL-17 and STAT3β deficient mice. Treatment of CD3 in co-cultured mesanigal cells and NKT cells induced mRNA expression of IL-17 receptor and secretion of IL-17 and IL-12p70 as well as inflammatory cytokines like IL-2, TNFa and IL-6. Blocking of IL-17 receptor reduced these inflammatory reactions.

All together, the data suggest that Th17 cells play an important role in the pathogenesis of experimental cresecentic GN. IL-17 and STAT3 might be a feasible target in protecting renal injury in the disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2798**

**C4d Deposition in Thrombotic Microangiopathy Following Allogeneic Stem Cell Transplantation: Evidence for Humoral Graft Versus Host Disease?** Jamie S. Chua,[1] Danielle Cohen,[1] Khadro Jama,[1] Stefan P. Berger,[2] Johan W. De Fijter,[3] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Pathology, Leiden University Medical Center, Leiden, Netherlands; [2]Nephrology, Haga Teaching Hospital, The Hague, Netherlands; [3]Nephrology, Leiden University Medical Center, Leiden, Netherlands.*

Thrombotic microangiopathy (TMA) is one of the most severe complications of allogeneic stem cell transplantation. Mortality is high, around 50% and the pathogenesis is poorly understood. We hypothesize that TMA in this clinical setting could be the result of humoral activity in graft versus host disease (GVHD), causing classical complement activation, endothelial damage and finally, thrombosis.

Deposition patterns of C4d in renal tissue of 5 patients who died of TMA following allogeneic stem cell transplantation were investigated by staining with a polyclonal anti-C4d antibody. A properdin stain was used to determine presence of alternative pathway activity. Five patients who died of rapidly progressive TMA of various causes served as controls. All patients had end stage renal disease at time of death.

All patients with TMA following allogeneic stem cell transplantation had extensive glomerular and arteriolar deposition of C4d. Arteriolar positivity was associated with presence of arteriolar microthrombi in all 5 cases. In control patients C4d staining was negative. Properdin staining was negative in all cases.

Glomerular and arteriolar C4d deposition was present in all patients with TMA following allogeneic stem cell transplantation, and this pattern was not observed in any of the control patients. Presence of C4d may be a witness of an antibody mediated component of GVHD, causing severe endothelial damage which leads to TMA. As a diagnostical tool, C4d staining on a renal biopsy in patients with TMA associated with stem cell transplantation,

may be helpful in distinguishing those patients with a humoral component of GVHD. Additionally, this finding may have therapeutic implications in view of possible treatment with anti-CD20 or anti-C5 therapy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2799**

**Mesenchymal Stromal Cells Suppress Primary T-Helper 17 (Th17) Differentiation as Well as Re-Activation of Th17 Cells from Injured Kidney**
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**Background and Aims:** Interleukin 17-producing T-helper cells (Th17 cells) are implicated in AKI, glomerulonephritis and transplant rejection. Mesenchymal stromal cells (MSC) are immunosuppressive with potential to treat inflammatory and immune-mediated disease. The effects of MSC on Th17 differentiation and activation *in vitro* and in mouse unilateral ureteral obstruction (UUO) were studied. **Methods:** Th17 differentiation was induced by activation of CD4+ T-cells in the presence of IL-6, TGFβ1, anti-IFNγ and anti-IL-4. Bone marrow-derived MSC were added in varying ratios. MSC/Th17 co-cultures were analyzed by ELISA and multi-color flow cytometry. UUO was carried out in adult mice for 72 hours. Cell suspensions were prepared from obstructed and control kidneys, enriched for CD45+ cells by magnetic separation then stimulated *in vitro* with anti-CD3 followed by IL-17 ELISA of culture supernatants. **Results:** MSC inhibited primary Th17 induction in a dose-dependent manner as evidenced by reduced IL-17 intra-cellular staining and decreased IL-17 secretion upon re-stimulation in the absence of MSC. Conditioned medium from MSC/Th17 co-cultures strongly inhibited fresh Th17 cultures - an effect that was reversed by indomethacin or a selective COX-II inhibitor and reproduced by addition of PGE2. T-cell/MSC contact under Th17 skewing conditions was associated with production by MSC of high concentrations of PGE2. MSC suppressed anti-CD3-induced IL-17 secretion by effector-memory Th17 cells from acutely obstructed kidneys. MSC inhibition of intra-renal Th17 cells was also reversed by COX inhibition. **Conclusions:** MSC potently suppress primary differentiation of Th17 cells as well as the reactivation of Th17 cells from obstructed kidney. The effect is mediated by contact-dependent production by MSC of a prostanoid (likely PGE2) and has potential therapeutic applications for renal diseases involving Th17 activation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2800**

**Cross-Species Transcriptional Network Analysis Defines Shared Inflammatory Responses in Murine and Human Lupus Nephritis**
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Lupus nephritis (LN) is a significant cause of morbidity and mortality in lupus. Several immunomodulators effective in murine LN models failed human trials, raising concerns about the relevance of these models in human LN. We used a transcriptional network comparison to define similarities and differences between LN murine models and human LN.

Affymetrix based expression profiles from 3 LN mouse models (NZB/W, NZM2410, NZW/BXSB; n=40) and human renal biopsies (n=47) were analyzed with Bibliosphere software. Transcriptional networks were compared using the *T*ool for *A*pproximate *L*arg*E* graph matching (TALE) to define cross-species conserved functional interactions. Pathway analysis of 17 nodes shared by human and 3 murine models highlighted macrophage activation pathways ($p<0.01$).

To confirm that the signatures were derived from resident or infiltrating cells, gene expression profiles of macrophages isolated from NZB/W kidneys were compared with murine and human LN kidney profiles. 406 transcripts were concordantly regulated between NZB/W kidney isolated macrophages and whole kidneys. Of those, 132 transcripts were regulated in the human LN kidneys, consistent with an mRNA macrophage-derived expression. Network analysis of the shared murine-human renal macrophage signature defined Anxa2 as one of the major hubs. MESH filter "macrophage activation" on the network highlighted Lyn and CD44.

Treatment response of tissue resident macrophages was assessed in NZB/W mice in which complete remission was induced with cyclophosphamide/CTLA4Ig/anti-CD154. Lyn, Anxa2, and CD44 mRNA levels were significantly repressed in kidney resident macrophages by triple therapy.

Our transcriptional analysis of renal tissue and kidney derived macrophages defined key regulators of inflammation shared between murine and human LN. Key nodes in the murine macrophage inflammatory response were affected by triple therapy, representing potential therapeutic targets in humans.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2801**

**Intrinsic APRIL and BLyS Production in Human Lupus Nephritis (LN)**
Matthias A. Neusser,[1] Maja Lindenmeyer,[1] Ilka Edenhofer,[1] Stefanie Gaiser,[1] Matthias Kretzler,[2] Stephan Segerer,[1] Clemens D. Cohen.[1] *[1]Nephrology, University of Zurich, Zurich, Switzerland; [2]Nephrology, University of Michigan, Ann Arbor.*

Introduction: APRIL and BLyS, two B cell survival factors, play an important role in B cell maturation and activation. Large clinical trials for systemic lupus erythematosus are underway investigating interference with B cell function by targeting these molecules. However, the local expression of APRIL and BLyS has not been studied in detail in kidneys with LN.

Methods: We analyzed the mRNA expression of APRIL, BLyS and the corresponding receptors BCMA, TACI and BAFF-R in microdissected human biopsies with proliferative LN (n=25) and compared it with pretransplant biopsies of living donors (n=9). To identify the cells expressing the respective proteins in the kidney, APRIL, BLyS and BAFF-R were also studied by immunohistochemistry in renal biopsies with proliferative (n=21) or membranous (n=8) LN.

Results: APRIL and BLyS mRNA levels were increased in glomeruli of patients with proliferative LN (12 ± 22- [$p<0.05$] and 30 ± 47-fold [$p<0.01$], respectively). Tubulointerstitial expression of APRIL, BLyS, BCMA, and TACI was also elevated (13 ± 25- [$p<0.01$], 58 ± 94-[$p<0.01$], 136 ± 332-[$p<0.01$], and 109 ± 305–fold [$p<0.05$], respectively) and BAFF-R showed low basal expression. APRIL stained prominently in glomeruli with proliferative, but not membranous LN and the pattern was consistent with mesangial cells. An accumulation of CD68 positive cells was present in glomeruli in association with APRIL expression. APRIL, BLyS and BAFF-R could also be localized to interstitial inflammatory cells.

Conclusion: This is the first study to provide detailed data on local expression of APRIL and BLyS in glomeruli and tubulointerstitium of human proliferative LN. Future studies should aim to clarify intrarenal effects of APRIL and BLyS inhibition.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2802**

**Treatment with Anti-IgA Fc Receptor (FcαRI) Antibody Prevents Progression of Lupus Nephritis in Pristane Mice**
Chunbei Liu,[1] Yutaka Kanamaru,[1] Tomonari Watanabe,[1] Nobuhiro Tada,[2] Yusuke Suzuki,[1] Satoshi Horikoshi,[1] Yasuhiko Tomino.[1] *[1]Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan; [2]Research Institute for Diseases of Old Age, Juntendo University Faculty of Medicine, Tokyo, Japan.*

The Fc receptor for IgA (FcαRI), with an inhibitory immunoreceptor tyrosine-based activatory motif (iITAM) in the cytoplasmic domain, down-regulates humoral immune responses and modulates the risk of autoimmunity in animal models. The model of progressive lupus nephritis in autoimmune disease mice induced by pristane was used to evaluate whether FcαRI targeting late in the disease course can affect progression to renal failure. FcαRI/FcRγ transgenic mice (Tg) were treated with intraperitoneal (IP) injections of either vehicle or MIP-8a, anti-FcαRI Fab, three times a week from week 8 to 36 of age. MIP-8a improved proteinuria levels, markers of glomerular injury, deposition of immunoglobulins, and serum cytokine levels, and reduced the amount of F4/80 macrophages, CD3 lymphocytes in the interstitium and glomeruli. MIP-8a affected serum anti-nuclear antibody (ANA) in the pristane mice. This was associated with reduced serum titers of immunoglobulin IgG2a but not IgG1, IgG2b, and IgG3. Furthermore, MIP-8a reduced the extent of interstitial fibrosis as evaluated by interstitial masson-trichrome staining and type I collagen deposits. This is the first evidence that, in advanced lupus nephritis, FcαRI targeting through iITAM can halt disease progression and improve renal function by selective inhibition of leukocyte recruitment and renal inflammation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2803**

**Th17 Responses in the Experimental Lupus Nephritis**
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Th17 cells, secreting potent proinflammatory cytokine IL-17, are emerging as a major player in several autoimmune diseases like multiple sclerosis, previously known as a Th1 mediated disease. It has been suggested that Th17 cells are also involved in the pathogenesis of lupus nephritis (LN). In the present study, we examined the Th17 response in the experimental LN mice.

The experimental LN was induced by injection of lymphocytes from (C57BL/6xDBA/2J) F1 hybrids into wild C57BL/6 mice. The transferred donor lymphocytes were observed in the recipient glomeruli. Induction of LN was confirmed by increased BUN and serum creatinine levels, the development of proteinuria, and renal pathology (mesangial proliferation and glomerular crescents formation accompanied with CD3 cell infiltration in mice kidney). CD69, activated T cell marker, and intracellular IL-17 expression increased in the spleen of LN mice. Inflammatory cytokine/chemokine MCP-1, IL-6, INF-γ mRNA increased in LN mice spleen. Th17 related cytokines, IL-17, IL-23 and IL-27 mRNA levels also increased in the spleen. In the LN kidney, IL-17 receptor (IL-17R), IL-27 and STAT3

mRNA all increased. The expression of intracellular IL-17 protein also increased in the LN mice kidney. In addition, immunohistochemical study showed that IL-17 and STAT3, the essential transcriptional factor of Th17 cell differentiation, were observed only in the disease-induced mice kidney. Treatment of CD3 in co-cultured mesanigal cells and NKT cells induced the secretion of inflammatory cytokines like IL-2, TNFα and IL-6. The expression of IL-17R mRNA and secretion of IL-17 and IL-12p70 also increased in the co-cultured cells. Blocking of IL-17R reduced these inflammatory reactions.

Taken all together, Th17 response was activated in the experimental LN. Th17 related molecules like IL-17, IL-17R, STAT3 might be the novel therapeutic target of LN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2804**

**Comparison of Mycophenolate Mofetil and Rapamycin on Inflammatory and Fibrotic Mediators in NZB/W Mice with Active Nephritis** Daniel Tak Mao Chan, Chen Zhu Zhang, Mel Chau, Susan Yung. *Department of Medicine, The University of Hong Kong, Hong Kong.*

Rapamycin might have a role in the treatment of lupus nephritis because of its immunosuppressive and anti-proliferative effect. This study compared the effect of rapamycin and mycophenolate mofetil (MMF) on inflammatory and fibrotic mediators in a murine lupus nephritis model.

Female NZB/W mice with established nephritis and proteinuria >3g/L were randomized to receive treatment with vehicle alone (control), MMF (100mg/kg/d), or rapamycin (3mg/kg/d) for 2, 6 and 12 weeks (n=6 for all time points in each group), and clinical parameters and renal histology assessed.

Albumin-to-creatinine ratio and circulating anti-DNA antibodies increased progressively in control mice, and these abnormalities were ameliorated in both MMF and rapamycin treated groups ($P$<0.01 for both compared to control mice after 12 weeks). Mice treated with MMF or rapamycin demonstrated reduced glomerular expansion (glomerular tuft area: 4252.15±324.15, 2645.76±564.21 and 2648.21±468.32μm$^2$ for control, MMF and rapamycin respectively, $P$<0.01 for control vs MMF or rapamycin after 12 weeks treatment), and decreased interstitial fibrosis as well as tubular atrophy compared to control mice. Both MMF and rapamycin significantly reduced glomerular IgG and C3 deposition and this was associated with the abrogation of intra-glomerular expression of CD4, CD8, CD19 and MAC-1. Renal expression of IL-6, TGF-β1, fibronectin and collagen type I was reduced to near normal levels in MMF treated mice, whilst tubular expression of the aforementioned fibrotic markers was still significant in mice treated with rapamycin for 12 weeks.

These results suggest that while both MMF and rapamycin ameliorate disease manifestations in lupus nephritis, rapamycin might be less effective in suppressing tubulointerstitial expression of fibrosis markers compared with MMF.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2805**

**Effect of Anti-IL6 Monoclonal Antibody Therapy in the Treatment of Established Experimental Autoimmune Glomerulonephritis in the CD1 Mouse** John Reynolds,[1] Jill Wechsler,[1] Diane Marshall,[2] Stevan G. Shaw,[2] Richard E. Gelinas,[3] H. Terence Cook,[1] Charles D. Pusey.[1] *[1]Renal Section, Division of Medicine, Imperial College London, United Kingdom; [2]UCB Celltech, Slough, United Kingdom; [3]Battelle Seattle Research Center, Seattle.*

Experimental autoimmune glomerulonephritis (EAG) can be induced in CD1 mice by immunization with the recombinant NC1 domain of the alpha 3 chain of type IV collagen (α3(IV)NC1). In this murine model of EAG, CD1 mice develop circulating and deposited anti-glomerular basement membrane (GBM) antibodies, and focal necrotizing glomerulonephritis with crescent formation by week 12 after immunization. Previous unpublished studies from our group have demonstrated that anti-IL6 monoclonal antibody (mAb) is effective in the prevention of EAG in the CD1 mouse. The aim of this study was therefore to examine the role of anti-IL6 mAb therapy in the treatment of established disease. Groups of CD1 mice with EAG (n=10) were given a weekly subcutaneous injection of anti-IL6 mAb (54E07), or an irrelevant mAb (101.4, positive control) at a dose of 30mg/kg, starting at week 6 after immunization, when disease was established. Animals given the anti-IL6 mAb showed a significant reduction in the urinary albumin/creatinine ratio (control 6.9mg/mmol vs anti-IL6 mAb 4.1mg/mmol, p<0.04), glomerular abnormalities (control 54% vs anti-IL6 mAb 33%, p<0.006) and glomerular T cells (control 3.9/glomerulus vs anti-IL6 mAb 2.2/glomerulus, p<0.0003) by week 12, when compared to positive controls. No significant reduction was observed in the levels of circulating antibodies directed towards α3(IV)NC1, or in the intensity of deposits of IgG on the GBM, suggesting an effect on cell-mediated immunity. The results from this study demonstrate that anti-IL6 mAb therapy is effective in the treatment of established EAG. This confirms the importance of IL-6 in the development of glomerulonephritis, and implies that IL-6 may represent a valid therapeutic target in the treatment of human glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2806**

**MHC-DRB1 Alleles in PR3-ANCA Disease: A Highly Immunogenic Role for the HLA DRB1*15 Allele** Yali Cao,[1] Jia Jin Yang,[1] John Schmitz,[2] Susan L. Hogan,[1] Donna O. Bunch,[1] Yichun Hu,[1] Caroline E. Jennette,[1] Elisabeth Berg,[1] Frank C. Arnett,[3] J. Charles Jennette,[2,1] Ronald J. Falk,[1,2] Gloria A. Preston.[1,2] *[1]UNC Kidney Center, UNC-CH; [2]Dept of Pathology, UNC-CH, Chapel Hill, NC; [3]Dept of Internal Medicine, UT Health Science Center, Huston, TX.*

MHC class II molecules present a range of peptides for recognition by T cell receptors of CD4+T helper cells and in presenting self antigens stimulating the maturation of autoantibody expressing B cells and autoimmune disease. The objective here was to genotype Caucasian and African American patients to establish whether PR3-ANCA and/or MPO-ANCA are HLA-linked disorders.

By RPE analysis, strikingly, the allele linked with the greatest risk for PR3-ANCA disease in African Americans was *DRB1*15*($p=5.52x10^{-11}$). In the Caucasian patient group *DRB1*15* was linked also with PR3-ANCA serology (p=0.0001). RPE analysis of alleles associated with MPO-ANCA serology in African Americans showed a significant correlation with *DRB1*16* (DR2 serologic group). In the Caucasian group, no *DRB1* allele was significantly associated with MPO-ANCA serology.

The odds ratio that *DRB1*15* is a risk factor for African Americans to develop PR3-ANCA disease is 73.3 ($p=2.3x10^{-9}$). *DRB1*15* is also a risk factor in Caucasians with an odds ratio of 2.2 (p=0.008), as is *DRB1*14*, with an odds ratio of 5.9 (p=0.007). A disproportionate number of African American patients carried the *DRB1*1501* allele of Caucasian descent (50% versus 13%), rather than the *DRB1*1503* allele of African descent. All Caucasians were of the *DRB1*1501* genotype.

Functionally, DRB1*1501 protein bound specific sense-PR3 peptides and specific complementary-PR3 peptides in an *in vitro* assay, in agreement with *in silico* predictions. Peripheral neutrophils/monocytes from patients homozygous for *DRB1*1501*, expressed surface DRB1 proteins, after TNF-alpha exposure, that similarly bound the same specific peptides, while cells from a patient carrying the *DRB1*04*14* alleles bound complementary-PR3 but not the sense-PR3 peptides. The data support that *HLA-DRB1*15* is a risk factor for PR3-ANCA disease, especially impacting African American patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2807**

**Anti-Plasminogen Antibodies in Patients with ANCA-Associated Vasculitis Compromise Fibrinolysis and Are Related to Hallmark Histopathological Renal Lesions** Annelies Evaline Berden,[1] Sarah Nolan,[2] Hannah L. Morris,[2] Rogier M. Bertina,[3] Nico H. Van Tilburg,[3] Dianhdra Dyane Erasmus,[1] Ernst C. Hagen,[4] Donal P. Hayes,[5] Jan A. Bruijn,[1] Caroline O. S. Savage,[2] Ingeborg M. Bajema,[1] Peter Hewins.[2] *[1]Pathology, Leiden University Medical Center, Leiden, Netherlands; [2]Renal Immunobiology, Medical School, University of Birmingham, Birmingham, United Kingdom; [3]Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, Netherlands; [4]Internal Medicine, Meander Medical Center, Amersfoort, Netherlands; [5]Pathology, Meander Medical Center, Amersfoort, Netherlands.*

Antibodies recognizing plasminogen were correlated with venous thrombotic events in PR3-ANCA vasculitis. We investigated the prevalence and functionality of these antibodies in UK and Dutch AAV patients.

AAV-IgG and healthy control IgG was screened by ELISA. Eighteen of 74 (24.3%) UK and 10/38 (26.3%) Dutch AAV-IgG were anti-plasminogen antibody (anti-PLG) positive compared to 0/50 and 1/61 (2%) controls ($P$ < 0.001). Anti-PLG antibodies were detected in PR3- and MPO-ANCA positive patients. Furthermore anti-tissue plasminogen activator (tPA) antibodies were demonstrated in 13/74 (17.6%) patients. Anti-tPA antibodies associated with anti-PLG antibodies: 7/18 (39%) anti-PLG antibody positive patients were also anti-tPA antibody positive versus 6/56 (11%) anti-PLG antibody negative patients ($P$ = 0.011). Eighteen of 74 AAV-IgG but no control IgG retarded in vitro fibrinolysis ($P$ <0.001) and this associated with anti-PLG and/or anti-tPA antibody positivity. Only 4/18 AAV-IgG retarding fibrinolysis harbored no such antibodies. Dual positive samples retarded fibrinolysis to the greatest extent. Patients with anti-PLG antibodies had higher percentages of glomeruli with fibrinoid necrosis ($P$ < 0.05) and cellular crescents ($P$<0.001) than patients without and had more severely reduced renal function.

Concluding, anti-PLG and anti-tPA antibodies occur in AAV and are associated with functional inhibition of in vitro fibrinolysis. Seropositivity for anti-PLG antibodies correlates with renal lesions and renal function. Conceivably, fibrinolysis-enhancing therapies might benefit a subset of AAV patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2808**

**Tolerance to Myeloperoxidase and Resistance to Autoimmune Anti-MPO Nephritis Is Maintained by Thymic Deletion and Peripheral Regulatory T Cells** Diana S. Tan, Kim O'Sullivan, A. Richard Kitching, Stephen R. Holdsworth. *Centre for Inflammatory Diseases, Department of Medicine, Monash Medical Centre, Monash Univeristy, Victoria, Australia.*

The role of thymic deletion and peripheral regulatory T cells (Tregs) in developing and maintaining tolerance to myeloperoxidase (MPO) was assessed in C57BL/6 mice. Immunohistogical colocalisation confirmed that MPO was expressed in an AIRE independent manner by medullary thymic epithelial cells in mouse and human thymi but was absent in MPO-/- mice. Immunisation of MPO+/+ mice with MPO in CFA induced

autoreactive anti-MPO T cells in draining lymph nodes (LN). The number of responder LN cells was higher in MPO-/- mice (ELISPOT IFNγ: 202±45 vs 526±26/$10^6$ LN cells; IL-17A: 136±42 vs 333±43/$10^6$ LN cells; both p<0.001). ANCA titres were also higher in MPO-/- mice confirming the role of thymic deletion of autoreactive anti-MPO T cells in repertoire generation. To assess peripheral tolerance in normal mice, PC61 mAb was used to deplete CD4+CD25+ Tregs in normal mice that were then immunised with MPO in CFA. Depletion of Tregs resulted in elevated anti-MPO ANCA titres (control IgG: 0.8±0.1 vs PC61: 1.2±0.4 $OD_{450nm}$; p<0.001) and MPO-specific T cell responses (IFNγ 41±49 vs 207±67/$10^6$ and IL-17A 28±20 vs 104±52/$10^6$ LN cells; both p<0.001). Fewer Tregs (CD4+Foxp3+) were seen in the draining LN of PC61-depleted mice (9.7±0.5% vs 6.1±0.8 LN cells; p<0.001). Glomerulonephritis (GN) was triggered by low dose anti-GBM antibody to induce glomerular neutrophil degranulation and MPO deposition. Proteinuria was enhanced in Treg-depleted mice (2.1±0.3 vs 6.7±4.2mg/24hrs; p<0.01) as was glomerular injury (0.8±2.1 vs 29±22% segmental necrosis; p<0.001). Glomerular leukocyte recruitment was also increased in Treg-depleted mice, with increased glomerular CD4+ T cells (0.8±0.4 vs 1.5±0.5 cells per glomerular cross-section (c/gcs)), macrophages (0.9±0.3 vs 1.6±0.4 c/gcs), and neutrophils (1.5±0.7 vs 2.5±0.7 c/gcs; all p<0.01). Thus both central tolerance through thymic deletion and peripheral tolerance maintained by Tregs are important in the induction and maintenance of tolerance to MPO. They also confer resistance to the development of autoimmune anti-MPO GN.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2809

**IgA1 Antibodies Specific for Galactose-Deficient IgA1 (Gd-IgA1) Are Elevated in Sera from Patients with IgA Nephropathy (IgAN)** Koshi Yamada,[1,2] Hitoshi Suzuki,[1,2] Stacy D. Hall,[1] Zina Moldoveanu,[1] Bruce A. Julian,[1] Yasuhiko Tomino,[2] Jiri F. Mestecky,[1] Jan Novak.[1] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]Juntendo University Faculty of Medicine, Tokyo, Japan.*

Patients with IgAN have elevated serum levels of Gd-IgA1 that is recognized by naturally occurring anti-glycan antibodies, leading to formation of immune complexes that deposit in the glomeruli to induce renal injury. We have shown previously that anti-glycan IgG antibodies that recognize Gd-IgA1 are elevated in serum of patients with IgAN. However, a possible role of IgA1 antibodies in binding Gd-IgA1 has not been studied. To assess the presence and levels of IgA1 antibodies specific for Gd-IgA1, we have developed a novel dot-blot assay using Fab fragment of Gd-IgA1 (Ste) myeloma protein that contained a portion of the hinge region with the attached *O*-glycans. We also prepared a variant of the Fab fragment, by adsorption on immobilized lectin from *Helix aspersa* (HAA), that had predominantly fully galactosylated *O*-glycans. Following application of samples with normalized concentration of IgA, the detection of IgA1 binding to Fab of IgA1 was achieved by Fc-specific anti-IgA antibody and was evaluated densitometrically. Using this assay, we measured IgA1 anti-Gd-IgA1 antibody levels in sera from 24 patients with biopsy-proven IgAN, 24 disease controls (patients with non-IgAN renal diseases), and 10 healthy controls. Our results showed that sera from IgAN patients had higher levels of IgA1 antibodies binding to the Fab fragment of Gd-IgA1 (Ste) compared to those from healthy controls and disease controls (p<0.05 and p<0.0001, respectively). Binding of IgA1 from IgAN patients but not that from healthy controls was glycan-specific, as evidenced by the reduced binding to the Fab fragment with predominantly fully galactosylated *O*-glycans (p<0.01). Furthermore, we have identified Gd-IgA1-specific IgA1 in supernatants of Epstein-Barr virus-immortalized cell lines from patients with IgAN. These results prove the feasibility to further characterize these IgA1 antibodies at the molecular level and assess the roles of anti-glycan IgG and IgA1 isotypes in IgAN.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2810

**Excessive Premature Ageing of Circulating T Cells in Endstage Renal Disease Patients** Michiel G. H. Betjes, Elly L. E. A. De Wit, Nicolle H. R. Litjens. *Nephrology, Erasmus Medical Center, Rotterdam, Netherlands.*

**Background:** Progressive loss of renal function is associated with clinical signs of decreased T cell immunity. Changes in the number and differentiation of circulating T cells are probably contributing significantly to the ESRD-related immune deficiency. In this study we have tested the hypothesis that ESRD-related changes of the T cell compartment are in fact compatible with the concept of premature ageing.

**Patients and Methods:** The differentiation profile of circulating T cells of young (18-40 years) and old (60-80 years) ESRD patients (n=120) were compared to age-matched healthy controls. Expression of CD45RO combined with CCR7 (differentiation markers), and CD28 and CD57 (ageing markers) were used to characterize T cell subsets within the CD4pos and CD8pos T cell compartment. In addition, the naïve T cell compartment was analyzed in more detail for the presence of recent thymic emigrants (CD31pos cells), homeostatic proliferation and susceptibility for apoptosis.

**Results:** In the young age group the absolute number of CD4+ and CD8+ naive T cells from ESRD patients were on average decreased by 60%, compared to their age-matched healthy controls. T cells from ESRD patients showed an age-dependent decrease in naive T cells and recent thymic emigrants which exceeded normal age-related changes by at least 2-3 fold. This was accompanied by a significantly increased percentage of dividing naive T cells in ESRD patients (0.6% vs 0.3 %, p<0.05), indicating increased homeostatic proliferation. This increased proliferation was specifically observed in the CD31neg naive T cell population. The memory T cell compartment of both CD4pos and CD8pos T cells showed a dramatic shift towards terminal T cell differentiation with loss of CD28 expression and increased expression of CD57. Activation-induced apoptosis, but not apoptosis of resting T cells, was significantly increased in all T cell subsets of ESRD patients.

**Conclusions:** A remarkable premature ageing of T cells is present in ESRD patients which may underlie their immunedeficiency. Increased activation-induced T cell apoptosis could be a cause or consequence of premature ageing.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2811

**Biological Activities of IgA1-Containing Immune Complexes from Pediatric Patients with IgA Nephropathy (IgAN)** Jan Novak,[1] Leona Raskova-Kafkova,[1,4] Hitoshi Suzuki,[1,3] Milan Tomana,[1] Karel Matousovic,[1,5] Rhubell T. Brown,[1] Stacy D. Hall,[1] John T. Sanders,[2] Theodore Matthew Eison,[2] Zina Moldoveanu,[1] Lea Novak,[1] Zdenek Novak,[1] Richard Mayne,[1] Bruce A. Julian,[1] Jiri F. Mestecky,[1] Robert J. Wyatt.[2] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]University of Tennessee Health Sciences Center, Memphis, TN; [3]Juntendo University Faculty of Medicine, Tokyo, Japan; [4]Palacky University, Olomouc, Czech Republic; [5]Charles University, Prague and Plzen, Czech Republic.*

Sera of patients with IgAN contain immune complexes (IC) composed of galactose-deficient IgA1 (Gd-IgA1) bound by anti-glycan antibodies. We isolated from sera of pediatric IgAN patients IC of different size and determined their effect on cultured human mesangial cells. Our results showed that IC with molecular mass >800 kDa containing Gd-IgA1 stimulated cellular proliferation, while smaller IC were inhibitory. Addition of stimulatory and inhibitory IC to cultures of human mesangial cells differentially altered phosphorylation patterns of three major tyrosine-phosphorylated proteins of molecular mass 37, 60, and 115 kDa. The stimulatory IC transiently increased tyrosine-phosphorylation of the 37-kDa protein and decreased phosphorylation of the other two proteins, whereas the inhibitory IC increased phosphorylation of all three proteins. Furthermore, we investigated the influence of IgA1-containing IC from sera of children with IgAN with clinically active or inactive disease on the expression of IL-6 and IL-8 genes by mesangial cells. Our RealTime RT-PCR results showed that the IC from patient with active disease stimulated mesangial cells to express the two cytokine genes at higher levels than did the IC from patient with inactive disease. Moreover, stimulatory IC increased production of the extracellular matrix protein laminin. These data indicate that sera of pediatric IgAN patients contain biologically active IC with Gd-IgA1 and that their level and/or biological activity may reflect clinical activity of the disease.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2812

**Dual Non-HLA Antibodies Simultaneously Targeting Endothelial AT1- and ETA Receptors Induce Vascular Proliferation and Coagulation** Melanie Naether,[1] Aurélie Philippe,[1] Rusan Catar,[1] Andreas Eisenreich,[2] Gabriela Riemekasten,[3] Ursula Rauch-Kröhnert,[2] Duska Dragun.[1] *[1]Nephrology and Intensive Care Medicine, Charité Campus Virchow; [2]Cardiology and Pulmology, Charité Campus Benjamin-Franklin; [3]Rheumatology, Charité Campus Mitte, Berlin, Germany.*

Transplant recipients and patients with vascular autoimmune pathologies harbouring non-HLA-antibodies directed against Angiotensin II type 1 (AT1R-Abs) and Endothelin-1 type A receptors (ETAR-Abs) are at increased risk for early onset of microvasculopathy. We hypothesized that these non-HLA antibodies may actively contribute to vascular obliteration by induction of complement-independent mechanisms and sought to investigate mechanisms responsible for intravascular coagulation and proliferation responses.

AT1R-Ab and ETAR-Ab positive IgG fraction isolated from patients with obliterative vasculopathy or control IgG served for signal transduction and transcription factor activation studies, as well as for coagulation and proliferation assays in human microvascular endothelial cells.

Both autoantibodies were biologically active as they induced stress-kinase ERK1/2 phosphorylation which could be blocked by respective receptor inhibitors. AT1R- and ETAR-Abs specifically triggered activation of transcription factor Ets-1 down-stream from ERK1/2, as confirmed by phosphorylation, chromatin immunoprecipitation and electromobility shift assays followed by cell proliferation. Pharmacological inhibition of the upstream kinases of ERK1/2 established a direct link between ERK1/2, Ets-1 and endothelial proliferation.

We also detected increased tissue factor expression, a gene target of Ets-1. AT1R- and ETAR-Abs also enhanced tissue factor procoagulatory activity of endothelium determined in coagulation assay.

Anti- AT1- and ETA receptor autoantibodies may directly contribute to the key mechanisms involved in pathogenesis of obliterative vasculopathy and represent a link between the increased vascular responsiveness, intravascular coagulation and proliferation responses. Dual receptor pharmacologic targeting should add to current immunosuppressive regimens in patients harbouring non-HLA antibodies directed against vascular receptors.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2813**

**Altered Gene Silencing in Leukocytes of Lupus Nephritis Patients** Elizabeth R. Blyth, Jia Jin Yang, Dominic J. Ciavatta, Susan L. Hogan, Yichun Hu, Gloria A. Preston, J. Charles Jennette, Ronald J. Falk, Keisha L. Gibson. *UNC Kidney Center, UNC, Chapel Hill, NC.*

ANCA disease patients aberrantly express neutrophil granule genes PR3 and MPO due to depleted histone H3K27me3 modifications and loss of normal gene silencing. A subset of patients with lupus nephritis also exhibit aberrant leukocyte PR3 and MPO gene expression. The objective here is to determine the underlying factors attributable to changes in gene expression in lupus; specifically, can expression be linked to positive ANCA titers? Is necrosis in the renal biopsy a factor? Is aberrant transcription in lupus nephritis initiated by epigenetic changes similar to those observed in ANCA disease?

Increased PR3 and/or MPO mRNA expression (2 SD above mean of healthy controls) was present in patients with lupus nephritis (27/68), but not RA (n=46) or IBD (n=40). All lupus patients were negative for ANCA titers. There was no difference in PR3 or MPO expression between lupus patients with histological evidence of necrosis (n=44) versus those with no necrosis (p=0.56). Expression did not differ based on race, age or SLEDAI score.

Patients with lupus nephritis had significantly lower levels of PR3 compared to patients with ANCA disease (p=0.009) but comparable levels of MPO (p=0.95). Statistical analysis of MPO to PR3 ratios comparing all disease groups was significant (p<0.0001, F-test), with a higher ratio in lupus nephritis than ANCA.

Epigenetic studies (ChIP) of four lupus patients determined that H3K27me3 was depleted at the MPO gene and reduced at PR3 compared to MYO-D, which is transcriptionally silent in neutrophils; however, there was slight enrichment at the PR3 gene compared to mock immunoprecipitated samples. These data suggest that MPO is not epigenetically silenced in lupus, while PR3 may be silenced in a fraction of neutrophils. Thus, regulation of MPO and PR3 is altered in individuals with lupus nephritis, but the stimuli for this modulation of gene silencing deviates from ANCA disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2814**

**TLR4 and TLR9 Enhance Experimental Lupus Induced by Pristane** Shaun Andrew Summers, Oliver M. Steinmetz, A. Richard Kitching, Stephen R. Holdsworth. *Centre for Inflammatory Diseases, Monash University, Melbourne, Victoria, Australia.*

**Aim:** To define the role of Toll-like receptor (TLR) 4 and TLR9 in experimental systemic lupus erythematosus (SLE), induced by pristane.

**Methods:** To determine TLR4 and TLR9 function in experimental SLE we administered pristane to C57BL/6 wild-type (WT), TLR4-/- and TLR9-/- mice (both on BL/6 backgrounds). Systemic cellular immune responses and serum auto-antibodies, including total immunoglobulin (IgG), anti-dsDNA and anti-RNP IgG were assessed after 8 months. Functional renal injury, albuminuria, glomerular injury and proliferative glomerulonephritis were studied. Glomerular IgG and complement, deposition were assessed by direct immunoflourescence.

**Results:** Analysing systemic immune responses demonstrated decreased IFNγ splenocyte production in TLR4-/- and TLR9-/- mice (WT 236±59, TLR4-/- 39±39, TLR9-/- 50±25pg/ml, p<0.05). In TLR4-/- mice IL-17A (49±11 vs. 16±4pg/ml, p<0.05) and IL-6 (489±114 vs. 36±8pg/ml, p<0.05) production was also decreased compared to WT mice. Total serum immunoglobulin (IgG) levels were decreased in TLR4-/- and TLR9-/- mice. Compared to WT mice, TLR4-/- mice demonstrated decreased titres of anti-dsDNA IgG (0.22±0.06 vs. 0.13±0.04 $OD_{450}$ p<0.05) and anti-RNP IgG autoantibody levels (0.61±0.08 vs. 0.18±0.03 $OD_{450}$ p<0.05). TLR9-/- mice demonstrated a selective decrease in anti-RNP IgG titres (0.08±0.01 $OD_{450}$ p<0.01 vs. WT).

All mice developed progressive renal injury. After 8 months albuminuria was decreased in TLR4-/- and TLR9-/- mice (WT 432±97, TLR4-/- 150±30, TLR9-/- 117±61µg/24 hours, p<0.05). Proliferative glomerulonephritis and histological injury was attenuated in TLR4-/- and TLR9-/- mice. Using a semi-quantative scoring system direct immunoflourescence demonstrated decreased glomerular IgG (WT 1.4±0.1, TLR4-/- 0.9±0.1, TLR9-/- 0.9±0.1, p<0.001) and complement deposition (WT 1.8±0.1, TLR4-/- 1.3±0.1, TLR9-/- 1.3±0.1, p<0.001) in TLR4-/- and TLR9-/- mice.

**Conclusion:** Both TLR4 and TLR9 enhance the development of lupus autoimmunity and lupus nephritis. While TLR9 deficiency results in a selective decrease in Th1 associated cytokines and anti-RNP IgG, TLR4 deficiency results in a generalised decrease in immune responses.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2815**

**Anti-dsDNA Antibodies from Patients with Lupus Nephritis Bind to Annexin II on Human Mesangial Cells** Susan Yung, Kwok Fan Cheung, Qing Zhang, Daniel Tak Mao Chan. *Department of Medicine, The University of Hong Kong, Hong Kong.*

Lupus nephritis is characterized by the production of anti-dsDNA antibodies and proliferative glomerulonephritis. The mechanism through which anti-dsDNA antibodies interact with resident renal cells remains to be elucidated. We and others have previously demonstrated that human anti-dsDNA antibodies can bind to human mesangial cells (HMC) without the need of bridging chromatin material. In this study, we characterized the cross-reactive antigen(s) on HMC that mediate the binding.

Human polyclonal anti-dsDNA antibodies were isolated from patients with lupus nephritis using Protein A-Sepharose followed by DNA-cellulose affinity chromatography. The binding of anti-dsDNA antibodies to HMC was assessed by flow cytometry and cellular ELISA. HMC plasma membrane fractions were subjected to Western blotting, probed with purified anti-dsDNA antibodies, and analyzed with MALDI-TOF spectrometry to identify 'cross-reactive' membrane proteins. Renal biopsies were examined with immunohistochemistry.

Limited trypsin but not DNase treatment of HMC significantly reduced anti-dsDNA antibody binding ($P$<0.05). Anti-dsDNA antibodies predominantly bound to a cell surface antigen with molecular weight of ~36kDa. This band was identified as annexin II by MALDI-TOF spectrometry. The binding activity between anti-dsDNA antibodies and annexin II correlated with disease activity. Glomerular annexin II expression was significantly increased in active lupus nephritis compared with controls and non-lupus kidney diseases ($P$<0.05), and co-localized with IgG and C3 deposition.

Our data demonstrate that annexin II on the plasma membrane of HMC mediates anti-dsDNA antibody binding.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2816**

**Defects in B Cell Tolerance in BWF1 Mice with Severe Lupus Nephritis** Qihua Fan,[1,2] Amy G. Clark,[1,2] Melissa L. Weston,[1,2] Mary H. Foster.[1,2] *[1]Medicine, Duke University Medical Center, Durham, NC; [2]Medicine, VAMC, Durham, NC.*

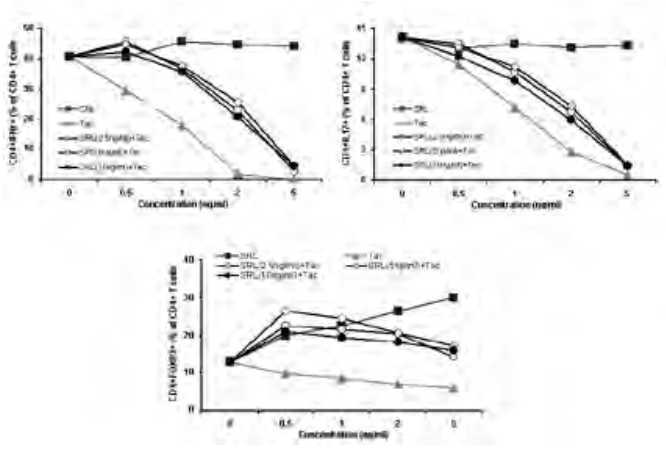
Understanding the basis of lupus genetic susceptibility will provide new insight into disease mechanisms and a basis for novel therapies. Because autoantibody (autoAb) mediated nephritis is a major cause of morbidity in lupus, we examined genetic control of B cell tolerance. Through successive backcrossing, we established a single lupus autoAb transgene (Tg) on multiple lupus-prone strains, each of which carries a unique constellation of susceptibility loci that predispose to disease resembling human SLE with severe glomerulonephritis. Previously we showed that in healthy B6 mice and MRL lupus mice, B cells expressing the Tg, which encodes a dominant anti-laminin Ig heavy chain, are tightly regulated by clonal deletion, receptor editing, and anergy. Tolerance was also maintained in Tg BXSB lupus mice despite increased B cell numbers, whereas detection of serum Tg autoAb in Tg NZB lupus mice suggested partial loss of tolerance. Herein we report the fate of Tg B cells in (NZBxNZW)F1, or BWF1, mice with aggressive lupus (values refer to splenic B cells, Tg vs non-Tg littermates). 79% of cells express surface Tg vs 3% background in non-Tg mice. Mean spleen B cell count is 3.2-fold lower in Tg mice (7.1±4.1 vs 23.0±10.6, millions±SD, p<0.001), consistent with substantial deletion. Nonetheless, B cell depletion is less striking than the 8.3-, 6.8-, and 5.1-fold difference previously observed in B6, MRL, and BXSB Tg mice, respectively. Receptor editing as gauged by increased %lambda expression on spleen B cells is not detected (4.2±2.3% vs 5.8±2.7%, p=NS). Evidence of anergy in Tg cells is limited: in vivo B cell %BrdU+ is higher in Tg mice after 30 days (48.6±4.0% vs 35.7±4.1%, p<0.05); however, there is no difference in ex vivo proliferation in response to Ig crosslinking or LPS, and minimal difference in surface activation marker expression. Serum Tg autoAb levels are low but detectable (0.050±0.03 vs 0.010±0.04, p=0.004, mean OD±SD). We conclude that clonal deletion is intact in BWF1 lupus mice, but that at least a subset of surviving autoreactive B cells are functional and express autoreactivity.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2817**

**Human Allospecific Th1, Th17 and Treg Are Differentially Affected by Calcineurin and mTOR Inhibitors** Lorenzo G. Gallon,[1] Nader Najafian,[2] Anton I. Skaro,[1] Giovanna La Monica,[1] Joseph Ross Leventhal,[1] Luting Xu.[1] *[1]Nephrology, Northwestern University; [2]Harvard Medical School.*

Subpopulation of T helper cells (Th1, Th17, and Treg) play a major role in promoting rejection or can facilitate regulatory immune-mechanisms for the development of a pro-tolerant state towards the transplant. Little is known about the impact of immunosuppressive (IS) drugs on allospecific T cell subpopulations. Methods: Alloreactve CD4+ T cells were first generated in MLR culture with responding CD4+CD45RA+ nave T cells and allogeneic CD14+ monocytes. Alloreactive CD4+ T cells, from the primary MLR, were enriched and re-stimulated by co-culturing with autologous CD14+ monocytes in the presence of antigenic stimulation of anti-CD3 to increase the frequency of Th1,Th17 and Treg cells. Two different IS agents, Tacrolimus (Tac) and Sirolimus (SRL) were added to the second culture alone or in combination at different concentrations. The effects on the generation of Th1, Th17, Treg cells from alloreactive memory T cells (CD4+CD45RO+) were then determined by intracellular cytokine staining. Results: Tac at low doses, significantly blocked the productions of IFN- (Th1) and IL-17 (Th17), while SRL even at high concentration (10ng/ml or 20ng/ml) had minimal effect on IFN- and IL-17 production (see top panels of figure). FOXP3 expression (Treg) was markedly increased in SRL compared to Tac (see bottom panel). When Tac and SRL were used in combination and at different concentrations, we found that Tac at 2-5 ng/ml with SRL at 2.5-10ng/ml achieved the maximal effect in inhibiting the production of IFN- and IL-17 while maintaining a high level of FOXP3 expression.



Conclusions: Tac and SRL have differential effects on subpopulation of T helper cells. These findings can help to guide the clinical use of IS drugs to promote Treg expansion while controlling Th1 and Th17 responses.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2818

**A Role for the ChemR23/Chemerin Axis in the Recruitment of Plasmacytoid Dendritic Cells in Lupus Nephritis** G. Castellano, Giuseppe De Palma, A. Loverre, G. Grandaliano, Francesco Paolo Schena. *Renal Unit, University of Bari, Bari, Italy.*

Plasmacytoid dendritic cells (pDC) play a pivotal role in driving the autoimmune response in SLE. Recent data showed that pDC infiltrate the kidney of patients with lupus nephritis (LN), but the factors regulating the pDC recruitment at renal level are unknown. Chemerin is the recently identified natural ligand of ChemR23, a receptor expressed by pDC. The aim of this study was to investigate the possible role of the ChemR23/Chemerin axis in the recruitment of pDC in LN.

The presence of ChemR23 and Chemerin were determined by immunohistochemical and immunofluorescence analysis of kidney sections from 10 patients with LN. Chemerin transcript levels were quantified using Real-time RT-PCR and Chemerin protein production was assessed by ELISA in human renal proximal tubular epithelial cells (RPTEC). Chemerin-dependent trans-endothelial migration of pDC was examined in transwell systems.

Quantification of percentage of ChemR23+ staining area revealed a significative increase of ChemR23+ pDC in class III-IV patients (4.4±1.1%) compared to class I-II patients (1.1±2.1%). ChemR23+ pDC clearly surrounded tubular epithelial cells and frequently localized around the glomeruli, but rarely within them. In addition, we found ChemR23+ pDC associated with renal vessels. In accordance, we identified Chemerin associated to tubular epithelial cells. The intensity of Chemerin staining was significantly higher in class IV LN, indicating an increased production in patients with worse kidney involvement. Chemerin was also associated to Podoplanin+ lymphatic endothelial cells. RPTEC express specific Chemerin mRNA, and produced Chemerin in vitro (25 ng/ml). in additioni, Chemerin was significantly down-regulated by TNF-α and IFN-γ after 48h of activation (reduction of 80% and 58% respectively). Interestingly, only TNF-α was capable to induce the functionally active form of renal Chemerin, resulting in an efficient recruitment of pDC.

Therefore, we hypothesize that the Chemerin/ChemR23 axis may play a pivotal role in the recruitment of pDC within the kidney in patients affected by LN.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2819

**Interleukin-1 Receptor-Associated Kinase-M Prevents Lupus Nephritis by Suppressing Toll-Like Receptor-7 Signaling** Maciej Lech, Claudia Kantner, Hans J. Anders. *Medizinische Poliklinik, University of Munich, Munich, Germany.*

SLE is a heterogenous autoimmune syndrome deriving from multiple gene polymorphisms of immunoregulatory genes. Loss of function muations in genes that regulate Toll-like receptor-7 signaling have been shown to affect SLE and lupus nephritis. The interleukin-1 receptor-associated kinase (IRAK)-M suppresses innate immune activation in bacterial sepsis, hence, we hypothesized a similar role for IRAK-M in lupus nephritis.

*Irak-m*-deficiency converted the mild autoimmune phenotype of C57BL/6-lpr/lpr mice into a massive lymphoproliferative syndrome with severe lupus nephritis. *Irak-m*-deficiency induced a number of interferon-related genes, cytokines and plasma cell survival factors in spleen dendritic cells of these mice. *Irak-m*-deficient C57BL/6-lpr/lpr mice revealed expansion of autoreactive T cells, dysfunctional regulatory T cells, and plasma cells which was associated with increased lupus autoantibody production and immune complex disease. TLR7 antagonism completely abrogated this phenotype in-vivo consistent with IRAK-M-mediated suppression of TLR7 signaling in-vitro. In addition, distinct haplotype polymorphisms of the human IRAK-M gene were associated with systemic lupus in a cohort of 840 lupus patients and 1218 controls.

These data identify a previously unknown function of IRAK-M, i.e. suppression of autoimmunity, and vice versa, mutant IRAK-M as a genetic risk for SLE and lupus nephritis.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2820

**Role of TH9 Cells in Nephrotixic Serum Nephritis** Kathrin Eller, Philipp Eller, Gert J. Mayer, Alexander R. Rosenkranz. *Internal Medicine IV, Innsbruck Medical University, Austria.*

Very recently, TH9 cells have been described as a new subpopulation of T helper (TH) cells, which have the capacity to act either pro- or anti-inflammatory when activated. Their marker cytokine interleukin (IL)-9 promotes on the one hand the development of TH17 cells and on the other hand the activity of regulatory T cells.

To evaluate the impact of TH9 cells on nephrotoxic serum nephritis (NTS), NTS was induced in either IL-9 knock-out (ko) or wild-type (wt) controls. Mice were followed for 7 or 14 days and evaluated for albuminuria, histologic changes, immune cell infiltration in the kidney, cytokine profiles of the kidney and lymph node.

IL-9 ko mice displayed significantly decreased disease indices such as albuminuria and histologic changes compared to wt controls 7 and 14 days after induction of NTS. Furthermore, significantly decreased numbers of infiltrating CD4 T cells and F4/80+ cells as well as CD68+ macrophages were detected in IL-9 ko mice, whereas no difference in the infiltration of CD8+ T cells was found 14 days after NTS induction. Kidney infiltration of neutrophils was significantly decreased in kidneys of IL-9 ko mice 7 days after induction of NTS. Cytokine profiling in the kidneys revealed significantly decreased amounts of IFN-gamma, IL-10 and IL-17 in the kidneys of IL-9 ko mice as compared to wt controls 14 days after induction of NTS. In the regional draining lymph nodes the cytokines IL-6, IFN-gamma, IL-10 and IL-17 were significantly decreased in IL-9 ko mice, whereas the regulatory T cell marker FoxP3 was increased. Mast cells, as detected by mast cell tryptase, were slightly diminished in lymph nodes of IL-9 ko mice 7 days after NTS induction.

Thus, the TH9 cell population is crucial in the pathogenesis of NTS. They have the potential to tip the immune balance towards a TH1 and TH17 response, whereas Treg activity was blocked.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2821

**Circulating Endothelial Biomarkers and the Activity of ANCA Associated Vasulitis (AAV)** Wei-Xin Hu, Ying-Hua Chen, Zheng-Zhao Liu, Hai-Tao Zhang, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China.*

During vascular inflammation, activated endothelial cells express various membrane molecules, which facilitate the binding of circulating leukocytes. Damaged endothelial cells may detach from the endothelium to form circulating endothelial cells (CECs). Therefore, CECs and the plasma concentration of soluble molecules are supposed to reflect the degree of AAV activity, which has not been widely studied. This study investigates CECs, soluble endothelial biomarkers (vWF, thrombomodulin, VCAM-1 and E-selectin) in patients with active and remission stage. We aim to document the relationship between various endothelial markers and the activity of AAV. 51 patients with AAV in active phase (M:20, F:31, mean age 47.8+17.4y, BVAS score 13.0+2.9), 31 in remission phase (M:12, F:19, mean age 48.2+16.9, BVAS score 1.0+1.7), 20 healthy controls were studied. Endothelial cells were isolated from peripheral blood by use of Dynabeads coated with antibodies against CD146. Plasma vWF, thrombomodulin(TM), VCAM-1 and E-selectin were detected with ELISA. Results showed that CECs, vWF, E-selectin and TM levels, except VCAM-1 were much higher in the active phase of AAV than controls ($P<0.01$). No significant differences of endothelial markers were found among AAV patients in remission and controls. CECs and plasma TM levels were significantly higher in patients with active AAV than patients in remission ($P<0.01$), yet plasma vWF and E-selectin levels showed no significant differences between patients in active phase and patients in remission.

| | AAV in active(n=51) | AAV in remission(n=31) | Control(n=20) |
|---|---|---|---|
| CECs(cell/ml) | 30± 12## | 17±6** | 15± 2 |
| TM(ng/ml) | 9.4± 7.0## | 5.9± 3.2** | 4.9± 1.3 |
| vWF% | 200.8± 28.4## | 177.4± 54.8 | 155.5±25.7 |
| E-selectin(ng/ml) | 88.6± 45.4## | 76.4± 36.6 | 51.1± 14.9 |
| VCAM-1(ng/ml) | 1800.5± 1147.5 | 1496.9± 744.9 | 1420.4± 372.4 |

AAV in active or in remission *vs* control, ##: $P$ <0.01; AAV in remission *vs* AAV in active, ** : $P$<0.01

In conclusion, Circulating endothelial cells and plasma thrombomodulin level are potential biomarkers in evaluating the activity of ANCA associated vasulitis.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2822

**ANCA Stimulated Neutrophils Release BLyS and Promote B Cell Survival *In Vitro*** Neil J. Holden,[1] Julie M. Williams,[2] Lorraine Harper,[1] John Gordon,[1] Caroline O. S. Savage.[1] *[1]School of Immunity and Infection, University of Birmingham, United Kingdom; [2]Wellcome Trust Clinical Research Facility, Queen Elizabeth Hospital Birmingham, United Kingdom.*

The presence of B cell infiltrates within the kidney has been reported in a number of inflammatory renal diseases, including the ANCA-associated vasculitides. These cells are likely to promote continued inflammation and may represent a niche for auto-antibody production. We sought to determine a role for ANCA activated neutrophils in promoting B cell survival, through the release of BLyS (B Lymphocyte Stimulator). Neutrophils

isolated from healthy donors were assessed for BLyS expression by flow cytometry and release by capture ELISA. Both non primed neutrophils treated with TNF-α (10ng/ml) or fMLP (1μM) and TNF-α (2ng/ml) primed cells treated with ANCA (200μg/ml) exhibited a significant increase in BLyS surface expression, within 30 minutes, which returned to basal levels following 2 hours culture. BLyS was also detected in cell supernatants supporting the notion that BLyS can be shed from the neutrophil surface. To determine whether ANCA activated neutrophils support B cell survival *in vitro*, supernatants collected from ANCA treated neutrophils (2 hours) were added to the B cell lymphoma cell line L3055 (10%): L3055 cells were cultured for 48 hours with low serum (2.5%) to induce apoptosis and cell death was measured by measuring annexin V binding expression. Supernatants from ANCA IgG but not normal IgG treated neutrophils significantly increased B cell survival, equivalent to a BLyS at 10ng/ml. Using CFSE to label actively dividing B cells, we were also able to demonstrate that neutrophil supernatants added to L3055 cell culture were able to significantly increase cell proliferation over 72 hours. In summary, we have shown that ANCA specifically causes the release of BLyS from activated neutrophils and that supernatants from activated neutrophils can support B cell survival *in vitro*. The work highlights BLyS as a potential therapeutic target to inhibit ANCA induced inflammation and may be particularly relevant in light of proposed uses of novel anti-BLyS monoclonal antibody therapies to treat a number of autoimmune diseases.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2823

**Identification of Structural Isomers of IgA1 Hinge-Region *O*-Glycopeptides: Implications for Pathogenesis of IgA Nephropathy (IgAN)** Kazuo Takahashi,[1,3] Hitoshi Suzuki,[1,4] Archer D. Smith,[1] Knud Poulsen,[2] Mogens Kilian,[2] Yukio Yuzawa,[3] Yoshiyuki Hiki,[3] Bruce A. Julian,[1] Jiri F. Mestecky,[1] Matthew B. Renfrow,[1] Jan Novak.[1] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]Aarhus University, Aarhus, Denmark; [3]Fujita Health University, Toyoake, Japan; [4]Juntendo University Faculty of Medicine, Tokyo, Japan.*

IgA1 with galactose (Gal)-deficient hinge-region (HR) *O*-glycans plays a pivotal role in the pathogenesis of IgAN. To characterize the pathogenic forms of IgA1, *O*-glycan microheterogeneity and attachment sites should be analyzed, as each HR has 9 potential *O*-glycosylation sites. We have developed mass spectrometric protocol using 3 bacterial IgA-specific proteases and trypsin combined with electron capture dissociation (ECD) to localize all *O*-glycosylation sites in IgA1. Using IgA1 (Mce1) myeloma protein as a model Gal-deficient IgA1 protein, we prepared HR glycopeptides and fractionated them by on-line liquid chromatography (LC) and analyzed them by Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) and tandem MS (MS/MS). Some of the HR glycopeptides exhibited multimodal-peak distribution in LC, indicating possible structural isomers. Off-line LC was used to separate the structural isomers of HR glycopeptides, and the sites of glycan attachment and glycan compositions were determined by activated-ion (AI)-ECD MS/MS. The attachment of an *O*-glycan alternatively to T233 or T236 produced structural isomers, including those with Gal-deficient glycans. Further analyses indicated that similar isomeric glycoforms exist in normal human serum IgA1. Furthermore, LC-extracted ion chromatogram indicated the presence of isomeric structures of Gal-deficient GalNAc in IgA1 HR from serum IgA1 and IgA1 secreted by IgA1-producing cells from patients with IgAN. These findings represent the first definitive identification of structural isomeric IgA1 *O*-glycoforms, including two unique Gal-deficient forms. Future studies will determine whether distinct Gal-deficient structural isomers are differentially recognized by anti-glycan antibodies and, thus, are relevant to IgAN pathogenesis.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2824

**Melatonin Ameliorates Experimental Murine Membranous Nephropathy: Immunomodulatory, Anti-Oxidative, and Anti-Apoptotic Effects** Chia-Chao Wu,[1] Jin-Shuen Chen,[1] Shih-Hua P. Lin,[1] Yuh-Feng Lin,[2] Pauling Chu.[1] *[1]Division of Nephrology, Department of Internal Medicine, Tri-Service General Hospital, Taipei, Taiwan; [2]Division of Nephrology, Department of Internal Medicine, Shuang-Ho Hospital, Taipei, Taiwan.*

Therapeutic agents for membranous nephropathy (MN) remain ill-defined. Melatonin is the major secretory product of the pineal gland and displays multifunctional properties including the regulation of circadian and seasonal rhythms, antioxidation reactions and immune modulation. Based on the immunosuppressive properties of melatonin, we we assessed the efficacy of malatonin therapy for MN. MN mice were induced with intravenous injections of cationic bovine serum albumin. Three groups of mice were administered low dose melatonin (2mg/kg/bw), high dose melatonin (20mg/kg/bw), or phosphate-buffered saline via subcutaneously injection daily. Disease severity, cytokine profiles, immunoglobulin production, oxidative stress and apoptosis were determined. Mice treated with high dose melatonin displayed a significant reduction in proteinuria and a marked amelioration of glomerular lesions, accompanied by attenuated complement activation. The subpopulation of immune cells, especially proportion of CD19+ B cells and CD25+ T cells, were significantly modulated compared with those of mice in the other two groups. Oxidative stresses in the serum and kidneys, as well as apoptosis, were also significantly reduced in high dose melatonin -treated mice. Cytokine mRNA expression in the kidney indicated that melatonin not only decreased the expression of proinflammatory cytokines, but also increased the expression of anti-inflammatory cytokines. Melatonin therapy may ameliorate experimental MN via multiple pathways, including immunomodulatory, anti-oxidative, and anti-apoptotic effects. Melatonin should be considered a potential therapeutic intervention in MN in the future.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2825

**Cancer Associated Membranous Nephropathy: Analysis of Anti-$PLA_2R$ Autoantibodies and Glomerular IgG Subclass Deposits** Shi-Jun Li,[1] Wei-Song Qin,[1] Ming-Chao Zhang,[1] Chun-Xia Zheng,[1] Ke Zuo,[1] Hui-Ping Chen,[1] Yan Wu,[1] Laurence H. Beck,[2] David J. Salant,[2] Zhi-Hong Liu.[1] *[1]Research Insitute of Nephrology, Jinling Hospital, Nanjing University of Medcine, Nanjing, Jiangsu, China; [2]Boston University School of Medicine, Boston, MA.*

Membranous nephropathy (MN) might be associated with cancer in adult patients, but the characteristics of cancer associated MN are unknown. We studied the clinical pathologic features of MN patients associated with cancer and the relationship between MN and cancer.

MN patients who developed cancer at the time of renal biopsy or within a year were involved in this study. The clinical and pathologic data of those patients were analyzed. Anti-$PLA_2R$ (M-type phospholipase $A_2$ receptor) autoantibodies in serum and the glomerular IgG subclass deposits were examined.

Ten patients developed solid tumors of various types, including carcinomas of lung (5 cases), stomach (2 cases), colon (1 case), larynx (1 case), and tongue (1 case). All of the patients presented proteinuria and edema; 8 patients showed nephrotic syndrome. Anti-$PLA_2R$ autoantibodies were detected in the serum of 3 patients; IgG1 (IgG4 negative) was the dominant IgG in the glomerular deposits of 8 patients. However, the glomerular IF intensities of IgG4 deposits were the same as IgG1 in 2 patients with Anti-$PLA_2R$ autoantibodies. During follow-up, in 7 patients without Anti-$PLA_2R$ autoantibodies, complete remission of the proteinuria was seen in 2 patients associated with tumor remission; three patients died of cancer; the remaining 2 patients had persistent proteinuria without tumor resection. 3 patients with Anti-$PLA_2R$ autoantibodies had persistent or relapse of proteinuria after resection of the tumor.

In conclusion, the clinical and pathologic features of cancer-associated MN are not significantly different from those of idiopathic MN. Most of patients have no Anti-$PLA_2R$ autoantibodies in the sera and have glomerular IgG1 deposits, which indicates that the pathogenesis of cancer-associated MN differs from that of idiopathic MN. The patients with Anti-$PLA_2R$ autoantibodies in the sera and glomerular IgG4 deposits may suggest the coincidence of cancer and idiopathic MN.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2826

**Anti-Phospholipase A2 Receptor Antibody in Chinese Membranous Nephropathy Patients** Wei-Song Qin,[1] Laurence H. Beck,[2] David J. Salant,[2] Cai-Hong Zeng,[1] Zhao-Hong Chen,[1] Zhi-Hong Liu.[1] *[1]Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China; [2]Boston University School of Medicine, Boston, MA.*

Membranous nephropathy (MN) is a common cause of idiopathic nephrotic syndrome in Chinese patients. The M-type phospholipase A2 receptor (PLA2R) is the first autoantigen identified in adult idiopathic membranous nephropathy. In this research, the prevalence of autoantibody against PLA2R was explored in Chinese patients with primary and secondary MN.

Idiopathic MN (n=60), lupus MN (n=20), HBV associated MN (n=16), mercury-induced MN (n=10) and tumor associated MN (n=10) were diagnosed by kidney biopsy in Nanjing. Anti-PLA2R antibody in serum was detected by Western blotting under non-reducing conditions using protein extracts from normal human glomeruli and was checked with recombinant PLA2R. Sheep anti-human IgG4 was used as primary antibody and HRP-donkey anti-sheep IgG as secondary antibody.

Among 60 Chinese patients with idiopathic MN, 49 of them were detected of Anti-PLA2R autoantibody. The reactive serum specimens recognized PLA2R in extracts of human glomeruli and in extracts of HEK cells expressing recombinant PLA2R. Anti-PLA2R autoantibodies in serum samples from patients with membranous nephropathy were mainly IgG4, the predominant immunoglobulin subclass in glomerular deposits.

Anti-PLA2R antibody was detected in one MN patient with lupus, one MN patient with HBV and two MN patients that had been exposed to mercury. Anti-PLA2R antibody was also detected in three MN patients with cancer of lung, larynx and stomach respectively.

Anti-PLA2R antibody in Chinese membranous nephropathy

| MN | n | Anti-PLA2R | % |
|---|---|---|---|
| Idiopathic MN | 60 | 49 | 81.7% |
| Lupus MN | 20 | 1 | 5.0% |
| HBV-MN | 16 | 1 | 6.3% |
| Mercury induced MN | 10 | 2 | 20% |
| Tumor associated MN | 10 | 3 | 30% |

Anti-PLA2R autoantibody was positive in a majority of Chinese patients with idiopathic MN, which indicates that PLA2R is a major target antigen in primary MN Chinese patients. Although anti-PLA2R was also detected in a small number of patients with apparent secondary MN, we cannot rule out the possibility that this is a coincidental occurrence.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2827**

**Endotoxin-Tolerance Monocyte Profile in Minimal Change Nephritic Syndrome (MCNS): Role in Increased Susceptibility to Bacterial Infections**
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MCNS is often complicated by bacterial infections, contributing significantly to the morbidity of this benign disease. The pathogenic mechanisms responsible for the increased susceptibility to bacterial infections are not well understood. We have previously shown that lymphocyte interleukin (IL)-13 gene expression was upregulated during relapses in children with MCNS. This was associated with downregulation of proinflammatory cytokines, IL-8 and tumor necrosis factor (TNF)-a, in lipopolysaccharide (LPS)-stimulated monocytes, as well as decreased expression CD14, suggestive of an anti-inflammatory effect of IL-13. This study aimed to identify the 'gene signature' in monocytes isolated from MCNS patients, in both remission and relapse. Monocytes were isolated from the patients using MACS-monocyte Isolation Kit II and cultured for 4 hours with and without LPS. Monocyte RNA from 5 patients in relapse and remission were converted to cRNA and hybridized into Illumina Human Ref 8 chips. Gene ontology (GO) and pathway analysis were carried out using MetaCore™. The transcription profile of unstimulated monocytes from patients with MCNS in relapse revealed >2-fold change in expression in 734 genes. GO analysis showed genes involved in inflammatory response (*IL-1R1, IL-6, LTA, TNF*) were greatly upregulated, including interferon-inducible genes (*IRF4, IRF7, IFI6, IFI27, IFI35, IFI44, SERPING1, Mx1, OAS1, OAS2, OAS3, OASL, CXCL9, CXCL10*), antiviral genes (*DDX58*) and genes involved in STAT1 pathway. However, following LPS stimulation, genes that were usually responsive to LPS (*CD86, IL-1, IL-6, TNF*) were downregulated, exhibiting a refractory state of monocytes. In conclusion, our results demonstrated the bipolar nature of monocytes in MCNS patients following infection-triggered relapse, with the presence of an inflammatory profile *in-vivo*, but which appeared refractory to LPS stimulation suggesting an anti-inflammatory or endotoxin-tolerance profile. This could explain the increased susceptibility to infections during MCNS relapses.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2828**

**Activation of Toll-Like Receptor 3 Induces Proteinuria and Glomerular CD80, and Increases Urinary CD80 in Mice**
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We have reported that children with biopsy-proven minimal change disease express CD80 in their podocytes and excrete high levels of CD80 (also known as B7.1) in their urine during active nephrotic syndrome. We also reported that polyIC, a Toll-like receptor 3 (TLR3) ligand, increases CD80 mRNA and protein in cultured human podocytes dose-dependently, with actin re-organization and a reduction in synaptopodin expression.

To determine the effect of polyIC in the kidney, C57BL6/J mice (male, 6 weeks-old) underwent systemic injection of polyIC or PBS. Urine, blood and kidney tissue were collected at 6 h, 24 h, 1 week and 2 weeks. Mice injected with polyIC developed significant proteinuria compared to PBS injected controls with a peak at 6 h. CD80 production in the kidney was measured by ELISA, and was significantly increased in mice injected with polyIC at 6 and 24 h (2.9 fold increase vs. PBS), with increased CD80 mRNA expression peaking at 6 h (16 fold increase vs. PBS). Urinary CD80 concentration was significantly increased in mice with polyIC. In pathological examination, glomeruli from mice injected with polyIC were normal (PAS staining). To examine the effect of systemically injected polyIC in the glomeruli, we isolated the glomeruli from mice injected with polyIC or PBS at 6 h, and examined the mRNA expression of CD80, CTLA-4, IL-10 and synaptopodin by QPCR. In the glomeruli, CD80 and IL-10 were significantly increased (CD80, 2.9 Fold vs. PBS) with a mild increase of CTLA-4. Synaptopodin in the glomeruli was decreased significantly.

Our study demonstrates that systemically administered polyIC can induce transient proteinuria and urinary CD80 excretion with increased glomerular CD80 and reduced synaptopodin expression as in cultured human podocyte. These findings may be relevant to the pathogenesis of minimal change disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2829**

**Mice with Higher Angiotensinogen (Agt) Copies Show Exacerbation of *In Situ* HIV-Associated Nephropathy (HIVAN)**
Dileep Kumar,[1] Sandeep Magoon,[1] Deepti D. Torri,[1] Hersh Goel,[1] Swapna Sayeneni,[1] Ashwani Malhotra,[1] Praveen N. Chander,[2] Pravin C. Singhal.[1] *[1]Medicine, Long Island Jewish Medical Center, New Hyde Park, NY; [2]Pathology, New York Medical College, Valhalla, NY.*

Incidence of AIDS-associated complications has reportedly declined since the advent and utilization of highly active anti-retroviral therapy (HAART). Moreover, HIV-infected patients treated with HAART show higher longevity and a normal life style. It has been expected that in post-HAART era, the spectrum of HIV-related diseases will also change. We hypothesized that after an HIV-1-induced initial injury, adverse environment such as elevated levels of Ang II may exacerbate *clinically occult* (minimal or submicroscopic) HIV renal lesions incurred during the period of significant viral loads may progress into an overt HIVAN phenotype despite use of HAART in the later period. We further hypothesized that not only timing of the institution of HAART but also the host factors may determine the development of an overt HIVAN phenotype.

Vpr mice (a kind gift From Prof. Jeffery Kopp, NIH) were bred with FVB/N mice with four angiotensinogen (Agt) copies to generate Vpr mice with 2, 3, and 4 Agt copies. By three weeks, doxycycline (doxy)-fed Vpr mice (2 Agt copies) display either minimal or no microscopic renal lesions, whereas, six weeks of doxy-treatment is required to develop an overt HIVAN phenotype. Eight weeks old Vpr mice (five mice per group) with variable copies of angiotensinogen (Vpr-Agt 2, Vpr-Agt 3 and Vpr-Agt 4) were fed drinking water containing doxy for three weeks. After three weeks, doxy was withheld from the drinking water. These mice were sacrificed at the end of 6 wks; kidneys were isolated and evaluated for renal histology.

Vpr-Agt-2 mice revealed no glomerular lesions and only mild tubular dilatation without any microcyst formation. Vpr-Agt-3 mice showed mild mesangial expansion and minimal tubular dilatation; whereas, Vpr-Agt-4 mice showed glomerular sclerosis albeit, mild and moderate tubular dilatation.

These findings indicate that higher Ang II levels allow the progression of *clinically occult* renal lesions into an overt HIVAN phenotype.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2830**

**Exogenous ATP Metabolism by Human CD39 Overexpression Limits Adriamycin Nephropathy: Role for Both Local Expression and Circulating Cells Including Tregs**
Yuan Min Wang,[1] Jennifer L. Mcrae,[3] Simon C. Robson,[4] Anthony J. F. d'Apice,[3] Peter J. Cowan,[3] Geoff Yu Zhang,[1] Min Hu,[1] Tania Polhill,[1] Yiping Wang,[2] Ya Wang,[2] Vincent W. S. Lee,[2] Guoping Zheng,[2] David C. Harris,[2] Karen M. Dwyer,[3] Stephen I. Alexander.[1] *[1]Centre for Kidney Research, Children's Hospital at Westmead, The University of Sydney, Sydney, NSW, Australia; [2]Centre for Transplantation and Renal Research, University of Sydney at Westmead Millennium Institute, Sydney, NSW, Australia; [3]Immunology Research Centre, St. Vincent's Health, Melbourne, VIC, Australia; [4]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.*

**Background:** CD39 is a dominant ectoenzyme that degrades ATP to AMP and is highly expressed on *Foxp3*+ regulatory T cells (Tregs) in both mice and humans. Human CD39 transgenic mice on the BALB/c background have been generated. **Aims:** The aim of the study was to evaluate a key Treg molecule CD39 *in vivo* in Adriamycin Nephropathy (AN) then to define where CD39 exerted its effect. **Methods:** Human-CD39 transgenic mice (CD39Tg) and wild type mice (WT) were treated with ADR. Then mixed chimeric mice were generated and AN was induced in four groups of mice as donors→ recipients (WT→Tg, WT→WT, Tg→WT, Tg→Tg). Finally adoptive transfer of CD25+ and CD25- T cells isolated from both CD39Tg and WT in AN was performed. **Results:** CD39Tg mice were protected from renal injury. Urinary protein and serum creatinine were significantly reduced, and there was significantly less glomerulosclerosis, tubular damage, and less fibrosis in, compared with WT ($P<0.05$ and $p<0.01$). Mixed chimeras showed that tissue and circulating CD39 was most effective in protecting against renal injury; though local and circulating expression alone also provided protection compared to WT to WT mice. Expression of hCD39 on CD25- cells was protective as were WT CD25 Tregs and hCD39 CD25+ Tregs. **Conclusions:** CD39 protects against AN. Tissue expression of CD39 and circulating CD39 including that on Tregs contributes to this protection. This supports a role of purines as mediators of injury and the key role of enzymes involved in purine metabolism.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2831**

**Cytoskeleton Proteins Promote HIV Accumulation by Expressing DC-Specific ICAM-3-Grabbing Nonintegrin (DC-SIGN) Expression by Human Podocytes**
Dileep Kumar, Divya Salhan, Shabina Rehman, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.*

The occurrence of the collapsing variant of glomerulosclerosis in HIVAN (HIV-associated nephropathy) patients highlighted the role of HIV-1 in the development of characteristic podocyte phenotype. However, the involved mechanism of HIV-1 entry is far from clear. Recently, we demonstrated that DC-SIGN facilitated HIV-1 entry into conditionally immortalized human podocytes (CIHPs) (J Am Soc Nephrol 2009). Similarly, DEC-205 acted as a receptor for HIV-1 entry into human tubular cells (J Am Soc. Nephrol. 18:780-787, 2007). In the present study we examined the role of cytoskeleton proteins in podocyte viral accumulation.

CIHPs were incubated with R5 and X4 HIV-1 primary strains for two hours, followed by trypsiniztion and repeated washings. Subsequently, intracellular viral presence was assayed by p24 ELISA, RT-PCR, and electron microscopic studies at different time periods. CIHPs rapidly internalized R5 and X4 HIV-1 primary strains via endocytosis, without establishing a productive infection. Electron microscopic studies confirmed the presence of HIV-1 in podocytes. Pre-treatment with anti-DC-SIGN antibody inhibited HIV-1 entry into podocytes. Similarly, CIHPs transfected with siRNA/DC-SIGN showed attenuated HIV-1 entry into podocytes.Pretreatment of CIHPs with both colchicine and cytochalasin B increased podocyte HIV-1 accumulation and thus, showed the role of actin and microtubules in the podocyte viral trafficking. Since Brefeldin A (an inhibitor of the Golgi-ER pathway) also enhanced podocyte HIV-1 accumulation, it seemed that Golgi-ER pathway might be linked to podocyte viral sorting. To determine the involved mechanism of cytoskeleton protein-

mediated enhanced podocyte viral accumulation, we evaluated the effect of colchicine, cytochalasin B and Brefedin A on podocyte expression of DC-SIGN. Interestingly, CIHPs treated with colchicine, cytochalasin B, and Brefedin A showed a two-fold increase in DC-SIGN expression. We conclude that cytoskeleton proteins promote podocyte HIV-1 accumulation by enhancing podocyte expression of DC-SIGN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2832**

**B7-1 Mediated Danger Signalling in Podocyte Injury in IL-13 Overexpression Rat Model of Minimal Change-Like Nephropathy (MCN)** Chang Yien Chan,[1] Tarun K. Maheshwari,[1] Jinmiao Chen,[2] Caroline G. L. Lee,[1] Subhra K. Biswas,[2] Henry Yang,[2] Hui Kim Yap.[1] *[1]Pediatrics, National University of Singapore, Singapore; [2]SIgN, BMSI (A*Star), Singapore.*

We have previously shown that *interleukin-13 (IL-13)* overexpression in rats can result in MCN accompanied by podocyte injury with downregulation of slit diaphragm proteins. This was associated with increase in glomerular expression of IL-13 receptor subunits and B7-1 at both gene and protein level. This study examined the *in-vitro* effect of IL-13 on podocyte B7-1 expression, and subsequently profiled the differentially regulated molecules in the glomerular B7-1 danger signaling pathways that resulted in podocyte effacement in the *IL-13* overexpression rat model of MCN. RNA isolated from conditionally immortalized murine podocyte cell line (H-2Kb-tsA58), unstimulated and stimulated with mouse recombinant IL-13, were used for real-time PCR analysis. RNA from the glomeruli of 6 control and 6 *IL-13* transfected rats with MCN were reverse transcribed and hybridized into Illumina Rat Ref12 microarray chips. Gene ontology and pathway analysis were carried out using MetaCore™. *In-vitro* experiments on IL-13-stimulated podocytes showed significant upregulation of *IL-13Rα2* and *B7-1* (0.00056±0.00015 and 1.05±0.49 respectively), compared to controls (0.000077±0.000015 and 0.59±0.36 respectively) ($p<0.001$). Microarray analysis of the glomeruli showed 1120 differentially expressed genes with >1.5-fold change in expression. Genes involved in *B7-1* transcription, *JAK3*, *PIK3cd*, and *Rel*, and podocyte cytoskeleton remodeling were upregulated in the glomeruli of nephrotic rats. This was associated with downregulation of podocyte slit diaphragm molecules, *NEPH2*, *nephrin*, *podocin*, *JAM4*, *cadherin-1*, *cadherin-11*, and *cadherin-16*, actin cytoskeleton molecules *ZO-1*, *Nck2*, *Synpo*, *MAGI-2*, *α-catenin* and *α-actinin-4*, podocyte basal and apical membrane domain protein complex molecules, *dystroglycan*, *α3β1 integrin*, *GLEPP1* and linkage molecule *Ezrin*. In conclusion, our results suggest that IL-13 can act directly on podocytes by activating the B7-1 danger signaling pathway which then acts on cytoskeleton and slit diaphragm molecules causing podocyte effacement.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2833**

**Human Proximal Tubule Epithelial Cells Modulate Autologous Immunity** Kerry (Kathrein) E. Roper, Xiangju Wang, Helen G. Healy, Ray Wilkinson. *Renal Laboratory, Dept. of Renal Medicine, Royal Brisbane and Women's Hospital/Queensland Health, Brisbane, Queensland, Australia.*

**Background:** The role of proximal tubular epithelial cells (PTECs) in the immunology of renal disease has largely been deduced through animal models and transformed cell lines. We aimed to understand if PTECs mediate human autologous immunity in renal pathology by similar pathways.

**Methods:** Primary PTECs and peripheral blood mononuclear cells (PBMC) were collected from patients undergoing nephrectomies at RBWH. PBMC and purified T and B lymphocytes were cultured with activated autologous PTECs in conditions mimicking immunological signaling ie specific Ag (TT, KLH), cross-linking Ab (CD3/CD28, IgM) and mitogen (PHA, PWM). Responses were monitored by proliferation, cytokine production and surface Ag expression.

**Results:** PTECs activated by IFNγ expressed 5-10 fold more PD-L1 and up to 5 fold more HLA-DR then resting PTECs. Our findings indicate that these activated autologous PTECs significantly suppress the proliferation response of PBMC, CD4+, CD8+ T cell and B cell populations to PHA and/or cross-linking Abs and that this was partially reversed with the addition of blocking antibodies to PD-L1. CD4+ proliferative responses to both naïve and recall Ags were also decreased. Interestingly, maturation/activation markers, particularly CD69 and CD38, were increased in the presence of PTECs. Cytokine profiles were also modulated by autologous PTECs, with decreased expression of IFNγ, IL-2, IL-10 and TNF and an increased expression of IL-4 in most cell populations. There was no significant effect on the levels of Treg cells in any of our cultures.

**Discussion:** We have shown that the inhibitory effect of activated PTECs on both T and B cell proliferation occurs in response to cross-linking Ab and specific Ag, as well as polyclonal activation through mitogen stimulation. Together our results suggest that PTECs act to modulate human autologous immunity via complex interactions with immune cells and this is partially mediated by PD-L1. Further dissection of the mechanism of PTECs modulation of autologous immune responses may prove beneficial for clinical intervention or monitoring in renal medicine.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2834**

**Renoprotective Effects of Kv1.3 Blocker in Rats with Anti-Glomerular Basement Membrane Glomerulonephritis** Toshitake Hyodo,[1] Takashi Oda,[1] Keishi Higashi,[1] Kojiro Yamamoto,[1] Taketoshi Kushiyama,[1] Naoki Oshima,[1] Soichiro Miura,[2] Hiroo Kumagai.[1] *[1]Department of Nephrology, National Defense Medical College, Tokorozawa, Saitama, Japan; [2]Department of Internal Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan.*

We previously reported that the blocker (Psora-4) against a voltage-gated K channel, Kv1.3, could reduce the level of proteinuria and the ratio of crescentic glomeruli in the model of rats with anti-glomerular basement membrane (GBM) glomerulonephritis (GN). In the present study, we investigated the mechanisms for this renoprotective effect of Psora-4 by analyzing phenotypes of renal infiltrating cells. Flow cytometric analysis of kidney cells of anti-GBM GN rats revealed that most of the $CD4^+$ T cells showed the effector memory Tcell ($T_{[sup]EM[/sup]}$) phenotype ($CD45RC^-CD62L^-$). Furthermore, flow cytometric analysis of T cells isolated by magnetic cell sorting from peripheral blood (PB) and from kidney samples of anti-GBM GN revealed that T cells from the kidney samples were mostly $CD62L^-$ cells while those from PB included numerous $CD62L^+$ cells. A higher intensity of Kv1.3 expression was observed on the $CD62L^-$ T cells (corresponding to $T_{[sup]EM[/sup]}$) than on the $CD62L^+$ T cells (corresponding to naive or central memory T cells). We also confirmed the expression of Kv1.3 channels on isolated T cells from kidney samples of anti-GBM GN by double immunofluorescence staining. These results suggest that Kv1.3 channels expressed on $T_{[sup]EM[/sup]}$ play a critical role in the pathogenesis of rat crescentic GN, and therefore, Psora-4 might be more specific and efficient treatment for patients with rapidly progressive GN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2835**

**Renal-Associated NLRP3 Mediates Renal Dysfunction Following Ischemia/Reperfusion Injury** Pieter J. Bakker,[1] Ingrid Stroo,[1] Loes Butter,[1] Richard A. Flavell,[2] Sandrine Florquin,[1] Jaklien Leemans.[1] *[1]Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; [2]Immunobiology, Yale University School of Medicine, New Haven, CT.*

Renal ischemia/reperfusion injury (IRI) can be characterized as a sterile inflammation where danger-associated molecular patterns are released that subsequently activate Toll-like receptors and Nod-like receptors. It has previously been established that the NLRP3-dependent inflammasome contributes to renal inflammation in IRI. NLRP3 -/- mice have decreased mortality in case of lethal IRI. NLRP3 is expressed on inflammatory cells and also on renal tubular epithelial cells. To date, it is not yet known what the role is of renal- or leukocyte-associated cells NLRP3 in IRI.

A bone marrow transplantation was carried out. C57Bl/6J wild-type (wt) or NLRP3 -/- mice were subjected to 2 doses of sub-lethal irradiation followed by an i.v. injection of splenocytes and bone marrow cells, either derived from wt or NLRP3 -/- mice. Six weeks after bone marrow transplantation, IRI was induced by clamping renal arteries and mice were sacrificed the following day. Blood was collected via heart puncture. Kidneys were formalin fixed and paraffin-embedded. Kidney sections were used for immunohistochemistry.

Apoptosis (Caspase-3+) and proliferation (Ki67+) of tubular epithelium was similar in all groups. In concordance, tubular necrosis (PAS-D scores) was also equal in all groups. Despite equal damage, influx of leukocytes differed between groups. Nalp3-/- bone marrow, Nalp3-/- parenchyma (ko→ko) mice had a significant lower influx of granulocytes (Ly6+) and macrophages (F4/80+) compared to wt-bone marrow, wt-parenchyma (wt→wt) mice. Both chimeras (wt→ko and ko→wt) showed a tendency of lower granulocyte and macrophage influx. Finally, creatine levels were significantly lower in ko→ko mice and wt→ko compared to wt→wt mice.

In conclusion, renal-associated NLRP3 deficiency limits renal dysfunction but not leukocyte-associated NLRP3 deficiency following IRI. Moreover, a NLRP3 deficiency-related reduction of macrophage and granulocyte influx is observed.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2836**

**Investigating Lymphocyte Function during Tubulointerstitial Injury in the Native Kidney** Victoria J. Ingham, Neil S. Sheerin. *Institute of Cellular Medicine, Newcastle University, Newcastle-upon-Tyne, United Kingdom.*

Tubulointerstitial (TI) inflammation and fibrosis is seen in progressive renal failure irrespective of aetiology. If infiltrating T lymphocytes demonstrated clonal proliferation within injured kidney, this would suggest a break in self tolerance and evidence for the role of TI lymphocytes in non-immunologically mediated renal injury.

Methods

The unilateral ureteric obstruction (UUO) model was used. There are 22 T Cell Receptor (TCR) Variable genes on the β chain (TRVβ) however only one is expressed by any T cell. Real time PCR with primers specific to the 22 TRVβ genes was used to assess restriction of TRVβ gene usage in kidney compared to spleen. Over expressed TRVβ gene PCR products were sequenced. Lymphocytes were extracted from digested UUO kidney and sorted by FACS into CD4+ and CD8+ populations and sequenced.

Results

T cells expressing TRVβ3 were over represented in the UUO kidney compared to the spleen. This was not seen in normal animals. In each day 7 and 14 UUO kidney 20-30% of TCR sequences expressing TRVβ3 were identical. Such a clonal population was not seen in UUO spleen. In normal kidney there were also identical T cells accounting for around 1/3rd of all lymphocytes expressing TRVβ3, again not seen in normal spleen.

Multiple identical TCR sequences were demonstrated in the sorted CD8+ population from UUO kidney.

Short, identical amino acid sequences in the CDR3 region were found in the clonal T cell population of both UUO and normal kidneys.

Conclusions

In day 7 and 14 UUO kidneys a large clonal population of T cells were seen, suggesting loss of tolerance to self and possibly renal autoantigen. This was less evident at day 28 and may be due to epitope spreading or chemokine mediated recruitment of polyclonal T cells. The clonal population of T cells within normal kidney may represent an anergic or regulatory population of T cells.

The T cell clone from both UUO and normal kidneys had an identical amino acid motif within the CDR3 region, suggesting T cells in normal kidney may proliferate in response to injury. Understanding the role of lymphocytes in non-immunologically mediated renal injury may guide therapeutic strategies in chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2837**

**Regulatory Renal Dendritic Cells Provide Innate Cytoprotection to Podocytes in Endotoxemia** Bettina Burnworth,[1] Nicole Kahoud,[1] Leslie A. Bruggeman,[2] Kelly D. Smith,[1] Peter J. Nelson.[1] *[1]University of Washington, Seattle, WA; [2]MetroHealth Medical Center, Cleveland, OH.*

Mechanisms of peripheral tolerance in the kidney that protect podocytes from injury during auto-inflammatory insults are poorly understood; however, within other non-lymphoid tissues, surveying conventional dendritic cells present at steady-state (ssDCs) perform this role, producing regulatory cytokines that maintain local tissue homeostasis during the innate phases of peripheral tolerance. Thus, we asked whether ssDCs ameliorate podocyte injury and albuminuria during endotoxemia, a model system to decipher inflammatory *versus* regulatory innate responses. Specific pathogen free (SPF) mice exhibited a dose-dependent threshold to albuminuria from LPS. In contrast, SPF mice depleted of renal ssDCs developed significant albuminuria with focal podocyte foot process effacement from normally sub-proteinuric LPS. Parallel examination of intra-renal IL-10 showed that normal kidneys, but not kidneys minus renal ssDCs, significantly up-regulated IL-10 from sub-proteinuric LPS. Opposite the cytokine response by inflammatory GM-CSF-derived DCs, renal ssDCs challenged with LPS *ex vivo* secreted high IL-10-to-TNF-α ratios, confirming renal ssDCs as a key regulatory source of intra-renal IL-10. Because heme oxygenase- 1 (HO-1) is a mediator of innate cytoprotection by IL-10, we investigated this candidate mechanistic pathway for podocytes *ex vivo*. Quiescent podocytes expressed IL-10 receptor and responded to soluble IL-10 by inducing HO-1, abrogating activation of NF-κB in podocytes by LPS. Together, these results suggest that renal ssDCs are regulatory and provide innate cytoprotection to podocytes, setting an important threshold to proteinuria during auto-inflammatory insults. Research is ongoing to determine whether the IL-10-HO-1 axis or additional pathways are responsible for setting this threshold.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2838**

**Analysis of Aberrant *O*-Glycosylation of IgA1 in Patients with IgA Nephropathy (IgAN): Bacterial IgA-Specific Proteases and High-Resolution Mass Spectrometry (MS)** Hitoshi Suzuki,[1,2] Kazuo Takahashi,[1] Zina Moldoveanu,[1] Bruce A. Julian,[1] Mogens Kilian,[3] Knud Poulsen,[3] Yasuhiko Tomino,[2] Jiri F. Mestecky,[1] Matthew B. Renfrow,[1] Jan Novak.[1] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]Juntendo University Faculty of Medicine, Tokyo, Japan; [3]Aarhus University, Aarhus, Denmark.*

IgA1 in the circulation of patients with IgAN is aberrantly glycosylated; some of the multiple *O*-glycans in the hinge region (HR) are galactose (Gal)-deficient. These aberrant glycans are recognized by anti-glycan antibodies, resulting in formation of pathogenic immune complexes. *O*-glycans on IgA1 secreted by IgA1-producing cells from patients with IgAN (IgAN cells), but not from controls, is Gal-deficient with sialylated or terminal GalNAc, due to decreased β1,3-galactosyltransferase and elevated α2,6-GalNAc-sialyltransferase II enzyme activities in IgAN cells. It is not known whether the Gal deficiency occurs randomly or preferentially at specific sites and which sites are recognized by anti-glycan antibodies. To address this question, we analyzed glycosylation and IgG binding of IgA1 produced by IgA1-secreting cell lines from IgAN patients and healthy controls. *O*-glycosylation of these proteins was analyzed by lectin- and antibody-western blotting after digestion with a panel of bacterial IgA-specific proteases that cleave different sites in the HR. In IgA1 from IgAN patients, Gal-deficient glycans, predominantly in the middle part of the HR with a fraction also in the C-terminal part, bound IgG antibody. For high-resolution MS analysis, IgA1 proteins were digested with an IgA-specific protease followed by trypsin. Three to six *O*-glycans per HR with up to three Gal-deficient sites were detected. Relative abundance of glycopeptides was independently calculated by area under the curve from extracted ion chromatogram. Sialylated glycopeptides with Gal-deficient GalNAc were elevated in IgA1 from IgAN patients. In summary, Gal-deficient glycans are localized at specific sites of the HR and are recognized by anti-glycan antibodies. These sites can be localized by western blotting after digestion with a panel of bacterial IgA proteases and by high-resolution MS analysis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2839**

**Synthesis of Galactose (Gal)-Deficient IgA1 *O*-Glycans by GalNAc-Transferase 2 and Pathogenesis of IgA Nephropathy (IgAN)** Kazuo Takahashi,[1] Milada Horynova,[2] Milan Raska,[2] Stacy D. Hall,[1] Archer D. Smith,[1] Bruce A. Julian,[1] Zina Moldoveanu,[1] Jiri F. Mestecky,[1] Matthew B. Renfrow,[1] Jan Novak.[1] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]Palacky University in Olomouc, Olomouc, Czech Republic.*

In IgAN, IgA1 with Gal-deficient *O*-glycans in the hinge region (HR) is in the circulating immune complexes (IC) and in mesangial immunodeposits. Terminal *N*-acetylgalactosamine (GalNAc) in the Gal-deficient IgA1 is recognized by anti-glycan antibodies, resulting in formation of IC that may deposit in the kidney. HR of IgA1 has up to 6 of the 9 potential *O*-glycosylation sites occupied; some of these glycans may be Gal-deficient. *O*-glycosylation of IgA1 is initiated by a GalNAc-transferase (GalNAc-T), namely GalNAc-T2. As its abnormal localization or activity in IgA1-producing cells can lead to production of Gal-deficient IgA1, it is important to understand the kinetics and site-specificity of this enzyme. We produced recombinant GalNAc-T2 using insect cells and developed an *in vitro* system to study the kinetics of site-specific glycosylation using high-resolution mass spectrometry (MS). Time course of the enzyme reaction with synthetic HR (sHR) (VPSTPPTPSPSTPPTPSPSC) as acceptor was monitored by liquid chromatography (LC) coupled with Fourier transform ion cyclotron resonance (FT-ICR) MS and the glycosylated sites were determined by tandem MS. Our results showed that the number of GalNAc residues added to the sHR increased with time, the nonglycosylated sHR was completely consumed in 10 min; within 15 min, sHR had 3 to 6 GalNAc residues attached. Glycosylation sites were consistent with the sites previously described (VPS***T***PP***T***P***S***P***S***TPP***T***PSPSC; underlined S/T residues were glycosylated), and included those that are Gal-deficient in IgA1 (marked in bold and italics). Moreover, the two side-by-side S-T sites were alternatively glycosylated by one GalNAc each and were the last to be glycosylated. In summary, these data show that GalNAc-T2 can add GalNAc to all glycosylated sites in HR of IgA1, including those that are Gal-deficient. Thus, detailed studies of GalNAc-T2 kinetics and specificity will provide new information relevant to the pathogenesis of IgAN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2840**

**TLR2 Induces Th17 Nephritogenic Myeloperoxidase Autoimmunity, While TLR9 Drives Th1 Autoimmunity** Shaun Andrew Summers, Oliver M. Steinmetz, Poh-Yi Gan, A. Richard Kitching, Stephen R. Holdsworth. *Medicine, Monash Medical Centre, Melbourne, Victoria, Australia.*

**Aim:** To define a link between infections and ANCA vasculitis, we explored the role of Toll-like receptor (TLR) 2 and TLR9 in experimental anti-myeloperoxidase (MPO) induced autoimmunity and glomerulonephritis.

**Methods:** To determine the role of TLR2 and TLR9 in anti-MPO autoimmunity C57BL/6 wild type (WT) mice were immunized with MPO alone, MPO+TLR2 ligand (L), Pam3CSK4, or MPO+TLR9(L), Cpg-ODN. Immune responses were studied on day 6 and 28. Anti-MPO glomerulonephritis was triggered by a sub-nephritogenic dose of nephrotoxic serum (NTS), glomerular injury was assessed 4 days later.

**Results:** Six days after immunization with MPO alone, MPO+TLR2(L) or MPO+TLR9(L) antigen-specific systemic production of IFNγ was increased only in mice immunized with MPO+TLR9(L) (MPO alone 336±291, MPO+TLR2(L) 830±172, MPO+TLR9(L) 3588±588pg/ml, $p<0.001$ compared to all other groups). Conversely, IL-17A production was increased only in WT mice immunized with MPO+TLR2(L), (MPO alone 24±10, MPO+TLR2(L) 1394±470, MPO+TLR9(L) 312±109pg/ml, $p<0.05$ compared to all other groups). There was no difference in IL-4 production between the groups. On day 28, IFNγ production in mice treated with MPO+TLR9(L) and IL-17A production in mice treated with MPO+TLR2(L) remained significantly elevated. On day 28 anti-MPO IgG was also increased in mice immunized with MPO and either TLR(L). Administration of a sub-nephritogenic dose of NTS resulted in increased albuminuria, (MPO alone 0.4±0.2, MPO+TLR2(L) 4.7±1.6, MPO+TLR9(L) 6.2±1.7mg/24hours, $p<0.05$) and histological renal injury in mice immunized with MPO + either TLR(L). TLR2(L)+MPO induced glomerulonephritis was neutrophil mediated and attenuated after treatment with monoclonal anti-IL17A neutralizing antibody (24±2% vs. 16±2% abnormal glomeruli, $p<0.05$). TLR9(L)+MPO induced glomerulonephritis was predominantly macrophage mediated, injury was attenuated after administration of anti-IFNγ therapy (25±3% vs. 18±1% abnormal glomeruli, $p<0.05$).

**Conclusion:** TLR2 induces Th17 mediated autoimmunity to MPO with neutrophil mediated glomerular injury. Conversely TLR9 induces Th1, macrophage mediated glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2841**

**Different Disease Phenotypes by Activation of TLR9 on Dendritic Cells (DC) and B Cells in Murine IgA Nephropathy** Tadahiro Kajiyama, Yusuke Suzuki, Hitoshi Suzuki, Satoshi Horikoshi, Yasuhiko Tomino. *Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan.*

[Background and Purpose]

Pathological roles of dendritic cells (DC) and B cells in mucosal immunity have been discussed. Recently, we demonstrated that Toll-like receptor 9 (TLR9), especially in

mucosa, has a crucial role in the pathogenesis of both human and murine IgA nephropathy (IgAN) (J Am Soc Nephrol 19: 2384-95, 2008). In the present study, we examined whether activation of TLR9 on mucosal DC and B cells contributes in different ways to the pathogenesis of murine IgAN using different cell-specific types of CpG-ODN, which is a ligand of TLR9.

[Methods]

For this study, we used three CpG-ODN, types A, B and C, which bind to TLR 9 on DC, B cells and both cells, respectively. IgAN prone mice (gddY) at 4 weeks of age were each nasally challenged with 10 ug of types A, B and C CpG-ODN at weekly intervals for 8 consecutive weeks. Blood and urine samples were collected before and after each administration. At 8 weeks after mucosal immunization, kidney samples were also collected and evaluated histopathologically.

[Results]

Urinary protein was aggravated after each administration of CpG-ODN in all mice. Serum IgA-IgG2a immune complexes were elevated in mice treated with types A and C, while serum IgA was increased in mice treated with type B. Interestingly, the mice treated with type A predominantly showed mesangial proliferative glomerular lesions, while sclerotic glomerular damage mainly with sclerotic lesions was observed in those treated with type B, suggesting that different disease phenotypes were induced.

[Conclusion]

It appears that the mucosal activation of TLR9 on DC and B cells may induce different types of glomerular damage in murine IgAN.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2842

**Colon Cancer-Burden Experimental Rats Develop Marked Proteinuria; a Possible Research Tool for Paraneoplastic Glomerulopathy** Shin-Ichi Takeda,[1] Junko Chinda,[1] Akihiko Numata,[1] Takashi Murakami,[2] Masafumi Takahashi,[2] Eiji Kusano.[1] *[1]Division of Nephrology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan; [2]Division of Bioimaging Sciences, Center for Molecular Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan.*

**Background.** Cancer patients occasionally develop renal disorders independently of direct tumor burden. In fact, a recent study (Kidney Int 70:1510-,2006) demonstrated that 10% of patients with membranous nephropathy had malignancies. We have also reported a case of crescentic glomerulonephritis who showed amelioration of renal impairment after resection of rectal cancer (Nephrol Dial Transplant Plus 3:330-,2010). However, this clinical entity remains mysterious, especially due to the lack of research tool. **Aim.** The objective of this study is to investigate whether cancer-bearing rats develop overt glomerulopathy. **Methods.** RCN-9 rat colon cancer cells ($1x10^7$) were injected into male 6-to-8-week-old F344 rats (n=6) and immunodeficient F344 rats (nude rats; n=3) via the portal vein. Urinalysis and histological examinations were performed in comparison with the control rats (n=6) that received vehicle-injection. **Results.** Metastatic growth of RCN-9 cells exclusively in the liver was observed in the cancer-injected rats, whereas direct invasion into the kidney was not evident (even microscopically). All of the cancer-injected F344 rats showed marked proteinuria (up to 158.0 mg/day) by 6 wks after cancer-injection in contrast to the controls (no more than 25.0 mg/day; $p<0.01$). Although microscopic morphological change was not evident, a large quantity of IgG was delineated by immunohistochemistry in the glomerular basement membrane zone of the proteinuric rats. Of particular interest is that none of the nude rats showed proteinuria despite of cancer growth, suggesting that T-cell-mediated immune responses are involved. **Conclusions.** As expected from the clinical perspectives, the cancer-bearing rats developed features of glomerulopathy such as proteinuria and deposition of immunological substance. Consequently, the present animal model should provide a new platform to address the underlying mechanism of paraneoplastic glomerulopathies.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2843

**The ASC-Dependent Inflammasome Protects Against Tubular Injury and Induces Inflammation Following Progressive Renal Injury** Wilco Pulskens,[1] Loes Butter,[1] Gwendoline J. D. Teske,[1] Nike Claessen,[1] Richard A. Flavell,[2] Fayyaz Sutterwala,[3] Sandrine Florquin,[1] Jaklien Leemans.[1] *[1]Pathology, AMC, Netherlands; [2]Immunobiology, Yale University; [3]Internal Medicine, University of Iowa.*

Tubulo-interstitial injury is a common finding in the chronically failing kidney and characterized by inflammation and fibrosis. The intracellular inflammasome contributes to the induction of inflammation through maturation of effector cytokines. Although the mechanism of activation is still unclear, several endogenous danger ligands are identified that can activate the inflammasome upon tissue injury. Furthermore, it is known that inflammasome components, NLRP3 and ASC are expressed in both inflammatory and epithelial cells. However, the role of the inflammasome in progressive renal injury is yet unknown.

C57Bl/6J wild type and ASC-/- mice (n=7/group) were subjected to unilateral ureter obstruction (UUO) by a permanent double ligation of the right ureter. Mice were subsequently sacrificed 1, 3, 7 and 14 days post-UUO to determine renal injury, inflammation and renal fibrosis. Renal mRNA levels were determined by quantitative RT-PCR and renal cytokines were measured by ELISA.

Following UUO, ASC mRNA remained constitutively present, whereas a strong increase of NLRP3 mRNA was observed compared to contralateral kidneys. Interestingly, ASC-/- mice displayed significantly enhanced levels of renal injury 1, 3 and 7 days post-UUO compared to wild type mice. In addition, ASC-/- mice had significantly less apoptotic tubular epithelial cells (t=14, and a tendency at t=3). Moreover, ASC deficiency resulted in reduced granulocyte influx (t=1 and 14), a reduced renal IL1β concentration (t=1) but did not affect accumulation of macrophages or myofibroblasts, total collagen deposition or renal TGFβ concentration. Preliminary data revealed that NLRP3-/- mice also had enhanced tubular injury compared to wild type mice 1, 3 and 7 days post-UUO.

The ASC-dependent inflammasome protects against tubular injury following UUO, associated with less apoptic epithelial cells. Moreover, the ASC-dependent inflammasome contributes to renal inflammation but does not affect fibrosis.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2844

**PPARδ Agonist Exerts Anti-Inflammatory Effect in Renal Proximal Tubular Cells** Xu Yang,[1,2] Shinji Kume,[1] Keiji Isshiki,[1] Masami Kanasaki,[1] Shin-Ichi Araki,[1] Toshiro Sugimoto,[1] Ping Han,[2] Detian Li,[2] Daisuke Koya,[3] Masakazu Haneda,[4] Hiroshi Maegawa,[1] Takashi Uzu.[1] *[1]Medicine, Shiga University of Medical Science, Otsu, Shiga, Japan; [2]Medicine, ShengJing Hospital of China Medical University, Shen Yang, China; [3]Medicine, Kanazawa Medical University, Kahoku-Gun, Ishikawa, Japan; [4]Medicine, Asahikawa Medical College, Asahikawa, Hokkaido, Japan.*

PPARs are the nuclear receptor family of ligand-inducible transcription factors, and exert multiple effects in various organs. PPARα and γ agonists have renoprotective effects in proteinuric kidney disease, but the therapeutic potential of PPARδ agonist remains unclear. Thus, we examined a renoprotecitve effect of GW501516, a PPARδ agonist, in free fatty acid (FFA)-bound albumin-overload model, a model to evaluate tubulointerstitial lesion in proteinuric kidney disease, and tried to indentify its molecular mechanism by in vitro study. Mice were fed on either standard diet (SD) or SD with GW501516, and intraperitoneally injected with FFA-bound albumin or PBS for 4 d. In SD group, FFA-bound albumin caused tubular damages and macrophage infiltration, and increased mRNA expression of inflammatory cytokines, TNFα and MCP-1. These alterations were all prevented by the treatment with GW501516 in vivo. Furthermore, we identified the molecular mechanism underlying the anti-inflammatory effect of GW501516 by using the cultured proximal tubular cells. GW501516 attenuated both TNFα- and FFA-bound albumin-induced MCP-1 expression through the inhibition of TAK1 activation, a downstream molecule of both toll-like receptor-4 and TNFα receptor, and subsequent NFkB(p65) nuclear translocation. These results indicate that PPARδ agonist exerted anti-inflammatory effect in renal tubular cells both in vivo and in vitro and may serve as a therapeutic candidate to attenuate the tubulointerstitial lesion in proteinuric kidney disease.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2845

**The Antigens Recognized by Anti-Glomerular Basement Membrane Antibodies Undetectable by Enzyme-Linked Immunosorbent Assays** Zhao Cui,[123] Xiao-Yu Jia,[123] Zhen Qu,[123] Rui Yang,[123] Juan Zhao,[123] Ming Hui Zhao.[123] *[1]Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China; [2]Institute of Nephrology, Peking University, Beijing, China; [3]Key Laboratory of Renal Disease, Ministry of Health of China, Beijing, China.*

**Background:** Cases with anti-glomerular basement membrane (GBM) disease have been reported with linear deposition of IgG along GBM, but undetectable anti-GBM autoantibodies in circulation by ELISA. We speculated that the structure of antigens recognized by these antibodies may contribute to the negative results of ELISA.

**Methods:** Sera from four patients were collected, with typical linear deposition of IgG along GBM but no anti-GBM reactivity by ELISA. Circulating anti-GBM antibodies were detected by indirect immunofluorescence. Antigen specificity was identified by ELISA and Western-blot analysis, using purified bovine α(IV)NC1 and recombinant human α1~α5(IV) NC1 as antigens. The conformational structure of antigens was investigated by Western-blot analysis by preparing antigens under different conditions.

**Results:** The presence of circulating anti-GBM antibodies was confirmed by indirect immunofluorescence with linear deposition of IgG towards the cryptic epitopes along GBM on normal kidney sections. These antibodies did not recognize purified bovine α(IV)NC1 or recombinant human α1, α2, α4 or α5(IV)NC1 by ELISA and Western-blot analysis, but could blot recombinant human α3(IV)NC1 under non-reducing non-boiling condition, under such condition, the conformational epitope(s) on α3(IV)NC1 were thought to be preserved. When α3(IV)NC1 was prepared under reducing condition with β-mercaptoethanol and/or boiled to destroy the disulfide bonds, the binding with these antibodies disappeared.

**Conclusion:** Anti-GBM autoantibodies in circulation could recognize the cryptic and conformational dependent epitopes on α3(IV)NC1, which were pathogenic and may contribute to the negative results of ELISA.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2846**

**RNase 7 Expression in the Human Kidney and Urinary Tract** John D. Spencer, Andrew L. Schwaderer, Kirk M. McHugh, David S. Hains. *The Research Institute, Nationwide Children's Hospital, Columbus, OH.*

**Background**: Urinary tract infections (UTI) are a common bacterial infection. While many consider the urinary tract sterile, little is known how the body maintains sterility. Recent studies stress the importance of antimicrobial peptides (AMP) in preventing infection. Ribonuclease 7 (RNase7) is an important AMP that has been studied in the skin. RNase7's role in the epithelium of the urinary tract is unknown.

**Objective**: To characterize gene and protein expression of RNase 7 in the human kidney and urinary tract

**Design/Methods**: *Gene expression*: We isolated RNA from human kidney, ureter, and bladder tissue harvested from non-infected surgical pediatric patients. Isolated mRNA was reverse transcribed and quantified using real-time PCR. *Protein expression*: RNAse 7 expression was localized using immunohistostaining. To examine RNase7 protein expression in the urine, we developed a sandwich ELISA using two distinct antibodies to RNase7 and normalized urine concentrations to mg of creatinine.

**Results**: *Gene expression*: Constitutive *RNase7* mRNA expression was detected in human kidney, ureter, and bladder tissue. Absolute quantification using real-time PCR and a standard curve revealed that *RNase7* is expressed (ng RNase7/ 10 ng total RNA) in the renal cortex at 270, outer medulla 190, and renal pelvis 170. *Protein expression*: Immunohistochemistry localized RNase 7 to the urothelium of the bladder, ureter, and a subset of cells in the collecting duct. Specifically, immunofluorescence localized RNase7 to intercalated cells of the collecting duct. RNase 7 was detected in non-infected human urine samples. Control urine normalized to urine creatinine demonstrated RNase7 protein expression ranging from 1.5 to 6.6 (mcg/mg creatinine) with a mean SD of 4.1 +/- 1.9.

**Conclusion**: Our results characterize the expression a novel AMP, RNase 7, in the human kidney and urinary tract. *RNAse7* is expressed in the kidney, ureter, and bladder. Within the kidney, mRNA expression is greatest in the renal cortex. This potent AMP is specifically expressed in intercalated cells. In addition to maintaining acid-base homeostasis, intercalated cells have a role in the innate defense of the urinary tract.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2847**

**Ontogeny of the Goodpasture Autoantigen: Structural Alterations of Collagen IV Decrypting NC1 Autoepitopes during Glomerular Aging** Dorin-Bogdan Borza,[1] Yoshikazu Sado,[2] Wentian Luo.[1] *[1]Dept of Medicine (Nephrology), Vanderbilt University School of Medicine, Nashville, TN; [2]Shigei Medical Research Institute, Okayama, Japan.*

In Goodpasture (GP) disease, autoantibodies targeting the noncollagenous (NC1) domain of α3(IV) collagen in the glomerular basement membrane (GBM) mediate rapid progressive glomerulonephritis. How GP autoantibodies are elicited is unclear since their autoepitopes are cryptic within the NC1 hexamers formed by canonical assembly of α3α4α5(IV) collagen in the GBM. Because GP autoantibodies bind to the GBM of adults but not infants, we tested the hypothesis that the native GP autoantigen arises from age-dependent structural alterations of α3(IV) collagen. We found that α3-α5NC1 epitopes cryptic in the infant GBM were naturally unmasked in the adult GBM, which also contained a higher proportion of NC1 monomers. These differences were due to the occurrence in the adult but not infant GBM of distinct collagen IV isoforms, characterized by predominantly monomeric NC1 domains not assembled into hexamers, which included α3NC1 isoforms with naturally decrypted GP autoepitopes. Similar age-associated alterations of GBM collagen IV were observed in squirrel monkeys but were minimal in mice. These findings establish that structurally distinct isoforms of collagen IV are naturally produced and accumulate in the GBM during glomerular aging, without eliciting autoimmune pathology in most individuals. However, α3(IV) collagen isoforms with decrypted autoepitopes have the hallmark features of the autoantigen inciting the production of GP autoantibodies that they natively bind, presumably due to a breach in peripheral immune tolerance.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2848**

**Therapeutic Potential of Adipose-Derived Stem Cells for Anti-GBM Glomerulonephritis** Kazuhiro Furuhashi, Naotake Tsuboi, Hansu Kim, Takayuki Katsuno, Yosuke Saka, Takenori Ozaki, Waichi Sato, Enyu Imai, Yasuhiko Ito, Seiichi Matsuo, Shoichi Maruyama. *Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.*

**Introduction:** We have reported that adipose tissue-derived stem cells (ASCs), or mesenchymal stem cells (MSCs) form fat, promote regeneration in a rat model of acute kidney injury. More recently, MSCs have been shown to modulate immune reaction, suggesting that ASCs can be applied to the treatment for immune-mediated diseases. In the present study, we examined the renoprotective effects of ASCs focusing on their immunomodulatory properties.

**Methods:** Necrotizing crescentic glomerulonephritis was induced in WKY rats by intraperitoneal injection of anti-rat GBM mAb. Rat ASCs or control media were intravenously given everyday from day 0 through day 5. Renal function and renal histology were assessed on day 7. For cell tracking experiment, ASCs were stained with CFSE, and then were injected into animals.

**Results:** Intravenous injection of ASCs significantly prevented rats from renal dysfunction and proteinuria after administration of anti-GBM IgG. The number of glomeruli with crescent formation was significantly decreased in the ASCs group compared to control group. Interestingly, infiltration of ED2-positive immunoregulatory macrophages (M2) in glomerular crescents was increased in ASCs group despite comparable number of ED1-positive cells (M1) to control group. IL-10 concentration in renal cortex in diseased animal was higher in ASC group than in control group. IL10 concentration correlated closely with the number of ED2. Prominent number of CSFE-positive exogenous ASCs was observed in lung and spleen in contrast to their faint infiltration into kidneys, suggesting that ASCs can centrally demonstrate their immunoregulatory properties.

**Conclusion:** ASCs exert profound immunoregulatory properties especially on macrophages and ameliorate glomerular injury in a rat model of anti-GBM glomerulonephritis. The present study suggests that ASCs may provide a novel therapeutic approach for human crescentic glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2849**

**Tolerance Mechanisms Regulating Goodpasture Anti-Collagen Autoimmunity** Amy G. Clark,[1,2] Melissa L. Weston,[1,2] Mary H. Foster.[1,2] *[1]Medicine, Duke University Medical Center, Durham, NC; [2]Medicine, VAMC, Durham, NC.*

Organ deposition of autoantibodies against the NC1 domain of the alpha3 chain of type IV collagen leads to severe kidney and lung injury in Goodpasture Syndrome and anti-GBM nephritis. The origin of these highly pathogenic autoantibodies remains unknown. Recent analysis of an anti-alpha3(IV)NC1 H+L chain autoantibody transgenic model revealed that anti-alpha3(IV)NC1 B cells are normally regulated by receptor editing and deletion, suggesting that these tolerance mechanisms must be overcome or bypassed for disease initiation. To better define the mechanisms and role of receptor editing in autoimmune nephritis, we adopted a genetic approach. Editing is a process by which the autoreactive B cell receptor is rendered non-autoreactive by replacement of either the Ig heavy or light chain with a second chain generated by additional gene rearrangements at the Ig loci. We eliminated such rearrangements by breeding anti-alpha3(IV)NC1 Ig transgenic mice with MuMT and kappa-KO mice bearing targeted deletions of the endogenous (non-transgenic) IgM heavy chain and Ig kappa light chain, respectively. Spleen B cells were enumerated in the mutant transgenic progeny (millions±SD, n=4/group): 22.6±6.7, k+/Mu+; 24.7±4.6, kKO/Mu+; 5.0±1.7, k+/MuKO; and 2.5±0.8, kKO/MuKO, where +=sufficiency and KO=deficiency. Flow cytometry confirmed transgene Ig expression, and in kKO/Mu+ transgenic mice revealed lambda light chain editing. Thus, extensive editing involving both Ig chains occurs during development of anti-alpha3(IV)NC1 B cells, without which most cells are deleted. Nonetheless, recovery of IgM+kappa+ cells expressing the anti-alpha3(IV)NC1 transgene from kKO/MuKO mice indicates escape or persistence of a small population of potentially nephritogenic B cells.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2850**

**Deletion of Inhibitory Fc gamma Receptor 2B on Myeloid Cells Rather Than B Cells Enhances Murine Immune Complex Glomerulonephritis** Ruth M. Tarzi,[1] Javier Martín Ramírez,[2] Phoebe E. H. Sharp,[1] Charles D. Pusey,[1] H. Terence Cook,[1] Sjef Verbeek.[2] *[1]Imperial Kidney and Transplant Institute, Imperial College London, London, United Kingdom; [2]Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands.*

Fc gamma receptor 2B (CD32) is an inhibitory receptor for the Fc portion of IgG. It is expressed on B cells, where it regulates antibody production, and myeloid cells, where it regulates inflammatory responses to immune complexes. Nephrotoxic nephritis (NTN) is a model of immune complex glomerulonephritis, induced by the injection of sheep anti-mouse glomerular basement membrane antibody to preimmunized mice. We have created mice lacking CD32 on a pure C57BL/6 background, and confirmed that these mice have enhanced disease in NTN. In order to determine the relative contributions of CD32 on B cells and myeloid cells in NTN, we have induced NTN in mice with reduced FcgR2B expression on selective cell types, generated by crossing floxed FcgRIIB mice ($FcgRIIB^{fl/fl}$) with cell type specific Cre expressing transgenic mice. In $FcgRIIB^{fl/fl}$ X CD19Cre mice FcgR2B was reduced by 90% on B cells. FcgR2B expression was reduced 90% in Gr1+ neutrophils and 60% in Mac1+ cells using Lysozyme M Cre. Five days after induction of NTN, mice with myeloid deletion of CD32 ($LysMCreCD32^{-/-}$) and the full knockout $CD32^{-/-}$ mice had clinical signs of disease, whilst the $CD19CreCD32^{-/-}$ and floxed (WT) mice appeared well. There was significantly more glomerular thrombosis in the $LysMCreCD32^{-/-}$ and full $CD32^{-/-}$ mice compared with the $CD19CreCD32^{-/-}$ mice ($p<0.01$ and $p<0.05$ respectively). $CD19CreCD32^{-/-}$ mice had no more glomerular thrombosis than WT. Similarly, serum urea was raised in the full $CD32^{-/-}$ and $LysMCreCD32^{-/-}$ mice compared with the $CD19CreCD32^{-/-}$ mice ($p<0.05$), whilst the $CD19CreCD32^{-/-}$ mice had no more disease than the floxed (WT) mice.

Our results show that in this non-autoimmune model of immune complex glomerulonephritis, myeloid rather than B cell expression of the inhibitory Fc gamma receptor is responsible for protection from disease. These results give direct in vivo evidence for a protective role of the inhibitory Fc gamma receptor on myeloid cells in glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2851**

**Contact between Neighbouring Podocytes Forming Tight Junctions Precedes Glomerular Basement Membrane Thickening and Foot Process Effacement in Early Experimental Crescentic Glomerulonephritis** Lena Succar,[1] David J. Nikolic-Paterson,[2] David C. Harris,[1] Ross A. Boadle,[3] Gopala K. Rangan.[1] *[1]Renal Medicine Dept, Westmead Millennium Institute, The University of Sydney, Westmead Hospital, Sydney, NSW, Australia; [2]Nephrology Dept , Monash Medical Centre, Monash University, Clayton, VIC, Australia; [3]Electron Micropscopy Unit, Institute of Clinical Pathology & Medical Research, Westmead Hospital, Sydney, NSW, Australia.*

Podocytes contribute to crescents by forming bridges between the glomerular capillary tuft and Bowman's capsule in crescentic glomerulonephritis (CGN). However, the early ultrastructural alterations of podocytes in CGN have not been characterized. This study aims to investigate podocyte alterations by transmission electron microscopy (TEM) in early rat anti-GBM CGN. Anti-GBM was induced in pre-immunized male Wistar-Kyoto rats by sheep anti-rat GBM serum i.v.i. day 0 (d0) and examined d1,2,3,5,7,14. GBM-thickness; foot process effacement (FPE) assessed by filtration slit frequency(FSF)/µm GBM length; distances between neighbouring podocytes and podocyte to parietal basement membrane (PBM) were measured on randomly selected glomeruli in TEM digital images (anti-GBM, n=50; control n=25 glomeruli/time-point). On d1, there was widespread formation of focal contacts between podocyte cell bodies, resembling tight junctions at high magnification (20000x). Microvillous transformation at site of podocyte contact was frequent. By morphometric analysis, neighbouring inter-podocyte distance decreased (1.5±0.4; 2.4±0.2µm; mean±SEM; P<0.01 for anti-GBM vs. control groups) despite normal foot processes and GBM-thickness. By d2, inter-podocyte distance decreased further (1.1±0.2; 2.2±0.2 µm; P<0.01), GBM-thickness increased 4-fold (P<0.01) and FPE was segmental. By d5, FPE was extensive (1.5±0.1; 2.2±0.07 FSF/µm) accompanied by podocyte-bridge formation, as shown by decrease in podocyte to PBM distance (1.7±0.1; 3.4±0.9 µm; P<0.01). At d7 and d14 glomeruli had fibrinoid necrosis and cellular crescents. Podocyte cell bodies form focal contact prior to formation of podocyte-PBM bridges, suggesting potential cross-talk between neighbouring podocytes early in CGN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2852**

**Rapamycin Causes Alterations of Podocytes Even without Proteinuria** Kostas Stylianou,[1] Ioannis Petrakis,[1] Vasiliki Mavroeidi,[1] Stavros Stratakis,[1] Theodora Katsarou,[1] Eleftheria A. Vardaki,[1] Spyros Stratigis,[1] Kostas Perakis,[1] John Kyriazis,[2] Eugene Daphnis.[1] *[1]Nephrology, University of Crete, Heraclion, Greece; [2]Nephrology, General Hospital of Chios, Chios, Greece.*

Introduction: Rapamycin can cause or ameliorate proteinuria. This contrasting behavior maybe due to either the dose used or the duration of administration. The current study was undertaken to examine whether or not rapamycin exerts direct toxicity on podocytes.

Methods: Balb/c mice were given either 3 different rapamycin doses for 1 week (low dose LD:1, intermediate dose ID:1.5 and high dose HD:3mg/kg/day i.p, -dose model) or an ID of rapamycin (1.5mg/kg) for 3 different periods (1, 4 and 8 weeks, time model). Six Balb/c mice injected with the rapamycin solvent (DMSO) served as controls.

Kidney morphology was examined by photon and electron microscopy. Expression of nephrin, podocin, Akt-kinase and Ser473pAkt was examined by WB, RT-PCR and IF.

Results

None of the mice developed proteinuria. Nevertheless, we noticed a dose and time dependent increase in the mean foot process width (FPW). On the first week FPW was as follows: controls 335nm, LD:385nm, ID:420nm, HD:420nm. FPW further increased from 420nm on week-1 to 456nm on week-2, and finally improved on week-8 (401nm). Both Podocin and Nephrin mRNA levels showed a significant decrease on week-1 (3 times lower vs. control, p<0.001), which improved on week-4 and was restored to normal on week-8. Nephrin and podocin protein levels didn't show significant alterations on WB or IF. Akt phosphorylation increased (fourfold vs. controls), in the HD and the week-4 groups (p<0.005).

Conclusions

Although rapamycin induced proteinuria was not detected, significant alterations were noticed in podocytes architecture, slit diaphragm proteins transcription and glomerular Akt activation. These alterations happened early on treatment and improved in the long term, indicating that a rapamycin "escape phenomenon" may exist.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2853**

**Different Podocyte Expression of Apelin in Focal and Segmental Glomerulosclerosis and Minimal Change Disease** Jan U. Becker,[1] Friedrich Modde,[1] Mario Schiffer,[2] Clemens L. Bockmeyer,[1] Catherine Meyer-Schwesinger,[3] Verena Broecker.[1] *[1]Medizinische Hochschule Hannover, Institut für Pathologie, Hannover, Lower Saxony, Germany; [2]Medizinische Hochschule Hannover, Klinik für Nieren- und Hochdruckerkrankungen, Hannover, Lower Saxony, Germany; [3]Universitätsklinikum Hamburg-Eppendorf, III. Medizinische Klinik, Hamburg, Germany.*

INTRODUCTION:

Apelin is a peptide which is known to directly induce contraction in smooth muscle cells and as a cofactor for VEGF.

Recently we found glomerular expression of Apelin and its receptor APJ exclusively in podocytes. To investigate the role of Apelin and APJ in podocytes, its expression in renal biopsies with podocyte injury and in normal kidneys was examined.

METHODS:

36 formalin fixated and paraffin embedded biopsies, 12 minimal change disease (MCD), 10 focal segmental glomerulosclerosis (FSGS), 3 IgA glomerulonephritis, 4 membranous glomerulonephritis, 4 diabetic glomerulosclerosis and 3 normal controls were examined. Immunhistochemistry for Apelin and APJ was quantified as positive and negative.

mRNA levels of Apelin and APJ were quantified by real time PCR in laser micro-dissected glomeruli as relative expression levels compared to normal controls.

RESULTS:

All normal kidneys (3/3) and most of the FSGS biopsies (8/10) had positive Apelin immunostaining of podocytes, while most MCD biopsies (9/12) were negative (p=0.0102). In contrast relative Apelin mRNA levels were significantly higher in glomeruli of MCD compared to FSGS (p=0,0409).

Immunostains and real time PCR of APJ showed positive podocytes in all specimens examined and no differences in mRNA levels.

CONCLUSION:

Podocytes in most cases of FSGS seem to retain the normal Apelin immunostaining while it is lost in most cases of MCD.

This contrasts with significantly increased mRNA levels of Apelin in MCD compared to FSGS.

Taken together, these data could indicate more rapid secretion of Apelin by podocytes in MCD, making it undetectable by immunostaining and a secretion defect with retained immunoreactivity in FSGS.

Further studies are needed to explore the possibility of impaired Apelin secretion by and autocrine effects of Apelin on podocytes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2854**

**Upregulation of Calcineurin Is Involved in the Development of PAN Nephropathy, an MCNS Model** Masayuki Tomita, M. D. Murad Hossain, Mihoko Yamazaki, Hiroshi Kawachi. *Department of Cell Biology, Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.*

We have previously reported that the treatment with a calcineurin inhibitor (CNI), tacrolimus reduced the amount of proteinuria in Thy1 GN (Kidney Int 61:1139, 2002). We have also reported that the podocyte dysfunction is involved in the development of proteinuria in Thy1 GN (Kidney Int 60:2192, 2004, JASN 14:3111, 2003). Recently it is reported that another CNI stabilizes the actin cytoskeleton of podocyte, and protect podocyte from injury. These observations indicated that the alteration of calcineurin is involved in podocyte injury. In this study, first we analyzed whether the alteration of calcineurin is involved in the development of PAN nephropathy, a mimic of MCNS, and we also analyzed the effect of CNI on PAN nephropathy. At the peak of proteinuria in PAN nephropathy (day 10), the glomerular expression of calcineurin remarkably increased (519% to normal). Then, we analyzed the effect of tacrolimus on proteinuria in PAN nephropathy. The rats were treated with tacrolimus (0.2 mg/kg) from one day before PAN injection. The tacrolimus treatment reduced the amount of proteinuria (day 10: 207 ± 25 mg/day vs. 378 ± 34, P<0.01, day 24: 17 ± 6 vs. 75 ± 15, P<0.01). The staining of nephrin was clearly reduced in the vehicle-treated control group, but the reduction of nephrin staining was inhibited by the tacrolimus treatment (Score (0-4): 1.68 ± 0.15 vs. 1.15 ± 0.10, P<0.05). Next, the protective effect of tacrolimus was analyzed with cultured podocyte (The cells were kindly donated by Dr. Mundel). The cells were treated with PAN (5 mg/mL) for 72 hr in the presence or absence of tacrolimus (100 nM). Treatment with PAN increased the mRNA expression of calcineurin (817%) and FKBP12 (524%) in cultured podocyte. PAN caused cortical F-actin ring formation and stress fiber attenuation in those cells. Treatment with tacrolimus prevented PAN-induced cytoskeletal rearrangement in podocyte. In conclusion, calcineurin is involved in the development of proteinuria in PAN nephropathy, and CNI has a protective effect for podocyte.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2855**

**Significant Role of Glomerular CD44 Expression in Adriamycin-Associated Nephropathy** Takayuki Okamoto, Satoshi Sasaki, Yasuyuki Sato, Hironobu Ito. *Pediatrics, Hokkaido U Grad Sch Med, Sapporo, Japan.*

***PURPOSE (INTRODUCTION).***

Pivotal functions of cell adhesion molecule CD44 include signal transduction of osteopontin (OPN) and macrophage migration inhibitory factor (MIF). Because recent studies have indicated significant roles of OPN and MIF in the progression of glomerulosclerosis, we examined the roles of CD44 as a key molecule for these cytokines using adriamycin (ADR)-associated nephropathy.

***Methods.***

Male 12-week-old Balb/c mice and MIF-deficient Balb/c mice were used. After the injection of 12mg/kg of ADR at day 0, histological and functional data were sequentially collected from day 1 to day 28. Glomerular expression of CD44, OPN, and MIF were immunohistochemically evaluated together with the markers for podocytes and parietal epithelial cells (PECs).

***RESULTS.***

Wild mice showed focal reduction of the expression of podocyte markers, synaptopodin and podocin, from day 10. Preceding the reduction of the podocyte marker expression, CD44 staining became positive in claudin-1-positive PECs in a few corticomedullary

glomeruli from day 7. Thereafter the number of CD44-positive cells increased in both PECs and proliferating glomerular cells with an upregulation of the staining intensity. OPN also showed positive staining in some PECs from day 14. Confocal microscopic analysis showed perinuclear OPN expression in most PECs with perimembranous CD44 expression; moreover, in a few PECs in the glomeruli showing segmental reduction of podocyte markers, OPN was co-localized with CD44 in focal deposits at the leading edge of the cells, and these findings are likely to be implicated in migratory effects of CD44/OPN interaction. MIF-deficient mice showed significantly less histological and functional injuries after ADR injection. In addition, the CD44 and OPN expression was significantly less in MIF-deficient mice compared to wild mice and perimembranous co-localization of CD44/OPN was not observed.

***CONCLUSION.***

CD44 is a key molecule that reflects podocyte injuries in the progression of focal segmental glomerulosclerosis. CD44-mediated cell motility may have a significant role in PEC migration through the interactions with functionally related molecules such as OPN and MIF.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2856**

**TRPC6 Expression Was Suppressed by 1, 25-Dihydroxyvitamin D3 in the Rat Kidney of Puromycin Aminonucleoside Nephropathy** Houqin Xiao, Yong Zhang, Qinhong Zhang. *Department of Nephrology, Taihe Hospital of Yunyang Medical College, Shiyan, Hubei, China.*

Accumulating evidence suggests that vitamin D is renoprotective. However, the precise mechanisms and the molecular targets by which active vitamin D exerts its beneficial effects remain obscure. Mutations of TRPC6 gene as cause for hereditary FSGS as well as the localization of TRPC6 at the slit diaphragm indicate that TRPC6 plays a pivotal role in genetic and acquired forms of proteinuric kidney disease. The objective of this study was to evaluate the TRPC6 expression effect of active vitamin D on rats with puromycin aminonucleoside (PAN) nephropathy.

PAN rat model were constructed by consecutive three times intravenous injection PAN of 100 $mg.kg^{-1}$ body weight every ten days and 1,25-dihydroxyvitamin D3 ($0.2\mu g.kg^{-1}.d^{-1}$) were gavaged. The rats were sacrificed at one and three months after PAN injection respectively. 24-hour urinary protein, renal function and morphologic parameters in glomeruli were evaluated. Expressions of nephrin, TRPC6, TGF-β1, VDR mRNA were examined by RT-PCR. The protein expression and location of nephrin, TRPC6, VDR were decided by western blot and confocal microscope respectively.

PAN administration caused serious proteinuria, hydroperitoneum, hyperlipoidemia, hypoproteinemia and glomerular sclerosis. Compared with vehicle controls, 1, 25-dihydroxyvitamin D3 significantly attenuated proteinuria, gloumeruslerosis, restored renal function after PAN administration. In PAN model rat, TRPC6 protein expression were significant increased and merged with synaptopodin on one months and decreased on three months with a granular patter. Compared with vehicle controls, TRPC6 expression was significantly decreased and VDR expression was significantly increased after 1, 25-dihydroxyvitamin D3 treament. Significantly negative correlation was found between TRPC6mRNA and VDR mRNA. In addition, 1, 25-dihydroxyvitamin D3 suppressed renal TGF-β1 expression, restored the loss of nephrin after podocyte injury.

In conclusion, 1, $25(OH)_2 D_3$ can significant attenuate the PAN-induced podocyte injury and proteinnuria. The beneficial effects of 1, $25(OH)_2 D_3$ on podocyte may be partly attributable to suppression of TRPC6.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2857**

**The Balance of Thymosin β4 and Its Metabolite Ac-SDKP Regulates Profibrotic Factors** Yiqin Zuo, Haichun Yang, Li-Jun Ma, Agnes B. Fogo. *Pathology, Vanderbilt University, Nashville, TN.*

We previously showed that the G-actin sequestering protein thymosin β4 (Tβ4) is remarkably increased in the obstructed kidney in the unilateral ureteral obstruction (UUO) model of tubulointerstitial fibrosis. Tβ4, which is postulated to be profibrotic, is degraded by prolyl oligopeptidase (POP) to the anti-fibrotic Ac-SDKP peptide. Our study further revealed that inhibition of POP shifted the balance of Tβ4 and Ac-SDKP and exacerbated fibrosis in obstructed kidneys. We now investigated whether the altered balance of Tβ4 vs Ac-SDKP affects profibrotic molecules in the UUO kidneys.

Male C57BL/6 mice underwent UUO and were treated as follows: UUO without treatment, UUO+POP inhibitor (S17092, 40mg/kg per day, by gavage), UUO+Tβ4 (150μg/d, i.p.), UUO+combination (POP inhibitor and Tβ4), and UUO+Ac-SDKP (1.6 mg/kg/d, delivered by minipump). Mice were sacrificed at day 5.

POP activity was significantly lower in the obstructed kidneys of mice treated with POP inhibitor or combination (POP inhibitor, 2.1±0.3; combination, 5.7±1.6; vs untreated UUO, 27.6±2.3 pmol/min*mg tissue, both $p<0.05$; Ac-SDKP, 31.9±1.2, pNS vs untreated UUO). Consequently, the Ac-SDKP concentration was significantly lower in POP inhibitor and combination treatment groups but dramatically higher in Ac-SDKP group vs. untreated UUO (POP inhibitor, 0.66±0.08; combination, 0.86±0.09; Ac-SDKP, 2.41±0.08; vs. untreated UUO, 1.40±0.19 pmol/mg tissue, $p<0.05$). Neither POP activity nor Ac-SDKP was affected by Tβ4 treatment (21.2±1.6 pmol/min*mg tissue and 1.09±0.12 pmol/mg tissue, respectively). Plasminogen activator inhibitor (PAI-1), transforming growth factor (TGF)-β1, Tβ4, collagen I and III expression assessed by real time PCR were all dramatically reduced vs. untreated UUO by Ac-SDKP treatment. By contrast, PAI-1, TGF-β1 and collagen III expressions were significantly increased by POP inhibitor.

Our study suggests that exogenous administration of Ac-SDKP inhibits profibrotic factors. We propose that a shift from thymosin β4 to Ac-SDKP is crucial in modulating profibrotic molecules and eventually determining tubulointerstitial fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2858**

**Topoisomerase 2 (Top 2)-DNA Covalent Complex Mediated Adriamycin-Mediates Podocyte Injury** Pu Duann,[1] Lisa Y. Lyu,[3] Ling-Mei Chiang,[2] Elias A. Lianos.[1] *[1]Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ; [2]Pediatrics, Chang-Gung Memorial Hospital, Keelung, Taiwan; [3]Pharmacology, Robert Wood Johnson Medical School, Piscataway, NJ.*

Adriamycin (ADR, doxorubincin) is a frequently used anticancer drug. In the mouse ADR causes proteinuria due to podocyte injury, an underlying mechanism being overproduction of reactive oxygen species (ROS). ADR kills tumor cells by complexing with the ADR specific target, Topoisomerase(Top)-2α.There are two Topoisomerase isozymes: a) Top2α, which is induced in actively proliferating states and is highly abundant in cancer cells, and b) Top2β, which appears only in terminally differentiated cells, e.g. adult cardiocytes and podocytes. We previously reported that the tumoricidal effect of ADR is mediated by trapping Top2-DNA covalent adducts (Top2 cleavage complexes) while ADR-mediated cardiotoxicity is mediated through Top2β. In the present study, we tested the hypothesis that Top2β is the target for ADR-mediated podocyte injury. In a culture system, we observed DNA damage after ADR treatment in wild type controls but not Top2β KO (Top2β-/-) cells, suggesting that DNA-damage is Top2β mediated. The proteasome core component 20S has been shown to work with Ump1/POMP. Using POMP-siRNA we demonstrated that etoposide (a Top2 drug)-mediated Top2β downregualtion was completely abrogated, suggesting the involvement of the 20S proteasome in mediating drug-induced Top2β downregulation. In addition, POMP siRNA treatment blocked the etoposide-induced DNA damage signal γ-H2AX suggesting that proteasomal degradation of Top2β can lead to permanent DNA breakage and activation of double strand break (DSB) signals (e.g. γ-H2AX) Dexrazone, an established ADR chemical inhibitor, antagonized formation of Top2α and Top2β-DNA covalent adducts, and blocked ADR-mediated DNA damage. In ADR-treated mice, we observed (immunohistochemistry) Top2-beta level induction. These observations point to Top2β as a mediator of ADR-mediated cytotoxicity in podocytes by: 1) interfering with formation of Top2-DNA covalent complex, and 2) depleting Top2β through the proteasome pathway.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2859**

**Matrix Metalloproteinase-12 (MMP-12) Deficiency Attenuates Crescent Formation in Anti-GBM Glomerulonephritis** Abu P. Abraham,[1,2] Greg Tesch,[1,2] Frank Yuanfang Ma,[1,2] William Richard Mulley,[1,2] David J. Nikolic-Paterson.[1,2] *[1]Nephrology, Monash Medical Centre, Clayton, Victoria, Australia; [2]Medicine, Monash University, Clayton, Victoria, Australia.*

MMP-12 is an enzyme that can cleave various extracellular matrix proteins and is required for macrophage infiltration and pulmonary fibrosis in experimental emphysema. We have shown previously that MMP-12 is highly up-regulated in experimental anti-GBM disease. The aim of this study was to determine whether MMP-12 (macrophage elastase) is required for glomerular macrophage infiltration and crescent formation in anti-GBM glomerulonephritis. Accelerated anti-GBM disease was induced in groups of 5 to 6 MMP-12 gene deficient mice (MMP-12-/-) and wild type C57BL/6J (WT) controls, which were killed 12 days after serum injection. WT and MMP-12-/- mice developed glomerular damage and glomerular tuft adhesions to Bowman's capsule. Both groups developed severe proteinuria. WT mice also developed crescents characterized by macrophage infiltration, epithelial cell proliferation, fibrin deposition and Bowman's capsule rupture. In contrast, MMP-12-/- mice were protected from crescent formation (3.1±1.4% vs. 24±2.7% in WT; $P<0.01$). This was associated with a significant reduction in macrophage infiltration in both glomeruli (1.4±0.2 vs. 3.1±0.2 CD68+ cells/gcs; $P<0.01$) and the interstitium (16±2 vs. 26±3 CD68+ cells/hpf), with a 90% reduction in renal TNF-α mRNA levels in MMP12-/- mice. Semi-quantitative assessment also showed a reduction in peri-glomerular macrophage infiltration in MMP12-/- compared to WT mice (1.5±0.2 vs. 2.5±0.2; $P<0.01$). The degree of tubular damage was reduced in MMP-12-/- mice based upon KIM-1 mRNA levels (60% reduction vs WT; $P<0.05$). In conclusion, these data demonstrate that endogenous MMP-12 is required for glomerular crescent formation in anti-GBM glomerulonephritis. This most likely operates via macrophage recruitment and activation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2860**

**Lymphangiogenesis during the Development of Chronic Kidney Disease in Rat Anti-GBM Glomerulonephritis** Maki Tanabe, Akira Shimizu, Yukinari Masuda, Emiko Fujita, Shinya Nagasaka. *Pathology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

Lymphangiogenesis is the growth and formation of new lymphatic vessels. In renal diseases, lymphatic vessels develop during tubulointerstitial fibrosis. However, little is known about the lymphangiogenesis during the development of glomerulonephritis (GN) with chronic kidney injury. Progressive necrotizing and crescentic GN was induced in rats by intravenous injection of anti-GBM antibody. We assessed the distribution and pathologic characterizations of lymphatic vessels during the progression to end stage renal failure. In addition, we examined the expression of VEGF-C in the kidney. After anti-GBM antibody injection, necrotizing and crescentic GN developed with mecophage infiltration by day 7.

Newly formed necrotizing lesions reoccurred by week 3 with the development of glomerular sclerosis. Subsequently, intersitial fibrosis developed with mononuclear cell infiltration. Before anti-GBM antibody administration, podoplanin+ lymphatic vessels were located mainly around arcuate arteries. In renal cortex, a few lymphatic vessels were present around interlobular arteries, but not around glomeruli. However, after induction of anti-GBM GN, several podoplanin+ branches extended to cortex and located around inflamed glomeruli by week 2. At week 2, glomerular infiltrated macrophages expressed VEGF-C, and glomerular VEDG-C was upregulated by western blotting, suggesting that inflamed glomeluri induced lymphangiogenesis in interstitium before the development of interstitial fibrosis. Subsequently, interstitial fibrosis developed with irregular enlarged and increased number of lymphatic vessels. In this phase, VEGF-C expressed injured tubular epithelial cells and infiltrating cells. Importantly, lymphatic vessels during lymphangiogenesis in kidney were characterized by immunostaining with podoplanin+, Prox-1+, MIB-5 (proliferation maker)+ and type IV collagen (basement membrane)(-) vessels. In conclusion, in severe glomerular inflammation may lead to interstitial lymphangiogenesis by VEGF-C expression in glomeluar inflammation, before the development of interstitial fibrosis with VEGF-C expression in tubules and infiltrating cells.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2861

**Coronin 1a Deficiency Reduces T-Cell Recruitment in Anti-GBM-Nephritis** Christoph Daniel,[1] Laura Königer,[1] Christina Grigo,[1] Silke Meister,[3] Charlotte Starke,[3] Reinhard Voll,[3] Christian Hugo.[2] *[1]Nephrology and Hypertension, University of Erlangen, Erlangen, Germany; [2]Nephrology, University of Dresden, Dresden, Germany; [3]Immunology, University of Erlangen, Erlangen, Germany.*

Coronins are cytoskeletal, actin binding proteins involved in regulation of actin bundling. Using microarray analysis we identified Coronin 1a as a potential marker for glomerular reserve/precursor cells repopulating the glomerulus after severe mesangial injury. To characterise Coronins role in early anti-GBM nephritis we investigated disease progression 7 days after disease induction using coronin 1a deficient mice. We analysed renal function, histological changes and inflammation. In addition, inflammatory cells were analysed in blood, spleen, regional lymph nodes and bone marrow using flow cytometry. Interestingly, IgG antibody titers generated against the disease inducing sheep antibody were markedly reduced in Coronin 1a deficient mice (ELISA OD: 1,7±0,4 vs. 0,2±0,11) compared to wt mice. In contrast, number of IgG-secreting plasma cells was similar in both groups. Only in bone marrow, B220 positive B-cells were significantly lower in coronin 1a deficient mice compared to wt controls. Renal infiltration with CD4 positive T-cells of wt mice was about 4 times higher in anti-GBM-nephritic mice whereas the increase in Coronin 1a deficient mice was only the half (20,3±4,8 vs. 9,8±1,1 cells per high power field). Significantly lower CD4 positive T-cells were also detected in all investigated compartments. In contrast, percentage of CD8 positive T-cells was significantly lower in the nephritic kidney (13,1±2,0 vs. 8,8±2,4 cells per high power field) and regional lymph node (11,1±2,3% vs. 3,8±1,5%) but comparable in blood, and spleen. However, both coronin 1a deficient and wt mice developed anti-GBM-nephritis with comparable crescent formation and only a tendency to reduced proteinuria in the Coronin 1a deficient mice.

In conclusion, during early GBM-nephritis Coronin 1a is involved in regulation of T-cells and humoral response with minor effects on kidney function and renal damage.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2862

**Effects of Long- and Short-Term Treatment with Imatinib in the Progression of Renal Dysfunction and Fibrosis in Established Experimental Anti-GBM Nephritis** Masayuki Iyoda, Takanori Shibata, Yoshihiro Kuno, Yuki Hirai, Tadao Akizawa. *Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan.*

Introduction: Imatinib (IM) is a selective tyrosine kinase inhibitor that can block PDGF receptor (PDGFR) activity. In this study, whether long-term or short-term IM treatment (Tx) could prevent the progression of chronic kidney disease (CKD) was investigated in order to determine whether IM may have potential to treat patients with CKD. Methods: Nephrotoxic serum nephritis (NTS-N) was induced in Wistar-Kyoto (WKY) rats on day 0. Groups of animals were given either IM (25 mg/kg) or vehicle daily by intraperitoneal injection, from day 7 to day 49 for 43 days in the long-term Tx study (Long Tx), and from day 7 to 20 for 14 days in the short-term Tx study (Short Tx); all rats were sacrificed at day 50. Vehicle-treated groups received an equal volume of sterile water. Seven, female, 14-week-old WKY rats were used as normal controls (WKY-NTS (-)). Proteinuria (U-P), serum creatinine (Cr), and body weight (BW) were measured periodically. Morphological investigations were performed at sacrifice. Results: Cr levels were significantly lower in the IM-treated rats than in the vehicle-treated rats in the Long Tx group (Long Tx: 0.39 ± 0.01 vs. 0.28 ± 0.01 mg/dL, $p < 0.01$; Short Tx: 0.40 ± 0.05 vs. 0.35 ± 0.03 mg/dL, NS). When compared to vehicle Tx, IM-treated rats had reduced U-P at the end of the study (Long Tx: 175.38 ± 15.71 vs. 103.86 ± 17.79 mg/day, $p < 0.001$; Short Tx: 161.72 ± 9.79 vs. 131.93 ± 9.69 mg/day, $p < 0.05$). Imatinib Tx also decreased kidney weight (Long Tx: 2.09 ± 0.04 vs. 1.68 ± 0.03 g, $p < 0.001$; Short Tx: 1.91 ± 0.07 vs. 1.75 ± 0.05 g, $p < 0.05$; 1.63 ± 0.03 g in WKY-NTS (-)) and reduced glomerulosclerosis and tubulointerstitial damage scores. Renal cortical mRNA for collagen type I (collagen type I/GAPDH mRNA: Long Tx: 9.92 ± 2.52 vs. 2.14 ± 0.40, $p < 0.01$; Short Tx: 11.84 ± 1.48 vs. 5.31 ± 0.96, $p < 0.01$) was also significantly decreased in the IM-treated group. Conclusion: These results suggest that IM may prove useful in limiting the progression of chronic renal disease to end-stage renal failure.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2863

**p53 Deficiency Exacerbates Progression of Immune-Mediated Glomerulonephritis** Ryota Kimura,[1] Ryota Shirai,[1] Yuki Udagawa,[1] Yuki Tokui,[1] Takashi Okude,[1] Shiro Ueda,[1] Yoshihiko Ueda,[2] Osamu Yokosuka,[3] Makoto Ogawa,[4] Yuki Hamano.[4] *[1]Graduate School of Pharmaceutical Sciences, Chiba University, Japan; [2]Dokkyo Medical University Koshigaya Hospital, Japan; [3]Graduate School of Medicine, Chiba University, Japan; [4]Chiba University Hospital, Japan.*

**Objective:** p53 tumor suppressor is a key regulator of the response to cellular stress or DNA damage. p53 activation leads to cell cycle arrest, apoptosis, or senescence. p53 has been shown to be an important regulator of renal tubular cell viability in the animal models of ischemic and toxic acute kidney injury. In theses models, p53 activation induced proapoptotic pathways in renal tubular cells which contributed to kidney dysfunction. In this study, we investigated whether genetic inhibition of p53 provided a protective effect in a model of immune-mediated glomerulonephritis.

**Methods:** p53-null (KO) and wild-type (WT) mice were immunized subcutaneously with normal rabbit IgG in complete Freund's adjuvant, followed by intravenous injection of rabbit anti-mouse GBM serum. On days 6 and 9, blood and urine samples were collected and mice were sacrificed for the histological assessment.

**Results:** Upon induction of anti-GBM glomerulonephritis in WT mice, p53 expression was upregulated within the glomeruli and tubulointerstitium. Unexpectedly, the serum creatinine level and urine albumin excretion were significantly increased in KO mice compared with WT mice. KO mice displayed significant increase in the number of proliferating mesangial cells and macrophages in the glomeruli and proliferating macrophages in the tubulointerstitium, mesangial expansion and interstitial fibrosis. The expression of CDK2, cyclin E1 and E2 was upregulated in the cortex of KO mice compared with WT mice. In contrast, there were no significant differences in the number of apoptotic cells in the glomeruli and tubulointerstitium and expression of TNF-α and Bcl-2 in the cortex between KO and WT mice.

**Conclusion:** p53 plays an important role in altering renal inflammation by inhibiting proliferation of intrinsic and infiltrating cells in the glomeruli and tubulointerstitium and has a protective effect against progressive glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2864

**Clearing of Apoptotic Mesangial Cells and Associated Debris by Mesenchymal Stem Cells: A Crucial Step in Mesangial Repair Monitored with a 6D (Dimensional) – Live Cell Model** Jiamin Teng, Elba A. Turbat-Herrera, Guillermo A. Herrera. *Pathology, Nephrocor, Tempe, AZ.*

**Background:** Mesangial repair remains a poorly understood, yet very important step in glomerular recovery after an injurious event. Mesangial damage is often associated with mesangial cell (MC) apoptosis. Repair of the damaged mesangium may require exogenous cellular elements that can repopulate and rebuild the injured mesangial areas.

**Materials and methods:** An in-vitro platform using a 6D cell live model allows monitoring the sequential steps that must take place for mesangial repair to occur. Rat mesangial cells (RMCs) were cultured on Matrigel loaded glass bottomed multi-well plates with 10% FBS/RPMI 1640 until confluence. MCs were then made quiescent by incubating them first with 0.5% FBS/ RPMI for 48 hours and afterwards with glomerulopathic light chains (GLCs) (10 ug/ml, 48 hours X 2) purified from the urine of patients with light chain deposition disease and AL(light chain-associated) amyloidosis. Rat mesenchymal stem cells (RMSCs) were stained with PKH-2 fluorescence dye (green) and / or with Lysotracker Texas Red (red) and placed 96 hours later into the wells of GLCs-treated MCs for up to 2 weeks. MC cultures were maintained alive and 15 different locations were monitored simultaneously with sequential photos every 15 minutes using this system.

**Results:** Before the RMSCs were added to the system, most if not all MCs were in various stages of apoptosis. The various steps in the apoptotic process were monitored. RMSCs rapidly migrated towards the areas with the most damage and phagocytosed the fragments of apoptotic cells and debris. Not until mostly all of the damaged mesangial cells and surrounding debris, including damaged matrix, were cleared, the RMSCs began differentiating and transforming into mature MCs, establishing contacts with adjacent cells, and producing new matrix.

**Conclusions:** RMSCs actively participate in disposing of the damaged MCs and surrounding affected matrix. This appears to be an initial crucial step for the repair of the mesangium to occur. The findings indicate that exogenous MSCs may play an important role in clearing the injured mesangium prior to remodeling.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2865

**PIASy Regulates SM α-Actin Expression by Interacting with E12 in Mesangial Proliferative Glomerulonephritis** Kazuo Torikoshi,[1] Makoto Araki,[1] Takeshi Matsubara,[1] Akira Mima,[1] Takahiro Hirano,[2] Hideharu Abe,[2] Atsushi Fukatsu,[1] Hidenori Arai,[3] Toshio Doi.[2] *[1]Nephrology, Kyoto University, Kyoto, Japan; [2]Clinical Biology and Medicine, Tokushima University, Tokushima, Japan; [3]Innovation Unit for Near Future System and Technology, Kyoto University, Kyoto, Japan.*

α-Smooth muscle actin (SMA) is known to be a crucial marker for phenotypic transformation of glomerular mesangial cells. The SMA promoter contains two E-boxes, where the E2A binds and regulates the transcription.In this study, we tried to identify

a novel E2A binding protein and to examine its role in glomerulonephritis.Yeast two-hybrid screening using the cDNA library derived from mouse mesangial cells identified PIASy as an E12-interacting protein. The binding of PIASy to E12 was confirmed by co-immunoprecipitation in COS7 cells overexpressing both proteins. A small interfering RNA (RNAi) for PIASy significantly increased SMA mRNA expression, while RNAi for E12 reduced its expression in mesangial cells. Overexpression of E12 enhanced the SMA promoter activity approximately by 3 times, and the increase was blocked by co-transfection of PIASy in mesangial cells. Next, we examined the effects of TGF-β on PIASy in mesangial cells. The levels of PIASy mRNA were increased 12, 24, 48 hours after administration of 1ng/ml of TGF-β (1.36±0.32, 1.38±0.17, 1.72±0.22, respectively, $p<0.05$, vs. control). TGF-β also increased the levels of E12 and SMA in a time dependent manner consistent with PIASy. In vivo, we utilized an acute model of mesangial proliferative glomerulonephritis, Thy1 glomerulonephritis. Quantitative RT-PCR showed that glomerular PIASy, E12, TGF-β, and SMA mRNA were increased at days 6 (1.81±0.30, 2.95±0.62, 3.54±1.8, 32.4±30.3, respectively). Glomerular PIASy protein was also significantly increased at days 6 (1.77±0.45). Immunohistochemical analysis confirmed that PIASy was expressed in the mesangial area mainly at day 6. These results suggest that PIASy plays a critical role in experimental mesangial proliferative glomerulonephritis by modulating SM α-actin expression upon the interaction with E12.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2866

**A Mouse Model of Spontaneous Mesangio-Proliferative Glomerulonephritis Linking Hyperlipidemia and Renal Disease** Harini Bagavant, Yogesh M. Scindia, Amanda Doran, Seshagiri Rao Nandula, Saleh Mohammad, Paromita Dey, Alexis Cutchins, Dominika Nackiewicz, Stephanie Oldham, Coleen Mcnamara, Umesh Deshmukh. *Division of Nephrology and 2 Cardiovascular Medicine, University of Virginia, Charlottesville, VA.*

Purpose: Hyperlipidemia is associated with chronic kidney disease, but per se does not cause renal failure. Animal and human studies suggest that renal disease in hyperlipidemia is determined by additional factors. We describe a mouse model of C57BL/6 (B6) mice, genetically deficient in Apolipoprotein E ($ApoE^{-/-}$) and a transcription regulator "Inhibitor of differentiation 3" ($Id3^{-/-}$) that can be used to investigate these factors.

Methods: It was recently shown that $ApoE^{-/-}Id3^{-/-}$ double knockout (DKO) develop hyperlipidemia and accelerated atherosclerosis. To study renal disease, male and female mice were maintained on regular mouse chow and sacrificed at 16 and 24wks. Renal pathology was evaluated by light and electron microscopy. Cell infiltrates and immune complex deposits were studied by immunostaining. Mesangial cell proliferation was studied by staining for Ki67 antigen and mesangial matrix expansion by fibronectin deposits. Proteinuria and serum creatinine levels were monitored for renal function. Gene expression in isolated glomeruli was studied using Affymetrix microarrays.

Results: DKO mice develop glomerular immune complex deposits, macrophage infiltration, and mesangio-proliferative glomerulonephritis (GN). DKO mice also develop loss of renal function. This was not seen in hyperlipidemic $B6.ApoE^{-/-}$ mice or normolipidemic $B6.Id3^{-/-}$ mice. Glomerular gene expression analyses indicate a dominant contribution of macrophage products in GN.

Conclusions: Results from DKO mice suggest that genetic factors like Id3 deficiency influences development of renal disease in hyperlipidemia. Recently, participants of a Diabetes Heart Study showed linkage of a functionally deficient Id3 SNP variant with atherosclerosis. Preliminary analyses also showed an association of the same variant with reduced glomerular filtration rate. Thus, pathways regulated by Id3 may also be important in human renal disease associated with elevated lipids.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2867

**Impairment of Macrophage Function Resulting from Fc Receptor gamma Chain (FcRγ) Deficiency Causes the Development of Lipoprotein Glomrulopathy (LPG)** Kenji Ito, Maho Watanabe, Hitoshi Nakashima, Yasuhiro Abe, Takao Saito. *Div. of Nephrol. and Rheumatol., Dept. of Int. Med., Fukuoka University, Japan.*

[Purpose] LPG is a unique renal disease characterized by intraglomerular lipoprotein thrombi. In human, several novel apolipoprotein E(apoE) mutations and dyslipidemia were identified in patients with LPG. While, Kanamaru et al. reported LPG developed in chronic GVHD induced FcRγ-deficient mice. We attempted to clarify which has a principle role in development of LPG, dyslipidemia or FcRγ insufficiency. [Methods] Human apoE3 gene was introduced by adenovirus-mediated gene transfer into 20 week-old apoE and FcRγ double knockout(DKO), apoE knockout(AKO) and wild type(WT) mice, respectively. Blood sample were collected each time point, and kidneys were dissected after 3 weeks of apoE3 introduction. The specimens were stained with Oil Red O after lipid fixation in osmium tetroxide and were immuno-stained with CD68. For evaluation of macrophage function, peritoneal macrophages harvested from each genotype mice were cultured with oxidized LDL, and observed by light microscopy with Oil Red O stain. [Results] LPG-like lesions were observed in DKO and AKO, and the positive rate was significantly higher in DKO than that of AKO. The number of CD68 positive cells in glomerulus was significantly less in DKO than that in AKO. The oxidized-LDL uptake of macrophage was significantly reduced in DKO compared with macrophage from AKO. Serum total cholesterol(TC) levels of AKO and DKO were significantly higher than that of WT at baseline. After apoE3 introduction, serum TC levels were improved to normal level but triglyceride levels transiently increased in both mice. [Conclusion] Similar serum lipid alterations by human apoE3 administration were seen in DKO and AKO. While macrophage functions such as migration to glomeruli and uptake of oxidized LDL were significantly impaired in DKO. The fact that LPG was clearly frequently developed in DKO rather than AKO suggests that FcRγ impairment plays more important role in the development of LPG than dyslipidemia does. These results indicate that the pathogenesis of LPG may be related to macrophage dysfunction.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2868

**Impaired Glomerular Adaptability Against Subtotal Nephrectomy after Conditional Knock-Out of Platelet-Derived Growth Factor Receptor (PDGFR)-β in Mice** Taizo Nakagawa,[1] Kumi Ichikawa,[1] Hidenori Yamazaki,[1] Takeru Hamashima,[2] Jie Shen,[2] Yoko Ishii,[2] Fumihiro Tomoda,[1] Hiroshi Inoue,[1] Masakiyo Sasahara.[2] *[1]The Second Department of Internal Medicine, University of Toyama, Toyama, Japan; [2]Department of Pathology, University of Toyama, Toyama, Japan.*

Background and Aim: Mesangial cell functions are critically regulated by PDGFR-βsignals. At ASN-Renal Week 2009, we have reported about systemically depleted PDGFR-β mice (Deletant). After 2 weeks of subtotal nephrectomy (SN), Deletant have shown increase of albuminuria. So we have hypothesized that the glomeruli have altered response to reduce renal mass under PDGFR-β depleted condition. To inspect the hypothesis, we have analyzed the pathology of remnant kidney(RK) at this phase.

Method: Deletant were obtained by giving tamoxifen to the mutant mice harboring both the PDGFR-β floxed allele and the transgenes encoding Cre recombinase with a tamoxifen-sensitive mutated estrogen receptor at 4 weeks of age. Mice with conserved PDGFR-β expression (Floxed) were used as controls. SN was carried out at 13 weeks of age, and RK were harvested after 2 weeks of SN.

Result: In RK of Floxed, the glomerular structures were preserved with dilated capillary lumina. On the other hand, in Deletant, abnormal glomeruli were focally detected, in which glomerular cells were distributed at higher density. In morphometry, although glomerular size similarly increased in both lines, Deletant included glomeruli with higher ratio of glomerular cell number for glomerular size (cellular density). As a result, the average of cellular density was significantly higher in Deletant than in Floxed. In electron microscopical observations (EM), mesangial cells formed branch-like structures to support the capillary lumina in Floxed. In contrast, collapsed glomeruli with hypertrophic podocytes were detected in Deletant. These changes were detected in 11 glomeruli of Deletant but none of Floxed within 34 glomeruli which were randomly selected from 4 mice of each line.

Conclusion: These glomerular changes were similar to collapsing glomerulopathy. This indicates the inhibition of PDGFR-β could induce impaired glomerular adaptability to massive nephron loss.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2869

**Proximal Tubular Injury and Rapid Formation of Atubular Glomeruli in Adult Mice with Unilateral Ureteral Obstruction: A New Look at an Old Model** Robert L. Chevalier, Michael S. Forbes, Barbara A. Thornhill. *Pediatrics, University of Virginia, Charlottesville, VA.*

Unilateral ureteral obstruction (UUO) is widely used as a model of progressive renal injury and fibrosis, with the mouse as the species of choice to study molecular mechanisms. While many renal disorders, including diabetes, lead to widespread formation of atubular glomeruli (ATGs) (JASN 19:197-206, 2008), identification of ATGs has been limited by the use of labor-intensive techniques (microdissection and serial sectioning). In rat models of renal disease (Heymann nephritis and polycystic disease), ATGs evolve over 6-8 months. Adult male mice were subjected to sham operation or complete UUO for 1 to 14 days. Kidneys were removed for determination of cell death (TUNEL), autophagy (LC3B) and collagen (Sirius); tubular atrophy and ATGs were identified by loss of *Lotus tetragonolobus* lectin staining from the epithelial cells of Bowman's capsule (normally present in 86%). After 7 days UUO, only 56±7% of glomeruli remained lectin-positive ($p<0.001$ vs. sham), and by 14 days, this fraction decreased to 18.5±3% ($p<0.001$ vs. sham and 7-day UUO). Integrity of the glomerulotubular junction of kidneys following 14 days UUO was validated in serial sections of 125 glomeruli: only 15% retained normal connections with proximal tubules (correlating with 18.5% lectin-positive); 46% were connected to atrophic proximal tubules, and 39% were ATGs. As a result of UUO, epithelial cells of the glomerulotubular junction die by apoptosis, autophagy and necrosis. Remaining cells at the glomerulotubular junction become flattened, the lumen collapses, followed by separation of glomerulus from tubule. While interstitial collagen deposition is largely limited to the medulla, and accounts for less than 5% of the fractional volume of the kidney, after 14 days of UUO over 80% of proximal tubules are atrophic or have separated from glomeruli. Thus, in addition to studying fibrogenic pathways in the mouse model of UUO, attention should be focused on the integrity of the glomerulotubular junction, which can be rapidly measured by Lotus lectin staining. This approach should lead to new insight in the pathogenesis of chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2870**

**Acute Kidney Injury during Hepatic Failure in Rats** Akira Shimizu, Yukinari Masuda, Shinya Nagasaka, Emiko Fujita. *Pathology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

Acute kidney injury (AKI) is a common complication in the acute liver dysfunction, posing an enormous obstacle to treatment efficiency and patient survival. However, little is known about the pathomechanisms of AKI during the development of acute liver dysfunction, in part due to the lack of an animal model. In this study, we characterize a rat model of AKI during the development of acute liver dysfunction following liver transplantation. Orthotopic liver transplantation was performed from DA (RT1a) to Lewis (RT1l) rats without immunosspression. In this combination, animal will die around day 11 with severe acute liver dysfunction. We studied kidney samples at day 5, day 7, and day 9 to 11, focusing on the cellular infiltrate and tubular injury. In addition, we also examined capillary injury in interstitium with the alteration of angiogenic growth factors.In the liver graft, a progressive acute cell- and antibody-mediated rejection developed leading to irreversible graft failure by day 11. During the progression of rejection of hepatic graft, acute liver dysfunction developed (T-Bil 6.4±2.8). During the development of liver dysfunction, AKI gradually developed in rats and is characterized by renal tubular degeneration with loss of brush border, KIM-1 expression, and severe disruption of renal proximal tubule epithelial filamentous-actin. In interstitium, focal interstitial edema occurred with neutrophil and mononuclear cell infiltration. In peritubular interstitial capillaries, endothelial dysfunction developed with decrease expression of eNOS. In addition, downregulation of VEGF, angiopoietin-1, and angiopoietin-2 was detected by immunohistochemistry and western blotting in renal cortex. In conclusion, AKI developed in rats during the development of acute liver dysfunction and was characterized by renal tubular degeneration and peritubular interstitial capillary endothelial dysfunction with decrease expression of angiogenic growth factors. Our rat model of AKI during liver injury closely mimics human AKI associated with acute liver injury and may be useful in delineating the mechanisms and potential therapies for this common clinical condition.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2871**

**A Murine Model of Phosphate Nephropathy** Philipp Eller, Kathrin Eller, Alexander R. Rosenkranz. *Innsbruck Medical University.*

Hyperphosphatemia is crucial since it correlates with all-cause mortality in patients with end stage renal failure. Acute hyperphosphatemia can result from ingestion of excessive amounts of phosphate-containing laxatives and results in an acute renal failure due to tubular precipitation of calcium phosphate deposits forming crystals of hydroxyapatite.

Db/db leptin-receptor deficient mice, which develop obesity and type II diabetes mellitus, were uninephrectomized at the age of 5 weeks and were either fed with standard chow or phosphorus-rich diet for the next 8 weeks. After 8 weeks renal cryosections of db/db mice treated with phosphorus-rich diet showed abundant tubular casts of calcium phosphate crystals with a strong histochemical reaction in the von Kossa stain as compared to controls. The acute degenerative tubular changes were accompanied by peritubular infiltration of CD4+, CD8+ T cells and macrophages as well as by interstitial fibrosis. Serum-phosphate levels significantly increased, whereas serum-calcium levels decreased in db/db mice treated with phosphorus-rich diet as compared to db/db mice on standard diet. Interestingly, db/db mice on phosphorus-rich diet had less body weight, significantly lower fasting blood glucose levels and a lower HOMA insulin resistance index. Moreover, they performed a significantly improved glucose as well as insulin tolerance test and had significantly smaller adipocyte diameters in the visceral adipose tissue. Significantly decreased numbers of CD8+ T cells, monocytes/macrophages, but increased numbers of CD4+FoxP3+ T cells were detected in the visceral fat of mice fed with a phosphorus-rich diet. Contrary, no difference in the size of subcutaneouse adipocytes or in the infiltration of inflammatory cells into the subcutaneous fat was observed between db/db mice on phosphorus-rich or standard diet. Additionally, mice with acute phosphate nephropathy displayed significantly thicker myocardial septal wall and smaller interior diameter of the left ventricle.

We here provide a murine model of phosphate nephropathy, which can be used for future pharmacological and pathophysiological studies analyzing the effect of hyperphosphatemia on renal, metabolic and cardiovascular phenotypes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2872**

**Activation of Hypoxia-Inducible Factor Protects Against Cyclosporine A Induced Chronic Nephrotoxicity** Sewon Oh,[1] Jeongmyung Ahn,[2] Yun Mi Lee,[1] Su Jung Ha,[1] Ho Jun Chin,[1] Dong Wan Chae,[1] Ki Young Na.[1] *[1]Department of Internal Medicine, Seoul National University Bundang Hospital; [2]Department of Internal Medicine, Maryknoll Hospital, Busan, Democratic Peoples Republic of Korea.*

**Background:** Chronic cyclosporine A (CsA) nephropathy is characterized by interstitial fibrosis, arteriopathy and inflammatory cell infiltration. The main mechanism of Chronic CsA nephropathy is a chronic hypoxic injury and promotes apoptosis on renal tubular cells. Hypoxia-inducible factor (HIF) is a transcription factor which allows cells to adapt hypoxia, and it is known as a target to protect kidney from hypoxia. We investigated the hypothesis that HIF activation could attenuate the renal injury in chronic CsA nephropathy.

**Methods:** Male Sprague Dawley rats kept on a 0.05% low salt diet were treated with CsA for 28 days (15mg/kg/day, subcutaneous). Rats received a continuous infusion of cobalt chloride (CoCl) (10mg/kg/day) and dimethyloxalyglycine (DMOG) (100mg/kg/day) via osmotic minipump during the whole experimental period to activate HIF.

**Results:** CoCl or DMOG infusion did not significantly improve creatinine clearance (0.040± 0.004; CsA, 0.035±0.004; CoCl, 0.032±0.005 ml/min/100g; DMOG) and proteinuria (77.59±41.48; CsA, 55.82±7.03; CoCl, 33.82±8.44 mg/day; DMOG). Rat kidney treated with CoCl or DMOG showed the improvements of arteriopathy (1.75±0.25; CsA, 0.75±0.14; CoCl, 0.75±0.14; DMOG, $P<0.05$ vs. CsA) and tubulointerstitial fibrosis (2.83±0.58 vs. 1.25±0.35, CsA vs. DMOG; $P<0.05$). CoCl or DMOG increased renal HIF-1α protein, as manifested by western blot of nucleus extracts in the kidneys. Immunohistochemical evaluation revealed that the number of infiltrating glomerular and interstitial macrophages were significantly higher in CsA treatment group and this increase was attenuated by CoCl (12.94±2.04 vs. 4.62±0.99, CsA vs. CoCl, $P<0.05$) and DMOG treatment(1.3±0.85, $P<0.05$ vs. CsA). Enhanced apoptotic cell death in CsA- treated rat was decreased with CoCl treatment (76.03±13.11 vs. 10.65±7.28, CsA vs. CoCl , $P<0.05$).

**Conclusion:** Activation of HIF by CoCl or DMOG attenuates renal injury through anti-inflammatory and anti-apoptotic effect.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2873**

**Tubular Angiogenic Response after Glomerular Injury Is Modified by Pigment Epithelium Derived Factor** Davide Pietro Cina,[1] Hui Xu,[2] Limin Liu,[3] Laszlo Farkas,[4] Daniela Farkas,[4] Peter Margetts.[3] *[1]Medicine, University of Toronto, Toronto, ON, Canada; [2]Nephrology, Xiangya Hospital, Changsha, China; [3]Medicine, McMaster University, Hamilton, ON, Canada; [4]Internal Medicine, Virginia Commonwealth University, Richmond, VA.*

Peritubular vascular changes and hypoxia in the setting of glomerular injury may explain subsequent tubulointerstitial injury and fibrosis. We used a common model of glomerular injury in rats in order to assess vascular changes and to identify potential factors associated with regulating the angiogenic response. Further, we evaluated the effect of hypoxia on cultured renal tubular epithelial cells (HK2). Anti-Thy1.1 antibody administration (1 or 4 weekly doses) led to a dose dependent renal damage characterized by glomerulonephritis, elevated serum urea, and tubulointerstitial fibrosis. Using laser capture microdissection, we demonstrated an early induction of fibrogenic and angiogenic factors in the glomeruli and a subsequent dysregulated angiogenic response in the tubulointerstitial compartment in animals that developed glomerulonephritis. This was associated with peritubular capillary rarefaction assessed by CD34 and CD31 staining. Proximal tubules of anti-Thy1.1 treated animals demonstrated an increase in the angiostatic pigment epithelial derived factor (PEDF). Temporally associated with PEDF expression was a transient downregulation of tubular hypoxia inducible factor (HIF)1α. In HK2 cell culture, hypoxia induces HIF1α expression and this was down regulated by PEDF. Hypoxia also induced prolyl hydroxylase (PHD) 2 and PHD3 expression, but PHD expression was not differentially regulated by PEDF. PEDF expression was not regulated by hypoxia, and transforming growth factor β significantly suppressed PEDF expression.

In anti-Thy1.1 glomerulonephritis, there is aberrant tubular angiogenesis associated with glomerular injury and tubulointersititial fibrosis. We showed that PEDF may be involved by downregulating HIF1α. Further work is needed to elucidate the mechanism of PEDF upregulation and action in the tubules.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2874**

**TGFβ Differentially Regulates Expression of Cadherins in Proximal Tubule Cells** Nileshkumar Shah,[1] Iain Macphee,[2] Mark Edward Dockrell.[1] *[1]SW Thames Institute for Renal Research, London, United Kingdom; [2]Renal Medicine, St George's, University of London, London, United Kingdom.*

Adherens junctions and their constituent cadherins mediate adhesion between cells and maintain local intracellular concentrations of scaffolding and signaling molecules. They are key in regulating differentiation and polarisation of epithelial cells. E-cadherin (CDH1) has been much studied in the loss of renal epithelial integrity and the development of tubulointerstitial fibrosis, particularly in the context of TGFβ1. Our current studies of expression of cadherins in human biopsies (submitted in a separate abstract) indicate that CDH1 is not expressed in the human proximal tubule but that CDH2 (N-cadherin) and CDH6 (K-cadherin) are the predominant isoforms there.

Hence, we have investigated the CDH expression and regulation by TGFbeta. in human proximal tubule epithelial cells (PTEC) in culture.

Three cell models were used; primary cultures, SV 40 transformed HKC, and HPV transformed HK-2, cells. Cells were grown to 75% confluence and treated with TGFβ1 in a dose (1.25-5.0 ng/ml) and time (1-72 h) dependent manner. CDHmRNA was studied by RT-qPCR; and protein by immunoblotting.

In HKC CDH1 protein was readily detectable along with CDH2 but not CDH6. In contrast, in primary PTEC all 3 CDHs were detected. qPCR demonstrated higher expression of CDH6 and lower expression of CDH1 in primary cells compared to HKC. HK-2 cells had a similar profile to primary cells.

CDH1 mRNA and protein was down-regulated by TGFβ1 in all cell lines, as expected.

In contrast CDH2 was consistently up-regulated in all models.

In Primary cells TGFβ1down-regulated CDH6 protein but this was not reflected by reduction in mRNA. In contrast, in HK-2 cells CDH6 was upregulated both at protein and mRNA level.

Our results show that although TGFβ1 does down regulate CDH6 in primary cultures, unlike CDH1 this is not transcriptionaly regulated and therefore may not involve traditional TGFβ1 signaling. The TGFβ1-inudced increase in CDH2 is consistent with

the association of CDH2 with a migratory phenotype. The "natural" CDHs of the human proximal tubule do not respond to TGFβ1 in a manner predicted from murine or existing cell culture models.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2875**

**Altered Expression of the LIM Protein Hic-5 in Glomerular and Tubular Cells during Unilateral Ureteral Ligation** Nick Hornigold,[1] Madeleine Anne Vernon,[2] Jeremy Hughes,[2] Andrew F. Mooney.[3] *[1]CRUK Clinical Research Centre, St James's University Hospital, Leeds, West Yorkshire, United Kingdom; [2]Queen's Medical Research Institute, University of Edinburgh, Edinburgh, Scotland, United Kingdom; [3]Renal Unit, St James's University Hospital, Leeds, West Yorkshire, United Kingdom.*

The LIM protein, Hic-5, has an established role in experimental glomerular scarring (KI 2010). In vitro, mesangial cell attachment to collagen I upregulates Hic-5, initiating a pro-sclerotic phenotype with increased susceptibility to apoptosis (ASN 2008). Hic-5 is also expressed in normal distal tubular cells but its role here is unknown. We have now studied the tubular and glomerular expression of Hic-5 during unilateral ureteral ligation.

Rats underwent unilateral ureteral ligation by standard methods and were sacrificed at days 0, 7, 14 and 21. Immunohistochemical analysis of Hic-5 expression was undertaken and quantified by a blinded observer.

At day 0, there was no glomerular Hic-5 expression, as previously reported, but widespread expression in distal tubular cells. During progression of the disease, this pattern reversed. Using semi-quantitative scoring on a scale of 0-5, Hic-5 expression in glomeruli increased from 0.05 +/- 0.1 at day 0, to 0.14+/-0.02 at day 7, 1.57+/- 0.39 at day 14 and 1.69+/- 0.81 at day 21 despite no obvious changes in glomerular morphology.

To assess tubular expression, consecutive random cortical cross-section high-power fields were counted for Hic-5 positive cells. These changed from 140.6 +/- 7.7 at day 0, to 29.5 +/- 9.5 at day 7, 43.7 +/- 20.7 at day 14 and 36.8 +/- 3.8 at day 21.

These data suggest an important role for Hic-5 in tubular disease as well as glomerular disease. Experiments are underway to understand the consequences for tubular cells of reduced Hic-5 expression.

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Disclosure of Financial Relationships: nothing to disclose

**SA-PO2876**

**A Novel Benzylidene-Thiazolidinedione with Specific and Partial Agonist Activity on PPARγ** Angélica Amorim Amato,[1] Senapathy Rajagopalan,[5] Bruno M. Carvalho,[2] Mario Saad,[2] Marie Togashi,[1] Luiz A. Simeoni,[1] Igor Polikarpov,[3] Maria Carmo Alves Lima,[4] Suely L. Galdino,[4] Ivan R. Pitta,[4] Paul Webb,[5] Kevin Phillips,[5] Francisco R. Neves.[1] *[1]Pharmaceutical Sciences, University of Brasilia, Brasilia, Federal District, Brazil; [2]Internal Medicine, State University of Campinas, Campinas, Sao Paulo, Brazil; [3]Physics, University of Sao Carlos, Sao Carlos, Sao Paulo, Brazil; [4]Antibiotics, University of Pernambuco, Recife, Pernambuco, Brazil; [5]Center for Diabetes Research, The Methodist Hospital Research Institute, Houston, TX.*

Thiazolidinediones (TZDs) can improve whole body insulin sensitivity and ameliorate glucose metabolism in many animal models of obesity and diabetes, and also in humans. Despite the established therapeutic value of TZDs, their clinical use is limited by a number of adverse effects. Aiming to identify novel peroxisome proliferator-activated receptor-gamma (PPARgamma) ligands, synthetic benzylidene-TZDs were screened for PPAR agonist activity using reporter gene assay in U-937 human pro-monocytes. GQ-16 (5-(5-bromo-2-methoxy-benzylidene)-3-(4-methyl-benzyl)-thiazolidine-2,4-dione) was the only compound found to have agonist activity on PPARgamma. This compound displayed features of a partial and specific agonist for the receptor. It also repressed TNF-alpha promoter in human mesangial cells in a concentration-dependent manner, with reduced potency but with a comparable maximal response to rosiglitazone. GQ-16 bound PPARgamma with reduced affinity when compared to rosiglitazone, induced a protease digestion pattern similar to rosiglitazone, and SRC1 recruitment similar to troglitazone. The adipogenic potential of GQ-16 was less pronounced than that of rosiglitazone in vitro. Studies in obese Swiss mice indicated GQ-16 treatment results in insulin sensitization comparable to rosiglitazone, but with no weight gain. The cocrystal structure of GQ-16 with PPARgamma ligand-binding domain revealed a different binding mode when compared to that of rosiglitazone. These data establish GQ-16 as a novel PPARgamma agonist with promising therapeutic utility with the potential for less weight gain.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2877**

**Eicosapentaenoic Acid Restores Diabetic Tubular Injury through Regulating Oxidative Stress and Mitochondrial Apoptosis** Sekiko Taneda,[1] Kazuho Honda,[1] Chiharu Aoki,[1] Saeko Kanai,[1] Kosaku Nitta,[2] Hideaki Oda,[1] Kenta Uto.[1] *[1]Department of Pathology, Tokyo Women's Medical University; [2]Department of Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.*

**Objective:** The present study was designed to elucidate the mechanism of early diabetic tubular injury which is important in the pathogenesis of diabetic nephropathy, and to examine a therapeutic potential of dietary eicosapentaenoic acid (EPA) for the prevention of diabetic nephropathy. **Research design and methods:** Utilizing streptozotocin (STZ)-induced diabetic mice, albuminuria and the extents of histological injuries were monitored at 2 weeks after diabetes induction. Reactive oxygen species (ROS) production, apoptosis and hypoxia in the kidney were evaluated by immunohistochemistry and western blotting. In vitro study was performed using rat proximal tubular cells (NRK-52E) to confirm the protective effects of EPA for methylglyoxal (MG)-induced ROS generation and mitochondrial apoptosis. **Results:** The extents of albuminuria and histological tubular injuries were significantly lower in EPA-treated diabetic mice compared to the untreated diabetic mice. The levels of lipid peroxidation product (4-hydroxy-2-nonenal), oxidative DNA damage (8-hydoxy-deoxyguanosine), and mitochondrial apoptosis (TUNEL, caspase-9, cleaved caspase-3 and cytochrome C release) in the tubular cells were also significantly lower in EPA-treated diabetic mice. Furthermore, HIF-1α expression was significantly up-regulated in kidney tissues from EPA-treated mice than from untreated diabetic mice, although both diabetic groups were at similar levels of pimonidazole. NRK-52E cells incubated with MG induced ROS overproduction and apoptosis, and both of which were significantly reduced by the EPA treatment. **Conclusions:** Our results indicated that the ROS generation and mitochondrial apoptosis were involved in hyperglycemia-induced tubulopathy and EPA has a beneficial effect by suppressing ROS formation and apoptosis partly through the augmentation of HIF-1α response in diabetic tubular injury.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2878**

**Sildenafil, a Phosphodiesterase Type 5 Inhibitor, Attenuates Diabetic Nephropathy in Non-Insulin-Dependent Otsuka Long-Evans Tokushima Fatty Rats** Yoshihiro Kuno, Masayuki Iyoda, Takanori Shibata, Yuki Hirai, Tadao Akizawa. *Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan.*

Background. It is well established that the pathogenesis of diabetic nephropathy (DN) is associated with abnormalities of renal nitric oxide (NO) generation. Many of the biologic actions of NO are mediated by cGMP, which is rapidly degraded by phosphodiesterases (PDE). In this study, we evaluated the renoprotective effects of sildenafil (SIL), an inhibitor of PDE-5, in type 2 diabetic rats. Methods. Male Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a non-insulin-dependent diabetes model, and Long-Evans Tokushima Otsuka (LETO) rats, a non-diabetic control, were treated with either SIL (2.5 mg/kg in drinking water) (OLETF-SIL) or undosed water (OLETF-CON) for 28 weeks, starting at 20 weeks of age; all rats were sacrificed at week 48. Albuminuria, BP, and BW were measured periodically. Results. SIL treatment significantly decreased albuminuria (36.19 ± 6.35 vs. 48.01 ± 8.21, $p < 0.05$), attenuated glomerular hyperfiltration, and resulted in a decrease in glomerular hypertrophy (glomerular size: 9921.71 ± 266.60 vs. 10856.60 ± 206.65 mm2, $p < 0.05$), in addition to a reduced glomerulosclerosis score (semiquantitative score: 0.89 ± 0.13 vs. 1.42 ± 0.13, $p < 0.05$) and a dramatic decrease in the number of glomerular and tubulointerstitial PCNA-positive cells in OLETF rats (PCNA+ cells in glomerulus: 0.043 ± 0.02 vs. 6.68 ± 0.37, $p < 0.0001$; PCNA+ cells in tubulointerstitium (0.5mm2): 4.60 ± 0.50 vs. 63.90 ± 16.00, $p < 0.001$). This was accompanied by a significant reduction in renal cortical mRNA levels of collagen types I ($p < 0.05$) and III ($p < 0.05$). The increased mRNA levels of matrix metalloproteinase (MMP)-2, MMP-9, tissue inhibitors of MMPs (TIMP)-1, and TIMP-2 in the OLETF rats were significantly or partially attenuated by SIL treatment (MMP-2, $p < 0.01$; MMP-9, $p < 0.01$; TIMP-1, $p < 0.05$; TIMP-2, NS, OLETF-SIL vs. OLETF-CON). Conclusions. This study suggests that SIL attenuated DN by its potent antiproliferative effects and by regulating extracellular matrix by affecting the balance between MMPs and their inhibitors.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2879**

**Precedent Anti-Inflammation and Suppression of Subsequent Interstitial Fibrosis in the Kidney by Non-Smooth Muscle Calponin (Calponin-h2) in Diabetic Mice Are Independent of Both Glucose Metabolism and Expression of Its Related Molecules** Kiyoko Inui, Takahiro Nakayama, Yoshihiko Inoue, Ashio Yoshimura. *Division of Nephrology, Showa University Fujigaoka Hospital, Yokohama, Kanagawa, Japan.*

Neutral calponin (Calponin-h2, CN2) is a non-smooth muscle isoform, and suppresses smooth muscle cell migration in addition to physiological roles in cytoskeletal organization. We established CN2 transgenic (CN2-Tg) mouse with the CRE-loxP site-specific recombination system, in that CN2 expression is induced only during the treatment of cadmium sulfate (0.5mg/kg BW/day) and we studied participation of CN2 in diabetic interstitial injury. Diabetes was induced by streptozocin (STZ, 100mg/kg BW x 2 times/3days) on both of CN2-Tg mouse and wild type ones (WT). Cadmium sulfate intraperitoneal treatment was started 5 days before the first STZ injection. Nephrectomy was done on 8 and 30 days after disease induction as well as on day 0 (n=6 in each). Immunostaining showing below was performed for evaluation of interstitial inflammation and all data were evaluated by computer-analysis system. The interstitial cell proliferation (ki-67$^+$ cells/high power field, hpf) was significantly suppressed in CN2-Tg at day 8 (3.7±0.5 (m±SE) in CN2-Tg, vs 5.7±0.4 in WT, $p<0.05$), and reduction of macrophages recruitment in the interstitium (F4/80$^+$ cells/hpf) was induced in CN2-Tg at day 8 (3.7±0.1 vs 6.9±1.3 , $p<0.02$), and day 30 (2.2±0.7 vs 5.4±0.5, $p<0.01$). Interstitial fibropsis was also significantly suppressed in CN2-Tg. There was no significant diffetrence in serum levels at day 8 for glucose (500.9±25.2 vs 444.3±76.9 mg/dl), insulin (0.5±0.02 vs 0.5±0.03 ng/ml), reptin (68.3±2.5 vs 68.4 ±2.6 pg/ml) and adiponectin (37.9±4.1 vs 48.0±4.0 ng/ml), those were all

studied by EIA. In conclusion, CN2-Tg mice showed precedent anti-inflammatory property and suppressed subsequent development of interstitial renal fibrosis in diabetes mellitus, those were independent of glucose metabolism and expression of its related molecules.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2880**

**Identification of Periostin as a Novel Matricellular Protein Linked to Progression of Glomerulonephropathies** Kontheari Sen,[1] Maja Lindenmeyer,[1,2] Ariana Gaspert,[3] Felix H. Eichinger,[4] Matthias Kretzler,[4] Stephan Segerer,[2] Clemens D. Cohen.[1,2] *[1]Institute of Physiology, University of Zurich, Zurich, Switzerland; [2]Division of Nephrology, University Hospital Zurich, Zurich, Switzerland; [3]Institute of Surgical Pathology, University Hospital Zurich, Zurich, Switzerland; [4]Department of Medicine, University of Michigan, Ann Arbor.*

Matricellular proteins (MP) are known to be involved in the pathogenesis of chronic nephropathies. To identify MP contributing to the progression of glomerulonephropathies (GN), glomerular gene expression was studied by microarrays on patients with focal-segmental glomerulosclerosis (FSGS, n=19), membranous GN (MGN, n=21), minimal change disease (MCD, n=5) and confirmed by real-time RT-PCR on additional biopsies. Immunohistochemistry (IHC) was performed on routine kidney biopsies.

Fifteen out of 19 known MP were found to be induced on transcriptional level in proteinuric GN. The highest induction was seen for periostin (POSTN), specifically in the progressive diseases FSGS and MGN. Real-time RT-PCR on glomerular samples confirmed the POSTN mRNA induction in progressive GN (lupus nephritis (LN, n=20) 12.9±33.8, $p<0.001$, FSGS (n=16) 2.3±1.9, $p<0.05$, MGN (n=14) 1.9±1.9, $p<0.1$). POSTN mRNA expression showed a negative correlation with renal function in a larger set of glomerular and tubulointerstitial specimen ($r=-0.18$, p=8.1E-03, $r=-0.47$, p=6.9E-14, respectively; n=221). By IHC on healthy kidneys a discrete positivity for periostin was found in the glomerular tuft, surrounding the vascular pole, and along the Bowman's capsule; no expression in the tubulointerstitium was detected. A prominent mesangial periostin staining was present in biopsies with progressive GN. Furthermore, periostin was found in areas of interstitial fibrosis. Co-immunofluorescene for smooth muscle actin and periostin revealed no overlap but a clear proximity suggesting mesangial cells (MC) as the source of glomerular periostin. This was supported by the detection of periostin by western blot on a MC line and its supernatant.

In sum, periostin is constitutively expressed in healthy glomeruli and is linked to progression of GN and renal failure.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2881**

**Adiponectin Regulates Bone Marrow-Derived Fibroblasts in Renal Fibrosis** Song-Chang Lin, Gang Chen, Feixia Dong, Jie Du, William E. Mitch, Yanlin Wang. *Department of Medicine-Nephrology, Baylor College of Medicine, Houston, TX.*

Progressive renal fibrosis is the final common manifestation of chronic kidney disease resulting in irreversible loss of kidney parenchyma and renal function. Myofibroblasts play a critical role in the pathogenesis of renal fibrosis. Their origin remains controversial. Recent evidence suggests that bone marrow-derived fibroblasts can be detected in the kidney leading to renal fibrosis. In this case, the stimulus for recruitment of bone marrow-derived fibroblasts into the kidney is not clearly defined. Adiponectin (APN) is a circulating adipokine that can influence the migration of progenitor cells. Since APN levels are elevated in chronic kidney disease, we investigated the role of adiponectin in regulating the uptake of bone marrow-derived fibroblast into the kidney in a murine model of renal fibrosis induced by unilateral ureteral obstruction (UUO). Wild type (WT) and adiponectin knockout (APN-KO) mice were subjected to UUO and maintained for up to 2 weeks. Bone marrow-derived fibroblasts, identified as cells positive for both CD45 and collagen I or cells positive for both CD11b and collagen I, were accumulated in obstructed kidneys of WT mice in a time-dependent manner, reaching a peak at 5 days after UUO, which were significantly reduced in obstructed kidneys of APN-KO mice. Furthermore, the number of bone marrow-derived myofibroblasts that are positive for both CD45 and α-SMA were significantly decreased in obstructed kidneys of APN-KO mice compared to those of WT mice. Compared to contralateral kidneys, obstructed kidneys of WT mice showed increases in type I collagen and α-SMA as determined by immunohistochemical staining and Western blot analysis and total collagen as detected by picrosirius red stain. All of these parameters were attenuated in obstructed kidneys of APN-KO mice. These data indicate that APN can play a significant role in the recruitment and activation of bone marrow-derived fibroblasts during the pathogenesis of renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2882**

**Analysis of GDNF-Inducible Zinc Finger Protein 1 Expression in Human Diseased Kidney** Shoji Saito,[#1,#2] Shoichi Maruyama,[#1] Seiichi Matsuo,[#1] Masahide Takahashi.[#2] *[1]Nephrology, Nagoya University, Nagoya, Aichi, Japan; [2]Tumor Pathology, Nagoya University, Nagoya, Aichi, Japan.*

The glial cell-line-derived neurotrophic factor (GDNF)-RET signaling pathway plays an important role in kidney development. We have previously identified a novel zinc finger protein, GZF1 (GDNF-inducible zinc finger protein 1), whose expression was induced in the human neuroblastoma cell line TGW expressing RET by GDNF stimulation and also detected in mouse metanephric kidney. In the present study, we examined the immunohistochemical expression of GZF1 in normal human kidney and various kidney diseases including chronic kidney disease (CKD) and cancers, and assessed the clinical significance of GZF1 expression. In the normal kidney, GZF1 was highly expressed only in the proximal tubular epithelial cells that were also positive for angiotensin-converting enzyme. We also evaluated GZF1 expression in various kidney diseases including membranous nephropathy, minimal change nephrotic syndrome, IgA nephropathy, diabetic nephropathy, acute tubular necrosis and ANCA-related glomerulonephritis. We found that decreased expression of GZF1 was associated with an increase in tubulointerstitial damage and serum creatinine levels. In addition, GZF1 expression was undetectable or very low in most cases of renal cell carcinomas and Wilms tumors. These findings suggest that GZF1 represents a new marker for renal proximal tubules and that there is an inverse correlation between the expression level of GZF1 and tubular function.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2883**

**Expression of P311 and Transforming Growth Factor-β1 in the Inflammatory Injured Human Kidneys** Feng Ping Wang, Zi Li, Junming Fan, Ping Fu. *Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

***Background:*** By binding to the transforming growth factor-β1 (TGF-β1) latency associated protein (LAP), P311 can induce myofibroblast phenotype in NIH3T3 cells *in vitro*, which suggested P311 involves in the fibrosis progression. This study was to detect the expression of P311 and TGF-β1 in the inflammatory injured human kidneys. ***Methods:*** 57 immunoglobulin A nephropathy (IgAN) patients, 11 membranoproliferative glomerulonephritis(MPGN) patients, 10 minimal-change nephrotic syndrome(MCNS) patients, 10 lupus nephropathy (LN) patients and 10 controles from partial nephrectomy were included. Clinical data from these patients at renal biopsy are shown in Table 1. In IgAN, the relevant clinical data of subgroups according to Katafuchi pathological cumulative scores were expressed in Table 2. P311 and TGF-β1 protein expression were detected by immunohistochemistry. Clinical data such as proteinuria quantification, serum creatinine level were analysized. The relationship between P311 and TGF-β1 in IgAN was analysized. ***Results:*** In normal human kidneys, anti-P311 antibody and anti-TGF-β1 antibody detected negative or weak immunoreactivity in renal tubular cells (Fig.1: a, b). In inflammatory injured human kidneys, intense P311 immunoreactivity was localized to distal tubules and collecting ducts (Fig.2: a,c,e,h), whereas TGF-β1 showed intense staining in both distal and proximal tubules (Fig.2: b,d,e,g). In addition, there was significant difference in P311 protein expression among different pathological grading subgroups in IgAN (Table 3; Fig.3). P311 protein expression in tubulointerstitial tissue was correlated with TGF-β1 in IgAN ($r = 0.921$, $P < 0.001$). ***Conclusions:*** These results indicate P311 and TGF-β1 was expressed in inflammatory injured human kidneys. P311 might be a key cytokine and be involved in the progression of renal fibrosis by binding to the TGF-β1 LAP.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2884**

**Role of Epithelial Mesenchymal Transdifferentiation (EMT) in Pathogenesis of Collapsing Focal Segmental Glomerulosclerosis in Children** Hanan K. Tawadrous,[1] Raafat Gorgy,[3] Sonal Bajaj,[1] Anthony D. Nicastri,[1] Morris J. Schoeneman,[1] Pravin C. Singhal,[2] Anil K. Mongia.[1] *[1]Pediatrics, Downstate Medical Center, Brooklyn, NY; [2]Medicine, Long Island Jewish Medical Center, New Hyde Park, NY; [3]Medicine, Trinitas Regional Hospital, Elizabith, NJ.*

*Background:* Collapsing focal segmental glomerulosclerosis ( CFSGS) is characterized by severe nephrotic syndrome, collapse and sclerosis of the glomerular tuft with prominent podocyte alterations and extensive tubulointerstitial lesions. Although there is an agreement on glomerular proliferative phenotype, the type of cells contributing to this phynotype remains controversial. Epithelial mesenchymal trans differentiation (EMT) is a process in which renal epithelial cells lose their epithelial phenotype and attain new characteristic features of mesenchymal cells. EMT has been reported to contribute to the progression of renal fibrosis in animal models. Increase expression of proliferating cell nuclear antigen (PCNA), α-smooth muscle actin(α-SMA), fibroblast-specific protein-1(FSP1) increase in EMT.

*Design:* We studied the role of EMT in the pathogenesis of collapsing type of FSGS. To determine the transformation of epithelial cells to myofibroblasts, we immunolabeled renal cortical sections from 8 patients with CFSGS and 3 controls for -SMA, PCNA, FSP1, E-cadherin. The number of positive cells in 10 random fields was counted, and the mean numbers of cells were calculated per glomerulus and per tubule. The results were compared with historical controls.

*Results:* Our data showed an increase of α- SM-actin, PCNA, FSP-1 positive stain in CFSGS pts in comparison to the controls. SMA means per glomerulus (SMA 28.6±8.75 vs1.5±1.1 p 0.0007 , PCNA17.3±6.58 vs 1.8±1.2, p 0.0035 FSP1 2.42 ±0.76 vs 0.5± 0.1 p 0.002) and per tubules SMA 17.42 ± 6.63 vs 1.5±1.1 p0.002 PCNA 6.16±3.05 vs 1.4±0.5p 0.009 FSP1 2.22±0.76 vs 1.25±1 p_0.11. E-cadherin was diminished for glomerulus 4.21±2.68and per tubules 1.66±1.41p <000.1

*Conclusion:* We conclude that renal cell EMT plays a role in the development of collapsing FSGS and increased intensity of SMS, PCNA, or FSP might correlate with *Conclusion:* We conclude that renal cell EMT plays a role in the development of collapsing FSGS and increased expression of SMS, PCNA, or FSP might correlate with intensity of the disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2885**

**Oral Iron Chelator – Deferiprone Prevents Nephrogenic Systemic Fibrosis in Mice** Chhanda X. Bose,[1] Sudhir V. Shah,[1] Kim M. Hiatt,[2] Sundararaman Swaminathan.[1] *[1]Nephrology, University of Arkansas for Medical Sciences, Little Rock, AR; [2]Dermatopathology, University of Arkansas for Medical Sciences, Little Rock, AR.*

**Background**: Nephrogenic Systemic Fibrosis (NSF) is associated with tissue accumulation of iron. Gadolinium contrast agents induce iron mobilization. However, role of endogenous iron in the pathophysiology is NSF has never been examined before. We examined this by using an oral iron chelator- deferiprone in a murine model of NSF.

**Methods:** In BalbC mice, we performed staged 5/6 nephrectomy to induce chronic kidney disease (CKD). Omniscan was administered intravenously, in various dosages (0.1, 0.5 and 2.5 mmol/Kg) and frequency (5 days a week to thrice a week) to CKD and sham-operated mice. Mice were sacrificed at varying time points (4 weeks to 18 weeks) after completing the Omniscan treatments. Once the murine NSF model was established, experiments were repeated in which oral iron chelator-deferiprone was administered along with IV Omniscan in a subgroup of mice. After 4-18 weeks of treatment, histologic analyses were performed.

**Results:** Omniscan treatment induced ulceration and sloughing of tail. No overt skin changes were observed in other areas of the skin. Progressive weight loss was observed in the Omniscan-treated mice. Early skin biopsies revealed mast cell and macrophages accumulation and increase in dermal collagen. Low doses (0.1 mmol/Kg) of Omniscan) induced NSF-like skin changes in CKD mice evaluated at 18 weeks. Iron chelator treated mice exhibited less tail ulceration, decreased weight loss with preservation of adipose tissue and diminished mast cell and macrophage infiltration and substantially decreased dermal fibrosis.

**Conclusion:** Prevention of NSF in this mice model suggests an important role for iron in the pathogenesis of gadolinium toxicity and NSF.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2886**

**Cutaneous Toxicity of Various Gadolinium Chelates in a Rat Model of Nephrogenic Systemic Fibrosis** Nathalie Fretellier,[1] Patrick Bruneval,[2, 3] Chantal Mandet,[2] Nejma Bouzian,[1] Jean-Marc Idee.[1] *[1]Research Department, Guerbet, Roissy, France; [2]INSERM U970, INSERM, Paris, France; [3]Pathology, University Paris Descartes, Paris, France.*

Nephrogenic systemic fibrosis (NSF) is a severe side effect of gadolinium chelates (GC) used as contrast agents for MRI in patients with end stage renal failure. The aim of this study was to test the cutaneous toxicity of various GC in a sensitized rat model of NSF.

Chronic renal failure was induced in male Wistar rats by 5/6 nephrectomy. Rats were sensitized by high phosphate diet (1%). Rat groups (n = 8/group) received single daily intravenous injections for 5 days either of saline for the control group or various GC at 2.5 mmol/kg (mL/kg of body weight): gadodiamide (Omniscan®), gadoterate (Dotarem®), gadobenate (MultiHance®), or gadobutrol (Gadovist®). Skin biopsies were performed at days 1, 8, and 25 ( or at premature animal sacrifice). Clinical and histological analysis were assessed blindly.

In all the rat groups, no early skin lesion were observed at days 1 and 8. In the gadodiamide-treated group, ulcerative and squamous skin eruptions appeared in 5 rats out of 8, from Day 8 until Day 23, and worsened in 4 animals or improved in 1 rat. Four rats were sacrificed for ethical reasons. No clinical skin lesions were observed in the other GC-treated groups or in controls. Skin histology was abnormal in 6 rats out of 8 in the gadodiamide-treated group, while no histological skin changes were observed in the other groups. Lesions consisted of bands of inflammatory dermal fibrosis. ED1-positive macrophages accumulated in the involved dermis and expressed TGFβ1. They were associated with an increased density of fibroblasts expressing TGFβ1, CD34, and S100A4 (equivalent to FSP1). Interestingly no myofibroblasts expressing α-smooth muscle actin were detected. In all the other GC-treated groups and in controls, histological and immunohistochemical patterns were normal.

In conclusion, the nonionic, linear GC gadodiamide is associated NSF-like skin lesions in hyperphosphoremic rats with advanced chronic renal failure.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2887**

**A Mineralocorticoid Receptor Antagonist Prevents Chronic Cyclosporine A Nephropathy in the Rat** Finn Thomsen Nielsen,[1,2] Boye Jensen,[1] Pernille B. Hansen,[1] Peter Bie.[1] *[1]Inst. of Molecular Medicine, University of Southern Denmark, Odense, Denmark; [2]Nephrology Clinic P, Copenhagen State Hospital, Copenhagen, Denmark.*

Aldosterone is suspected to aggravate cyclosporin A (CsA)-induced nephrotoxicity. We hypothesized that the mineralocorticoid receptor (MR) blocker eplerenone (EPL) prevented chronic CsA-induced renal functional decrease and tissue injury in rats.

Sprague-Dawley rats received CsA (15 mg/kg/day i.p.) and/or EPL (100 mg/kg/day p.o.) for 12 w. At 11 w, arterial and venous catheters were implanted. Rats were housed individually and were able to move freely during blood sampling and on-line measurement of BP. Inulin was infused for 24 h. At termination, one kidney was rapidly frozen and the contralateral kidney was perfusion fixed and divided in 1 mm slices, embedded in paraffin. Random sections were stained with PAS. Stereological analysis allowed blinded calculation of the volumes of tubuli ($V_{tub}$) and of the interstitial space ($V_{int}$).

CsA treatment showed significantly lower $V_{tub}$ and larger $V_{int}$ compared to controls ($p<0.001$); these changes were attenuated by EPL treatment ($p<0.001$). Hyaline vacuolization in tubules and vascular depositions in arterioles were seen in CsA treated rats but less pronounced after combination therapy. Inulin clearance, plasma sodium and potassium, or kidney weight/body weight ratio displayed no significant changes between groups. CsA reduced weekly body weight gain: 13.4±5.2 vs. 23.0±5.0 g, increased mean day-time BP: 125.8±6.4 vs. 108.0±9.8 mmHg and mean night-time BP: 124.3±6.6 vs.112.4±6.1 mmHg compared to controls ($p<0.01$ in all cases). These changes were prevented by EPL treatment: weight gain: 22.2±4.0 g, mean day-time BP: 109.7±7.5 mmHg and mean night-time BP: 112.4±6.1 mmHg (NS compared to controls). P-renin was elevated in CsA-treated groups compared to controls ($p<0.05$), P-aldosterone was higher in the EPL-treated group compared to both the CsA-alone treated group and controls ($p<0.02$). Whole blood CsA concentrations were not different between groups.

MR blockade by EPL prevented kidney tissue injury and hypertension by chronic CsA. It is concluded that the aldosterone-MR pathway contributes to chronic CsA-mediated nephrotoxicity.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2888**

**Inhibition of Renin Retards Progression of HIVAN** Dileep Kumar,[1] Ankita Sagar,[2] Shabina Rehman,[1] Ashwani Malhotra,[1] Praveen N. Chander,[2] Pravin C. Singhal.[1] *[1]Medicine, Long Island Jewish Medical Center, New Hyde Park, NY; [2]Pathology, New York Medical College, Valhalla, NY.*

Inhibition of the production of Ang II as well as blockade of AT1 receptors has been reported to slow down the progression of HIVAN. However, the role of renin inhibition has not been examined in the progression of HIVAN. We asked whether HIVAN mice had enhanced expression of renin. If yes, would the inhibition of renin would modulate the progression of HIVAN.

Six Vpr mice were either fed doxycycline (doxy) or vehicle in their drinking water for six wks (6 wks of doxy will allow them to develop HIVAN). Subsequently, kidneys were harvested and RNA was extracted, followed by probing for renin. A total of 24 (aged six wks) Vpr mice were fed doxycycline for six weeks followed by kidney biopsy for confirmation of the development of HIVAN and baseline kidney lesions. Subsequently, mice in groups of six were administered either normal saline or aliskiren (50 mg/Kg/day, by miniosmotic pump) for either 4 weeks (protocol A) or eight weeks (protocol B). At the end of experimental periods, blood and urine samples were collected and kidneys were harvested for renal histology. Vpr mice fed doxy showed 2-3 fold greater expression of renin expression. All mice showed overt HIVAN phenotype in the form of FSGS and microcystic dilatation of tubules at the end of the doxy therapy. In protocol A, mice receiving aliskiren showed 24.2% increase in number of sclerosed glomeruli (from the baseline value at 6 wks doxy) when compared to 139.2% increase in sclerosed glomeruli in saline receiving mice ($P<0.01$). In protocol B, mice receiving aliskiren showed 26.4% increase in number of sclerosed glomeruli (from the baseline value at 6 wks doxy) when compared to 124.6% increase in sclerosed glomeruli in saline receiving mice ($P<0.01$). Similar pattern was noted in the progression of tubular injury in both the protocols. Aliskiren also diminished urinary protein creatinine ratio (Protocol A, saline, 51.5 vs. aliskiren, 8.8, $P<0.01$). These findings indicate that renin inhibition slows down the progression of HIVAN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2889**

**Aldosterone Contributes to the Progression of Renal Lesions in HIV-Associated Nephropathy (HIVAN) Mice** Dileep Kumar,[1] Ankita Sagar,[2] Divya Salhan,[1] Ashwani Malhotra,[1] Praveen N. Chander,[2] Pravin C. Singhal.[1] *[1]Nephrology, North Shore LIJ Health System, Great Neck, NY; [2]Pathology, New York Medical College, Valhalla, NY.*

Aldosterone has been reported to modulate the progression of renal lesions in several models of chronic kidney diseases. Since renin-angiotensin-aldosterone has been reported to play a role in the pathogenesis of HIVAN, we examined the contributory role of aldosterone in the pathogenesis of HIVAN.

To evaluate the effect of aldosterone on HIVAN, three weeks old control (FVB/N) or Tg26 mice (n=6) were administered either normal saline (FVBN, CS;Tg26, TgS), aldosterone (2 µg/day, FVBN, CA; Tg26, TgA), spironolactone (an aldosterone antagonist, FVBN, CSP; Tg26, TgSP), or aldosterone + spironolactone (FVBN, CA-SP;Tg26, TgA-SP) via miniosmotic pumps for four weeks. Every week blood pressure (BP) was recorded. At the end of the experimental period, blood and urine samples were collected and kidneys were harvested for grading the severity of renal lesions.

TgA mice showed higher ($p<0.01$) blood pressure when compared with TgS (TgA, 159/114; TgS, 118/80 mm Hg). However TgA-SP showed comparable blood pressure to TgS. Similarly, TgA mice showed higher urinary protein creatinine ratio (UP/CR) than to TgS (TgA, 38.5; TgS 27.5; CS, 6.9); whereas, spironolactone attenuated this effect of aldosterone on proteinuria. CS showed segmental sclerosis only in 2.5 ± 1% glomeruli and 0 to 1 + dilatation of tubules. In contrast, CA showed segmental glomerulosclerosis in 22.5 ± 2% of glomeruli. TgS displayed 12. 8 ± 2% segmentally sclerosed glomeruli and grade 2-3 dilated tubules; on the other hand, TgA showed 32.7 ± 3% segmentally sclerosed glomeruli and grade 3-4 dilated tubules. However, TgSP showed only 9.1 ± 0.8% segmentally sclerosed glomeruli and 0 to 1 dilated tubules. Since administration of aldosterone accelerated progression of HIVAN and inhibition of aldosterone by spironolactone slowed down the progression of HIVAN, it suggests that aldosterone contributes to the pathogenesis of HIVAN.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2890**

**Sirolimus Retards Development and Progression of Renal Lesions in HIV-Associated Nephropathy (HIVAN) by Altering HIV-1 Gene Transcription**
Dileep Kumar, Divya Salhan, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.*

Sirolimus has been demonstrated to modulate the development of renal lesions in animal models of glomerulosclerosis such as diabetic nephropathy as well as cystic diseases such as polycystic kidney disease. Interestingly, HIVAN is characterized by both glomerulosclerosis and microcystic tubular dilatation of tubules. On that account, we hypothesize that sirolimus would also modulate the development of renal lesions in HIVAN. To prove our hypothesis we have used HIV-1 transgenic mice (Tg26).

Four weeks old Tg26 animals (n=6) were administered either vehicle or sirolimus (5 mg/kg/day, intra-peritoneally) for 14 days (Group A). In another set of experiments, 4 wks old Tg26 mice (n=6) were administered either vehicle or sirolimus for two weeks followed by no treatment for the next two weeks (Group B). At the end of experimental periods, total RNA was extracted and HIV-1 gene transcription was measured by real time PCR. Renal lesions were graded for sclerosis and tubular dilatation. In *in vitro* studies, human podocytes were transduced either with HIV-1 or vector, followed by treatment with either vehicle of sirolimus for 72 h. Subsequently RNA was isolated and HIV-1 gene expression was measured by RT-PCR.

In group A, the vehicle treated mice showed 1+ to 2+ glomerulosclerosis and 2+ to 3+ tubular dilatation, two sirolimus-treated mice showed no glomerular sclerosis and only mild dilatation of tubules and one sirolimus treated mouse showed 0 to 1+ glomerulosclerosis and 1+ to 2+ tubular dilatation. In group B, vehicle treated animals showed two-fold advanced glomerular and tubular lesions vs. sirolimus-treated animals. Renal cortical sections of sirolimus treated animals showed attenuation of PCNA + ive cells, expression of TGF-β, α-SMA, cTGF, VEGF-R1, and MCP-1 when compared with vehicle-treated animals. Sirolimus also decreased transcription of HIV-genes both in renal tissue as well as in HIV-1 transduced podocytes. These findings indicate that sirolimus-induced altered HIV-1 gene transcription could be contributing to its disease modulating effects in HIVAN mice.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2891**

**Coumarin-Induced Intimal Plaque Calcification in ApoE Knock-Out Mice**
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Vascular calcification (VC) is generally regarded as an independent risk factor for cardiovascular morbidity and mortality. Coumarin treatment has been demonstrated to increase medial elastocalcinosis in both experimental animal models and man by impairing the vitamin K-dependent matrix Gla-protein (MGP), a potent inhibitor of VC. However nothing is known about a potential effect of coumarin treatment on atherosclerosis. We used an animal model for atherosclerosis and investigated the effect of coumarin on intimal plaque calcification. ApoE-/- mice received a western diet for three month to induce atherosclerotic plaques. Next, they were randomized to receive a diet containing vitamin K (VK; 1.5mg/g) or VK plus warfarin (VK&W; 1.5 mg/g & 3.0 mg/g). Animals were sacrificed at baseline (t = 0), one week or four weeks after treatment. Mice treated with VK&W had significantly more VC as measured by both atomic absorption spectroscopy (quantitative) and von Kossa staining (qualitative). Moreover, in the intimal plaque of coumarin treated animals a substantial part of the intimal cells displayed a chondrocytic phenotype. This was consistent with the presence of collagen type II, a marker for chondrocytes, and von Kossa staining. Coumarin also induced phosphatidylserine (PS) exposure of endothelial cells covering the plaque, as measured by in vivo annexin A5-biotin binding.

This is the first study demonstrating that coumarin treatment causes an increase of intimal plaque calcification; moreover, the plaque calcification was associated with an increase of apoptotic cells, suggesting accelerated progression towards a vulnerable plaque.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2892**

**Dedifferentiation of Medial Smooth Muscle Cells Is Associated with Transplant Vasculopathy in Rat Renal Allografts**
Miriam Boersema,[1] Kirankumar Katta,[2] Heleen Rienstra,[3] Gerjan Navis,[2] Jacob Van den Born,[2] Jan-Luuk Hillebrands,[1] Eliane R. Popa.[1] *[1]Pathology and Medical Biology, UMC Groningen; [2]Nephrology, UMC Groningen; [3]Cell Biology, UMC Groningen, Netherlands.*

**Background.** Chronic transplant dysfunction (CTD) is the leading cause of renal allograft loss. CTD is, among others, characterized by transplant vasculopathy (TV), i.e. occlusive neointima formation with smooth muscle cells (SMCs) resulting in ischemic graft failure. We recently demonstrated α-SMA$^+$ neointimal SMCs in rat renal allografts to be solely graft-derived. In the current study we hypothesized that neointimal SMCs are derived from dedifferentiated medial SMCs by analyzing SMC (de)differentiation marker gene expression levels in microdissected tissue. Dedifferentiated SMCs are able to migrate, proliferate and secrete matrix molecules thereby forming a putative source for neointimal cells.

**Methods.** Using laser dissection microscopy neointimal and medial tissue was isolated from Dark Agouti (DA)-to-Wistar Furth renal allografts (n=5, with CTD & TV) and DA-to-DA isografts as well as non-transplanted DA renal tissue (control groups, both n=5, no CTD & TV). Low density qPCR was used to analyze gene expression of SMC (de)differentiation markers.

**Results.** Compared to both control groups, medial expression of SMC-specific differentiation marker SM22α was downregulated, suggesting dedifferentiation. In line with this, KLF4 (inducer of dedifferentiation) was upregulated. Furthermore, medial expression of the matrix molecules Coll1α1 and Coll4α1 and the profibrotic factors TGF-β1 and CTGF was increased in allografts (vs. both controls). Compared to medial SMCs in allografts, neointimal SMCs displayed a further decrease and increase of SM22α and KLF4 expression, respectively. PDGF-B was solely expressed in the neointima, whereas its receptor PDGFRB was upregulated in the allograft media (vs. media both controls).

**Conclusions.** Medial SMCs in rat renal allografts are dedifferentiated thereby potentially providing a source for neointimal SMCs. Neointimal PDGF-B expression might serve as a chemoattractant for medial SMCs. Prevention of medial SMC dedifferentiation is a potential target for prevention of TV.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2893**

**Cardioprotection in Chronic Kidney Disease – The Role of Erythropoietin**
Sebastian Markus Schaefer,[1] Nadezda Koleganova,[1] Grzegorz Piecha,[1] Eberhard Ritz,[2] Marie-Luise Gross.[1] *[1]Institute of Pathology, University of Heidelberg, Heidelberg, Germany; [2]Dept. Internal Medicine, University of Heidelberg, Heidelberg, Germany.*

Cardiovascular disease is the primary cause of mortality in patients with chronic kidney disease (CKD). Heart remodeling develops in CKD comprising of interstitial fibrosis and capillary loss.

12-weeks old male Sprague-Dawley rats were randomized to 5/6 nephrectomy (NX) or sham operation and subsequently received murine erythropoietin (2.5 µg/kg/week), enalapril (12 mg/kg/day), erythropoietin plus enalapril, erythropoietin plus dihydralazine (25 mg/kg/day), or vehicle for 16 weeks. Volume density of capillaries, interstitium, and fibrocytes as well as length density of capillaries were analysed in the myocardium using stereology.

Left ventricle fractional shortening (by echocardiography) was reduced in vehicle treated NX (66.3%) compared with sham-op (81.2%) and this was ameliorated by erythropoietin (72.6%) and prevented by enalapril (80.6%). Capillary length density was lower in vehicle treated NX (3631±466 mm/mm3) compared to sham-op (4264±442), and the capillary rarefaction was prevented in NX treated with erythropoietin plus enalapril (4298±576) and reduced in NX treated with enalapril (3908±383), and erythropoietin plus dihydralazine (3949±355), but not with erythropoietin (3697±565; ANOVA $p<0.02$). In parallel expression of the p47phox NADPH oxidase was higher in untreated NX and most effectively reduced in NX treated with erythropoietin plus enalapril. In basal condition, there was no difference between the groups regarding myocardial hypoxia, reflected by pimonidazole staining.

Erythropoietin in combination with enalapril additively reduce cardiac fibrosis and microvessel disease in 5/6 nephrectomized rats presumably by decreasing myocardial oxidative stress.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2894**

**Erythropoietin Does Not Prevent Albuminuria in 5/6 Nephrectomized Rats**
Firas Aldebssi,[1] Grzegorz Piecha,[1] Nadezda Koleganova,[1] Sebastian Markus Schaefer,[1] Eberhard Ritz,[2] Marie-Luise Gross.[1] *[1]Institute of Pathology, University of Heidelberg, Heidelberg, Germany; [2]Dept. Internal Medicine, University of Heidelberg, Heidelberg, Germany.*

Chronic kidney diseases progress over time irrespective of the primary insult. Erythropoietin not only promotes erythrocytes production, but also acts as an anti-apoptotic cytokine. We hypothesize that treatment with erythropoietin in subtotally nephrectomized rats may slow down the progression of renal damage.

12-weeks old male Sprague-Dawley rats were randomized to 5/6 nephrectomy (NX) or sham operation and subsequently received murine erythropoietin (2.5 µg/kg/week), enalapril (12 mg/kg/day), erythropoietin plus enalapril, erythropoietin plus dihydralazine (25 mg/kg/day), or vehicle for 16 weeks.

The albumin excretion was significantly higher in untreated NX (48.6±43.0 mg/24h) compared to sham-op (0.8±0.7), and this was reduced in NX treated with enalapril (3.6±3.1) and erythropoietin plus enalapril (12.7±10.2) but was not changed in NX treated with erythropoietin (50.1±34.7) and erythropoietin plus dihydralazine (85.2±59.9). Systolic blood pressure was higher in NX treated with erythropoietin but not in NX treated with erythropoietin plus enalapril and erythropoietin plus dihydralazine compared to untreated NX. Treatment of NX rats with erythropoietin resulted in lower expression in the kidney of p47phox NADPH oxidase, glutathione peroxidase 3, eNOS, iNOS, and β2 subunit of the soluble guanyl cyclase.

Treatment with erythropoietin failed to decrease albuminuria in 5/6 nephrectomized rats regardless of the blood pressure.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2895**

**BAMBI Is Expressed in Endothelial Cells and Is Regulated by Lysosomal/ Autolysosomal Degradation** Sandhya Xavier,[1] Victoria Gilbert,[1] Maria Pia Rastaldi,[3] Anand C. Reddy,[1] Stefanie Krick,[1] Dmitrij Kollins,[1] Clemens D. Cohen,[2] Erwin P. Bottinger,[1] Detlef O. Schlondorff.[1] *[1]Mount Sinai School of Medicine, New York, NY; [2]University of Zurich, Zurich, Switzerland; [3]Milan, Italy.*

BAMBI (**B**MP and **A**ctivin **M**embrane **B**ound **I**nhibitor) is considered to influence TGFß and Wnt signaling, and thereby fibrosis. Surprisingly data on cell-type specific expression of BAMBI are not available. **Purpose**: examine the localization, the gene regulation, and protein turnover of BAMBI in kidneys. **Methods and Results**: By immunofluorescence microscopy and by mRNA expression BAMBI is restricted to endothelial cells of the glomerular and peritubular capillaries, and of arteries and veins in both murine and human kidneys. TGFß upregulated mRNA of BAMBI in murine glomerular endothelial cells (mGEC). LPS did not downregulate mRNA for BAMBI in mGEC or in HUVECs. BAMBI mRNA had a half-life of only 60 min and was stabilized by cycloheximide indicating post-transcriptional regulation due to AU-rich elements, which we identified in the 3' untranslated sequence of both the human and murine BAMBI gene. BAMBI protein turnover was studied in HUVECs with BAMBI over-expression using a lentiviral system. Serum starvation as an inducer of autophagy caused marked BAMBI degradation, which could be totally prevented by inhibition of lysosomal and autolysosomal degradation with bafilomycin, and partially by inhibition of autophagy with 3-methyladenine, but not by proteasomal inhibitors. Rapamycin activates autophagy by inhibiting TOR, and resulted in BAMBI protein degradation. Both serum starvation or rapamycin increased the conversion of the autophagy marker LC3 from LC3-I to LC3-II and also enhanced costaining for BAMBI and LC-3 in autolysosomal vesicles. **Summary:** 1. BAMBI localizes to endothelial cells in the kidney and to HUVECs. 2. BAMBI mRNA is regulated by post-transcriptional mechanisms. 3.BAMBI protein is regulated predominantly by autolysosomal and lysosomal degradation. **Conclusion**: The endothelial localization and the quick turnover of BAMBI may indicate novel, yet to be defined, functions of this modulator for TGFß and Wnt protein actions in the renal vascular endothelium in health and disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2896**

**Conditional Ablation of Glycogen Synthase Kinase 3b in Postnatal Mouse Kidney** Rujun Gong, Lance D. Dworkin. *Brown Medical School, Providence, RI.*

Glycogen synthase kinase (GSK) 3 is a ubiquitously expressed serine/threonine kinase existing in two isoforms, GSK3a and GSK3b. Aside from the long-recognized role in regulating insulin signal transduction and glycogen biosynthesis, a growing body of evidence has coined GSK3b as a master control molecule of NFkB activation and renal injury. Nevertheless, previous data are less conclusive because they relied greatly on small molecule inhibitors, which lack selectivity and barely distinguish between the GSK3 isoforms. In addition, early embryonic lethality of the global knockout of GSK3b precludes the interrogation of the biological role of GSK3b in adult kidney. To circumvent this issue, the Cre/loxP system for tissue-specific gene targeting was employed to generate a conditional knockout mouse model in which the GSK3b gene was specifically deleted in kidney cortical tubules at postnatal mature stage. Kidney specific ablation of GSK3b resulted in a phenotype no different from the control littermates. Knockout mice were viable and exhibited normal gross appearance, behavior, and development as well as normal kidney physiology in terms of kidney function, urine albumin excretion and urine concentrating ability. Of note, apart from normal glomerular and tubulointerstitial morphology, kidneys from knockout mice demonstrated more glycogen accumulation in the brush border of renal cortical tubules as assessed by both periodic acid-Schiff staining for light microscopy and direct biochemical assay, consistent with an elevated glycogen synthetic activity as evidenced by diminished inhibitory phosphorylation of glycogen synthase that occurred subsequent to GSK3b ablation. This finding was further validated by electron microscopic observations of increased deposition of glycogen particles in renal tubules of knockout mice, suggesting that GSK3a could not fully compensate for the loss of GSK3b in regulating glycogen metabolism in the kidney. In summary, kidney specific ablation of GSK3b results in increase renal tubule glycogen accumulation but has minimal effects on kidney function and histology. These mice should provide a powerful tool to uncover the role of GSK3b in the pathophysiology of kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2897**

**Peritoneal Adipocyte Is Not Just an Innocent Bystander but Act as a Pivotal Cell of PD-Induced Peritoneal Membrane Damages** Talerngsak Kanjanabuch,[1,2] Sirigul Kanchanabuch,[2] Wasin Manuprasert,[2] Somchai Eiam-Ong.[1] *[1]Kidney&Metabolic Disorders Research Center, Chulalongkorn University, Thailand; [2]Medicine, Chulalongkorn University, Thailand.*

Continuous exposure of the peritoneal membrane to unphysiologic PD fluids (PDF) during long-term dialysis results in peritoneal damages. The role of adipocytes in PDF-induced membrane injury was investigated in both *in vivo* and *in vitro* studies.

**Methods:** 20 male Sprague-Dawley rats were subjected to twice daily perioneal injections with 4.25%PDF or 0.9% NSS. After 4- and 12-wk injections, histologies of omental&parietal peritoneal fats were assessed. Omental-derived primary pre-adipose cell and murine 3T3-L1 pre-adipocytes were used for *in vitro* study. Differentiations of both pre-adipocytes to adipocytes were induced by addition of an adipogenic hormonal cocktail. Fully differentiated adipocytes were incubated with conventional glucose-based, 1.1%amino acid, and 7.5%polyglucose PDF for 12 hr. The cell injuries and cell death were assessed. Cytokine and matrix protein expressions, including TGF-β, PAI-1, and collagen I were investigated using quantitative real time PCR and ELISA techniques.

**Results:** In *in vivo* studies, submesothelial fat tissues had increased adipocyte size at 4 and 12 wk when compared with control. This occurred in correspondence with thickening of submesothelial loose CNT layer and up-regulation of TGF-β. Adipocytes cultured with conventional PDF showed time and dose-dependent morphological changes, which were accompanied by cell injury, apoptosis, and loss of differentiation markers. Using of 7.5%polyglucose and 1.1%amino acid solutions significantly abrogated all of above changes in response to the PDF. TGF-β, PAI-1, and collagen type I were up-regulated in fully differentiated adipocytes after incubation with conventional PDF but were attenuated by changing to non glucose-based PDF.

**Conclusion**: Peritoneal fat cells are not just an innocent bystander but act as a pivotal cell of PD-induced peritoneal membrane injuries which may be mediated partly by manipulating TGF-β and PAI-1 expressions, and matrix production. Using of non glucose-based PDF can prevent PD-mediated fat cell injury.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2898**

**Transcutaneous Measurement of Renal Function in Freely Moving Rats** Daniel Schock-Kusch,[1] Qing Xie,[1] Yury Shulhevich,[2] Jürgen Werner Hesser,[2] Dzmitry Stsepankou,[2] Maliha Sadick,[3] Stefan Koenig,[4] Friederike Hoecklin,[5] Johannes Pill,[6] Norbert Gretz.[1; 7] *[1]Medical Research Centre; Medical Faculty Mannheim; University of Heidelberg, Germany; [2]Experimental Radiation Oncology, Medical Faculty Mannheim University of Heidelberg, Germany; [3]Freudenberg Forschungsdienste KG D-69465 Weinheim, Germany; [4]Freudenberg MekTek Europa GmbH 69465 Weinheim, Germany; [5]Institute for Clinical Radiology and Nuclear Medicine, Medical Faculty Mannheim, University of Heidelberg, Germany; [6]Roche Diagnostics GmbH, Sandhofer Str. 116, Mannheim, Germany; [7]Institute for Medical Technology of the University Heidelberg and the University of Applied Science Mannheim, Germany.*

A convenient method for the assessment of renal function using exogenous clearance marker is still missing. We describe a novel device, allowing determination of renal elimination kinetics of FITC-labelled renal markers in freely moving rats. The device excites an injected fluorescent marker and measures the concentration dependent fluorescent signal. Data transfer from the device to a host computer is accomplished by a RF sending device. The small size of the device allows measurements on the back of a rat. In contrast to classical clearance procedures the method is independent of blood and/or urin samples, moreover, as the clearance is measured in awake rats it is not influenced by anaesthesia as other published transcutaneous methods. As proof of principle, comparative measurements of transcutaneous and plasma elimination kinetics of FITC-sinistrin were performed in seven healthy, freely moving rats. Results show highly comparable elimination rate constants (m: transcutaneous: -0.026 ± 0.004 min-1; plasma: -0.027 ± 0.002 min-1) and elimination half-lifes (t1/2: transcutaneous: 27.3 ± 3.9 min; plasma: 25.3 ± 1.5 min). These data demonstrate that renal function can be measured in freely moving rats.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2899**

**Development of a Novel Mass Spectrometry-Based Method for Serum Creatinine Measurement in Rodents** Kevin Wyatt McMahon,[1] Masoud Zabet-Moghaddam,[2] Sharma S. Prabhakar.[1] *[1]Internal Medicine, Texas Tech University HSC, Lubbock, TX; [2]Center for Biotechnology and Genomics, Texas Tech University, Lubbock, TX.*

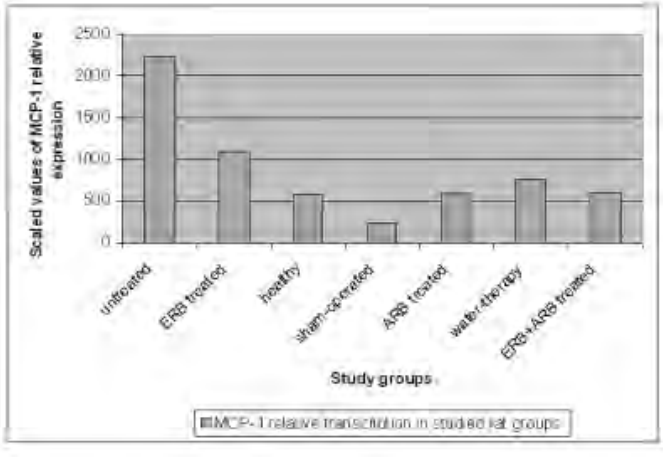
The measurement of serum creatinine in rodents has been plagued by interference from confounding compounds. We have developed a simple method for measuring creatinine in rat serum using matrix-assisted laser desorption/ionization- time of flight (MALDI-TOF) mass spectrometry. Sera (n=6) were collected from obese ZSF rats at 26 weeks of age given either plain water or water containing curcumin (1 mg/L – Pandey et al., ASN abstract, 2009). The calibration curve (R2= 0.997) was obtained using 2H3-creatinine as an internal standard (IS) by mixing a constant amount of IS with various amounts of creatinine (3.5-226 pg). The amount of 3.5 pg indicates 5-fold improvement in detection compared to similar approach using LC-MS. Sample preparation involves mixing one µL of serum sample with of a known amount of internal standard (99 µL, at C= 4 µM) followed by brief centrifugation through a centrifugal filter (Amicon Ultra-0.5 mL, 3,000 MWCO). The flow-through samples are then mixed with α-cyano-4-hydroxycinnaminic acid (CHCA) as the MALDI-matrix and subjected to MALDI-analysis. Spectra showed a strong peak at m/z= 114 Da corresponding to the mass of protonized creatinine, indicating that the sample clean up method adequately removed confounding molecules. The serum creatinine level was then calculated using the calibration curve. Using our new method, serum creatinine levels were found to be lower in obese ZSF rats given curcumin-containing compared with those given plain water, consistent with reported results. In addition, use of a creatinine autoanalyzer to measure serum creatinine showed good correlation with the results using our method ($r2 = 0.74$, $p<0.05$) indicating the reliability of the method. This simple, sensitive and fast technique could be a useful alternative to both enzymatic and HPLC-based methods. To our knowledge, this is the first time that MALDI has been suggested as a method for measuring serum creatinine in rodents.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2900**

***CCL2/MCP-1* and *NF-κB* Gene Transcription in Remnant Kidneys after Physical Exercise** Mai Ots-Rosenberg,[1] Ülle Pechter,[1] Kylli Kõlvald,[1] Jana Uhlinova,[1] Ingrid Kalev.[2] *[1]Internal, University of Tartu, Estonia; [2]General and Molecular Pathology, University of Tartu, Estonia.*

Physical exercise has been shown to have positive influence on physical capacity, hypertension, left ventricular function etc. We have shown the positive effect of aquatic exercise on the chronic kidney disease (CKD) progression. Aim of the study was to investigate the effects of aquatic exercise to the gene expression in experimental CKD and compare with endothelin and angiotensin receptor blockers treatment. Wistar rats were divided into matched groups after renal ablation and studied during 12 weeks. Exercise group was subjected to thermoneutral water immersion and swimming without exhaustion 30 min daily. Chronic studies of systolic blood pressure and urinary protein excretion rate were performed. QPCR was performed for the transcription of intrarenal *CCL-2/MCP-1* and *NF-κB*. For quantification of rat *CCL2/MCP-1*, β-actin (*NF-kB*) mRNA we used a SYBR Green real-time quantitative RT-PCR method based on the TaqMan fluorescence method with the ABI Prism 7000 Sequence Detection System. Results revealed that the degree of hypertension was significantly higher in sedentary animals (177,8±4,9 vs 135,5±5,8). Proteinuria was reduced significantly (2,9±0,7 vs 10,1±3,1) in trained compared to sedentary rats. Deterioration of renal function were ameliorated significantly after physical activity.




*CCL2/MCP-1* renal abundance were all increased in the remnant kidney of the rats without physical activity and were reduced significantly by aquatic exercise treatment. QPCR transcription differences between study groups (Mann-Whitney test). *NF*κ*B* transcription was not significantly suppressed by treatments. Conclusion. Aquatic exercise improves the course of experimental CKD. These results point on the additional renoprotective properties of long-term water immersion and daily exercise in CKD rats.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2901**

**PTH Effects on Osteocytes in Uremia** Michael Pazianas,[1] Rachel M. Locklin,[1] Philippa Hulley,[1] Stelios A. Panagoutsos,[2] Ploumis Stavros Passadakis,[2] Graham Russell,[1] Vassilios A. Vargemezis.[2] *[1]The Botnar Research Centre and Oxford University Institute of Musculoskeletal Sciences, University of Oxford, Oxford, United Kingdom; [2]Nephrology, Thrace University, Alexandroupolis, Greece.*

A central role for osteocytes in the physiology of bone metabolism has been suggested. Osteocytes express the PTH receptor 1. Therefore, we assessed the effects of serum from long-term dialysis patients (pts) with high (78.8 to 81 pmol/l) or 'normal' PTH (1.1-6.9 pmol/l) & healthy controls (CTRs) on the growth of osteocyte-like MLO-Y4 cells in culture. The biochemical bone profile of the serum was remarkable for high FGF-23 (range: 816-1368 RU/mL) & low $1,25(OH)_2D_3$ (range: 22-43 pmol/l) concentrations for all of the uremic pts compared to the CTRs (FGF-23: 32-99 RU/mL & $1,25(OH)_2D_3$: 91-131 pmol/l). Serum calcium & phosphate in the 'normal' PTH (n-PTH) group were similar to those of the CTRs & elevated in the high PTH (h-PTH) group. 5% serum from dialysis pts with either n-PTH or h-PTH levels reduced significantly the cell number & viability within 24 hours compared to CTRs. Furthermore, the cell numbers were lower in the n-PTH than the h-PTH group & the number of normal cells in the n-PTH group was generally less than half that of the CTRs. However, adding back 40 pg/ml of recombinant 1-34 PTH (equivalent to the concentration in cultures using serum from h-PTH pts) failed to reverse the discrepancy in cell numbers between n-PTH & h-PTH osteocyte cultures. Analysis of the cultured cells by nick translation assay, which specifically measures the proportion of apoptotic cells, showed no increase in apoptosis in cultures fed with serum from dialysis pts up to 72 hours. **U**remic serum from dialysis pts reduced the growth of osteocyte-like MLO-Y4 cells through an apoptosis-independent mechanism. Although differences were seen between cultures treated with h-PTH & n-PTH serum, this may not to be the main factor affecting osteocyte survival in uremic conditions.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2902**

**Osteocyte Pathways Are Altered Early in *Jck* Mice, a CKD-MBD Model** Yves Sabbagh,[1] Wen Tang,[1] Stephen O'Brien,[1] Lucy A. Phillips,[1] Susan Ryan,[1] Luciene M. dos Reis,[2] Fabiana G. Graciolli,[2] Vanda Jorgetti,[2] Rosa Ma Moyses,[2] Shiguang Liu,[1] Susan Schiavi.[1] *[1]Endocrine, Genzyme Corp, Framingham, MA; [2]Nephrology Div, University of Sao Paulo, Sao Paulo, Brazil.*

Chronic kidney disease-mineral bone disorder (CKD-MBD) is defined by abnormalities in mineral and hormone metabolism, bone histomorphometric changes and/or the presence of vascular or soft tissue calcification. Emerging evidence suggests that features of CKD-MBD may occur early in disease progression and limited data raises the possibility that decreases in bone formation and/or mineralization may precede the onset of high turnover bone disease. To identify early pathophysiological changes in bone, we utilized the *Jck* mouse, a genetic model of polycystic kidney disease that exhibits progressive biochemical features of CKD-MBD. In our current study we investigated temporal bone changes in *Jck* relative to wild-type (WT) mice from 6 through 18 weeks of age. The development of osteitis fibrosis was observed as early as 9 weeks based on increased BFR in *Jck* ($0.57\pm0.04\mu m^3/\mu m^2/yr$) relative to WT mice ($0.36\pm0.02\mu m^3/\mu m^2/yr$) with a significant increase in bone volume by 12 wks (WT 8.9±0.4% vs *Jck* 6.8±0.5%). Parameters associated with mineralization rates were not substantially altered in *Jck* mice at any time point indicating a mineralization defect is absent in these mice. To capture the early molecular and cellular events in the progression of CKD-MBD we have begun to examine specific biochemical pathways associated with osteocyte function and bone remodeling beginning with analysis of the osteocyte specific Wnt-antagonist, sclerostin (SOST). The number of SOST positive osteocytes was significantly elevated in *Jck* mice at 9 weeks of age (48±9%) prior to the appearance of osteitis fibrosis, compared to WT age-matched mice (23±9%). A significant drop in the number of osteocyte positive cells was observed in *Jck* mice at 18 weeks (12±5%). As PTH rises, SOST expression may be down-regulated further contributing to high turnover disease. These data provide evidence for early bone changes in osteocytes that may negatively influence osteoblast function during progression of CKD-MBD.

Disclosure of Financial Relationships: Employer: Genzyme Corporation; Ownership: Genzyme Stock.

**SA-PO2903**

**Osteocytic Osteolysis Is More Active in Woven Bone in Patients with Secondary Hyperparathyroidism** Aiji Yajima,[1] Masaaki Inaba,[2] Shigeru Otsubo,[3] Yuko Iwasa,[1] Yoshihiro Tominaga,[4] Kosaku Nitta.[5] *[1]Nephrology, Towa Hospital, Tokyo, Japan; [2]Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan; [3]Nephrology, Sangenjaya Hospital, Tokyo, Japan; [4]Transplant Surgery, Nagoya Second Red Cross Hospital, Nagoya, Japan; [5]Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.*

Purpose; Woven bone is fragile and is abundant in patients with secondary hyperparathyroidism. We investigated whether or not osteocytic osteolysis is more active in woven bone as compared with lamellar bone.

Methods; Ten hemodialysis patients with secondary hyperparathyroidism (age; 59.1 ± 8.4 years, duration of dialysis; 11.3 ± 7.5 years, serum intact PTH; 1264.5 ± 416.8 pg/ml) were investigated. The lacunae were classified into three groups (Renal Week 2009) and the parameters were calculated as follows; Woven or lamellar bone volume referent number of Lc with a predominantly eroded surface (ES) (N.ES.Lc/Wo.BV or N.ES.Lc/Lm.BV; N/mm$^2$), number of Lc with a predominantly quiescent surface (QS) (N.QS.Lc/Wo.BV or N.QS.Lc/Lm.BV; N/mm$^2$), and number of Lc with a predominantly osteoid surface (OS) (N.OS.Lc/Wo.BV or N.OS.Lc/Lm.BV; N/mm$^2$). Thereafter, the relationships between these parameters were analyzed by Wilcoxon's signed rank test.

Results; In woven bone, N.ES.Lc/Wo.BV was significantly greater than N.ES.Lc/Lm.BV (465.2 ± 88.5 versus 123.9 ± 66.8 N/mm$^2$, P=0.007). And N.ES.Lc/Wo.BV was greater than N.OS.Lc/Wo.BV (465.2 ± 88.5 versus 37.1 ± 87.0 N/mm$^2$, P=0.007) and N.QS.Lc/Wo.BV (465.2 ± 88.5 versus 67.0 ± 57.2 N/mm$^2$, P=0.007). N.OS.Lc/Wo.BV was not different from N.OS.Lc/Lm.BV(37.1 ± 87.0 versus 2.3 ± 5.1 N/mm$^2$, P=0.144). Both N.OS.Lc/Wo.BV and N.OS.Lc/Lm.BV was 0 N/mm$^2$ in 7 patients.

Conclusions; Osteocytes apparently resorb woven bone actively as compared with lamellar bone in patients with secondary hyperparathyroidism. It seems that bone formation is dependent on osteoblast function in these patients, but bone formation by the osteocyte may be activated by the reduction of serum PTH levels (JBMR in press).

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2904**

**Bone Collagen Degeneration and Calcification Disorder Are Critical Phenomena in Adenine-Induced Renal Failure Rat** Chiharu Aoki, Kazuho Honda, Kenta Uto, Saeko Kanai, Sekiko Taneda, Hideaki Oda. *Department of Pathology, Tokyo Women's Medical University, Tokyo, Japan.*

**Background and Objective:** Molecular mechanism of renal osteodystrophy (ROD) is still uncertain and closely associated with the pathogenesis of chronic kidney disease-mineral bone disorder (CKD-MBD). The present study is designed to elucidate the essential phenomena in ROD focusing on the bone matrix collagen and its calcification process using adenine-induced renal failure rat model.

**Methods:** Renal failure was induced in 8-week-old male SD-Jcl rats by feeding 0.75% adenine-containing diet for 6 weeks. Serum parameters, body weight and bone

histopathology were examined at 2, 4, 6 weeks after adenine administration. Micro CT (μCT) analysis and stress analysis of bone were also evaluated.

**Results:** Serum creatinine, inorganic phosphate and intact PTH were progressively increased, and body weight, serum calcium and 1, 25$(OH)_2D_3$ were decreased concomitantly. The bone morphometric parameters showed increased bone formation (osteoblast surface / bone surface) and resorption (osteoclast surface / bone surface) until 2 weeks with increased peritrabecular fibrosis. Thereafter, bone resorptive parameters oppositely decreased at 4 weeks with massive increasement of osteoid tissue, indicating a severe calcification disorder. The μCT analysis of the femur showed decreased bone mineral density and increased osteoporosity in trabecular bone (control: 168mg/cm$^3$, adenine: 78.5mg/cm$^3$), and in cortical bone (control: 753mg/cm$^3$, adenine: 486mg/cm$^3$). Furthermore flexural strength was decreased in femur (control: 232N, adenine: 150N). Scanning electron microscopy revealed that an irregularity of collagen fiber arrangement and width at 6 weeks accompanying disappearance of periodic striation of collagen fibers. Western blot analysis of OPN and collagen I showed impaired production from 4 weeks to 6 weeks.

**Conclusion:** Ultra-structual abnormalities of bone matrix collagen associated with osteoblast dysfunction preceded a severe calcification disorder of the bone in the experimental model of ROD. The calcification process regulated by osteoblast/bone matrix interaction can be a clue for the pathogenesis of CKD-MBD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2905**

**Association of Secondary Hyperparathyroidism with High Mortality and Morbidity in Dialysis Patients** Patricia T. Goldenstein, Lilian P. F. Carmo, Fernanda O. Coelho, Karina F. Pinheiro, Rosa Ma Moyses, Vanda Jorgetti. *Nephrology, University of São Paulo, São Paulo, Brazil.*

Bone abnormalities are found almost universally in patients with CKD requiring dialysis. Studies have shown associations between disorders of mineral metabolism and fractures, cardiovascular disease, and mortality. We retrospectively evaluated 300 outpatients with CKD on dialysis that were sent to a referral center for CKD-MBD from January 2005 to December 2009. The aim of this study was to compare morbidity and mortality in patients with secondary hyperparathyroidism (SHPT) that were submitted or not to parathyroidectomy (PTx). Their mean age was 48 ± 13 yrs, median dialysis time was 54.5 (36-108) months, 54% were female, and only 10% diabetic. Pain was the most common symptom (63%), followed by fracture (13.2%). Serum Ca, P and alkaline phosphatase were 9.9 ± 0.9 mg/dL, 5.5 ± 1.7 mg/dl, 208 U/L (120 - 442), respectively, whereas median PTH and 25vitamin D were 1217 pg/ml (IQR ; 656-1815) and 24 ng/mL (16-35). SHPT, defined as PTH >500, was detected in 82% of them. Patients with a severe SHPT (PTH ≥ 800 pg/ml) compared to those with PTH < 800 pg/ml more commonly presented pain (67 vs. 52% ; p<0.05), whereas a similar fracture rate was found between them (14 vs. 8% ; ns). PTx was indicated in 73% of the patients, but only performed in 38% of the patients with severe SHPT. PTx was associated with a decreased mortality (12.2% in PTx vs. 30.7% in non-PTx patients, RR = 0.51 ; 95% CI = 0.27-1.02 ; p = 0.05). In this study, the low prevalence of patients with low PTH levels in our cohort suggests that they are less often symptomatic. On the other hand, our results confirm the high morbidity and mortality associated with SHPT, emphasizing the need for early referral, as well as for other therapeutic interventions that would certainly benefit our patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2906**

**Impaired Cortical Bone in Pre-Dialysis CKD Patients Is Even More Marked in Those with Fragility Fracture** Stephanie Boutroy, Thomas L. Nickolas, Emily Stein, Adi Cohen, Elizabeth Shane. *Columbia University Medical Center, New York, NY.*

Cortical (Ct) bone comprises the majority of the appendicular skeleton and supports the majority of axial loads. Although alterations of Ct bone have been reported in CKD patients, there are no methods to depict accurately the microstructural properties of Ct bone in vivo. We applied a new quantitative computational method to high-resolution peripheral quantitative computed tomography (HR-pQCT, Scanco Medical AG, resolution ~82μm) images of the distal radius and tibia to measure both intra-cortical porosity (CtPo) and cortical thickness (CtTh) (Burghardt et al and Nishiyama et al - JBMR 2010). Our goal was to evaluate Ct bone characteristics at these sites in pre-dialysis CKD patients and controls and to determine whether CtTh and CtPo discriminate between CKD patients with and without a history of fragility fracture (FX).

We evaluated 3 groups of 17 patients: a control group (68±10 yrs) and CKD patients with (74±9 yrs) and without FX (73±8 yrs), matched for gender, race and ethnicity. Each patient was scanned at the radius and tibia by HR-pQCT and CtPo and CtTh were measured.

The groups did not differ by age, height and weight. eGFR and intact PTH did not differ between CKD patients with and without FX (respectively eGFR=24[13:44] and 28[17:46]; PTH=54[27:283] and 58[36:95], median[IIQ]). At the tibia, CtTh did not differ between CKD patients and controls (-7%, p=0.4). However CtPo was significantly higher in CKD patients (40%, p=0.004). CKD with FX tended to have thinner Ct bone than CKD without FX (-12%, p=0.08); although CtPo was 13% higher, the difference was not significant (p=0.3). At the radius, CtPo was 32% higher (p=0.2) and CtTh 16% lower (p=0.06) in CKD patients than controls. CKD with FX had significantly lower CtTh (-19%, p=0.02) than CKD without FX, but CtPo did not differ.

Using a novel method to assess CtPo and CtTh, we confirmed that the integrity of Ct bone is impaired in pre-dialysis CKD patients compared to controls. In addition, lower radius CtTh differentiated CKD patients with FX from those without FX. Larger, prospective studies are needed to determine whether Ct bone impairment predicts FX in CKD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2907**

**The Pathogenesis of the CKD-MBD in Stage 2 CKD** Yifu Fang, Suresh Mathew, Keith A. Hruska. *Division of Pediatric Nephrology, Washington University School of Medicine, St. Louis, MO.*

In CKD, osteoblastic differentiation of cells in the neointima causes calcification of atherosclerotic plaques stimulated by hyperphosphatemia and inhibited by Pi binders. Vascular calcification (VC) is an important cause of CV morbidity in CKD. Hyperphosphatemia induces the expression of osterix, an osteoblast specific transcription factor, in the aorta. However, the CKD-MBD begins early in CKD as shown by in studies demonstrating abnormal trabecular architecture by HR qCT in stage 2 CKD patients (Bacchetta J et al JBMR 2009), increased FGF-23 (Pereira RC et al Bone 2009), and decreases in vascular smooth muscle contractile phenotype markers (Kokubo et al, JASN 2009). The loss of both the anabolism of bone and the vascular smooth muscle phenotype before abnormalities of mineral metabolism in the early phases of the CKD-MBD make it necessity to define a new molecular regulatory system underlying the condition. Here we tested the hypothesis that CKD induces the CKD-MBD prior to the onset of positive phosphate balance and stimulates vascular calcification in early CKD. *Ldlr-/-* mice fed high fat diets were subjected to renal cortical electrocautery and contralateral nephrectomy at 14 weeks of age to produce CKD –MBD, euthanasia was at 28 weeks. A relatively mild reduction in the glomerular filtration rate was seen with 76 % of normal (GFR (ml/min/kg): 1.36±0.13 (CKD) vs. 1.79±0.39 (WT) P<0.01) at 22 weeks of age (equal to stage-2 CKD). Serum assays of BUN, Ca, Pi and PTH were normal. CKD stimulated accumulation of aortic Ca levels at 28 weeks of age (0.52±0.19 (CKD) vs. 0.26±0.14 (wt) and 0.32±0.23 (sham) mg/g dry weight, p<0.05). Osteoblast surfaces and bone formation rates were reduced, which showed an inverse relationship between VC. Dickkoff -1 (Dkk-1), a circulating inhibitor of bone formation, levels were increased from normal wt 1868±772 or sham 1650±882 to 3132±1590 (pg/ml, p<0.01). Increased serum levels of FGF-23 were also detected (1154±763 (CKD) vs. 342±126 (WT) or 299±164 (sham) pg/ml, P<0.01). We conclude that the CKD-MBD is stimulated early in response to kidney injury with decreased bone formation, increased VC, and elevated FGF23 and Dkk-1 levels.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2908**

**Wnt Pathway Inhibition: Another Actor in CKD-MBD Pathophysiology?** Fabiana G. Graciolli,[1] Rodrigo B. Oliveira,[1] Luciene M. dos Reis,[1] Ana L. E. Cancela,[1] Lilian Cuppari,[2] Maria Eugenia F. Canziani,[2] Aluizio B. Carvalho,[2] Yves Sabbagh,[3] Vanda Jorgetti,[1] Susan Schiavi,[3] Rosa Ma Moyses.[1] *[1]Nephrology Division, University of Sao Paulo, Sao Paulo, Brazil; [2]Nephrology Division, Federal University of Sao Paulo, Sao Paulo, Brazil; [3]Renal and Endocrine Sciences, Genzyme Corp, Framingham, MA.*

CKD bone disease is complex and multifactorial. Recent studies have demonstrated that some proteins, such as Sclerostin (SOST) and DKK1 (Dickkopf-1), are able to inhibit bone formation in other diseases. We asked whether these proteins are involved in CKD-MBD pathophysiology. Toward this end, we measured the serum levels of SOST and DKK1 in 40 predialysis CKD patients [50 ± 11 yrs., creatinine clearance (CrCl) = 35 ± 16 ml/min] that were randomized to receive calcium acetate (Ca) or sevelamer hydrochloride (Sev) during 6 weeks. At baseline, serum SOST was elevated [0.8 ng/ml (0.4-1.1); ref. range = 0.3 (0.2-0.4)], as well as DKK1 [17.1 ng/ml (14.5-20.7); ref. range = 0.9 (0.3-2.3)]. We found significant correlation between SOST and serum FGF 23 (R= 0.48), phosphate (P; R=0.35) and CrCl (R=-0.43), as well as between DKK1 and P (R=0.39) and leptin (R=0.46). After 6 wks on P binder therapy, we observed a significant decrease of SOST in the Sev-treated patients (0.83 vs. 0.65 ng/ml; p < 0.05), whereas this effect was not observed in the Ca-treated patients (0.8 vs. 0.79 ng/ml, ns). These data suggest that, in CKD, there is an impairment of the Wnt pathway, confirmed by the elevated levels of SOST and DKK1. In addition, these preliminary results open the possibility of modulating their serum levels through the use of P binders.

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**SA-PO2909**

**Plasma Fetuin-A May Not Be Associated with Inflammatory Status in CKD Stages 3 & 4** Martin L. Ford,[1] Edward R. Smith,[2] Laurie A. Tomlinson,[3] Chakravarthi Rajkumar,[1] Stephen Holt.[4] *[1]Brighton & Sussex Medical School, Brighton, United Kingdom; [2]Dept of Clinical Biochemistry, Brighton & Sussex University Hospitals NHS Trust, Brighton, United Kingdom; [3]Clinical Pharmacology Unit, Addenbrooke's Hospital, Cambridge, United Kingdom; [4]Dept of Nephrology, Brighton & Sussex University Hospitals NHS Trust, Brighton, United Kingdom.*

Introduction

Fetuin-A, a 62kDa protein, is synthesized in the liver. Fetuin-A is as an inhibitor of ectopic calcification, but is also thought to be a negative acute-phase reactant. Low circulating fetuin-A concentrations have been associated with aortic stiffening and adverse cardiovascular outcomes in both pre-dialysis and dialysis Chronic Kidney Disease (CKD). This association is potentially confounded by inflammation

Methods

170 stable outpatients with CKD stages 3 & 4 were enrolled in a prospective study of cardiovascular risk. Plasma and serum samples were taken on recruitment and stored at -70°C. Plasma fetuin-A was measured by ELISA (Biovendor, CZ). Serum hsCRP was

measured using particle enhanced nephelometry (Siemens, UK). Serum IL-1β & IL-6 & TNF were measured using ELISA (RnD Systems, UK).

Results

Mean MDRD eGFR was 32 (±11ml/min/1.73m²). Age 69 (±12 yrs). Male:female 119:51 . Non:diabetic:diabetic 128:42. Mean fetuin-A was 0.236 (±0.072g/l). Fetuin-A was significantly higher in the diabetic patients compared to the non-diabetics (0.290 ± 0.080g/l v 0.218 ± 0.060g/l, p<0.001)

Median hsCRP was 5.66 (IQR 5.01mg/l), median IL-1β 2.97 (IQR 1.30pg/mL), median IL-6 6.51 (IQR 5.02pg/mL) and median TNF was 16.72 (IQR 9.38 pg/mL). Fetuin-A did not correlate with log hs-CRP (r=0.045, p=0.559) logIL-1β (r=0.081, p=0.295), logIL-6 (r=-0.019, p=0.804) or logTNF (r=0.054, p=0.488). Subgroup analysis using fetuin-A by tertile or diabetic status did not demonstrate any significant relationship with inflammatory mediators.

Log hsCRP correlated significantly with log IL-1β (r= 0.183, p=0.017) and IL-6 (r=0.158, p=0.039) but there was no significant correlation with TNF (r=-0.139, p=0.070).

Conclusions

In this cohort of stable pre-dialysis CKD patients, fetuin-A was not associated with sensitive markers of inflammation.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2910

### Role of Osteoprotegerin (OPG) in Predicting Loss of Foot Pulse and Development of Foot Ulcer in Type 1 Diabetic Patients with and without Diabetic Nephropathy

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The bone-related peptide osteoprotegerin is produced by vascular cells and is involved in the process of vascular calcification previously shown to predict mortality and cardiovascular events. We investigated the predictive value of plasma OPG in relation to development of foot complications in patients with type 1 diabetes (T1DM) with and without diabetic nephropathy.

Prospective observational follow-up study of 397 type 1 diabetic patients with overt diabetic nephropathy (243 men; age [mean ± SD] 42.1 ± 10.5 years, duration of diabetes 28.3 ± 8.9 years, GFR 76 ± 33 ml/min/1.73 m²) and a control group of 176 patients with longstanding type 1 diabetes and persistent normoalbuminuria (105 men; age 42.6 ± 9.7 years, duration of diabetes 27.6 ± 8.3 years). p-OPG was measured by ELISA.

The median OPG for the 573 patients was 2.8(1.3-11.4)µg/L.

During 15.5 (0.2-17.0) years (median (range)) of follow-up, 107 (40%) with OPG levels above the median *vs*. 76 (27%) below developed a foot ulcer, $p$=0.001. This corresponds to a hazard ratio (HR) of 1.7[1.2-2.2] and covariate adjusted (sex, age, nephropathy status, smoking, $HbA_{1c}$, systolic BP, eGFR, and CRP) HR 1.5[1.0-2.1],($p$=0.04). Furthermore, 42 (16%) patients with higher OPG *vs.* 15 (5%) with lower lost foot pulse, adj. HR 2.2[1.0-4.6],($p$=0.04). Similarly, 51 (18%) with higher OPG levels *vs*. 21 (7%) with lower had vascular surgery or amputation performed, HR 2.9[1.7-5.0],($p$<0.001), however after adjustment this was no longer significant ($p$=0.53). In contrast, OPG levels were not related to loss of vibration perception or development of a Charcot foot, ($p$=0.11 and 0.27, respectively).

In conclusion, OPG remained an independent predictor of lost foot pulse and development of foot ulcer during follow-up in patients with T1DM.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2911

### Prevalence and Risk Factors for Coronary Artery Calcification Progression over Five Years in Pre-Dialysis CKD Patients

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Coronary artery calcification (CAC) is common in chronic kidney disease (CKD). Not all patients demonstrate CAC despite a similar risk profile. We sought to determine risk factors for CAC progression (increase in CAC ≥ 30 Agatston units or increase in Agatston CAC category) in 95 stage 3-5 CKD patients over 5 years. At baseline, mean eGFR 25 ml/min/1.73m², 58% male, 40% diabetic, and 82% had BMI > 25 kg/m². [sup]M[/sup]edian baseline CAC was 83 *vs* 184 at study end (P<0.0001). 63% of participants had progressive CAC; progressors were older (68 *v* 58 years; p < 0.0001), had lower diastolic blood pressure (70 *v* 77 mmHg; p=0.002); higher fasting glucose (7.2 *v* 5.8 mmol/L; p=0.02), higher serum osteoprotegerin (OPG) (4.5 *v* 3.6 pmol/L; p=0.02) and lower serum fetuin (0.64 *v* 0.58 g/L; p=0.02). Baseline CAC score was important in determining risk of CAC progression. An adjusted logistic regression model with CAC progression as the dependent variable, and baseline CAC categories as covariates (CAC = zero as the referent group), CAC score categories of 101 to 400 and > 401 were associated with an increased risk of CAC progression (CAC 101-400: OR 27.2; 95% CI 2.4 to 312; p=0.008), (CAC ≥ 401: OR 5.1; 95% CI 0.97 to 27.4; p=0.056). In a separate multivariable logistic regression model adjusted for traditional cardiovascular risk factors (as a composite score) and eGFR, both serum OPG, and low serum fetuin (OR 0.008; 95% CI 0.000 to 0.387; p=0.01) were associated with CAC progression. There was a significant interaction between OPG and BMI, so that the greatest risk of progressive CAC was demonstrated in those who had higher levels of each variable (OR 1.4; 95% CI 1.11 to 1.8; p=0.005). In summary, CAC progression was common in many patients, yet individuals largely protected from CAC at baseline remained protected over 5 years. Important inhibitors to vascular calcification were demonstrated as pertinent factors in predicting those at risk for CAC progression.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2912

### Vitamin D Receptor and Osteoprotegerin Gene Promoter Polymorphisms Are Not Associated with Coronary Artery Calcification in Stage 3-5 CKD

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In CKD patients, derangements within the vitamin D endocrine system may incite the process of vascular calcification. Moreover, vascular calcification inhibitor function (such as osteoprotegerin (OPG)) is defective. Genetic polymorphisms have been identified in the vitamin D receptor (VDRP) and OPG gene promoter region (OPGP). Data on the association between VDRP and osteopenia are conflicting, but suggest the presence of BsmI bb may be protective. Furthermore presence of the 950 T→C allele of the OPG gene promoter region has been associated with increased vascular intima-media thickness. Given the association between osteoporosis and vascular calcification, we hypothesized 1) presence of the BsmI *bb* allele may protect against vascular calcification, 2) the OPG *CC* genotype might be associated with increased vascular calcification, 3) a combination of BsmI *BB/Bb* and 950 T→C might be associated with a greater risk of vascular calcification. We enrolled 95 stage 3-5 CKD patients (58% male, 40% diabetic) and identified the prevalence of OPG 950 T→C and VDR BsmI polymorphisms. We investigated whether particular genotypes were associated with varying severity of coronary artery calcification (CAC), FGF-23 or 25-OH D.

| Variable | OPG TT/TC n=70 | OPG CC n=23 | Bsm BB/Bb n=66 | Bsm bb n=28 |
|---|---|---|---|---|
| CAC median | 136 | 200 | 157 | 188 |
| 25 Vitamin D | 59.4 | 56.5 | 60.1 | 54.2 |
| log FGF 23 | 2.2 | 2.1 | 2.2 | 2.2 |
| OPG | 4.2 | 3.4 * | 3.75 | 4.74 * |
| PTH | 15.3 | 16.4 | 14.3 | 18.9 |
| Calcium | 2.3 | 2.3 | 2.3 | 2.2 |
| Phosphorus | 1.27 | 1.28 | 1.25 | 1.32 |

* P < 0.05

For hypothesis #3, CAC, FGF-23 and 25-OH D were compared in individuals with the combination of BsmI *BB/Bb* and 950 T→C genotypes (n=19) *v* BsmI *bb* and *CC* (n=75). No differences were identified, although patient numbers were limited. In summary, in this cohort of CKD patients, approximately 30% of patients possessed recessive genotypes of OPG and VDR polymorphisms. No differences were demonstrated with respect to the polymorphism and CAC, or FGF-23 and 25-OH D. However, lower OPG levels were observed in those with the OPG *CC* genotype.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2913

### Assessment of Bone Quality in Renal Transplant Patients by DXA and µCT

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Renal transplant patients (RTP) are at risk of developing bone abnormalities due to preexisting renal osteopathy and immunosuppressive therapy. The changes in bone structure and their pathogenesis are poorly described so far.

Bone quality was investigated in 29 RTP with stable graft function (mean eGFR 54±16 ml/min) in the context of an interventional study aimed at correction of metabolic acidosis. Bone mineral density (BMD) was assessed by dual energy x-ray absorptiometry (DXA) and compared to cancellous bone micro-architecture of iliac crest bone biopsies analyzed by micro-computed tomography (µCT). Follow-up (FU) DXA measurements and biopsies were performed 1 year after baseline (BL) evaluation.

Cumulative BL and FU assessments resulted in 46 pairs of DXA and µCT measurements. Comparative analysis by linear regression of BMD at lumbar spine, hip and femoral neck determined by DXA versus µCT revealed no significant correlations. None of the other micro-architectural parameters including cancellous bone volume density, bone surface density, connectivity density, trabecular number, separation and thickness obtained by µCT correlated with BMD from DXA measurements. BL and FU DXA measurements revealed individual changes in absolute BMD ranging from -5 to +11% for all sites and patients. Nevertheless, these relative alterations did not result in significant shifts in the WHO classifications of osteoporosis (T-score, Z-score). In contrast, FU biopsies of 18 patients showed prominent quantitative micro-architectural changes ranging from -42 to +288% versus BL for some parameters.

In conclusion, DXA and µCT analyses revealed changes in bone mineral density and cancellous bone micro-architecture of RTP within a 1 year FU. The findings suggest that assessment by DXA does not adequately reflect the type, degree and dynamics of structural bone alterations in these patients. Further studies will clarify which method is more suitable to evaluate bone quality to predict fracture risk in RTP.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2914**

**Sclerostin Serum Levels Correlate Positively with Bone Mineral Density and Structure in Hemodialysis Patients** Daniel Cejka, Danielle Diarra, Martin Haas. *Department of Nephrology and Dialysis, Medical University Vienna, Vienna, Austria.*

Context

Sclerostin is a soluble inhibitor of wnt signaling, which is crucial for bone biology. Sclerostin is down-regulated by the parathyroid hormone (PTH). Secondary hyperparathyroidism in dialysis patients might influence bone metabolism through regulation of Sclerostin.

Objective

We investigated whether Sclerostin levels in dialysis patients are influenced by iPTH, and whether Sclerostin is associated with bone turnover, structure and mass.

Design: Cross-sectional study

Setting and patients

76 dialysis patients and 45 healthy controls were included in this study. Sclerostin, Dkk-1, intact PTH (iPTH), bone alkaline phosphatase (bAP), type I collagen c-terminal telopeptide (CTX) and vitamin D were measured in all participants. Dual x-ray absorptiometry (DXA) of the lumbar spine, femoral neck, distal radius, and high resolution peripheral quantitative computed tomography (HR-pQCT) at the tibia and radius were performed in 37 dialysis patients.

Results

Dialysis patients had significantly higher Sclerostin levels than healthy controls (1257 pg/ml vs. 415 pg/ml, $P<0.001$). A significant correlation was found between Sclerostin and gender ($R=0.41$), iPTH ($R = -0.28$), 25(OH)D3 ($R=0.27$) and calcium ($R=0.25$) in the bivariate analysis. Gender and iPTH remained significantly associated with Sclerostin in the multivariate analysis. Sclerostin was associated with bone mineral density of the lumbar spine ($R=0.46$), femoral neck ($R=0.36$) and distal radius ($R=0.42$). Significant positive correlations between Sclerostin and HR-pQCT measurements were found predominantly in trabecular structures. Dkk-1 was neither related to bone measures nor to serologic parameters.

Conclusion

Sclerostin is increased in end stage renal disease. Sclerostin is positively associated with bone mineral density and bone structure in dialysis patients, and may prevent bone loss in renal osteodystrophy.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2915**

**Novel Markers for Renal Osteodystrophy (ROD)** Vincent Brandenburg,[1] Gunter B. Wolf,[2] Gabriele Lehmann.[2] *[1]Cardiology, RWTH University Hospital Aachen, Aachen, Germany; [2]Nephrology, University Hospital Jena, Jena, Germany.*

**INTRODUCTION AND AIMS:** Different subtypes of renal osteodystrophy are associated with significant morbidity. Bone biopsy is the gold-standard for the diagnosis of ROD subtypes. We evaluated the role of novel serum biomarkers for ROD diagnosis in ESRD patients.

**METHODS:** 56 ESRD pts underwent iliac bone biopsy for histomorphometry. In parallel serum samples were analyzed for fibroblast growth factor 23 (FGF23), osteoprotegerin (OPG), sclerostin, PTH, bone alk phos (BAP), TRAP5b, cross-linked N-terminal telopeptide of type I collagen (NTX) and other serum markers of bone metabolism. The aim of the study was to investigate the prognostic value of novel biochemical markers in the diagnosis of ROD subtypes. Serum sclerostin was measured by ELISA**.**

**RESULTS:** We investigated 56 Causasian ESRD pts (54 HD pts, 70% males; median age 50 yrs, median time on dialysis 35 months (range 2 to 119), diabetic nephropathy in 20%. Low-turnover ROD (LTO) was present in 20%, high turnover ROD in 80% - mild osteitis fibrosa in 43% and severe osteitis fibrosa in 37%. Osteocyte counts per bone volume correlated significantly with other cellular bone metabolism makers (ObS/BS and OcS/BS). Moreover, osteocyte counts were significantly higher in HTO than in LTO and correlated positively with FGF23 but not sclerostin in serum. Median serum sclerostin level was 0.99 ng/mL (range 0.14 to 2.98, median of normal range 0.71) in the entire group. Only in females sclerostin levels were significantly different depending on ROD subtype: higher in LTO than in HTO. In the entire group, ROC analyses revealed AUC with $p<0.05$ for PTH, TRAP5b, NTX and BAP for differentiation of LTO from HTO. Mean levels of these markers were all significantly higher in HTO pts than in LTO.

**CONCLUSIONS:** Osteocyte count per bone volume is markedly increased in HTO compared to LTO bone biopsies. In general, serum levels of osteocyte markers FGF23 and sclerostin do not help in differentiating ROD subtypes in HD pts. In addition to PTH, BAP, and TRAP5b, NTX serum levels may also play a role as a biomarker for ROD in ESRD.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2916**

**Raman Spectrum Properties and Morphological Characteristics in Human Uremic Bone Samples** Junichiro J. Kazama,[1] Yoshiko Iwasaki,[2] Hideyuki Yamato,[3] Ichiei Narita.[1] *[1]Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; [2]Biological Science, Oita University of Nursing and Health Science, Oita, Japan; [3]Blomedical Research Laboratories, Shinjuku, Japan.*

Raman spectroscopy is a non-destructive examination method, which detects molecular/crystal structural properties by analyzing frequencies of incoming laser beam to and scattering ray from the target sample. We compared Raman spectroscopic properties and morphological characteristics determined by bone histomorphometry in biopsied human uremic bone samples. Iliac bone samples were obtained from 48 CKD5D patients (M22:F26, 50.8±14.7 yo, HD duration 38.1±66.1M). Samples were embedded in MMA resin, and conventional bone histomorphometry was performed on the processed sections. Coherent laser beam was shot into the surface of remaining block, and the specific Raman spectrum was obtained by analyzing scattering ray. The area of .354 mm square with highest phosphate content was designated for the analyses. In this study, hydroxyproline/proline ratio (H/P), mineral/matrix ratio (M/M), mature collagen cross-links/immature collagen cross-links ratio (M/I) and hydoxylapatite crystallizing ratio (HC) were analyzed. In result, conventional bone histomorphometry categorized those 48 samples into 5 groups (mild change (ML) 11, adynamic bone (AB) 13, osteitis fibrosa (OF) 11, osteomalacia (OM) 6, mixed type (MX) 7). Significant negative correlations were found between FbV/TV and H/P($p<.01$, $r^2=.150$), FbV/TV and M/I($p<.005$, $r^2=.177$), intact PTH and H/P($p<.05$, $r^2=.130$), intact PTH and M/I($p<.005$, $r^2=.175$), OV/BV and M/I ($p<.005$, $r^2=.185$), respectively. OM showed lower M/M than others. ML and AB showed higher M/I, however, HC was low in AB. The relationships between PTH·FbV/TV and H/P·M/I suggested the disturbed matrix protein maturation in woven bone. Mild abnormal mineralization was found in calcified bone besides at calcified front in OM, which may also be related to immaturity of matrix protein. No matrix protein abnormality was detected in AB, while the development of hydroxyl apatite crystal was disturbed.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2917**

**Sclerostin – A Marker Superior to iPTH for Assessment of Bone Turnover in Dialysis Patients** Hartmut H. Malluche,[1] Daniel Cejka,[2] Johann Herberth,[1] Adam Branscum,[3] Danielle Diarra,[2] Martin Haas,[2] Marie-Claude M. Faugere.[1] *[1]Nephrology, Bone and Mineral Metabolism, University of Kentucky, Lexington, KY; [2]Nephrology, Medical University of Vienna, Vienna, Austria; [3]Biostatistics, Statistics and Epidemiology, University of Kentucky, Lexington, KY.*

**Background:** Sclerostin, a protein produced by osteocytes and circulating in blood, was recently identified as a component of parathyroid hormone (PTH) signal transduction. The current study was conducted to compare the value of determinations of blood concentrations of sclerostin versus intact PTH (iPTH) for assessment of bone turnover in stage 5 chronic kidney disease patients on dialysis (CKD-5D).

**Materials and Methods:** In a cross sectional study, 60 CKD-5D patients underwent bone biopsies after tetracycline double labelling followed by bone histomorphometry. Blood samples were drawn at time of bone biopsy. Levels of sclerostin were determined by ELISA and iPTH by IRMA. Associations between biochemical and histomorphometric parameters were evaluated by correlation and multiple linear regression analyses.

**Results:** Serum levels of sclerostin and iPTH correlated negatively ($rho=-0.34$, $p=0.01$). In unadjusted and adjusted analyses, serum sclerostin levels were strongly associated with histomorphometric parameters of bone turnover at the tissue level (measured by activation frequency [Ac.f] and bone formation rate/bone surface [BFR/BS]; Ac.f. β-estimate -0.000248, $P<0.001$; BFR/BS β-estimate -0.000235, $p=0.003$), while serum iPTH levels showed stronger associations than sclerostin with cellular parameters of bone formation and resorption (number of osteoblasts / bone perimeter β-estimate 0.0017, $p<0.001$ and number of osteoclasts / bone perimeter β-estimate 0.000809, $p<0.01$).

**Conclusions:** In CKD-5D patients, determinations of iPTH give information on number of bone forming and resorbing cells. Bone turnover at the tissue level is the result of both, number and activity of bone forming and resorbing cells. In the studied patient cohort, sclerostin is superior to iPTH for predicting bone turnover at the tissue level.

Disclosure of Financial Relationships: Research Funding: Vifor; Honoraria: Shire, Genzyme; Scientific Advisor: Shire, Vifor, Novartis.

**SA-PO2918**

**Low Levels of 25-Hydroxyvitamin D and High Levels of Fibroblast Growth Factor-23 Are Associated with Calcitriol Deficiency in Patients with Severe CKD** Jessica B. Kendrick,[1] Alfred K. Cheung,[2,4] James S. Kaufman,[3] Tom H. Greene,[4] William L. Roberts,[4] Gerard John Smits,[1] Michel B. Chonchol.[1] *[1]University of Colorado Denver Health Sciences Center, Denver, CO; [2]VASLCHCS, Salt Lake City, UT; [3]VA Boston Healthcare System, Boston, MA; [4]University of Utah, Salt Lake City, UT.*

Purpose: Factors other than loss of renal mass may contribute to low calcitriol levels. We tested the hypothesis that high plasma fibroblast growth factor-23 (FGF-23) levels are associated with decreased calcitriol levels independent of 25-hydroxyvitamin D (25(OH) D) and kidney function.

Methods: This study was conducted on 1099 patients with severe CKD not on dialysis who participated in the HOST study. Calcitriol, 25(OH)D, intact parathyroid hormone levels

(iPTH), and FGF-23 levels were measured in stored plasma samples. Analysis was done using multiple linear regressions.

Results: Participants had a mean (SD) age and eGFR of 69±11 years and 18 ±6.5 mL/min/1.73m2, respectively. The median (IQR) calcitriol, 25(OH)D, iPTH and FGF-23 levels were 18 [12-26] pg/mL, 19 [13-27] ng/mL, 147 [90-246] pg/mL and 380 [216-945] RU/mL, respectively. In univariate [(R2 =0.20; p< 0.0001 for 25(OH)D and R2 =0.16; p< 0.0001 for FGF-23)] and multivariable analyses, 25(OH)D and FGF-23 were the strongest determinants of calcitriol levels. Coefficients shown in Table 1 represent changes in calcitriol levels per unit increase of the variable.

Table 1

| Independent Variables | β (95% CI) | P-value |
|---|---|---|
| eGFR (mL/min/1.73m2) | 0.007 (0.005, 0.009) | <0.0001 |
| Log10 25(OH)D (ng/mL) | 0.45 (0.40, 0.50) | <0.0001 |
| Log10 FGF-23 (RU/mL) | -0.13 (-0.16, -0.11) | <0.0001 |
| Log10 iPTH (pg/mL) | 0.09 (0.06, 0.12) | <0.0001 |
| Calcium (mg/dl) | -0.01 (-0.03, 0.01) | 0.2 |
| Phosphorus (mg/dl) | -0.016 (-0.03, -0.01) | 0.0005 |

Conclusion: Low 25 (OH) D and high FGF-23 levels are strongly associated with low calcitriol levels in patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2919

**Bone Turnover Markers Discriminate Fracture Status Non-Dialysis CKD Patients** Thomas L. Nickolas,[1] Elzbieta Dworakowski,[1] Valerie Thomas,[1] Emily Stein,[1] Adi Cohen,[1] Stephanie Boutroy,[1] Ryan Chauncey,[1] Mary B. Leonard,[2] Serge Cremers,[1] Elizabeth Shane.[1] *[1]Medicine, Columbia Univeristy, NY, NY; [2]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA.*

It is not known if bone turnover markers (BTMs) predict fracture (FX) in patients with predialysis chronic kidney disease (CKD). To address this question, we compared BTMs and measures of areal bone mineral density (aBMD) by dual energy X-ray absorptiometry (DXA) and bone geometry and microarchitecture by high resolution pQCT (HRpQCT, resolution ~82µm) for discrimination of FX in predialysis CKD.

Intact parathyroid hormone (iPTH), markers of bone formation (bone alkaline phosphatase [BSAP] and osteocalcin [OC]) and resorption (tartrate-resistant acid phosphatase 5b [Trap5b] and C-terminal telopeptides [CTX]) were measured in CKD patients with (n=26) and without (n=56) FX. aBMD was measured at the one-third and ultradistal radius (1/3R and UDR), total hip (TH), femoral neck (FN) and lumbar spine (LS). Cortical area, density and thickness (Ctarea, Dct, CTh) and trabecular density, number, thickness and separation (Dtb, TbN, TbTh, TbSp) were measured by HRpQCT at the distal radius (RAD) and tibia (TIB). All comparisons were adjusted for age, gender, body mass index (BMI) and diabetes.

FX and nonFX groups did not differ by kidney function, gender, race or ethnicity. Patients with FX were older (74±10 vs. 69±8 years, p=0.03), had lower BMI (28±4 vs. 30±5 years, p<0.05) and were more likely diabetic (58% vs. 32%, p0.03). In the FX group, OC, Trap5b and CTX were 36%, 28% and 31% higher (all p<0.05). By DXA, aBMD was 20% and 14% lower at UDR and LS (p=0.007 and 0.04; respectively). By HRpQCT at the RAD, Dtb and TbTh were 25% and 14% lower (p=0.02 and 0.008; respectively) and at the TIB, Ctarea and CTh were 30% lower (both p<0.05). Other BTMs or imaging measures did not differ. Areas under the ROC curves ranged from 0.61 (CTX) to 0.76 (UDR BMD); none differed significantly from a reference of LS BMD (AUC 0.70).

BTMs discriminate FX status in patients with CKD comparably to DXA or HRpQCT. Longitudinal studies are required to determine if BTMs can predict FX in patients with predialysis CKD.

Disclosure of Financial Relationships: Consultancy: Abbott DiagnosticsResearch Funding: Abbott Diagnostics: A study of NGAL in the Emergency Room; Patent: Columbia University has licensed NGAL to Abbott and Biosite.

## SA-PO2920

**Tests of Neuromuscular Function Discriminate among Those with and without Fractures in Chronic Kidney Disease** Charmaine E. Lok,[1,2] Sarah West,[2] Sophie Jamal.[2,3] *[1]Toronto General Hospital, Toronto, ON, Canada; [2]University of Toronto, Toronto, ON, Canada; [3]Women's College Hospital, Toronto, ON, Canada.*

**Background**: Fractures occur at a high frequency in patients with chronic kidney disease (CKD), with ~40% having sustained a fracture prior to initiating dialysis.

**Purpose:** Tests of neuromuscular function (NMT) have discriminated fracture status in dialysis patients; however, the ability of NMT to discriminate among fractured and non-fractured patients with stages 3 to 5 CKD has not been reported.

**Methods:** Baseline data from an ongoing prospective observational study of adult patients with stages 3-5 (pre-dialysis) CKD was used to determine if tests of neuromuscular function, as indicated by the timed up and go test (TUG), and the 6 minute walk test (6MW), could discriminate between those with and without self-reported low trauma fractures occurring after age 40. Results are expressed as areas under the receiver operating characteristic curves (AUC) with 95% confidence intervals (CI).

**Results:** Data was available for 71 men and 45 women. They were primarily Caucasian (62.9%) with a mean age of 62±16 yrs and weight of 78.7±17.6 kg. The most common cause of CKD was diabetes (43.4%). The mean eGFR (MDRD) was 19.1±11.0 ml/min/1.73m$^2$, with most (85.1%) patients categorized as stage 4 or 5 CKD. Almost half (46.4%) had a history of fractures and 34.6% reported a fall in the past year. Additionally, over half (54.5%) of all patients were sedentary, with less than 25% reporting that they engage in activity that causes them to sweat. The TUG was able to discriminate among those with and without fractures (AUC: 0.65 [95% CI: 0.49-0.81]), as was the 6MW test (AUC: 0.71 [95% CI: 0.53-0.90]). There were no statistical differences in the performance characteristics of the TUG and 6MW.

**Conclusions**: Among adult patients with stages 3 to 5 CKD not on dialysis, the majority of patients are inactive. Tests of neuromuscular function, i.e., the TUG and 6MW, are able to discriminate among those patients with and without fractures occurring after the age of 40. NMT is an easy and cost effective way to identify patients at risk of fractures for whom preventative strategies may be beneficial.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2921

**Osteoprotegerin Is Associated with Cardiac Dysfunction and Vascular Damage in Predialysis Patients** Shirley Yumi Hayashi,[2] Marcelo M. Nascimento,[1] Astrid Seeberger,[1] Britta Lind,[2] Anna Bjällmark,[2] Miguel C. Riella,[3] Lars-Åke Brodin,[1] Bengt Lindholm.[1] *[1]School of Technology and Health, KTH, Royal Institute of Technology, Stockholm, Sweden; [2]Baxter Novum & Renal Medicine Karolinska Institute, Stockholm, Sweden; [3]Pontifícia Universidade Católica do Paraná.*

Atherosclerosis and vascular calcification(VC) contribute to the high risk of cardiovascular disease (CVD) in chronic kidney disease (CKD) patients. Osteoprotegerin (OPG) has been associated to VC and mortality in CKD patients. The objective of the present study was to evaluate the relationship between VC markers and cardiac dysfunction parameters in pre-dialysis patients.. Fifty four (34 male, 60.0 ±11.2 yr) CKD patients stages 3 and 4 were evaluated. VC markers( fetuin and OPG) were analysed as well as cardiac function through conventional echocardiography and measurements of myocardial velocities using tissue Doppler echocardiography (TDE). Moreover, atherosclerosis was evaluated by measurements of carotid intima media thickness, and vascular elasticity using a new described technique called ultrasound strain imaging which measures the deformation or strain of common carotid artery by speckle tracking technique. No significant difference in OPG levels were observed between patients with CKD stage 3-4 (6.9±2.5 vs. 7.1±2.5 pmol/l)(p>0.05).Furthermore, OPG levels were negatively correlated with isovolumetric contraction velocities (IVCv) (Rho -0.45, p<0.001), peak systolic velocities (Rho -0.37, p<0.01) and positively with left ventricular (LV) end diastolic pressure (E/E´) (Rho 0.34, p<0.05), indicating the possible association between OPG and cardiac dysfunction. OPG levels were positively associated with IMT (Rho 0.48, p<0.001), common carotid artery strain (Rho – 0.32, p<0.05) and strain rate (-0.32,p<0.05). On the other hand, no associations were found between fetuin, cardiac function and vascular damage.In a stepwise multiple regression model, OPG was an independent predictor of IVCv, IMT,carotid strain and strain rate. In conclusion, in pre-dialysis patients OPG showed significant association with cardiac dysfuntion and vascular damage.These results suggest its role on VC and CVD in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2922

**Bone Histomorphometry and Biochemical Markers across CKD Stages** Katia R. Neves,[1] Rosa Ma Moyses,[1] Fellype Barreto,[2] Daniela Veit Barreto,[2] Cristianne Tomiyama,[2] Andrea Higa,[2] Fabiana G. Graciolli,[1] Luciene M. dos Reis,[1] Maria Eugenia F. Canziani,[2] Vanda Jorgetti,[1] Aluizio B. Carvalho.[2] *[1]Nephrology, Universidade de São Paulo, Brazil; [2]Nephrology, Universidade Federal de São Paulo, Brazil.*

CKD-MBD is characterized by early changes in bone histology, as well as in serum biochemistry. However, few studies have simultaneously evaluated biochemical markers and bone histomorphometry across CKD stages. In a cross-sectional study, we evaluated 148 adult CKD patients (CKD 2-5), and observed a progressive decrease in serum Ca, and a progressive increase in serum P, alkaline phosphatase (AP), PTH and FGF 23.

Bone Histomorphometry analysis disclosed decreased bone formation and mineralization in dialysis patients despite higher PTH levels. No differences were seen in bone volume (BV/TV).

| | CKD 2-3 (n=32) | CKD 4 (n=18) | CKD 5 (n=98) |
|---|---|---|---|
| Age | 50.0±9.5 | 54.4±11.8 | 48.5±13.1 |
| iPTH | 75 (42-89)[a,b] | 272 (120-398) | 242(113- 589) |
| iCa | 1.3±0.0[a,b] | 1.3±0.1 | 1.2±0.1 |
| P | 3.6 (3.2-3.8)[a] | 4.5 (4.0-4.8)[a] | 6.7(6.2-7.8) |
| AP | 109 (66-160)[a] | 119 (77-164)[a] | 203(158-282) |
| 25-OH Vitamin D | 42.3±19.9[a,b] | 25.0±10.3 | 31.9±15.7 |
| FGF 23 | 43 (25-62)[a] | 84 (48-138)[a] | 18,260(5,705-45,298) |
| BV/TV | 17.6 (12.9-21.1) | 17.9 (10.6-22.2) | 16.5 (12.2-21.9) |
| BFR | 0.005 (0.005-0.02) | 0.01 (0.007-0.04)[a] | 0.0004 (0.0004-0.03) |
| Mlt | 113 (31-655)[a] | 103 (33-273) | 656 (40-656) |

Mean ±SD except those in median (interquartile range). P < 0.05: a vs. CKD 5; b vs. CKD 4

Bone formation rate (BFR/BS) correlated with FGF 23 (R = -0.20), age (R = -0.24), PTH (R = 0.20) and Cr clearance (R = -0.16), whereas mineralization lag time (Mlt) correlated with FGF 23 (R= 0.22), age (R = 0.22), Ca (R = -0.16) and Cr clearance (R = -0.24). In conclusion, the natural course of CKD-MBD does not seem to influence BV/TV, but is associated with effects on bone turnover and mineralization. Although a significant correlation was found between some biochemical markers and bone turnover and mineralization, the exact role of some of them, such as FGF 23, requires further investigation.

Disclosure of Financial Relationships: Research Funding: Genzyme Co Fresenius Medical Care.

**SA-PO2923**

**Higher Estradiol (E2) and Lower Sex Hormone Binding Globulin (SHBG) Levels in CKD Are Associated with Greater Bone Mineral Density (BMD) Compared with Controls: A CRIC Study** Takayuki Hamano, Matthew T. White, Thomas L. Nickolas, Raymond R. Townsend, Harold I. Feldman, Lucy W. Kibe, Michael Sulik, Mary B. Leonard. *CRIC.*

Sex hormone associations with estimated GFR (eGFR) and BMD have not been established.

This cross-sectional study examined 487 controls and 249 CRIC subjects [eGFR: median 47], age 21-80 yrs, excluding those on sex hormones, raloxifene, prednisone, or bisphosphonates. BMD was measured by DXA at the 1/3rd and ultradistal radius, and lateral lumbar spine. Total E2, testosterone (T), SHBG, 1-84PTH, and BSAP levels were obtained.

Total E2 levels were greater ($p < 0.001$) and SHBG levels were lower in CKD ($p < 0.05$) compared with controls, adjusted for age, sex, race, BMI, T levels and sex-age interactions. CKD effects were comparable in men and women. Within CKD, serum E2 levels were negatively associated with eGFR ($p < 0.001$), adjusted for age, sex, race, and T levels. BSAP levels were independently and negatively associated with E2 ($p < 0.01$), and positively associated with PTH ($p < 0.001$), adjusted for sex, race, and age in CRIC and controls.

Compared to controls, CKD participants had higher BMD Z-score at 1/3 radius and lumbar spine.

| **Distal 1/3 Radius Z-score** | **CKD Effect [95%C.I.]** | **P-value** |
|---|---|---|
| unadjusted | 0.30 [0.15, 0.46] | <0.0001 |
| adjustment for BMI | 0.22 [0.05, 0.38] | <0.01 |
| adjustment for BMI + E2 | 0.19 [0.12, 0.36] | 0.04 |
| **Lateral Spine Z-score** | **CKD Effect [95%C.I.]** | **P-value** |
| unadjusted | 0.56 [0.39, 0.73] | <0.0001 |
| adjustment for BMI | 0.46 [0.28, 0.63] | <0.0001 |
| adjustment for BMI + E2 + SHBG | 0.43 [0.24, 0.61] | <0.0001 |

Adjustment for BMI, E2 and SHBG attenuated the CKD effect. At the ultradistal radius, E2 was associated with greater BMD and SHBG with lower BMD, in CKD and controls. These data suggest that greater bioavailable E2 levels in CKD partly explain the higher BMD. The positive effect of E2 on BMD was independent of BSAP at radius, implying the beneficial effect of E2 was not mediated only through reduced bone turnover.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2924**

**Predictive Value of Biomarkers for Bone Turnover in ESKD** Hartmut H. Malluche,[1] Ezequiel R. Bellorin-Font,[2] Eudocia Rojas,[2] Aluizio B. Carvalho,[3] Patrick C. D'Haese,[4] Tilman B. Drueke,[5] Manuel A. Ferreira,[6] Vanda Jorgetti,[7] Sharon M. Moe,[8] Stuart M. Sprague.[9] *[1]Univ of Kentucky, Lexington, KY; [2]Hospital Universitario de Caracas, Caracas, Venezuela; [3]Federal Univ of São Paulo, São Paulo, Brazil; [4]Univ of Antwerp, Antwerp, Belgium; [5]Hôpital Necker, Paris, France; [6]Hospital Curry Cabral, Lisbon, Portugal; [7]Univ of São Paulo, São Paulo, Brazil; [8]Indiana Univ, Indianapolis, IN; [9]NorthShore Univ HS, Evanston, IL.*

Management of CKD-MBD requires assessment of bone turnover. The utility of serum biomarkers to discriminate bone turnover is unclear. An international cross-sectional, retrospective study to determine whether serum biomarkers could assess bone turnover was performed in 647 HD patients. PTH, intact (i) & whole (w), bone-specific alkaline phosphatase (BSAP), and procollagen type-1 N-terminal propeptide (P1NP) were measured in serum drawn at the time of bone biopsy. Bone turnover was determined as BFR/BS. Receiver operator curves were developed to assess minimal discriminating (AUC>0.70) or diagnostic (AUC>0.85) ability. Median iPTH, wPTH, BSAP and P1NP were different among categories of turnover ($p<0.0001$). None of the biomarkers achieved an AUC>0.70 to separate low or high from normal except for BSAP which discriminated low from normal (AUC>0.738). The combination of iPTH or wPTH and BSAP discriminated low from normal (AUC 0.702 & 0.726, respectively). Neither combination discriminated high from normal turnover. iPTH, BSAP, P1NP, and combination of iPTH and BSAP were able to discriminate high from nonhigh BFR/BS (AUC>0.70-0.743). Discrimination between low and nonlow BFR/BS was achieved with iPTH, wPTH, BSAP, and the combination of BSAP with either iPTH or wPTH (AUC>0.701-0.757). There were differences between iPTH, wPTH, BSAP, and P1NP across BFR/BS; no marker singly or in combination was robust enough to diagnose low, normal or high turnover. There was significant ROC based discrimination in differentiating low from nonlow and high from nonhigh, however, it did not reach acceptable levels for cross sectional diagnosis. Further prospective studies are required to assess their value for trend analysis.

Disclosure of Financial Relationships: Research Funding: Vifor; Honoraria: Shire, Genzyme; Scientific Advisor: Shire, Vifor, Novartis.

**SA-PO2925**

**Serologic Diagnosis of Bone Turnover after Kidney Transplantation** Danielle Diarra,[1] Daniel Cejka,[1] Martin Haas.[1] *[1]Internal Medicine III, Nephrology, Medical University Vienna, Vienna, Austria; [2]Internal Medicine, KH der Elisabethinen Linz.*

BACKGROUND. Current guidelines suggest to maintain PTH levels in the upper range of normal in kidney transplant recipients with CKD stages 3-5T. Evidence in support of this recommendation is lacking.

METHODS. Bone biopsies were performed in 39 kidney transplant recipients. Renal osteodystrophy (ROD) was classified according the Delling classification as either type I (fibrosteoclasia and marrow fibrosis), type II (osteoidosis with low bone turnover) or type III (osteoidosis and fibrosis with increased bone turnover). Serologic bone markers (c-telopeptide [CTX], osteocalcin [OC], bone alkaline phosphatase [bAP]), intact (i) PTH, electrolytes and vitamin D status were obtained. Serologic values of patients with normal histology and ROD type II were combined for receiver operating characteristic (ROC curve).

RESULTS. Mean estimated GFR was 49±37ml/min. Nine patients had normal bone morphology, 13 ROD type II and 17 ROD type III. Median iPTH level did not differ between the groups and was above normal in all. There was no difference in median eGFR, median serum levels of calcium and phosphate, and median vitamin D or calcitriol levels between the groups. Patients with ROD type III had significantly higher CTX values than patients with normal bone histology, and significantly higher OC and bAP values than both other groups. The ROC curve of OC had the highest area under the curve (0.90) followed by bAP (0.82), PTH (0.73) and CTX (0.73). An OC level of 46ng/ml had a sensitivity of 88% and a specificity of 85% to differentiate high turnover from normal/low turnover ROD.

CONCLUSION. Measurement of intact PTH is an unsuitable means to assess bone turnover in post-transplant renal osteodystrophy and should not be used for treatment considerations. Osteocalcin has the best predictive value for the diagnosis of increased bone turnover in transplant recipients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2926**

**Sensitivity and Specificity of Guideline PTH Targets To Differentiate High and Low Bone Turnover** Sharon M. Moe,[1] Ezequiel R. Bellorin-Font,[2] Aluizio B. Carvalho,[3] Patrick C. D'Haese,[4] Tilman B. Drueke,[5] Hongyan Du,[6] Manuel A. Ferreira,[7] Hartmut H. Malluche,[8] Stuart M. Sprague,[6] Vanda Jorgetti.[9] *[1]Indiana Univ, Indianapolis, IN; [2]Hospital Universitario de Caracas, Caracas, Venezuela; [3]Federal Univ of São Paulo, São Paulo, Brazil; [4]Univ of Antwerp, Antwerp, Belgium; [5]Hôpital Necker, Paris, France; [6]Univ of Chicago, Evanston, IL; [7]Hospital Curry Cabral, Lisbon, Portugal; [8]Univ of Kentucky, Lexington, KY; [9]Univ of São Paulo, São Paulo, Brazil.*

To determine whether guideline values for PTH can differentiate high and low turnover (TO) bone disease, an international cross-sectional analysis of PTH targets to predict bone TO was performed. Bone biopsy and serum PTH (intact [i] & whole [w]) were analyzed in 647 subjects. PTH was stratified based on KDOQI (150-300 pg/ml) and KDIGO (2-9 times upper limit of normal [ULN]) targets. TO was based on BFR/BS being either low or high. KDOQI iPTH of <150 pg/mL had sensitivity/specificity of 68.6/61.2% for low TO. iPTH >300 pg/mL had a sensitivity/specificity of 58.0/77.7% for high TO. For KDIGO, an iPTH of less than 2X ULN had a sensitivity/specificty of 65.0/67.3% for low TO. An iPTH >9X ULN had a sensitivity/specificty of 37.0/85.8% for high TO. A wPTH of <2X ULN had a sensitivity/specificity of 73.5/56.7% whilst a wPTH of greater than 9X ULN had a sensitivity/specificity of 30.7/87.9% for low and high TO, respectively. Calculated ROC's predict the optimal iPTH for low TO would be <103.7 pg/mL with an averaged sensitivity/specificity (AUC 0.701) and to predict high TO would be an iPTH >242.7 pg/mL (AUC 0.701). A wPTH <47.1 pg/mL would predict low TO (AUC 0.712), however, there was not a wPTH that would achieve the minimally acceptable criteria (AUC >0.7) to predict high TO. Thus, the iPTH cutoffs for KDOQI and KDIGO had relatively high specificity, but low sensitivity to differentiate high TO, but were inadequate to predict low TO. However, wPTH can be predictive only of low TO with a threshold lower than proposed by KDIGO and KDOQI. In summary, iPTH can predict both low and high TO, but the diagnostic performance of this biomarker to reflect bone TO remains suboptimal.

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**SA-PO2927**

**Cortical Bone Analysis in CKD Patients – A New Approach** Catarina Carvalho,[1] Katherine Wesseling-Perry,[2] Isidro B. Salusky,[2] R. C. Pereira.[2] *[1]Nephrology, Hospital Sao Joao, Porto, Portugal; [2]Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

ROD diagnoses are defined by trabecular bone parameters. Cortical bone is the major determinant of bone strength, but the relationship between cortical bone, trabecular bone, and biochemical parameters in CKD pts remains unknown. Thus, 22 pts age 10.4±0.7 yrs treated with CCPD underwent double labeled BBx. Cortical bone was compared to biochemical values and traditonal trabecular TMV parameters. S-values were: Ca 9.3±0.2 mg/dl, P 5.7±0.2 mg/dl, Alk P-tase 380±41 IU/l, 1st PTH-IMA 524±86 pg/ml. Cortical porosity (Po) and osteonal osteoid surface (OS) were related to Ca (r= -0.30 and r= -0.42;

p=0.05), PTH (r=0.43 and r=0.40; p<0.01), and Alk P-tase (r=0.48 and r=0.48; p<0.01). Trabecular BFR correlated with PTH (r=0.88; p<0.01) and Alk P-tase (r=0.68; p<0.01) and trabecular osteoid volume (OV) with Ca, PTH and Alk P-tase (r= -0.63, r= 0.93, r=0.61; p<0.01).

| Cortical Parameter | Trabecular Parameter | | | | | | |
|---|---|---|---|---|---|---|---|
| | Bone Volume (BV/TV) | Osteoid Volume (OV/BV) | Osteoid Surface (OS/BS) | Osteoid Thickness (O.Th) | Bone formation rate (BFR/BS) | Eroded Surface (ES/BS) | Mineralization Lag Time (MLT) |
| Channel Diameter | NS | NS | NS | NS | NS | NS | NS |
| Osteon Diameter | NS | NS | NS | NS | NS | NS | NS |
| Porosity | NS | 0.518 P=0.000 | 0.490 P=0.001 | 0.509 P=0.000 | 0.457 P=0.001 | NS | 0.509 P=0.000 |
| Cortical Thickness | NS | NS | NS | NS | NS | NS | NS |
| Bone Formation Rate | NS | NS | NS | NS | NS | NS | NS |
| Osteoid Volume | 0.324 P=0.034 | 0.344 P=0.024 | 0.331 P=0.030 | 0.362 P=0.017 | 0.344 P=0.027 | 0.486 P=0.001 | NS |
| Osteoid Surface | NS | 0.488 P=0.001 | 0.425 P=0.005 | 0.368 P=0.015 | 0.358 P=0.021 | NS | NS |
| Eroded Surface | NS | NS | NS | NS | NS | 0.423 P=0.006 | NS |

Po and cortical OV correlated with trabecular bone formation, likely due to the action of PTH on both cortical and trabecular bone. Lack of correlation between cortical and trabecular BFR suggests that additional factors modulate cortical modeling in pediatric CKD. Understanding of cortical abnormalities may have therapeutic implications in the prevention of skeletal morbidity of CKD.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2928

**FRAX and Fracture Risk in Patients with Chronic Kidney Disease** Sophie Jamal,[1] Lisa Langsetmo,[2] Sarah West,[1] Sandhya S. Thomas,[3] Ryan Chauncey,[3] Thomas L. Nickolas.[3] *[1]Medicine, University of Toronto, Toronto, ON, Canada; [2]Statistics, McGill University Research Institute, Montreal, QC, Canada; [3]Medicine, Columbia University, NY, NY.*

Half of osteoporotic (OP) fractures (Fx) occur in patients with bone mineral density (BMD) above the OP diagnostic threshold. The FRAX risk assessment tool uses both BMD and other clinical risk factors for Fx to improve absolute Fx risk determination. In chronic kidney disease (CKD), BMD poorly identifies Fx risk. We hypothesized FRAX would improve Fx risk assessment in CKD.

We combined data from two cross-sectional studies of men and women, ≥18 yrs with pre-dialysis CKD Stages 3 to 5. We evaluated the ability of FRAX, with and without femoral neck (FN) BMD and assuming no prior Fx history, to discriminate prevalent OP Fx status. We also evaluated the ability of FRAX, with and without BMD and including prior clinical Fx, to determine prevalent spine Fx. The discrimination of age alone for Fx was used as a reference. Results are presented as AUCs with 95% confidence intervals (CI).

We enrolled 109 men and 81 women of whom 187 had BMD measurements. Mean age was 66.5 yrs, BMI was 28.5 kg/m2, and FN BMD was 0.722 g/cm2; 49 had OP Fx, 44 had spine Fx, 20 had a family history of Fx, 19 were taking steroids, 3 had RA, 15 were smokers and one person consumed ≥3 units of alcohol/day. FRAX both with and without BMD and excluding prior clinical Fx discriminated OP Fx status with AUC=0.67 (95% CI:0.58-0.75) and AUC=0.63 (95% CI:0.54-0.71) respectively, compared to AUC=0.60 (95% CI:0.52-0.69) for age. FRAX with and without BMD and including prior clinical Fx discriminated spine Fx status with AUC=0.66 (95% CI:0.57-0.75) and AUC=0.70 (95% CI:0.62-0.78), respectively, compared to AUC=0.68 (95% CI:0.59-0.77) for age.

FRAX, with or without BMD, had moderate ability to discriminate Fx status in pre-dialysis CKD patients. For spine Fx, FRAX performed similar to age. For clinical Fx, evaluation of other clinical risks for Fx modestly improved Fx risk assessment. Larger, longitudinal studies are needed to determine if assessment of clinical risk factors for Fx, in addition to BMD, improve Fx prediction in CKD.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2929

**Bone Mineral Density and Bone Structure Discriminate among Those with and without Fractures in Chronic Kidney Disease** Sophie Jamal,[1,2] Sarah West,[2] Charmaine E. Lok.[2,3] *[1]Women's College Hospital, Toronto, ON, Canada; [2]University of Toronto, Toronto, ON, Canada; [3]Toronto General Hospital, Toronto, ON, Canada.*

**Purpose:** The ability of bone mineral density (BMD) by dual x-ray absorptiometry (DXA) and bone structure by high resolution peripheral quantitated computed tomography (HR-pQCT) to discriminate among fractured and non-fractured patients with stages 3 to 5 chronic kidney disease (CKD) has not been well studied.

**Methods:** Baseline data from an ongoing prospective study of adult patients with stages 3-5 CKD was used to determine if BMD by DXA (Hologic) at the lumbar spine (LS), total hip (TH), ultradistal radius (UDR); and/or bone cortical area, density & thickness, trabecular density, thickness, separation & number by HR-pQCT (XtremeCT) at the radius could discriminate between those with and without self-reported low trauma fractures occurring after age 40. Results are expressed as areas under the receiver operating characteristic curves (AUC) with 95% confidence intervals (CI).

**Results:** Data was available for 71 men and 45 women. They were primarily Caucasian (62.9%) with a mean age of 62±16 yrs and weight of 78.7±17.6 kg. The most common cause of CKD was diabetes (43.4%). Almost half (46.4%) had a history of fractures and 34.6% reported a fall in the past year. BMD by DXA was able to discriminate among those with and without fractures at all sites (AUC for LS: 0.72 [95% CI: 0.61-0.84]; AUC for TH: 0.74 [95% CI: 0.61-0.88]; AUC for UDR: 0.69 [95% CI: 0.57-0.81]). HR-pQCT also performed well for cortical measures (AUC for area: 0.63 [95% CI: 0.49-0.78]; density: 0.65 [95% CI: 0.53-0.78] and thickness: 0.65 [95% CI: 0.53-0.78]) as well as trabecular measures (AUC for density: 0.69 [95% CI: 0.55-0.82]; number: 0.73 [95% CI: 0.61-0.86]; thickness: 0.65 [95% CI: 0.53-0.78], and separation: 0.69 [0.56-0.83]). There were no statistical differences in the performance characteristics of any of these tests.

**Conclusions**: Among adults ≥40 years old with stages 3 to 5 CKD not on dialysis, BMD by DXA and HR-pQCT parameters are able to successfully discriminate among those patients with and without self-reported low trauma fractures.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2930

**Characterization of Bone Loss in Patients with Chronic Kidney Disease Stage 5 on Dialysis (CKD 5 D) by High Resolution Peripheral Quantitative Computed Tomography (HR-pQCT)** Armando Luis Negri,[1] Elisa Elena Del Valle,[1] María B. Zanchetta,[1] Marcelo Puddu,[2] Roberto Barone,[3] Cesar Bogado,[1] José R. Zanchetta.[1] *[1]Nephrology, Instituto de Investigaciones Metabolicas, Buenos Aires, Argentina; [2]FMC-Caballito, Buenos Aires, Argentina; [3]STR Hurlingham, Hurlingham, Argentina.*

Patients with CKD 5D are at increased risk of fragility fractures. Recently HR-pQCT, a new non-invasive 3D bone imaging modality has been developed, to more accurately estimate bone quality and strength. The aim of this study was to assess whether volumetric bone mineral density (vBMD) and bone microstructure are impaired in dialysis patients using HR-pQCT bone imaging (XtremeCT, Scanco Medical AG) at the tibia and radius. HRpQCT has a resolution of <100um versus 350um of the standard pQCT machines allowing better analysis of trabecular microstructure. We examined 48 CKD 5D patients, 20 men and 28 women; mean time in dialysis was 65.6 ± 51 months. Mean age was 58.6 ± 11.1y for men and 53.5 ± 9.8 y for women. Mean serum iPTH level was 699 pg /mL (range: 79 - 2453 pg/ml). Both CKD men and women experienced significant trabecular (Tb) and cortical (Ct) impairment. CKD men vs. healthy controls at the radius: Tb vBMD: 138.9+/-45.8 vs. 188.4+/- 29.8 mg HA/cm(3)(p=0.001); Tb BV/TV(Bone volume/Total volume): 0.11±0.03 vs 0.15±0.02 (p=0.001); Tb number 1.72+/-0.39 vs. 2,00+/-0.27 mm(-1) (p=0.03) and Tb separation 564+/-307 vs. 429+/-67 mum. Cortical parameters in CKD men were also significantly decreased compared with healthy controls at the tibia: Ct thickness (ThK) 859+/- 260 vs. 1251+/-404mum (p=0.002); Ct vBMD 783.5±102 vs. 853.6± 56.2 mgHA/cm$^3$ (p=0.03); Ct area 90.3 ± 36.3 vs. 140.8 ± 32.5 mm$^2$ (p<0,001). Tibial CtThK had a positive significant correlation with Tb vBMD (r=0.62; p<0.001), Tb number (r=0.64; p<0.001) and a negative correlation with Tb separation (r=-0.55; p<0.001). In conclusion, this study shows an important impairment of trabecular microstructure in CKD 5D patients in addition to important alterations in cortical parameters with good correlations between them. Further longitudinal studies should be performed to validate HR-pQCT as a useull tool for predicting the fracture risk in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2931

**Diagnostic Usefulness of Bone Mineral Density in Predicting Vertebral Fracture and Abdominal Aortic Calcification in CKD Stage 5D Patients** Soichiro Iimori,[1] Sei Sasaki,[2] Yusuke Tsukamoto.[1] *[1]Department of Nephrology, Shuwa General Hospital, Kasukabe-shi, Saitama, Japan; [2]Department of Nephrology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.*

[Background] We previously reported that bone mineral sensity (BMD) was useful in predicting symptomatic any fracture (ASN 2009, abstract No. SA-PO2814). Since many cases of asymptomatic vertebral fracture (VF) co-existed, we analyzed usefulness of BMD by DEXA on VF and also examined the relationship with abdominal aortic calcification (AAC).

[Methods] Hemodialyzed patients (n=485) were enrolled from April/2003 to March/2008. Annual lateral abdominal X-ray was utilized to diagnose VF and AAC. Laboratory data was chosen from immediate previous examination before the X-ray showed VF or AAC (non-VF case), otherwise the first data.

[Results] 29 cases of VF and 277 cases of AAC were recorded. (1) Between VF and non-VF there was no difference in diabetes prevalence, dialysis vintage, hemoglobin, calcium, phosphorus nor intact PTH, but was different in female (<0.01), younger (p<0.001), lower BMI (p<0.01) and lower serum albumin (p<0.001). Among BMD measurements, 1/3 distal radius (1/3R), lateral lumbar spine (LS), femoral neck (FN) and femoral trochanter (FT) showed significant differences between the two groups (p<0.01). (2) ROC analysis on predicting VF, AUC was significant in FN (0.83), FT (0.78), 1/3R (0.72) and LS (0.67). When this result was stratified by gender, AUCs increased at FN (0.9), FT (0.83) and 1/3R (0.75) in male but decreased in female (FN: 0.73, FT: 0.70, 1/3R: 0.68). (3) Logistic regression analysis revealed lower BMD T-score at 1/3R (OR 0.63), LS (OR 0.7) and FN (OR 0.26) were significant predictor of VF (p<0.01). (4) Lower BMD T-score at 1/3R

(OR 0.87), LS (OR 0.80) and FN (OR 0.68) were significant predictor of AAC ($p<0.01$), and the presence of VF also predicted AAC (OR 6.04, $p<0.05$). (adjusted by age, gender, HD vintage and diabetes)

[Conclusions] BMD at femoral neck (t-score≤-2.4) was the best useful predictor of fracture not only in symptomatic any fracture but also in asymptomatic VF. This study suggested that loosing bone mineral caused vascular calcification in CKD stage 5D.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2932

**Comparison of Changes in DXA and Peripheral Quantitative Computed Tomography (pQCT) Measures of Bone Mineral Density (BMD) Following Renal Transplantation (RTxp)** Mary B. Leonard,[1] Hesham Shaban,[2] Babette Zemel,[1] Debbie Foerster,[1] Simin Goral,[2] Felix Wehrli.[2] *[1]Children's Hospital of Philadelphia; [2]University of Pennsylvania, .*

The structural basis of bone loss after RTxp has not been established. QCT provides discrete measures of trabecular and cortical volumetric BMD (vBMD, $g/cm^3$) while DXA measures areal BMD (aBMD, $g/cm^2$) of superimposed cortical and trabecular bone. QCT and DXA were compared at RTxp and 6 months later in 29 subjects (age 41±11 yr). Tibia QCT measures included trabecular vBMD in the metaphysis and cortical vBMD and thickness in the diaphysis. DXA aBMD was measured in the AP and lateral spine and total hip. Race, sex, and age-specific Z-scores were generated using reference data in 540 controls. Trabecular and cortical vBMD, and lateral spine and hip aBMD Z-scores decreased significantly.

| Z-Scores | Baseline | 6 Months | p |
|---|---|---|---|
| pQCT | | | |
| Trabecular vBMD | -0.63 ± 1.02 | -0.78 ± 1.02 | 0.0001 |
| Cortical vBMD | 0.14 ± 1.70 | -0.04 ± 1.54 | < 0.01 |
| Cortical Thickness | -0.72 ± 1.38 | -0.73 ± 1.45 | 0.70 |
| DXA | | | |
| AP Spine aBMD | -0.27 ± 1.42 | -0.44 ± 1.27 | 0.26 |
| Lateral Spine aBMD | -0.07 ± 1.28 | -0.40 ± 1.39 | 0.001 |
| Total Hip aBMD | -0.80 ± 0.96 | -0.91 ± 0.95 | 0.04 |

At baseline the significant correlations between QCT and DXA measures were: trabecular vBMD with AP spine ($r=0.46$; $p<0.01$), lateral spine ($r=0.50$, $p<0.01$) and hip aBMD ($r=0.67$, $p<0.0001$); cortical vBMD with AP spine aBMD ($r=0.51$; $p < 0.01$); and cortical thickness with AP spine ($r=0.58$, $p<0.001$), lateral spine ($r=0.40$, $p=0.04$) and hip aBMD ($r=0.56$, $p<0.001$). Correlations at 6 months were similar. Changes in trabecular vBMD were associated with changes in hip aBMD ($r=0.46$, $p<0.02$) but not with changes in AP or lateral spine aBMD. Changes in cortical vBMD and thickness were not associated with changes in DXA aBMD. These data demonstrated good correlations at each time point between QCT and DXA BMD. Total hip aBMD showed the strongest association with trabecular vBMD. Moreover, only total hip aBMD demonstrated good agreement with trabecular vBMD for changes over time.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2933

**International Assessment of TMV Classification of Bone Biopsy in ESKD** Stuart M. Sprague,[1] Hongyan Du,[1] Thomas L. Manley,[2] Aluizio B. Carvalho,[3] Patrick C. D'Haese,[4] Tilman B. Drueke,[5] Manuel A. Ferreira,[6] Vanda Jorgetti,[7] Sharon M. Moe,[8] Hartmut H. Malluche,[9] Ezequiel R. Bellorin-Font.[10] *[1]NorthShore Univ HealthSystem, Evanston, IL; [2]NKF, NY, NY; [3]Federal Univ of São Paulo, São Paulo, Brazil; [4]Univ of Antwerp, Antwerp, Belgium; [5]Hôpital Necker, Paris, France; [6]Hospital Curry Cabral, Lisbon, Portugal; [7]Univ of São Paulo School of Medicine, São Paulo, Brazil; [8]Indiana Univ School of Medicine, Indianapolis, IN; [9]Univ of Kentucky Medical Center, Lexington, KY; [10]Hospital Universitario de Caracas, Caracas, Venezuela.*

To determine whether serum biomarkers could predict bone disease, an international, multi-center, cross-sectional, retrospective analysis of the correlation between serum biomarkers and histologic bone disease was performed. Subjects (n=647) had a bone biopsy within the previous 10 years and blood obtained at the time of the biopsy; stored continuously at ≤ -20°C. PTH, intact (i) and whole (w), bone-specific alkaline phosphatase (BSAP), and procollagen type I intact N-terminal propeptide (P1NP) were measured in a central laboratory. Bone histomorphometery was classified using the KDIGO TMV system based on bone volume/tissue volume (BV/TV), mineralization lag time (MLT), osteoid thickness (Oth), and bone formation rate (BFR/BS). Demographic data, biochemistries (including original iPTH [OiPTH]) and treatment information at the time of the biopsy were collected. All 3 PTH assays were highly correlated (iPTH vs wPTH, $r=0.926$; iPTH vs OiPTH, $r= 0.629$; wPTH vs OiPTH, $r=0.670$; $p < 0.001$). Bone biopsy were classified by Mineralization (M), Turnover (T), and Volume (V). 55% of the biopsies demonstrated abnormal mineralization; 25% with abnormal mineralization, low turnover, and low volume; 17% with high turnover and 7% with normal bone. A weak correlation was observed between TMV score and BSAP ($r=0.333$, $p < 0.0001$), whereas no association between TMV score and other biochemical parameters. TMV classification of bone as described by KDIGO appears to be a valid means of classifying bone lesions; however, the biomarkers assessed in this study could not discriminate between various bone lesions.

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## SA-PO2934

**Vertebral Bone Mineral Density Is Associated with Histomorphometric Bone Structure Parameters but Not with Serum Bone Markers in Hemodialysis Patients** Graziella M. Leme,[1] Ricardo Carneiro,[2] Rosa Ma Moyses,[3] Vanda Jorgetti,[3] Raul D. Santos Filho,[2] Carlos Eduardo Rochitte,[2] Maria Eugenia F. Canziani,[1] Aluizio B. Carvalho.[1] *[1]Nephrology, Federal University of São Paulo, São Paulo, São Paulo, Brazil; [2]Heart Institute (INCOR), University of São Paulo, São Paulo, São Paulo, Brazil; [3]Nephrology, University of São Paulo, São Paulo, São Paulo, Brazil.*

**Introduction**: Renal osteodystrophy (ROD) is a common feature in hemodialysis (HD) patients. ROD is frequently accompanied by low bone mass which can be assessed by vertebral computed tomography (vCT). The aim of this study was to evaluate the relationship between vertebral bone mineral density (BMD) assessed by vCT with serum bone markers and bone biopsy in HD patients.

**Methods:**One hundred patients (48.513 ys; 65% male; 57% white; mean length on HD: 31±24.8 mo) were evaluated. They underwent vCT, undecalcified bone biopsy and serum bone markers. Vertebral BMD was obtained at the trabecular bone of the middle section of one thoracic vertebra at the level of aortic arch, expressing in Housefield Units (HU). Transiliac bone biopsy was analyzed by histomorphometry (Osteomeasure®).

**Results:** Intact-PTH was 359.3±319.8pg/mL, osteoprotegerin 172.3±70.3pg/mL, deoxypyridinoline (DPD) 108.9±142.4nmol/mL, bone-specific alkaline phosphatase (BAP) 29.4±29.4U/L and 25OHvitamin D 31.8±15.5ng/dL. Bone volume (BVTV) was 17.4±6.4%, trabecular separation (Tb.SP) 602±238µ, trabecular thickness (Tb.Th) 113.2±19.6µ and trabecular number (Tb.N) 1.5±0.72/mm.

Correlations of vertebral BMD with serum bone markers and histomorphometric bone structure parameters

| | r | p |
|---|---|---|
| intact-PTH | 0.13 | 0.23 |
| OPG | - 0.13 | 0.23 |
| DPD | 0.21 | 0.46 |
| BAP | 0.06 | 0.58 |
| 25OHD | - 0.15 | 0.89 |
| BVTV | 0.41 | <0.001 |
| Tb.Sp | - 0.35 | 0.001 |
| Tb.Th | 0.34 | 0.001 |
| Tb.N | 0.31 | 0.004 |

**Conclusions**: vCT is a useful tool for the evaluation of bone structure in HD patients. However, it does not give any information regarding bone metabolism in that population.

Disclosure of Financial Relationships: nothing to disclose

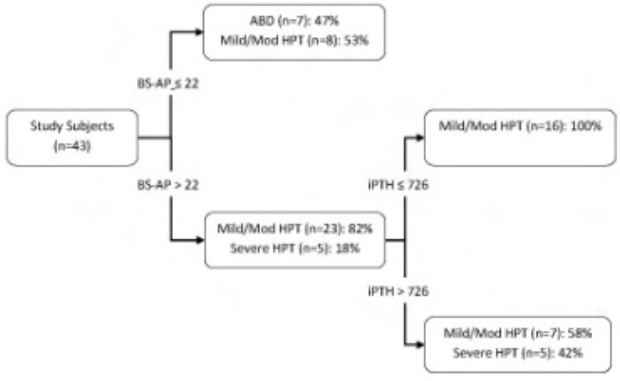
## SA-PO2935

**Determining the Optimal Predictor of Bone Histology in African-American (AA) Hemodialysis (HD) Subjects: A Classification and Regression Tree (CART) Analysis** Carol L. Moore, Beth Adams, Jerry Yee. *Nephrology/ Hypertension, Henry Ford Hospital, Detroit, MI.*

**Background:** Bone biopsy is the gold standard for determining bone histology. Non-invasive measures of bone activity include iPTH, CAP, CIP, total alkaline phosphatase (T-AP) and bone-specific alkaline phosphatase (BS-AP). We conducted CART analysis to determine which is the best predictor for classification of biopsy findings.

**Methods:** Bone biopsies were obtained in stable AA HD subjects. Lab markers for CKD-MBD were collected at time of biopsy. CART analysis determined the strongest predictors of biopsy findings and classified subjects based on the cutoff points found by the analysis.

**Results:** Pts (n 43) had an average age of 53.7 (11.6) yrs, dialysis vintage of 40.4 (24.5) mos, 30% with diabetes and 51% male. Biopsy results were classified as either adynamic bone disease (16%), mild/moderate (72%) and severe (12%) hyperparathyroidism (HPT). At the time of biopsy, mean iPTH was 225, 566, and 975 pg/mL ($p=0.006$), mean CAP was 125, 321, 534 pg/mL ($p=0.009$), mean CIP was 101, 245, 442 pg/mL ($p=0.010$), mean total alkaline phosphatase (T-AP) was 75, 150, 246 IU/L($p=0.001$) and mean bone-specific alkaline phosphatase (BS-AP) was 16, 34, 64 ng/mL ($p<0.0001$). The strongest predictor of biopsy findings was BS-AP. When BS-AP was > 22, the iPTH was useful in further classifying biopsy findings. With BS-AP > 22 and iPTH ≤ 726, 100% of subjects had mild/moderate HPT.




**Conclusion:** These results indicate that BS-AP, not iPTH, was the best predictor of biopsy findings with an optimal cut at 22. iPTH was able to further classify biopsy findings in those with a BS-AP > 22. No subjects with adynamic bone disease were found when BS-AP was >22. In those with BS-AP >22 and iPTH ≤ 726, all had mild/moderate HPT. BS-AP is an inexpensive, readily available assay that can assist clinicians in guiding therapy for CKD-MBD.

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## SA-PO2936

**Paricalcitol Reduces Bone Alkaline Phosphatase (BAP) in Patients with Diabetic Nephropathy: Results from the VITAL Study** Daniel W. Coyne,[1] Dennis L. Andress,[2] Michael Amdahl,[2] Utpaul Audhya,[2] Eberhard Ritz,[3] Dick De Zeeuw.[4] *[1]Washington University; [2]Abbott Laboratories; [3]University of Heidelberg; [4]University Medical Center Groningen.*

**Introduction:** Observational studies in CKD patients show elevated levels of serum alkaline phosphatase and PTH predict all-cause mortality and elevated BAP predicts cardiovascular (CV) outcomes independent of PTH levels. Because treatment with vitamin D receptor activators to lower PTH associates with improved survival in CKD, we examined BAP after paricalcitol therapy to determine if the expected BAP reduction is primarily PTH-independent.

**Methods:** VITAL was a randomized, double-blind clinical trial that enrolled 281 subjects with type 2 diabetic nephropathy (DN) and iPTH 35 to 500 pg/mL. Subjects were randomized to placebo (n=93), 1 µg (n=93) or 2 µg paricalcitol (n=95) daily for 24 weeks to assess change in urinary albumin. Serum PTH and BAP were measured monthly and again 60 days after drug withdrawal.

**Results:** The mean baseline BAP and PTH were 25 U/L and 98 pg/mL and were evenly distributed among the groups. 52% of subjects had a PTH <70 pg/ml. Analyses of change from baseline in BAP and an evaluation of PTH dependence are shown in Table 1. Sixty days after stopping paricalcitol mean BAP levels remained significantly lower than baseline in the 1 and 2 µg groups (15% and 10%, P< 0.001 and P=0.003 vs. placebo) while mean PTH had risen to 28% and 18% above baseline levels (P=NS vs. placebo in both groups).

**Conclusion:** Paricalcitol has a direct effect on BAP reduction in DN CKD patients by PTH-independent mechanisms. Mean BAP reduction was sustained for as long as 60 days after stopping therapy despite the return of PTH levels to baseline. Future studies should examine a potential role for paricalcitol to reduce CV outcomes by a BAP-dependent mechanism.

Table 1. Analyses of Change from Baseline in BAP and an Evaluation of PTH Dependence

| | N | Mean % Change in BAP from baseline | P value (vs. Placebo) | Change in iPTH and BAP Correlation Coefficient* | P value |
|---|---|---|---|---|---|
| Placebo | 75 | -3% | | 0.17 | 0.154 |
| Paricalcitol 1 µg | 75 | -26% | P<0.001 | 0.27 | 0.017 |
| Paricalcitol 2 µg | 73 | -27% | P<0.001 | 0.45 | <0.001 |
| Subjects with baseline PTH <70 pg/mL | | | | | |
| Placebo | 41 | 2% | | 0.16 | 0.379 |
| Paricalcitol 1 µg | 36 | -20% | P<0.001 | -0.14 | 0.428 |
| Paricalcitol 2 µg | 39 | -26% | P<0.001 | 0.07 | 0.672 |
| Subjects with baseline PTH ≥70 pg/mL | | | | | |
| Placebo | 34 | -8% | | 0.26 | 0.141 |
| Paricalcitol 1 µg | 39 | -33% | P<0.001 | 0.22 | 0.178 |
| Paricalcitol 2 µg | 34 | -34% | P<0.001 | 0.36 | 0.035 |
| **Path Analysis of Change in BAP** | | **Coefficient** | | **% of Effect** | **P value** |
| Direct Effect | | -4.6106 | | 55% | <0.0001 |
| Indirect Effect through Change in iPTH | | -2.5163 | | 55% | <0.0001 |

*Spearman Rank-Order Correlation Coefficient

Disclosure of Financial Relationships: Consultancy: Abbott, AMAG, INEOS, Sanofi Aventis, Pharmacosmos, Shire, WatsonResearch Funding: Abbott, Amgen, J&J, Roche, Kureha, Merck; Honoraria: Abbott, AMAG, Sanofi Aventis, Pharmacosmos, Watson.

## SA-PO2937

**Racial Differences in the Clinical Use of Cinacalcet among Hemodialysis Patients** Britt B. Newsome,[1] Craig Solid,[3] David A. Zaun,[3] Ryan D. Kilpatrick,[2] Jiannong Liu,[3] Kimberly M. Nieman,[3] Wendy L. St. Peter.[3,4] *[1]Denver Nephrologists, P.C., Denver, CO; [2]Amgen, Inc., Thousand Oaks; [3]Chronic Disease Research Group, MMRF, Minneapolis, MN; [4]Univ. of Minnesota.*

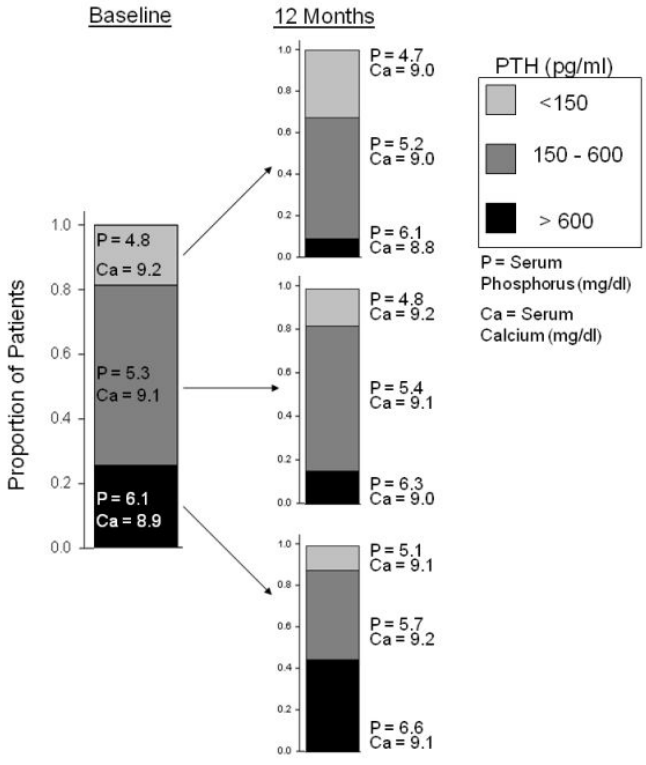
**Background:** African American (AA) patients with End Stage Renal Disease (ESRD) on dialysis are known to have more severe secondary hyperparathyroidism (SHPT) as reflected by higher levels of parathyroid hormone as well as requirements of higher doses of activated vitamin D. Despite these prior observations, racial differences in the clinical use of cinacalcet in the control of SHPT have not been examined. **Methods:** Data for this retrospective cohort study were from DaVita Inc. for 45,589 prevalent hemodialysis patients in August 2004 linked to Centers for Medicare and Medicaid Services data with follow-up through July 2007. Patients were included who had Medicare as primary payer and used IV vitamin D in baseline period (Aug. 1, 2004-Oct. 31, 2004). Patients with modality change, kidney transplant before Nov. 1, 2004 were excluded. Patients were grouped by parathyroid hormone (PTH) level <150, ≥150-≤300, >300-≤600, >600 pg/mL at baseline. Adjusted Cox regression was used to predict time to cinacalcet initiation, titration and discontinuation. **Results:** Of the 25,250 patients included in this analysis, 9474 (37.5%) were AA, 13632 (54.0%) were white (W) and 2144 (8.5%) were other race. Among cinacalcet users, 49.8% were AA. Among AA, 40.3% were prescribed cinacalcet compared to 24.4% of C and 24.7% of individuals classified as other race. Within each PTH strata, a greater percentage of AA, compared to the other race groups, was prescribed cinacalcet, and AA were prescribed higher doses. After covariate adjustment, AA, compared to W, were more like to be initiated on cinacalcet [AA:W hazard ratio (HR) 1.17; 95% confidence interval (CI) 1.11, 1.23] and less likely to be discontinued (AA:W HR 0.78; 95% CI 0.70, 0.85). There was no significant difference between the two race groups with respect to up titration of cinacalcet (AA:W HR 1.08; 95% CI 0.99, 1.18). **Conclusion:** AA patients more commonly are prescribed cinacalcet and at higher initial doses than other race groups in the management of SHPT.

Disclosure of Financial Relationships: Ownership: NephroceuticalsResearch Funding: Research grants from Amgen, Genzyme, Genentech and Shire pharmaceuticals.

## SA-PO2938

**Parathyroid Hormone (PTH) and Phosphorus (P) Trends after Cinacalcet Initiation in Hemodialysis (HD) Patients** Ryan D. Kilpatrick,[1] David A. Zaun,[2] Craig Solid,[2] Britt B. Newsome,[3] Jiannong Liu,[2] Kimberly M. Nieman,[2] Wendy L. St. Peter.[2,4] *[1]Amgen, Inc., Thousand Oaks, CA; [2]Chronic Disease Research Group, MMRF, Minneapolis, MN; [3]Denver Nephrologists, P.C., Denver, CO; [4]U of MN.*

**Background:** Cinacalcet response, i.e. change in secondary hyperparathyroidism (SHPT) lab values, has not been well characterized in real-world settings. Two response indicators may be disease severity at cinacalcet initiation and need for upward dose titration. We explored descriptive trends in PTH and P over 12 mos following cinacalcet initiation by baseline PTH category to describe real-world response. **Methods:** Data were from 45,589 prevalent DaVita Inc. HD patients (pts) in facilities on 8/2004. Pts had Medicare as primary payer and used IV vitamin D during baseline (8/04-10/04). 7,674 pts were identified who initiated cinacalcet between 11/04-8/07. To determine trends, Cinacalcet users remaining on therapy for ≥12 mos (n=4,177) were examined. Cohort was separated into pts who remained on their initial dose for first 12 mos (n= 2,196) and pts who had ≥1 upward dose titration (n = 1,981). Monthly median PTH and mean P values pre-initiation and in each mo. for 12 mos were calculated and stratified by baseline PTH (≥150-≤300, >300-≤600, >600 pg/mL). **Results:** Reductions in absolute PTH and P were larger for pts initiating at PTH levels >600 pg/mL. Pts receiving dose titration(s) had higher pre-initiation PTH and P levels and smaller reductions in PTH over 12 mo. period as compared to no-titrate pts. Overall, PTH and P reductions were sustained over 12 mos and for higher PTH initiators, continued decline in PTH and P was observed, possibly due to addition or adjustment of other therapies. **Conclusion:** Despite larger reductions in PTH and P for pts initiating in PTH >600 group, after 12 mos, PTH and P values remained higher than in pts initiating at lower PTH.




Disclosure of Financial Relationships: Employer: Department of Biostatistics and Epidemiology, Amgen, Inc.

**SA-PO2939**

**Persistent Secondary Hyperparathyroidism: The Relationship between FGF23 and Cinacalcet** Magdalene M. Assimon,[1] Roy Mathew,[2] Darius Mason.[1] *[1]Albany College of Pharmacy & Health Sciences, Albany, NY; [2]Stratton VAMC, Albany, NY.*

Fibroblast growth factor 23 (FGF23) regulates phosphate excretion, vitamin D metabolism and directly reduces parathyroid hormone (PTH) secretion. PTH levels may remain elevated in dialysis patients despite elevated FGF23 levels and use of therapy to control secondary hyperparathyroidism (SHPT). Limited data exists regarding the association between cinacalcet and FGF23 in persistent SHPT. The aim of this study was to explore the association between cinacalcet use, FGF23 and SHPT in hemodialysis (HD) patients.

**Methods:** Adults receiving HD for ≥3 months were enrolled in this cross-sectional study. Patient demographics, laboratory values and medication lists were obtained. Pre-dialysis levels of intact FGF23 were measured by ELISA. Stepwise linear regression was used to identify factors associated with FGF23.

**Results:** Subjects' (n=118, 57.6% male, 46.6% diabetic etiology) mean age = 63.5±16.3 years, HD vintage = 3.3±2.9 years and log FGF23 = 2.50±0.36 pg/ml. Log FGF23 levels positively correlated with calcium [r=0.25, p=0.0063], phosphorus [r=0.56, p<0.0001], PTH [r=0.28, p=0.0022] and HD vintage [r=0.26, p=0.0026] and negatively correlated with age [r=-0.27, p=0.0006]. Log FGF23 levels were higher in cinacalcet users (n=38, mean dose = 51.5±41.3 mg/day, mean length of use = 6.9±6.3 months) compared to cinacalcet non-users (n=80) [2.62±0.41 pg/ml vs. 2.44±0.31 pg/ml, p=0.0185]. Use of cinacalcet [β=0.14, p=0.0052], calcium [β=0.19, p<0.0001], phosphorus [β=0.11, p<0.0001] and dialysis vintage [β=0.27, p=0.0013] were associated with log FGF23. Patients taking cinacalcet had higher median (IQR) PTH levels [431.7 (321.8–669) pg/ml vs. 301.7 (173.4-368.6) pg/ml, p=0.006] and received greater doses of IV calcitriol [3.6±3.6 mcg/week vs. 2.0±2.1 mcg/week, p=0.0185] compared to non-cinacalcet users.

**Conclusions:** Log FGF23 was positively associated with cinacalcet use, calcium, phosphorus and HD vintage. Subjects taking cinacalcet had worse SHPT and required larger doses of calcitriol, despite a 6.9 month mean duration of cinacalcet therapy. Cinacalcet use may be a surrogate marker for refractory SHPT resulting in the observed association with log FGF23.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2940**

**Comparison of Lanthanum Carbonate with Calcium Carbonate on Biomarkers of Bone Turnover in Hemodialysis Patients: A Cross-Over Study** Tatsunori Toida,[1] Keiichi Fukudome,[1] Shouichi Fujimoto,[2] Kazuhiro Yamada,[2] Susumu Chiyotanda,[1] Kazuo Kitamura.[2] *[1]Chiyoda Hospital, Hyuga, Miyazaki, Japan; [2]Dialysis Division, University of Miyazaki Hospital, Miyazaki, Japan.*

Lanthanum carbonate (LC) is a new non-calcium-containing phosphate binder. However, there are few comparative studies of the efficacy on bone turnover between LC and calcium carbonate (CaC). The aim of this cross-over study was to compare the effects of these drugs on biomarkers of bone turnover in hemodialysis (HD) patients.Fifty patients undergoing maintenance HD with bicarbonate dialysate containing 2.5 mEq/L of calcium were investigated. Following washout (2 weeks), these patients were randomized (1:1) to receive LC or CaC for 3 months. Thereafter, patients underwent a second washout (2 weeks) and switched to the alternative binder for 3 months. In addition of Ca, P and intact PTH, several bone turnover markers were measured using ELISA kits. Use of other phosphate binders and cinacalcet were prohibited during the study periods.Clinical characteristics and laboratory data were not different between group LC and CaC at the start of the study. Serum P levels were equally decreased in both groups (0M vs 3M; LC, 7.1+/-1.7 vs 5.5+/-1.3, p < 0.01; CaC, 7.1+/-1.8 vs 5.6+/-1.2 mg/dL, p < 0.01), but Ca levels were clearly increased in group CaC (0M vs 3M; 8.7+/-0.8 vs 9.1+/-0.7mg/dL, p < 0.01) 3 months after the treatment. The concentrations of intact PTH at pre- and post-treatment were not different between both groups, but the reduction with CaC was greater than with LC (CaC vs LC, -73.9+/-80.2 vs -12.9+/-78.7 pg/mL, p < 0.001). The levels of serum BAP and TRAP5b were significantly elevated in group LC (0M vs 3M; BAP, 23+/-8.9 vs 35+/-20 U/L, p < 0.001; TRAP5b, 768+/-304 vs 992+/-457 mU/dL, p < 0.01 ), but not in group CaC. In both groups, serum FGF23 levels were significantly decreased 3 months after the treatment, while osteoprotegerin levels did not change during the study periods. In conclusion, LC effectively reduced serum P levels in HD patient and raised the bone turnover without the change of PTH levels. LC may have advantages in the treatment of CKD-MBD in comparison with CaC.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2941**

**The Effects of Magnesium Carbonate/Calcium Acetate on Experimental CKD-MBD** Guaraciaba O. Ferrari,[1] Juliana C. Ferreira,[1] Katia R. Neves,[1] Cássia M. Puci,[1] Raquel T. Cavallari,[1] Wagner V. Dominguez,[1] Fabiana G. Graciolli,[1] Luciene M. dos Reis,[1] Jutta Passlick-Deetjen,[2] Vanda Jorgetti,[1] Rosa Ma Moyses.[1] *[1]Nephrology, Universidade de Sao Paulo, Brazil; [2]Fresenius Medical Care, Germany.*

Adequate phosphate (P) control is still a challenge in CKD-MBD. In this study, we evaluated the effects of a new P binder on parathyroids, bone and vascular calcification (VC). Rats were fed normal chow or an adenine(AD) diet for 4 wks, followed by 4 weeks of therapy with Ca (calcium acetate); CaMg [Ca/magnesium carbonate (Osvaren®)]; or Untreated. After that, we performed biochemical and bone histomorphometric analyses, as well as von Kossa staining of aortic sections and observed that P binders decreased fractional excretion of P (FeP) and FGF23. In the CaMg group, Mg was slightly elevated, whereas iPTH and FGF23 were lower than in the other AD-groups. P binders therapy was able to decrease bone resorption, but with a trend to an increase in mineralization defect. Despite a lower PTH, CaMg group presented an increase in parathyroid PCNA positive cells. VC was less commonly observed in CaMg than in Ca-treated rats (1/9 vs. 3/10).

| | Control Diet | Untreated | CaMg | Ca |
|---|---|---|---|---|
| Ionized calcium(mmol/L) | 1.15±0.08[b] | 1.07±0.07 | 1.07±0.11 | 1.12±0.10 |
| iPTH(pg/ml) | 125±79[bc] | 630±354[c] | 362±122 | 688±778 |
| Mg(mg/dL) | 1.8±0.1[abc] | 2.4±0.4[ac] | 3.2±0.6 | 2.6±0.4[c] |
| P(mg/dL) | 5.6±0.9[abc] | 7.4±0.7 | 7.6±1.2 | 7.2±1.7 |
| FeP(%) | 9.1±4.8[abc] | 21.2±10.9[ac] | 2.5±1.5 | 3.7±4.0 |
| PCNA(cells/mm2) | 2.4±4.1[bc] | 31.4±35.4[ac] | 201.2±137.0 | 4.0±9.8 |
| Bone volume(%) | 27.4±5.0 | 31.1±6.7 | 30.3±9.3 | 26.8±11.7 |
| Osteoid Thickness (μm) | 1.9±0.4[ac] | 2.6±1.3[a] | 5.3±2.8 | 7.3±2.4 |
| Bone Formation Rate(μm3/μm2/d) | 0.04±0.04 | 0.11±0.08 | 0.05±0.03 | 0.05±0.03 |
| Mineralization Lag Time(d) | 7±6[a] | 36±57 | 44±26 | 59±47 |

Mean ± SD; P< 0.05 = a vs Ca; b vs untreated; c vs CaMg.

In conclusion, in this model of CKD-MBD, CaMg was able to decrease PTH, FGF23 and VC prevalence compared to Ca. The mechanism that underlies the Mg effects on parathyroid proliferation should be elucidated in further studies.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2942**

**Comparison of Oral and Intravenous Alphacalcidol in Chronic Hemodialysis Patients** Myriam Lessard, Annie-Claire Nadeau-Fredette, Robert Bell, Jean-Philippe Lafrance, Vincent Pichette, Michel Vallee. *Nephrology, Hopital Maisonneuve-Rosemont, Montreal, QC, Canada.*

BACKGROUND: It is well known that activated vitamin D is the mainstay of treatment for secondary hyperparathyroidism in chronic hemodialysis patients. However, the optimal route of administration of activated vitamin D is still debated.

OBJECTIVES: The aims of our study were to compare efficacy and side effects of intravenous (IV) versus oral administration of alfacalcidol in hemodialysis. Our secondary objective was to determine if oral administration has a cost-effectiveness advantage compare to IV administration.

METHODS: 88 chronic hemodialysis patients receiving IV alfacalcidol 3 times a week for at least 3 months were included in the study. All were switched to the same dose of oral alfacalcidol administered 3 times a week in the hemodialysis unit. Alfacalcidol dose was then adjusted according to K/DOQI recommendations. Annual costs and nursing time were calculated for both oral and IV routes of administration. Parameters at baseline and 3 months after the switch were compared using a Student's paired t-test.

RESULTS: Mean patient age was 64 years old and 43% were males. Three months after switching from IV to oral alfacalcidol, PTH levels significantly decreased from 754 to 538 pg/mL (p<0.001), total calcium levels increased from 9.36 to 9.60 mg/dL (p=0.001), whereas phosphorus levels remained stable (from 4.61 to 4.86 mg/dL, p=0.19). Compared to IV, oral administration was associated with an annual cost reduction of 187 000 $CAN and an annual nursing time reduction of 25 days.

CONCLUSION: Our study suggests that switching from IV to oral administration of alfacalcidol during hemodialysis sessions may lead to better secondary hyperparathyroidism control. Finally, oral administration of alfacalcidol has a major cost advantage and reduces the nursing time required.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2943

**Effect of Cinacalcet on the Change of Bone Mineral Density and Bone Markers in Hemodialysis Patients with Secondary Hyperparathyroidism** Yuki Tsuruta,[#1] Kazuhiro Okano,[#1] Naoki Kimata,[#2] Takashi Akiba,[#2] Kosaku Nitta.[#1] *[1]Department of Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan; [2]Departmente of Blood Purification, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Cinacalcet markedly reduces serum intact parathyroid hormone (iPTH) in hemodialysis (HD) patients with secondary hyperparathyroidism (SHP). Previous studies reported that HD patients have higher risk for hip fracture and higher rate of 1 year mortality after the fracture compared with general population. Parathyroidectomy reduces serum iPTH levels and increases bone mineral density (BMD) in HD patients. However, there is few reports about effect of cinacalcet on BMD and bone markers in HD patients.

Patient and method: We performed a 1 year cohort study included 21 HD patients who had serum iPTH more than 300pg/ml under conventional therapies included vitamin D. The patients with or without cinacalcet treatment are defined as cinacalcet (10 patients) and control (11patients) group, respectively. BMD of femoral neck and alkaline phosphatase (ALP) were measured at baseline and after 1 year treatment.

Result: There is significant reduction of serum iPTH and ALP levels for cinacalcet group, not for control group. BMD significantly increased 6.4% per year for cinacalcet group and decreased 6.2% per year for control group (p=0.0001). The changes of serum ALP levels during observation has negative correlation with the changes of BMD ($R^2$=0.629, p<0.0001).

Bone mineral density and bone markers

| | | Cinacalcet(n=10) | | | Control(n=11) | |
|---|---|---|---|---|---|---|
| | Before | After | p value | Before | After | p value |
| BMD | 0.573±0.125 | 0.604±0.109 | 0.0082 | 0.644±0.150 | 0.606±0.155 | 0.0011 |
| iPTH | 687.8±268.4 | 163.8±109.0 | <0.0001 | 316.7±106.2 | 334.4±163.8 | NS |
| ALP | 657.7±344.4 | 297.3±105.3 | 0.0096 | 326.0±126.5 | 361.4±173.5 | NS |

Conclusion: One year treatment of cinacalcet for HD patients with SHP reduced serum iPTH levels markedly and increased BMD of femoral neck. Furthermore, our study suggests that dynamics of ALP levels are a useful predictor for change of BMD in HD population.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2944

**Calcium (Ca) and Phosphate (P) Changes in Diabetics Receiving Moderate to High Doses of Paricalcitol: Results from the VITAL Study** Daniel W. Coyne,[1] Dennis L. Andress,[2] Utpaul Audhya,[2] Michael Amdahl,[2] Eberhard Ritz,[3] Dick De Zeeuw.[4] *[1]Washington University; [2]Abbott; [3]University of Heidelberg; [4]University Medical Center Groningen.*

**Introduction:** Increased serum P and Ca associate with increased mortality in CKD patients. CKD-related low calcitriol levels associate with poor outcomes, while use of vitamin D receptor activators (VDRA) associates with improved outcomes. Calcitriol therapy carries risks of elevated P and Ca. This analysis examined subjects receiving a selective VDRA, paricalcitol, for albuminuria reduction, to determine risk of elevated P and Ca.

**Methods:** VITAL was a randomized trial enrolling 281 type 2 diabetic subjects with CKD corrected serum Ca level <9.8 mg/dL and iPTH 35 to 500 pg/mL. Subjects received placebo (pbo, n=93), paricalcitol 1 µg (n=93), or paricalcitol 2 µg (n=95) daily for 24 weeks. Dose reductions to TIW occurred if elevated calcium was confirmed or if PTH <15 pg/ml.

**Results:** 43% of subjects had PTH in the normal range at baseline: mean PTH in pbo, 1µg and 2 µg groups were 105.3±91.2 pg/mL, 97.4±77.2 pg/mL, and 90.9±65.1 pg/mL. PTH increased 23% in the pbo group and declined by 21 and 48% in the1 µg and 2 µg groups (p<0.001, both). Mean changes in P and Ca during treatment are shown in Table 1. Despite increases in Ca, only 1 subject in the 1 µg group and 3 subjects in the 2 µg group experienced hypercalcemia, versus 1 in pbo (P=NS). Dose reductions of paricalcitol occurred in 14 and 42% in the 1 µg and 2 µg groups, primarily for PTH suppression.

**Conclusions:** Paricalcitol resulted in significant but modest increases in Ca and few episodes of hypercalcemia despite meaningful reductions in PTH consistent with previous studies. The finding that urinary P excretion was unchanged despite potent PTH suppression with paricalcitol suggests intestinal P absorption may not be enhanced by this selective VDRA.

Table 1. Change from Baseline to Last On-Treatment Observation in Ca and P

| | Placebo Mean (SD) | Paricalcitol 1 µg Mean (SD) | Paricalcitol 1 µg p vs. placebo | Paricalcitol 2 µg Mean (SD) | Paricalcitol 2 µg p vs. placebo |
|---|---|---|---|---|---|
| Phosphorus | | | | | |
| Serum, mg/dL | -0.09 (0.64) | -0.60 (0.62) | 0.005 | 0.32 (0.66) | <0.001 |
| Urine, g/24 hr | -0.61 (0.32) | -0.67 (0.27) | 0.125 | -0.12 (0.55) | 0.366 |
| Calcium | | | | | |
| Serum, mg/dL | 0.02 (0.44) | 0.16 (0.49) | 0.039 | 0.47 (0.58) | <0.001 |
| Urine, mg/24 hr | -2.42 (24.76) | 21.68 (39.49) | 0.003 | 24.17 (70.52) | <0.001 |

Disclosure of Financial Relationships: Consultancy: Abbott, AMAG, INEOS, Sanofi Aventis, Pharmacosmos, Shire, WatsonResearch Funding: Abbott, Amgen, J&J, Roche, Kureha, Merck; Honoraria: Abbott, AMAG, Sanofi Aventis, Pharmacosmos, Watson.

## SA-PO2945

**Pharmacokinetics of Alendronate Sodium in End-Stage Renal Disease Patients** Methee Chanpitakkul, Amnart Chaiprasert. *Department of Medicine, Phramongkutklao Hospital, Bangkok, Thailand.*

Osteoporosis is a worldwide public health problem. Bisphosphonates (e.g. alendronate sodium) are widely used for the treatment of osteoporosis. Alendronate sodium is not recommended in patients with creatinine clearance less than 35 mL/min due to lack of experience in renal failure. Until now, pharmacokinetics of alendronate sodium in end-stage renal disease (ESRD) patients are unknown.

**Objective**

To study the pharmacokinetics of oral alendronate sodium in ESRD.

**Method**

ESRD patients treated with regular hemodialysis three times a week for at least three months with no residual urine were enrolled. Exclusion criteria were history of drug allergy to alendronate sodium, intact parathyroid hormone < 150 pg/mL. After fasting at least 10 hours, the day after hemodialysis, single oral alendronate sodium 70 mg was taken. The plasma samples were drawn at 0, 0.5, 1, 2, 3, 4, 6, 8, and 10 hours after medication and were analyzed for alendronate concentration by high performance liquid chromatography method.

**Results**

Seven ESRD patients were studied. All were male, mean±SD age 54.1±5.9 years. The baseline calcium, phosphate, albumin and intact parathyroid hormone were 9.36±0.95 mg/dL, 5.13±1.59 mg/dL, 4.36±0.33 g/dL and 228.4±66.1 pg/mL, respectively. Pharmacokinetics of alendronate sodium in ESRD patients were as following; maximal concentration ($C_{max}$) 80.3±51.7 ng/mL, time of $C_{max}$ ($T_{max}$) 0.89±0.32 hour, area under the curve ($AUC_{0-inf}$) 195.6±93.3 ngxh/mL and half-life 4.36±4.99 hours. Clearance of alendronate, non-renal and non-dialysis, divided by fractional of bioavailability was 469.2±287.0 L/h.

**Conclusion**

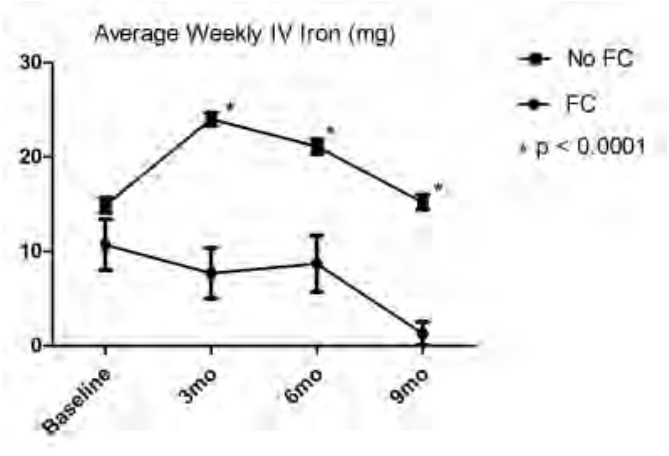
Pharmacokinetics of oral alendronate sodium in end-stage renal disease patients are different from studies of healthy subjects.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2946

**Prolonged Use of Ferric Citrate (FC) as a Phosphate Binder Reduces IV Iron Use in Pts with ESRD** Mohammed Sika,[1] Marvin V. Sinsakul,[2] Robert M. Niecestro,[3] Shou-Shan Chiang.[4] *[1]Vanderbilt University, Nashville, TN; [2]Edmund Lewis&Assoc, Chicago, IL; [3]Keryx Biopharmaceuticals; [4]Shin Kong Wu Ho-Su Memorial Hospital, Taiwan.*

FC is being evaluated as a phos binder in pts with ESRD. Studies have shown possible iron absorption with short-term use of FC. After completing a double-blind, placebo-controlled, dose-ranging study for 28 days, pts enrolled in an open-label extension study at a single center. Pts were started on 2-6gm of FC (500mg) used to maintain phos at 3.5-5.5mg/dL. IV iron and erythropoeitin stimulating agents(ESA) were continued based on protocol. Hct, Fe, ferritin, TSAT, average weekly IV iron and ESA dosages were recorded at baseline, 3, 6 and 9 mo. 28 pts (9M/19F, age 54±14yrs) received FC and followed for 306±85 days and were compared to 385 ESRD pts not on FC. The average dose of FC at 3,6,9 mo. was 4.8±1.4, 4.7±1.5, and 4.8±1.5 gm/d. Pts who received FC had a significant decrease in IV iron use compared to pts not receiving FC, while maintaining iron parameters and Hct (Figure,Table). Pts receiving FC tended to use less ESA compared to those that did not receive FC. We observed that long term use of FC as a phosphate binder not only controls phos, but may also maintain all iron parameters allowing for a decrease in IV iron and ESA use over time and this warrants further study.




FC vs. No FC

| Parameter | Baseline | 3mo | 6mo | 9mo |
|---|---|---|---|---|
| phos(mg/dl) | 5.6±0.3vs5.4±0.1 | 5.5±0.3vs5.1±0.1 | 5.2±0.2vs5.3±0.1 | 5.2±0.2vs4.9±0.1 |
| ferritin(ng/ml) | 520±62vs556±17 | 744±122vs435±15 | 654±66vs482±16 | 781±73vs599±19 |
| Fe(mcg/dl) | 88±7vs78±2 | 94±8vs73±1 | 82±7vs77±2 | 88±7vs74±2 |
| TSAT(%) | 39±4vs37±1 | 42±4vs33±1 | 39±4vs34±1 | 46±4vs37±1 |
| Hct(%) | 31±1vs32±0 | 31±2vs30±0 | 32±2vs31±0 | 33±2vs32±0 |
| ESA(IU/wk) | 2964±525vs3345±169 | 3192±634vs4320±170 | 2826±608vs3947±176 | 2526±547vs3597 ±158 |

mean±SE

Disclosure of Financial Relationships: Research Funding: Keryx Biopharmaceuticals.

### SA-PO2947

**Short Term Effect of 25-Hydroxy Vitamin D Supplementation in Hemodialysis-Patients** Jean-Christophe Szelag, Alejandra Lenz, Myriam Pastural, Carlos Cardozo, Nouredine Boumendjel, Ignace Mpio, Elias Abdullah, Walid Arkouche. *AURAL Villon, AURAL, Lyon, France.*

Vitamin D deficiency is involved in overall morbidity of ESRD patients. We studied the frequency of 25-hydroxyvitamin D deficiency and the effects of its correction in 148 HD-patients (58 women, 90 men, 61,4yr) for a period of 3 months

**Patients and methods:** Usual parameters of mineral metabolism was determined on monthly (total calcium, phosphorus) or quarterly (25-OHvitaminD (2+3), PTH) basis between winter and the end of last spring. Every treatment of interest (oral calcium, sevelamer hydrochloride, lanthanum carbonate, cinacalcet, calcitriol, and cholecalciferol) were listed during the study. 25-OHvitamin D deficiency (< 30 ng/mL) was treated with cholecalciferol (100000IU one or twice a month) or calcidiol (600 to 1000 IU/day). A supplementation was already proposed in 56 % of the patients before the study.

**Results:** The frequency of vitamin D deficiency at month 0 was 66,8% (99 patients) in spite of a supplementation in 49,4% of the cases. At month3, the institution or the intensification of the supplementation to 81 of these patients were associated with an increase of vitaminD levels (17,4+/-6,6 vs 32,3+/-14,9 ng/mL, p<0,0001, Wilcoxon rank test). Supplementation was associated with a slightly but significantly decrease of PTH level (423+/-322,9 *vs* 381,3+/-289,9 ng/L, p=0,0317). We observed a better control of serum phosphorus (1,58+/-0,5 vs 1,47+/-0,46 mmol/L, p=0,012) without any change on serum calcium (2,18+/-0,17 *vs* 2,18+/-0,16 mmol/L, p=0,76). There was no significant change of CRP ( 11,8+/-17,1 vs 8,9+/-9,8 mg/L, p=0,26) and serum albumine (38,9+/-3,6 vs 38,5+/-3,7 g/L, p=0,065). The only significant change in treatment was a reduction of alfacalcidiol dose and administration (58 to 53%). In patients without deficiency (49, supplementation 70%), 25-OHvitaminD, serum phosphorus and PTH remained stable.

**Conclusion:** This short term single center study confirms the high frequency of vitamin D deficiency in HD-patients. The supplementation with cholecalciferol or calcidiol allowed a decrease of PTH level, a better control of serum phosphorus with a reduction of calcitriol administration.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2948

**Discovery of a Calcimimetic with Differential Effects on PTH and Calcitonin Secretion** Charles M. Henley III,[1] James R. Davis,[1] Sean Morony,[1] Wei Fan,[1] Monica Florio,[1] Banghua Sun,[1] Edward Shatzen,[1] William G. Richards,[1] Christopher Fotsch,[3] Jeff D. Reagan.[2] *[1]Metabolic Disorders, Amgen, Inc., Thousand Oaks, CA; [2]Metabolic Disorders, Amgen, Inc., South San Francisco, CA; [3]Medicinal Chemistry, Amgen, Inc., Thousand Oaks, CA.*

Calcimimetics are positive allosteric modulators of the calcium-sensing receptor (CaSR). Activation of the CaSR inhibits the secretion of parathyroid hormone (PTH), stimulates the secretion of calcitonin (CT) and decreases blood ionized calcium ($Ca^{2+}$). Cinacalcet, a second generation calcimimetic, is used therapeutically to reduce serum PTH, calcium, phosphate and calcium x phosphorous product in patients with secondary hyperparathyroidism on dialysis. The purpose of this research was to identify molecules that have divergent activities from current calcimimetic effects in uremic rats with partial ($1/6^{th}$) renal function. Towards this end, we developed a third generation calcimimetic and determined the molecular pharmacological properties of it using an operation model of allosteric modulation/agonism. We also measured compound effects on serum PTH, calcitonin and blood ionized $Ca^{2+}$ in 5/6 nephrectomized rats. We observed that the new molecule effectively reduced PTH levels without promoting CT secretion and thus had limited effects on $Ca^{2+}$ homeostasis in this uremic rodent model. These studies demonstrate the possibility of developing a calcimimetic with divergent activities on CT secretion. Whether these activities result from utility of different signaling pathways remains to be determined.

Disclosure of Financial Relationships: Employer: Amgen, Inc.; Ownership: Stock options and stock ownership in Amgen, Inc.

### SA-PO2949

**Continuous Erythropoietin Receptor Activator (CERA) Ameliorates Sepsis-Induced TLR4 Pathway-Mediated Downregulation of AQP2 Expression and Acute Kidney Injury** Camila Eleuterio Rodrigues,[1] Talita R. Sanches,[1] Rildo A. Volpini,[1] Maria Heloisa M. Shimizu,[1] Patricia Semedo Kuriki,[3] Niels Olsen Saraiva Camara,[2, 3] Antonio C. Seguro,[1] Lucia Andrade.[1] *[1]Nephrology Department, University of São Paulo, São Paulo, SP, Brazil; [2]Immunology Department, University of São Paulo, São Paulo, SP, Brazil; [3]Nephrology Department, UNIFESP, São Paulo, SP, Brazil.*

During bacterial sepsis, TLR4 expression mediates interstitial leukocyte infiltration and tubular injury. TLR4 activation on tubular epithelial cells stimulates the NFkB pathway, leading to the production of cytokines that downregulate AQP2 in kidney medulla. In sepsis, erythropoietin (EPO) can be renoprotective, although the mechanism has yet to be elucidated. We used a cecal ligation and puncture (CLP) model to investigate the role that CERA, an EPO with a unique pharmacologic profile and long half-life, plays in sepsis-related AKI. Wistar rats were randomly divided into 3 groups: control (sham-operated); CLP; and CLP+CERA. CERA (5 µg/kg BW, i.p.) was administered 24 h before CLP. We measured creatinine clearance (Ccr), urinary volume (UV), MAP, serum Ht, and FENa. Immunoblotting for NFkB, TLR4, AQP2 and NKCC2 was performed in kidney tissue. Serum IL-2, IL-1b, IL-6, IFN-γ and TNF-α were measured by multiplex cytokine detection. Data are expressed as mean ± SEM.

| | Control (n = 5) | CLP (n = 7) | CLP+CERA (n = 7) |
|---|---|---|---|
| Ccr (mL/min)/100gBW | 0.73±0.05 | 0.32±0.10[a,b] | 0.70±0.07 |
| MAP (mmHg) | 112±2.5 | 111±11.2 | 115±8.3 |
| UV (mL/24h) | 11.9±5.3 | 4.6±1.5[b] | 16.3±3.3 |
| Ht (%) | 42±1.3 | 41±1.8 | 38±1.8 |
| FENa | 0.26±0.03 | 0.69±0.13[c,d] | 0.30±0.09 |
| NFκB | 100±0.0 | 158±4.8[a,b] | 110±10.0 |
| TLR4 | 100±0.0 | 170±7.9[c,d] | 120±14.7 |
| AQP2 | 96.8±2.6 | 36.7±4.2[e,f] | 101.3±3.0 |
| NKCC2 | 95.7±2.5 | 20.6±3.1[e,f] | 98.7±7.2 |

[a]p<0.01 vs. Control; [b]p<0.01 vs. CLP+CERA; [c]p<0.05 vs. Control; [d]p<0.05 vs. CLP+CERA; [e]p<0.001 vs. Control; [f]p<0.001 vs. CLP+CERA

Levels of all measured cytokines were lower in CLP+CERA rats than in CLP rats. CERA renoprotection is TLR4-dependent and is partly due to inhibition of the inflammatory response.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2950

**Amplification of Nrf2 Signaling by CDDO-Imidazolide Improves Outcomes in Ischemic Acute Kidney Injury in Mice** Manchang Liu,[1] Narsa Machireddy,[1] Thomas W. Kensler,[2] Michael B. Sporn,[3] Lorraine C. Racusen,[1] Sekhar P. Reddy,[1] Hamid Rabb.[1] *[1]Medicine, Environmental Health Sciences and Pathology, Johns Hopkins University, Baltimore, MD; [2]University of Pittsburgh, Pittsburgh, PA; [3]Dartmouth Medical School, Hanover, NH.*

Rationale: Acute kidney injury (AKI) caused by ischemia-reperfusion (IR) is a major clinical problem in both native and transplanted kidney. Oxidative stress induced by IR contributes to the pathogenesis of AKI. We have shown that genetic disruption of Nrf2 transcription factor enhances susceptibility to experimental ischemic AKI in mice (*Kidney Int*:76:277-85, 2009). We hypothesized that amplification of Nrf2 signaling with the synthetic compound CDDO-Imidazolide (CDDO-Im) would confer protection against AKI. Methods: CDDO-Im (30 µmol/kg bw) or vehicle was administered to mice by gavage at 24 h and 3 h prior to and at 24 h after undergoing 30 min bilateral kidney ischemia followed by reperfusion for 72 h. Serum creatinine (SCr) was measured at 0 h, 24 h, 48 h and 72 h post ischemia and the kidney was examined for histology and genes expression. Results: Mice treated with CDDO-Im had significantly improved renal function after ischemia compared to vehicle-treated IRI mice (SCr in mg/dL: CDDO-Im vs. vehicle, 1.36 ± 0.67 vs. 3.82 ± 0.16, p=0.017, n=5). Tubular injury in CDDO-Im treated mice was less severe than vehicle treated group (necrosis: CDDO-Im vs. vehicle, 43% ± 16 vs. 11% ± 6, p=0.06; Regeneration: 30% ± 8 vs. 74% ± 13, p<0.05). CDDO-Im significantly improved survival of mice from AKI (Survival time: CDDO-Im vs. vehicle, 72 h ± 0.0 vs. 57.6 h ± 10.5, p<0.05 by Log-Rank test). To verify that CDDO-Im activated Nrf2-dependent gene transcription in the kidney as the likely mechanism of protection, we analyzed Nrf2 target gene expression at 72 h post ischemia in the kidney of mice treated with CDDO-Im or vehicle. CDDO-Im significantly increased the expression of *Gclc*, *Gpx2*, and *Hmox1*genes compared to vehicle (p<0.01, n=3). Conclusion: These data suggest that amplification of Nrf2 signaling by a synthetic triterpenoid CDDO-Im confers protection against AKI, and exploiting this pathway could provide a novel therapeutic approach to ischemic AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2951**

**Calpastatin Overexpression Decreases Sepsis-Induced Organ Damage** Lara Zafrani,[2] Xuzhen Hu,[1] Zachary Dezman,[1] Hua Zhou,[1] Asada Leelahavanichkul,[1] Joelle Perez,[2] Emmanuel Letavernier,[2] Takayuki Tsuji,[1] Robert A. Star,[1] Laurent Baud,[2] Peter S. Yuen.[1] *[1]NIDDK, NIH, Bethesda, MD; [2]INSERM U702, Hopital Tenon, Paris, France.*

Mortality from sepsis is increased dramatically when complicated by multiple organ failure. Multiple pathophysiologic mechanisms have been proposed for sepsis-induced organ damage: systemic inflammation, necrosis/apoptosis, and lymphocyte apoptosis. Calpains, calcium-activated cysteine proteases, can increase inflammation, apoptosis and necrosis. Therefore, we hypothesized that inhibition of calpains might have a protective effect on multiple organ function in sepsis. To test our hypothesis, we used a model of polymicrobial sepsis induced by cecal ligation and puncture (CLP) in wild type (WT) mice and transgenic (TG) mice overexpressing calpastatin, a calpain-specific inhibitor. In WT mice, sepsis transiently increased calpain activity in kidney, liver and spleen peaking at 6 hr after CLP surgery. CLP increased plasma calpain activity at 6h (0.05 vs 0.21U) in WT mice. All subsequent studies compared CLP in WT vs TG mice. Plasma calpain was significantly lower in TG vs WT mice (0.15 vs 0.21U). Tissue calpain activity in TG mice was significantly diminished at 6 hr vs WT. Serum LDH levels were decreased at 6h in TG mice (2208±600 U/L versus 3600±816 U/L), consistent with a contribution of calpain to cellular death. We then determined whether calpain promotes generalized tissue damage or has differential effects on individual organs. TG mice had lower BUN (101±11 mg/dl vs 75±12 mg/dl in WT and TG mice, respectively) 24 hr after CLP surgery. Injury was also less extensive in TG liver (including hepatocyte glycogen stores, focal necrosis) and spleen (lymphocyte apoptosis, as measured by caspase 3 antibody staining). Furthermore, serum IL-6 was significantly decreased after sepsis in TG mice. These results indicate that calpains are activated early in septic plasma, kidney, liver, and spleen and can enhance the severity of multiorgan failure in sepsis. Therefore, systemic inhibition of calpains is beneficial with respect to sepsis outcome, and plasma calpain may be an early sepsis biomarker.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2952**

**Peptide Bβ15-42 Attenuates Kidney Ischemia/Reperfusion Injury and Improves Renal Allograft Survival** Inga Soerensen,[1] Song Rong,[1] Nathan D. Susnik,[1] Faikah Gueler,[1] Nelli Shushakova,[1] Peter Petzelbauer,[2] Hermann G. Haller,[1] Roland Schmitt.[1] *[1]Department of Nephrology, Hannover Medical School, Hannover, Germany; [2]Medical University Vienna, Austria.*

Renal ischemia/reperfusion (IR) injury is a major cause of acute kidney injury in native kidneys, and it is associated with delayed graft function, increased allograft rejection and reduced graft survival in renal transplantation. In this study we tested the efficacy of the Bβ15-42, a fibrin-derived peptide known to ameliorate cardiac IR injury, in a murine model of IR caused by renal artery clamping and in allogenic kidney transplantation (C57BL/6 into BALB/c). Intravenous Bβ15-42 was administered during surgery at the time of renal reperfusion. Animal survival and renal function were monitored for 7 days in the IR model and for 28 days in the transplantation model. Additional mice from both models were used to study renal pathology employing histology, immunohistochemistry, qPCR and immunoblot. Treatment with Bβ15-42 in the IR injury model resulted in improved 1-week survival and better renal function. Similarly, allograft recipients with intravenous Bβ15-42 treatment showed a significantly better survival during the 28 day follow-up. Recipient survival was further improved if grafts were additionally flushed with a Bβ15-42 containing solution prior to implantation (60% survival vs. 10% in the control group). Renal pathology revealed that IR kidneys and allografts from Bβ15-42 treated mice had less infiltrating leukocytes and lower levels of endothelial activation at day 1 and 6. This was associated with an improved integrity of tubular epithelium. Flow cytometry and mixed lymphocyte reaction assays analyzing recipient splenocytes at day 6 post-transplantation showed that protective effects of Bβ15-42 were associated with reduced alloreactive T cell priming. In summary we demonstrate that Bβ15-42 treatment provides protection from renal IR injury by inhibiting early inflammatory processes such as leukocyte infiltration and endothelial activiation. In the renal transplantation model these early effects lead to an attenuated subsequent alloimmune response and to a significant prolongation of allograft function.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2953**

**Economic Evaluation of Different Treatment Modalities in Acute Kidney Injury** Monique M. Elseviers,[1] Delphine De Smedt,[2] Lieven Annemans.[2] *[1]Univ. Antwerp, Belgium; [2]Ghent Univ., Belgium.*

The SHARF4 study investigated outcome of acute kidney injury (AKI) in 1303 ICU patients. A cost-effectiveness evaluation of conservative treatment (CONS), intermittent (IRRT) and continuous renal replacement therapy (CRRT) was performed.

An area under the curve model was used, whereby patients were simulated using time-to-event data, combined with direct medical costs per day. SHARF4 contains data on hospitalization costs, length of stay and hospital mortality. In addition follow up cost, mortality and SF-36 data were available for 2 years. All analyses used PASW Statistics 18 and were corrected for disease severity using the SHARF score. To calculate the Quality Adjusted Life Years (QALYs), the SF-36 raw scores were converted to a utility value, representing the quality of a life year by using Braziers' algorithm. The outcome is described as the ratio of the incremental costs to the incremental QALYs, also called the incremental cost-effectiveness ratio (ICER). In Belgium, a treatment is considered cost-effective if this ratio does not exceed 30,000 € per QALY.

Analyses indicated that the mortality rate, the cost and the length of stay differed significantly between treatment modalities during hospitalization. During the follow-up period however, no significant difference in mortality rate and cost per day could be observed. Utility values, which improved gradually after hospital discharge, revealed no significant differences, neither during hospitalisation nor during follow up time, between the three treatment options. CONS was associated with a cost of 34,090 EUR and 0.49 QALYs. CRRT was the most expensive therapy with a cost of 51,664 EUR and 0.52 QALYs. Compared to CRRT, IRRT was less expensive (43,711 EUR) however IRRT also led to less QALYs (0.46). The ICER of CRRT vs. IRRT was 131,604 EUR/QALY. The ICER of CRRT vs. CONS was 651,318 EUR/QALY.

In conclusion this study indicated that the additional cost of approximately 7,952 EUR associated with CRRT versus IRRT generates only a minor increase in QALYs of 0.06 making CRRT cost-ineffective compared to IRRT. Additionally, both RRT modalities were more expensive and IRRT even less effective than CONS.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2954**

**Endogenous IL-10 Attenuates Cisplatin Nephrotoxicity: Role of Dendritic Cell IL-10** William Brian Reeves, Raghu K. Tadagavadi. *Division of Nephrology, Penn State College of Medicine, Hershey, PA.*

Sterile inflammation is associated with tissue injury and organ failure. Recent studies indicate that certain endogenous cytokines and immune cells may limit tissue injury by reducing immune-mediated inflammatory responses. Cisplatin is a commonly used anticancer chemotherapeutic agent but causes acute kidney injury and dysfunction. We recently showed that renal dendritic cells attenuate cisplatin-induced kidney injury by reducing inflammation. Here we investigated the effect of endogenous IL-10 and dendritic cell IL-10 in cisplatin-mediated kidney injury. Cisplatin treatment caused increases in renal IL-10R1 expression and STAT3 phosphorylation. IL-10KO mice showed earlier and greater increases in BUN and serum creatinine after cisplatin treatment compared with WT mice, indicating that endogenous IL-10 ameliorates kidney injury in cisplatin nephrotoxicity. Renal expression levels of the chemokines IP-10, KC and MCP-1 were significantly higher in IL-10KO mice than WT mice. Renal infiltration of IFNγ-producing neutrophils was markedly increased in IL-10KO mice compared with WT mice. However, neutralization of IFNγ did not affect cisplatin-induced renal injury. Cisplatin treatment caused an increase in IL-10 expression by renal dendritic cells. The role of dendritic cell-derived IL-10 in cisplatin nephrotoxicity was investigated using a conditional cell ablation approach. Mixed chimeric mice lacking IL-10 in dendritic cells showed moderately greater renal dysfunction than chimeric mice positive for IL-10 in dendritic cells, suggesting that dendritic cell-derived IL-10 provides partial protection from cisplatin nephrotoxicity. These data demonstrate that endogenous IL-10 reduces cisplatin nephrotoxicity and associated inflammation. Moreover, a portion of the protective effect of dendritic cells in cisplatin nephrotoxicity is mediated by IL-10 produced by dendritic cells themselves. In addition, although IFNγ-producing neutrophils are increased in the absence of IL-10, IFNγ production itself does not contribute to cisplatin-induced renal injury.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2955**

**Protection Against Sepsis-Induced Acute Kidney Injury (AKI) Including the Inhibition of p53 by Pifithrin-α (PIF)** Rildo A. Volpini, Ana C. de Bragança, Talita R. Sanches, Maria Heloisa M. Shimizu, Lucia Andrade, Antonio C. Seguro. *Nephrology, School of Medicine, University of São Paulo, São Paulo, São Paulo, Brazil.*

AKI is a clinical problem typically associated with sepsis. Sepsis-induced AKI (SI-AKI) involves ischemic and toxic injury to the renal tubule epithelium. Sepsis is also a transcriptional activator of p53 which controlls the activation of Bax and p21. We investigated the role of PIF, a specific inhibitor of p53, in SI-AKI using a cecal ligation and puncture (CLP) model. Wistar rats were divided into 3 groups: Control (n=5); CLP (n=8); and CLP+PIF (n=8). PIF was given via 3 i.p. injections (2.2 mg/kg): 24h and 1h before CLP and 24h after CLP. We measured blood pressure, inulin clearance (CIn) and renal interstitial area (RIA) at 24h after CLP. Kidney tissue samples were also submitted to immunohistochemistry for p53, Bax, ED1 and PCNA, as well as to immunoblotting for cytoplasmic p21.

| Variable | Control | CLP | CLP+PIF |
|---|---|---|---|
| CIn (mL/min/100g BW) | 0.84±0.04 | 0.46±0.06[a] | 0.67±0.10[c] |
| MAP (mmHg) | 108.0±2.08 | 91.75±3.65[b] | 103.43±3.43[d] |
| p53 (score) | 0.56±0.07 | 0.80±0.05[a] | 0.52±0.06[d] |
| ED1 (+cells/grid field) | 4.52±0.25 | 8.3±0.12[b] | 4.87±0.66[e] |
| PCNA (+cells/grid field) | 1.27±0.27 | 3.08±0.25[a] | 1.79±0.36[c] |
| p21 (% of control) | 98.96±2.11 | 59.67±1.66[b] | 82.77±3.62[e] |
| RIA (%) | 5.44±0.88 | 12.55±0.72[b] | 8.97±0.32[b,e] |

[a] p<0.01 vs. Control; [b] p<0.001 vs. Control; [c] p<0.05 vs. CLP; [d] p<0.01 vs. CLP; [e] p<0.001 vs. CLP. Data are expressed as mean±SEM. Score range from 0 to 4. Grid field, 0.087 μm$^2$.

PIF treatment improved the GFR and MAP; the RIA increased in the CLP group but not in the CLP+PIF group. In the CLP+PIF group, the p53 staining score and the infiltration by macrophages were lower. PIF improved p21 expression, resulting in fewer PCNA+ cells. Our results show that PIF treatment protected the renal function of SI-AKI animals, and that it did so by suppressing p53 and inducing p21, thereby reducing cell proliferation. Our data provide a novel description of the mechanisms by which sepsis causes kidney injury. FAPESP 06/56320-0.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2956**

**Administration of Allogeneic, Human Mesenchymal Stem Cells to Phase I Human Study Subjects Does Not Elicit an Immune Response, but Does in Rats with AKI** Anna Gooch,[1] Ping Zhang,[1] Zhuma Hu,[1] Florian E. Toegel,[2] Christof Westenfelder.[1,3] *[1]Medicine, University of Utah and VA Medical Centers, Salt Lake City, UT; [2]Medicine, Cornell College of Medicine, New York, NY; [3]Physiology, University of Utah, Salt Lake City, UT.*

Human Mesenchymal Stem Cells (hMSC) do not express DR and blood group antigens, and are negative for co-stimulatory CD40, -80 and -86, predicting that infused allogeneic MSC should not elicit an antibody response. However, since hMSC are routinely cultured in FBS, retained bovine antigen may induce an allo-response. To avoid this possibility, we cultured hMSC without animal products for our recently completed Phase I Clinical Trial in which allogeneic hMSC were infused to open heart surgery patients beeing at high risk for post-op AKI (Nat Rev Nephrol 6:179, 2010). The administration of hMSC was safe, and no study subject developed post-op AKI or CKD, while ~ 20% of closely matched case controls experienced post-op AKI and CKD. We obtained identical beneficial outcomes in rats in which AKI was treated with allogeneic rat MSC (Stem Cells Dev 18: 475, 2009). Although unlikely, it is unknown whether hMSC administered in a xenogeneic protocol (hMSC into rats with AKI) are similarly hypoimmunogenic. Accordingly, hMSC from the Phase I Trial were first incubated with study subject sera collected 40 days post cell infusion. Serum exposed hMSCs were then incubated with FITC-labeled anti-human IgG antibodies and FACS analyzed, which failed to detect, in all subjects, antibodies to allogeneic hMSC. Next, rat sera, collected 14 days after AKI treatment with hMSC, were tested in identical fashion, using FITC labeled anti-rat IgG. This demonstrated, in all animals, robust antibody responses to hMSC. In summary, allogeneic hMSCs cultured without animal serum, infused at incremental doses, do not elicit an antibody response in humans, while they do in rats, the latter despite MSCs' known inhibitory effects on antibody production. In conclusion, these data confirm the immunologic safety of clinically used allogeneic hMSC when cultured without animal serum, while xenogeneic uses of these cells appear not advisable.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2957**

**Platelet Inhibition by Clopidogrel Protects Against Renal Ischemia-Reperfusion Injury** Joris J. Roelofs, Gwendoline J. D. Teske, Nike Claessen, Sandrine Florquin. *Department of Pathology, Academic Medical Center, Amsterdam, Netherlands.*

*Background*

Acute Kidney Injury (AKI) is a life-threatening condition, which is often the result of ischemia-reperfusion (I/R) injury. For patients in shock, I/R is associated with a mortality rate of more than 50%. Currently, treatment options remain largely supportive. Two important hallmarks of I/R injury are activation of coagulation and inflammation, which synergistically cause tissue damage.

Although blood platelets are best known for their pivotal role in hemostasis, mounting evidence shows that platelets are active players in various inflammatory processes as well.

*Aim*

The aim of this study was to examine the role of platelets during experimental I/R injury.

*Methods*

C57Bl6/J mice (n=8 per group) were treated with Clopidogrel or saline for 5 days, after which renal ischemia was induced by clamping off both renal arteries for 45 minutes, followed by reperfusion. Mice were killed 24hrs after I/R.

Effectiveness of platelet inhibition was evaluated by standardized tail bleeding. Renal tissue damage was determined on PAS stained paraffin sections. Renal function was determined by measuring BUN and plasma creatinine. Infiltrating neutrophils were counted manually in Ly6-G immunostained sections.

*Results*

Treatment with Clopidogrel resulted in longer bleeding times during standardized tail bleeding experiments, confirming the effectiveness of platelet inhibition by Clopidogrel.

Clopidogrel treatment resulted in significantly lower levels of renal tissue damage, accompanied by significantly lower BUN and creatinine levels than control mice after I/R (BUN: 23.5 ± 4.1 (Clopidogrel) vs. 38.7 ± 5.2 (control) mmol/L – creatinine: 33.4 ± 9.3 (Clopidogrel) vs. 86.8 ± 12.9 (control) µmol/L; $P<0.05$).

Clopidogrel treated mice showed significantly lower numbers of infiltrating neutrophils after I/R than control mice (15.2 ± 4.2 (Clopidogrel) vs. 58.6 ± 11.7 (control) Ly6G positive cells/HPF; $P<0.01$).

***Conclusion***

We conclude that platelet inhibition by Clopidogrel results in effective protection against renal I/R injury. Therefore, platelet inhibition may prove to be an interesting novel therapeutic intervention for patients with ischemia-induced AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2958**

**Abstract Withdrawn**

**SA-PO2959**

**Conditional Knockout of Smad1 Ameliorates Glomerulosclerosis in Progressive Glomerulonephritis** Makoto Araki,[1] Hideharu Abe,[3] Kazuo Torikoshi,[1] Akira Mima,[3] Takeshi Matsubara,[1] Noriyuki Iehara,[1] Atsushi Fukatsu,[1] David J. Salant,[4] Hidenori Arai,[2] Toshio Doi.[3] *[1]Department of Nephrology, Kyoto University, Kyoto, Japan; [2]Department of Human Health Sciences, Kyoto University, Kyoto, Japan; [3]Department of Clinical Biology and Medicine, Tokushima University, Tokushima, Japan; [4]Boston University Medical Center, Boston, MA.*

A link between mesangial cell proliferation and subsequent glomerulosclerosis has been established in progressive glomerular injuries. However, its molecular mechanism has not been fully elucidated. We have reported that Smad1 is a key signaling molecule which regulates the transcription of type IV collagen (Col IV) in mesangial matrix expansion. We also showed that Smad1 is downstream of platelet-derived growth factor (PDGF)-BB in an acute model of glomerulonephritis. In this study we tried to delineate the signaling cascades involved in glomerulosclerosis by using conditional Smad1 knockout mice. C57BL/6 mice (7 weeks old) were injected nephrotoxic serum (NTN). After 21 days, mice were sacrificed, and the kidneys were removed for the analysis. In NTN mice, Col IV and PDGF-BB expression was increased in glomeruli along with induction of Smad1/5 phosphorylation. We then used the Cre-loxP system and Rosa22-Cre ERT2mice to conditionally knock out Smad1 by feeding them with tamoxifen. In Rosa22-CreERT2; Smad1fl/- mice (Smad1CKO), we found 70 % less expression of Smad1 than wild type (WT) mice in the kidneys. PAM-positive areas in Smad1CKO mice were significantly smaller than those in WT mice, which was consistent with the Col IV mRNA expression. We also examined the relationship between Smad1 and PDGF-BB in Smad1 CKO, and found that both mesangial proliferation and sclerosis were significantly reduced along with the decreased expressions of phosphorylated Smad1/5 and PDGF-BB. In conclusion, Smad1 also plays a pivotal role for development of in glomerular sclerosis in progressive glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2960**

**The Protective Effect of N-Acetylcysteine in Rats with Sepsis Submitted to Non-Lesive Mechanical Ventilation** Renata Campos, Maria Heloisa M. Shimizu, Rildo A. Volpini, Ana C. de Bragança, Fernanda D. T. Q. S. Lopes, Clarice R. Olivo, Antonio C. Seguro. *Nephrology, School of Medicine, University of Sao Paulo, São Paulo, São Paulo, Brazil.*

Patients with severe sepsis frequently present acute kidney injury (AKI) and needs mechanical ventilation. Experimental studies showed a protective effect of the antioxidant, N-acetylcysteine (NAC), in AKI models. We evaluated the effects of NAC on renal and pulmonary function in CLP-rats (cecal and ligation puncture) submitted to non-lesive mechanical ventilation (tidal volume: 8 ml/kg, PEEP: 6 $cmH_2O$ and $FiO_2$: 50%) for 2h. Three groups of male Wistar rats were studied 24h post-CLP: control, CLP and CLP+NAC. NAC was given in drinking water (4.8g/L) from 2 days before CLP until 24h after CLP, when we measured inulin clearance (GFR, ml/min/100gBW). In a 2nd set of experiments by using a Flexi-Vent, airway resistance (RAW), pulmonary elastance (HTIS), pulmonary resistance (GTIS) and airway peak pressure (PP) were determined. Data are mean±SEM. GFR was lower in CLP (0.45±0.08, n=12) when compared with 6 control-rats (0.83±0.04, p<0.01). NAC treatment prevented this alteration (0.78±0.12; p<0.01; n=6). As described in table 1, all ventilatory parameters were increased in CLP-rats and were normalized in CLP+NAC animals.

| Groups | RAW ($cmH_2O$.s/ml) | HTIS ($cmH_2O$/ml) | GTIS ($cmH_2O$/ml) | PP ($cmH_2O$) |
|---|---|---|---|---|
| Control (n=6) | 0.014±0.001 | 1.73±0.20 | 0.39±0.02 | 9.4±0.20 |
| CLP (n=6) | 0.028±0.001 * | 2.71±0.14 ♦∞ | 0.53±0.02 † | 12.1±0.20 † |
| CLP+NAC (n=6) | 0.015±0.001 | 2.08±0.15 | 0.40±0.01 | 9.1±0.18 |

*p<0.001, †p<0.01 vs. other groups; ∞p<0.01 vs. control, ♦p<0.05 vs. CLP+NAC.

The beneficial effect of NAC on respiratory mechanics was associated with a decrease in the perivascular edema evaluated by lung histology (CLP+NAC=12,4±0.7 µm; CLP=18.9±1.71 µm, p<0.001). Survival analysis followed every 12h after CLP for a total of 72h showed that NAC improved survival in septic rats (66% vs. 24%, p<0.01). In conclusion, NAC has a protective effect in respiratory mechanics and renal function of septic rats and reduces mortality.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2961**

**Fluid Balance, Diuretic Use, and Mortality in Critically Ill Patients with Acute Kidney Injury: A Secondary Analysis of the Fluid and Catheter Treatment Trial** Morgan E. Grams,[1] Michelle M. Estrella,[1] Josef Coresh,[1] Roy G. Brower,[1] Kathleen D. Liu.[2] *[1]Johns Hopkins University; [2]University of California, San Francisco.*

We evaluated whether fluid balance or diuretic use is associated with mortality in critically ill patients with acute kidney injury (AKI). Using data from the Fluid and Catheter Treatment Trial (FACTT, NEJM 2006; 354: 2564-75), a multicenter, randomized, controlled trial evaluating a conservative vs. liberal fluid management strategy in 1000 patients with acute lung injury, we evaluated the association between fluid management, fluid balance, and diuretic use with 90-day mortality in patients developing AKI, defined as a 50% or 0.3 mg/dl increase in serum creatinine by study day 4. Of the 300 patients with AKI, 244 patients survived 7 days of protocolized therapy and were included in the analysis. Baseline characteristics were similar between the 107 patients in the liberal group and the 137 patients in the conservative group. Positive fluid balance was significantly associated with mortality in both the crude analysis and that adjusted for demographics, severity of illness, day of AKI, diuretic dose, and randomization group (adjusted odds ratio [OR] 1.79 per L/day, 95% CI: 1.38-2.32, p<0.001). Higher furosemide doses had a protective effect on mortality in the unadjusted analysis (OR 0.45 per 100 mg/day, 95% CI: 0.28-0.75, p=0.002) but no significant effect after adjustment for fluid balance. There was no threshold dose above which mortality increased. The fluid management strategy had no significant effect on mortality by either analysis. Stratification by fluid management and AKI severity, and repeat analysis including the full cohort of patients with AKI (N = 300), showed similar results. We conclude that fluid balance is strongly associated with mortality in AKI, independent of liberal or conservative fluid management. Diuretic therapy, even in relatively high doses, had no adverse impact on short-term patient survival in patients with AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2962**

**Acute on Chronic Renal Failure in Rats Is Treatment Resistant to Erythropoietin but Effectively Reversed by Mesenchymal Stem Cells: Relevance to Clinical Trials** Christof Westenfelder,[1,2] Anna Gooch,[1] Ping Zhang,[1] Zhuma Hu,[1] Leanne Swenson,[1] Jon D. Ahlstrom,[1] Florian E. Toegel.[3] *[1]Medicine, University of Utah and VA Medical Centers, Salt Lake City, UT; [2]Physiology, University of Utah, Salt Lake City, UT; [3]Medicine, Cornell College of Medicine, New York, NY.*

A vast variety of interventions protect kidney function and accelerate repair in healthy animals with normal renal function. However, EPO, IGF-I or ANP and others, all renoprotective in healthy animals, have not been helpful in patients with AKI. We believe that this difference in outcomes is due to the fact (1) that the efficacy of such interventions was not tested in experimental models with important co-morbidities, such as underlying CKD, a known risk factor for clinical AKI; and (2) that pharmacological agents can only temporarily target a limited number of the complex components of AKI's pathophysiology & repair mechanisms, while Mesenchymal Stem Cells (MSC) are able to beneficially affect the entire spectrum of involved processes. These issues were tested in rats with stable CKD (5/6th NX; decreased function, proteinuria, hypertension). AKI (pedicle clamp) was superimposed on CKD, and rats were either treated with human EPO (300 U/kg sc x 3 days, renoprotective in normal rats with AKI) or with 2x10E6/kg of allogeneic MSCs infused post reflow (renoprotective in normal rats with AKI). Controls received vehicle. EPO was ineffective while MSC infusion significantly protected renal function and accelerated repair. Preliminary efficacy data from our recently completed Phase I Trial show that MSC treatment of open heart surgery patients with underlying CKD prevented post-op AKI and subsequent CKD, while ~20% of closely matched case controls developed AKI & progressively lost renal function. Conclusion: these data demonstrate that pre-clinical studies with novel interventions should be first investigated in clinically relevant animal models, e.g., with underlying CKD, before they are tested clinically. This has generally not been done. In addition, MSC-based therapies appear to be superior to pharmacological interventions both in animals and study subjects with underlying CKD.

Disclosure of Financial Relationships: Consultancy: Allocure, Inc.

**SA-PO2963**

**The Effect of Endogenous (Physiologic) and Exogenous (Pharmacologic) Angiotensin 1-7 (Ang1-7) on Ischemia/Reperfusion-Induced Acute Kidney Injury (I/R-AKI), and Its Associated Adaptive Responses** Arnaldo F. Lopez-Ruiz, Kiran B. Chandrashekar, Ruisheng Liu, Luis A. Juncos. *Medicine-Nephrology, University of Mississippi Medical Center, Jackson, MS.*

Ang1-7 has vasodilator and cytoprotective effects, that protect against chronic renal injury. However, its effect on I/R-AKI is unknown. We determined the effects of endogenous and exogenous Ang1-7 on I/R-AKI, and its resulting renal inflammation, apoptosis and expression of cytoprotective factors. Male rats were treated daily for 3 days with vehicle, A779 (Ang1-7 antagonist; 1120 Ug/kg ip) or Ang1-7 (560 Ug/kg ip), and then subjected to 40 minutes of I/R. They were followed for 3 days post I/R during which time they continued to receive their treatment. We determined the extent of AKI by measuring renal function (plasma creatinine) and injury (urine NGAL). In addition, we measured renal inflammation (TNFα) and apoptosis (cytochrome-C), as well as adaptive cytoprotective factors, heme oxygenase-1 (HO-1), vascular endothelial growth factor (VEGF), and prostacyclin (PGI2).

| | Creat mg/dl | NGAL UI/ml | TNFα pg/ug | Cyt-C ng/ug | HO-1 ng/ug | VEGF pg/ug | PGI2 ng/ml |
|---|---|---|---|---|---|---|---|
| SHAM | 0.6±0.03 | 120±5 | 0.4±0.1 | 0.2±0.02 | 0.1±0.1 | 33±1.2 | 2.3± 0.1 |
| I/R-Veh | 1.9±0.1* | 1730±3* | 4.9±0.3* | 4.2±0.4* | 0.7±0.03* | 8.7±0.6* | 0.7± 0.07 |
| I/R-A779 | 3.3±0.1*# | 2265±141*# | 4.6±0.4* | 4.2±0.03* | 0.65±0.05* | 10.7±0.7* | 0.6±0.04 |
| I/R-Ang1-7 | 1.2±0.04*# | 629±42*# | 2.4±0.2*# | 2.8±0.1*# | 1.1±0.1*# | 18±0.6*# | 3.5±0.06 |

Data: Mean±SEM, *p < 0.05 vs. Sham; #p< 0.05 vs. I/R-AKI; (n=6/group)

Blocking endogenous Ang1-7 exacerbated I/R-AKI (creatinine and NGAL increases were augmented), without altering the expression of the renal inflammatory or cytoprotective factors. Whereas, administering exogenous Ang1-7 blunted I/R-AKI, reduced renal inflammation and apoptosis while increasing HO-1, PGI2 and VEGF. Our results suggest that endogenous and exogenous Ang1-7 reduce I/R-AKI; however, the predominant mechanisms for the physiologic vs pharmacologic Ang1-7-induced protection may differ.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2964**

**Isoflurane Anesthesia Activates Intestinal Sphingosine Kinase To Protect Against Renal Ischemia Reperfusion Induced Liver and Intestine Injury** Minjae Kim,[1] Sang Won Park,[1] Jinu Kim,[1] Mihwa Kim,[1] Vivette D. D'Agati,[2] H. Thomas Lee.[1] *[1]Anesthesiology, Columbia University, New York, NY; [2]Pathology, Columbia University, New York, NY.*

Acute kidney injury (AKI) often leads to multi-organ dysfunction and systemic inflammation. We recently showed that AKI led to hepatic dysfunction mediated by cytokine release from the small intestine. We also previously showed that the volatile anesthetic isoflurane (Iso) protects against renal ischemia-reperfusion injury (IRI) via sphingosine kinase-1 (SK1) activation. We aimed to determine whether Iso protects against renal IRI-induced liver and intestinal injury and the mechanisms involved in this protection. After pentobarbital (PB) anesthesia and right nephrectomy, mice were subjected to 30 min of left renal ischemia followed by exposure to 4 h of equi-anesthetic doses of PB or Iso and then allowed to awaken from anesthesia. Mice exposed to PB after renal IRI developed severe AKI (Cr=2.39±0.05, N=10, p<0.01) and hepatic injury (ALT=238±18, N=10, p<0.01) compared with sham mice (Cr=0.47±0.03, N=6; ALT=61±8, N=4) 24 h after injury. The rise in ALT was associated with focused peri-portal hepatocyte necrosis, vacuolization, neutrophil infiltration, and pro-inflammatory mRNA upregulation. Iso exposure protected against AKI (Cr=1.61±0.17, N=8, p<0.01) and reduced hepatic injury (ALT=140±16, N=7, p<0.05) compared to PB exposure. Mechanistically, Iso induced liver protection via induction of small intestinal crypt SK1 as SK1 mRNA, protein expression, and enzymatic activity all increased. Intestinal sphingosine-1-phosphate (S1P) levels also increased with Iso exposure. We confirmed the importance of SK1 as mice treated with an SK inhibitor (SKI-II) or mice deficient in SK1 enzyme were not protected against hepatic and intestinal dysfunction with Iso exposure. Taken together, our model of renal IRI caused rapid hepatic and intestinal injury in mice. We show that Iso protects against this renal IRI-induced injury via upregulation of SK1/S1P in small intestinal crypts. Modulation of the SK1/S1P pathway may have important therapeutic implications to reduce extra-renal complications arising from AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2965**

**Serum Cystatin C as a Biomarker of Sepsis-Induced Acute Kidney Injury in Mice** Asada Leelahavanichkul, Hua Zhou, Christoph Eisner, Lingli Li, Xuzhen Hu, Takayuki Tsuji, Kent Doi, Jurgen B. Schnermann, Robert A. Star, Peter S. Yuen. *NIDDK, NIH, Bethesda, MD.*

Mortality from sepsis is increased dramatically when complicated by acute kidney injury (AKI); hence, early detection of AKI is important in septic patients. Unfortunately, serum creatinine (Scr) has several limitations that delay recognition of AKI. We sought to explore the usefulness of serum cystatin C (CysC), which has been proposed as an alternative biomarker. We measured CysC, Scr, and BUN and compared them to a gold standard FITC-inulin GFR in CD-1 mice before and 3, 6, 12, and 18 hr after cecal ligation and puncture (CLP) induced sepsis. Bilateral nephrectomy (BN) and bilateral ureteral obstruction (BUO) were also used to determine the renal contribution to CysC handling, alone or in combination with CLP. CysC peaked more rapidly (12 hrs) than Scr or BUN after CLP, BN, or BUO; Cys C also correlated better than Scr or BUN with inulin GFR at 12 or 18 hrs after sepsis. Additionally, CysC was higher in BN model than BUO, confirming renal clearance by the nonexcreting kidney. Both CysC and Scr decreased after CLP/BN and CLP/BUO (vs CLP with 2 normal kidneys) despite increases in inflammatory and non-renal organ damage markers. This suggests decreased production, increased clearance and/or increase in volume of distribution (Vd). We measured CysC clearance and Vd following injection of exogenous CysC at 12 h after CLP (when CysC was otherwise stable). We found that CysC clearance was increased in sepsis with BN with a similar Vd, suggesting that sepsis increases extrarenal clearance. We conclude that CysC was a better overall kidney injury biomarker than Scr or BUN for sepsis AKI but still not an ideal marker because sepsis increases non-renal clearance by an unknown mechanism. Direct injury markers might be a more sensitive approach to the early detection of early sepsis-induced AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2966**

**Combining Early and Late Remote Ischemic Preconditioning Strategies Confers Greater Tissue Protection Than Early Remote Ischemic Preconditioning Alone** Kieran Mccafferty, Conor Byrne, Julius Edward Kieswich, Martin J. Raftery, Magdi Yaqoob. *William Harvery Research Institute, Queen Mary university London, London, United Kingdom.*

Remote ischemic preconditioning (RIPC) is a process by which brief sub lethal episodes of ischaemia and reperfusion to an organ render distant organs resistant to subsequent lethal injury. RIPC confers 2 distinct phases of protection, the early phase (0-4h) and a late phase (24-72h). It is unknown whether additional protection can be achieved by summating early and late RIPC strategies. We hypothesized that combining these 2 strategies could reduce AKI following renal ischemia reperfusion injury.

Methods

Male Wistar rats were divided into 2 groups: Group 1 underwent a RIPC protocol consisting of 3 cycles of 5 minutes ischemia and reperfusion to the left leg using occlusion and reperfusion of the femoral artery. Group 2 underwent a sham RIPC protocol. 48 hours later both groups underwent a RIPC protocol as before, immediately followed by a right nephrectomy and 45 minute left renal artery occlusion to induce severe AKI. After a further 48 hours the animals were sacrificed and blood was taken for analysis.

Results:

| | Group 1 (n=8) | Group 2 (n=7) | p value |
|---|---|---|---|
| Creatinine (umol/l) | 243 (185-379) | 408 (366-549) | 0.05 |
| Urea (mmol/l) | 43.8 (36.7-51.2) | 63.9 (52.8-84.3) | 0.02 |
| Phosphate (mmol/l) | 3 (2.93-3.47 | 5.15 (4.44-6.07) | 0.01 |
| Base deficit (mEq/l) | 2.5 (0.05-4.25) | 4.5 (3.3-10.3) | 0.08 |

All values given as median (25th-75th Centile), with p values calculated using the Mann-Whitney test

The summation preconditioned (Group 1) had significantly better renal function than group 2 which had received only early RIPC.

Group 1 had 40% a lower median plasma creatinine, a 30% lower plasma urea, a 40% lower plasma phosphate and a 44% lower base deficit.

Conclusions

These results show for the first time that summation RIPC provides additional tissue protection when compared to early RIPC phase alone. Summation preconditioning may be an advantageous strategy for providing additional tissue protection in future clinical trials of RIPC, particularly applicable in the arena living kidney donation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2967**

**P2X$_7$ Receptors Mediate a Deleterious Renal Epithelial-Fibroblast Crosstalk** Shougang Zhuang. *Department of Medicine, Rhode Island Hospital and Warren Alpert Medical School of Brown University, Providence, RI.*

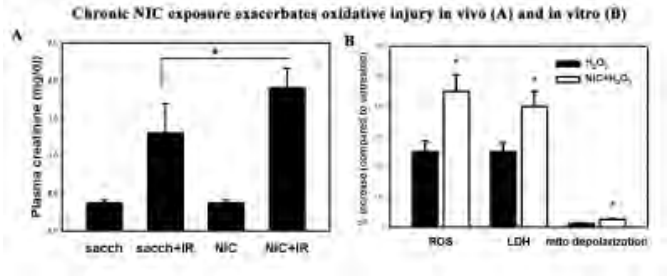
Peritubular fibroblasts in the kidney are the major erythropoietin (EPO) producing cells and also contribute to renal repair following acute kidney injury (AKI). Although a reduced number of renal fibroblasts is frequently observed in the interstitium adjacent to damaged tubular epithelium in the early phase of AKI, the underlying mechanism remains unknown. In this study, we tested the hypothesis that damaged renal epithelial cells directly induce renal interstitial fibroblast death via release of intracellular ATP and activation of purinergic signaling. Exposure of a cultured rat renal interstitial fibroblast cell line (NRK-49F) to necrotic renal proximal tubular cell (RPTC) lysate or supernatant induced NRK-49F cell death by apoptosis and necrosis. A high level of ATP was detected in necrotic RPTC supernatant. Depletion of ATP with apyrase or inhibition of P2X purinergic receptor with PPADS blocked the deleterious effect of necrotic RPTC supernatant. P2X$_7$, an ATP-sensitive purinergic receptor, was not detected in cultured NRK-49F cells but was inducible by necrotic RPTC supernatant. Treatment with A438079, a highly selective P2X$_7$ receptor inhibitor, or knockdown of P2X$_7$ receptor with siRNA, also diminished renal fibroblast death induced by necrotic RPTC supernatant. Conversely, over-expression of P2X$_7$ receptor potentiated this response. Collectively, these findings provide strong evidence that damaged renal epithelial cells can directly induce renal interstitial fibroblast death. The findings further suggest that this deleterious crosstalk arises via ATP action on the P2X$_7$ receptor.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2968**

**Chronic Nicotine Exposure Exacerbates Acute Renal Ischemic Injury** Istvan Arany,[1] Samira C. Grifoni,[2] Jeb S. Clark,[1] Luis A. Juncos.[2] *[1]Pediatrics, University of Mississippi Medical Center, Jackson, MS; [2]Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS.*

The association between long-term smoking and renal dysfunction has only been recently recognized. Smoking-mediated damage of the renal proximal tubules contributes to tubulointerstitial injury and progression to chronic renal disease but it may also exacerbate acute kidney injury (AKI). Nicotine (NIC) is believed to link smoking to injury of the kidney. Here, we tested the hypothesis whether chronic NIC exposure exacerbates renal ischemia/reperfusion (IR) injury in mice. Accordingly, 10-14-week-old male C57BL/6J mice were administered by NIC (200 mg/ml in 2% saccharine) in their drinking water for 3-4 weeks resulting in high levels of cotinine in the serum and the kidneys of mice that were comparable to that found in chronic smokers. The control group received 2% saccharine. Acute renal ischemic injury (15 minutes of warm ischemia followed by 24 hours reperfusion) was significantly higher in the NIC group compared to the controls as revealed by serum creatinine and renal KIM-1 expression. Also, the extent of oxidative stress (malondialdehyde content of the kidney and renal HO-1 expression) was significantly higher in the NIC group. *In vitro* experiments on cultured mouse renal proximal tubule cells (TKPTS) recapitulated this phenomenon: chronic (24 hours) exposure to NIC exacerbated hydrogen peroxide ($H_2O_2$)-induced reactive oxygen species (ROS) production, LDH release, mitochondrial depolarization, cell permeability and collapse of the actin cytoskeleton. Pre-treatment with the antioxidant N-acetyl-cysteine (NAC) attenuated NIC-induced injury *in vitro*. These results suggest that smoking increases oxidative stress in the kidney and thus, smokers are likely exhibit more severe response to acute kidney injury than non-smokers.




Disclosure of Financial Relationships: nothing to disclose

**SA-PO2969**

**Increased Renal CYP4A Expression and 20-HETE Production Promotes Resistance to Renal Rschemia-Reperfusion Injury in Rats** Brodie Marthaler,[1] Sarah M. White,[1] Scott K. Van Why,[2] Michael R. Garrett,[1] Richard J. Roman,[3] Kevin R. Regner.[1] *[1]Nephrology, Medical College of Wisconsin; [2]Pediatrics, Medical College of Wisconsin; [3]Pharmacology and Toxicology, University of Mississippi Medical Center.*

20-Hydroxyeicosatetraenoic acid (20-HETE) is a product of cytochrome P450 4A (CYP4A) mediated metabolism of arachidonic acid. We previously demonstrated that analogues of 20-HETE mitigate renal ischemia-reperfusion injury (IRI) in rats. However, the role of endogenous 20-HETE in renal IRI has not been well characterized. In the present study, we first determined whether IRI alters endogenous renal tissue levels of 20-HETE in Sprague-Dawley rats. Compared with sham-operated controls, renal 20-HETE levels were significantly increased following 30 min of renal ischemia. Renal 20-HETE returned to control levels at 24 hrs reperfusion. To determine whether endogenous 20-HETE plays a protective role following renal ischemia, we compared the susceptibility of SS.5LEW 4A+ and SS.5LEW 4A- congenic rats to renal IRI. These congenic strains were previously generated by introgression of a region on rat chromosome 5 that includes (4A+) or excludes (4A-) the CYP4A genes from the Lewis rat to the Dahl S (S) genetic background. The renal expression of CYP4A protein and the production of 20-HETE is greater in the 4A+ strain compared with the S and 4A- strains. Baseline renal function was similar in the S, 4A+ and 4A- strains. Following 30 min bilateral renal ischemia and 24 hrs reperfusion, plasma creatinines in the S, 4A- and 4A+ rats were $3.8 \pm 0.4$, $4.1 \pm 0.4$, and $1.4 \pm 0.5$ mg/dl, respectively. Renal 20-HETE levels were significantly higher in the 4A+ compared to the 4A- rats following ischemia. Administration of the 20-HETE antagonist, 6,15-20-HEDE, abolished the resistance to renal IRI in the 4A+ rats (plasma creatinine at 24 hrs reperfusion = $4.0 \pm 0.5$ mg/dl). Taken together, these data indicate that over expression of CYP4A protein and increased renal 20-HETE production confers resistance to renal IRI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2970**

**Pharmaceutical Induction of Heme Oxygenase 1 by Retinoic Acid Ameliorates Ischemic Acute Kidney Injury** Li-Wen Lai,[1] Kim-Chong Yong,[1] Serrine S. Lau,[1] Terrence J. Monks,[1] Yeong-Hau Howard Lien.[2] *[1]Pharmacology and Toxicology, University of Arizona, Tucson, AZ; [2]Medicine, Arizona Kidney Disease and Hypertension Center & University of Arizona, Tucson, AZ.*

Heme oxygenase 1 (HO-1) is the major stress protein in kidney and controlled up-regulation of HO-1 has been shown to exert anti-oxidant and anti-inflammatory activities and renoprotection against acute kidney injury (AKI). All-trans retinoic acid (ATRA), an active vitamin A metabolite, has multiple biological functions including cell growth and differentiation. We investigated protective effects of ATRA against ischemic AKI in mice. A single dose of ATRA (1mg/kg, i.p.) given 3-6 h prior to ischemia reperfusion injury (IRI) provided renoprotection with improved renal function (BUN: 19±1 vs. IRI: 121±19; serum Cr: 0.25±0.05 vs. IRI: 0.99±0.17, $p<0.01$), reduced tubular necrosis scores, neutrophil infiltration, and renal proinflammatory cytokine TNF-$\alpha$ mRNA abundance at 24 h of reperfusion. Importantly, ATRA treatment alone transiently induced HO-1 (3-4 folds increase in mRNA in 1-3 hrs). Moreover, a HO inhibitor, stannous mesoporphyrin, administered 90 min and 30 min before ischemia, completely abolished ATRA-mediated renoprotection, suggesting that HO-1 is the key effector of ATRA-mediated renoprotection. In contrast to the modest ATRA mediated HO-1 induction, IRI-mediated HO-1 induction is fulminant: 14-fold, 94-fold, and 4-fold at 1, 4, and 24 hr of reperfusion, respectively. ATRA pretreatment reduced IRI-mediated HO-1 induction by 70%, 55%, and 100% at 1, 4, and 24 hr of IRI, respectively. The expression of HO-2, a constitutively expressed HO was unchanged by ATRA, IRI or the combination treatment. Dual immunofluorescence

staining studies revealed that the induction of HO-1 occurs mainly in the proximal tubular cells. In conclusion, we have discovered ATRA as a novel pharmaceutical inducer for HO-1, demonstrated the potent efficacy of ATRA in protection against ischemic AKI at dosages commonly used in humans, and identified HO-1 as the key underlying effector of such protection. ATRA could be developed into a preventive agent for ischemic AKI and other AKI such as contrast nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2971

**Pretreatment with Sodium Cromoglicate Attenuates Renal Ischemia/ Reperfusion in Rats** Felipe Caetano Beraldo, Marilda Mazzali. *Division of Nephrology, State University of Campinas UNICAMP, Campinas, Sao Paulo, Brazil.*

Mast cells [MC] are rarely found in normal kidney. However, studies have shown the increase of these cells in inflammatory models of acute and chronic kidney injury, as well as in exta-renal models of ischemia reperfusion [I/R]. Blockade of MC degranulation was associated with regression of injury in different models. The aim of this study was to investigate the effects of pre treatment with sodium cromoglicate [SC] in inflammation and cell proliferation in a model of renal I/R in rats. Methods: 36 male Wistar rats were assigned to 9 groups with 4 animals each: 4 control groups, 4 treated groups and Sham. I/R was obtained by bilateral renal arterial clamping during 45 minutes. Treated animals received 160 mg/kg of SC, IP, immediately after reperfusion. Animals were sacrificed at days 1, 3, 5 and 7 after reperfusion. Renal function was measured by serum creatinine. Acute tubular necrosis [ATN] was analyzed by PAS staining. The proliferative response and inflammatory infiltration were quantified by immunostaining. Results: No difference in renal function was observed between treated and untreated animals. SC treated animals showed less severe ATN, with a marked reduction in tissue lesion at day 5 [3.5±0.6 vs 1.0±0.7 percent of denuded tubules, C5 vs T5, $p<0.05$]. Inflammatory cells followed the same pattern, with a significant lower macrophage infiltration after 1 and 5 days after I/R in treated animals [55.8±1.2 vs 26.3±0.9 cells/mm2, C1 vs T1; 121.87±5.9 vs 44.37±1.6 cells/mm2, C5 vs T5, $p<0.05$]. T cell infiltration was significantly reduced in early time points [D1] in treated animals [53.37±0.15 vs 26.87±0.5 cells/mm2, C1 vs T1, $p<0.05$]. The proliferative response to I/R injury was also more intense in non-treated groups, early after reperfusion [326.87±4.0 vs 216.25±2.4 cells/mm2, C1 vs T1, $p<0.05$]. Conclusions: The administration of a mast cell degranulation inhibitor, SC, prior to reperfusion attenuates ATN, inflammation and cell proliferation in an experimental model of renal ischemia reperfusion, suggesting that mast cell proteases can have a pivotal role in inflammatory cells attraction and maintenance of interstitial balance after acute ischemic injury.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2972

**Aldosterone Mediates Renal Dysfunction and Arteriolopathy in Cyclosporine (CsA) Nephropathy** Daniela Trujillo-Silva, Victoria Ramirez, Cristinoc Cruz, Rosalba Pérez-Villalva, Norma Bobadilla. *Molecular Physiology Unit, Instituto de Investigaciones Biomedicas, UNAM and Instituto Nacional de Ciencias Médicas y Nutrición Salvado Zubiran, Mexico, Mexico.*

We previously shown that aldosterone (Aldo) blockade prevented renal dysfunction and reduced structural injury in the model of chronic CsA nephrotoxicity, under low sodium diet (LSD) used to activate RAAS. To dissect the specific contribution of Aldo in inducing renal injury, rats treated with CsA on normal salt diet, received Aldo by infusion pumps to reach those levels found in rats feeding with LSD.

Twenty male Wistar rats were divided in: group receiving vehicle (V), rats receiving Aldo (175 µg/dia), rats treated with CsA (10 mg/Kg) and group simultaneously treated with CsA+Aldo for 15 days. Mean arterial pressure, glomerular filtration rate (GFR) and renal blood flow (RBF) were measured, and the percentage of arteriolopathy (Art) was quantified. In addition, Kim1 and TGFβ mRNA levels were evaluated and urinary $H_2O_2$ was assesed.

Results

| Group | Serum Aldo pg/ml | RBF ml/min | GFR ml/min | Urinary H2O2 nM/24h | Art % | Kim1/18s | TGFβ/18s |
|---|---|---|---|---|---|---|---|
| V | 332 ± 44 | 7.2 ± 0.7 | 2.2 ± 0.3 | 0.8 ± 0.2 | 5 ± 1 | 1 ± 0.1 | 1 ± 0.1 |
| Aldo | 4513 ± 254 *† | 4.8 ± 0.1*† | 1.0 ± 0.1*† | 1.3 ± 0.2 | 7 ± 2 | 1.3 ± 0.2 | 1.9 ± 0.2* |
| CsA | 1442 ± 121 | 6.9 ± 0.4 | 2 ± 0.2 | 3.1 ± 0.7*Ψ | 15 ± 1* | 4.4 ± 0.2*†Ψ | 1.5 ± 0.1 |
| CsA+ Aldo | 6175 ± 69*† | 3.7 ± 0.5*† | 0.8 ± 0.2*† | 4.6 ± 0.5*Ψ | 23 ± 1*† | 18 ± 4.3*†Ψ | 3.6 ± 0.6*†Ψ |

*$p<0.05$ vs. V, † $p<0.05$ vs. CsA Ψ $p<0.05$ vs. Aldo

Aldo infusion was associated with a significant reduction of RBF and GFR, together with an elevation of TGFβ mRNA levels. Low dose of CsA induced Art, an effect that was related with an increment in oxidative stress, as well as in TGFβ and Kim1. Aldo potentiated CsA toxicity, thus a greater reduction of GFR, together with enhanced arteriolopathy and TGFβ and Kim1 mRNA levels, were observed.

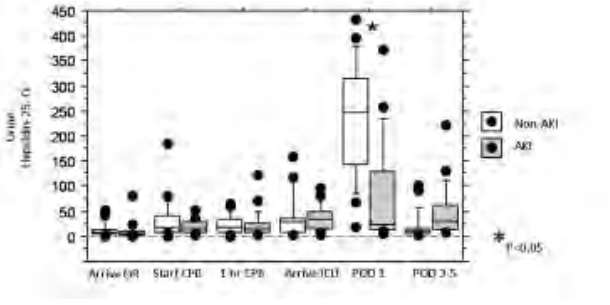
These results show that aldosterone contributes to renal dysfunction and structural injury in CsA nephrotoxicity, in absence of elevated angiotensin II, through increasing oxidative stress and TGFβ up-regulation.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2973

**Is Hepcidin-25 Protecting from Acute Kidney Injury Following Cardiopulmonary Bypass?** Martina Reslerova,[2] Julie Ho,[1,2] Claudio Rigatto.[2] *[1]Manitoba Centre for Proteomics and Systems Biology, University of Manitoba, Winnipeg, MB, Canada; [2]Section of Nephrology, University of Manitoba, Winnipeg, MB, Canada.*

Acute kidney injury (AKI) is a serious complication of cardiopulmonary bypass (CPB) resulting in increased short- and long-term morbidity and mortality. Urinary hepcidin-25 has been identified and shown to be elevated non-AKI versus AKI patients undergoing CPB using semi-quantitative mass spectrometry techniques (SELDI TOF-MS). The goal of this study was to validate these findings utilizing quantitative ELISA. In this prospective cohort, 250 CPB patients underwent a nested, case-controlled analysis of urinary hepcidin-25. Urines from Patients with (n=22) and without (n=22) AKI were collected at six time points: pre-operative, start of CPB, 1 hour on CPB, arrival to ICU, post-operative day 1 and days 3-5. Hepcidin-25 was found to be significantly elevated in non-AKI versus AKI patients at post-operative day 1,confirming the previously reported semi-quantitative mass spectrometry findings.




We postulate that hepcidin-25 may be protective in renal ischemia-reperfusion injury via iron sequestration mechanisms and that further studies are warranted to determine its overall utility as a biomarker in AKI.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2974

**Pre-Conditional but Not Post-Conditional HIF Activation Protects Against Acute Ischemic Kidney Injury** Zhendi Wang, Gunnar Schley, Carsten Willam, Kai-Uwe Eckardt, Wanja M. Bernhardt. *Department of Nephrology and Hypertension, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany.*

**Background.** Inhibition of the Prolyl Hydroxylase Domain-Proteins (PHD) 6 hours prior to renal injury has been shown to protect the kidney via activation of hypoxia-inducible transcription factors (HIF) and HIF target genes. It remains unclear whether HIF activation at the time of injury (post-conditioning) also affects renal function.

**Methods.** A model of warm renal ischemia/reperfusion injury (IRI) was used in Sprague-Dawley rats. Right nephrectomy was performed before clamping the left renal artery for 40 minutes. Groups were treated with either 25mg/kg or 50mg/kg b.w. of a PHD-Inhibitor (PHD-I) or vehicle (Veh) 6 hours prior to ischemia (pre-conditioning) or with 25mg/kg of the PHD-I at the beginning of reperfusion. Serum creatinine were measured before, 24 hours and 72 hours after initiation of IRI. The rats were sacrificed at 72 hours and left kidneys were processed for histology and mRNA expression studies. mRNA levels of HIF target genes, including erythropoietin (EPO) were determined using real-time-PCR.

**Results.** Pre-conditional activation of HIF by the PHD-I significantly improved serum creatinine levels in comparison to Veh ($p<0.05$), with 25mg and 50mg/kg b.w. having the same effect, while no differences in renal function were found between Veh and PHD-I application just prior to reperfusion.

EPO was found to be strongly upregulated after PHD-I treatment compared to Veh with significantly higher expression in response to 50 mg /kg compared to 25 mg/kg group.

**Conclusion.** In order to achieve renal protection against ischemic injury the PHD-I had to be applied before the ischemic insult and was ineffective when given just prior to reperfusion. These findings are in line with the concept that PHD-I exert their protective effects through accumulation of HIF target gene products, with time requirements for increased transcription and translation of HIF dependent genes. Moreover increased expression of EPO at a higher dosage of the PHD-I did not lead to better preservation of kidney function, suggesting that PHD-I mediated protection is not proportional to EPO gene expression.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2975**

**Over-Expression of Protein Kinase G Reduced Ischemia/Reperfusion Induced Renal Injury** Yanzhang Li, Shuxia Wang. *Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY.*

Protein Kinase G (PKG) is a cGMP-dependent protein kinase that can be activated by cGMP. There are two PKG genes: PKG-I and PKG-II. Alternatively spliced exons of PKG-I results in two specific PKG-I isoforms: PKG-1α and PKG-1β. Recently, it has been shown that PKG-1α attenuates necrosis and apoptosis in cardiomyocytes following ischemia / reoxygenation. However, whether PKG has protective role in ischemia/reperfusion (I/R) induced kidney injury is unknown. Therefore, we performed the following studies to test whether PKG can protect I/R induce kidney injury by using PKG transgenic mice that are generated by our lab. Overexpression of the constitutively active PKG-I was achieved in PKG transgenic mice. The kidney I/R injury was induced in PKG transgenic mice and wild type control mice by removing the right kidney and clamping the pedicle of left kidney for 45min. After 24 h of reperfusion, the mice were sacrificed and left kidneys were harvested for further analysis. Our data demonstrated that the expression of PKG-Iα in the kidney was down-regulated in control mice after I/R injury as compared to sham group. Kidney functional and histological analysis showed that I/R induced kidney injury was significantly attenuated in PKG transgenic mice as compared to control mice. Together, our studies suggest that increasing PKG activity protects ischemia/reperfusion induced kidney injury.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2976**

**The Sustained Renal NFκB Activation Contributes to Acquired Resistance in Cisplatin-Induced Acute Kidney Injury** Jinghui Luo,[1] Hideo Yasuda,[1] Tomoyuki Fujikura,[1] Akihiko Kato,[2] Yoshihide Fujigaki,[1] Akira Hishida.[1] *[1]First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; [2]Division of Blood Purification, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.*

The activation of NFκB after cisplatin administration has been shown to be implicated in acute tubular injury. The role of NFκB in acquired resistance in cisplatin-induced acute kidney injury, in which the rechallenge with the same dose of cisplatin day 14 after the first dose of cisplatin induced significantly less injury in rats, remains to be elucidated. Rats injected with cisplatin (5mg/kg) were divided into 3 groups: followed by the same dose injection of cisplatin day 14 (group1), the same dose injection of cisplatin day 14 with daily injections of pyrrolidine dithiocarbamate (PDTC) (a functional NFκB inhibitor, 80mg/kg/day) from day 12 (group2), and the same volume injection of vehicle day 14 with daily injections of PDTC (80mg/kg/day) from day 12 (group3). The renal NFκB activation after the first injection of cisplatin was sustained until day 14, which was attenuated by PDTC administration. Serum creatinine and renal apoptosis, evaluated by TdT-mediated dUTP nick-end labeling, was worsened day 17 and 19 in group 2 when compared to group 1.The expression of PCNA, a DNA repair marker, and heat shock protein (HSP) 72 were decreased form day 14 to day 17 in group 2 when compared to group1. The renal toxicity of PDTC was not found in group 3. These data imply that the sustained activation of NFκB after cisplatin injection contributes to acquired resistance in cisplatin-induced acute kidney injury via assembly of PCNA and HSP72.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2977**

**When Should We Stop Dialysis in Acute Kidney Injury: An Assessment of Peritoneal Dialysis, Urine Output, and Urinary NGAL in the Setting of Congenital Heart Surgery** Alyssa A. Riley,[1] Joshua Blinder,[1] John L. Jefferies,[1] David P. Nelson,[2] Stuart Goldstein.[2] *[1]Baylor College of Medicine, Houston, TX; [2]Cincinnati Children's Medical Center, Cincinnati, OH.*

Acute kidney injury (AKI) after cardiopulmonary bypass (CPB) to correct congenital heart disease (CHD) is common. We use early continuous peritoneal dialysis (CPD) to prevent fluid overload and further cardiac compromise in oliguric pts. The effect of CPD on AKI recovery is unknown, thus indications for discontinuing CPD are unclear. While change in urine output (UOP) may suggest recovery, urinary biomarkers are earlier, more sensitive indicators of AKI than serum creatinine, may or may not be affected by CPD, and have not been evaluated to predict AKI recovery. We aim to determine how CPD affects UOP recovery and if urinary NGAL (UNGAL) levels are associated with CPD provision and/or UOP recovery. Pts <90 days old undergoing CPB treated with CPD are randomized, matched for CHD physiology. At the clinical time of CPD discontinuation (DC) determination, pts either 1) DC CPD or 2) continue CPD for an additional 24 hours. UOP (ml/kg/hr) and total output (TOP) (urine, dialysate, chest tubes) (ml/kg/hr) is measured throughout the study period. UNGAL is assessed 6 hour post-CPB, then at 12 hour intervals until CPD catheter removal. Eleven pts have been randomized, 6 DC and 5 continuing CPD. At 8 hour intervals after randomization, average UOP (ml/kg/hr) and TOP (ml/kg/hr) were compared (Table 1). Median UNGAL levels did not differ in the 24 hours after randomization (Table 2). Our data show that while UOP recovers significantly after CPD discontinuation, UNGAL levels are not affected. We suggest that decreasing UNGAL levels may provide earlier evidence of renal recovery and be used to guide discontinuation of dialysis.

**Table 1.** Average Urine and Total Fluid Output After Randomization

| | DC CPD | | Continue CPD | | |
|---|---|---|---|---|---|
| | UOP (ml/kg/hr) | ±SD | UOP (ml/kg/hr) | ±SD | p-value |
| Hr 1-8 | 3.9 | 0.8 | 0.9 | 0.1 | <0.001 |
| Hr 9-16 | 5.2 | 0.4 | 1.8 | 0.3 | <0.001 |
| Hr 17-24 | 5.5 | 0.8 | 2.1 | 0.3 | <0.001 |
| | TOP (ml/kg/hr) | ±SD | TOP (ml/kg/hr) | ±SD | p-value |
| Hr 1-8 | 4.5 | 0.5 | 5.6 | 0.1 | 0.5 |
| Hr 9-16 | 5.6 | 0.4 | 5.4 | 0.2 | 0.4 |
| Hr 17-24 | 5.8 | 0.8 | 4.7 | 0.3 | 0.8 |

**Table 2.** Median Urinary NGAL Pre/Post Randomization

| | DC CPD | | Continue CPD | |
|---|---|---|---|---|
| Time (hr) | Median NGAL/Cr (ng/mg) | (25%, 75%) | Median NGAL/Cr (ng/mg) | (25%, 75%) |
| -18 | 319 | (94, 673) | 218 | (106, 1259) |
| -6 | 180 | (75, 352) | 149 | (61, 999) |
| 0 | Randomization | | | |
| 6 | 255 | (120, 375) | 26 | (81, 507) |
| 18 | 207 | (93, 380) | 183 | (113, 384) |
| | p = 0.24 | | | |

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2978**

**Repulsive Guidance Molecule C Is an Early Predicted Biomarker in Diagnosis of Acute Kidney Injury** Guang-Huar Young,[1] Vincent Wu,[2] Hung-Bin Tsai,[3] Wen-Je Ko.[1] *[1]Traumatology, National Taiwan University Hospital, Taipei, Taiwan; [2]Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; [3]Internal Medicine, Buddhist Dalin Tzu Chi General Hospital, Chiayi, Taiwan.*

**Introduction:**

Free iron plays roles in models of ischemia-reperfusion (I/R) induced acute kidney injury (AKI) which elevated the production of reactive oxygen species (ROS). The up-regulated of hepcidin modulated iron homeostasis and prevent the perpetuation of ROS injury. Repulsive guidance molecule c (RGMc) is a key regulator of hepcidin and is up-regulated in conditions of iron deficiency/hypoxia.

**Methods:**

Urinary RGMc was identified via proteomic method from cardiopulmonary bypass (CPB)-associated AKI according to RIFLE criteria. Male wistar rats received unilateral renal I/R injury, serum and urine samples were collected on several post-ischemic reperfusion time points (40 min, 3 h, 6 h, 12 h, 24 h and 48 h). Human proximal renal tubular cells (HK2) and mice distal renal tubular cell (DCT) were treated with $H_2O_2$-induced injury.

**Results:**

RGMc and hepcidin were detected at rat kidney tubules by PCR, western blotting and immunochemistry staining. Both RGMc and hepcidin were up-regulated in renal I/R injury rats compared with sham-operated group on mRNA level in acute phase (40 min vs. sham operated in RGMc: 1.52 fold, $p<0.05$; 3 hr vs. sham operated in hepcidin: 2.9 fold, $p<0.05$). Here we demonstrate that RGMc is up-regulated prior to hepcidin on mRNA level in rat kidney under I/R injury. Moreover, we show that urinary RGMc excretion in protein level was increased significantly in early phase of I/R injury rat (3 hr vs. shamed operated: 2.42 fold) and CPB-associated AKI patients (post-operative 24 hr urine from patients with AKI vs. without AKI: 2.07 fold, $p<0.05$). Renal tubule cell treated with additional recombinant RGMc could stimulate hepcidin gene expression under $H_2O_2$-induced injury.

**Conclusion:**

Here we identified at the renal tubules, hepcidin and RGMc were expressed and elevated after I/R injury. We further found that urinary RGMc was elevated at CPB associated AKI patients. These finding indicate that hepcidin and RGMc are early biomarkers in acute kidney injury.

Disclosure of Financial Relationships: Employer: National Taiwan University Hospital.

**SA-PO2979**

**L-NAME Abolishes Compensatory Mechanisms of Potassium ($K^+$) Secretion in Acute Kidney Injury Due to Ischemia and Reperfusion** Eliana Rachamimov,[1] Vered Avidan,[1] Hannah Wald,[2] Dvora Rubinger.[1] *[1]Nephrology and Hypertension Services, Hadassah University Medical Center, Jerusalem, Israel; [2]Gene Therapy Department, Hadassah University Medical Center, Jerusalem, Israel.*

During renal ischemic-reperfusion injury (IRI), increased nitric oxide (NO) production may affect tubular transport. The large-conductance $Ca^{+2}$-activated $K^+$ (BK) channels mediate flow-stimulated $K^+$ secretion.The present study was undertaken to define the role of NO system on $K^+$ handling and on BK channels expression in rats with IRI after bilateral renal artery occlusion, without (L-NAME (-)), and with (L-NAME (+)) pretreatment with the non-selective NO synthase inhibitor L-NAME. Plasma (P) creatinine levels were 160± 53 and 188± 40 µmol/l at 24hr and 62±30 and 279±61 µmol/l at 48hr reperfusion (p NS and p<0.001), in L-NAME (-) and L-NAME (+), respectively. L-NAME (+) animals were oligo-anuric, and had higher P $K^+$ and aldosterone levels than L-NAME (-). P $K^+$ levels were in correlation with urine flow rate (r=0.531, p<0.05). In L-NAME (-), IRI was associated with significant increases in protein (immunoblotting) and tissue (immunohistochemistry) expression of BK α subunit (functional channel) in renal cortex (3-4 fold), medulla and papilla (2-3 fold), and in colon (2 fold). These increases were blunted in L-NAME (+). The expression of FXYD4 (CHIF) , a protein involved in $K^+$ transport, was decreased in the kidney and increased in the colon in L-NAME (-) . In L-NAME (+) a more severe decrease in the renal expression and a decrease in colonic expression of FXYD4 was noted at 24hr reperfusion. The protein and tissue expression of α and β subunits of Na, K- ATPase were also markedly decreased after IRI, especially in L-NAME (+). These results show: 1.IRI is associated with significant increases in renal and colonic BK channel and colonic FXYD4 expression to maintain $K^+$ balance; 2. L-NAME administration is associated with delayed recovery of renal function, protracted hyperkalemia and abolishment of compensatory increases in BK channel and FXYD4; 3.These alterations are not related to aldosterone. Thus, in IRI, the compensatory increases in proteins involved in $K^+$ secretion are dependent on the integrity of NO system.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2980**

**Renal Metabolism of IL-6 Is Impaired in Acute Kidney Injury (AKI), but Not Pre-Renal Azotemia, in Mice** Nilesh Ahuja, Chris Altmann, Ana Andres-Hernando, Sarah Faubel. *Renal, University of Colorado Denver, CO.*

The proinflammatory cytokine IL-6 is increased in the serum of patients with AKI and predicts increased mortality and prolonged mechanical ventilation. The aim of this study was to examine the renal handling of IL-6. Since numerous proteins, including cytokines, are filtered by the glomerulus and metabolized by the proximal tubule (PT), we hypothesized that serum and urine IL-6 would increase in AKI due to impaired PT metabolism. Mouse models of pre-renal azotemia after 0.5 mg IP furosemide injection (no tubular injury) and ischemic AKI (tubular injury) (22 minutes of bilateral renal pedicle clamp) were studied. Renal histology 6 hours after furosemide injection was normal but was characterized by patchy tubular necrosis in AKI. BUN (mg/dL) was 15±1 in vehicle, 52±3 in pre-renal azotemia (P<0.0001, N=9-10), 24±1 in sham operated, and 60±1 in AKI (P<0.0001 vs sham; P=NS vs pre-renal azotemia, N=5-10). Urine IL-6 increased 6 hours after AKI but not pre-renal azotemia. To determine if circulating IL-6 appears in the urine in AKI, 200 ng of recombinant human (h)IL-6 was injected IV 5 hours post-procedure and urine collected for 1 hour; urine hIL-6 increased in AKI, but not pre-renal azotemia. Serum hIL-6 (pg/mL) was determined 1 hour post-injection and was 323±68 after vehicle injection, 394±40 in pre-renal azotemia (P=NS vs vehicle, n=3-4), 265±57 after sham operation, 4,609±1052 after AKI (P<0.001 vs sham, N=3-4), and 16,115±862 after bilateral nephrectomy (P<0.0001 vs sham, N=3-4). These data demonstrate that IL-6 elimination is intact in mice with functional kidneys (vehicle, pre-renal azotemia, and sham operation) but is greatly impaired in mice with impaired or absent kidney function (AKI and bilateral nephrectomy). To examine IL-6 metabolism, hIL-6 was added to the media of normal and hypoxic isolated PT; in normoxic PT, media IL-6 (pg/mL) was 773±22, in hypoxic PT media, IL-6 was 869±44 (P<0.05). In summary, reduced PT metabolism of IL-6 results in increased serum and urine IL-6. Impaired IL-6 metabolism leading to increased serum IL-6 may contribute to the deleterious systemic effects and increased mortality associated with AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2981**

**A Cross-Talk Pathway between Epithelial Adenosine Transporters (ENT1) and Vascular A2B Adenosine Receptors Conveys Potent Protection from Acute Kidney Injury** Julee Dalton,[1] Jessica Bauerle,[1] Hartmut Osswald,[2] Holger Eltzschig,[1] Almut Grenz.[1] *[1]Dept of Anesthesiology, UC Denver, Denver, CO; [2]Dept of Pharmacology, University of Tuebingen, Tuebingen, Germany.*

Introduction: Previous studies indicated that extracellular adenosine generation and signaling protects from acute kidney injury (AKI) [1]. Once generated into the extracellular milieu, adenosine is rapidly cleared through uptake by equilibrative nucleoside transporters (particularly ENT1 and ENT2) [2-3]. Therefore, we hypothesized that targeting renal adenosine transporters could convey protection from ischemic AKI by enhancing extracellular adenosine signaling events.

Methods: Human proximal tubular cells (HK-2) were exposed to hypoxia to measure expression and function of renal adenosine transporters. Adenosine transport was assessed in vitro utilizing radio-actively marked adenosine derivates. Gene targeted mice ($ENT1^{-/-}$, $ENT2^{-/-}$ or for individual adenosine receptors) were studied in an ischemic model of AKI. Adenosine levels were measured via HPLC. Renal function was determined by FITC-labeled inulin clearance. Renal blood flow was measured by renal ultrasound.

Results: Initial studies of renal adenosine levels, or kidney function following treatment with the non-selective ENT-inhibitor dipyridamole revealed elevated renal adenosine levels in conjunction with kidney protection from ischemia. Molecular studies pointed towards a prominent role of ENT1 as main adenosine transporter of the kidneys. Consistent with these findings, studies utilizing gene-targeted mice for ENT1 or ENT2 confirmed a selective role for ENT1 in kidney protection from ischemic AKI. Additional studies in gene-targeted mice for individual adenosine receptors suggested that dipyridamole-dependent kidney protection involves signaling events through vascular A2B adenosine receptors (A2BAR) by increasing renal blood flow following ischemia particularly in the cortex.

Conclusion: Our results indicate a selective role for ENT1 in kidney protection from ischemia via enhancing vascular A2BAR signaling events, and suggest off-label use of dipyridamole to prevent or treat kidney injury from ischemia.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2982**

**Inhibition of Soluble Epoxide Hydrolase Does Not Prevent Acute Renal and Hepatic Injuries in Septic Mouse Model** Cuong D. Nguyen,[2] Toshinori Aoyagi,[2] Tianxin Yang,[2] Bradley C. Baird,[2] Bruce D. Hammock,[3] Alfred K. Cheung.[1,2] *[1]Medicla Service, VASLCHCS; [2]Medicine, University of Utah, Salt Lake City, UT; [3]Entomology, University of California, Davis, CA.*

In response to stimuli, arachidonic acid (AA) is metabolized by cytochrome *p*-450 epoxygenase to form epoxyeicosatrienoic acids (EETs). Soluble epoxide hydrolase (sEH) degrades EETs to a less biologically active product, dihydroxyeicosatrienoic acids (DHETs). Previous studies showed that EETs possess anti-inflammatory effects in the vasculature and particularly in the kidney. We therefore hypothesize that inhibition of sEH would prevent sepsis-induced acute kidney and hepatic injuries in a murine cecal ligation-and-puncture (CLP) model.

Male, 10-to-12-week-old C57BL/6 mice (n= 10) were treated subcutaneously with a sEH inhibitor, *trans*-4-[4-(3-Adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*-AUCB), at 1mg/kg for 1 day before and 2 days after the CLP procedure. Compared to the control group, *t*-AUCB treatment did not decrease mortality (survival rate 3/15 vs. 2/15, n=15) nor prevent hypotension (SBP 69.0 ± 13.0 mmHg vs. 61.3 ± 10.5 mmHg). Furthermore, there was no significant difference between the *t*-AUBC and control groups in plasma levels of creatinine (0.38 ± 0.06 mg/dL vs. 0.41± 0.08 mg/dL) or hepatic injury markers (ALT 258.2 ±7.5 U/L vs. 64.0 ± 9.0 U/L, AST 654.0 ± 38.5 U/L vs. 626.2 ± 46.8 U/L). Similarly, *Ephx2-/-* mice without the sEH expression did not provide the protective benefits from CLP-induced acute injuries.

Despite of previous reports on the anti-inflammatory effects of EETs, our data suggest that inhibition of sEH *in vivo* was not associated with attenuation of organ injury and mortality in CLP-induced sepsis model. This difference could potentially be attributed to the severity insults observed in the CLP model. Additional work will be needed to clarify the implications of the anti-inflammatory actions of EETs in acute kidney injury.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2983**

**Comparison of Oral and Intraperitoneal Administration of Exogenous Reduced Glutathione (GSH) on Mitochondrial GSH Levels in Rat Kidney Cortex and Medulla** Marianna J. Zamlauski-Tucker, Josef M. Hannah. *Physiology & Health Science, Ball State University, Muncie, IN.*

GSH is the principal antioxidant inside cells and increasing the GSH level protects the cell against oxidative stress. The present study was undertaken to determine whether oral adminstration of GSH is as effective as intraperitoneal (i.p.) administration of GSH in increasing the mitochondrial GSH level in kidney cortex and medulla from ~ 9 month-old female Lewis rats. The Oral Group rats (n = 6) were given GSH (625 mg/Kg body wt) via gavage for two weeks. The i.p. Group rats (n = 7) were given GSH (250 mg/Kg body wt) via i.p. injection for two weeks. The Control Group rats (n = 9) were not given any exogenous supplementation. The kidneys were harvested at the end of the treatment period and separated into cortical and medullary tissue sections. The sections were further separated into cytosolic and mitochondrial fractions by differential centrifugation. GSH levels in the fractions were determined using a spectrophotometric assay. Statistical comparisons were done using ANOVA followed by the Fisher's LSD post hoc test.

Table 1

| | | Oral Group | | i.p. Group |
|---|---|---|---|---|
| | | GSH - µmol/g kidney wet wt | | |
| Cytosol | | | | |
| | Cortex | 4.7 ± 0.2 [a] | | 9.4 ± 0.9 [a,b] |
| | Medulla | 3.1 ± 0.1 [a] | | 6.2 ± 0.7 [b] |
| Mitochondria | | | | |
| | | GSH - nmol/g kidney wet wt | | |
| | Cortex | 127± 10 [a] | | 121 ± 11 [a] |
| | Medulla | 162 ± 17 [a] | | 107 ± 6 [b] |

Data expressed as X ± SEM; a - significantly different (p < 0.05) from Control Group; b - significantly different (p < 0.05) from Oral Group

Oral administration was as effective as i.p administration of GSH in increasing GSH levels in mitochondria from kidney cortex and medulla. However, a significant increase in GSH levels in the cytosol from kidney cortex and medulla was observed only with i.p administraton of GSH. Oral GSH admininstration significantly decreased GSH levels in cytosol in both kidney cortex and medulla. Thus, the route of administration of exogenous GSH does have different effects on cytosolic GSH levels in rat kidney cortex and medulla.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2984

**Isolated Kidney Injury Aggravates Effects of Secondary Lung Insult in a Two-Hit Mouse Model** Rajit K. Basu,[1] Alvin G. Denenberg,[1] Hector R. Wong,[1] Prasad Devarajan.[2] *[1]Critical Care, Cincinnati Children's Hospital, Cincinnati, OH; [2]Nephrology, Cincinnati Children's Hospital, Cincinnati, OH.*

Acute kidney injury (AKI) independently increases morbidity and mortality in hospitalized patients, possibly by detrimentally affecting distal organ homeostasis. Renal ischemia alters neutrophil chemotaxis and water permeability in the lung, but little is known about host response to secondary pulmonary insult after AKI. Using a novel mouse model, we hypothesized that primary non-lethal kidney ischemia followed by a secondary direct lung insult would worsen host lung injury. From preliminary data, we determined that 30 minutes of bilateral renal pedicle clamping (AKI) optimally 'primed' lungs at 24 hours. Thus, our two-hit model incorporated surgery (sham or AKI) at time zero, intratracheal inoculations (IT) at 24 hours, and sacrifice at 48 hours. We compared 4 groups: Sham + IT saline (Sham+PBS), Sham + 50 µg lipopolysaccharide IT (Sham+LPS), AKI + PBS, and AKI + LPS. Creatinine levels increased in AKI groups, but all mice survived. Serum inflammatory cytokines KC and MCP-1 significantly increased with AKI+LPS versus Sham+LPS. Cytosolic kidney cytokine levels were not significantly different. Lung inflammation by myeloperoxidase (MPO) synergistically increased and lung cytosolic MCP-1 and MIP-2 trended upwards with AKI+LPS versus Sham+LPS. Taken together, we demonstrate that AKI leads to a sub-lethal renal "distress" state that primes a host for heightened responses to subsequent lung challenge, a likely under appreciated phenomenon which may often occur in the clinical arena.

Effects of AKI followed by LPS Challenge

| | Sham | | AKI | | |
|---|---|---|---|---|---|
| **Serum** | PBS | LPS | PBS | LPS | p value |
| Creatinine | 0.14 ± .03 | 0.19 ± .13 | 0.5 ± 0.2 | 0.3 ± .05 | 0.22 |
| KC | 414 ± 161 | 526 ± 52 | 601 ± 146 | 1730 ± 151 | <.001 |
| MCP-1 | 47 ± 19 | 48 ± 6 | 209 ± 104 | 260 ± 122 | 0.04 |
| **Lung** | | | | | |
| MPO | 70 ± 28 | 313 ± 112 | 99 ± 40 | 475± 56 | 0.001 |
| MIP2 | 159 ± 37 | 7908 ± 1344 | 225± 92 | 10160 ± 1711 | 0.08 |

n=5 except MPO where n=9. Creatinine (mg/dl), KC, MCP-1, MIP-2 (pg/ml), and MPO (units/100mg). p values: Sham/LPS to AKI/LPS

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2985

**Estrogen Alters Ischemia-Reperfusion Injury Induced Glomerular Barrier Failure *In Vivo* and *In Vitro*** Michael Hutchens,[1] Yasuhara Kosaka,[1] Paco S. Herson,[1] Patricia D. Hurn,[1] Sharon Anderson.[2] *[1]Anesthesiology & Perioperative Medicine, Oregon Health & Science University, Portland, OR; [2]Division of Nephrology and Hypertension, Oregon Health & Science University, Portland, OR.*

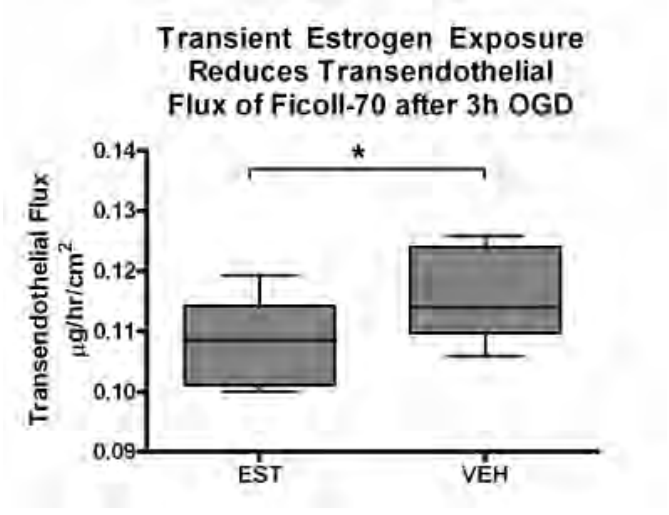
**Introduction:** Estrogen is renoprotective after cardiac arrest (CA). Investigation into renal ischemia has focused on tubular epithelium, but estrogen is active on endothelium. Glomerular endothelium forms part of the filtration barrier and is damaged by ischemia. We hypothesized that CA damages the glomerular barrier, and that estrogen protects it.

**Methods:** Ovariectomized female C57BL/6 mice received vehicle (VEH) and underwent sham or received VEH or estradiol (EST) and underwent 10 min CA, then cardiopulmonary resuscitation (CPR). 24h later, fluorescent-conjugated Ficoll-70(FITC-Ficoll), was given intravenously. 1h later, mice were killed, and BUN, creatinine, and urine fluorescence measured. Mouse glomerular endothelial cells were raised on membrane supports (Corning, Lowell, MA). Medium containing 1 uM EST or VEH was applied and cells subjected to 3h oxygen-glucose deprivation(OGD). Medium was then replaced with color/drug-free media with FITC-Ficoll in the upper chamber. After 4h, fluorescence of lower chamber media was measured.

**Analysis:** Data are reported as mean±SEM. Significance was set at $p<0.05$.

**Results:** CA/CPR increased glomerular ficoll-70 flux (sham 0.061±0.010, CA/VEH 0.510±0.033, CA/EST 0.390±0.072, mcg/hr, $p<0.05$ v sham, n=3-4/group) *in vivo*. There was a trend toward reduced flux in EST treated animals. *In vitro*, OGD increased macromolecular flux. EST treatment during OGD reduced flux compared with VEH (EST 0.1085±0.002, VEH 0.1159±0.003 g/cm$^2$,*=$p<0.05$, figure).

**Discussion:** We found that function of glomerular endothelium is altered after ischemia reperfusion. In addition we found that estrogen protects barrier function *in vitro*.




Disclosure of Financial Relationships: nothing to disclose

### SA-PO2986

**Application of NMR-Based Metabonomics in the Study of Rat Renal Ischemic-Reperfusion Injury** Nan Chen, Jun Wang, Xia Liu, Wen Zhang. *Department of Nephrology, Shanghai Ruijin Hospital, Shanghai, China.*

**Background:** Acute kidney injury is increasing obviously in recent years. The mechanism of acute kidney injury is still unclear. The metabolism of renal cell plays an important role in acute kidney injury and the study of renal metabolism is of great importance. The renal ischemia reperfusion injury rat model is the most often used animal model for acute kidney injury study. So we performed the metabonomics research in the ischemia reperfusion injury rat model to clarify the metabolic response to renal ischemic-reperfusion injury.

**Methods:** The rat renal ischemic-reperfusion model was studied by an integrated metabonomics strategy, utilizing $^{1}$H NMR and $^{31}$P NMR spectroscopy of kidney. Male Sprague-Dawley rats were subjected to either clamp of both sides of renal artery, sham operated (SO) surgery, or no treatment (n=5/group) and samples collected over 6 hours, 24 hours, 48 hours, 72 hours and 168 hours. The renal ultrastructure could be observed with transmission electron microscope. The data from NMR was analysed with SIMCA-P 10.5 software. The PCA map and OPLS-DA map were acquired. After data analysis by statistician the change of metabolite from renal tissue and serum were observed.

**Results:** A number of renal metabolic perturbations were observed in I/R rats compared with SO animals, including elevated isobutyrate, lactate, Sn-glycero-3-phosphocholine and hippurate while betaine, taurine, creatine and NADP were reduced. Scores plot of pattern recognition analysis were capable of distinguishing I/R from SO. The ATP level of renal tissue was decreased in the I/R group. By the same time the renal stucture was estimated under microscope with PAS and HE staining. The ultrastructure of renal tissue was detected by transmission electron microscope. The observed metabolic perturbations were consistent with the morphological change of kidney including the damaged membrane of renal tubular cell and the decreased density of mitochondria in tubular cells.

**Conclusions:** The observed changes in $^{1}$H and $^{31}$P NMR spectroscopy and morphology confirm the decreased energy metabolism and suggest the protection of mitochondria may be a strategy for prevention and treatment of AKI.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO2987

**Effect of DPP4 Inhibition on Ischemia Reperfusion Injury (IRI)-Induced Loss of Renal Function** Lorenzo Glorie,[1] Annelies De Beuf,[1] Anja Verhulst,[1] Marc E. De Broe,[1] Benjamin B. Vervaet,[1] Veerle Matheeussen,[2] Ingrid De Meester,[2] Patrick C. D'Haese.[1] *[1]Laboratory of Pathophysiology, University of Antwerp, Wilrijk, Belgium; [2]Laboratory of Medicinal Biochemistry, University of Antwerp, Wilrijk, Belgium.*

Inhibitors of dipeptidyl peptidase 4 (DPP4) exhibit a protective effect on IRI of heart and lung through increased half-life of DPP4 substrates. In this study, the effect of the commercially available DPP4 inhibitor vildagliptin (VG) on renal function after IRI was assessed.

This effect of VG was tested in a unilateral 30' IRI rat model, in which contralateral kidney was removed after release of renal pedicle clamp. Saline or VG (1 mg/kg and 10 mg/kg) was administered iv prior to sham operation or IR. Rats were sacrificed and the ischemic kidney was removed after 2h/12h/48h of reperfusion. Inhibition of DPP4 was confirmed by decreased DPP4 activity in serum and kidney segment homogenates. Measurement of serum creatinine revealed significant dose-dependent protection of renal function in VG vs saline treated IR rats (0.7 mg/dl and 1.3 mg/dl for 10 mg/kg and 1 mg/kg VG respectively, vs 1.9 mg/dl for saline at 12h reperfusion; p=0.0003 and p=0.0022, respectively). Interestingly, VG treatment did not have a significant effect on tubular morphology as shown by PAS-PCNA staining. mRNA expression of DPP4, SDF-1α (DPP4 substrate), CXCR4 (SDF-1α receptor), HO-1 (heat shock protein), IL-1β (pro-

inflammatory) and IL-10 (anti-inflammatory) was evaluated by real-time RT-PCR and showed increased expression of CXCR4 and SDF1α at 48h in VG treated sham-operated rats as compared to saline treated sham-operated rats, whereas this was not seen after IR. mRNA expression of DPP4, HO-1, IL-1β and IL-10 was not altered by administration of VG. Increased infiltration of macrophages was reported in IR rats treated with 10 mg/kg VG at 2h and 12h reperfusion as compared to the saline IR rats.

In conclusion, DPP4 inhibition with VG results in a significant functional protection against IRI, this however without having any effect on IR-induced morphological changes, suggesting other mechanisms (e.g. by hemodynamic effects) to be responsible for the protective effect on renal function.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2988**

**Spermidine/Spermine-N1-Acetyltransferase (SSAT) in Acute Kidney Injury in Humans and Rodents: Role in Pathophysiology and Use as a Biomarker** Kamyar A. Zahedi, Amit Govil, Sharon L. Barone, Charuhas V. Thakar, Manoocher Soleimani. *Medicine, University of Cincinnati, Cincinnati, OH.*

Renal ischemia reperfusion injury (IRI) is a major cause of acute renal failure (ARF) in native and the foremost cause of delayed graft function (DGF) in transplanted kidneys, and ranks among the leading causes of morbidity in hospitalized patients. The expression and activity of SSAT and spermine oxidase (SMO) increase in the kidneys of rodents subjected to renal IRI, indicating the onset of enhanced polyamine catabolism. Expression of SSAT in cultured cells leads to DNA damage and growth arrest. Furthermore, the inactivation of SSAT gene significantly reduces the severity of tubular damage and renal dysfunction in animals subjected to kidney IRI. Studies described here were designed to examine the role of SSAT expression by the proximal tubule epithelium and flavin adenine dinucleotide (FAD)-dependent polyamine oxidases, PAO and SMO, in the etiology of IRI. Our studies indicate that the specific ablation of SSAT gene in mouse renal proximal tubules and inhibition of PAO and SMO reduce the severity of renal IRI. In order to determine the clinical relevance of our experimental observations and assess the utility of measurement of urinary polyamine levels as biomarkers of renal injury we examined the expression of SSAT and SMO in the kidney and determined the putrescine levels in the urine of patients with DGF. Using specific antibodies and biopsy sections, immunohistochemical staining demonstrated that the expression of SSAT and SMO is significantly enhanced in the kidneys of patients with acute renal failure in DGF but not in non-ischemic models. Measurement of urinary putrescine, a metabolite of SSAT-mediated polyamine catabolism, indicated significant elevation in patients with DGF but not in patients with renal failure secondary to non-ATN causes. We propose that the activation of SSAT plays an important role in the pathogenesis of acute ischemic renal failure in humans and rodents. We further propose that SSAT expression in the kidney and measurement of its metabolite (putrescine) in the urine can be used as novel biomarkers in AKI.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2989**

**Cyclosporine-Induced Modifications of Proximal Tubule Actin Organization and tPA Expression May Contribute to the Interstitial Fibrosis during Chronic Allograft Dysfunction** Virginie De Merindol,[1,3] Elodie Mestre,[1,3] Marie Essig.[1,2,3] *[1]UMR-S850, Inserm, Limoges, France; [2]Nephrology Dialysis Transplantations, CHU Limoges, Limoges, France; [3]Université de Limoges, Limoges, France.*

Recent studies focusing on non-immunological actions of cyclosporine A (CsA) have demonstrated its role in the control of actin organization in podocytes. We investigated whether a similar effect can be observed in proximal tubular cells which possess a highly organized cytoskeleton and if this effect could be involved in the interstitial fibrosis of kidney grafts observed in the long term.

Methods: Pig proximal tubular cells (LLC-PK1 cells) were incubated for 24h with 5μM CsA. Cytoskeleton organization was analyzed by immunofluorescence. Expression and activity of extracellular matrix proteases [tissue-type plasminogen activator (tPA), plasminogen activator inhibitor (PAI-1) and metalloproteases (MMP2, MMP9)] and the role of NFAT and cofilin pathways were analyzed.

Results: In LLC-PK1 cells, CsA induced a strong rearrangement of the actin cytoskeleton with a reinforcement of the lateral network. As observed when the actin cytoskeleton of proximal cells is rigidified by mechanical stress (Essig et al, AJP, 2001), CsA-induced actin reorganization was associated with an inhibition of the expression and activity of tPA, independent of its inhibitor PAI-1. No modification of the expression and activity of MMP2 and MMP9 could be observed. CsA induced an increase in pNFAT-NFAT ratio, however the VIVIT peptide, a specific inhibitor of NFAT, did not reproduce the effects of CsA on actin and tPA expression. CsA also induced an increase in pCofilin/Cofilin ratio and the S3R peptide, a specific inhibitor of cofilin phosphorylation, increased tPA activity suggesting that the effects of CsA on actin and tPA expression are mediated through the modification of cofilin activity.

Conclusion: In proximal tubular cells, CsA induces reorganization of the actin network and inhibits tPA expression and activity independently of NFAT inhibition and maybe through modification of cofilin activity. The decrease in tPA activity may contribute to the interstitial fibrosis of the graft observed in the long term in CsA-treated patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2990**

**Molecular Patterns in Protocol Biopsies at 3 Months Predict Acute Rejection Episodes** Bernd Krüger,[1,2] Yi Luan,[1] Yi Cai,[1] Weijia Zhang,[1] Bernd Schroppel,[1,3] Barbara T. Murphy.[1,3] *[1]Mount Sinai School of Medicine, Division of Nephrology, New York, NY; [2]V. Medizinische Klinik, Universitätsklinikum Mannheim, Medizinische Fakultät der Universität Heidelberg, Mannheim, Germany; [3]Mount Sinai School of Medicine, The Transplantation Institute, New York, NY.*

Background: Protocol biopsies offer the potential to identify subclinical events that predate functional events. However, renal pathology can fail to predict future graft events. Microarrays offer the ability to examine intragraft activity at a molecular level and the potential to predict clinical events prior to clinical graft injury.

Methods: We analyzed 3 months post-transplantation protocol biopsies from the GoCAR study cohort. We performed microarray analysis (Affymetrix GeneChip Human Exon 1.0 ST Array) with subsequent hierarchical clustering on 48 protocol biopsies and examined the association between gene clusters and clinical outcomes. Follow-up time was at least 9 months. Statistical analyses were performed using the SPSS statistical package.

Results: Hierarchical clustering revealed two patient groups based on differential gene expression patterns in genes related to immune response, muscle/collagen/fibrosis, transport/metabolism, and cell adhesion. Demographic, and clinical parameters including kidney function and histopathological findings were not different between the different clusters at the time of biopsies. However, patients clustered to the group with the highest expression of immune response genes, had a significant higher risk of future acute rejection episodes (p=0.002).

Conclusion: These data suggest that molecular changes within the graft are a more sensitive marker of subsequent pathological events and may be potentially used to stratify patients prior to the development graft injury, allowing individualized tailoring of immunosuppressive therapies.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2991**

**Urinary B Cell Attracting Chemokine 1 (BCA-1) Is a Sensitive Marker of Acute Humoral Rejection** Wenhan Peng, Dajin Chen, Jianghua Chen. *The Kidney Disease Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.*

In previous studies, we found urinary fractalkine was a better marker of acute rejection, but urinary biomarkers of acute humoral rejection (AHR) were still unknown. The aim of this study was to investigate whether urinary B cell attracting chemokine 1 (BCA-1) was a useful noninvasive marker for detection of AHR in renal transplant recipients. Urinary C4d, fractalkine, chemokine monokine induced by IFN-γ (Mig), IFN-γ-inducible protein 10 (IP-10), granzyme B and perforin were also assessed. Urinary concentration of these markers was determined by ELISA technique in 323 recipients from June, 2001 to Dec, 2008 and 80 healthy controls. These urinary markers excreted by 115 patients with AR were at higher levels than those of healthy controls, 156 patients with stable renal function and 33 patients with chronic allograft nephropathy (P<0.001). Urinary fractalkine could also distinguish AR from acute tubular necrosis (N=19) (P<0.001). Only changes in urinary BCA-1 and C4d distinguished 37 patients with AHR from 78 patients with acute cellular rejection (ACR) (BCA-1: 20.99±3.57 vs. 1.99±0.41 ng/mmol creatinine, P<0.001; C4d: 27.21±6.13 vs. 11.62±2.22 μg/mmol creatinine, P=0.005, respectively). For distinguishing AHR from ACR, area under the conventional receiver operating characteristic curve of BCA-1 was greater than that of C4d (0.877 vs. 0.66). When BCA-1 was 5.64ng/mmol creatinine, the sensitivity was 83.3% and the specificity was 83.7% to diagnosis of AHR (P<0.001). The study shows that urinary BCA-1 and C4d were useful biomarkers for acute humoral rejection. The former was much better than C4d in predicting AHR.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO2992**

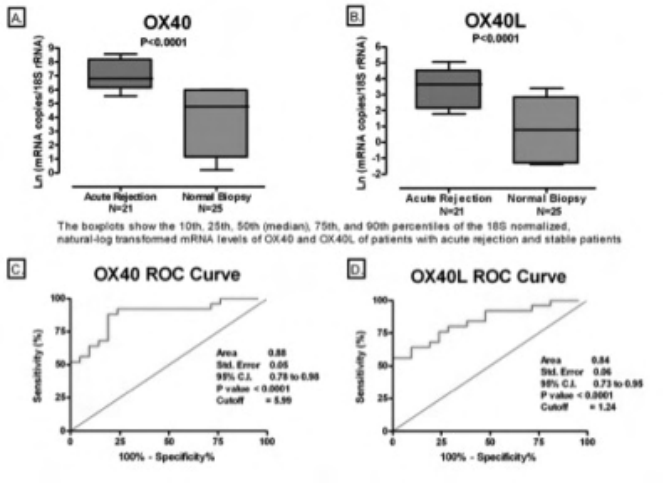
**Identification of High Levels of mRNA Encoding T Cell Co-Stimulatory Proteins OX40 and OX40L during Episodes of Acute Rejection of Human Renal Allografts** Michelle L. Lubetzky, C. Afaneh, Thangamani Muthukumar, Darshana M. Dadhania, Surya V. Seshan, Manikam Suthanthiran. *Division of Transplantation Medicine, New York Presbyterian-Weill Cornell, New York, NY.*

Acute rejection (AR) of human renal allografts remains a major cause of allograft failure. The development of novel urinary biomarkers for AR aids in diagnosis of rejection episodes. OX40 and its ligand (OX40L) are members of the TNFR superfamily and have been shown to be critical for T-cell co-stimulation and accelerated rejection in experimental models. Our goal was to investigate whether OX40 and OX40L contribute to AR in humans.

We studied urine samples from 21 patients with biopsy-proven AR and 25 patients with stable renal allograft function and normal biopsies. RNA was isolated from urinary cells, reverse transcribed to cDNA, preamplified with gene specific primers, and absolute mRNA copy numbers were quantified using RTQ- PCR assays.

The natural log-transformed mean (±SE) ratio of OX40 mRNA copies to 18S rRNA copies was higher in the urine of AR patients (6.98±0.26) compared to stable group (3.88±0.50) (P<0.0001) (Fig 1A) The ratio of OX40L mRNA to 18S rRNA was also higher in the urine of AR patients (3.37±0.28) compared to stable (0.72±0.41) (P<0.0001) (Fig 1B). Analysis of ROC curves demonstrated that AR can be predicted with a 88% sensitivity

and a 81% specificity using 5.99 as cutoff for OX40 mRNA (AUC: 0.88, P< 0.0001), and 56% sensitivity and 100% specificity using 1.24 as cutoff for OX40L mRNA (AUC: 0.84, P<0.0001) (Fig 1C & D).




Urinary cell mRNA levels of OX40 and OX40L are higher during episodes of AR in human renal allografts and can be noninvasive diagnostic biomarkers. Our report of altered levels of mRNAs for encoding OX40/OX40L in human transplantation advances these co-stimulatory molecules as potential drug targets in organ transplantation.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2993

**Unsupervised Analysis of Kidney Transplant Microarray Data Identifies Subgroups of Acute Rejection** Ken J. Park, Ying Huang, David L. Perkins. *Nephrology, University of California, San Diego, La Jolla, CA.*

**Introduction:** Based on multiple types of evidence, kidney transplant rejection is a heterogeneous diagnosis. We hypothesized that using unsupervised analysis of 2 publicly available kidney transplant microarray datasets, we could identify subsets of acute rejection (AR) based on molecular criteria of gene expression.

**Methods:** Data were downloaded from Gene expression Omnibus (GEO) microarray repository for "Paris" set (GSE9493) and from transplants.med.ualberta.ca for the "Alberta" set. Data was normalized with GCRMA in R and filtered by selecting genes with coefficient of variation of >10%. To build a classifier, we used the Alberta data as a test set. Significant genes were selected with p< 0.005 between AR and borderline TCMR (BL) vs. no rejection (NR) using t-test corrected with FDR. The number of clusters was determined using partition around medoids (PAM) for all AR and BL. Significant gene ontology (GO) biologic processes and KEGG pathways were identified using DAVID v6.7 (david.abcc.ncifcrf.gov/home.jsp). The classifier was then evaluated in the Paris validation set.

**Results:** 983 probe sets were significant between AR/BL vs. NR in the Alberta set. Clustering using PAM for AR and BL samples showed that two clusters had the best average silhouette width. 171 genes were significant between the two clusters. GO biologic processes and KEGG pathways were significantly different between the 2 subsets of rejection. Interestingly, comparison of histologic scores between the two groups showed increased glomerulitis and interstitial inflammation scores in the first cluster. Using the Paris set as an independent data set for validation, AR and BL samples also clustered into two groups using these 171 genes. Heat map showed differential expression of the genes between the 2 subsets. Importantly, Banff scores were higher in the group with higher expression of the classifier genes.

**Conclusion:** Based on comparison with histologic scores, unsupervised clustering of microarray data reveals two distinct groups of rejection which may represent different grades of severity of rejection.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2994

**Donor Specific Low Level C3d Fixing Anti-HLA Antibodies before Kidney Transplantation Are a Risk Factor for Early Rejection Episodes** Ulf Schoenermarck,[1] Markus Guba,[3] Teresa Kauke.[2] *[1]Medical Clinic I, Nephrology Div., University Hospital Munich-Grosshadern, Munich, Germany; [2]Laboratory of Immunogenetics, University Hospital Munich-Grosshadern, Munich, Germany; [3]Department of Surgery, University Hospital Munich-Grosshadern, Munich, Germany.*

Introduction:

The clinical relevance of anti-HLA antibodies only detected in solid phase assays (SPA) before kidney transplantation is discussed controversial. The aim of our study was to investigate the risk of early rejection in case of donorspecific low level C3d fixing (DSA+/C3d+) HLA-antibodies detected in pretransplant sera.

Patients and Methods:

219 consecutive patients with a deceased kidney allograft were retrospectively analysed. All patients were transplanted with a negative crossmatch. Patients with high immunological risk (e.g. with cytotoxic HLA-antibodies or with high level SPA+ antibodies) were not recommended for transplantation. 55/219 patients were positive in SPA before transplantation. SPA+ sera were measured by means of Luminex Single Antigen (LSA) beads with standard IgG conjugate and additionally with anti-C3d conjugate. Rejection episodes within the first 3 weeks after transplantation were correlated with the presence of DSA+/C3d+ HLA-antibodies.

Results:

Acute rejection (AR) occurred in 21/219 patients (9.6%) within the first 3 weeks after transplantation. AR was seen in 16/55 SPA+ patients (29%), but only in 5/145 SPA- patients (3.4%). DSA+/C3d+ HLA-antibodies were detected in 24/55 SPA+ patients (44%). AR occurred with higher frequency in patients with DSA+/C3d+ HLA-antibodies (13/24, 54%) than in DSA/C3d- patients (3/31, 9.7%; p<0.001).

Conclusion:

According to our preliminary data the presence of low level DSA+/C3d+ HLA-antibodies prior to transplantation is a risk factor for early rejection episodes. Further investigation using a combination of assays to determine specificity and function is necessary to evaluate the role of these low level HLA-antibodies on long-term graft survival.

Disclosure of Financial Relationships: Other Relationship: Travel grant from Astellas.

## SA-PO2995

**Molecules Selectively Expressed in T Cell-Mediated Rejection: Comparison with Antibody-Mediated Rejection** Declan G. de Freitas, Jeff Reeve, Joana Sellares, Konrad S. Famulski, Luis G. Hidalgo, Gunilla Einecke, Dina F. Badr, Banu Sis, Michael Mengel, Philip F. Halloran. *ATAGC, University of Alberta, Edmonton, AB, Canada.*

The alloimmune response damages organ allografts by either T cell-mediated rejection (TCMR) or antibody-mediated rejection (ABMR), which share features because both induce inflammation (e.g. interferon-gamma effects). Distinguishing TCMR from ABMR is important for understanding mechanisms and guiding treatment strategies.

Methods: We studied 403 consecutive kidney biopsies for cause in 315 patients by histopathology, microarray analysis and alloantibody status. We defined the molecules expressed in biopsies labelled C4d$^+$ ABMR and C4d$^-$ABMR to those labelled TCMR using both class comparison and Predictive Analysis of Microarrays (PAM). PAMR$^+$ cases were identified using a previously published rejection classifier.

Results: 28 cases of TCMR, 17 cases of C4d$^+$ABMR and 47 cases of C4d$^-$ABMR were identified using histopathology and HLA antibody status. In comparison, 30 cases of TCMR and 13 cases of C4d$^+$ABMR were identified when PAM was applied to the histological diagnoses. TCMR, defined both clinically and molecularly, was characterized by a higher burden of selected T cell transcripts (e.g. GZMK, CD8A, CD2) and macrophage transcripts (e.g. ADAMDEC1, CXCL13). Table 1 lists the top 10 genes in each class comparison. Few interferon-gamma induced transcripts were identified reflecting the rejection vs rejection analysis. Marked overlap occurred between the gene lists as determined by both class comparisons. CXCL13 and ADAMDEC1 were the top performing genes in all comparisons and were highly correlated with the lesions of TCMR. Thus although TCMR and C4d$^+$ABMR share features, TCMR can be distinguished by a combination of a high T cell transcript burden and selected macrophage genes.

| TCMR vs C4d$^-$ABMR (Histology and Antibody) | | | | | PAMR$^+$ TCMR vs C4d$^-$ABMR (Histology and Molecular Rejection Classifier) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ID | Sym | PBTs | Fold Change | FDR | ID | Sym | PBTs | Fold Change | FDR |
| 205242_at | CXCL13 | MMDC | 10.995 | 0.002 | 205242_at | CXCL13 | MMDC | 7.36 | 0.0226 |
| 206134_at | ADAMDEC1 | MMDC | 5.8 | 0.003 | 206134_at | ADAMDEC1 | MMDC | 4.272 | 0.0115 |
| 206666_at | GZMK | CAT | 3.186 | 0.004 | 206666_at | GZMK | CAT | 3.08 | 0.0021 |
| 211796_s_at | TRBC1 | CAT | 3.061 | 0.007 | 211339_s_at | ITK | CAT | 2.936 | 0.0047 |
| 205758_at | CD8A | CAT | 3.059 | 0.008 | 211796_s_at | TRBC1 | CAT | 2.845 | 0.0084 |
| 220485_s_at | SIRPG | CAT | 2.77 | 0.0005 | 205758_at | CD8A | CAT | 2.703 | 0.0116 |
| 205831_at | CD2 | CAT | 2.75 | 0.007 | 205831_at | CD2 | CAT | 2.668 | 0.0021 |
| 204416_x_at | APOC1 | MMDC | 2.733 | 0.0005 | 204416_x_at | APOC1 | MMDC | 2.656 | 0.0047 |
| 210972_x_at | TRAC | CAT | 2.661 | 0.004 | 210972_x_at | TRAC | CAT | 2.651 | 0.0047 |
| 213539_at | CD3D | CAT | 2.636 | 0.002 | 204891_s_at | LCK | CAT | 2.531 | 0.0047 |

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2996

**Urinary NGAL and IL-18 Predicts Graft Function within Hours after Renal Transplantation** Kalathil K. Sureshkumar,[1] Chirag R. Parikh,[2] Richard J. Marcus.[1] *[1]Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA; [2]Nephrology, Yale University, New Haven, CT.*

In renal transplantation, delayed graft function portends adverse long-term graft and patient survival. Early diagnosis is essential for any intervention to be successful. Serum creatinine is a poor maker for early diagnosis of allograft injury.

We performed a prospective observational cohort study of deceased-donor kidney transplant recipients to evaluate urinary neutrophil gelatinase-associated lipocalin (NGAL) and IL-18 as biomarkers for earlier prediction of adverse graft outcome. Graft function recovery was classified as delayed graft function (DGF-dialysis requirement within first week), slow graft function (SGF-<40% reduction in serum creatinine by post-operative day 3 and no dialysis requirement), or immediate graft function (IGF-remaining patients). Biomarker levels at different post-transplant time points in 89 patients are shown below.

| Urinary biomarker | Post-transplant hour | DGF(n=37) | SGF(n=26) | IGF(n=26) | p-value |
|---|---|---|---|---|---|
| NGAL (ng/ml) | 0 | 762(94-2832) | 576(99-2000) | 566(45-921) | 0.106 |
| | 6 | 947(213-3667) | 712(41-1658) | 262(18-1202)* | 0.001 |
| | 12 | 976(326-2993) | 554(27-2178) | 208(28-1104)* | <0.001 |
| | 24 | 1231(225-3560) | 552(49-2414) | 230(25-786)* | <0.001 |
| | 48 | 953(235-3021) | 296(38-1944) | 106(21-429)* | <0.001 |
| IL-18 (pg/ml) | 0 | 162.1(25.6-508.9) | 91.0(25.6-338.1) | 106.8(25.6-531.8) | 0.24 |
| | 6 | 219.2(53.1-946.4) | 65.2(25.6-2037.9) | 75.5(25.6-541.1) | 0.058 |
| | 12 | 351.5(77.3-2125.4) | 106.8(25.6-2247.6) | 98.1(25.6-417.0) | 0.005 |
| | 24 | 295.3(73.5-982.5) | 126.6(25.6-1143.2) | 73.2(25.6-939.6) | 0.002 |
| | 48 | 196.4 (53.9-938.1) | 106.9(25.6-711.5) | 69.2(25.6-939.6) | 0.017 |

Values expressed as median (10th to 90th percentile); *= p<0.05, IGF vs. SGF

Starting 6 hours post-transplant, urinary NGAL and IL-18 predicted DGF while NGAL also differentiated IGF from SGF. Our study supports the validity of these noninvasive tests for the early detection of allograft dysfunction which carries long-term adverse impact on graft and patient survival.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2997

**Renal Protection from Extreme Cold Ischemia in Hibernating Squirrels** Alkesh Jani, Elaine Epperson, Jessica Martin, Arijana Pacic, Sandy Martin, Danica Ljubanovic, Charles L. Edelstein. *U of Colorado.*

We have shown that cold ischemia (CI) results in increased caspase-3 activity, tubular apoptosis, and brush border injury (BBI) in mouse kidneys. The 13-lined ground squirrel (GS) undergoes hibernation and torpor, when core temperature (CBT) falls to 4C for 7-18 days, accompanied by profound bradycardia and low-level *in vivo* perfusion. Torpor is therefore a natural model of organ preservation. We hypothesized that torpid GS kidneys were protected from prolonged CI. Our aim was to determine if protection required low-level *in vivo* perfusion or was intrinsic to the kidney (ex vivo experiment). Methods: Radiotelemeters were implanted in 1 yr old GS, and CBT was remotely monitored. Animals were sacrificed during late torpor when CBT was 4C for ≥ 7 days. Both kidneys were then perfused with cold UW solution. For the *in vivo* experiment one kidney was immediately processed. For the *ex vivo* experiment, the contralateral kidney was perfused with UW and stored *ex vivo* in UW for 72 hours at 4C. % tubules with BBI and apoptotic cells/hpf were counted by a pathologist in a blinded manner. Caspase-3/7 activity was measured using fluorescent substrates Results (Table): Neither apoptotic cells/hpf nor caspase-3/7 activity differed significantly between *in*- and *ex vivo* kidneys. BBI was significantly worse in *ex vivo* kidneys but affected < 10 % of tubules. Conversion of pro- to active-form caspase-3 did not occur in either *in*- or *ex vivo* kidneys.

| | Apoptosis (cells/hpf) | BBI (% tubules affected) | Caspase-3/7 activity (nmol/min/mg) | Immunoblot | |
|---|---|---|---|---|---|
| | | | | Pro-form Caspase-3 | Active-form Caspase-3 |
| In vivo torpid kidney | 0.10 | 0 | 6.5 | ++ | ND |
| Ex vivo torpid kidney | 0.03* | <10 ** | 13.6* | ++ | ND |

* p =NS vs in vivo; **p < 0.0002 vs. in vivo; ND = not detected; n = 3 in all groups

Conclusion. We have shown that torpid kidneys are remarkably resistant to apoptosis during severe, prolonged CI both *in*- and *ex vivo*. The protection is associated with a failure to convert pro-caspase-3 to its active form. These findings indicate that torpid ground squirrel kidneys are intrinsically protected even *ex vivo* and are not reliant upon the low-level *in-vivo* perfusion.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2998

**Effect of Erythropoietin on Graft Function and Kidney Injury Biomarkers Following Deceased Donor Kidney Transplantation** Richard J. Marcus,[1] Sabiha M. Hussain,[1] Chirag R. Parikh,[2] Ngoc L. Thai,[3] Kalathil K. Sureshkumar.[1] *[1]Nephrology, Allegheny General Hospital, Pittsburgh, PA; [2]Nephrology, Yale University and VAMC, West Haven, CT; [3]Transplantation, Allegheny General Hospital, Pittsburgh, PA.*

There is evidence that erythropoietin attenuates ischemia reperfusion injury, a factor implicated in the development of delayed graft function (DGF) following kidney transplantation. The objectives of the current study are to assess the impact of epoetin alfa on: 1) DGF incidence and 2) levels of urinary biomarkers of acute kidney injury (AKI) following deceased donor kidney transplantation (DDKT).

Patients undergoing DDKT underwent double blinded randomization to receive either 40,000 units of epoetin alfa or saline into the ipsilateral iliac artery proximal to the allograft anastomosis site immediately after reperfusion. Patients were followed for 30 days. DGF was defined as the need for dialysis in the first post transplant week. Immediate graft function (IGF) was defined as no requirement of dialysis in the first post-transplant week and a creatinine fall of >40% by post operative day three.

Out of 72 patients enrolled into the study, 36 received epoetin and 36 received saline. DGF developed in 15/36 (42%) in the epoetin group compared to 17/36 (47%) in the saline group (p=0.635). IGF was observed in 12/36 (33%) and 6/36 (17%) in the epoetin and saline groups respectively (p=0.1025). The average number of dialysis treatments in the first post-transplant week were 1.3±2.0 and 1.4±2.0 in the epoetin and saline groups respectively (p=0.898).

IL-18 and NGAL were assayed in urine samples at post-transplant hours 0, 6, 12, 24 and 48. IL-18 and NGAL levels were similar between the groups at all time points.

Safety analysis of the data revealed the following: 7 cardiac related adverse events in the epoetin group compared to 6 in the saline group. There was 1 thrombosed allograft in the epoetin group and 2 peri-transplant hematomas in the saline group. There were no deaths.

In conclusion, epoetin alfa did not decrease the incidence of DGF but showed a trend towards an increase in IGF after DDKT. Epoeitin alfa had no influence on urinary biomarkers of AKI.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO2999

**Cellular Presensitization Converges with Ischemia Reperfusion Injury To Increase the Risk of Acute Rejection after Kidney Transplantation** Donald E. Hricik,[1] Aparna Padiyar,[1] Kenneth A. Bodziak,[1] Peter S. Heeger,[2] Joshua J. Augustine.[1] *[1]Medicine, University Hospitals Case Medical Center, Cleveland, OH; [2]Medicine, Mt. Sinai School of Medicine, New York, NY.*

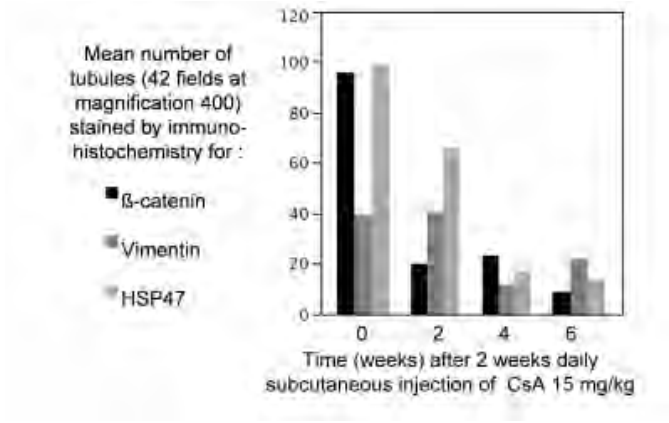
Recent studies suggest that an interplay between innate and alloimmunity can influence early outcomes after kidney transplantation. Innate immunity may be triggered by ischemia reperfusion injury (IRI). Alloimmune responses vary among individuals. Humoral alloimmunity is conventionally assessed by detecting preformed anti-HLA antibodies (panel reactive antibodies (PRA)). We previously have described the ELISPOT assay for interferon gamma as a measure of T cell alloimmunity. To determine the interplay between presensitization and IRI and their effects on early acute rejection, we studied 79 consecutive recipients of deceased donor kidney transplants from our center for whom pretransplant donor specific ELISPOT and PRA were available. Characteristics of the patients: age 46.1±12 yrs, 49% male, 50% African American, HLA mismatches 3.9±1.8, time on dialysis 47.2±31 mos. 28% had positive pretransplant ELISPOT (≥25/300K cells); 6% had peak PRA≥80%; 23% had delayed graft function (DGF) (need for dialysis in first week); 22% had biopsy-proven acute rejection (AR) in the first year. The incidence of AR was 39% vs 16% in patients with and without DGF (p=0.042), and 36% vs 16% in patients with and without a positive pretransplant ELISPOT. The incidence of AR was 67% for patients with a combination of DGF and a positive pretransplant ELISPOT vs 18% in those without DGF and with a negative ELISPOT (p=0.005). Logistic regression showed that the combination of DGF and a positive pretransplant ELISPOT was a significant correlate of AR (odd ratio 9.2, 95% CI 1.53-55.9, p=0.016) independent of age, ethnicity, gender, HLA mismatch, time on dialysis, PRA, or either DGF or a positive ELISPOT alone. The results suggest that heightened pretransplant cellular alloimmune responses measured by the ELISPOT assay for interferon gamma increase the risk of AR in patients with DGF. The ELISPOT assay may play an important role in pretransplant immune risk assessment, especially in patients at risk for DGF.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO3000

**Epithelial to Mesenchymal Changes Are Early and Reversible Markers of Cyclosporine Nephrotoxicity In Vivo** Pierre Galichon,[1,2,3] Nathalie Vittoz,[1,2] Emilie Cornaire,[2] Edith Baugey,[2] Sophie Vandermeersch,[2] Marie-Christine Verpont,[2] Laurent Mesnard,[1,2,3] Yi-Chun Xu-Dubois,[1,2] Alexandre Hertig,[1,2] Eric Rondeau.[1,2] *[1]UNTR, APHP, Paris, France; [2]U702, INSERM, Paris, France; [3]ED394, UPMC, Paris, France.*

A widely used immunosuppressant, cyclosporine A (CsA) conveys long-term nephrotoxicity (interstitial fibrosis and tubular atrophy) through unknown mechanisms. No specific marker is hitherto available to measure CsA toxicity in exposed individuals. In native and transplanted human kidneys, Epithelial to Mesenchymal Transition (EMT) markers are expressed by tubular epithelial cells (TEC) in various nephropathies and -in grafts- predict evolution towards interstitial fibrosis. We hypothesized that CsA could activate EMT pathway in TEC with subsequent renal fibrosis. We studied the kinetics of the expression of EMT markers β-catenin, vimentin, and HSP47 at the protein and mRNA level in the kidneys from rats injected 15 mg/kg/day of CsA or its vehicle for 2 weeks, and followed for 6 weeks after CsA wash-out. By 2 weeks, CsA had induced histological changes (tubular dilation and vacuoles) contemporarily of the expression of EMT markers by TEC. Interestingly, CsA wash out lead to gradual regression of these tubular lesions, however EMT markers were persistently expressed 4 and 6 weeks later in those tubules that were surrounded by de novo fibrosis. Angiotensin 2 and endothelin receptor antagonists were tested for their ability to prevent CsA-induced EMT, but to no avail. Our study suggests that EMT markers could help to identify and localize ongoing CsA-induced toxicity in TEC. Whether or not EMT fully occurs in human kidneys is still debated, yet the durable expression of EMT markers by rat TEC exposed to CsA in vivo, and the coincidence of fibrosis around those TEC, encourages to further explore their value as markers of CsA toxicity and their role in human graft fibrosis.




Disclosure of Financial Relationships: nothing to disclose

**SA-PO3001**

**Agonistic Antibodies to the Angiotensin AT1-Receptor after Kidney Transplantation** Martina Guthoff,[1] Falko Fend,[2] Silvio Nadalin,[3] Nils Heyne.[1] *[1]Dept. of Diabetes and Endocrinology, Angiology, Nephrology and Clinical Chemistry, University of Tuebingen, Tuebingen, Germany; [2]Dept. of Pathology, University of Tuebingen, Tuebingen, Germany; [3]Dept. of General-, Visceral- and Transplantation Surgery, University of Tuebingen, Tuebingen, Germany.*

Agonistic antibodies to G-protein coupled receptors form an alternate pathway of receptor activation, distinct from ligand interaction. AT1-receptor agonistic antibodies (AT1-RAA) have been described in the setting of preeclampsia, arterial hypertension and kidney transplantation. We report clinical course and histopathological findings of early antibody-mediated rejection (AMR) and malignant hypertension in a renal allograft recipient secondary to non-HLA AT1-RAA.

A 48-yr. old patient underwent first kidney transplantation in a standard immunologic risk situation. Despite primary function, the patient became anuric on day 2 with loss of diastolic flow in the transplanted kidney. Serial renal biopsies revealed massive congestion of large vessels and glomeruli with progressive endothelialitis and atypical positive C4d staining, sparing peritubular capillaries. Plasmapheresis and a course of corticosteroids was initiated without improving renal function. Subsequently, malignant hypertension developed requiring i.v. antihypertensive therapy. No donor-specific HLA antibodies were detectable. In search of non-HLA antibodies, agonistic antibodies to the angiotensin AT1-receptor were detected.

Within 48 hours upon initiation of AT1-receptor blockade by losartan, malignant hypertension and allograft perfusion normalised and the patient developed polyuria. Renal outcome at nine months is excellent under continuous AT1-receptor blockade. Follow-up biopsy revealed no signs of AMR.

In conclusion, non-HLA AT1-RAA are a rare cause of AMR in kidney transplantation. Underlying pathomechanisms include endothelial activation, interaction of recipient preformed AT1-RAA with naïve donor endothelium and altered AT1 receptor signalling. Both, functional hemodynamic and histopathological alterations are responsive to AT1-receptor blockade. Screening for AT1-RAA prior to transplantation could identify patients at risk for non-HLA AMR.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3002**

**The Effect of Cyclosporine-Induced Renal Injury on *Klotho* Gene Expression in Reduced Renal Mass** Hye Eun Yoon,[1] ShangGuo Piao,[2] Jungyeon Ghee,[2] Ji-Hyun Song,[2] Seok Joon Shin,[1] Chul Woo Yang.[2] *[1]Internal Medicine, Incheon St. Mary's Hospital, The Catholic University of Korea, Incheon, Republic of Korea; [2]Transplant Research Center, The Catholic University of Korea, Seoul, Republic of Korea.*

We have previously shown that *Klotho* expression is suppressed in an experimental model of chronic cyclosporine (CsA) nephropathy (Transplantation 2010 in press), but it is unclear whether decreased *Klotho* expression is influenced by CsA toxicity per se or is secondary to renal insufficiency independent from CsA. Under a low-salt diet, mice underwent sham-operation (Sham) or 5/6 nephrectomy (5/6NX), and were daily administered subcutaneously olive oil (1mg/kg) or CsA (30mg/kg) for 4 weeks. We performed Western blot and immunohistochemistry for *Klotho* in kidney. The results showed that KLOTHO significantly decreased in the Sham+CsA (41.0±5.2%) or 5/6NX groups (33.7±7.1%) compared with the Sham group (111.3±11.3%), and further decreased in the 5/6NX+CsA group (9.2±4.3%) compared with the Sham, Sham+CsA or 5/6NX groups ($P < 0.05$). This finding clearly demonstrates that KLOTHO expression is decreased in kidney with reduced mass or chronic CsA nephropathy, and addition of CsA treatment further decreases it. In conclusion, reduction in KLOTHO expression observed in chronic CsA nephropathy depends upon CsA-induced renal injury.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3003**

**Diminished Met Signaling in Podocytes Contributes to the Development of Podocytopenia in Transplant Glomerulopathy** Putri Andina Agustian,[1] Mario Schiffer,[2] Wilfried Gwinner,[2] Irini Tossidou,[1] Clemens L. Bockmeyer,[1] Verena Broecker,[1] Jan U. Becker.[1] *[1]Medizinische Hochschule Hannover, Institut für Pathologie, Hannover, Lower Saxony, Germany; [2]Medizinische Hochschule Hannover, Klinik für Nieren- und Hochdruckerkrankungen, Hannover, Lower Saxony, Germany.*

Introduction:

Transplant glomerulopathy (TxG) can show secondary focal and segmental glomerulosclerosis (FSGS). While TxG is generally accepted to be primarily due to glomerular endothelial cell (GEC) damage, FSGS in native kidneys is caused by podocytopenia. The present study shall demonstrate podocytopenia in TxG and examine the role of decreased paracrine Met activation on podocytes by decreased HGF secretion of damaged GECs in the development of podocytopenia in TxG.

Methods:

Podocytes were counted in 10 zero hour biopsies and specimens with and without TxG.

HGF/Met signaling was examined in immunostains and quantitative RT-PCR in a set of biopsies from 10 patients with TxG including the diagnostic biopsy (DiagnBx) and two preceding biopsies (1stPrevBx and 2ndPrevBx). The effect of HGF on immortalised mouse podocytes was examined in vitro.

Results:

Podocyte counts were lower in TxG, glomerular size larger, than in the specimens without TxG.

Less of the two preceding biopsies of the patients than of the controls contained phospho-Met(Tyr1349) positive podocytes (2/8 vs. 7/7, p=0.0070; 4/9 vs. 9/9, p=0.0249). Glomerular HGF mRNA levels were lower in the 1stPrevBx of the patients (0.049 ± 0.083 vs. 0.284 ± 0.331; p=0.0185).

HGF stimulation of podocytes caused phosphorylation of AKT and ERK and induction of XIAP in vitro.

Conclusion:

Decreased podocyte density, caused by a combination of podocyte loss and glomerular hypertrophy is a feature of TxG. Decreased antiapoptotic Met signaling in podocytes, probably due to decreased HGF secretion by GECs could contribute to podocyte loss and FSGS in TxG.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3004**

**Subclinical Rejections and Inflammation Are Still Prevalent after Renal Transplantation** Willy Aasebo,[1] Anna Reisaeter,[2] Karsten Midtvedt,[2] Finn P. Reinholt,[2] Christina Dörje,[2] Hallvard Holdaas.[2] *[1]Akershus University Hospital, Oslo, Norway; [2]Oslo, University Hospital, Rikshospitalet, Oslo, Norway.*

**Background**. Although the prevalence of subclinical rejections has declined with "modern" immunosuppressive regimes including tacrolimus and mycophenolate mofetil (MMF) there is a great variability in reported incidence.

**Objective:** In a large transplant unit we introduced in 2009 protocol biopsies at week 6 after renal transplantation. We wanted to assess the incidence of subclinical rejection in renal recipients receiving regimen consisting of basiliximab, cyclosporine/tacrolimus, MMF and prednisolone. CNI allocation to Cya or Tac was based on age, body mass index, and pretransplant oral glucose test.

**Results:** A total of 262 surveillance renal transplant biopsies were performet. We excluded patients with donor specific antibodies, AB0 incompatibility, combined pancreas and kidney and biopsies for cause (n=83). Biopsies were classified according to Banff criteria. In the remaining 179 low-risk patients with protocol biopsy during one year 10% of the patient had subclinical rejections (1 case of subclinical humoral rejection). Including borderline findings 36% of the patients had sign of inflammation/rejection at week 6.

Subclinical rejections

| | Negative | Borderline | Rejection |
|---|---|---|---|
| | n = 115 | n = 46 | n = 18 |
| Recipient age | 56.9 (13.5) | 50.9 (14.6) | 51.4 (14.5) |
| Donor age | 49.0 (15.5) | 52.1 (17.7) | 43.4 (16.0) |
| HLA-mismatch | 2.5 (1.4) | 3.3 (1.1) | 2.9 (1.4) |
| Living donor, number (%) | 42 (36.5) | 21 (45.7) | 6 (33.3) |
| Creatinine, µmol/L | 118 (35) | 125 (59) | 129 (42) |
| Protein/creatinine ratio | 30 (31) | 30 (33) | 76 (95)• |
| Interstitial fibrosis (0-3) | 0.56 (0.53) | 0.84 (0.42) | 1.14 (0.68)• |
| Cyclosporine as CNI | 73 (64) | 30 (65) | 11 (61.1) |

• = p<0.05

**Conclusion:** Biopsy findings of subclinical rejections were frequent in a selected low risk transplant population 6 weeks after transplantation. Although the presence of interstitial fibrosis overall was moderate patients with subclinical rejection demonstrated significantly more fibrosis than patients with no or borderline rejections. Subclinical rejection was associated with an increase in protein excretion.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3005**

**Matrix-Gla-Protein, Fetuin and Osteopontin in Calcification of Renal Allografts** Johan Lorenzen M. Lorenzen, Hermann G. Haller, Wilfried Gwinner. *Nephrology, Hanover Medical School, Hanover, Germany.*

Background: Calcification of renal allografts is not uncommon in the first year after transplantation and is related to hyperparathyroidism. It is associated with an impaired long-term outcome (Am J Transplant 2005;5:934-41).

Aim of this study is to examine surrogate factors in blood and urine which are related to renal allograft calcification.

Methods: We analyzed blood and urine samples of 30 patients with and 30 patients without allograft calcification taken at 6 weeks, 3 and 6 months after transplantation. To dissect factors for calcification besides hyperparathyroidism, patients were matched for their parathyroid hormone levels. Analyses included serum and urine electrolytes, Matrix-Gla protein, fetuin, FGF-23 in serum/plasma and osteopontin (OPN) in urine. Patient demographical data, cold ischemia time, initial graft function and donor characteristics were comparable between the two groups.

Results: In patients with calcification, matrix-Gla protein levels were significantly higher (25%) at 6 weeks compared to control patients without calcification, but decreased thereafter, reaching levels that were 27.5% lower than that of the controls at 6 months. There was no difference in FGF-23 levels. Fetuin levels were higher in patients with calcification (16.2% at 6 weeks; p=0.021 and 22.9% at 3 months; p=0.06) (Fig 1). Urinary OPN was considerably lower compared to the control group at 6 weeks after transplantation (by 66.4%; p<0.001), but increased by 6.2-fold, reaching values 75.6% over the controls at 6 months after transplantation. Using immunohistochemistry we could demonstrate that Matrix-Gla protein, osteopontin and fetuin are specifically expressed in the vicinity of calcified areas of renal allografts. Vitamin D and PTH levels were not different between the groups.

Conclusions: The striking differences in serum Matrix-Gla-protein and fetuin, and urinary OPN point to an important involvement of these factors in the process of allograft calcification.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3006**

**Exploring Kidney Microbiome and Its Role in Transplant (Graft) Survival** Vasuki N. Venkat, Ying Huang, David L. Perkins. *Division of Nephrology-Hypertension, University of California, San Diego, San Diego, CA.*

Metagenomics is a powerful tool for microbial and viral discovery,as nucleic acids can be isolated directly from environmental samples and sequenced without a priori knowledge or having to culture or clone. Advances in DNA sequencing technologies such as high throughput sequencing methods allow for the relatively inexpensive and rapid acquisition of millions of base pairs of high quality sequence data from various metagenomic communities. Using these methods, our objective is to identify the renal microbiome following kidney transplantation, and investigate their role in graft function and survival. Hypothesis: Changes in the metagenome following transplantation modulate renal injury and function. Urine provides a nonvasive biological sample to study the kidney microbiome. Methods: Urine collected from normal control and transplanted kidneys was pretreated with polyethylene glycol to concentrate the viral like particles (VLPs). Next,the VLPs were extracted by performing cesium chloride density gradient ultracentrifugation. Nucleic acids were then extracted by formamide/Cetyltrimethylammonium bromide methods using the standard Sambrook's protocol. The resulting purified DNA was then sequenced using Illumina Genomic DNA preparation kit. Using our purification protocol, we identified particles staining for DNA by epifluoresence microscopy, while no particles were detected in the supernatant from the same subject prior to centrifugation.

Results: DNA sequencing results from the preliminary samples indicate the presence of viruses both in renal transplant and normal controls. The following viruses have been identified in the urine samples collected after transplantation - BK polyomavirus, JC polyomavirus, Simian virus 12, Simian agent 12, Simian virus 40, Encephalomyocarditis virus, Macacine herpesvirus and Pepino mosaic virus. The significance of these viruses as related to their contribution towards graft survival is yet to be studied.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3007**

**DSAb Positive Acute Cellular Rejection Is Associated with Increased Risk of Subsequent Antibody Mediated Rejection, Transplant Glomerulopathy and Allograft Loss** Michelle Willicombe, Jack W. Galliford, Paul Brookes, Adam Mclean, Candice A. Roufosse, H. Terence Cook, Tom Cairns, David Taube. *Imperial College Kidney and Transplant Institute, London, United Kingdom.*

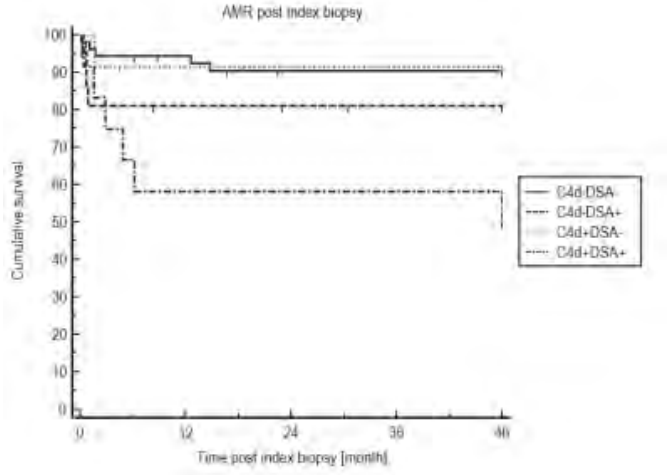
Although most T cell rejection [TCR] is rapidly and successfully reversed, there are a subgroup of patients who develop recurrent rejection and lose their allografts.

In this study, we have used C4d staining and Luminex single antigen bead screening for donor specific antibodies [DSAbs] to identify patients with TCR who may be at increased risk of further rejection and graft loss.

We retrospectively identified 100 patients [m: f, 76:24; mean age 43.96 ±13.11years; deceased: live donor 54:46; first: regraft 86:14; mean HLA mismatch 3.48 ±1.72] with histologically proven TCR. All patients received monoclonal antibody induction with tacrolimus, mycophenolate mofetil [MMF] and a steroid sparing regime. ACR was treated conventionally with iv methyl prednisolone and oral steroids. Mean follow up post TCR was 18.2 ±12.66 months.

54/100 patients were C4d-DSAb-, 22/100 patients were C4d-DSAb+, 12/100 were C4d+DSAb- and 12/100 were C4d+DSAb+. 3 year patient survival was similar in all groups. Compared with C4d-DSAb- patients, C4d+DSAb+ patients had inferior 3 year allograft survival [90.7%, 58.3%, p=0.023] and an increased risk of subsequent AMR [90.4%, 50.0%, p=0.005, Figure 1].

5/12 [41.67%] C4d+DSAb+ patients compared with 3/54 [5.56%] DSAb-C4d- went on to develop transplant glomerulopathy [p=0.0034].



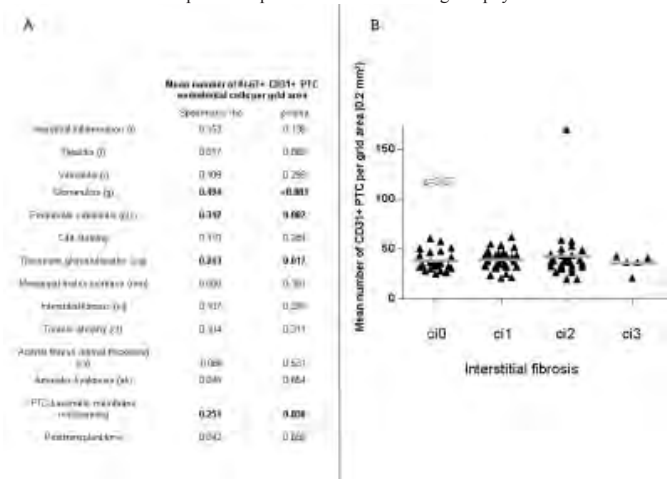

This is the first reported study showing that DSAb screening at the time of TCR predicts subsequent AMR, transplant glomerulopathy and graft failure. DSAb+ patients with TCR may benefit from enhanced surveillance and pre emptive augmented immunosuppression.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3008**

**Peritubular Capillary Endothelial Cell Cycling Is Selectively Increased in Antibody-Mediated Rejection of Kidney Transplants, but Not in Other Diseases** Stephen Adebayo Osasan, Jessica Chang, Yasemin Ozluk, Michael Mengel, Philip F. Halloran, Banu Sis. *University of Alberta, Edmonton, AB, Canada.*

Microcirculation endothelium is the main target of antibody-mediated rejection (ABMR). We hypothesized that ABMR is associated with a greater endothelial repair response compared to other diseases operating in kidney transplants. We related peritubular capillary (PTC) endothelial cell cycling and density of PTCs to histopathological Banff lesions and diagnoses, and whole-genome microarrays in 96 non-selected kidney transplant biopsies for cause. We performed double-immunostaining to label PTCs with anti CD31 and cycling cells with anti Ki-67. The PTC density and proliferating PTC endothelial cells were quantified by counting the number of CD31+ PTCs and Ki-67+CD31+ PTC endothelial cells, respectively, in the entire cortical biopsy area. The PTC endothelial cell cycling was selectively increased in C4d positive ABMR and C4d negative ABMR, but not in other diseases i.e. TCMR, acute tubular necrosis, glomerulonephritis (p=0.005). Increased PTC endothelial cell proliferation correlated with microcirculation lesions: glomerulitis, peritubular capillaritis, transplant glomerulopathy, and PTC basement membrane multilayering, but not with other lesions or C4d staining. Furthermore, transcript sets representing the molecular burden of active ABMR (endothelial cell-, macrophage-associated transcripts, IFNG regulated transcripts) correlated with increased PTC endothelial cell cycling (p<0.05). However, the PTC density did not differ among diagnoses and was not correlated with time post-transplant or lesions including atrophy and fibrosis.



Our results indicate that endothelial repair response is selectively increased in kidneys with ABMR, and there is no evidence for reduced PTC density among diseases, even with ABMR or significant scarring.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO3009

**Relationship between Albuminuria and Allograft Pathological Changes Associated with Diabetic Kidney Disease** Izumi Nyumura,[1] Tetsuya Babazono,[1] Kazuho Honda,[2] Yasuhiko Iwamoto.[1] *[1]Department of Medicine, Diabetes Center, Tokyo Women's Medical University, Tokyo, Japan; [2]Department of Pathology, Tokyo Women's Medical University, Tokyo, Japan.*

**Background and aims:** Albuminuria is a fundamental manifestation of diabetic kidney disease (DKD); however, information regarding relationship between urinary albumin excretion and recurrence and severity of DKD in the renal allograft has been scarce. We therefore conducted this study to highlight the relationship between urinary albumin excretion and clinical or histological parameters associated with DKD in renal allograft in diabetic patients.

**Materials and methods:** 37 diabetic renal allograft recipients who underwent allograft biopsies 2 to 16 years after transplantation were studied. Patients were classified into the following two groups according to the urinary albumin-to-creatinine ratio (ACR) at kidney biopsy: those with normo- or microalbuminuria (ACR < 300 mg/g, N=21) and those with macroalbumiruia (ACR ≥ 300 mg/g, N=16). Histological glomerular changes including mesangial/glomerular areas and glomerular capillary number were analyzed by a computer-assisted image analyzer. Thickness of glomerular basement membrane (GBM) was evaluated by an electron microscopy.

**Results:** There were no significant differences between the two groups in terms of recipient age, posttransplant duration, blood pressure and hemoglobin A1C. Serum creatinine was significantly higher in macroalbumiruic patients than normo- and microalbuminuric patients. In the univariate correlational analysis, ACR was significantly related with serum creatinine (rs=0.42, p=0.01). Pathological changes in the allograft that are characteristic of DKD; *i.e.*, mesangial/glomerular areas, glomerular capillary number, and thickness of GBM were not different in the two groups. In contrast, the grade of tubulo-interstitial lesions were significantly higher in macroalbuminuia group (p=0.005).

**Conclusions:** Albuminuria was not associated with the clinical and histological parameters of DKD but with the renal function and tubulo-interstitial lesions of renal allograft in diabetic transplanted patients.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO3010

**Effect of Cholecalciferol Supplementation on Renal Allograft Structural and Functional Deterioration** Marie Courbebaisse,[1] Yi-Chun Xu-Dubois,[2] Eric Thervet,[3] Gerard Friedlander,[4] Christophe M. Legendre,[3] Eric Rondeau,[2] Nicolas Palllet.[3] *[1]Nephrology and Dialysis Unit, Hopital Tenon APHP, Paris, France; [2]Transplantation Unit, INSERM U702, Hopital Tenon APHP, Paris, France; [3]Transplantation Unit, Hopital Necker APHP, Paris, France; [4]Renal Physiology Unit, Hopital Necker APHP, Paris, France.*

Beneath their important role in mediating calcium homeostasis, vitamin D receptor (VDR) agonists inhibit renin angiotensin system, inflammation and fibrosis. Renal models of chronic kidney injury and some clinical observational studies showed that VDR agonists could afford nephroprotection. The aim of this study is to test whether vitamin D3 (cholecalciferol, CLC) supplementation may confer structural and functional nephroprotection during the first year after renal transplantation. We analyzed glomerular filtration rate (GFR) using iohexol plasma clearance, urinary procollagen III aminoterminal propeptide excretion (uPIIINP), tubular vimentin expression and nuclear beta-catenin staining as markers of epithelial to mesenchymal transition (EMT) by immunohistochemistry and Banff scores of interstitial fibrosis (IF) and tubular atrophy (TA) at 3 months (M3) and M12 post transplantation in 32 renal transplant recipients (RTR) supplemented with CLC between M3 and M12 (100 000 UI every other week during 2 months and 100 000 UI every other month thereafter) and compared with an historical control group of 32 RTR. Clinical and biological characteristics of the two groups were similar at M3. At M12, serum 25(OH)vitamin D was higher (31.8±7.1 vs 16.6±6.6 ng/ml, p<0.0001) and serum parathormone was lower in the treated group. During the follow-up period, we found no difference regarding the incidence of biopsy proven acute rejection, new onset diabetes or hypertension between both groups. Mean GFR and uPIIINP remained stable and similar in both groups. Between M3 and M12, IF/AT and EMT scores increased similarly in the two groups. We show that CLC supplementation in these stable RTR does not prevent EMT, IF and TA. Our results suggest that this dosage of CLC does not afford major nephrotection during the first year after renal transplantation.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO3011

**The Effect of Bafilomycin A1 on Apoptosis and Autophagy in Cold Preservation Ischemia** Kultigin Turkmen, Ali Akcay, Jessica Martin, Iram Zafar, Quocan Nguyen, Dong Won Lee, Zhibin He, Alkesh Jani, Charles L. Edelstein. *Univ of Colorado.*

Apoptosis and autophagy are the two fundamental types of genetically controlled cell death. The aim of our study was to determine the connection between autophagy and apoptosis in a mouse model of cold preservation ischemia (CPI). C57BL/6 mice weighing 20-25 g were used. Kidneys were perfused via the left ventricle with cold University of Wisconsin (UW) solution with/without bafilomycin A1 (2 mg/kg), an autophagy inhibitor. One kidney was preserved in $4^0$ for 48 hours and the other kidney was the control. Immunoblotting was used to detect LC-3, ATG-5 and Beclin-1 as markers of autophagy and BID, Bax and Caspase-3 as markers of apoptosis. Caspase-3 activity was measured using fluorescent substrates. Table 1 shows the results of immunoblotting and caspase activity. In summary, in CPI, proapoptotic markers BID, Bax and caspase-3 and autophagy markers LC3, ATG-5 and beclin-1 are increased. Bafilomycin inhibits both apoptosis and autophagy markers. Bafilomycin may prove useful in cold preservation of organs for transplant.

Table 1

| Markers | Vehicle 0 hr | Vehicle 48 hr | Bafilomycin A1 2mg/kg 48 hr | P value |
|---|---|---|---|---|
| Caspase-3 (active 17 kDa) | + | +++ | 0 | P<0.005 |
| BID (22 kDa) | 0 | ++++ | 0 | P<0.005 |
| Bax (20 kDa) | + | +++ | 0 | P<0.005 |
| LC-3-I (19kDa) | + | +++ | 0 | P<0.05 |
| LC-3-II (active 17kDa) | + | +++ | 0 | P<0.05 |
| ATG-5 (33 kDa) | + | ++++ | 0 | P<0.005 |
| Beclin-1 (55 kDa) | + | +++ | 0 | P<0.005 |
| Caspase-3 (nmol/min/mg) | 4.0 | 9.6 | 4.3 | P<0.05 |

+, 1-fold increase; +++, 3-fold increase; ++++, 4-fold increase

Disclosure of Financial Relationships: nothing to disclose

### SA-PO3012

**Primary Glomerular Disease among 1006 Adult Kidney Transplant Recipients: A Single Center Experience** Aaron M. Dommu, Rupesh R. Mehta, Thangamani Muthukumar, Meredith Aull, Surya V. Seshan, Manikkam Suthanthiran. *Cornell University, New York, NY.*

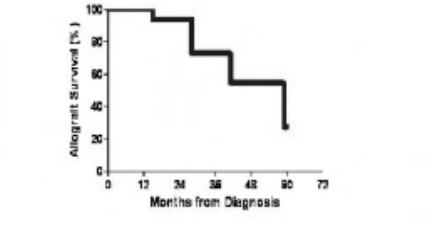
Our purpose was to identify the incidence of primary glomerular disease (PGD) in our transplant center and its impact on graft outcome.

We performed a chart review of all for-cause adult kidney transplant biopsies from our pathology records from 2003-2008 to look for PGD as the diagnosis. Allograft survival was defined as the time from diagnosis of PGD to ESRD. Patients (pts) who did not reach the endpoint were censored at their last follow up.

There were 1006 pts transplanted at our center during 2003-2008. A total of 451 for-cause biopsies were performed on 294 pts (29%). PGD was the 6th most frequent diagnosis (6%), after ATN (25%); Cellular Rejection (19%); CNI toxicity (14%); AMR (11%); and IFTA (7%). In the 19 pts with post-transplant PGD, the diagnosis was made at median of 9.5 months (range: 0-66) after transplantation. The mean (±SD) age was 41±15 years. 47% of the pts received living donor transplants. The median creatinine was 2.20 (range: 0.7-6.3) mg/dl and proteinuria was 4.3 (range: 0.7-22) gm/day. Ten of the 19 pts had native kidney biopsies prior to their transplants.

| Patient | Age | Sex | Race* | ESRD Cause | Donor Type | Allograft Biopsy | Graft Loss |
|---|---|---|---|---|---|---|---|
| 1 | 33 | F | H | FSGS | Deceased | FSGS | No |
| 2 | 20 | M | AA | FSGS | Living Related | FSGS | No |
| 3 | 12 | M | C | FSGS | Deceased | FSGS | No |
| 4 | 60 | M | AA | FSGS | Living Related | FSGS | No |
| 5 | 21 | M | AA | FSGS | Living Unrelated | FSGS | No |
| 6 | 30 | M | C | FSGS | Living Related | FSGS | No |
| 7 | 60 | M | AA | unknown | Deceased | FSGS | No |
| 8 | 18 | M | H | unknown | Deceased | FSGS | No |
| 9 | 60 | M | AA | unknown | Deceased | FSGS | Yes |
| 10 | 45 | M | AA | unknown | Deceased | FSGS | Yes |
| 11 | 36 | M | AA | unknown | Living Related | FSGS | Yes |
| 12 | 28 | F | H | unknown | Living Related | FSGS | No |
| 13 | 31 | F | C | unknown | Deceased | Minimal Change | No |
| 14 | 30 | M | H | Membranous | Deceased | Membranous | No |
| 15 | 40 | M | H | Membranous | Living Related | Membranous | No |
| 16 | 66 | M | C | unknown | Living Unrelated | Post infectious GN | No |
| 17 | 55 | M | C | unknown | Deceased | Post infectious GN | Yes |
| 18 | 44 | F | H | MPGN | Living Related | MPGN | Yes |
| 19 | 40 | M | O | IgM nephropathy | Deceased | IgM nephropathy | No |

* AA-African-American, C-Caucasian, H-Hispanic, O-Other




Five pts (26%) reached ESRD after a median of 40 months from the diagnosis (range: 16-49). The 3 year allograft survival after the diagnosis of PGD for the entire cohort was 73%. One pt died with a functioning allograft. The remaining 13 living pts with a functioning graft had a median creatinine of 1.7 (range: 0.9-5.1) mg/dl and median proteinuria of 0.64 (range 0-7.4) gm/day at most recent follow-up, 25 months (median) from the diagnosis. FSGS was the most common diagnosis and was found in 12 pts (63%).

PGD constitutes 7% of histologic diagnoses among transplant pts who undergo for-cause biopsies, and FSGS is the commonest lesion. A significant proportion of pts with PGD lose their graft.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3013**

**Cigarette Smoking Effects on a Chronic Cyclosporine Nephrotoxicity Model**
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The aim of this study was to assess the effects of cigarette smoking (CS) on chronic cyclosporine (CsA) nephrotoxicity. Male rats were exposed to CS (smoking chamber) or sham procedure (smoking chamber without CS) for 10 min, twice a day for 20 weeks (w). From the 16 to the 20th w rats received low salt diet and on the 17th w they were given CsA 2.5 mg/day or vehicle (VH) for 3 w. Final groups were CS+VH, sham+VH, CsA+CS (CsACS) and CsA+sham (CsA). On day 141, GFR (inulin clearance, ml/min/100g), RBF (Doppler ultrasound, ml/min), RVR (mmHg/ml/min), tubulointerstitial fibrosis (TIF, score 0-4), CsA blood level (ng/ml) and immunohistochemistry for α-SMA, nitrotyrosine (Ny) and vimentin (Vi) were assessed.

Results for rats receiving VH with or without CS were identical, so they were presented as a single group. Data (mean±SE) were analyzed by one-way ANOVA with post-test. CsA caused GFR decrease (0.53±0.05 vs. 0.83±0.15 in VH, p<0.001), which was enhanced by CS (0.39±0.03, p<0.05 vs VH and CsA). CsA alone or with CS decreased RBF (CsA 3.81± 0.59, CsACS 3.91±0.59 and VH 6.68±1.0, p<0.01 VH vs. CsA and CsACS) and increased RVR (26.7 ± 3.2 in CsA, 26.1 ± 2.5 in CsACS and 16.8 ± 3.1 in VH, p<0.001, VH vs CsA and CsACS). CsA promoted TIF, not affected by CS: CsA 0.21 ± 0.08, CsACS 0.17±0.03 and VH 0.03 ± 0.02 (p<0.001, CsA and CsACS vs VH). CS did not affect CsA blood levels (CsA 1888 ± 334 and CsACS 1601 ± 577). CsA enhancement of TI α-SMA was not influenced by CS (CsA 1.7±0.4, CsACS 1.8±0.3 and VH 0.77±0.3, p<0.001, CsA and CsACS vs VH). CS increased CsA-induced α-SMA expression in the periglomerular area (CsA 0.52±0.1 vs. VH 0.27±0.1, p<0.05 and CsACS 1.0±0.4, p < 0.001 vs VH and p<0.01 vs CsA). CsACS rats increased the expression of Ny when compared to CsA and VH (CsACS 2.96±0.3, CsA 2.45±0.2 and VH 2.35±0.4, p<0.01 CsACS vs. VH and p<0.05 CsACS vs. CsA). Only CsA-treated rats expressed VI (CsA 0.49±0.6, CsACS 0.44±0.4 and VH 0).

In conclusion, CS aggravated significantly CsA-induced GFR decrease, periglomerular structural lesion and oxidative stress in a model of chronic CsA nephrotoxicity.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3014**

**The Response of *Klotho* Gene Expression Against Cyclosporine-Induced Renal Injury**
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*Klotho* gene is an antiaging gene which has an antioxidant property. This study evaluated the response of *Klotho* gene expression against cyclosporine (CsA)-induced renal injury with different dosages and durations of CsA treatment.

First, under a low-salt diet, mice were daily administered olive oil (1mg/kg) and CsA (7.5mg/kg, 15mg/kg, and 30mg/kg) subcutaneously for one for 4 weeks. Second, mice were daily administered olive oil and CsA (30mg/kg) for one or 4 weeks. We performed RT-PCR, Western blot and immunohistochemistry for *Klotho* in kidney.

Short-term (1week) treatment with low-dose CsA (7.5mg/kg and 15mg/kg) increased *Klotho* expression in a dose-dependent manner, but long-term (4weeks) treatment with low-dose CsA decreased it. In contrast, treatment with high-dose CsA (30mg/kg) decreased *Klotho* expression in a time-dependent manner.

The response of *Klotho* expression was different against CsA-induced renal injury according to the dosages and durations of CsA. This suggest that adaption occurs in low-dose CsA by upregulating *Klotho* gene, but chronic CsA nephrotoxicity occurs in high-dose CsA by downregulating *Klotho* gene.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3015**

**The Molecular Phenotype of Six-Week Protocol Biopsies from Human Renal Allografts Reflects Prior Injury but Does Not Predict Outcome**
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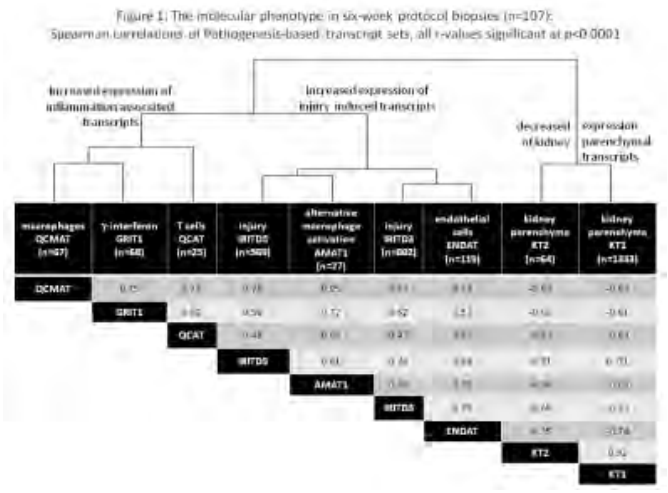
The rationale for protocol biopsies is to detect disease processes earlier, i.e. subclinically and thus putatively at a stage more amenable to treatment. We assessed the molecular phenotype in 107 six-week protocol biopsies from human renal allografts using Affymetrix microarrays. We summarized genome-wide expression data as pathogenesis based transcript sets (PBTs) reflecting inflammation, injury and loss of function in the kidney parenchyma. The molecular phenotype emerged as a robust correlation of increased inflammatory PBTs with increased injury PBTs and decreased expression in transcripts sets associated with normal nephron function.




Correlating this molecular phenotype to clinic-pathological variables before, at, and after the six-week protocol biopsy revealed it to be strongest associated with prior injury i.e. delayed graft function (r=0.41, p<0.001). A significant correlation between the six-week molecular phenotype with progression in interstitial fibrosis / tubular atrophy and persistence of inflammation between six-week and six-month protocol biopsies was found which did not translate into functional deterioration or allograft loss.

We conclude that in renal allografts the six-week subclinical molecular phenotype reflects prior injury to the graft but does not predict future outcome.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3016**

**Early High Dose Erythropoietin in ECD or NHBD Kidney Transplant Recipients Does Not Influence the Rate of Delayed Graft Function**
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Non-heart-beating donor (NHBD) and extended criteria donor (ECD) renal transplant recipients are at increased risk of delayed graft function (DGF) in the post-operative period. Both human and animal models suggest that high dose erythropoietin (EPO) may confer protection from reperfusion injury. However, the efficacy and safety of high doses of EPO in renal transplant recipients has not been established.

We performed a single centre, randomised, double-blind, placebo-controlled trial in 39 recipients of an ECD or NHBD kidney. Patients randomised to the treatment group (n=19) received 3 IV injections of erythropoietin beta (33,000iu each), beginning prior to reperfusion and at 24 and 48 hrs post-operatively. The control group (n=20) received 0.9% saline. DGF was defined as the need for dialysis post-transplantation.

At baseline, there were no differences in patient age, donor characteristics or ischaemic time between the two groups. DGF occurred in 10/19 (52%) EPO recipients and 11/20 (55%) placebo recipients (p=0.88). The median number of dialysis episodes did not differ between groups (1 vs 1.5, EPO vs placebo, p=0.78). Rejection at 3 months occurred in 26% of EPO treated patients and 15% of placebo patients (p=0.28). Renal function was similar in both groups at 3 months (152.4umol/l ± 60.9 *vs* 175.6umol/l ± 83.68, $p$=0.33) and 1year (144.3umol/l ± 54.5 *vs* 150.6umol/l ± 64.6, $p$=0.78). Two deaths with a functioning graft occurred after 6 months in the placebo group due to septic complications. One graft was lost in the EPO group due to severe renal artery stenosis after 9 months. High dose erythropoietin was well tolerated with no drug related adverse events noted. Mean arterial pressure was similar at the end of the 3rd infusion (median 97mmHg for both groups). Haemoglobin levels and EPO usage were similar between the groups at all time points.

High dose erythropoietin administered IV peri-operatively was safe but did not confer protection from delayed graft function or influence graft function at 3 months or 1 year.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3017**

**Urinary Microchimerism Detection Predicts Allograft Injury**
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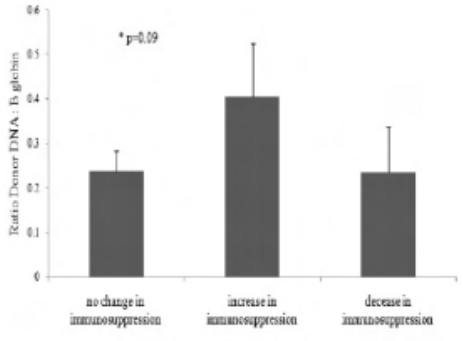
Renal transplantation is the treatment of choice for many patients with end stage renal disease. Significant progress has been achieved in improving short-term outcomes, but long-term graft survival has only marginally improved. One reason for this is a lack of tools to accurately monitor for allograft injury. As transplantation is a form of iatrogenic chimerism, soluble donor DNA released as a result of allograft injury could serve as a quantifiable marker for rejection.

We prospectively recruited renal allograft recipients to provide urine at the time of biopsy procedures. Urine was processed for total genomic DNA and assayed for donor and total DNA by quantitative PCR. Donor DNA was amplified using HLA specific

primers non-shared with the recipient, total DNA with beta globin primers. Using change in immunosuppression as a clinical marker of biopsy outcome, we categorized the donor DNA:total DNA ratios into 3 groups (no change, immunosuppression either increased or decreased).

At this interim, clinical data and urinary chimerism results are available for 60 events in 48 patients. The majority of renal biopsies were performed for cause (n=54). Thirty-three recorded events were associated with no management change; 20, increase in immunosuppression (because of concern for rejection); 7, decrease in immunosuppression. There was no difference in creatinine between the groups. Interestingly, the mean urinary donor DNA:total DNA ratio was 0.24 (sem 0.05) in patients with no change in immunosuppression compared to 0.41(sem 0.12) in patients with increases in immunosuppression, *p*=0.09.




These preliminary results suggest a role for monitoring urinary donor-derived DNA as a marker of allograft injury post transplantation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3018**

**Peritubular Capillary Basement Membrane Multilaminations in Renal Allografts: Typical but Not Pathognomonic for Antibody Mediated Injury** Georgios Liapis,[1] Harsharan K. Singh,[1] Vimal K. Derebail,[2] Tomasz Kozlowski,[3] Volker Nickeleit.[1] *[1]Pathology, UNC, Chapel Hill, NC; [2]Medicine, UNC; [3]Surgery, UNC.*

Peritubular capillary basement membrane laminations (PTCL) in transplants (tx) are thought to indicate chronic antibody mediated rejection. We aimed at determining the diagnostic significance of PTCL.

547 renal biopsies were analyzed (360 native,187 tx). PTCL was evaluated by electron microscopy, and cases with major changes (≥5 circumferential basement membrane layers - PTCL group B) were identified. PTCL-B was correlated with histologic findings/diagnoses and donor specific antibody titers (DSA).

PTCL-B was seen in 6.4% of native kidneys. It significantly correlated only with thrombotic microangiopathies (39% showing PTCL-B changes), and chronic tissue injury ($p<0.05$). In contrast, 36% of transplant biopsies showed PTCL-B ($p<0.05$), in particular late after grafting (5% in year-1, 61% after year-10). PTCL-B was associated with acute and chronic rejection (all $p<0.05$ by univariate analysis): peritubular capillaritis (61%), tx glomerulopathy (64%), glomerulitis (75%), endarteritis (38%), C4d positivity (61%), chronic vascular rejection (67%), elevated DSA (48%), and calcinurin-inhibitor toxicity (CNI 36%). PTCL-B was most tightly correlated with chronic antibody and/or T-cell mediated rejection (38-79% of cases, $p<0.05$). In a logistic regression model, the odds ratio (OR) for PTCL-B was 3.7 in cases with C4d positivity and 3.8 in biopsies taken >24 months post grafting (control group OR 0.24). Using the control group as reference, a diagnosis of CNI toxicity raised the OR for PTCL-B to 11, of acute cellular and/or antibody mediated rejection to 5-16, and chronic inactive or active cellular and/or antibody mediated rejection to 12-76.

PTCL-B is mainly found in tx biopsies post year-1 and in chronic active T-cell and/or antibody mediated rejection. Although PTCL-B is typical for an antibody mediated alloresponse, it is not pathognomonic. Between 21% and 62% of chronic rejection episodes do not show PTCL-B. However, if present, PTCL-B can serve as an adjunct marker of "rejection" and help avoid diagnoses of "CAN" or "IFTA".

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3019**

**Non-Immunological Factors and Non-Classical HLA Class I in Chronic Allograft Injury** Hitoshi Yokoyama. *Division of Nephrology, Kanazawa Medical University, Uchinada, Ishikawa, Japan.*

Background. Although the risk for morbidity and mortality is studied in subjects with renal transplantation, there are limited data to access the allograft survival in dyslipidemia, adiponectins and non-classical HLA class I. Methods. We investigated the alteration of estimated glomerular filtration rate (eGFR) based on the new 3-variable GFR-estimating equation for Japanese (194 x Serum Creatinine $(SCr)^{-1.094}$ x $Age^{-0.287}$ x 0.739, if female), and factors affecting the eGFR. We studied 120 adult subjects (age 19- to 72-year old; 77 males, 43 females; 23 cadaveric donors) for since January 2004. We examined clinical backgrounds, HLA matching, ischemic times, treated drugs, blood pressure, body mass index, and blood chemistry including lipids, total and high-molecular adiponectin (ADPN) levels, and circulating HLA-G5. Results. During the study periods, 5 years renal survival rate was 89.2%, and 15 subjects dropped out, because of end-stage renal failure in 6, death with a functioning kidney in 7, and moving in 2. Initial mean eGFR was 50.3 ml/min/1.73 $m^2$. The alteration of eGFR (ΔeGFR) was at -2.2 ml/min/1.73 $m^2$/year, which was higher in living donors than those of cadaveric donors (-2.8 vs. 0.1 ml/min/1.73 $m^2$/year, $p=0.002$). In single analyses, both LDL-C/HDL-C ratio below 2.0 and statin treatment decreased ΔeGFR at -1.4 and -1.0 ml/min/1.73 $m^2$/year, respectively. Serum high-molecular ADPN levels were reversely correlated with both eGFR and ΔeGFR (-0.240, $p=0.013$; -0.210, $p=0.031$, respectively). On the other hand, higher circulating HLA-G5 was selected as a renoprotective factor as well as statins in multivariate regression analysis (0.213, $p=0.038$; 0.258, $p=0.013$, respectively). The estimated equation of ΔeGFR (ml/min/1.73 $m^2$/year) was -6.710 + 2.138 x [donor type] (if cadaveric donor, 2; if living donor, 1) + 1.941 x [statin] (if treated by statin, 1) + 0.010 x [HLA-G] (serum HLA-G5 levels, U/ml) ($r=0.478$, $p<0.001$). Conclusion. Higher circulating HLA-G5 and statin could preserve the renal function judged by ΔeGFR in Japanese transplanted subjects, however. Total and high-molecular ADPN levels were reversely correlated with eGFR and ΔeGFR reported as an ADPN paradox.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3020**

**Everolimus (E) vs Enteric Coated Mycophenolate Sodium (EC-MPS): Proteomic and Pathologic Aspects** Elisabetta Bertoni,[1] Gianmarco Ghiggeri,[2] Marco Prunotto,[2] Solange Moll,[2] Maurizio Salvadori.[1] *[1]Renal Unit, Careggi University Hospital, Florence, Italy; [2]Division of Nephrology, Gaslini Hospital, Genova, Italy.*

Recent observations ascribe proteinuria after PSI use to apoptosis both of tubular and podocyte cells. Aim of this study was to compare by proteomic approach and protocol biopsies the effect of E vs EC-MPS in renal transplant (tx) recipients.

In a prospective, randomized, monocentric study 19 renal tx patients were enrolled to receive either E (8) or EC-MPS (11). E patients received high dose everolimus (C0 8-10 ng/ml) with CyA very low dose and steroids. The other group received EC-MPS (720 mg bid) with CyA standard dose and steroids. Urinary parameters were followed using proteomics for 1 year (quantitative assays, two dimensional electrophoresis, MALDI-TOF, Western blot).A protocol biopsy was performed at day 15 after tx. Immunochemistry for CD3,CD68, VEGF-A,Ki67 was performed. Apoptosis was evaluated by TUNEL. CD3,CD68,Ki67 expression was evaluated through computerized morphometrical analysis.

EC-MPS patients had higher DGF rate:RR=1.43, acute rejection rate :RR=1.41, lower eGFR at 1 year (66.18±4.5 vs 79.25±9.9 ml/min). E patients presented an increase of proteinuria reaching the highest level 15 days after tx ($p<0.03$).Proteomics showed in E patients an unselected increment of the high/medium and low molecular weight proteins. Biopsies in EC-MPS group showed a moderate/severe inflammation of lymphocytes and histiocytes. Expression of CD3 and CD68 on total area evaluated by morphometry was lower in E patients than EC-MPS patients: CD3 was 1.9±0.4% vs 4.4±0.5%,$p<0.05$ and CD68 was 5±1% vs 14±2%,$p<0.05$. Ki67 revealed a higher proliferation of tubular cells in the EC-MPS group: 2±1.3% vs 0.2±0.3% of total tubular cells in E patients;$p<0.05$. TUNEL showed a large number of glomerular and tubular apoptotic cells in the E group.

Our data confirm: a)E therapy with low CyA dose is safe and effective; b)EC-MPS vs E is associated to a higher RR of DGF and BPAR; c)Proteinuria in E group reversed after the first month to nornal levels; d)Biopsies documented a higher apoptosis in E patients as possible cause of proteinuria and higher immunological reactivity in EC-MPS patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3021**

**Renal Allograft Loss in the First Post-Operative Month: Causes and Consequences** Paul J. Phelan, James Lineen, Frank J. O Brien, Peter J. Conlon. *Department of Nephrology, Beaumont Hospital, Dublin, Ireland.*

Early transplant failure is a devastating outcome after kidney transplantation. We report the causes and consequences of deceased donor renal transplant failure in the first 30 days at our centre between January 1990 and December 2009.Controls were adult deceased donor transplant patients in the same period with an allograft that functioned greater than 30 days. Multi-factorial analysis was performed to test for independence of early allograft failure on patient outcome in the presence of several confounding variables. The incidence of early graft failure in our series of 2381 consecutive deceased donor transplants was 4.6% (n=109). The causes of failure were allograft thrombosis (n=48; 44%), acute rejection (n=19; 17.4%), death with a functioning allograft (n=17; 15.6%), primary non-function (n=14; 12.8%) and other causes (n=11; 10.1%). Mean time to allograft failure was 7.3 days. There has been a decreased incidence of all cause early failure from 7% in 1990 to <1% in 2009. Patients who developed early failure were older and had longer cold ischaemia times when compared to patients with allografts lasting > 30 days. Early allograft failure was strongly associated with reduced patient survival ($p<0.001$). In conclusion, early renal allograft failure is associated with a survival disadvantage but has thankfully become less common in recent years.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3022**

**Association between Interleukin 3 Gene Polymorphisms and Acute Rejection Following Kidney Transplantation** Dongyoung Lee,[2] Se-Bin Song,[1] Ju-Young Moon,[2] Kyung-Hwan Jeong,[1] Chun-Gyoo Ihm,[1] Tae Won Lee.[1] *[1]Internal Medicine, Division of Nephrology, KyungHee University Medical Center, Seoul, Republic of Korea; [2]Internal Medicine, Division of Nephrology, KyungHee East-West Neo Medical Center, Seoul, Republic of Korea.*

Acute rejection (AR) after kidney transplantation resulting from alloimmune responses has a negative effect on graft survival. AR is mainly caused by T-cell immune responses in renal allograft and many cytokines contributes to the activation of T-cell. Interleukin (IL) 2, 4, and 7 are well known cytokines related to AR. Many reports showed that single nucleotide polymorphisms (SNPs) of these cytokines can affect the occurrence of AR. IL3, which is secreted by activated T cells, contributes to T-cell activation and can mediate AR in kidney transplantation. This study aimed to investigate the association between SNPs of *IL3* and the occurrence of AR because there is no report about that. We analyzed three SNPs of *IL3* (rs181781, rs2073506, and rs40401) among 330 renal recipients, 60 of whom had developed AR. SNPs of *IL3* gene are one exonic SNP (rs40401) and two regulatory SNPs (thought to be the promoters, rs181781; rs2073506). The genotyping of the 60 AR patients and the 270 patients without AR demonstrated a significant relationship between genotype frequencies and the SNPs. The occurrence of AR was associated with rs181781 ($P$ = .021, codominant model; $P$ = .041, dominant model), rs2073506 ($P$ = .001, codominant model; $P$ = .0012, dominant model), and rs40401 ($P$ = .018, recessive model). Among haplotypes, a haplotype (AAT) showed a significant association with AR ($P$ = .0033). Our results suggest that *IL3* gene polymorphisms are associated with the development of AR.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3023**

**Blood Oxygen Level-Dependent MR Imaging (BOLD MRI) Early Post Kidney Transplantation Could Predict Acute Renal Rejection and Allograft Function** Ying Xu, Fei Han, Jianghua Chen. *The Kidney Disease Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.*

**Purpose:**

Our previous study has concluded that blood oxygen level-dependent magnetic resonance imaging (BOLD MRI) could be a valuable method to discriminate between acute rejection and acute tubular necrosis by measuring tissue oxygen bioavailability in early kidney allograft dysfunction [1]. Mean R2* values in the medullary regions (MR2*) of transplanted kidneys with BOLD-MRI could represent the oxygenation state of renal allograft. This study was designed to identify the significance of BOLD MRI in prediction of acute renal rejection and long-term allograft function.

**Methods:**

Eighty-two patients with normal functioning grafts underwent BOLD MRI within 2 to 3 weeks post kidney transplantation. According to our previous results, they were divided into two groups: lower medullary R2* value group (MR2*<14.9/sec, n=23) and higher medullary R2* value group (MR2*>14.9/sec, n=59). The clinical data during the follow-up time (45.9±6.9 months) were recorded from our electronic database.

**Results:**

The baseline data of two groups had no significant difference. Patients with lower medullary R2* values had higher acute rejection rates than those with higher medullary R2* values in the first 6 months post transplantation, but the difference between two groups was not significant (17.39% vs 8.47%, P=0.259). The simplified equation of MDRD calculated estimated Glomerular filtration rate (eGFR) at the end of follow-up was inferior in lower medullary R2* value group than higher medullary R2* value group (77.14±22.76 ml/min vs 89.29±24.02 ml/min, P=0.037).

**Conclusions:**

Blood oxygen level-dependent MR imaging (BOLD MRI) could predict acute renal rejection and long term allograft function for patients with normal functioning grafts early post kidney transplantation.

**Reference:**

1.Han Fei, Xiao Wenbo, Xu Ying, Wu JY, Wang QD, Wang HP, Zhang MM, Chen JH. The significance of BOLD MRI in differentiation between renal transplant rejection and acute tubular necrosis. Nephrol Dial Transplant 2008, 23(8): 2666-72.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3024**

**Impact of Luminex Solid Phase Screening on Recipient Selection and Renal Allograft Outcomes** Abdelaziz A. Elsanjak, Nabil Mohsin, Isabelle G. Wood, Helen Mah, Nidyanandh Vadivel, Edgar L. Milford, Anil Chandraker. *Brigham & Women's Hospital, Boston, MA.*

Aim: The aim of this study was to identify whether allocation of deceased donor kidneys by luminex solid phase screening of renal transplant candidates leads to a reduction of rejection episodes and an improvement in allograft function at one year.

Methods: Charts of 145 deceased donor kidney allograft recipients, transplanted between 12/2004 and 09/2009 were reviewed retrospectively. At the time of transplant, complement-dependent cytotoxic T-cell screening was used for 101 patients (transplanted between 12/04 - 04/07) and luminex solid phase screening technique was used for 44 patients (transplanted between 05/07-09/09). Of the 101 patients transplanted prior to adoption of luminex solid phase screening,73 stored pre-transplant sera sample were available for re-screening by luminex.

Result: Twenty five percent of patients (25 of 101) of the pre-luminex patients who underwent deceased donor renal transplantation had an episode of acute allograft rejection within one year, compared to 9% (4 out of 44) of patients in the post-luminex period. Of the 73 samples available in the pre-luminex patients only 7 had a rejection episode within one year (28 other samples including 18 patients with rejection were unavailable). Out of these 7 patients with allograft rejection, 4 had class I donor specific antibodies by luminex. However 12 (18%) out of 66 patients who were free from rejection were also noted to have class I DSA. The mean 1 year creatinine of pre luminex screened kidney transplant recipients was 2.0 mg/dl compared to 1.75 mg/dl in post luminex kidney transplant recipents.

Conclusion: We conclude that the introduction of luminex solid phase screening as a method for allocation for deceased donor kidney transplants, significantly reduced one year renal allograft acute rejection rates, and improved overall allograft function. It should however be noted that had luminex solid phase screening been in use in our center during the period 12/2004 through 5/2007, 18% of the 66 patients who were rejection free within the first year of transplantation would have been excluded from transplantation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3025**

**Secondary Hyperparathyreoidism in Renal Transplant Recipients** Christian Marx,[1] Martin Busch.[2] *[1]Nephrological Center and Dialysis, Nordhausen, Germany; [2]Internal Medicine/ Nephrology, Friedrich Schiller University, Jena, Germany.*

**Introduction:** The calcimimetic Cinacalcet may be used to treat secondary hyperparathyreoidism occuring during chronic kidney disease (CKD). After kidney transplantation (TX) hyperparathyreoidism often persists or relapses. Presently there is little data on the off-label use of cinacalcet in these patients. **Patients and methods:**We retrospectively examined the course of intact parathyroid hormone (iPTH), the occurrence of hypercalcemic episodes and prescription of cinacalcet in 54 renal transplant recipients (18 females, 36 males) over a period of 36 months after TX. **Results:** At the time of TX, the K/DOQI- target values for iPTH in patients with CKD-stage 5 (150-300ng/l) were only met in 12/ 54 patients (22%). 5 patients had very low iPTH-levels (<35ng/l). 11/ 54 patients (20%) were partially or totally parathyreoidektomized. 3 of them developed iPTH-levels of >300ng/l within 3 years after TX. Patients with an iPTH of 150-300 ng/l at time of TX were 14-fold more likely to have an iPTH of >300ng/l after TX compared to patients with iPTH <150ng/l at the time of TX. Patients with an iPTH of >300ng/l at the time of TX were 20-fold more likely to have a persisting iPTH of >300ng/l after TX. The probability to develop hypercalcemia and the need for cinacalcet treatment were 8- and 11-fold increased after TX in those having an initial iPTH >300 ng/l compared to patients with iPTH <150ng/l. **Discussion:** Patients reaching the K/DOQI- target values for iPTH are at risk for a worsening of hyperparathyreoidism. Therefore for patients on the waiting list new target values could be necessary. Already prior to TX a straight therapy of hyperparathyreoidism is required. Hyperparathyreoidism may also relapse after parathyreoidectomy in transplant recipients. The treatment with drugs such as cinacalcet is a safe and effective option in transplant recipients with hypercalcemia and hyperparathyreoidism.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3026**

**Intravenous Iron Supplementation for Kidney Transplant Recipients** Benaya Rozen-Zvi, Uzi Gafter, Ruth Rahamimov. *Neprology and Hypertension Department, Rabin Medical Center, Petah Tikva, Israel.*

Background:

Iron deficiency is common among kidney transplant recipients (KTR), but data concerning the efficacy and safety of intravenous (IV) iron in this population are sparse. We aimed to evaluate the effect of IV iron in a cohort of KTR who were treated with at least one dose of IV Iron sucrose.

Methods:

Data from KTR who had been treated with IV iron sucrose were retrospectively analyzed. Hemoglobin level was determined at the time of the first iron dose infusion and at three month to evaluate efficacy. The efficacy analysis included only patients who were not treated with erythropoietin or were treated with a stable dose for at least 3 months. Safety data included adverse events, hospitalizations infections and creatinine values before and after the IV iron treatment. Data are presented as mean ±SD. Paired t-test was used for comparisons.

Results:

Between 1/2000 and 12/2009 eighty four patients were treated with 709 doses of IV iron, with average dose of 766±363 mg at 3 month. Mean age was 49.9±14.1 years and 83% had eGFR less than 60 ml/min/1.73 $m^2$. Mean transferrin saturation (TSAT) was 13.9±8%, 81% had TSAT lower than 20%. Five episodes of adverse events were reported, all resolved without treatment. There were no reports of anaphylactic reactions and all patients resumed treatment with IV iron without further events. In the 60 patients who were included in the efficacy analysis hemoglobin level rose from 10.01±1.42 gr/dL to 11.24±1.56 mg/dL at 3 months ($p<0.001$). Creatinine levels were stable at three month (2.36±1.23 to 2.42±1.5 ($p=0.40$)) and there was no increase in hospitalizations and infections.

Conclusion:

IV iron was safe and effective in KTR with iron deficiency and anemia. To the best of our knowledge, this is the largest series of IV iron treatment in kidney transplant recipients. Controlled trials should evaluate IV iron in this important and distinctive group of patients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3027**

**Absence of Influence of Polymorphism -308A *TNFA* on Renal Graft Histopathologycal Findings in Mexican Mestizo Adults One Year after Transplant** Jose De la Cruz Moreira-Hernandez,[1] Caridad Aurea Leal,[2] Francisco Ramos Solano,[1] Eliseo Portilla-de Buen,[2] Benjamin Gomez-Navarro.[1] *[1]Nephrology and Transplant, Hospital de Especialidades del Centro Medico de Occidente, IMSS, Guadalajara, Jalisco, Mexico; [2]Biomedical Research Center West, Hospital de Especialidades del Centro Medico de Occidente, IMSS, Guadalajara, Jalisco, Mexico.*

BACKGROUND: Despite improvements in medical and pharmacological management chronic allograft nephropathy (CAN) remains the main cause of graft loss. Tumor necrosis factor alpha (TNFa) plays a central role as proinflammatory molecule. We analyzed the possible influence of polymorphism -308A of gene *TNFA*, which is known to produce high levels of TNFa, on the presentation of acute rejection or CAN currently interstitial fibrosis and tubular atrophy.

OBJECTIVE: To explore if the presence of high-production *TNFA* genotype or alleles in renal graft recipients is associated to acute rejection or CAN one year after transplant in a defined population.

MATERIALS AND METHODS: A cross-sectional study in 131 Mexican mestizo patients (89 male) 16 years or older who received a related living donor graft between January 2008 and March 2009. All had pre-implant biopsy. Variant -308A of gene *TNFA* was genotyped using allele-specific polymerase chain reaction. Clinical data were obtained from patient charts and the effect of the polymorphism on acute rejection or CAN was analyzed with Pearson´s and Spearman´s correlations, adjusting for demographic, biochemical and clinical variables.

RESULTS: At one year after transplant, 41 (31.0%) patients had acute rejection and 62 (47.3%) had calcineurin inhibitor toxicity. Overall, 57 (43.5%) patients showed CAN, but with mean creatinine 1.27 ± 0.79 mg/dl and mean GFR measured by creatinine clearance 77.32 ± 22.67ml/min. No influence of the polymorphism was found on any of the variables studied. Correlation with CAN was only found for calcineurin inhibitor toxicity ($r = 0.202$, $p = 0.05$) and acute cellular rejection ($r = 0.285$, $p = 0.005$).

CONCLUSIONS: Polymorphism -308A*TNFA* has no influence on acute rejection or chronic graft nephropathy one year after transplant in Mexican mestizo patients from Western Mexico.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3028**

**Is It Useful To Study Renal GFR and ERPF Determined by Renal Scintigram in the First Week To Predict Kidney Graft Function?** Nuno Figueiredo. *Nephrology, Hospitais Universidade Coimbra, Coimbra, Portugal.*

Introduction-The evaluation of glomerular filtration rate (GFR) and effective renal plasmatic flux (ERFP) are important methods in the management kidney transplant recipients.

Methods-From January to October of 2009, a renal scintigram was performed in the first week post-transplant in 63 patients, and the GFR and the ERPF were determined by DTPA-Tc99 and Tc99-MAG-3 respectively. They were followed up until 31/05/2010. Then we assessed the correlation between the observed GFR and RPF determined in the first week and the serum creatinine at the present time.

Results: Sample characterized by 63 kidney transplanted patients, with a mean age of 50,55 +/- 13,46 years, 97,1% were Caucasian and 2,9% Africans. The mean donor age was 52,48+/-16 years, 62 transplants were from deceased donors and 54,3% were from extended criteria donors(ECD). The mean cold ischemia time was 16,75+/- 4,9 hours. The mean HLA match was 2(28,6%), with a minimum of 0 in 8,6% and a maximum of 5 in 4,3%. About 55,6% received induction (36,5% Thymoglobulin and 19,1% Basiliximab). All had a calcineurin inhibitor (61,9% FK and 38,1% cyclosporine). Among the 63 patients, it was only possible to assess Tc99-MAG-3 ERPF in 46 patients. The mean DTPA-Tc99 GFR was 56,7 +/-29 ml/min/1,73M2. The overall mean ERPF was 344+/- 199.4 ml/min/1,73M2. At the present time the mean serum creatinine is 1,42 +/- 0,77 mg/dL. In our results there was no correlation between ERPF and the serum creatinine at the present time. We also did not find any correlation with the donor age, cold ischemia time or ECD ($p>0.05$). However we found a strong negative correlation between DTPA-Tc99 GFR with serum creatinine at the present time ($p<0.05$, Spearman coefficient factor of -0.455) and donor age ($p<0.01$, Pearson coefficient factor of -0.529).

According to our results the assessment DTPA GFR in the first week seems to be a good predictor of graft function however the same conclusion was not observed for effective renal plasmatic flux.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3029**

**Effect of Donor Vasopressor Support on Renal Allograft Function** Prasanna S. Srinagesh, William Yang, Aniruddha V. Palya, Parmish Lalit Kohli, Alden Michael Doyle, Karthik M. Ranganna. *Medicine, Drexel University College of Medicine, Philadelphia, PA.*

The outcome of cadaveric renal allografts procured from donors requiring inotropic support has been a subject of controversy. Prior studies have shown poorer outcomes in renal allografts obtained from donors requiring inotropic support, whereas one recent study reported beneficial effects on acute allograft rejection, and graft survival after cadaveric renal transplantation. Our study aims to determine whether the number of inotropic agents required by the donor prior to procurement affects both the short and long term outcomes of the renal allograft.

We reviewed data on recipients who recieved cadaveric renal transplantation from 2000 to 2004. There were 134 recipients from donors who were on donor inotropic support ranging from the use of one agent to three or more. Kidney function based on serum creatinine levels was followed at regular intervals. At 1 month follow up, serum creatinine was elevated in those recipients who received renal allografts from donors requiring 3 or more inotropic agents, compared to those who required inotropic support with only one agent. At 3 months post-transplantation, serum creatinine levels corresponded very closely regardless of the number of inotropic agents used in the donor. The difference in kidney function was not significant at 12 month follow-up.

| | Number of Pressors 1 | Number of Pressors 2 | Number of Pressors 3 | P value |
|---|---|---|---|---|
| Recipient Age M:F | 61.2 : 58.2 | 51.9 : 50.1 | 51.3 : 54.7 | |
| Gender M: F | 40 : 18 | 42 : 10 | 18 : 6 | |
| Donor Start Creatinine | 1.38±0.9 | 1.2±0.82 | 1.15±0.7 | 0.002 |
| Donor Final Creatinine | 2.30±2.29 | 1.57±1.4 | 1.71±1.0 | 0.106 |
| 1 Month | 2.95±2.45 | 2.84±2.56 | 3.24±3.38 | 0.85 |
| 3 Month | 2.22±1.6 | 2.13±1.7 | 2.31±1.42 | 0.90 |
| 6 Month | 2.08±1.31 | 2.24±2.29 | 2.16±1.31 | 0.91 |
| 12 Month | 2.22±1.63 | 1.93±1.01 | 1.88±0.8 | 0.46 |
| DGF M : F | 23 : 12 | 23 :4 | 13 : 5 | 0.18 |

Our study concludes that although initial kidney function may be lower in the initial post-transplantation period in those requiring more inotropic support, long term outcomes in these groups were relatively similar.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3030**

**Light Microscopic Features Do Not Distinguish between C4d Negative and C4d Positive Biopsies with Advanced Transplant Glomerulopathy** Kuang-Yu Jen, Jean L. Olson, Zoltan G. Laszik. *Department of Pathology, University of California San Francisco, San Francisco, CA.*

**Background:** Chronic antibody-mediated rejection with transplant glomerulopathy (TxGP) is an increasingly recognized cause of late allograft loss. Although the pathologic changes of TxGP are well characterized it is still uncertain whether light microscopic morphologic findings in patients with advanced C4d-negative TxGP reveal any distinguishing features from those with C4d positivity.

**Design:** The aim of the study is to quantitatively assess morphologic variables in 20 consecutive transplant kidney biopsies featuring advanced TxGP (Banff 07 cg3) with first time TxGP diagnosis. Eleven C4d positive and 9 C4d negative biopsies were scored for glomerular, tubulointerstitial, and vascular lesions according to the recommendations of Banff 07 Classification of Renal Allograft Pathology. The percentage of glomerular capillary double contours (CDC) in the most severely affected glomerulus was also recorded in each case. In addition, the proportion of non-globally sclerotic glomeruli featuring TxGP was calculated. The morphological findings in the C4d negative and C4d positive biopsies were correlated with each other and also with relevant clinical variables.

**Results:** Demographic and clinical variables, including post transplantation time and serum creatinine levels were similar in the C4d negative and C4d positive groups. None of the morphological variables of the Banff classification were statistically different in the two groups. Furthermore, the proportion of the non-globally sclerotic glomeruli affected by TxGP was similar between the two groups. However, the extent of the glomerular CDC (97.2%) in the most severely affected glomeruli in the C4d negative biopsies was significantly higher than in the C4d positive biopsies (69.5%) ($p=0.01$).

**Conclusion:** Our results indicate that the light microscopic features are highly similar in transplant biopsies showing advanced TxGP with and without C4d positivity. The difference observed in the extent of glomerular CDC in the two groups should be verified in a larger cohort of biopsies.

Disclosure of Financial Relationships: nothing to disclose

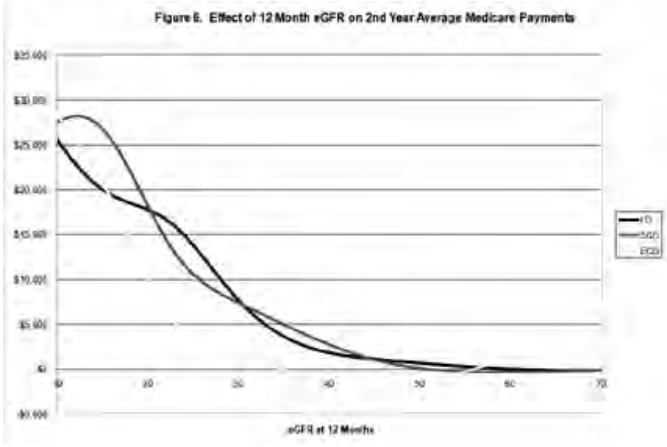
**SA-PO3031**

**Impact of Renal Function on Health Care Costs by Donor Type Following Kidney Transplantation in Medicare Patients** Mark Schnitzler,[1] Digisha Trivedi,[2] Krista L. Lentine,[1] Gilbert J. Litlaien.[2] *[1]Saint Louis University; [2]BMS.*

**Purpose:** To determine effects of renal function on cost by donor type.

**Methods:** The study included all recipients of single-organ kidney transplants in the United States between 1995 and 2003 recorded in the United States Renal Data System (USRDS) database. The predictor variable was renal function as measured by estimated Glomerular Filtration Rate (eGFR), calculated using the abbreviated Modification of Diet in Renal Disease equation including serum creatinine, age, gender and race. Estimated GFR in mL/m per 1.73 m2 was considered as a continuous variable at 1 year post-transplant. The total cost measure was actual payments made by Medicare for all health care services and was evaluated at yearly intervals up to three-years post-transplant. Linear regression was used to develop separate models for total costs during the second and third years post-transplant for each donor type.

**Results:** There were 32,681 LD, 49,551 SCD, and 5,343 ECD kidney transplant recipients included. Lower eGFR was a statistically significant predictor of increased cost of care during the second year post-transplant for each donor type (LD, $p<0.0001$; SCD, $p<0.0001$; ECD, $p=0.0001$) (See Figure). Similar eGFR/cost relationships were observed in the third post-transplant year for LD ($p<0.0001$) and SCD ($p<0.001$)While the . ECD eGFR/cost relationship was similar in the third post-transplant year as well, it was not statistically significant. The lack of significance for ECD is likely due to the skewed nature of cost data and small sample size.




**Conclusions:** In the three-year post-transplantation, there is a strong relationship between renal function as measured by eGFR and health care costs. This relationship is similar across donor types. Strategies that help patients maintain their eGFR at higher levels may provide cost savings to society.

Disclosure of Financial Relationships: Consultancy: BMS; Ownership: Xynthisis, LLPResearch Funding: Novartis, BMS.

### SA-PO3032

**The Complete Correction of Post-Transplant Anemia Improves Graft Survival: Results of a Multicentre Randomized Study** Gabriel Choukroun,[1] Nassim Kamar,[2] Bertrand Dussol,[3] Isabelle Etienne,[4] Azmi Al Najjar,[5] Philippe Lang,[6] Antoine Thierry.[7] *[1]SUD Hospital; [2]Rangueil Hospital; [3]Conception Hospital; [4]Bois Guillaume Hospital; [5]Bretonneau Hospital; [6]Henri Mondor Hospital; [7]Miletrie Hospital, France.*

Retrospective studies suggested that chronic allograft nephropathy (CAN) might progress more rapidly in pts with post-Tx anemia. CAPRIT is a multicentre, randomized study, to investigate the effect of SC epoetin beta to normalize Hb values (target Hb 13.0-15.0 g/dl, Gr A, n = 63), compared with partial correction of anemia (target Hb 10.5-11.5 g/dl, Gr B, n = 62) on the progression of CAN.

125 pts (mean age 48.9 yrs, 61M) were included. They were on Tx for at least 12 months, with a Hb less than 11.5 g/dl and an estimated GFR (MDRD formula) between 50 to 20 ml/min at inclusion. Baseline Hb level and eGFR were similar in both groups, 10.4 ± 0.9 g/dl and 10.6 ± 0.7 g/dl for Hb, 34.4 ± 10.0 ml/min and 33.7 ± 10.8 ml/min for eGFR in Gr A and B. After 12 months, Hb level was 13.4 ± 4.1 g/dl in Gr A, 11.2 ± 0.9 g/dl in Gr B ($p < 0.001$) ; 12.9 ± 2.1 g/dl and 11.3 ± 1.1 g/dl respectively at the end of the 2-yrs follow-up ($p < 0.001$). At one yr, eGFR was significantly higher in Gr A (35.9 ± 17.2 vs 30.8 ± 12.1 ml/min, $p < 0.025$). This difference was also significant after 2 yrs (32.6 ± 12.7 vs 27.9 ± 13.7 ml/min; $p < 0.05$). 16 pts reach ESRD before the 2 yrs follow-up, 3 in Gr A and 13 in Gr B ($p < 0.01$). 4 pts died during the study, 1 in Gr A and 3 in Gr B. The Kaplan-Meier cumulative death-censored graft survival was 20 % higher in Gr A ($p < 0.01$). At the end of the study, 89 % of pts in Gr A and 61 % of pts in Gr B were on epoetin beta. Weekly doses were 5600 UI and 4600 UI respectively, by SC. There was no significant difference in the incidence of adverse events between groups and no severe side effect was related to the treatment by epoetin beta. The number of cardiovascular events was low and similar between groups.

This prospective randomized study is the first to demonstrate that targeting a serum Hb level of 13 g/dl or above had a nephroprotective effect in kidney transplant recipient.

Disclosure of Financial Relationships: nothing to disclose

### SA-PO3033

**3 Year Results of a Randomised Controlled Trial of Alemtuzumab and Low Dose Tacrolimus Monotherapy Compared with Daclizumab, Tacrolimus and Mycophenolate Mofetil** Kakit Chan, Jack W. Galliford, Dawn Goodall, David Taube, Adam Mclean. *Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London.*

In this study, we present the 3 year results of our randomised controlled trial [(RCT) Clinical Trials.gov: NCT00246129] of Alemtuzumab induction and low dose Tacrolimus [Tac] monotherapy, compared with Daclizumab induction, conventional dose Tac and Mycophenolate Mofetil [MMF] in renal transplantation.

Recruitment into this trial was completed in April 2008 and mean follow up is 37.5 months.

82 patients [54m, 28f; mean age 47.3+13.4 years] received Alemtuzumab, low dose Tac [0.1mg/kg; target level 5-8 ng/mL] and 41 patients [27m, 14f; mean age 47.0+10.6 years] received Daclizumab, Tac [0.15mg/kg; target level 8-12 ng/mL] and MMF [target level: 1.5-3.0 mg/L]. Both groups received our steroid sparing regime [prednisolone 60mg daily day 1-3; 30mg daily day 4-7 and then stopped] .

Rejection was diagnosed by biopsy and treated with steroids and the addition of MMF to the Alemtuzumab group.

Table 1 shows that patient, graft and rejection free survival were similar in the 2 groups. There was no difference in the cumulative risk of rejection and no increased late rejection in the Alemtuzumab group.

Allograft function [MDRD eGFR] was 5.6 ml/min [95%CI: 2.3,8.9; p=0.001] and 4.4 ml/min [95%CI: 2.0, 6.7, p<0.001] better at 1 and 3 years. [Bootstrap method] in the Alemtuzumab group

75.4% and 69.7% of the Alemtuzumab group remained on Tac monotherapy at 1 and 3 years.

Infection rates [positive bacterial and viral isolates, expressed as incidence/100 patient months] were similar in both groups.

This RCT shows that at 3 years, Alemtuzumab induction and low dose Tac monotherapy provides excellent patient and allograft, rejection and infection free survival similar to a conventional Daclizumab, Tacrolimus and MMF protocol.

Furthermore, allograft function is significantly better in the Alemtuzumab, low dose Tac group.

| | | Alemtuzumab | Daclizumab |
|---|---|---|---|
| Patient Survival | 3 year | 97.4% | 97.5% |
| Graft survival | 3 year | 92.2% | 97.6% |
| Rejection free survival | 3 year | 88.5% | 79.5% |
| MDRD eGFR [Mean±1SD] | 3 year | 52.0±15.1 | 47.6±18.4 |
| Infection free survival | 1 year | 70.0% | 60.0% |

Disclosure of Financial Relationships: nothing to disclose

### SA-PO3034

**African-American vs. Non-African American *De Novo* Renal Transplant Recipients Receiving Everolimus Versus Mycophenolate: Results from a Pooled Analysis** Fuad S. Shihab,[1] Diane M. Cibrik,[4] S. P. Mulgaonkar,[2] Dharmesh Patel.[3] *[1]University of Utah, School of Medicine; [2]Saint Barnabas Medical Center; [3]Novartis Pharmaceuticals Corporation; [4]University of Michigan.*

African-american (AA) de novo renal transplant recipients (RTs) are considered at higher risk for graft loss and rejections than non-African-americans (nonAA). Everolimus (EVR) comparative risk assessment of AAs vs. non-AAs between EVR and MPA is warranted.

Data on 2004 RTs from three EVR studies B201(N = 588), B251(N = 583), and A2309 (N = 833), were analyzed to identify impact of AA vs. non-AA on efficacy outcomes across both EVR dosing groups and mycophenolate (MPA) control groups. EVR groups received either 1.5 mg/day, or 3 mg/day; with either standard (SD-CsA) or reduced dose cyclosporine (RD-CsA). All control groups received MPA (1.44g/day) with ST-CsA. Composite efficacy end-point for the pooled analysis was graft loss, death, treated biopsy-proven acute rejection, or lost to follow up. Odds ratios (ORs) were calculated using a logistic regression model with treatment, study and AA (Yes or No) as independent variables.

MPA-treated RTs who experienced a composite end-point were 44.6% vs 30.7% for AA vs. non-AA subgroups respectively. EVR 1.5 mg-treated RTs who experienced a composite endpoint for AA vs. nonAA, similarly, were 44.1% vs. 31.2%; for EVR 3.0 mg values were 41.2% and 31.4%.

Likelihood of a Composite End-Point Event based on Treatment or AA vs nonAA

| | Odds Ratio (OR) | 95% C.I. | P-value |
|---|---|---|---|
| EVR 1.5 vs. MPA | 1.019 | 0.8090, 1.2835 | NS |
| EVR 3.0 vs. MPA | 1.0077 | 0.8006, 1.2684 | NS |
| AA vs. non-AA | 1.7915 | 1.3515, 2.3748 | P<0.0001 |

In this pooled data analysis in over 2000 RTs EVR treatment vs MPA resulted in similar composite end-point incidence events across AAs and non-AAs confirming no treatment effect. This result is expected as these studies were powered for non-inferiority. However, overall risk for experiencing a composite end-point in AA RTs was significantly higher compared to non-AA RTs. This pooled analysis confirms that AA recipients are at higher risk for poorer efficacy outcomes, irrespective of treatment.

Disclosure of Financial Relationships: Employer: University of UtahResearch Funding: Novartis; Honoraria: Novartis.

### SA-PO3035

**Benefits and Risks of Surveillance Biopsies in Stable Renal Transplant Recipients** Prashant Pendyala,[1] Neha Nainani,[1] Kathleen M. Tornatore,[2] Mareena Susan Zachariah,[1] Rocco C. Venuto.[1] *[1]Renal and Pancreas Transplant Unit, Erie County Medical Center and University at Buffalo, Buffalo, NY; [2]Department of Pharmacy Practice, School of Pharmacy & Pharmaceutical Sciences, University at Buffalo, Buffalo, NY.*

**AIM:** To assess the value of surveillance biopsies (SB) in the management of stable renal transplant recipients (RTR).

**METHODS:** SB are being used commonly in RTR for early detection and treatment of subclinical acute rejection and of interstitial fibrosis and tubular atrophy. Biopsies carry the risk of various complications. We assessed the frequency with which unsuspected diagnoses were detected in the SB and the occurrence rate of complications. In this prospective study, RTR underwent SB at 3, 9-12 and 24 months (mo) after transplantation. SB performed between the years 2006-2008 were included.

**RESULTS:** A total of 202 biopsies were performed on 98 RTR. Twenty nine (14.3%) subclinical rejections (SC REJ) were detected. Nine (4.4%) biopsies showed acute calcineurin (CNI) toxicity and 19 (9.4%) showed disease recurrence (DIS R). A total of 45 (22.2%) treatment changes (RX) resulted from SB. A total of 16 (7.9%) hospital admissions followed the SB due to bleeding. Eight patients had an increase of creatinine of 0.3mg/dl or more that reverted to baseline. No grafts were lost and no patient needed surgical

intervention. SB were sub analyzed based on the time post transplant. There were 83 biopsies at 3 mo, 74 at 9-12mo and 45 at 24mo. In the 3 mo SB, 13 had SC REJ, 7 had DIS R and 5 had CNI toxicity. There were a total of 19 RX (23%) and the complication rate was 3.6%. At 9-12 mo 14 SC REJ and 9 DIS R were detected and 4 had CNI toxicity. These findings directly resulted in 21 (28%) RX with a complication of 8.1%. RX resulted in histologic improvement in the majority of the cases. At 24 mo biopsies, there were only 2 SC REJ and 3 DIS R. There were 4 (8.8%) RX whereas 7 (15.5%) RTR had complications.

**CONCLUSION:** SB done up to Year 1 are useful in the management of RTR and were associated with minimal morbidity. The risk benefit ratio for Year 2 SB requires further definition as complications at this time might outweigh the benefit from the SB.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO3036

**Renal Function of an Everolimus Based Therapy after Calcineurin Inhibitor Withdrawal in Maintenance Renal Transplant Recipients One Year after Conversion** Claudia Sommerer,[1] Klemens Budde,[1] Wolfgang Arns,[1] Stefan Kramer,[2] Eva-Maria Vogel,[2] Frank B. Pietruck,[1] Thomas Rath,[1] Hermann G. Haller.[1] *[1]The APOLLO Study Group; [2]Novartis Pharma, Nuremberg, Germany.*

Objective: Renal function, safety and efficacy of an Everolimus regimen was assessed 12 months after Calcineurin-Inhibitor (CNI) withdrawal in maintenance kidney allograft patients (pts).

Methods: In an open-label, randomized, controlled, multi-center study 93 renal maintenance pts with a stable renal function (creatinine < 2.5mg/dl) and on a therapy consisting of CNI, Enteric-coated mycophenolate sodium (EC-MPS) with or without steroids were randomized to either continue CNI treatment (n=47) or b) convert to an Everolimus based (n=46) regimen.

Results: 93 pts were randomized to either Everolimus or CNI group (29 CsA/17 Tac; C0: CsA 98.0±25.6ng/mL; Tac 5.7±2.2ng/mL). Mean of years since the most recent transplantation was higher in the Everolimus compared to the CNI group (7.0±5.2 vs. 5.8±5.6). Renal function expressed as calculated GFR (Nankivell method) improved from conversion to M12 by 3.6mL/min/1.73m2 in favor of the Everolimus regimen (ns). The observed GFR slope from conversion to M12 was +3.4[-0.2;+7.0] for Everolimus and -0.2[-3.9;+3.5] mL/min/1.73m2 for CNI patients (ns). One death occurred in both groups and no graft loss or BPAR was observed in either group. Proportion of pts discontinued study regimen due to AE was 15(32.6%) in the Everolimus and 5(10.6%) in CsA group. Safety parameters related to study medication are listed below.

| | CNI | Everolimus |
|---|---|---|
| Hyperlipidaemia[%] | 0 | 6.5 |
| Total Cholesterol[mmol/L] | 5.2 | 6.1 |
| LDL[mmol/L] | 3.1 | 3.7 |
| HDL[mmol/L] | 1.3 | 1.5 |
| Triglycerides[mmol/L] | 2.6 | 2.5 |
| Anaemia[%] | 0 | 6.5 |
| Leucopenia[%] | 2.1 | 10.9 |
| Thrombocytopenia[%] | 0 | 4.3 |
| Mouth ulceration[%] | 0 | 26.1 |
| Diarrhoea[%] | 4.3 | 6.5 |
| Any infections[%] | 53.2 | 63.0 |
| Serious infections[%] | 10.6 | 17.4 |

Conclusions: Late conversion to an Everolimus/EC-MPS treatment in maintenance renal transplant pts after CNI withdrawal leads to a better renal function. Overall late conversion was safe although more side effects were reported. For a complete risk/benefit assessment a long term follow-up is needed which will be attempted.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO3037

**Dosing Modifications Are Required for Concomitant Administration of Colchicine and Cyclosporine** Matthew W. Davis,[1] Suman Wason,[1] Jennifer L. Digiacinto.[2] *[1]URL Pharma, Inc, Philadelphia, PA; [2]Salamandra, LLC, Bethesda, MD.*

**PURPOSE:** Cyclosporine potently inhibits P-glycoprotein membrane transporter and CYP3A4 enzyme required for metabolism of colchicine. This study was conducted to determine whether cyclosporine substantially increases exposure to colchicine when the two are coadministered, as may occur in renal transplant patients who develop gout.

**METHODS:** Twenty-four healthy volunteers received single-dose colchicine (0.6 mg) alone and in combination with single-dose cyclosporine (100 mg). On Day 1, volunteers received colchicine. Blood samples for pharmacokinetic analysis were collected predose and 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, 24.0, 36.0, 48.0, 72.0, and 96.0 hrs postdose. After a 14-day washout, volunteers (fasted conditions) received colchicine plus cyclosporine followed by blood sample collection for pharmacokinetic assessment as described.

**RESULTS:** Twenty-three volunteers completed the study. One volunteer discontinued because of elevated creatine kinase that resolved without treatment. Coadministration of colchicine with cyclosporine resulted in 224% increase in colchicine mean $C_{max}$ compared with colchicine alone (8.82 ng/mL *versus* 2.72 ng/mL, respectively). Colchicine $AUC_{0-\infty}$ increased approximately 215% when coadministered with cyclosporine (47.3 ng·hr/mL *versus* 15.0 ng·hr/mL for colchicine alone). Colchicine $T_{max}$ was unaffected by coadministration with cyclosporine. Total apparent oral clearance of colchicine decreased by 72% following coadministration compared with colchicine alone (13.4 L/hr *versus* 48.2 L/hr, respectively). Across the study, the most common adverse event following colchicine administration was headache (n=4, 16.7%). All adverse events were mild to moderate in severity.

**CONCLUSIONS:** These findings indicate a significant drug interaction occurs when colchicine and cyclosporine are coadministered. Therefore, dose reduction for colchicine (for prophylaxis, reduction to 0.3 mg [1/2 tablet] once daily or once every other day; for acute flare, 0.6 mg [1 tablet] repeated no earlier than every third day) should be made in patients requiring coadministration of colchicine and cyclosporine.

Disclosure of Financial Relationships: Employer: I am the Chief Medical Officer of URL Pharma; Ownership: I have stock options in URL Pharma; Patent: I have two patents pertaining to colchicine dosing with clarithromycin. I do not receive royalties. These patents have been assigned to URL Pharma.

## SA-PO3038

**A Multicenter Experience of Kidney Transplantation in ANCA-Associated Vasculitis (AAV) in the Era of Modern Immunosuppression** Duvuru Geetha,[1] Alfonso Eirin,[2] Karin A. True,[3] Maria V. Irazabal-Mira,[2] Ulrich Specks,[2] Philip Seo,[1] Patrick H. Nachman,[3] Fernando C. Fervenza.[2] *[1]Johns Hopkins University, Baltimore, MD; [2]Mayo Clinic, Rochester, MN; [3]UNC Kidney Center, Chapel Hill, NC.*

We report our experience with a multicenter cohort of AAV patients who underwent kidney transplantation (KTX) at our institutions from January 1996 to March 2010. A total of 85 patients (45M/40F; mean age 49 years) received a KTX for ESRD secondary to biopsy proven microscopic polyangiitis (n=39), Wegener's granulomatosis (n=42), or renal limited vasculitis (n=4). Twenty-four patients were transplanted preemptively and 61 were on dialysis. Sixty-nine patients received a living donor KTX and 16 received a deceased donor KTX after a mean of 24 months from the start of dialysis. All patients were in clinical remission at time of KTX, with 57/85 (67.0%) in remission for more than 12 months. Fifty-eight patients received induction therapy. Sixty-four patients received maintenance immunosuppression with prednisone, mycophenolate mofetil and tacrolimus. Patients were followed up for a median of 64 months after KTX. At the time of KTX, 29 patients were ANCA-positive. Nine relapses occurred in 8 patients (6 WG; 2 MPA) resulting in graft lost in only one. Five of these patients were ANCA positive at the time of transplant. The vasculitis relapse rate was 0.2 per 1000 patient-years. There were 23 rejection episodes in 13 patients. Graft loss resulted from non-compliance with immunosuppression in 3 patients and from unrelated causes in 4. Six patients developed BK nephropathy. The median serum creatinine at 1 year was 1.3 mg/dl in the 75 patients who had more than one year of follow-up. The median serum creatinine at last follow up was 1.4 mg/dl. There was no difference in serum creatinine at last follow- up between patients with WG versus MPA (1.2 versus 1.3, P=0.09). Eight patients had post transplant malignancies (3 skin cancer, 1 bladder cancer, 1 renal cell carcinoma, 1 gastric carcinoma, 1 lung carcinoma, 1 head and neck carcinoma). KTX is a safe and effective option for treating ESRD secondary to AAV and relapses are rare with current immunosuppression regimens.

Disclosure of Financial Relationships: nothing to disclose

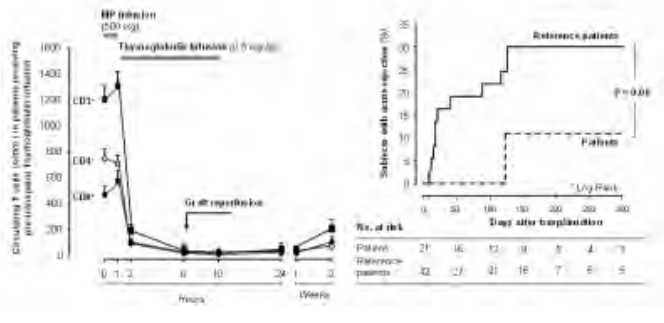
## SA-PO3039

**Pre- vs Post-Surgery Low-Dose Rabbit Thymoglobulin (RATG) Plus Basiliximab Induction in Renal Transplantation: A Prospective, Matched Cohort Study** Alessia Gennarini, Paolo Cravedi, Maddalena Marasa', Annalisa Perna, Giuseppe Remuzzi, Piero Ruggenenti. *Department of Medicine and Transplantation, Ospedali Riuniti di Bergamo and Mario Negri Institute for Pharmacological Research, Bergamo, Italy.*

Background: In high-risk kidney transplant recipients, low-dose RATG plus basiliximab prevented acute rejection as effectively as standard RATG induction, but was safer and cheaper (CJASN 2006,1:546).

Methods: In a single-center, prospective, matched-cohort study, we compared the outcome of 21 consecutive patients induced with a 6-day low-dose (0.5 mg/kg/day) RATG course started on average 6 h before graft reperfusion with that of 42 gender- and age- (±5 years) matched reference-patients given the same RATG course started post-surgery. All subjects were managed by the same team and received the same concomitant therapy with basiliximab (20 mg on day 0 and 4 post-transplant), cyclosporine and mychophenolate mofetil or azathioprine, without steroids. Each couple of reference patients had the same observation period of corresponding patient.

Results: Baseline characteristics, including HLA matching and cold ischemia time, of study groups were comparable. At graft reperfusion, all patients had their CD3+, CD4+ and CD8+ T cells fully depleted from the circulation.



Over a median (interquartile range) follow-up of 126 (40-198) days, 1 patient (4.8%) vs 11 reference patients (26.2%) had a biopsy-proven acute rejection. No patient compared to 5 reference-patients (11.9%) required dialysis therapy within 1 week post-transplant. Both treatment strategies were well tolerated.

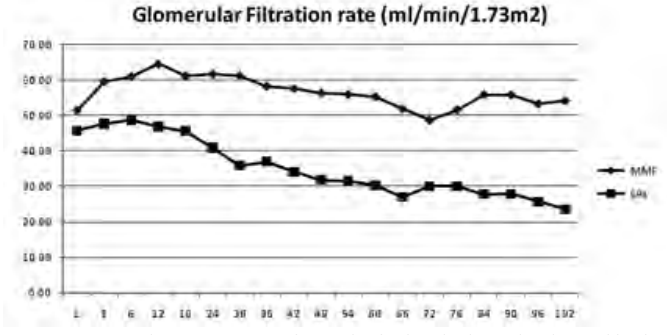
Conclusions: In renal graft recipients, pre-transplant RATG infusion further enhanced the protective effect of low-dose RATG plus basiliximab induction against acute allograft rejection and delayed graft function, possibly through T cell depletion at the time of grafting.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3040**

**Long Term Renal Allograft Function and Graft Survival Comparing Two Pred-Free, CNI Based Maintenance Immunosuppression: FK/MMF vs. FK/SRL** Lorenzo G. Gallon,[1] Darshika Chhabra,[1] Nader Najafian,[2] Pranav Dalal,[3] Gaurav R. Shah,[3] Joseph Ross Leventhal,[1] Anton I. Skaro.[1] *[1]Medicine/Nephrology, Northwestern University Medical School, Chicago; [2]Medicine/Nephrology, Harvard Medical school, Boston; [3]Medicine, Mt Sinai Hospital, Chicago.*

Background and Methods: The present prospective single center study was designed to compare the long term impact on renal allograft survival and function of two tacrolimus (Tac) based, pred-free maintenance immunosuppressive protocols: Tac/Sirolimus (SRL) vs. Tac/MMF. Renal transplant patients, after given induction with IL2-RA and rapid steroid elimination, were prospectively randomized to Tac/SRL (n=37) or Tac/MMF (n=45). Mean post-transplant follow-up 8.5 yrs. During the entire study, the following data were collected: patient and graft survival, incidence of ACR, glomerular filtration rate (GFR) at different time points post-tx, incidence of infections, malignancies, cardiovascular events, hypertension, hyperlipidemia, post-tx anemia and DM. Results: There were no significant differences between the two groups when comparing pt demographics and characteristics except for the mean age of donor that was significantly lower in the Tac/MMF than in the Tac/SRL group (3311 vs.3913 yrs, p=0.02). The Kaplan-Meier graft survival rates at 8.5 years post-tx were significantly different ( 91.1% in the Tac/MMF vs. 70.3% in the Tac/SRL, p=0.02). GFR measured at different time-points post-tx (up to 66 months post-tx) was consistently lower in the Tac/SRL than in Tac/MMF and the slope of GFR decline per month was significanltly steeper (p<0.0001) in the Tac/SRL vs. Tac/MMF.




Conclusions: The present prospective randomized study shows that the combination Tac/SRL in a pred-free regimen is associated with significantly lower long term graft survival and significantly worse renal transplant function when compared to the combination Tac/MMF.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3041**

**Alemtuzumab Induction in Renal Transplantation: A Single Center Experience** Jeannie P. Co,[1] Jessica G. Lucas,[1] Imran Dosani,[1] Uzoamaka T. Nwaogwugwu,[1] Rahul Bhardwaj,[1] Richard J. Marcus,[1] Kalathil K. Sureshkumar,[1] Sabiha M. Hussain,[1] Tina Y. Ko,[1] Ngoc L. Thai.[2] *[1]Nephrology, Allegheny General Hospital; [2]Transplantation, Allegheny General Hospital, Pittsburgh, PA.*

Alemtuzumab is increasingly used as an induction agent in renal transplantation. We aimed to compare retrospectively the safety and efficacy of induction with alemtuzumab, rabbit antithymocyte globulin (r-ATG) and basiliximab in renal transplant recipients on a calcineurin inhibitor-based steroid withdrawal protocol. Patients received induction with either single dose of alemtuzumab 30 mg intra-operatively, 4 consecutive 1.5 mg/kg doses of r-ATG or basiliximab 20 mg intra-operatively and on post-operative day 4. Results are as follows:

| | Alemtuzumab (n=71) | r-ATG (n=71) | Basiliximab (n=71) | p-value |
|---|---|---|---|---|
| DGF (%) | 27 | 28 | 38 | 0.286 |
| AR rate (%) | 13 | 11 | 28 | 0.013 |
| Number of Infections | 29 | 20 | 28 | 0.226 |
| LOS (days) | 6.2 | 7.1 | 8.1 | 0.013 |
| Admissions/patient/6mos | 0.6 | 0.9 | 0.9 | 0.226 |
| 1-year graft survival (%) | 85 | 85 | 87 | 0.371 |
| 1-year patient survival (%) | 97 | 99 | 96 | 0.426 |

DGF=delayed graft function; LOS=length of stay; AR=acute rejection

Leukopenia defined as a WBC count <4400/μL was observed in 24%, 16% and 2.8% among alemtuzumab, r-ATG, and basiliximab groups respectively (p=0.001) at post-operative day 7. WBC counts were similar at all other time points. Incidence of thrombocytopenia (defined as platelet count <145,000/μL) was similar between the groups at all time points. The reported cases of skin cancers were 0, 3 and 4 and solid tumors were 1, 1 and 0 in the 3 groups respectively. Cost analysis showed significant financial advantage with induction using alemtuzumab ($9,621) compared to r-ATG ($17,737) and basiliximab ($14,340).

In conclusion, alemtuzumab induction is associated with similar graft and patient outcomes in renal transplantation when compared to r-ATG and basiliximab. Alemtuzumab induction was also associated with lower AR rates and shorter LOS compared to basiliximab. Cost benefit analysis favored alemtuzumab use. Therefore, we feel that alemtuzumab provides a safe and effective approach to induction therapy in a cost-conscious health care environment.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3042**

**Does Rituximab Usage Improve the Likelihood of Recurrent FSGS Response?** Carlos E. Araya, Eduardo H. Garin, Richard E. Neiberger, Vikas R. Dharnidharka. *Pediatric Nephrology, University of Florida, Gainesville, FL.*

**Background:** Recurrence of primary FSGS in the allograft occurs in 30 to 50% of cases and is associated with early graft failure. Therapies are not well established and in many cases fail to induce remission. Rituximab has been used in recurrent FSGS with variable success.

**Methods:** We analyzed the existing literature in children and adults with recurrence of FSGS who were treated with rituximab, to determine factors associated with a response to treatment. Analyses were performed with Chi-Square testing or Fisher's exact test for categorical variables and Mann-Whitney test for non-normally distributed variables. Variables with p value <0.10 in univariate analysis for a relationship with development of a primary outcome were entered into multivariate logistic regression analysis.

**Results:** 34 renal transplant patients (16 pediatric) were treated with a mean of 3 rituximab doses (range 1-6). By univariate analysis for two outcomes (no response to therapy vs any response to therapy), a lower number of rituximab infusions (p = 0.008) and a normal serum albumin level (p = 0.039) were associated with a higher frequency of response to rituximab therapy. The patients who improved with therapy were younger at the time of transplant (19.2 vs 28.5 years), but this did not reach statistical significance (p = 0.095). By univariate analysis for 3 outcomes (no response, partial and complete remission) a lower number of rituximab infusions (p=0.03), shorter time to rituximab treatment from relapse (p = 0.02), and normal serum albumin (p = 0.007) were associated with a higher frequency of achieving remission. In multivariate analyses only a normal serum albumin level was significantly associated with a positive response to rituximab therapy (p = 0.007).

**Conclusion:** Adjuvant therapy with rituximab for recurrence of FSGS may be beneficial in only a small subgroup of patients and the response will likely be evident after the initial medication doses. A normal serum albumin is the main determinant of response.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3043**

**Risk for Delayed Graft Function in *De Novo* Renal Transplant Recipients Receiving Everolimus Versus Mycophenolate: Results from a Pooled Analysis** S. P. Mulgaonkar,[1] Diane M. Cibrik,[2] K. Mange.[3] *[1]Saint Barnabas Medical Center; [2]University of Michigan; [3]Novartis Pharmaceuticals Corporation.*

Delayed graft function (DGF) is a risk factor impacting efficacy outcomes in de novo renal transplant recipients (RTs). Everolimus (EVR) is an immunosuppressant used in cyclosporine (CsA) reduction and withdrawal protocols, and comparative risk assessment for DGF between EVR and MPA is warranted.

Data from 2004 de novo renal transplant recipients (RTs) from three EVR studies B201(N = 588), B251(N = 583), and A2309 (N = 833), were analyzed to identify the impact of DGF on efficacy outcomes across both EVR dosing groups and mycophenolate (MPA) control groups. In all studies, EVR groups received either 1.5 mg/day, or 3 mg/day; with either standard or reduced dose CsA depending on the individual study protocol. All control groups received MPA (1.44g/day) with standard dose CsA. Steroids were given as per center practice. Composite efficacy end-point for the pooled analysis was graft loss, death, treated biopsy-proven acute rejection, or lost to follow up. Odds ratios (ORs) were calculated using a logistic regression model with treatment, study, and DGF (Yes or No) as independent variables.

MPA-treated RTs who experienced a composite end-point were 29.3 vs.56.6% for no-DGF vs. DGF subgroups respectively. EVR 1.5 mg and 3.0 mg-treated RTs who experienced a composite endpoint for no-DGF vs. DGF, similarly, were identical at 29.8 vs. 50.6%.

Likelihood of a Composite End-Point Event based on Treatment or DGF

| | Odds Ratio (OR) | 95% C.I. | P Value |
|---|---|---|---|
| EVR 1.5 vs. MPA | 0.9868 | 0.7820, 1.2452 | NS |
| EVR 3.0 vs. MPA | 0.9868 | 0.7825, 1.2445 | NS |
| DGF vs. no-DGF | 2.5686 | 1.9579, 3.3699 | P<0.0001 |

In this pooled data analysis in over 2000 RTs EVR treatment resulted in similar composite end-point events confirming no treatment effect. This result is expected as these studies were powered for non-inferiority. However, risk for experiencing a composite end-point in those RTs with DGF increased significantly compared to those RTs who did not have DGF. This pooled analysis confirms that DGF is a risk factor for poorer efficacy outcomes, irrespective of treatment.

Disclosure of Financial Relationships: Research Funding: Novartis Pharmaceuticals Corporation.

**SA-PO3044**

**IL2ra Induction Therapy in the Prevention of Acute Rejection with TAC/MPA-Based Immunosuppression: Differential Effect by Type of Donor**
Jane Gralla, Alexander C. Wiseman. *University of Colorado.*

**Background:** IL2 receptor anatagonist (IL2ra) induction therapy has an excellent safety profile and improved outcomes in randomized trials using CsA-based immunosuppression. However, there have been no large randomized trials or retrospective analyses examining the effect of IL2ra vs. no induction using tacrolimus and mycophenolate (TAC/MPA)-based therapy.

**Methods:** A retrospective analysis of the SRTR database of adult, primary kidney transplant recipients from 2000-2008 with initial immunosuppression of TAC/MPA/prednisone, who received IL2ra induction therapy or no induction therapy (N=28,686) was performed. Acute rejection at 1 year and graft and patient survival at 1 and 3 years were compared by type of donor.

**Results:** There was no significant difference in 1- or 3-year graft or patient survival for IL2ra vs. no induction therapy, overall or by deceased donor (DD) or living donor (LD). Acute rejection at 1 year was significantly lower with IL2ra (11.6%) vs. no induction therapy (13.0%, relative risk (RR) 0.89, p<0.001, number needed to treat to prevent one episode of acute rejection (NNT)=71). Acute rejection with IL2ra for LD's was 10.8% vs. 12.7% with no induction therapy (RR=0.85, p<0.001, NNT=53) and 12.2% vs. 13.2% for DD's (RR=0.92, p=0.047, NNT=100). Accounting for other risk factors for rejection, the impact of IL2ra remained significant only for LD's.

Table 1: Overall Graft and Patient Survival and Multivariable Analysis of Acute Rejection at 1 Year

| | 3-Yr Graft Survival | 3-Yr Patient Survival | RR of Acute Rejection with IL2ra (95% CI) |
|---|---|---|---|
| All Donors (N=28,686) | 87.5% vs. 87.8% (p=0.50) | 92.8% vs. 93.2% (p=0.16) | 0.90 (0.85-0.96) (p=0.001) |
| DD (N=16,305) | 84.4% vs. 84.5% (p=0.85) | 90.6% vs. 91.2% (p=0.20) | 0.95 (0.87-1.03) (p=0.23) |
| LD (N=12,381) | 91.8% vs. 91.8% (p=0.91) | 95.8% vs. 95.7% (p=0.73) | 0.82 (0.74-0.90) (p<0.001) |

**Conclusion:** The benefit of IL2ra induction with TAC/MPA/prednisone maintenance immunosuppression is less than reported for CsA (baseline acute rejection rates are lower), but may still be of value particularly in LD recipients.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3045**

**Bortezomib Therapy for Antibody-Mediated Rejection**
Masahiko Nagahama. *Division of Nephrology, Virginia Commonwealth Univ, Richmond, VA.*

**Introduction**: Although, it is true that abrogating antibody production is essential for antibody-mediated rejection (AMR), current therapies for AMR do not target the primary antibody producing cells, the plasma cells. Bortezomib is a first protesomal inhibitor, which is FDA approved, for the treatment of plasma cell-derived tumors. Only a few case reports and case series of Bortezomib therapy for AMR in renal transplant patients have been reported and there is little information available. The purpose of this case report is to describe the clinical experience with Bortezomib therapy for refractory AMR.

**Methods**: Two renal transplant patients with refractory AMR, who failed to alternate day 200 mg/kg intravenous cytomegalovirus immunoglobulin and plasmapheresis (CMVIG+PP) for 6 cycles, were treated with Bortezomib (1.3 mg/m$^2$, twice weekly for 4 doses) along with methylpredonisolone (250mg). Serial measurements of Flow cytometry crossmatch (FCXM) and donor-specific antibodies (DSA) were conducted by single antigen bead on Luminex platform.

**Results**: (Case 1) A 56 year-old African American (AA) male developed delayed graft function with AMR. He was treated with CMVIG+PP for 6 cycles. However, his FCXM remained positive with increased DSA levels (HLA A24: 10454 SFI; B32: 14426 SFI; B65: 13666 SFI, SFI:standard fluorescence intensity). He underwent Bortezomib therapy, which decreased DSA levels (HLA A24: 8368 SFI; B32: 11024 SFI; B65: 11141 SFI) and FCXM became negative. He came off dialysis and his serum Cr is stable for 6 months after Bortezomib therapy. (Case 2) A 44 year-old AA female developed AMR 6 months after transplant. She did not respond to CMVIG+PP for 6 cycles, and her FCXM remained positive with elevated DSA levels (HLA B44: 3199 SFI; DR15: 5957 SFI; DR4: 1778 SFI). Following Bortezomib therapy, FCXM was still positive and DSA levels were unchanged (HLA B44: 2332 SFI; DR15: 5921 SFI; DR4: 1000 SFI). Her renal function deteriorated and she returned to dialysis.

**Conclusion**: Bortezomib therapy may provide effective reduction in DSA levels and resolution of refractory AMR. However, this is not true for all patients and the prediction of sensitivity to this plasma cell targeted therapy should be investigated by clinical trials.

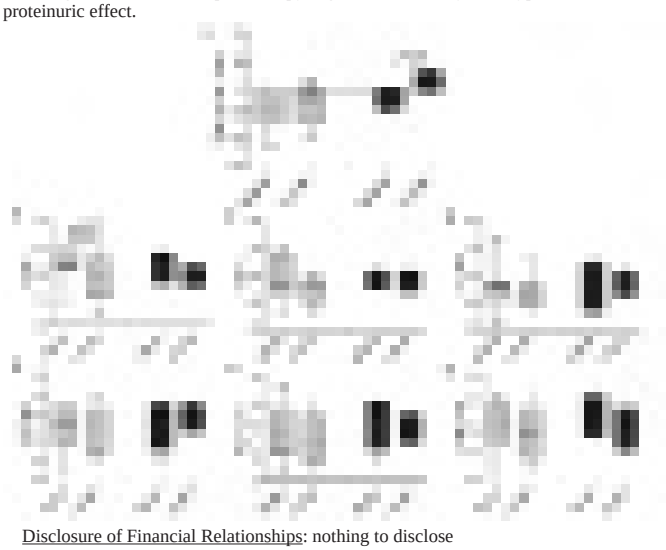
Disclosure of Financial Relationships: nothing to disclose

**SA-PO3046**

**6-Months Low-Sub-Therapeutic Dose of Valsartan-Therapy Improves Kidney β-ATP/Pi Levels in Stable Normotensive Kidney-Transplanted Patients: A 31P-MRS Study**
Roberto Bassi,[1,2] Paolo Fiorina,[1,2] Andrea Vergani,[1,2] Alessandro Del Maschio,[3] Antonio Secchi.[2] *[1]Nephrology Division, Transplantation Research Center, Children's Hospital/Harvard Medical School, Boston, MA; [2]Medicine, San Raffaele Scientific Institute, Milan, Italy; [3]Radiology, San Raffaele Scientific Institute, Milan, Italy.*

The effect of angiotensin receptor antagonists on kidney metabolism has not been fully studied. We took advantage of a new technique, 31P-Magnetic Resonance Spectroscopy (31P-MRS), to non-invasively study the effect of a low-sub-therapeutic dosage of the angiotensin receptor antagonist Valsartan treatment on kidney high-energy phosphate metabolism as evaluated with 31P-MRS. Twenty consecutive kidney-transplanted stable patients were enrolled and received Valsartan (80 mg/day) for six months or nothing. Kidney high-energy phosphate metabolism was evaluated both at baseline and after treatment by 31P-MRS with a 1.5T system (Gyroscan Intera Master 1.5 MR System; Philips Medical Systems, Best, the Netherlands). Valsartan-treated patients (n=9) showed a significant increase in ß-ATP/Pi ratio, a marker of kidney high-energy phosphate metabolism (baseline=1.03±0.08 vs. 6 months=1.26±0.07, p=0.03). In contrast, the ß-ATP/Pi ratio in the control group (n=11) did not change (baseline=0.85±0.10 vs. 6 months=0.89±0.08, ns). The improvement in ß-ATP/Pi ratio was not associated with a reduction in arterial blood pressure or in urinary albumin excretion.

Short-term and low-sub-therapeutic dose of Valsartan improves high-energy phosphate kidney metabolism in stable normotensive patients as assessed by kidney-localized 31P-Magnetic Resonance Spectroscopy regardless from any anti-hypertensive or anti-proteinuric effect.



Disclosure of Financial Relationships: nothing to disclose

**SA-PO3047**

**Outcomes of Kidney Transplant in Highly Sensitized Patients. Single-Center Experience**
Maria Joao Carvalho Azevedo Rocha,[1,2] António Cabrita.[1,2] *[1]Nephrology, Hospital Santo António- Centro Hospitalar do Porto, Porto, Portugal; [2]Transplant, Hospital Santo António- Centro Hospitalar do Porto, Porto, Portugal.*

Transplantation on hypersensitized patients (HSP) is a major challenge for any transplantation team. Portuguese law change in 2007, increased the incidence of (HSP) recipients from 1.3% to 7,6%.Our purpose is to analyze clinical outcomes of HSP.We retrospectively analyzed demographic and clinical data of all HSP transplanted between 2007 and 2009 in our unit.25 HSP (PRA>50%) 14 female,11 male,mean age 48,9±11,3 years received an isolated kidney graft.Mean dialysis time was 148,8±94,5 months.Average PRA was 70,8±17,1% by Luminex.Average donor age was 47,3±10y.CDC cross-match was negative in all cases.All received tacrolimus,MMF and prednisone,21 received ATG and 4 antiCD25 antibodies.Five patients received IVIG,one with plasmapheresis.Delayed graft function (DGF) occurred in 36%(75% in the antiCD25 group vs 28% in the ATG group; p=1,00).Six patients experienced one episode of acute biopsy proven rejection (ABPR),all antibody-mediated (AM),1 with superimposed cellular rejection.Time until ABPR was 16.6±6,9 days,4 patients presented acute graft dysfunction and 2 DGF.All received IVIG and plasmapheresis,one received ATG,3 received Rituximab.Discharge mean plasma creatinine (PCr) was1,83±0,9 mg/dL.Three allografts where lost,1 because of receptor death,1 primary non-function and 1 due to acute allograft thrombosis without AR.There were 36 hospital readmissions,13 caused by severe bacterial infection. With a mean follow-up of 24,9±9,4 months,22 allografts are functioning with a mean Pcr of 1,51(mg/dl] 0,77 mg/dL.The average PCr of patients who had ABPR is 2,31[]0,98 mg/dL and 1,1±0,44mg/dL in those who didn't(p=0,0015).HSP had a higher incidence of ABPR compared to the general transplant population(10,2%).Rejection episodes occurred soon after transplant and were antibody mediated.Despite this,there was no graft loss attributed to ABPR, although it seems to have a negative impact on graft function.Transplant is a good therapeutical option for these patients.Dessensitization therapies may further improve outcomes.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3048**

**A Randomized Clinical Trial Comparing Tacrolimus and Sirolimus Maintenance Monotherapy Following Kidney Transplantation** Monique E. Cho,[1] Xiongce Zhao,[2] Michael S. Ring,[1] Roslyn B. Mannon,[3] Allan Kirk.[4] *[1]Kidney Disease Branch, NIDDK; [2]Office of the Director, NIDDK, NIH, Bethesda, MD; [3]University of Alabama, Birmingham, AL; [4]Emory University, Atlanta, GA.*

Background: Both sirolimus (Rapa) and tacrolimus (FK) are effective in preventing renal allograft rejection when used as part of a multidrug regimen, but there have been no studies directly comparing these agents as monotherapies. Methods: In order to investigate whether there was a clear advantage to either drug, we enrolled 31 patients in a phase II trial using Thymoglobulin and methylprednisolone induction followed by combination therapy with both Rapa and FK for 6 months. At 6 months, those without rejection on protocol biopsy with good tolerance to both drugs were randomized either Rapa or FK monotherapy. Primary outcome was renal graft function over time, using linear mixed model to calculate the least mean square values of serum creatinine. The secondary outcomes included safety and tolerability profiles and renal graft biopsy results. Results: Out of 31 patients, 7 were randomized to Rapa and 8 to FK. The remaining 16 patients could not be randomized due to Rapa intolerance {oral ulcers (1); hyperlipidemia (2); arthropathy (3)}, rejection on protocol biopsy (4), or unrelated issues (6). The mean follow-up was 4 yrs post randomization. Their mean nadir serum Cr (mg/dL) was 1.03 in Rapa group and 1.17 in FK group and remained stable over 54 months without significant increase. There were no differences in the rates of biopsy diagnoses of IF/TA, rejection, or BK nephropathy in the two groups, based on a mean of 3.5 biopsies per patient over a mean of 1.4-year period. Serum triglycerides levels were significantly higher in the Rapa group between months 6-24, and the absolute lymphocyte count remained significantly lower in Rapa group between months 12-30. The incidence of post transplant diabetes was similar in both groups. Conclusion: In patients selected for their stability and tolerance to either drug, both Rapa and FK monotherapy provided similar prophylaxis from rejection without differing long-term consequences over a 4 year follow-up period. FK was associated with fewer dose limiting consequences.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3049**

**Everolimus Plus Reduced CsA Exposure: Preserved Efficacy and Renal Function from 12 to 24 Months** Fuad S. Shihab, Diane M. Cibrik, Yu Seun Kim, R. Walker, G. Zibari, C. Cornu-Artis, C. Panis, H. Jiang, Helio Tedesco-Silva. *For 2309 Study Group.*

Everolimus is an mTOR inhibitor that has immunosuppressive and anti-proliferative properties. The efficacy and safety of everolimus with cyclosporine (CsA) minimization was assessed in 833 de novo renal transplant recipients.

A2309 is a 24-month, randomized, multicenter, open-label study comparing 2 targets of everolimus (EVR) (C0 3 – 8 ng/mL or C0 6 – 12 ng/mL) with reduced CsA exposure versus a control group receiving enteric-coated mycophenolate sodium (MPA) 1.44g/day with standard exposure CsA. The endpoints at 24M were composite efficacy (incidence of graft loss, death, BPAR, loss to followup-LTF), plus renal function and safety comparisons between the EVR groups and the MPA control.

Donor and recipient characteristics were comparable between the groups. Around a 60% reduction in CsA exposure for both EVR groups continued to be maintained from 12M to 24M versus MPA control group (mean C0 at 24M: 52, 50 & 135ng/mL for EVR 3-8ng/ml, 6-12ng/ml & MPA groups, respectively). Mean 24M calculated GFR (MDRD) values were 52.2, 49.4 and 50.5 mL/min/1.73m2 for EVR 3-8, EVR 6-12 and MPA respectively, and comparable renal function was maintained from 12M to 24M.

Results for the ITT population at 24M by treatment group*. All values are n (%)

| | EVR 3-8ng/mL | EVR 6-12ng/mL | MPA 1.44g |
|---|---|---|---|
| Primary Composite Efficacy** | 91 (32.9) | 75 (26.9) | 76 (27.4) |
| Death | 9 (3.2) | 10 (3.6) | 8 (2.9) |
| Graft Loss | 16 (5.8) | 17 (6.1) | 11 (4.0) |
| Death, or Graft Loss | 23 (8.3) | 26 (9.3) | 18 (6.5) |
| Loss to follow up | 21 (7.6) | 14 (5.0) | 12 (4.3) |
| Treated BPAR** | 55 (19.9) | 42 (15.1) | 53 (19.1) |
| On-treatment Primary Composite Efficacy* | 72(26.0) | 56(20.1) | 74(26.7) |

**Only the first treated BPAR event included in composite score.

The incidence of notable AEs was higher in the EVR 6-12ng/mL group at 24M (75%), but the incidences for EVR 3-8ng/mL vs MPA were comparable, (68 vs 66%).

This analysis confirms that efficacy parameters and renal function achieved at 12M with a combination of EVR and 60% lower exposure to CsA as compared to MPA control were maintained at 24M.

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**SA-PO3050**

**ABO Incompatible Living Donor Kidney Transplantation (ABOI-KT) without Splenectomy: Single Center Experience** Jeongmyung Ahn,[1] Dong Ryeol Lee,[1] Mi Young Jeon,[2] Byung Chang Kim,[3] Jin M. Kong.[1] *[1]Division of Nephrology, Internal Medicine, Maryknoll Medical Center, Busan, Republic of Korea; [2]Department of Pathology, Maryknoll Medical Center, Busan, Republic of Korea; [3]Department of Clinical Laboratory, Maryknoll Medical Center, Busan, Republic of Korea.*

**Introduction:** ABOI-KT seems a valuable option to overcome global organ shortage. With the recent protocol with no splenectomy, excellent graft outcome equivalent to ABO-compatible transplantation has been reported.

**Method:** Twenty-one ABO-I KT without splenectomy has been performed in our center since 2007. Rituximab was given 1 month prior to transplantation. Plasmapheresis was done to lower anti-ABO antibody to ≤8 on transplantation day. Number of HLA mismatch was 3.86(0-6). Anti-IL2 antibody induction and tacrolimus-based triple drug was used. Median IgG anti-ABO antibody titer initial, on transplant day and at last follow up was 32(8-512), 2(1-8) and 2(1-32), respectively. Posttransplant plasmapheresis was done not routinely but selectively in 7 patients with high initial ABO antibody titer, rapidly rising antibody titer during 2 weeks posttransplantation or rising serum creatinine.

**Result:** Median follow up was 20(2-41) months. One episode of ABO-antibody mediated acute rejection occurred in a patient, recovered by treatment. One patient developed leukoencephalopathy at 10Mo. post transplantation. But, his neurologic problem recovered after immunosuppressant reduction. No patient or graft was lost to date. There was no major infection requiring hospitalization. **Conclusion:** ABOI-KT has excellent outcome, and is a good way to increase donor availability.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3051**

**One-Year GFR as a Surrogate for Long Term Allograft Survival: Estimated Versus Measured GFR** Nicolas Maillard,[1] Olivier Moranne,[2] Christopher R. Mariat.[1] *[1]Service de Néphrologie et Laboratoire d'Explorations Fonctionnelles Rénales, CHU Saint Etienne, Saint Etienne, France; [2]Service de Néphrologie et Département de Santé Publique, CHU Nice, Nice, France.*

An increasing number of clinical trials use one-year GFR as an endpoint to estimate the risk of long-term allograft dysfunction. Several registry studies have recently questioned the real performance of the estimated GFR as a valid surrogate marker since the main estimation formulas exhibited only weak predictive values. This point could be explained by the inability of these creatinine-based equations to accurately estimate the true GFR in this population. The aim of our study was to evaluate whether a direct measure of GFR by inulin clearance could be more efficient than the MDRD equation to predict long term allograft survival.

We retrospectively analyzed all transplant recipients with a minimum follow-up of 10 years who had a one-year post-transplantation inulin clearance measurement (measured GFR) and a MDRD assessment (estimated GFR). The respective performances of these GFR evaluations were compared using multivariate analysis with adjustment on main co-variables known to impact allograft survival (Cox proportional model and ROC curves analysis).

Two hundred and twenty nine patients were retained for the analysis. While the one year inulin-based GFR was significantly associated with the 10-year graft loss for the CKD stages 3 (Hazard Ratio 2.9; CI95% [1.1-8.2] and 4 (HR 6.9 [1.2-40.1]), the MDRD-assessed GFR was only for stage 4 (HR 5.6 [1.3-23.9]. The multivariate ROC analysis tended to confirm the superiority of the one-year measured GFR, albeit its predictive performance still remained limited (Inulin and MDRD Area Under Curve respectively 0.65 vs. 0.60; ns)

Our results suggest that the one-year measured GFR is a better predictive factor of the long term allograft outcome than the creatinine-based GFR estimation. However, this parameter, when considered alone, cannot be considered as a valid surrogate marker of the long term allograft survival. Whether other parameters (e.g. proteinuria, histology) combined to the GRF evaluation would improve the prediction needs to be studied.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3052**

**It's Not Who You Know, It's What Who You Know Knows: The Role of Dialysis Teams and Social Networks in Improving Kidney Transplant Parity** Teri Browne. *College of Social Work, University of South Carolina, Columbia, SC.*

PURPOSE: Research has shown that black dialysis patients are significantly less likely than their white peers to be evaluated and listed for a kidney transplant. The contribution of the present study is to investigate previously unconsidered social network factors that might influence the racial composition of kidney transplant-patient populations. METHODS: Surveying 228 black hemodialysis patients in Illinois, the following research questions were addressed using an original survey: (1) What is the role of social networks in providing information about kidney transplantation to black hemodialysis patients? (2) What is the relationship between social networks and a patient's likelihood of being seen at a kidney transplant center? RESULTS: 94% of patients surveyed were interested in a kidney transplant, and 98% percent had insurance that would pay for a kidney transplant, but only 9% were active on a transplant waiting list. Black hemodialysis patients with lower incomes were less likely to be seen at a kidney transplant center (OR 1.38, 95%CI: 1.09-1.76, p<.01), and patients who have people in their social network with information about kidney transplant are significantly more likely to be seen at a kidney transplant center. Specifically,

black dialysis patients who get informational social support from their dialysis team (OR 1.76, 95%CI: 1.5-2.1, p<.001) and social networks (OR 1.63, 95%CI: 1.2-2.3, p<.001) are significantly more likely to be seen at a kidney transplant center. IMPLICATIONS: Kidney transplant disparity is a multifaceted social problem, and considering black dialysis patients' social milieu can be complimentary to the important existing research regarding this public health crisis. The logistic regression models imply that correct information about a kidney transplant and success of being seen at a kidney transplant center can be differentiated on the basis of considering social network informational attributes and income. Dialysis health teams can augment patients' social networks through their own interventions or by linking patients with mentors or patient navigators.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO3053

**Peginterferon Alfa-2A Plus Ribavirin Combination Treatment in Chronic H C V Post Renal Transplantation: An Interim Analysis** Dujanah Hassan Mousa,[1] Faisal Sanai,[2] Hassan A. Aleid,[3] Hamad Ibrahim Al-Ashgar,[4] Khalid Abdulmohsen Almeshari,[3] Khalid I. Bzeizi.[2] *[1]Renal Department, Renal Transplant Program, Riyadh Military Hospital, Riyadh, Saudi Arabia; [2]Hepatology Department, Riyadh Military Hospital, Riyadh, Saudi Arabia; [3]Renal Transplant Program, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; [4]Hepatology Devision, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.*

Chronic liver disease has a significant impact on the survival of renal transplant recipients. At present, there is no safe and efficacious therapy of chronic HCV after renal transplantation. Interferon (IFN)-based therapy in renal transplant recipients remains a controversial issue, as it has been associated with a high risk of graft rejection and poor efficacy. Aim: To assess the safety and efficacy of PEG-IFN and ribavirin combination therapy in post-renal transplant HCV-infected patients. Methods: 28 adult renal transplant recipients at 2 centers in Saudi Arabia,12 months after surgery with confirmed HCV and evidence of histological disease were recruited in a prospective, pilot, open-label trial with PEGIFN-alpha-2a (135-180 micg/week) plus ribavirin (200-1200 mg/day)GFR base dose. Safety and laboratory assessments were performed periodically. more male (57%), mean age 47y, genotype 1&4,and mean HCV RNA 6.4 log10 IU/mL. Renal biopsy was performed in patients with a 20% increase in s creatinine. Results: 26 patients completed study. Dose reductions of PEGIFN and ribavirin were required for hematological side effects. Overall, 55.6%, 37% and 32% achieved EVR, end-of-treatment and SVR, respectively. No documented rejection episodes. Non-renal complications were seen in 3 patients.There was no difference in the pre-treatment and end-of-assessment serum creatinine. Conclusions: PEGIFN/ribavirin therapy in renal recipients is safe with no rejection episodes, but has limited efficacy in the treatment of chronic HCV.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO3054

**Area under the Curve (AUC) Bioequivalence (BE) of Mycophenolate Mofetil (MMF): CellCept® vs. Generic** Ian C. Doyle,[1] Ahmed M. Zikri,[2] William M. Bennett,[3] Michal J. Figurski,[4] Leslie M. Shaw.[4] *[1]School of Pharmacy, Pacific University Oregon; [2]Pharmacy Services; [3]Transplant Services, Legacy Good Samaritan Hospital; [4]Pathology Laboratory Medicine, University of Pennsylvania Medical Center.*

Purpose: Determine BE of generic MMF (Mylan) compared to CellCept (innovator MMF) in renal transplant (RT) recipients based on limited sampling mycophenolic acid (MPA) AUC.

Methods: During 5/09 to 4/10, we established an MMF therapeutic drug monitoring (TDM) program utilizing simultaneous multi-linear regression (MLR) (using SAS) and Bayesian kinetics (BAY) (using NONMEM). Currently, MLR and BAY models have been developed using CellCept. Since 5/09, 7 FDA-approved generic formulations of MMF have become available. Six RT patients were prospectively enrolled in an open-label, crossover design study to compare MPA AUCs of CellCept and the generic MMF formulation (Mylan) most often dispensed in our area. The 250mg capsule of each product was used. MPA levels were drawn at 0/40/240 min. and 0/40/120 min. for FK and CSA patients respectively. Patients consumed a minimum of 6 days of drug during crossover.

Results: MPA AUC

| Dose (mg) | CNI | Day | CellCept AUC BAY | Cellcept AUC MLR | Day | MMF AUC BAY | MMF AUC MLR |
|---|---|---|---|---|---|---|---|
| 500 BID | FK | 201 | 47.25 | 61.14 | 208 | 38.02 | 48.89 |
| 750 BID | FK | 64 | 86.77* | N/A* | 71 | 88.57 | 112.81 |
| 1000 BID | CSA | 57 | 38.67 | 39.77 | 64 | 38.53 | 44.51 |
| 500 BID | FK | 67 | 28.86 | 35.18 | 74 | 36.1 | 47.02 |
| 1000 BID | FK | 56 | 61.72 | 67.95 | 63 | 55.01 | 63.85 |
| 750 BID | FK | 82 | 28.52 | 35.16 | 76 | 43.82 | 62.87 |

* 240-minute sample not available (broken during shipping)

Conclusions: The FDA approves drugs as bioequivalent using data from healthy subjects who achieve a range of 80-125% AUC of innovator. MMF (Mylan) was approved as BE, with AUC 100% and Cmax 89% of CellCept. In our study, 4 of 6 patients had generic MMF AUC levels within 90%-110% AUC of innovator, and 5 of 6 patients were within 80-125%. Since AUC monitoring is subject to a high degree of intra- and inter-patient variability, AUC BE testing for RT patients is warranted. Current CellCept-based MLR and BAY models might not be applicable to patients taking generic MMF.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO3055

**1 Year Results of an Intensified Dosing of Enteric-Coated Mycophenolate Sodium in Renal Transplant Patients Results in Improved Efficacy without Compromising Safety: 1 Year Follow-Up Results** Klemens Budde,[1] Claudia Sommerer,[2] Petra Glander,[1] Toofan Ariatabar,[3] Stefan Kramer,[4] Eva-Maria Vogel,[4] Martin G. Zeier,[2] Wolfgang Arns.[3] *[1]University Hospital Charité, Berlin; [2]Nierenzentrum, University Hospital Heidelberg; [3]Transplantationszentrum, Dept. of Nephrology, Koeln-Merheim; [4]Novartis Pharma, Nuremberg, Germany.*

**Objective:** The influence of an intensified dosing (ID) regimen with enteric-coated mycophenolate sodium (EC-MPS) compared to EC-MPS standard dosing (SD) on efficacy and safety 12 months after renal transplantation was assessed.

**Methods:** De-novo kidney transplant recipients were treated with basiliximab, steroids and cyclosporine and randomized to EC-MPS standard (SD: 1440mg/d) or to EC-MPS intensified dosing regimen (ID: 2 weeks: 2880 mg/d; subsequent 4 weeks: 2160 mg/d; followed by 1440 mg/d). After completion of the core study at month 6, patients (pts) were included in an observational follow-up until month 12.

**Results:** 128 patients were randomized to either SD (n=65) or ID (n=63), 101 (78.9%) pts (n=49 ID vs. n=52 SD) completed the follow-up analysis at month 12. The incidence of BPAR was lower in the ID compared to the SD group (12(23%) vs. 3(6.1%); p= 0.025) at month 12 with one additional BPAR in ID vs 2 BPAR in SD pts in the follow-up period. Patient survival was 98.0% in the ID and 96.2% in the SD group, and 3 graft losses were observed in ID and 5 graft losses in SD pts. In the follow-up period the ID regimen was not associated with a higher rate of hematological side effects (11 ID vs. 15 SD pts). Slightly more infections (29 ID vs. 24 SD pts) and gastrointestinal symptoms (19 ID vs. 15 SD) were reported in the ID group. Safety parameters are listed below:

| | SD | ID |
|---|---|---|
| Anaemia [% of pts] | 12 | 8 |
| Leucopenia [% of pts] | 15 | 4 |
| Thrombocytopenia [% of pts] | 2 | 2 |
| Constipation [% of pts] | 2 | 4 |
| Diarrhea [% of pts] | 10 | 12 |
| Hemoglobin [g/dl] | 12.8 | 12.8 |
| Leukocytes [/nl] | 7.5 | 7.2 |
| CMV infection [% of pts] | 2 | 8 |
| BKV infection [% of pts] | 8 | 8 |
| Pneumonia [% of pts] | 4 | 6 |
| Serum creatinine [mg/dl] | 1.6 | 1.8 |

**Conclusion:** An intensified dosing regimen of EC-MPS was associated with a significantly lower BPAR rate at month 12 without compromising tolerability and safety.

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## SA-PO3056

**Steroid Withdrawal in Renal Transplant Recipients after Conversion from a Cyclosporine Based Immunosuppressive Regimen to Tacrolimus Monotherapy; a Prospective Multicentre Trial** Lars Backman. *Uppsala University Hospital, Dept of Transplantation surgery, Uppsala, Sweden.*

Inspite of the introduction of new immunosuppressive (IS) agents and regimens, steroids (st) are still used widely. St are associated with an increased incidence of diabetes, hypertension and hyperlipidemia, aggravated by the use of Cyclosporine (CsA). Aim: to evaluate whether conversion from a CsA based IS to Tacrolimus (Tac) monotherapy resulted in improvements of these side-effects.

**Methods:** 79 renal transplant recipients (58M/21F) from 9 centres, with mean age of 59 (range 25-80) years with CsA and st associated side-effects were included. The patients were converted to a Tac based regimen with the subsequent withdrawal of st with a f-u of 24 months. Baseline results were compared to 24-month results with paired t-test. Levels are expressed as mean ± SD.

**Results**: 62 of the 79 patients (78%) completed the study and 43 (69%) were completely off st after 24 months (nopred), whereas 16 were still treated with prednisolone (pred), 2.5-7.5 mg/day. 2 patients died (lymphoma and heart failure) and 3 grafts were lost. 10 additional patients were withdrawn. None experienced acute rejection or developed diab during the follow-up.

| | s-crea | s-chol | s-trig | BP |
|---|---|---|---|---|
| Pred BL(n=19) | 140 ±32 | 5.18 ±1.50 | 1.99 ±0.85 | 140/75 |
| Pred 24months | 167 ±69 | 4.96 ±1.04 | 1.89 ±0.95 | 135/77 |
| P value | 0.05 | ns | ns | ns |
| Nopred BL(n=43) | 127 ±37 | 5.33 ±1.54 | 1.76 ±0.86 | 137/80 |
| Nopred 24months | 134 ±45 | 4.58 ±1.28 | 1.53 ±0.85 | 135/80 |
| P value | 0.044 | 0.011 | 0.042 | ns |

**Conclusion:** Conversion from a Cyclosporine based IS was safe and well tolerated. No patient had acute rejection or developed diabetes. The conversion from Cyclosporine resulted in a significant decrease in s-chol. Discontinuation of pred and Tacrolimus monotherapy resulted in a significant decrease in both chol and trig levels with a lower, but increase in crea levels over 24-months. Conversion from Cyclosporine based IS in renal transplant recipients with the subsequent withdrawal of steroids was possible in 73% of the patients and may result in a more favourable cardiovascular risk profile after renal transplantation.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3057**

**Mycophenolate Mofetil (MMF) Therapeutic Drug Monitoring (TDM): Combined Multi-Linear Regression (MLR) and Bayesian (BAY) Area under the Curve (AUC) vs. Standard Care (SC)** Ian C. Doyle,[1] Ahmed M. Zikri,[2] William M. Bennett,[3] Leslie M. Shaw,[4] Michal J. Figurski.[4] *[1]School of Pharmacy, Pacific University Oregon; [2]Pharmacy Services; [3]Transplant Services, Legacy Good Samaritan Hospital; [4]Pathology Laboratory Medicine, University of Pennsylvania Medical Center.*

Purpose: Compare outcomes of renal transplant (RT) recipients following establishment of a mycophenolic acid (MPA) AUC TDM program to a concurrent fixed-dose, SC cohort.

Methods: From 5/09 to 4/10, 29 RT were prospectively enrolled into a study of CellCept®, having MPA levels monitored & doses adjusted to achieve AUC of 45 mg•hr/L (range 40-50). Initial MMF doses were 1000 mg BID for FK patients and 1500 mg BID for CSA patients. Limited sampling MPA levels were drawn on days 7, 30, & 60, at 0/40/240 min. and 0/40/120 min. for FK and CSA patients respectively. Final AUC was determined by averaging MLR and BAY AUCs. Doses were adjusted using AUC results, in conjunction with a transplant nephrologist. Side effects were assessed on AUC sampling days. Surveillance biopsies were performed, unless clinically contraindicated, at 6-8 weeks & 6 months post RT. SC patients were assessed via retrospective chart review (RT 4/09 to 11/09).

Results

| Measure | TDM d7 | TDM d30 | TDM d60 | SC d7 | SC d30 | SC d60 |
|---|---|---|---|---|---|---|
| n (ITT=29/group) | 26 | 24 | 24 | 27 | 27 | 27 |
| Mean MMF mg | 1010 | 844 | 728 | 964 | 894 | 827 |
| % at AUC 40-50 | 42 | 4 | 13 | - | - | - |
| % at AUC 30-60 | 65 | 38 | 58 | - | - | - |
| # dose changes per AUC | 13 | 16 | 11 | - | - | - |
| # dose decrease | 8 | 13 | 8 | 0 | 2 | 1 |
| Mean WBC | 8.64** | 5.66* | 5.5* | 11** | 6.49* | 5.7* |
| Mean Hgb* | 11 | 11.8 | 12.9 | 10.7 | 11 | 11.8 |
| GI intolerance* | 3 | 2 | 2 | 2 | 3 | 1 |
| BK viruria by 3 mo* | | | 4/24 | | | 2/26 |
| Acute Rej. cumulative by 8wk & 3mo* | | | 0/24, 3/24 | | | 3/27, 7/26 |

*NS, **p=0.017

Conclusions: The low rate of in-range AUC values on day 30 might indicate more frequent AUC monitoring is needed during this time period. The large proportion of MMF dose decreases on day 30 & 60 is consistent with literature that MPA AUC increases with time. Many patients can have MMF dose decreased without increasing risk of AR. MPA TDM is associated with a non-significant decreased rate of AR at 3 months. An increased rate of BK infection was observed.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3058**

**Maintaining a Short and Stable Waiting List for Renal Transplant Candidates** Hallvard Holdaas. *Department of Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway.*

**Introduction.** A universal and profound problem in nephrology is the increasing number of patients on waiting lists for renal transplantation. In most countries there is a critical organ donation shortage.

**Method.** We examined listing for renal transplantation and time on the waiting list for endstage renal disease (ESRD) patients in Norway during the last decade.

**Results**. The incidence of ESRD increased from 89 per million per year (pmp) in 2000 to 116 per million inhabitants per year in 2008. Mean age at start of renal replacement therapy was 65.2 years (9 – 88) and 64.8 years (7 – 95) in 2000 and 2008 respectively. During the years 2000 to 2006 the waiting list increased from 35 to 48 pmp with a transplant rate of 46.0 and 45.7 pmp respectively. Kidney grafts from living donors were stable in this period, 17 – 20 pmp. The supply from deceased donors was suboptimal and stable, 27.3 and 26.9 pmp in 2000 and 2006 respectively. However, after establishment of specific donor responsible doctors and a Governmental directive coupled with financial resources to hospitals providing deceased donors, the numbers of deceased donor increased to 35.4 in 2008 and 35.8 pmp in 2009. We also liberally accept extended criteria donors. Theare is also steady supply of living donors is provided by local nephrologist with no financial incentive to choose dialysis as the preferred treatment of ESRD. Overall transplantation rates in 2008 and 2009 were 58.3 and 60.9 pmp versus 46 pmp in 2000. The waiting list is now reduced to 37.3 pmp which is similar to 2000. The current median waiting time for first and secondkidney from deceased donor is about 10 months.

**Conclusion.** Sufficient supply for renal grafts may be obtainable by a combined utilization of kidneys from deceased and living donor sources. This national strategy for ESRD has resulted in a stable waiting list. Of all patients in ESRD in Norway 70% is transplanted and 30% in dialysis treatment.

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3059**

**Kidney Transplant Candidates' Information Needs and Perceptions about OPTN-Defined High Risk Donor Organs** Elisa J. Gordon,[1,2] Elizabeth A. Reddy,[1] Daniela Ladner,[1,2] John J. Friedewald,[2] Michael Ison.[2] *[1]Institute for Healthcare Studies, Northwestern University; [2]Surgery, Northwestern University, Chicago, IL.*

OPTN-policy requires special consent for recipients of organs from OPTN-defined "high risk" donors (HR) but it is unknown what information patients need to make an informed decision about accepting HR organs. We investigated kidney transplant candidates' understanding of, information needs about, and willingness to accept HR donor kidneys. A consecutive sample of kidney transplant candidates underwent a 20-minute telephone interview. Open-ended responses were analyzed for repetitive themes and patterns. The response rate was 83% (147/177 patients). Candidates perceived 'high risk donor' to mean that the donor: was older (39%), had a general health problem (33%), had an infectious disease (29%), used drugs or alcohol (8%), or that the donor kidney would not function well or match well with the recipient (7%). Candidates wanted to know "everything" about the HR donor's: risky behaviors (smoking, drinking, drug use) (41%), medical conditions (16%), age (8%), how donors died, how well the graft would function (17%) and match (7%), and the statistical likelihood of transmitting infectious disease (39%). Candidates were unwilling (47%) or unsure (21%) to accept a HR kidney because they were concerned about getting an infection and endangering health (31%), the kidney would not function well or last (29%), doing well on dialysis (21%), and do not have enough information (11%).

Table 1. Information Needs about HR Kidneys

| |
|---|
| "Wouldn't it be a good thing to know what it was that killed the donor? What made [the organ] high risk? How long would it last?" |
| "What's wrong with the organ and how can it effect your body … Will it be rejected? That would be the major thing. I don't want to go through two surgeries." |

Transplant candidates for other organs may differ in willingness to accept HR organs as they may not have alternative therapy but certain death. Greater efforts are needed to educate kidney transplant candidates about what HR donor organs are, how well they function, and the donor's behaviors to optimize their informed treatment decisions.

Disclosure of Financial Relationships: nothing to disclose

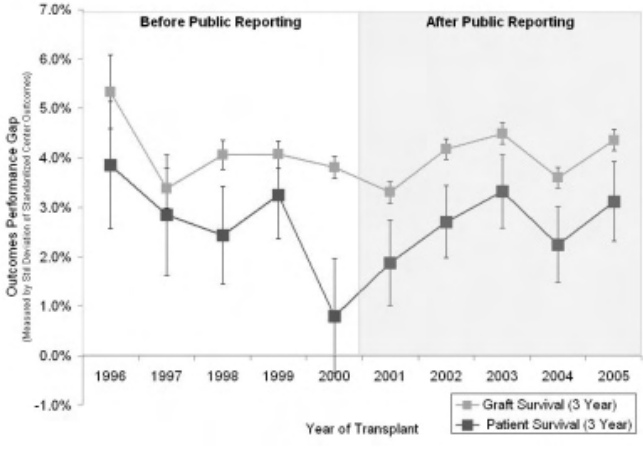
**SA-PO3060**

**Public Reporting Has Not Reduced the Performance Gap across Kidney Transplant Centers** Constantia Petrou,[1] Stefanos Zenios.[1,2] *[1]Culmini Inc; [2]Graduate School of Business, Stanford University.*

**Purpose:** To determine whether public reporting of kidney transplant center performance has reduced the outcomes gap across centers.

**Methods:** We used a mixed effects approach to provide robust estimates of the performance gap across transplant centers. Unlike current methods, this approach is not susceptible to generating extreme estimates of the performance gap due to random chance. Data were obtained from the USRDS. All adult patients who received a transplant between 1/1/95 and 9/30/05 were included. 3-year patient survival and 3-year graft survival were modeled using generalized linear mixed effect models with a logit link. Risk factors at the time of transplant were the fixed effects and transplant centers were the random effects. The outcomes gap across transplant centers was measured by the standard deviation of standardized outcomes derived from the models.

**Results:** Using the mixed effects method, the typical performance gap across centers was: 4.2 % for graft survival and 2.5% for patient survival (p-value < 0.01). Methods currently used in public reports exaggerated the performance gap: 8.6% gap for graft survival and 7.7% gap for patient survival. The effect of our more conservative estimate is clinically significant. If the performance gap would be completely eliminated (by worse centers matching the outcomes of the better ones), 25% of all graft failures and 20% of all deaths in the first three years after transplant could be averted. However, the figure below shows that the performance gap did not decrease after the introduction of public reports in 2001.



**Conclusions:** Public reporting of transplant center performance has not reduced the outcomes gap across centers. For all patients receiving a kidney transplant in any given year and followed for 3 years post-transplant, reducing this center gap is expected to save up-to 278 patient lives and 670 grafts.

Disclosure of Financial Relationships: Employer: Culmini Inc; Ownership: Culmini IncResearch Funding: Abbott Inc.

## SA-PO3061

**Treating Post Transplant Anaemia Does Not Affect Progression of Chronic Kidney Disease: A Pilot Randomised Controlled Trial** Taryn Pile,[1] Martin J. Raftery,[1] Magdi Yaqoob.[2] *[1]Department of Renal Medicine and Transplantation, Barts and the London NHS Trust, London, United Kingdom; [2]Department of Translational Medicine and Therapeutics, William Harvey Research Instituite, Queen Mary, University of London, United Kingdom.*

Background

Post Transplant Anaemia (PTA) affects 30-45% of renal transplant recipients. The treatment of PTA has not been well studied in randomised controlled trials (RCT). A pilot study to assess the effect of treatment with Epoetin beta on renal progression, blood pressure and proteinuria in anaemic renal transplant recipients was performed.

Methods

A RCT was performed in a single renal transplant centre. Renal transplant patients more than 3 months post transplant with an Hb between 9 and 11.5g/dl were recruited. The treatment arm received Epoetin beta (EB) starting at a dose of 50U/kg/week to achieve a target of 11.5-13.5g/dl. The No Treatment group (NT) was treated with EB if the Hb fell below 9g/dl. The primary end-points were progression of CKD, blood pressure and proteinuria.

Results

55 patients were recruited (NT N= 27, EB N=28) and were followed up for a median of 23.34 months. The demographics and baseline biochemistry was similar in both groups at initiation of the study. At the end of the study the Hb was significantly higher in the EB group (EB: 12.3 ± 0.18 vs. NT: 9.99 ± 0.22 g/dl, $p < 0.001$). Protein:Creatinine Ratio was similar in both groups (EB 45.74 ± 12.07 vs. NT: 34.8 ± 6.84 mg/mmol, p=0.64). Average of 3 blood pressures was not significantly different at the end of the study (SBP: EB 130.9 ± 2.1 vs. NT: 127.3 ± 2.2, p=0.45; DBP 82.95 ± 1.4 vs. NT: 82.1 ± 1.4 mmHg, p=0.48). Rate of progression, determined by slope of eGFR by MDRD7, was not significantly different between the two groups (EB: -0.09 ± 0.1 vs. NT: -0.12 ± 0.15 mLs/min, p=0.78).

Conclusion

The treatment of anaemic transplant patients with EB in this small pilot RCT did not have any significant effect on renal progression. There is however no evidence of harm done to these patients treated to a target of 11.5-13.5g/dl. A large, multi-centred RCT is warranted to study the effects of erythropoietin treatment in PTA.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO3062

**High-Dose Erythropoietin (EPO) and Graft Function after Cadaveric Kidney Transplantation** Carsten Hafer,[1] Jan T. Kielstein,[1] Thomas Becker,[3] Hermann G. Haller,[1] Danilo Fliser.[2] *[1]Internal Medicine / Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [2]Internal Medicine / Nephrology and Hypertension, Saarland University Medical Centre, Homburg / Saar, Germany; [3]Transplantation Surgery, Hannover Medical School, Hannover, Germany.*

**Background**: In experimental studies administration of erythropoietin (EPO) revealed tissue-protective effects and prevented renal tissue damage after ischemia-reperfusion injury.

**Methods**: We evaluated short- and long-term effects of high dose EPO on kidney function after renal transplantation in a prospective double-blind placebo controlled study. Ninety patients with CKD stage 5 D receiving a cadaveric kidney allograft were randomized to 3 x 40.000 IU EPO alpha (1st injection intra-arterial immediately after opening the transplant artery, 2nd and 3rd were given 3 and 7 days after successful transplantation) or placebo. Primary endpoint was estimated glomerular filtration rate (eGFR/CKD-EPI) 42 days after transplantation. Secondary endpoints were incidence of delayed graft function, i.e. dialysis dependency during the first 7 days after transplantation, and eGFR at 6 and 12 month.

**Results**: Eighty-eight patients were included in the intention to treat analysis. eGFR after 42 days was similar in both groups: 46.6 ± 28.3 ml/min (EPO) vs. 45.7 ± 21.6 ml/min (placebo), (p=0.78). The results were similar (p=0.24) after 12 months: 39.7 ± 3.4 (EPO) vs. 44.56 ± 3.6 ml/min (placebo). There was no significant difference between groups with respect to the incidence of delayed graft function (EPO: 10 of 44 pts. [22.7%]; placebo: 14 of 44 pts. [31.8%]). Transplant biopsies 6 weeks and 6 months after transplantation showed no significant differences in all histological indices. Number and severity of adverse events were comparable between both groups as was patient survival after 12 months, i.e. 97.7 % (EPO) vs. 95.5 % (placebo).

**Conclusion**: Administration of EPO after cadaveric kidney transplantation was well tolerated, but did not have a significant effect on graft histology or function in cadaveric allograft kidney transplants.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO3063

**Urinary NGAL-Ratios as a Non-Invasive Biomarker for Acute Tubular Injury in Kidney Transplant Patients** Jessica K. Kaufeld,[1] Wilfried Gwinner,[1] Irina Scheffner,[2] Hermann G. Haller,[1] Mario Schiffer.[1] *[1]Department of Nephrology, Medical School Hannover, Hannover, Germany; [2]Department of Statistics, Medical School Hannover, Hannover, Germany.*

Urinary neutrophil gelatinase-associated lipocalin (NGAL) has been recognized as an early and sensitive biomarker of kidney injury. It has been shown to predict dialysis and delayed graft function after kidney transplantation in deceased-donor kidney transplant recipients. In our study, we addressed the question whether urinary NGAL could predict the long-term graft function of renal allograft recipients (living-donor and deceased-donor) that were enrolled in the protocol-biopsy program of our center.

Protocol biopsies (pBx) were taken at 6 weeks, 3 months and 6 months after kidney transplantation. Urinary NGAL was measured in the spot urine at the time of the biopsy, normalized to urinary creatinine excretion and correlated to biopsy findings according to the current BANFF-classification such as acute tubular injury (ATI) and rejection as well as eGFR. Controls included 9 healthy individuals (2-kidney controls) and 12 individuals after kidney donation (1-kidney control) with normal kidney function. We screened 5 ICU patients with acute kidney failure to confirm the sensitivity of our assay.

Renal transplant recipients had 1.9-fold higher urinary NGAL ratios compared to the 2-kidney controls. Transplant patients with ATI or CNI-toxicity had only a numerical trend towards higher NGAL-ratios compared to those without. Patients with multiple findings of ATI did not have higher urinary NGAL compared to those with only one ATI finding in the pBx. No differences were found between moderate and severe ATI. Notably, when 1-kidney controls were analyzed, we found a significantly reduced urinary NGAL-ratio compared to 2-kidney controls (59%) and to the transplanted patients (32%). ICU-patients had 105-fold higher NGAL-ratios compared to the 2-kidney controls ($p<0.001$).

We conclude that urinary NGAL-ratios are apparently not sensitive enough as a non-invasive biomarker to monitor ATI in kidney transplant recipients. The differences seen between 1- and 2-kidney controls point to a confounding effect of renal mass on the urinary NGAL excretion.

Disclosure of Financial Relationships: nothing to disclose

## SA-PO3064

**Novel Quantitative Virtual Microscopy-Based Method To Evaluate GL-3 Inclusions in Renal Peritubular Capillaries in Patients with Fabry Disease** Laura M. C. Barisoni,[1] J. Charles Jennette,[2] Robert B. Colvin,[3] Sheela Sitaraman,[4] Jeff Castelli,[4] Pol Boudes.[4] *[1]New York University School of Medicine; [2]University of North Carolina; [3]Harvard Medical School; [4]Amicus Therapeutics.*

Introduction: Renal failure is a major cause of morbidity and mortality in patients with Fabry disease (FD). Levels of GL-3 cytoplasmic inclusions in peritubular capillaries (PTCs) of renal biopsies are considered the most useful pathologic measure of therapeutic efficacy. Previous trials of enzyme replacement therapy used a semi-quantitative light microscopy (LM) approach to measure GL-3 inclusions in PTCs; however, limitations in quantifying low levels of GL-3 using this method were identified. Here we describe a novel virtual microscopy (VM) quantitative method to measure GL-3 inclusions in PTCs.

Methods: Renal biopsies were collected from 17 patients with variable FD severity enrolled in Phase 2 studies of AT1001, a pharmacological chaperone in clinical development for the treatment of FD. Biopsy samples were scanned at 100X magnification into whole slide digital images to enable virtual annotation of PTCs and scoring of GL-3 inclusions. For each digitally-imaged biopsy sample, a minimum of 50 PTCs were annotated by one pathologist. GL-3 inclusions in each annotated PTC were recorded by the other two pathologists using identical duplicates of the annotated virtual images. The final score for each sample was calculated as the average number of GL-3 inclusions per PTC.

Results: Using the published, semi-quantitative LM method, two pathologists scored 4/17 and 7/17 pre-treatment samples as "0" (using a scale from 0-3+), making it difficult to assess the effect of therapeutic interventions. Using the new quantitative VM method, GL-3 inclusions were detected in all samples tested, and the average number of GL-3 inclusions at baseline per PTC varied between 0.2 and 4.3.

Conclusions: The VM-based quantitative scoring improves sensitivity of measuring GL-3 inclusions in PTCs. Other advantages include the ability to annotate images, allow multiple pathologists to access the same slides from remote locations, and create an auditable archive of data for clinical trials.

Disclosure of Financial Relationships: Consultancy: Amicus theraputics.

**SA-PO3065**

**Improved Gastrointestinal Symptoms and Health-Related Quality of Life after Conversion from Mycophenolate Mofetil to Enteric-Coated Mycophenolate Sodium in Renal Transplant Patients Receiving Tacrolimus**

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Background: The benefit of conversion from mycophenolate mofetil (MMF) to enteric-coated mycophenolate sodium (EC-MPS) in terms of gastrointestinal (GI) symptom burden and health-related quality of life (HRQoL) is unclear in renal transplant recipients receiving tacrolimus. This study was performed to determine whether tacrolimus-treated patients experience these benefits of EC-MPS conversion.

Methods: The study consisted of multi-center, open-label trial. Patients were categorized into two groups by a simple questionnaire for GI symptom. Patients with GI complaints were converted to equimolar EC-MPS (n=175) and the others continued on MMF (n=83). Gastrointestinal Symptom Rating Scale (GSRS) and Gastrointestinal Quality of Life Index (GIQLI) were evaluated at baseline and measured for one month. Patients and physicians completed Overall Treatment Effect (OTE) at one month.

Results: EC-MPS-converted patients had worse GSRS and GIQLI scores at baseline than MMF-continued patients (all $p < 0.01$). Significant improvements in GSRS and GIQLI scores were observed for EC-MPS patients at the end of one month, but MMF patients showed worsened GSRS scores (all $p < 0.05$). OTE scale indicated that EC-MPS-converted patients made more improvements in overall GI symptoms and HRQoL than MMF-continued patients did ($p < 0.001$).

Conclusions: In tacrolimus-treated renal transplant recipients with GI burdens, conversion from MMF to EC-MPS improves GI-related symptom and HRQoL.

Disclosure of Financial Relationships: Research Funding: The study was funded by Novartis Pharma.

**SA-PO3066**

**Validation of the Cystatin C as a Diagnostic Tool for Estimating Glomerular Filtration Rate in Donors One Year Post-Donation**

Miguel Angel González Alfaro,[1] Luz Adriana Balderas,[3] Mario Sandoval Sandoval,[1] Enrique Rojas-Campos,[2] Francisco Monteon,[1] Abel Puentes Camacho,[1] Benjamin Gomez-Navarro.[1] *[1]Nephrology and Organ Transplant, Hospital de Especialidades IMSS, Guadalajara, Jalisco, Mexico; [2]Clinical Researche Unit in Epididemiologic, Hospital de Especialidades IMSS, Guadalajara, Jalisco, Mexico; [3]Medical Research Unit In Renal Diseases, Hospital de Especialidades, Guadalajara, Jalisco, Mexico.*

Introduction: serum creatinine(SCr) is the most widely test, to estimate the glomerular filtration rate (eGFR). However SCr is less sensitive to detect early renal disease. Cystatin C (CyC) is a protein with a constant production rate, therefore is a potential good marker to estimate GFR.

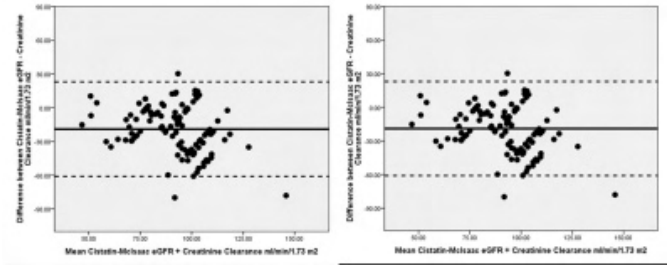
Aim: To determine the sensitivity, specificity of serum CyC as a marker of GFR in kidney donors vs CrCl and MDRD.

Methods: cross-sectional study of 125 stables kidney donors; to determine the levels of SCr, CrCl and CyC. Estimation of GFR by MDRD and McIsaac formulas (CyC) compared to CrCl, discordances between tests were done by Bland-Altman.

Cut-off 60ml/min

| | GFR McIsaac | GFR CrCl | GFR MDRD |
|---|---|---|---|
| Sensitivity | 44.82% | 13.79% | 13.79% |
| Specificity | 97.9% | 100% | 100% |
| PPV | 86.67% | 100% | 100% |
| NPV | 85.45% | 79% | 79% |

RESULTS. Sensitivity and other results are shown in table. Bland-Altman comparisons are shown in the graphic.



Conclusion: The correlation between estimated glomerular filtration rate by MDRD and McIssac calculated and the serum cystatin C is r=0,744 (P 0.000), with CrCl correlation drops to r=0,455 (P 0.000). Bland – Altman, showed that CcCl C vs Cystatin C underestimates and GFR MDRD vs Cystatin C overestimates GFR

Disclosure of Financial Relationships: nothing to disclose

**SA-PO3067**

**Everolimus Ascertain: Multivariate Analyses Identified High Baseline GFR as a Major Factor for Improving Renal Function**

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Early switch to an Everolimus (EVR) CNI elimination regime has demonstrated improved renal function in de novo renal transplant patients (RTs). This subanalysis of ASCERTAIN, a prospective, multicenter, open-label, three-arm study investigated the impact of CNI withdrawal or reduction by the addition of EVR in maintenance RTs on renal function (mGFR) at 24 months (M).

RTs (N=398) with renal impairment (est. GFR 30-70 mL/min) post-6M receiving CNIs, with MPA (72.6%) or AZA (27.4%) were randomized to Group (Gp) A (N=125): continued treatment with CNIs, GpB (N=128): EVR (8-12 ng/mL + CNI elimination, or GpC (N=145): EVR 3-8 ng/mL + CNI 70-90% reduction. An on-treatment (OT) univariate analysis of mGFR at 24M identified baseline risk factors. Further OT multivariate (MV) analysis of the factors showed the statistical significance of baseline mGFR, donor age and time since transplantation for mGFR at 24M across Gps. Baseline mGFR was a significant risk factor across treatment Gps.

Multivariate risk factor OT analysis of mGFR at 24M on per protocol population (N=385)

| RISK FACTOR | REGRESSION | P-VALUE |
|---|---|---|
| Recipient age (years) | -0.040 | 0.782 |
| Donor age (years) | -0.320 | 0.0089 |
| Time since transplantation (years) | -0.986 | 0.047 |
| History of diabetes mellitis (yes/no = y/n) | -0.060 | 0.989 |
| Calcium channel blockers at baseline (y/n) | 0.442 | 0.891 |
| Baseline mGFR mL/min/1.73m2 | 0.683 | <0.0001 |
| Lipid modifying agents at baseline (y/n) | -1.562 | 0.634 |
| Baseline urine protein/creatinine ratio (mg/mmol) | -0.066 | 0.271 |
| Baseline arteriolar hyaline thickening (lesion score ah) | -2.498 | 0.195 |
| Baseline severity grade of CAN | -2.240 | 0.279 |

This multivariate analysis identified time since transplantation and donor age as significant risk factors contributing to further reduction of GFR in maintenance RTs. A high baseline mGFR at time of EVR use was a significant benefit for GFR improvement at 24M.

Disclosure of Financial Relationships: Research Funding: Novartis Pharmaceuticals Corporation.

**PUB001**

**Effects of (+)-Catechin on the Progression of Diabetic Nephropathy in Rats** Sudha Chennasamudram,[1] Majid Moridani,[2] Shashi Kudugunti,[2] Tetyana L. Vasylyeva.[1] *[1]Department of Pediatrics, School of Medicine, Texas Tech University Health Sciences Center, Amarillo, TX; [2]Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, TX.*

**Purpose:** to investigate the role of (+)-catechin in the prevention of diabetic nephropathy in the streptozotocin-induced (STZ) diabetic rat.

**Methods:** Groups of control and STZ rats (n = 6) received 30-40 mg of (+)-catechin in the drinking water daily for 12 weeks. Additional groups received plain water. Urine was collected weekly and analyzed for albumin and creatinine. Plasma was collected weekly to monitor liver function (alanine aminotransferase [ALT]) and oxidative stress by assessing the thiobarbituric acid reactive substances (TBARS) test for lipid peroxidation and the concentration of free thiols. The effects of (+)-catechin on several inflammatory and growth factors were investigated. Lipid peroxidation and thiol content were also measured in liver and kidney tissue at necropsy.

**Results:** After 12 weeks of treatment, the 24-hours mean urinary albumin and albumin:creatinine ratio were significantly lower in diabetic rats treated with (+)-catechin compared to appropriate controls ($p < 0.05$). Additional indicators of renoprotection were observed, but none reached statistical significance. ALT was lower in the (+)-catechin-treated STZ animals. Lipid peroxidation was reduced four–fold and the thiol content was depleted by 51% in the control STZ group opposed to only 15% depletion in the (+)-catechin STZ group. Preliminary work indicated that Interleukin 6, tumor necrosis factor a, and soluble intercellular adhesion molecule-1 were elevated in the STZ model.

**Conclusion:** Our study demonstrated that (+)-catechin was non-toxic to liver and prevented oxidative stress in STZ diabetic rats. The renoprotective properties of (+)-catechin appeared to halt the progression of diabetic nephropathy in this model.

Disclosure of Financial Relationships: nothing to disclose

**PUB002**

**Mechanisms Critical in the Induction of Functional Renal Tubules** Katherine J. Kelly,[1] Jesus H. Dominguez,[1,2] Jizhong Zhang,[1] Barbara Kluve-Beckerman.[3] *[1]Medicine/Nephrology, Indiana University School of Medicine, Indianapolis, IN; [2]Medicine/Nephrology, Roudebush VA Medical Center, Indianapolis, IN; [3]Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN.*

Recovery from an acute renal insult recapitulates components of embryonic tubulogeneis, an elegant and complex process during which branching tubules that maintain solute and water homeostasis are formed. We have demonstrated robust expression of the acute phase reaction protein serum amyloid A (SAA) during tubule formation in embryogenesis and during regeneration after an acute renal insult in adult life. SAA expression in cultured renal tubule (NRK52E) cells induces the formation of functioning tubules. Recovery from established, experimental acute kidney injury induced by diverse insults in rats is accelerated by intravenous transplantation of renal tubule cells overexpressing SAA. The mechanism of tubule formation includes redistribution of the actin cytoskeleton with changes in cell shape, motility, adhesion and cell-matrix interaction. Decreased activation of extracellular signal regulated kinase (ERK) is associated with changes in the actin cytoskeleton. Tubule formation also results from increased proliferation of tubule cells expressing SAA. These studies support our hypothesis that serum amyloid A initiates a critical program in renal morphogenesis. They show that renal tubular cell re-differentiation is a complex response that involves changes in extracellular matrix as well as rearrangement of actin cytoskeleton. These effects might also take part in renal recovery following injury.

Disclosure of Financial Relationships: Ownership: Partial ownership of LLC providing microscopic imaging and consulting services.

**PUB003**

**Kidney Pericytes Stabilize Vascular Tubes in 3D Collagen Matrices** Claudia Schrimpf, Jeremy S. Duffield. *Renal Division, Brigham and Women's Hospital Harvard Institutes of Medicine, Boston, MA.*

We have recently described kidney pericytes, mural cells of the peritubular capillaries and their key role as myofibroblast progenitors (Humphreys B. D. et al. AJP 2010 176 (1); Lin S. L. et al. AJP 2008 173 (6)). However pericyte function in kidney tissue regeneration is unknown. To study their function in vasculogenesis and vascular stabilization after injury we developed a 3D assay of capillary tube formation in a collagen matrix. Capillary tubes are unstable and regress in the presence of serine proteases, but pericytes from other tissues are known to stabilize capillaries. We purified kidney pericytes, established and characterized primary cultures. Kidney pericytes attached to forming endothelial tubes and prevented capillary regression similarly to human vascular brain pericytes. Here we establish a hitherto unknown function for kidney pericytes that links pericyte detachment following injury to capillary rarefaction.

Disclosure of Financial Relationships: nothing to disclose

**PUB004**

**A Study of Nondiabetic Renal Disease (NDRD) in Type 2 Diabetes Mellitus (DM) Patients in Indian Population** Vishwanath Siddini, Sudarshan H. Ballal. *Nephrology, Manipal Hospital, Bangalore, Karnataka, India.*

**Introduction and Aims**:

Wide spectra of nondiabetic renal disease are reported to occur in patients with type 2DM. we evaluated the prevelance and spectrum of NDRD in biopsied Type 2 DM. its correlation with diabetic retinopathy(DR) and rapid decline of renal function was also studied. **Methods**:

Between January 2001 and june2010, 120 patients underwent renal biopsy based on standard clinical indicators for NDRD. Clinical records and hisyopathological reports were reiviewed.

**Results**:

Major indications for biopsy were rapidly progressive renal failure (RPRF) and proteinuria in absence of diabetic retinopathy. Renal histology showed pure NDRD in 44, mixed NDRD-Diabetic nephropathy in 43 and pure DN in 33. DR was seen in 5(11%), 24(55%) and 23(70%) in patients with pure NDRD, mixed NDRD-DN and pure DN respectively. Negative predictive value of DR for predicting NDRD was 86% (58/68)

Of total 87 cases with NDRD:

48(52%) were tubulointerstitial lesions namely acute interstitial nephritis (AIN) – 32, chronic interstitial nephritis- 9 and acute tubular necrosis- 7.

37(44%) were glomerular lesions namely PIGN-12, MGN-8, IgA Nephropathy -6, FSGS-5, MCNS-3, Lupus Nephritis-1 , Idiopathic crescentic GN -1, and amyloid -1.

3(4%) were vascular lesions namely Atheroembolic ARF – 2 and scleroderma-1

Total 86 patients were identified as having RPRF/unexplained advanced renal failure. Pure NDRD was seen in 26(29%), mixed NDRD-DN in 37(44%) and pure DN in 23(27%). Positive predictive value of RPRF in predicting NDRD was 73 %( 63/86). Of these 63 patients patients, commonest lesion was AIN-32(50%) followed by PIGN-12(19%), CIN-7(13%), the majority of which had background DN. Rest of lesions were ATN, atheroembolic, scleroderma, lupus and crescentic GN. History of NSAIDs/native medications was available in more than half of cases of AIN. Skin infection was seen in 70% of PIGN cases.

**Conclusions**:

Prevalence of NDRD was 72% in biopsied type 2 diabetics. 73% of patients with Type 2DM and RPRFshowed NDRD. High incidence of AIN is probably related to increasing use of NSAIDS/native medications in such patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB005**

**Polymyxin B: A Nephrotoxic Drug in Fact** Maria De Fatima Vattimo,[1] Bruce A. Molitoris.[2] *[1]School of Nursing, University of Sao Paulo, Sao Paulo, SP, Brazil; [2]Nephrology Division, Indiana University Purdue University, Indianapolis, IN.*

Polymyxin B (PmxB) is a nephrotoxic drug used to treat multiresistent Gram-negative bacteria infection. Its main side effect, the nephrotoxicity, reduces its clinical use. The objective of this study was to evaluate its nephrotoxicity and citotoxicity . Studies of renal function (creatinine clearance, crCl/100g), urinary peroxides (UP, nmol/g creat), TBARS (nmol/g creat), catalase activity (CA, nmol/degraded $H_2O_2$/mg total protein), thiols (nmol/mg total protein), LHD (%) and histology of renal tissue were performed in wistar rats. Cell culture studies evaluated apoptosis and viability (72h, Hoescht 33342,Acridine orange/Ethydium bromide, %). Mitochondrial function on LLC-$PK_1$ cells (MF, 4h, %) after the treatment with RhodaminePmxB (375 M) was also performed. Data are presented as mean±standard deviation (X±SD).

Results of renal function, lipid peroxidation and LLC-PK1 cell culture.

| Rats | crCL | UP | TBARS | CA | Thiols |
|---|---|---|---|---|---|
| CTL | 0.70±0.08 | 5.0±1.3 | 34.4±8.0 | 5.6±1.2 | 28.9±6.1 |
| PmxB | 0.30±0.33* | 35.6±9.9* | 98.1±18.0* | 1.2±0.4* | 15.7±1.3* |
| Cells | Apoptosis | Viability | LHD | MF | |
| CTL | 5±1 | 81±3 | 2.0±0.1 | 45.1±4.5 | |
| PmxB | 30±3* | 41±7* | 10.6±1.7* | 40.5±3.5* | |

* $p < 0.05$ vs Control (CTL)

PmxB induced acute kidney injury with the increment in UP and TBARS, and reduction in CA and thiols. It also increased the fractional interstitial area of renal tissue with acute tubule necrosis in renal cortex.The PmxB citotoxicity was confirmed with the increase in apoptosis, reduction in viability and elevation in DHL levels. MF was reduced and associated with the elevation of the dose, what suggests that the ATP dysfunction precedes cell injury. Thus, it can be concluded that PmxB induces acute kidney injury, with lipid peroxidation and renal cell death. Mitochondria plays a crucial role in the endogenous pathways of apoptosis in a dose paired manner.

Disclosure of Financial Relationships: nothing to disclose

**PUB006**

**Hsp70 Expression in Septic Critically Ill Patients and Relation with the Severity of Cases and Acute Kidney Injury** Itir Yegenaga,[1] Murat Kasap,[2] Aylin Kanli,[2] Nur Baykara.[3] *[1]Nephrology, Kocaeli University Medical School, Kocaeli, Turkey; [2]Medical Biology, Kocaeli University Medical School, Kocaeli, Turkey; [3]Intensive Care, Kocaeli University Medical School, Kocaeli, Turkey.*

Acute kidney injury (AKI) especially in sepsis in intensive care units (ICU) is an important problem with serious consequences. Recently it is suggested that HSP 70(heat shock) protein family which is known to rescue the cells against the stress, might play a crucial role on the severity of sepsis and worse output.

Aim of this study is to search if there is a relationship between HSP70 protein expressions in the cells of urine sediment and mononuclear cells in blood of AKI and non-AKI patients with sepsis in the ICU and compared to the healthy individuals.

Clinical features of patients were shown in Table 1.

Clinical feature of patients

| | Mean age/SD | Gender/male | Mean SOFA | Mean APACHE II |
|---|---|---|---|---|
| AKI(n=5) | 77,75+3,30 | 3 | 15,25+1,71 | 30,50+8,10 |
| Non-AKI(n=5) | 44,33+17,39 | 2 | 8,00+2,00 | 15,33+7,09 |

Western blotting was used to study HSP70 expression by using a monoclonal HSP70 antibody (Biovision, USA). HSP*70* gene expression was studied by quantitative real time PCR(polymerase chain reaction) using a 5'-FAM labeled hydrolysis probe.

Although HSP*70* expression was present by PCR; HSP 70 protein was not determined in the cells of urine sediment. But there were no differences between groups. In blood samples, HSP*70* expression was 2.8-fold up-regulated in non-AKF patients then AKF patients. Furthermore, HSP*70* expression in non-AKI patients were up regulated (1.2-fold), while a 1.6-fold down regulation was observed in AKI patients when compare to the healthy control group. However, to see the capacity of HSP*70* expression, samples were heated 42°C 30 min, HSP*70* induction were 19.6-fold, 61.1-fold and 91.9-fold up-regulated in non-AKI, AKF and control sample, respectively.

This finding might suggest that non-AKF patients were more capable of protecting their cells, and the level of basic HSP 70 level might be related to the severity of sepsis in human in vivo.

Disclosure of Financial Relationships: nothing to disclose

**PUB007**

**Overexpression of Cyclin-Dependent Kinase Inhibitor p18$^{INK4C}$ Protects Cisplatin-Induced Renal Tubular Epithelial Cell Injury** Zhang Yi,[1] Li Yuan,[2] Changlin Mei.[3] *[1]Nephrology Department of Changzheng Hospital, The Second Military Medical University, Shanghai, China; [2]Nephrology Department of Changzheng Hospital, The Second Military Medical University, China; [3]Nephrology Department of Changzheng Hospital, The Second Military Medical University, China.*

**Objective:**

We postulated that INK4 family members should have protective effects against AKI and used P18 as the main subject to explore its protective actions in cisplatin-induced AKI.

**Methods:**

We overexpressed P18 in renal tubular cells (LLC-PK1) and observed the effect of p18 overexpression on the LLC-PK1 cells by proliferation assay and the cell cycle analysis. Renal cell injury was induced by cisplatin (100μM) incubation for 24h and LDH release, caspase 3 activity and the splice fragments of caspase 3 and PARP, a caspase 3 substrate, were detected in the vehicle and p18 plasmid transfected cells after cisplatin administration. In order to identify the possible mechanism of p18 protection, we also detected the difference of endoplasmic reticulum stress (ERS) degree between the vehicle and p18 plasmid transfected cells after cisplatin administration.

**Results:**

After P18 plasmid transfection, P18 expression in LLC-PK1 cells increased markedly, and blocked the cell cycle and inhibited proliferation of LLC-PK1 cells. After cisplatin incubation, P18 overexpression did not affect cell necrosis significantly but reduced cell apoptosis markedly. Activation of caspase 3 and the splice fragments of caspase 3 and PARP were also reduced markedly in p18 plasmid transfected cells. Further, p18 overexpression reduced the upregulation of chaperone grp78, attenuated the degree of caspase 12 activation and decreased the level of PERK/eIF2α phosphorylation after cisplatin administration.

**Conclusions:**These results showed that P18, an INK4 family member, was also involved in cisplatin-induced renal cell injury and played protective actions via affecting the ERS pathway in addition to the cell cycle regulation.

Disclosure of Financial Relationships: nothing to disclose

**PUB008**

**P2X7R and Acute Tubular Injury** Nishkantha Arulkumaran,[1] Simona Deplano,[1] Reiko Hewitt,[1] Alex Dyson,[2] Robert J. Unwin,[3] Mervyn Singer,[2] H. Terence Cook,[1] Frederick W. K. Tam.[1] *[1]Department of Nephrology, Imperial College Kidney and Transplant Institute, London, United Kingdom; [2]Department of Intensive Care Medicine, University College London, London, United Kingdom; [3]Department of Nephrology, University College London, London, United Kingdom.*

Purpose:

The P2X7 receptor (P2X7R) plays a key role in inflammatory cytokine release (IL-1β, IL- 18) and apoptosis. We determined the pattern of renal P2X7R expression in 2 rat models of acute kidney injury (AKI). In vitro work was done ascertain the functional significance of the P2X7R.

Methods:

(1). Rat model of haemorrhage and resuscitation

Forty percent of circulating volume of anaesthetised rats was shed. After 30mins, shed blood was re-infused over 2- hours. Rats were given 21% (n=5) or 100% oxygen (O2) (n=8).

(2). Rat model of sepsis

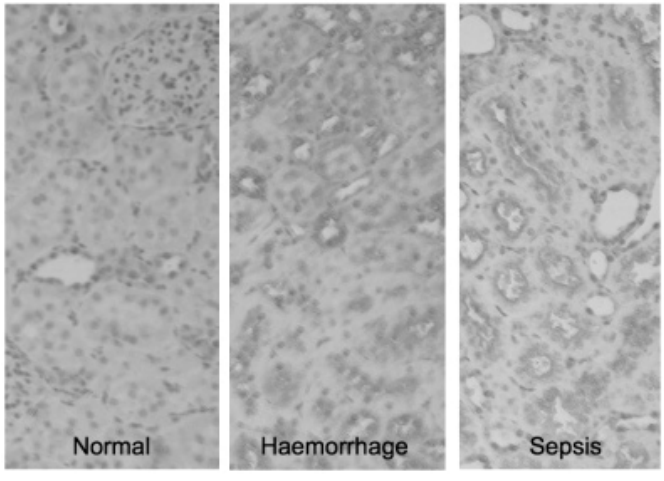
Sepsis was induced by injection of faecal slurry into the peritoneal cavity. Rats were fluid resuscitated for 6 hours.

(2). Cell culture

NRK 52E rat tubular epithelial cells were cultured. Cells were primed with LPS (1μg/ml) for 24 hours. Western blot was used to detect P2X7R and IL-1 expression.

Results:

(1). Immunohistochemistry (IHC) showed upregulation of P2X7R in renal tubules that correlated with the degree of acute tubular injury (see figure). There was no difference in acute tubular injury between rats receiving 21% and 100% O2.




(2). IHC demonstrated upregulation of P2X7R in renal tubules that correlated with the degree of acute tubular injury [figure 1].

(3). P2X7R protein was not detected under basal conditions. LPS stimulation resulted in a significant upregulation of P2X7R and pro- IL-1β protein as detected by Western Blot.

Conclusion:

Upregulation of P2X7R plays a crucial role in septic and ischaemic AKI. Tubular epithelial P2X7R expression has been demonstrated in both in vivo and in vitro work. Further work is required to determine the functional significance of tubular P2X7R.

Disclosure of Financial Relationships: nothing to disclose

**PUB009**

**Results of Open Heart Surgery in Jehovah´s Witnesses: Incidence of Acute Kidney Injury and Mortality Compared with a Control Group** Carmen Bernis,[1] Ana Perez de José,[1] Rosario Madero,[2] Pablo Alonso,[3] Juan Bustamante,[4] Jose-Antonio Sanchez-Tomero.[1] *[1]Nephrology, HUPrincesa, Madrid, Spain; [2]Biostatistics unit; [3]ICU; [4]Cardiac surgery, .*

Acute kidney injury (AKI) is one of the most serious complications occurring after cardiac surgery (ACS) and is associated with increased mortality. Cardiac surgery often requires blood transfusion but some patients, such as Jehovah´s Witnesses refuse to use blood products.

The objective of this study is to evaluate the incidence of AKI (defined by RIFLE and AKIN ) and the mortality in Jehovah´s Witnesses undergoing cardiac surgery

**Methods**

All Jehovah´s Witnesses undergoing cardiac surgery in a reference Hospital, between January 2003 and July 2009 were retrospectively evaluated (n=67). All these patients refused any form of blood transfusion by signing a special consent. Data were prospectively collected. We evaluate the incidence of AKI using RIFLE and AKIN classification and in-hospital mortality . The statistical package SPSS (version 15 for windows) was used. Crude and adjusted dates for age, sex, type of surgery and baseline renal function ( CKD< ó >60 ml/min) are expressed.

**Results**

67 patients (mean age 62.19 ± 10.9 years; 53.7% females; mean Cleveland Score 3.66 ± 1.6 and SRI score 2.13 ± 0.93, 83.6% valvular surgery and 16.4% coronary artery bypass) were evaluated. Mean baseline creatinine 1.06 ± 0.29 mg/dl and maximum creatinine 1.13 ± 0.32 mg/dl.

The incidence of AKI defined by AKIN classification in Jehovah´s Witnesses was 13.4% (n=9) and in the control group 16.6% (n=100) (p=0.603).Defined by RIFLE the incidence was 9% (n=6) in Jehovah´s Witnesses and 7.2% (n=43) in the control group (p 0.619). Mortality in Jehovah´s Witnesses was 10% (n=7) and in the control group 8% (n=48) (p 0.481). Crude and adjusted dates were not statistically significant

Mortality was 10.4% in Jehovah´s Witnesses and 8% in control group (p 0.48)

**Conclusions**

AKI incidence (defined by RIFLE or AKIN ) and mortality were similar in Jehovah´s Witnesses and in the control group.

Disclosure of Financial Relationships: nothing to disclose

**PUB010**

**Bif-1/Endophilin B1 in ATP Depletion-Induced Renal Tubular Cell Apoptosis** Sunggyu Cho,[1] Craig R. Brooks,[2] Zheng Dong.[1] *[1]Department of Cellular Biology and Anatomy, Medical College of Georgia and Charlie Norwood VA Medical Center, Augusta, GA; [2]Renal Division, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.*

Bif-1 was originally identified as a Bax interacting protein via yeast two hybrid screening. Due to its sequence and structural homology to endophilins, Bif-1 is also called endophilin B1. More recent studies suggest that in addition to Bax, Bif-1 may also regulate mitochondrial dynamics and autophagy. Whether Bif-1 contributes to the regulation of renal pathophysiology is unclear. Here we have established stable Bif-1 knockdown renal proximal tubular cell lines by transfecting shRNA. The knockdown cells were resistant to apoptosis following ATP-depletion. Mechanistically, we found that Bif-1-knockdown did not affect Bax translocation to mitochondria during ATP-depletion, but it prevented Bax oligomerization in mitochondria. As a result, cytochrome c release from mitochondria was partially blocked. The results have demonstrated evidence for a role of Bif-1 in renal cell injury and death.

Disclosure of Financial Relationships: nothing to disclose

**PUB011**

**Acute Trypanosoma cruzi Induce Renal Kidney Injury (AKI) in Mice** Gabriel Melo De Oliveira,[1] Nestor Schor.[2] *[1]Medicine-Nephrology Division, UNIFESP, São Paulo, São Paulo, Brazil; [2]Laboratorio de Biologia Celular, Fiocruz, Rio de Janeiro, Brazil.*

Experimental acute infection with Trypanosoma cruzi in mice promotes an intense myocarditis and other systemic changes. The aim of this study was to investigate the mechanisms of AKI in mice during experimental acute phase as well as the potential vulnerability of renal cells lines by the parasite. Infected mice showed significant increase ($p<0.05$) in creatinine (2.0 vs. 0.4 mg/dl,) and urea (40 vs.80 mg/dl) serum levels on the 6th day post infection (dpi) vs. control group. Also, histopathology analysis characterized AKI, but not related to the presence of the parasite. Proximal tubular injury presented cloudy swelling of the cytoplasm, increased tubular light and loss of cytoplasmic substance. The interaction between the parasite in renal cell lines (HMC, MDCK and LLC-PK1) was observed and it was disclosed that human mesangial cells (HMC) are the most resistant to infection with only 3.4 % infected cells, vs. 7.6% and 13%, respectively ($p<0.05$). However, HMC presented higher impairment, with 50% decreases in the viability and integrity after 72h of infection. Also, only HMC cells presented significant ($p<0.05$) increases in the NO expression (0.01 vs. 0.17 uM/mg), TNF-$\alpha$ (14.3 vs. 4.3 pg/dl) and IFN-$\gamma$ (5.7 vs. 106 pg/dl) vs. control group. Thus, these data suggested that AKI, in this model, are mainly due to inflammatory mediators released by parasite infection.

Disclosure of Financial Relationships: nothing to disclose

**PUB012**

**Effects of Pre-Treatment with a High Dose of Methylprednisolone on Renal Ischemia/Reperfusion Injury** Ida M. Fernandes,[1] Gloria E. Mendes,[1] Terezila Machado Coimbra,[2] Emmanuel A. Burdmann.[1,3] *[1]Sao Jose do Rio Preto Medical School, Sao Jose do Rio Preto; [2]University of Sao Paulo Medical School, Ribeirao Preto; [3]University of Sao Paulo Medical School, Sao Paulo, Brazil.*

**Introduction:** Renal ischemia is the most important cause of acute kidney injury. Methylprednisolone (MP) has been shown to protect against ischemia/reperfusion injury (I/R) in other organs. **Objective**: To examine the effects of MP in renal I/R. **Methods**: 24 male Wistar rats received 30mg/kg of iv MP or saline 1 hour before 30 min of renal ischemia (RI). They were divided into 3 groups (n=8, each): control (C, sham surgery, no RI), I/R (saline infusion pre-RI), and MP (MP infusion pre-RI). GFR (inulin clearance, ml/min/100g) and sodium fractional excretion (FENa, %) were assessed 2 days after I/R. In the same time, macrophages (ED-1), neutrophils (No), lymphocytes (Lo) and nuclear factor-kappa- B (NFκ-B) were assessed by immunohistochemistry and scored according to the extent of tubulointerstitial immunostaining in the cortex and outer medulla (OM) areas. Results (mean±SD) were compared by ANOVA followed by Bonferroni test ($p<0.05$). **Results:** GFR was 0.92±0.3 in the MP group, 0.90±0.3 in the C group, and 0.47±0.2ml in the I/R group, ($p<0.05$ vs. MP and C). The FENa was similar in the MP (0.19) and C groups (0.35), and higher in the I/R group (0.57, $p<0.05$ vs. MP and C). Tubulointerstitial staining for Lo was significantly more intense in the I/R group when compared with C and MP groups in the cortex (14.4±3 vs. 6.7 ±1 and 5.3±1.6, respectively, $p<0.05$ I/R vs C and MP) and OM areas (10.6±3 vs. 4.3±1 and 3.7±0.6, $p<0.05$ I/R vs. C and MP). Similar results were observed for OM staining of ED1 (9.8±3 in I/R vs. 4.6±1 in C and 4.1±2 in MP, $p<0.05$) and No (3.1±2 in I/R vs. 1.1±0.3 in C and 1.4±1 in MP, $p<0.05$). NFκ-B immunostaining was significantly more intense in the OM of the I/R group compared with the sham and the MP groups (0.6±0.3 in I/R vs. 0.03±0.03 in C and 0.1±0.1 in MP, $p<0.05$). **Conclusion:** These results demonstrated that pre-treatment with high doses of MP conferred protection against renal I/R and suggested that this action is likely related to modulation of I/R-induced inflammatory mechanisms.

Disclosure of Financial Relationships: nothing to disclose

**PUB013**

**Retroperitoneal Sarcoma Presenting as AKI Secondary to Bilateral Renal Artery Invasion** Amit Kumar Gupta,[BCM] Abdul A. Abdellatif.[BCM] *[1]Department of Medicine, Baylor College of Medicine, Houston, TX; [2]Department of Nephrology, Baylor College of Medicine, Houston, TX.*

62 yr female with baseline creatinine of 1 presented to the emergency department with sudden onset of anuria and 20-25 lbs weight loss in the last six months. Prior to presentation, she was started on Lisinopril 5 mg daily for hypertension. On physical examination blood pressure was mildly elevated to 150/86 with negative orthostatic changes. The laboratory findings were significant for: serum BUN 42mg/dl, Cr 5.1mg/dl, Sodium 126mmol/l, Potassium 5.4mmol/l, Serum osmolality 264, and calculated Fractional excretion on sodium was <1%. Urine analysis showed 50 RBC, 400mg/dl protein, no casts and urine osmolality of 229.

Lisinopril was discontinued and Doppler Renal Ultrasound to rule out renal artery stenosis showed the right kidney being 3 cm smaller than the left kidney and normal resistive indices. Within two days therapy, electrolyte abnormalities improved but the BUN and creatinine continued to get worse (BUN 78mg/dl and Cr 9.8mg/dl). Further laboratory workup including ANA, ANCA, C3, C4, Anti GBM antibodies was normal. Kidney biopsy was performed to rule out rapidly progressive glomerular nephritis which was normal.

Secondary to the high suspicion of a pre-renal etiology and to avoid the risk of Nephrogenic Systemic Fibrosis (NSF) a time-of-flight noncontrast MR angiogram was performed, which revealed an irregular, heterogeneous 3x5x5 cm mass in the para-aortic region encasing the origin of bilateral renal arteries and superior mesenteric artery (SMA). Flow in SMA and right renal artery was markedly reduced and complete cut-off of flow in left renal artery after its origin from aorta was noted.

CT guided biopsy of the mass revealed high grade liposarcoma. The mass was surgically excised with positive margins and vascular reconstruction was done. Patient recovered renal function after 6 weeks of intermittent dialysis with new baseline for creatinine of 2.5.

Conclusions:

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Noncontrast time-of-flight MR angiography is sensitive for evaluation of renal artery stenosis [90% sensitive compared to Renal angiogram] without the risk of NSF in patients with chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**PUB014**

**Henoch-Schonlein Purpura: Still a "Childhood" Disease?** Tarek Hamieh,[1] Manish K. Saha,[1] Vesselin Dimov.[2] *[1]Department of Medicine, Regions Hospital, University of Minnesota, Saint Paul, MN; [2]Department of Allergy and Immunology, Creighton University, La Vista, NE.*

Case Presentation: A 72-year-old female presented with one week history colicky abdominal pain, hematochezia, arthralgia and rash. The rash started on her elbows and extended to the legs and thighs. She denied recent use of antibiotics or anti-inflammatory medications. Patient was afebrile, normotensive, and palpable purpura was noted on the extensor surface of the arms and lower extremities. She had a normal hemogram and renal function. Urinalysis was positive for microscopic hematuria and trace proteinuria. Cryoglobulins were negative. Skin biopsy of the rash demonstrated leukocytoclastic vasculitis with IgA deposition on immunofluorescence consistent with Henoch-Schonlein Purpura. Patient's symptoms improved markedly with initiation of corticosteroids.

Discussion: Henoch-Schonlein Purpura (HSP) is a systemic vasculitis characterized by purpuric rash, abdominal pain, arthralgia, and renal involvement. It is characterized by the tissue deposition of IgA-containing immune complexes. Although it is commonly reported to be a "childhood" disease, it is increasingly reported in adults. The rash is typically purpuric and symmetric, often located on the extensor surfaces of the extremities. Renal involvement is common, and usually mild, however in severe cases, can progress to end-stage renal disease. When HSP is suspected, a urinalysis should be done promptly to check for active sediment consisting of red cell casts, which would suggest glomerulonephritis. Evidence suggests that glucocorticoids enhance the rate of resolution of the arthritis and abdominal pain and may prevent delayed-onset HSP nephritis. This case serves to illustrate the need to include HSP in the differential diagnosis in the appropriate setting (rash, arthralgias, renal involvement) when caring for adult population with abdominal symptoms or gastrointestinal bleeding.

Disclosure of Financial Relationships: nothing to disclose

**PUB015**

**Sex Differences in the Regulation of the Vasodilator/Vasoconstrictor Balance in the Renin Angiotensin System Contribute to Sex Differences in Renal Interstitial Fibrosis** Hong Ji, Wei Zheng, Jun Liu, Xie Wu, Bilkish Bajaj, Jeffrey Seiden, Kathryn Sandberg. *Medicine, Georgetown University, Washington, DC.*

Renal disease progression is faster in men compared to women in numerous non-diabetic diseases. This sex difference is also observed in models of chronic and acute renal disease including unilateral ureteral obstruction (UUO), a model of tubulointerstitial fibrosis. We investigated the expression of key components of the vasoconstrictor and vasodilator arms of the renin angiotensin system (RAS) male and female mice subjected to UUO. One week after surgery, there was no effect of UUO nor were there sex differences in renal angiotensin type 1 receptor mRNA [$AT_1R$ (AU): M-Sham, 1.10±0.51; M-UUO, 0.92±0.23; F-Sham, 1.28±0.36; F-UUO, 1.01±0.14]. Compared to the female, angiotensin converting enzyme (ACE) mRNA in the male was 3.1-fold ($p<0.01$) and 1.7-fold higher in the sham and UUO kidneys, respectively [ACE (AU): M-Sham, 2.59±0.34; M-UUO, 1.00±0.28; F-Sham, 0.83±0.08; F-UUO, 0.58±0.14]. The female down-regulated renal expression of renin mRNA after UUO to a 1.9-fold ($p<0.05$) greater extent than the male [Renin (AU): M-Sham, 0.91±0.02; M-UUO, 0.87±0.09; F-Sham, 1.51±0.24; F-UUO, 0.78±0.08]. Furthermore, UUO-induced a 2.4-fold ($p<0.05$) greater increase in renal ACE2 in the female compared to the male [(AU): M-Sham, 1.34±0.81; M-UUO, 1.54±0.29; F-Sham, 0.69±0.22; F-UUO, 1.64±0.04] and a 2.2-fold ($p<0.03$) greater increase in the $AT_2R$ in the female kidney compared to the male [$AT_2R$ (AU): M-Sham, 0.67±0.54; M-UUO, 0.88±0.50; F-Sham, 1.23±0.32; F-UUO, 2.68±0.27]. These data suggest the female kidney is protected from UUO-induced renal injury compared to the male because the female is able to down-regulate the vasoconstrictor arm (renin and ACE) and up-regulate the vasodilator arm (ACE2 and $AT_2R$) of the RAS cascade in response to the UUO insult to a greater extent than the male. Supported by NIA R01 AG019291.

Disclosure of Financial Relationships: nothing to disclose

**PUB016**

**Erythropoietin (EPO) Protects the Kidney Against the Injury and Dysfunction Caused by Ischemia-Reperfusion (I/R) in Diabetic Rats** Julius Edward Kieswich, Ananda Chapagain, Martin J. Raftery, Magdi Yaqoob. *Centre for Translational Medicine and Therapeutics, William Harvey Research Institute, London, United Kingdom.*

There is a growing body of evidence to suggest that EPO is tissue protective against I/R injury of kidneys, heart, brain and liver through its potent anti-apoptotic properties. Diabetic patients are at a high risk of acute kidney injury (AKI) following ischemic or nephrotoxic insults which are associated with increased morbidity and mortality.

We have previously shown that a single systemic administration of EPO pre-ischemia prevents AKI in rats and mice through the inhibition of apoptosis. The aim of this study was to investigate the ability of EPO to attenuate this injury in diabetic rats.

Rats weighing 200g were divided into four groups. Diabetes was induced in two of the groups through intraperitoneal injection of streptozotocin (50mg/kg). After 17 days EPO (300U/kg) was administered to one non-diabetic and one diabetic group. One hour after EPO injection, the I.R. protocol was performed. I/R. injury was achieved by clamping both renal arteries for 45 minutes.

The results showed that EPO reduced AKI similarly in both diabetic and non diabetic groups as evidenced by significant reductions in serum creatinine and urea levels ($p<0.05$). Ex vivo analysis of kidney protein by Western blotting showed upregulation of anti-apoptotic molecules such as Bcl-XL and XIAP in both EPO treated groups.

We conclude that EPO administration is equally effective in preventing I/R injury in both diabetic and non diabetic rats. The results of these investigations have implications for the prevention of AKI in diabetic patients undergoing high risk surgical procedures.

Disclosure of Financial Relationships: nothing to disclose

**PUB017**

**Contrast Induced Nephropathy Following Intravenous Contrast Administration: A Meta-Analysis** Judith Kooiman,[1] Sharif M. Pasha,[1] Olaf Dekkers,[1] Yvo W. J. Sijpkens,[2] Aart J. van der Molen,[3] Menno V. Huisman.[1] *[1]Department of General Medicine, Leiden University Medical Center, Leiden, Netherlands; [2]Department of Nephrology, Bronovo Hospital, The Hague, Netherlands; [3]Department of Radiology, Leiden University Medical Center, Leiden, Netherlands.*

**Introduction** Contrast induced nephropathy (CIN) is defined by an increase in serum creatinine > 25% or > 0.5 mg/dL 48-72 hours after administration of iodinated contrast media (CM). Although generally mild and reversible, in rare cases CIN can lead to a need for renal replacement therapy (RRT). In literature, the incidence of CIN ranges between 1-25%. The aim of this meta-analysis was to asses the incidence of CIN and the need for RRT in patients undergoing IV CM administration prior to CT-scanning.

**Methods** Studies evaluating renal function after IV CM administration published after 2000 were searched for in Medline, Cochrane, Web of Science and EMBASE.

Primary endpoint was the weighted pooled incidence risk of CIN defined by an increase in serum creatinine> 25% or > 0.5 mg/dL. Secondary endpoint was the incidence of RRT after CM administration. Prespecified subgroup analyses were performed in patients with diabetes mellitus (DM) and pre-existent renal failure. All analysis were performed in a random effects-model.

**Results** Twenty prospective and 20 retrospective studies including a total of 19585 patients were included. In 75% of studies including patients with chronic renal failure preventive hydration regimes were advised. The weighted pooled incidence risk of CIN was 7.2% (95% CI 6.0-8.9) in a random-effects model. There was considerable between-study heterogeneity ($I^2$ 87%). Renal replacement therapy was needed in 0.6% (95% CI 0.4-1.0) of patients following CM administration. The incidence for CIN was 10.4% (95% CI 7.4-14.5) in patients with chronic renal failure and 10.4% (95%CI 6.6-16.1) in patients with DM.

**Conclusion** CIN occurred in about 7% of patients after IV CM administration, which is in the lower range reported by previous studies. The incidence of CIN was higher in subgroups of patients diagnosed with chronic renal failure or DM. The need for RRT is rare and occurs in 0.6% of all patients after administration of IV contrast.

Disclosure of Financial Relationships: nothing to disclose

**PUB018**

**The Role of Bile Acids in Cryopreservation Injury of Kidney** Shunan Li,[1,2] Lawrence Wang,[1] Kajohnsak Noppakun,[1,2] Sandeep Gupta.[1,2] *[1]Stem Cell Institute; [2]Department of Medicine, University of Minnesota, Minneapolis, MN.*

**Background:** Prolonged cryopreservation of donor kidney decreases graft function and increases patient mortality. Kidney cells die by necrosis during cryopreservation and apoptosis during warm reperfusion phase. Delayed cell death by apoptosis during the reperfusion phase provides a window of opportunity for reducing cryopreservation injury. Tauroursodeoxycholic acid (TUDCA), a hydrophilic bile acid, prevents cell death by inhibiting apoptosis and upregulating survival pathways. We hypothesized that TUDCA can prevent cryopreservation injury by inhibiting apoptotic cell death.

**Objective:** The purpose of this study is to determine the protective effects of TUDCA in an in vivo model of cryopreservation injury of kidney.

**Methods:** Cell line: We used primary human renal proximal tubular epithelial cells (RPTEC) in a validated in vivo model of cryopreservation injury. Cryopreservation injury: RPTEC were grown to confluence in complete medium. The complete medium was subsequently replaced with UW (University of Wisconsin) cryopreservation solution with or without 50 to 1200 µM TUDCA, and the culture plates were incubated at 4°C for 48 hours. To simulate warm reperfusion phase of the transplantation, we replaced UW solution with complete medium and cultured cryopreserved cells for an additional 24 hours at 37°C. We determined apoptotic cell death by caspase-3 assay and upregulation of survival pathway by Western blot analysis. Caspase-3 Assay: We measured caspase-3 activity by using fluorometric substrate-Z-DEVD-Rhodamine 110. Survival pathway analysis: We determined activation of survival pathway by using specific antibodies against activated forms of p38, JNK, and ERK protein and standard Western blot techniques, and confirmed visual results by densitometry normalized for b-actin.

**Results:** Pretreatment of RPTEC with 150-1200 µM TUDCA significantly protected against cryopreservation injury by inhibiting caspase-3 activation and upregulating of ERK survival pathway.

**Conclusions:** TUDCA protected against cryopreservation injury and is a promising therapeutic agent for improving patient and graft outcome following cryopreservation of donor kidneys.

Disclosure of Financial Relationships: nothing to disclose

**PUB019**

**Effect of Mesenchymal Stem Cells on Renal Tubular Epithelial Cells' Self-Recovery in Mice under Ischemia/Reperfusion and Possible Mechanism** Nanmei Liu, Jinyuan Zhang. *Jimin Hospital of Shanghai.*

**Objective:** Establish mice's acute renal injury model,exogenous intervened by mesenchymal stem cells(MSCs),observing its effect on renal tubular epithelial cells'(RTECs) regeneration of model mice.

**Methods:**45 healthy male C57BL/6 mice were scattered into control group(15),I/R group(15, clamping bilateral renal pedicles and then reopening after 30 minutes), I/R+mMSCs group(15, the same to I/R group, meanwhile,injected mMSCs through caudal vein into the body). 1 day,2days,3 days,7 days,14 days after, killed them(3/each group). Detected Scr and BUN, mice kidneys were dyed with HE to observe their pathological changes, evaluated their acute tubular necrosis score.The proportions of RTECs' proliferation were assessed by IHC of PCNA, apoptosis were detected by TUNEL. the protein level of Caspase-3,Bcl-2 in renal tubules were detected by Western blot. The levels of TNF-α,IL-1β,MCP-1, IL-10, HGF, BMP-7 were detected by ELISA.

**Results:**Comparing with I/R group, the level of BUN and Scr in I/R+mMSCs were much lower,at the same time ,this group's renal tubule's pathological changes mitigated significantly. ATN score was also significantly lower. The immunohistochemical staining of PCNA demonstrated that the positive expression of RTECs was increased significantly after intervention of mMSCs($P<0.05$). I/R could induce RTECs' apoptosis, mMSCs' intervention could significantly relieve its apoptosis($P<0.05$ or $P<0.01$). Western blot analysis revealed:contrasted with I/R group, the level of Caspase-3 in I/R+mMSCs group decreased notably(1.16±0.33, $P<0.01$),while the level of Bcl-2 increased significantly(0.94±0.27, $P<0.01$). ELISA result showed that the levels of TNF-α,IL-1β,MCP-1 in I/R group's renal tissue homogenate were increased significantly,while the levels of IL-10,HGF,BMP-7 decreased notably. While mMSCs' intervention could change these cytokines levels to the opposite direction.

**Conclusion:**MSCs can contribute to RTECs' regeneration,inhibit their apoptosis.The possible mechansim may be that MSCs can regulate cytokines secretion in renal tissues under ischemia/reperfusion,and then contribute to RTECs'self-recovery by paracrine.

Disclosure of Financial Relationships: nothing to disclose

**PUB020**

**Significant Microalbuminuria May Portend AKI in Sepsis** Arghya Majumdar.[1] *[1]Nephrology, AMRI Hospitals, Kolkata, West Bengal, India; [2]Pharmacology, Jadavpur University, Kolkata, West Bengal, India.*

Introduction:

Sepsis leads to diffuse endothelial dysfunction; the glomerular manifestation of which is microalbuminuria (MA). Recent evidence indicates that the pathophysiology of sepsis induced acute kidney injury (AKI) is unique. Histo-pathological evidence of extensive glomerular capillary infiltration by leucocytes has been observed in septic AKI. Further, animal models of hyperdynamic sepsis have shown that AKI can occur in the absence of hypotension. These findings suggest that endothelial dysfunction may play a prominent role in septic AKI in comparison to patients of AKI due to other causes. Markers of endothelial dysfunction such as microalbuminuria may therefore have a potential diagnostic role in septic AKI.

Methods:

Prospective observational study in a 20 bed Intensive Care Unit (ICU) in a tertiary care hospital. MA estimated as spot urine albumin-creatinine ratio (ACR, mg/g) was measured on ICU admission. Between Jan 2007 and Dec 2008, 266 patients were recruited. Patients with ICU stay of less than 24 hrs and other confounding factors were excluded. Sepsis was diagnosed by the ACCP/SCCM criteria; AKI identified by the RIFLE criteria.

Results:

Of the 266 patients studied 130 patients had AKI as classified by the RIFLE criteria. Median age was 61.5 yrs, 61% were male, median APACHE II score was 15. The median level of ACR [204.1 mg/g, (IQR 119.7 – 402.0)] of patients with septic AKI (n=66) was significantly greater ($p<0.0001$) than the median ACR level of 67.1 mg/g (IQR 32.99 – 129.9) of patients who had AKI from other causes (n=70). Patients of sepsis who had developed AKI also had higher ACR than those who did not (n=51) [ACR 119.3 mg/g (IQR 37.02 – 354.3)] ($p=0.0137$).

Conclusions:

Microalbuminuria is a cheap point of care bed-side test which might help to preempt development of AKI in sepsis patients. This might help in guiding management strategies in remote resource poor areas and prioritizing transfer to a tertiary care centre with dialysis facility.

Disclosure of Financial Relationships: nothing to disclose

**PUB021**

**Preliminary Results of the Clinical Trial: Prevention of Contrast-Induced Nephropathy by Oral or Intravenous Hydration. Evolution of Renal Function According to Levels of Creatinine and Cystatin C** Paloma L. Martin Moreno,[1] Nuria Garcia-Fernandez,[1] Nerea Varo,[2] Laura Montero,[2] Francisco Javier Lavilla,[1] Jorge M. Nunez-Cordoba.[3] *[1]Nephrology, Clinica Universidad de Navarra, Pamplona, Navarra, Spain; [2]Laboratory of Biochemistry, Clinica Universidad de Navarra, Pamplona, Navarra, Spain; [3]Department of Preventive Medicine and Public Health, Clinica Universidad de Navarra, Pamplona, Navarra, Spain.*

Objective: To compare the efficacy of hydration with IV bicarbonate or oral serum in the prevention of contrast-induced nephropathy. To study the usefulness of Cystatin C for monitoring renal function.

Material and methods:

Prospective, randomized, single-centre trial in hospitalized non-diabetic patients with estimated glomerular filtration rate (eGFR) calculated by MDRD-4 $\geq$ 30 ml/min, undergoing procedures with contrast media.

Patients were randomized in three groups: G1: IV bicarbonate, G2: Oral serum and G3: control. Variables studied before and 24 hrs after: creatinine, cystatin C and eGFR.

Results:

So far 67 patients have been enrolled. There were no significant differences ($p>0.05$) between groups regarding their baseline characteristics. The change in creatinine, cystatin and eGFR 24 hrs after contrast media administration was non-significant ($p>0.05$) within as between groups.

| | G1 | G2 | Control |
|---|---|---|---|
| Creatinine (mg/dL) | | | |
| Baseline | 0.98 (0.35) | 0.8 (0.24) | 1.39 (1.56) |
| 24 h after | 1.01 (0.35) | 0.85 (0.3) | 0.86 (0.23) |
| Cystatin C (mg/L) | | | |
| Baseline | 0.83 (0.34) | 0.82 (0.3) | 0.66 (0.15) |
| 24 h after | 0.82 (0.35) | 0.83 (0.32) | 0.65 (0.15) |
| eGFR by MDRD-4 (ml/min) | | | |
| Baseline | 85.1 (27.78) | 109.05 (47.91) | 78.47 (28.16) |
| 24 h after | 84.57(30.48) | 101.1 (37.35) | 92.17 (20.15) |

Data expressed as means and standard deviation of means

Conclusions:

The effect of contrast media in renal function of hospitalized non-diabetic patients with eGFR $\geq$30 ml/min is non-significant after 24 hours independently of the protocol of hydration. Creatinine, cystatin C and eGFR could be similar parameters for monitoring renal function.

Disclosure of Financial Relationships: nothing to disclose

**PUB022**

**Preliminary Results of the Clinical Trial: Prevention of Contrast-Induced Nephropathy by Oral or Intravenous Hydration. Evolution of Markers Related to Oxidative Stress** Paloma L. Martin Moreno,[1] Nuria Garcia-Fernandez,[1] Nerea Varo,[2] Laura Montero,[2] Francisco Javier Lavilla,[1] Jorge M. Nunez-Cordoba.[3] *[1]Nephrology, Clinica Universidad de Navarra, Pamplona, Navarra, Spain; [2]Laboratory of Biochemistry, Clinica Universidad de Navarra, Pamplona, Navarra, Spain; [3]Department of Preventive Medicine and Public Health, Clinica Universidad de Navarra, Pamplona, Navarra, Spain.*

Objective: To study the erythrocyte superoxide dismutase (SOD) activity and levels of F2-isoprostanes in urine of patients who have received contrast media with different hydration protocols.

Material and methods:

Prospective, randomized, single-centre trial in hospitalized non-diabetic patients with estimated glomerular filtration rates $\geq$ 30 ml/min (eGFR) calculated by using the MDRD-4 equation, undergoing procedures with contrast media.

Patients were randomized in three groups: G1: IV bicarbonate, G2: Oral serum and G3: control. Variables studied before and after contrast media administration: erythrocyte SOD activity and F2-isoprostanes in urine.

Results:

So far 67 patients have been enrolled. There were no significant differences ($p>0.05$) between groups regarding their baseline characteristics, neither in the erythrocyte SOD activity nor in the level of F2-isoprostanes in urine within as between groups before and after contrast media administration.

Erythrocyte superoxide dismutase (SOD) activity and levels of F2-isoprostanes in urine before (baseline) and after contrast media administration

| | G1 | G2 | G3 |
|---|---|---|---|
| Erythrocyte SOD (U/mL) | | | |
| Baseline | 5 (3.92) | 4.64 (2.79) | 4.43 (1,14) |
| 4 h after contrast administration | 7.29 (13.39) | 7.58 (13.66) | 4.6 (1.68) |
| Isoprostanos F2 orina (pg/mL) | | | |
| Baseline | 11474.39 (11467.71) | 9726.11 (11358.03) | 18527.36 (18626.01) |
| 12 h after contrast administration | 11922.07 (11326.98) | 11110 (12328.13) | 12659.55 (12595.18) |

Data expressed as means and standard deviation of the means

Conclusions:

The administration of contrast media with different protocols of hydration to hospitalized non-diabetic patients with eGFR $\geq$ 30 ml/min does not bring about significant changes in the erythrocyte SOD activity or F2-isoprostanes levels in urine.

Disclosure of Financial Relationships: nothing to disclose

**PUB023**

**Preliminary Results of the Clinical Trial: Prevention of Contrast-Induced Nephropathy by Oral or Intravenous Hydration. Evolution of Serum and Urine Markers of Renal Damage: Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Interleukin-8 (IL-8)** Paloma L. Martin Moreno,[1] Nuria Garcia-Fernandez,[1] Nerea Varo,[2] Laura Montero,[2] Francisco Javier Lavilla,[1] Jorge M. Nunez-Cordoba.[3] *[1]Nephrology, Clinica Universidad de Navarra, Spain; [2]Laboratory of Biochemistry, Clinica Universidad de Navarra, Spain; [3]Department of Preventive Medicine and Public Health, Clinica Universidad de Navarra, Spain.*

Objective: To compare the effectiveness of IV and oral hydration in prevention of contrast-induced nephropathy through measurements of NGAL and Il-8.

Material and methods:

Prospective, randomized, single-centre trial in hospitalized non-diabetic patients with estimated glomerular filtration rate (eGFR) $\geq$ 30 ml/min calculated by MDRD-4, undergoing procedures with contrast media.

Patients were randomized in three groups: G1: IV bicarbonate, G2: Oral serum and G3: control. Variables studied serum and urine NGAL and IL-8.

Results:

So far 67 patients have been enrolled. There were no significant differences ($p>0.05$) between groups regarding their baseline characteristics, neither between groups in NGAL and serum Il-8 levels before and after contrast media administration ($p>0.05$). The change of urine Il-8 level after contrast media administration was significant ($p=0.033$) and different between groups ($p=0.035$).

NGAL and Il-8 levels

| | G1 | G2 | G3 |
|---|---|---|---|
| Serum NGAL (ng/ml) | | | |
| Baseline | 210.74 (229.99) | 201.05 (214.47) | 157.45 (40.49) |
| 4 h after | 276.39 (394.03) | 230.6 (313.06) | 159 (87.7) |
| Urine NGAL (ng/ml) | | | |
| Baseline | 76.79 (133.22) | 57.13 (91.51) | 105.67 (307.11) |
| 4 h after | 90.39 (162.36) | 46.57 (66.61) | 30.31 (62.09) |
| Serum Il-8 (pg/mL) | | | |
| Baseline | 151.52 (170.81) | 87.52 (116.54) | 164.95 (255.12) |
| 12 h after | 181.75 (199.12) | 77.43 (91.48) | 133.59 (227.01) |
| Urine Il-8 (pg/mL) | | | |
| Baseline | 175.75 (371.6) | 164.25 (285.96) | 395.92 (793.29) |
| 12 h after | 138.16 (211.44) | 192.46 (357.65) | 173.19 (321.68) |

Data expressed as means and standard deviation

Conclusions:

Urine Il-8 level changes in the first hours following administration of contrast media and this effect seems to depend on the protocol of hydration applied. This result is not observed in serum Il-8 or NGAL levels.

Disclosure of Financial Relationships: nothing to disclose

**PUB024**

**Sevoflurane Induced Severe Acute Renal Failure Requiring Hemodialysis** Penchala S. Mittadodla, Robert S. Gayner. *St Luke's Hospital, Bethlehem, PA.*

Drug induced nephrotoxicity is common in clinical practice and accounts for up to 25% of all cases of acute renal failure. It is important for clinicians to be aware of nephrotoxic drugs and take necessary steps to prevent renal insult. We report a case of Sevoflurane precipitated severe acute renal failure in a patient who had multiple operations and Sevoflurane exposure.

A 73-year old Caucasian male was admitted following a motor vehicle accident and found to have a large laceration to the right thigh requiring debridement. Two weeks later, his right thigh wound got infected with S. aureus and E. coli for which he was treated with vancomycin and piperacillin/tazobactam. Subsequently, he had further debridement of the wound under general anaesthesia with Sevoflurane. He had a CT scan of chest with contrast to investigate an episode of dyspnea one day prior to the debridement. He did not have any documented episode of hypotension during his entire hospital stay. He developed severe acute renal failure with an elevated BUN and creatinine of 40 mg/dl and 8.0 mg/dl respectively and ultimately required hemodialysis.

His past medical history included type 2 diabetes mellitus, hypertension and hypothyroidism but no evidence of chronic kidney disease with a baseline creatinine of 1.0 and an estimated GFR > 60 ml/min/1.73 sq m. His medications included glyburide, pioglitazone, synthroid and SQ heparin.

Physical exam was significant for bibasilar crackles, 2+ bilateral pedal edema with normal blood pressure and he was nonoliguric. Serologic workup was normal and LFTs/CPK were normal. Urinalysis was positive for trace blood and protein and negative for eosinophils. A renal ultrasound was normal. Renal biopsy revealed acute tubular necrosis.

Sevoflurane, an inhaled anaesthetic agent, is metabolised into Compound A, which can have a tubulotoxic effect leading to ATN. It can be worsened by nephrotoxic drugs like contrast dye, renal insufficiency, volume depletion, diabetes, heart failure, sepsis or age >60. Repeated exposure to Sevoflurane should be avoided especially, with recent use of contrast dye.

Disclosure of Financial Relationships: nothing to disclose

**PUB025**

**Overlapping Lupus Myocarditis and Nephritis: A Diagnostic and Therapeutic Challenge** Lawand A. Saadulla,[1] Giselle Baquero,[2] Navin Verma.[1] *[1]Nephrology, PSU/Hershey Medical Center, Hershey, PA; [2]Internal Medicine.*

We describe a case of a young woman presenting with fulminant heart failure, myocarditis and nephritis as first manifestation of SLE which represented a diagnostic and therapeutic challenge. To our knowledge a few similar cases have been reported in the literature.

A previously healthy 22 year old Hispanic female was transferred to our institution after she developed cardiogenic shock and hypoxemic respiratory failure requiring inotropic support and mechanical ventilation. Initial EKG showed non-specific T wave abnormalities with prolonged QT. Echocardiography demonstrated global hypokinesis with severe systolic dysfunction and left ventricular ejection fraction 15%. The patient developed a non-oliguric acute renal failure. Right heart catheterization was consistent with cardiogenic shock. Laboratory tests revealed high titers of ANA and dsDNA. Levels of C3 and C4 complement were markedly low. Clinical and laboratory findings were suggestive of lupus myocarditis as an etiology for her heart failure. A subsequent renal biopsy for her worsening acute renal failure showed diffuse proliferative glomerulonephritis. Treatment for SLE with pulse intravenous cyclophosphamide and methylprednisolone was initiated resulting in near complete organ recovery. The patient was discharged home to continue monthly cyclophosphamide maintenance therapy, with progressive improvement in her cardiac and renal function.

Acute lupus myocarditis leading to heart failure complicated by nephritis is uncommon but a potentially fatal initial manifestation of SLE. The diagnosis is challenging due to the higher prevalence of viral myocarditis and idiopatic dilated cardiomyopathy among patients presenting with newly diagnosed heart failure. Lupus myocarditis should be considered, diagnosed and treated promptly to avoid fatal consequences. Immunosuppressive agents are the mainstay therapy. The choice of regimen is controversial due to the paucity of literature on the treatment of lupus myocarditis overlapping with nephritis. Our patient responded well to combined cyclophophamide and steroid therapy, with induction of remission and improvement of her overall clinical condition.

Disclosure of Financial Relationships: nothing to disclose

**PUB026**

**Outcomes of Acute Kidney Injury in Bone Marrow Transplant Patients** Ankit Sakhuja, Abhishek Deshmukh, Nilay Kumar, Aaron T. Dall, Gagan Kumar, Rahul S. Nanchal. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

**Background:**

Acute kidney Injury (AKI) has shown to be independent predictor for adverse outcomes in many diseases. Bone marrow transplant (BMT) patients constitute a unique group of patients exposed to multiple drugs that can worsen kidney function. There is limited literature on the development of AKI in the BMT population. We sought to investigate the frequency and associated outcomes of patients with BMT developing AKI.

**Methods**

Using Nationwide Inpatient Sample 2007, patients aged 18 years and above, discharged with any diagnosis of bone marrow transplant were identified through appropriate ICD-9 codes. The primary outcomes measured were frequency of AKI, all cause in hospital mortality and length of stay (LOS). Pearson correlation and Chi square were used to compare the variables for unadjusted analysis and logistic regression was used to obtain adjusted odds ratios. $\alpha$ was set at 0.05.

**Results**

There were an estimated 44,630 BMT patients admitted to the hospital in 2007. Of those 8.5% developed AKI and 2.8% had pre-existing chronic kidney disease (CKD).

After multivariate logistic regression and controlling for demographic characteristics and co-morbid conditions, patients with BMT developing AKI had significantly higher in-hospital mortality (OR 2.95; 95%CI 2.18-4.02) when compared to those with normal kidney function. BMT patients with CKD had significantly higher frequency of AKI (OR 5.8; 95%CI 4.12-8.24).

LOS was 6.2 days longer (95%CI 5.1-7.4 days) in BMT patients with AKI when compared to those with normal renal function.

**Conclusion**In BMT patients, AKI predicts higher all cause in-hospital mortality and longer LOS.

Disclosure of Financial Relationships: nothing to disclose

**PUB027**

**Role of Statins in Prevention of Contrast Induced Nephropathy** Majed Samarneh, Norbert Shtaynberg, Morton J. Kleiner, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background:

Studies in animal models have suggested that there is a role for statins, acting as free radical scavengers, in the prevention of contrast-induced nephropathy (CIN). However, the effect in humans is not known.

Materials and methods

We carried out a retrospective chart review at our institution. 282 charts of patients who underwent a cardiac catheterization or a CT scan with an intravenous contrast were reviewed. Patients presenting with acute renal failure before the radiological study or who have end stage renal disease were excluded. We defined contrast-induced nephropathy as an increase in serum creatinine from baseline by greater than 0.5 mg/dL or an increase of 25% within 72 hours following the procedure.

Results

The Fisher's test was used to test for significant associations between categorical variables. The t-test or Mann-Whitney test was used as appropriate for continuous variables. Age, creatinine at baseline, and peak creatinine post percutaneous coronary intervention (PCI) were all significantly different between CIN groups. Subjects who had CIN were significantly older (69.6 +/- 11.9 vs. 63.2 +/- 14.7; p=0.0006), had significantly higher creatinine levels at baseline (1.6 +/- 0.84 vs. 1.14 +/- 0.98; p<.0001) and had significantly higher peak creatinine levels post-PCI (3.01 +/- 1.70 vs. 1.23 +/- 0.85; p<0.0001). Subjects with hypertension were 4.6 times more likely to develop CIN compared to subjects who did not have hypertension[95% CI: (2.4, 8.6). Subjects with all three risk factors, including hypertension, diabetes mellitus, and age greater than 65 were 11.6 times more likely to develop CIN. Patients who were on statin therapy were not protected from CIN (65 patients vs 92 patients; p=0.08).

Conclusion

Statin therapy was not effective in prevention of CIN in our study. However, these subjects who were on statins had higher prevalence of risk factors such as hypertension, age greater than 65, and a higher baseline creatinine which may have contributed to the higher incidence on CIN. A larger randomized sample size may elucidate the beneficial effects of statins in prevention of CIN.

Disclosure of Financial Relationships: nothing to disclose

**PUB028**

**Cardiopulmonary Bypass Causes Short Term, but No Long Term Alterations in Healthy Rat Kidney** Maria Schenk, Iryna V. Samarska, Hjalmar R. Bouma, Robert H. Henning, Leo E. Deelman. *Clinical Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands.*

**Background:** Cardiopulmonary bypass (CPB) is a commonly used technique in cardiac surgery. Clinical studies show both acute and long term effects on kidney function and a higher mortality in patients treated with CPB compared to patient treated without CPB. To obtain more insight into the pathogenesis of impaired renal function following CPB, we recently established a rat CPB model. In the present study we characterized the short-term effects of CPB on the kidney.

**Methods:** Rats underwent CPB or Sham procedure and were sacrificed at 60 min, 1, 2 and 5 days following the procedure. Subsequently, blood and urine samples were taken and the kidneys were removed. Renal gene expression was studied by Rt-PCR targeted to markers of ischemia, inflammation and fibrosis. Blood analyses were performed for several markers of the kidney function.

**Results:**

HO-1, TNF-alpha, Vcam, E-selectin and Pai-I gene expression were all upregulated at 60 min. after CPB. Immunohistochemistry demonstrated HO-1 upregulation in the renal tubuli. Almost all parameter normalized at 1 day after CPB. Cd68 expression and staining were upregulated at day 1 after CPB, indicating the influx of macrophages. Sham animals did not show significant changes. Blood analyses did not demonstrate impaired kidney function at any of the time points. Troponin levels were increased in the sham and CBP group at 60 min indicating cardiac damage by the procedure.

**Conclusion:** Our study demonstrates that CPB in healthy rats causes mainly acute alterations in the kidney, but no deterioration in kidney function. No long term effects were found in the healthy rat kidney. Long term effects in the kidney of patients are most likely caused by a combination of underlying pathophysiology and CPB treatment.

Disclosure of Financial Relationships: nothing to disclose

## PUB029

**Dose Dependent Renal Protection by iNOS Inhibition in Ischemic Acute Kidney Injury (iAKI) with Impact on Organic Cation Transporters** Reinhard Schneider, Marcus Meusel, Michael Kersten, Christoph Wanner. *University Hospital, Dept. Medicine, Div. Nephrology, Wuerzburg.*

Induction of inducible nitric oxide (NO) synthase (iNOS) is known as an important player in the pathomechanism of AKI. A dysbalance of NO isoforms, the postulated protective endothelial (e)NOS and the rather destructive inducible iNOS, might be causal. NO mediated effects e.g. on regulation of vasodilation, renal hemodynamics, glomerular filtration and tubuloglomerular feedback as well as inflammation is well described, but effects on alteration of tubular transporter mechanisms are lacking. Here we analyzed effects of iNOS inhibition by L-NIL [L-N6-(1-Iminoethyl)lysine] regarding iAKI damage and hypothesized impact on regulation of organic cation transporters (OCT).

Bilateral clamping of Aa. renales for 45 min followed by reperfusion resulted in iAKI in our animal model in rats. Beginning with ischemia L-NIL (in various doses) or saline was applied intraperitoneally. Corresponding sham served as control. Regarding renal functional parameters we determined inulin-/PAH-clearance and PAH netsecretion (NS) after iAKI. Cortical expression of eNOS and phos-eNOS(Ser1177) as counterpart of iNOS and expression of OCT1/2 was analyzed in kidney cortex.

Ischemia dependent reduction of inulin-/PAH-clearance and PAH-NS was improved by low dose application of L-NIL in our iAKI model. Using this dosage we detected an additional increase of eNOS overexpression while phos-eNOS(Ser1177) expression was not altered. Increased iNOS induction was unchanged by L-NIL. This was combined with a reversal of OCT1- as well as OCT2-downregulation using low dose L-NIL after iAKI.

Low dosage of L-NIL turned out to be protective in iAKI regarding renal functional parameters. eNOS upregulation might probably not be a the crutial factor of renal protection due to its unchanged activation via Ser1177 phosphorylation. iNOS inhibition itself seems to be a relevant issue, especially as no additional iNOS expression follows L-NIL application. Interestingly, abrogated OCT downregulation by L-NIL might indicate an important regulatory role of iNOS derived NO after iAKI, which has to be confirmed in future studies.

Disclosure of Financial Relationships: nothing to disclose

## PUB030

**Identification and Evaluation of Rat Urinary Biomarker Candidates for Drug-Induced Nephrotoxicity** Justyna Siwy,[1] William Mullen,[2] Harald Mischak,[1,2] Peter Rossing,[3] Parvaneh Espandiari,[4] Sharron R. Stewart,[4] Rodney L. Rouse,[4] Joseph Peter Hanig.[4] *[1]Mosaiques Diagnostics GmbH, Hannover, Germany; [2]University of Glasgow, Glasgow, United Kingdom; [3]Steno Diabetes Centre, Gentofte, Denmark; [4]U.S. Food and Drug Administration, Silver Spring.*

In an effort to identify biomarkers for drug-induced renal damage, we have previously identified potential urinary proteomic biomarkers for cisplatin-induced nephrotoxicity in rats, using the CE-MS (capillary electrophoresis coupled to mass spectrometry) technology. In a dataset consisting of 25 controls and 14 cases after treatment with cisplatin, 321 potential marker candidates could be identified as being significantly altered (p-value <0.05 as significance level after Benjamini and Hochberg multiple testing corrections). We now extend these findings by investigating the distribution of these biomarkers in urine from a longitudinal study of rats receiving gentamicin. Groups received three consecutive daily doses of 0, 75, 150, or 300 mg/kg gentamicin. Urine samples (4 per group) were collected on days 1, 5, 8, 13, 16, 20, 27, 34, and 41. All samples were analyzed using CE-MS. We analyzed the distribution of the 321 potential biomarkers of cisplatin-induced nephrotoxicity in urine samples from the gentamicin study. Thirty-two biomarker candidates were clearly associated with gentamicin induced injury, as indicated by significant changes following gentamicin treatment. In a second set of experiments, we examined the data for potential gentamicin-induced biomarkers of renal injury by identifying molecules with transient and dose-dependent changes following gentamicin administration. Of 368 candidates, 66 showed a transient and dose-dependent change. Of these, 6 were also indentified in the cisplatin study rendering these six excellent potential biomarkers for chemical-induced renal damage. We will disclose the identity of these potential biomarkers and give potential links to pathophysiology of renal injury. Collectively, these studies have demonstrated the potential of urinary proteomic biomarkers in assessing drug-induced renal damage and enabling advancements in preclinical safety testing.

Disclosure of Financial Relationships: Employer: mosaiques diagnostics GmbH.

## PUB031

**Non-Dilated Obstructive Uropathy: A Clinical Pitfall in the Investigation for Renal Failure** Dilip Unnikrishnan,[2] Hugo J. Villanueva,[1] Jinil Yoo.[1] *[1]Nephrology, Montefiore Medical Center North Division, Bronx, NY; [2]Nephrology Associates, Evansville, IN.*

Urinary tract obstruction is one of the important causes of acute renal failure (ARF) and failure to relieve obstruction expeditiously can permanently impair renal function. A dilated urinary system is used in most diagnostic procedures as a sign indicating obstruction. However, non-dilated obstructive uropathy (NOU) has been reported and is creating a clinical pitfall in the investigation for renal failure. We present a patient with NOU due to retroperitoneal fibrosis (RPF) who developed ARF requiring dialysis.

A 58-year-old male with hypertension on metoprolol presented with anuria. Renal ultrasound with doppler study showed normal sized kidneys without hydronephrosis. His renal functions worsened to serum creatinine (SCr) 14.5 mg/dL requiring hemodialysis. A retrograde cystoureterography was performed and demonstrated minor narrowing of the mid ureters without any ureteral obstruction. MRI showed no hydronephrosis but revealed a mantle of adenopathy encasing the abdominal aorta above its bifurcation. Because of strong clinical suspicion for obstructive uropathy, bilateral ureteric stents were placed. He subsequently underwent exploratory laparotomy, which confirmed the presence of RPF but no lymphadenopathy, and the open kidney biopsy showed histologic findings compatible with obstructive ARF. On the day of his discharge, renal functions improved to SCr 1.5 mg/dL.

NOU has been described with certain medications, in patients with RPF, and malignancies involving pelvis/ retroperitoneum. Our patient has RPF, idiopathic or related to use of beta blockers. About 2.5% patients with RPF are reported to have no dilatation of urinary system on sonogram. CT scan and MRI offer better anatomic delineation. Our case illustrates that a non-dilated urinary system on an imaging study or on retrograde cystoureterogram may not rule out the presence of obstruction. If clinical suspicion for urinary obstruction is high, expeditious further testing should be pursued to rule out obstructive ARF.

Disclosure of Financial Relationships: nothing to disclose

## PUB032

**Mitochondrial Injury and Tubular Apoptosis during Renal Ischemia-Reperfusion Are Suppressed in Bak-Knockout Mice** Qingqing Wei, Zheng Dong. *Department of Cellular Biology and Anatomy, Medical College of Georgia and Charlie Norwood VA Medical Center, GA.*

Renal tubular cell apoptosis via the intrinsic or mitochondrial pathway contributes to ischemic acute kidney injury (AKI). A key event of the mitochondrial pathway of apoptosis involves the activation of Bax and Bak, two multidomain proapoptotic Bcl-2 proteins. However, the specific roles played by Bax and Bak in mitochondrial injury and tubular cell apoptosis during ischemic AKI has not been established. In this study, we determined the role of Bak using gene-knockout mouse models. We tested two strains of mice with Bak knockout. One strain was purchased from Jackson Laboratory (B6 strain) and the other was derived from Bax(flox/flox)Bak(-/-) mice (BBC strain) . The mice were subjected to 30 min bilateral kidney ischemia followed by 48hrreperfusion. Compared with wild-type, the Bak-KO mice from both strains were significantly more resistant to ischemic AKI. For the B6 stain, Bak-KO mice showed BUN and serum creatinine of 229.8 and 2.32 mg/dL mg/dL vs.119.7 and0.91 mg/dL in wild-type mice. For the BBC strain, Bak-KO mice showed BUN and serum creatinine of 249.1 and 1.8 mg/dL vs. wild-type 114.2 and0.6 mg/dL. Ischemic AKI induced mitochondrial fragmentation in 41.4% proximal tubular cells in wild-type mice, which was suppressed to 15.9% in Bak-KO mice. Cytochrome c release was also significantly decreased in Bak-KO mice, so was tubular cell apoptosis. However, tubular necrosis shown by histological damage scores was not ameliorated in Bak-KO mice. Bak-KO mice survived better after ischemic AKI. Together, the results suggest that Bak mediates mitochondrial damage and tubular cell apoptosis, contributing to ischemic AKI.

Disclosure of Financial Relationships: nothing to disclose

## PUB033

**High Fat Diet Induced Proteinuria and Renal Insufficiency in Sprague-Dawley Rats** Jinn-Yang Chen. *Division of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan.*

Backgrounds

Obesity is established independent risk factors for the development of chronic kidney disease. The mechanisms obesity induced renal insufficiency is investigated.

Methods

Eight weeks male Sprague-Dawley rats with (Sham) or without unilateral nephrectomy (UNX) received 60% high fat diet (HFD) or standard chow for 3 months. The oral glucose tolerance test (OGTT) was performed in the morning after an overnight 10- to 12-h fast once every month. Fasting blood samples were taken for measurement of glucose, insulin, total cholesterol, triglyceride, creatinine, BUN, HDL cholesterol. Urine protein was measured with spot urine protein/creatinine ratio. The abdominal fat distribution and

area were measured by CT scan. After 3 months of treatments, all rats were sacrificed. Epididymal fat, liver, heart, kidney and muscle were removed for immunohistochemistry and real-time quantitative PCR.

Results

After 3 months of experiments, sham rats taking standard chow weight 582.13 ± 39.2 gm and HFD rats weight 685.5 ± 87.5 gm. HFD rats developed increased insulin resistance, serum creatinine, leptin, urine protein/creatinine ratio and decreased serum adiponectin. Rats receiving both NPX and HFD had largest increase of proteinuria. HFD rats also have increased visceral fat/subcutaneous fat ratio. Surprisingly, UNX rats have increased sensitivity to insulin. Light microscopic investigation showed a dilatation in blood vessels and Bowman's space, mononuclear cell infiltration, degeneration in nephrons, including glomerulosclerosis and tubular defects, and an increase in the connective tissue in the kidneys in the treatment group. UNX rat has significant heart fibrosis.

Conclusion

Fatty diet is responsible for the rats' obesity and may lead to proteinuria, renal insufficiency, renal deformities as a result of histopathological changes such as dilatation, tubular defects, inflammation and connective tissue enlargement of the kidney.

Disclosure of Financial Relationships: nothing to disclose

**PUB034**

**Effects of *Fangjihuangqitang* on the Expressions of MCP-1 in Renal Cortex of Adriamycin-Induced Nephropathy (AIN) Rats** Hongyu Chen, Qin Zhu, Yongyun Wang. *Nephrology, Hangzhou Hospital of Traditional Chinese Medicine (TCM), Hangzhou, China.*

**Aims:** The purpose of study is to observe effects of *fangjihuangqitang* on the expressions of MCP-1 in renal cortex of AIN rats.**Methods:** 21 rats were divided into three groups randomly (normal group, AIN group and treatment group). Adriamycin was given (6.5mg/kg) to the AIN group and treatment group through the caudal vein, while isometric physiological saline was injected to the normal group. Treatment group were feed with *fangjihuangqitang* as the same time, while normal group were feed with isometric physiological saline. The quantitative detection of 24-hour proteinuria was been done on the second and eighth week respectively. Renal cortexes were taken at the end of experimentation. The expression of MCP-1 was tested by immunohistochemisty and RT-PCR.**Results:**Two weeks later, the quantitations of proteinuria in normal group, AIN group, and treatment group were 19.10±3.93mg/d, 408.85±149.27mg/d, and 489.10±296.20mg/d in the experiment.The values of the AIN group and the treatment group were much higher than the normal group($P$<0.05). Eight weeks later, the quantitations of proteinuria in normal group, AIN group, and treatment group were 42.03±9.58mg/d, 615.33±48.32mg/d, and623.86±146.22mg/d, and there was no significant difference between AIN group and treatment group.Tested by immunohistochemisty, the expression of MCP-1 in renal cortex in normal group, AIN group and treatment group were 0.04±0.01, 0.11±0.01, and0.07±0.01. Tested by RT-PCR. The expression levels of MCP-1 in renal cortex in normal group, AIN group and treatment group were 13.05±0.27, 34.51±1.20, and28.57±0.11. Both the values of the AIN group and the treatment group were much higher than the normal group($P$<0.05), while the treatment group was much lower than the AIN group($P$<0.05).**Conclusion:** The expression of MCP-1 mRNA and protein in Adriamycin-induced nephropathic rats was abnormal. The increase of MCP-1 expression is likely to be related to the mass of proteinuria. The reason for *fangjihuangqitang* which has gotten some effects on chronic kidney disease is possibly related to decreasing expressions of MCP-1 in renal cortex.

Disclosure of Financial Relationships: nothing to disclose

**PUB035**

**The Role of Protease Activated Receptor 4 in Renal Fibrosis** Chee Kay Cheung,[1] Ai Yen Chin,[1] Timothy Scott Johnson,[2] Simon P. Hart,[1] Sunil Bhandari.[1] *[1]Centre for Clinical Sciences, Hull York Medical School, Hull, East Yorkshire, United Kingdom; [2]Sheffield Kidney Institute, University of Sheffield, Sheffield, South Yorkshire, United Kingdom.*

The protease activated receptors are a novel group of G-protein coupled receptors which have been implicated in a range of diseases including inflammatory, fibrotic and coagulation disorders. 4 receptors (PAR1-4) have been identified, which are activated by various proteases. PAR1 and 2 have been studied extensively in kidney but the role of PAR4 is unknown. In lung fibroblasts, our group previously showed that PAR4 was upregulated in response to inflammatory cytokines, and targeting PAR4 led to a reduction in fibroblast number. We aimed to see whether a similar process occurred in renal fibroblasts, in cell culture and a model of renal fibrosis.

Primary human renal fibroblasts, the NRK49F rat fibroblast cell line and the NRK52E rat tubular cell line were used to identify PAR4, by RT-PCR and Western blotting. A rat 5/6ths nephrectomy model was used to assess distribution of PAR4 in the kidney, by RT-PCR and immunohistochemistry.

Renal fibroblasts constitutively expressed PAR1 and 2, but not PAR3 and 4 mRNA. PAR4 mRNA levels were not significantly upregulated in response to tumour necrosis factor (TNF)-α or lipopolysaccharide (LPS) in primary renal fibroblasts and NRK49F cells. PAR4 mRNA was expressed in sham kidney (n=5) and was significantly upregulated in the 5/6ths nephrectomy model (n=6) at day 90 by 2.2 (SEM ± 0.4) fold. PAR4 stained positively in proximal and distal tubules in sham kidney and 5/6ths nephrectomy, and negatively in fibroblasts, with no difference in intensity of staining between conditions. PAR4 mRNA and protein expression were confirmed in NRK52E cells by RT-PCR and Western blotting.

We have demonstrated distribution of PAR4 in the kidney for the first time. PAR4 expression was not increased in renal fibroblasts in response to inflammatory mediators or in a model of CKD, thus is unlikely to be a suitable target for apoptosis. Studies are ongoing to assess the effects of PAR4 stimulation on tubular cells, specifically if this promotes pro-fibrogenic cytokine release, as demonstrated previously for PAR1 and PAR2.

Disclosure of Financial Relationships: nothing to disclose

**PUB036**

**Combined Losartan (L) and Furosemide (F) Treatment Is Less Renoprotective Than L+Hydrochlorothiazide (H) in the 5/6 Renal Ablation Model (Nx)** Simone R. Costa, Renata A. Souza, Carla Romagnoli, Marcela C. Morais, Claudia R. Sena, Camilla Fanelli, Bianca H. Ventura, Denise M. Malheiros, Clarice K. Fujihara, Roberto Zatz. *Univ of Sao Paulo, Brazil.*

We showed (AJP 292:F1810) that LH association arrested renal injury for at least 7 months in Nx. Here we investigated if equal protection can be obtained by combining L with F instead of H. Nx was performed in 59 Munich-Wistar rats. One month later, tail-cuff pressure (TCP, mmHg), albuminuria (ALB, mg/d), left kidney weight/body weight (LKW/BW), glomerular volume ($V_G$, x$10^6$µm$^3$), glomerulosclerosis index (GSI), interstitial macrophage infiltration (Mø, cells/mm$^2$) and tubulointerstitial (TI) proliferating cell nuclear antigen (PCNA+, cells/mm$^2$) were measured in 17 rats (**$Nx_{pre}$**). The remaining 42 rats were distributed into: **Nx** (untreated); **$Nx_{LH}$**, (L, 50 mg/Kg/d + H, 6 mg/Kg/d); and **$Nx_{LF}$** (L + F, 20 mg/Kg/d, the highest nontoxic dose). Results after 7 months (Mean ± SE, S, sham-operated, [a] p<0.05 vs. S; [b] p<0.05 vs. $Nx_{pre}$; [c] p<0.05 vs. Nx, [d] p<0.05 vs. $Nx_{LH}$):

| | TCP | ALB | LKW/BW | $V_g$ | GSI | Mø | TI PCNA+ |
|---|---|---|---|---|---|---|---|
| S | 149±3 | 28±6 | 0.49±0.1 | 0.7±0.1 | 1±1 | 36±5 | 26±5 |
| $Nx_{pre}$ | 198±5[a] | 91±8[a] | 0.53±0.1[a] | 0.8±0.1 | 16±3[a] | 104±10[a] | 142±16[a] |
| Nx | 200±14[a] | 203±26[ab] | 0.53±0.1[a] | 1.4±0.1[ab] | 328±38[ab] | 217±19[ab] | 163±21[a] |
| $Nx_{LH}$ | 146±5[bc] | 26±4[bc] | 0.46±0.1[bc] | 1.3±0.5[ab] | 12±4[abc] | 87±7[ac] | 34±3[abc] |
| $Nx_{LF}$ | 167±6[abcd] | 90±18[acd] | 0.56±0.1[ad] | 1.6±0.1[abd] | 38±12[acd] | 121±11[acd] | 86±10[abcd] |

Survival was 35% in Nx, 100% in $Nx_{LH}$ and 82% in $Nx_{LF}$. LH treatment arrested GSI, Mø and interstitial expansion, and reversed TCP, ALB, renal hypertrophy and TI hyperplasia. Though beneficial, LF treatment failed to normalize TCP or ALB, reverse renal growth or TI hyperplasia or detain GSI and INT progression. Mechanisms for the lower efficacy of LF may include increased glomerular stress from tuft hypertrophy and inflammation linked to TI hyperplasia. The clinical implications of these results remain to be investigated.

Disclosure of Financial Relationships: nothing to disclose

**PUB037**

**Up-Regulation of miR-29c Suppresses Tropomyosin 1a Expression in the Remnant Kidney Model with Short Term Activation of Hyopoxia-Inducible Factor** Yi Fang,[1] Xiaofang Yu,[1] Jiaming Zhu,[1] Xialian Xu,[1] Mingyu Liang,[2] Yong Liu,[2] Alison J. Kriegel,[2] Xiaoqiang Ding.[1] *[1]Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China; [2]Physiology, Medicial College of Wisconsin, Milwaukee, WI.*

MicroRNAs (miRNAs) are small regulatory RNA molecules that modulate the activity of specific mRNA targets and play important roles in a wide range of physiologic and pathologic processes. We hypothesized that miRNAs might be involved in the progression of CKD. After 5/6 subtotal nephrectomy, male Sprague-Dawley rats were treated with intraperitoneal injections of L-mimosine(L-Mim), an inhibitor of prolyl 4-hydroxylase domain (PHD), at a dosage of 50 mg/kg every other day from wk 5 to wk 12 to inhibit PHD activity. L-Mim treated rats had lower BUN, Scr and Ualb, higher Hb and less severe tubular interstitial injury than control. Nuclear staining of HIF-1α and HIF-2α in the tubulointerstitial area in the L-Mim group is stronger than that in the control group. We found miR-29c in renal cortex was up-regulated in L-Mim groups compared with the control using Agilent miRNA microarrays. Of the microRNAs and proteins that exhibited reciprocal changes in expression following the L-Mim treatment, miR-29c and tropomyosin 1a (TPM1), which is involved in stress fiber function, met the sequence criteria for microRNA-target interaction, which was later confirmed by 3'-untranslated region reporter analyses. Real time PCR re-confirmed that miR-29c levels in rat cortex was up-regulated after short term L-Mim treatment while TPM1 was down-regulated at the protein level. TGFb1 treatment (3 ng/ml, 24 hours) decreased miR-29c expression and up-regulated protein expression of TPM1 in human renal epithelial cells. Overexpression of miR-29c significantly attenuated TGF-b1 induced increase in TPM1 in vitro. The results suggest that short term stabilizing of HIF up-regulates miR-29c, which contributes to suppression of TPM1 and amelioration of CKD progression.

Disclosure of Financial Relationships: nothing to disclose

**PUB038**

**Introducing a CD for the Instruction of the Surgical Procedures Recommended for the Induction of a Mouse Model of Chronic Kidney Disease (CKD) (The So-Called Electrocautery Mouse Model)** Raymonde Gagnon. *Departrment of Medicine, McGill University Health Centre - Montreal General Hospital, Montreal, QC, Canada.*

The purpose of this CD is to facilitate the teaching of the 2 surgical procedures required for the induction of this proven mouse model of CKD i.e. electrocautery of the surface of the right kidney followed by a left nephrectomy 2 weeks later. The first author had been involved previously in the teaching of the 2 surgical procedures to various interested research groups. This new teaching tool includes the following 3 parts: 1) 200 professional

grade photographs of all aspects of the 2 surgical procedures (from skin-to-skin) and of important related information, e.g. the handling of the electrocautery apparatus; 2) an accompanying text provided detailed information related to the photographs and to a few drawings to accompany key photographs for special emphasis (i.e. the actual electrocautery procedure); and 3) additional relevant information on details of procedures used in our Center, particularly the special care of the mice immediately before and after the 2 surgical procedures. Ths mouse model of CKD presents distinct advantages in that it can provide different degrees of CKD (i.e. mild, moderate or severe) carried over various time periods as required by the chosen experimental protocol. By providing all the necessary information in this CD format, it is expected that CKD can be induced safely and efficiently in mice thereby promoting its use to conduct basic research in CKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB039**

**Effects of High Glucose and Mannose-Binding Lectin on the Activation of Lectin Complement Pathway in Human Renal Glomerular Endothelial Cells** Songmin Huang, Wenxing Fan, Wanxin Tang, Ping Fu, Fang Liu, Hongyu Qiu. *Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China.*

***Aim:*** To investigate the effects of high glucose and mannose-binding lectin (MBL) on the activation of lectin complement pathway (LCP) in human renal glomerular endothelial cells (HRGECs).

***Methods:*** HRGECs were guaranteed to culture in endothelial cell medium (ECM) *in vitro*. First, for the effects of high glucose on the activation of LCP, cells were cultured in ECM containing high glucose for different times (0-72 hours) or in different concentrations of glucose (0-60 mmol/L). Second, for the effects of exogenous MBL on the activation of LCP, cells were cultured in ECM plus exogenous MBL for different times (0-16 hours) or at different concentrations of MBL (0-8μg/ml). Third, for the effects of anti-human MBL monoclonal antibody (anti-MBL-mAb) or C1-esterase inhibitor (C1-INH) on the activation of LCP, cell-surface MBL, C3 and C5b-9 deposition were detected by flow cytometry, immunofluorescence and Western blot, respectively. NF-κB DNA-binding activity was evaluated by electrophoretic mobility shift assay (EMSA).

***Results:*** MBL or/and C3 deposition stimulated by high glucose were observed in both time and dose dependent manners. MBL or/and C3 deposition stimulated by exogenous MBL were also observed in both time and dose dependent increase manners, and peaked at 4 hours, then decreased. After treating with anti-MBL-mAb or C1-INH, both MBL and C3 deposition were significantly reduced. Treatment with anti-MBL-mAb or C1-INH could significantly attenuate the cell-surface C5b-9 staining, and obviously decrease NF-κB DNA-binding activity in HRGECs (all $P<0.05$).

***Conclusions:*** High glucose and exogenous MBL might mediate the activation of LCP in HRGECs, the mechanism by which may be associated with the cell-surface glycosylation and the level of exogenous MBL. C5b-9 deposition caused by the activation of LCP might strongly induce NF-κB DNA-binding activity in HRGECs, which may lead to endothelial cell injury. Both anti-MBL-mAb and C1-INH treatment could significantly inhibit the activation of LCP mediated by high glucose and exogenous MBL.

Disclosure of Financial Relationships: nothing to disclose

**PUB040**

**Erythropoiesis-Stimulating Agent Aggravates Vascular Endothelial Function in Normal and Chronic Renal Failure Rats** Chieko Ihoriya, Minoru Satoh, Naruya Tomita, Tamaki Sasaki, Naoki Kashihara. *Division of Nephrology and Hypertension, Department of Internal Medicine, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Recent studies show erythropoietin has a wide variety of nonhematopoietic effects. However, it is still controversial whether these activities are beneficial in terms of the cardio-vascular protections. Erythropoietin acts through the erythropoietin receptor (EpoR) present in erythroblasts and also in the vascular endothelium. The binding affinity to EpoR differs depending on the erythropoiesis-stimulating agent. The binding affinity with Darbepoetin a (D-EPO) is lower than that with erythropoietin (EPO), thus it is possible that D-EPO has different effect on endothelial function. Normal male SD rats were treated with either EPO (20 IU/kg/week) thrice weekly or D-EPO (0.1 μg/kg/week) once weekly for 4 weeks. Endothelial-dependent vasodilatory response, gene expression of ICAM1 and TNFa, and NADPH oxidase activity were then assessed. The dosages of EPO and D-EPO were determined so as to have no effect on hemoglobin level. Next, adenine-induced uremic rats were divided into five groups: (1) no treatment, (2) low dose EPO treatment (20 IU/kg/week), (3) high dose EPO treatment (600 IU/kg/week), (4) low dose D-EPO treatment (0.1 μg/kg/week), and (5) high dose D-EPO treatment (3.0 μg/kg/week). EPO or D-EPO was administered for 4 weeks and survival rate was evaluated. In normal SD rats, the acetylcholine-dependent vasodilatory response decreased significantly in both EPO and D-EPO treatment groups. NADPH oxidase activity increased in both groups. Aortic gene expression of ICAM1 and TNFa similarly increased in both groups. There was no difference between EPO and D-EPO. In adenine-induced uremic rats, body weight and blood pressure were similar in the five groups. All rats had high blood pressure due to chronic renal failure compared to normal rats. Some rats died within one week after treatment. High-dose EPO and D-EPO groups had a low survival rate. In conclusion, administration of EPO and D-EPO increased oxidative stress and impaired endothelial function in normal rats. Administration of high-dose EPO to chronic renal failure rats further worsened prognosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB041**

**Metabolomic Search for Biomarkers of the Effect of a Spherical Carbon Adsorbent by Liquid Chromatography/Electrospray Ionization-Tandem Mass Spectrometry** Kaori Kikuchi,[1] Yoshiharu Itoh,[1] Kenjiro Murakami,[1] Toshimitsu Niwa.[2] *[1]Kureha Corp., Tokyo, Japan; [2]Advanced Medicine for Uremia, Nagoya Univ. Graduate School of Medicine, Nagoya, Japan.*

We searched biomarkers of the effect of a spherical carbon adsorbent by metabolomic study using liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS).

Serum metabolites in normal and chronic renal failure (CRF) rats before and after administration of a spherical carbon adsorbent for 3 days were analyzed by LC/ESI-MS/MS and principal component analysis. Further, serum and urine levels of the biomarkers were quantified by the selected reaction monitoring of LC/ESI-MS/MS. Indoxyl sulfate was the first principal serum metabolite, which could differentiate CRF from both normal and adsorbent-administered CRF rats, followed by hippuric acid, phenyl sulfate and 4-ethylphenyl sulfate. CRF rats showed increased serum levels of indoxyl sulfate, hippuric acid, phenyl sulfate, 4-ethylphenyl sulfate and *p*-cresyl sulfate, and administration of the spherical carbon adsorbent for 3 days to the CRF rats reduced the serum and urine levels of the metabolites.

Indoxyl sulfate is the best biomarker of the effect of the spherical carbon adsorbent in CRF rats. Hippuric acid, phenyl sulfate and 4-ethylphenyl sulfate are suggested as the additional biomarkers.

Disclosure of Financial Relationships: Employer: Kureha Corp.

**PUB042**

**The Prevalence of Chronic Kidney Disease (CKD) among Elderly People in Jiangsu, China: A Population-Based Cross-Sectional Epidemiologic Study** Bi-Cheng Liu, Hong Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China.*

**Background:** Chronic kidney disease is a growing problem among aging populations. However, few studies have focused on the prevalence of CKD in the elderly population. This study was performed to survey the prevalence, awareness and the risk factors of CKD among the elderly Chinese people.

**Methods.** A total of 1316 adults aged 60 years or older was investigated based on the stratified randomized sampling method from two city of Jiangsu province, southeast China (664 from Huaian and 652 from Suzhou) . All were screened for morning spot urine albumin-to-creatinine ratio, serum creatinine (Scr), uric acid (UA), cholesterol (TCh), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and fasting blood glucose (FBG). Glomerular filtration rate (GFR) was estimated according to MDRD formula. CKD was defined as K/DOQI guideline.

**Results.** The overall prevalence of CKD was 32.3%. The distribution of CKD stages were as following: 16.6% at stage 1, 12.5% at stage 2, 3.0% at stage 3, 0.1% at stage 4, and 0.2% atstage 5. Albuminuria was detected in 30.2% of subjects, and the prevalences of microalbuminuria and macroalbuminuria were 29.3% and 0.9%, respectively. Reduced eGFR was found in 3.2% of all studied subjects. The awareness rate of CKD was only 9.6%. The logistic regression showed that age, gender, hypertension, systolic blood pressure (SBP), diabetes mellitus, fasting blood glucose, hypercholesterinemia, metabolic syndrome and hyperuricaemia were the independent factors related to the presence of CKD. Age, gender, hypertension, systolic blood pressure (SBP), fasting blood glucose and hyperuricaemia were independently associated with albuminuria.

**Conclusion.** This is the first community-based epidemiological study data on elderly Chinese people. The prevalence of CKD is 32.3%, while the awareness is only 9.6%, suggesting more attention should be paid to this specific group of people.

Disclosure of Financial Relationships: nothing to disclose

**PUB043**

**Heat Shock Protein 72 Induced by Heat Shock Treatment May Function as a Novel Therapeutic Target in the Prevention of Vascular Calcification** Tzong-Shi Lu,[1] Kenneth Lim,[1,2] Guerman Molostvov,[2] Daniel Zehnder,[2] Li-Li Hsiao.[1] *[1]Renal Division, Brigham and Women's Hospital, Boston, MA; [2]Clinical Science Research Insitute, Warwick Medical School, Coventry, United Kingdom.*

Vascular calcification (VC) is a significant contributor to cardiovascular mortality in patients with chronic kidney disease (CKD) and coronary artery disease (CAD). Osteo/chondrocytic transformation and simultaneous dedifferentiation of smooth muscle cells (SMC) are important in the pathogenesis of VC. Heat shock protein 72 (HSP72) is a cardio-protective inducible heat shock protein that functions as a molecular chaperone. However, little is known about its effects in VC. We have established a long-term calcification and heat shock treatment (HST) model using human aortic-smooth muscle cells (HA-SMCs); calcification was achieved using 5mM $CaCl_2$ and 5mM β-Glycerolphosphate for 21 days and for HST, daily hyperthermia treatment at 43ºC for 30 minutes. Our results show for the first time that induction of HSP72 by HST significantly prevented the development of calcification in HA-SMCs, and these effects were abolished by the HST inhibitor, quercetin, *in vitro*. We next showed that HSP72 suppressed osteo/chondrocytic transformation markers, core binding factor α-1, Osteocalcin and Alkaline Phosphatase. Furthermore, we show that HSP72 upregulated the expression of myocardin and serum response factor (SRF), regulators of SMC contractile genes; co-immunoprecipitation studies demonstrated the association of myocardin-SRF-HSP72 complex. We also describe for the first time marked reduction of HSP72 expression in arteries from patients with CKD and CAD, compared to healthy controls, *in vivo*. In addition, organ culture of arteries from CKD and CAD

patients, confirmed that despite initially reduced expression, retained their ability to induce HSP72 following HST at 41ºC for 30 minutes. In conclusion, our study showed that HSP72 may function as a central regulator of molecular pathways involved in the development of VC, by suppression of osteo/chondrocytic transformation and stabilization of smooth muscle cell phenotype. We suggest treatment strategies that up-regulates HSP-72 as a new approach to inhibit VC.

Disclosure of Financial Relationships: nothing to disclose

**PUB044**

**Renal Disease, Oxidative Stress, Inflammation Markers in Patients with Familial Lecithin-Cholesterol Acyltransferase Deficiency** Przemyslaw Miarka, Barbara Idzior-Walus, Marek Kuzniewski, Malgorzata Walus-Miarka, Wladyslaw Sulowicz, Przemyslaw Witek. *Dept of Nephrology, Metabolic Diseases, Jagiellonian University, Poland.*

Familial LCAT deficiency (FLD) is genetic disorder of lipid metabolism, characterized by low plasma HDL-cholesterol, proteinuria, haemolytic anaemia, corneal opacities. HDL functions including reduction of oxidative stress, anti-thrombotic, anti-inflammatory activity could be impaired in these patients. Usually patients with FLD develop renal failure during 3rd decade of life, but pathogenesis isn't known. There is growing evidence of inflammatory role, oxidative stress in development of renal disease. In study we assessed parameters of oxidative stress, inflammation and coagulation in 2 patients with LCAT deficiency due to Val309Met mutation of LCAT gene.

Material include 2 siblings with FLD (WX;SY) and control group. We also examined 5 other siblings, who were heterozygous (HTZ). Levels of IL6, IL10 and TNF were measured with ELISA, coagulation factorVII (FVII), and antithrombin III (A-TIII) activities by chronometric method and PAI-1 by chromogenic method.

Results: In 2 patients with FLD plasma thiobarbituric acid reactive substances (TBARS) levels were 1.55 times higher and paraoxonase (PON) activity was 2.4 times lower than in HTZ and controls. TNF and IL6 levels were lower and IL10 concentrations higher in FLD patients in comparison to healthy and HTZ persons.

FLD patients with renal disease are characterized by increased oxidative stress indicated by elevated TBARS and decreased PON activity. However, lower values of inflammatory cytokines, such as IL6, TNF and FVII and higher values of anti-inflammatory IL10 in comparison to controls were observed. The results indicate that low HDL levels are associated with low antioxidant defences, so oxidative rather than pro-inflammatory stress might be relevant in progression of renal disease in these patients.

| | WX | SY | HTZ | Controls | p |
|---|---|---|---|---|---|
| TRABS | 5.56 | 5.56 | 4.51 | 3.58 | 0.05 |
| PON | 285 | 415 | 421 | 698 | 0.01 |
| TNF | 2.86 | 2.71 | 2.52 | 3.58 | 0.05 |
| IL6 | 0.28 | 0.56 | 0.73 | 0.5 | 0.05 |
| IL10 | 0.63 | 2.0 | 0.65 | 0.17 | 0.05 |
| FVII | 76 | 68 | 126 | 112 | 0.05 |

Disclosure of Financial Relationships: nothing to disclose

**PUB045**

**Urinary NGAL as a Marker for Early Stages of Chronic Kidney Disease in 5/6 Nephrectomised Rats** Martin Skott,[1] Rikke Norregaard,[1,2] Tae-Hwan Kwon,[3] Jorgen Frokiaer,[1,2] Soren Nielsen.[1] *[1]Water & Salt Research Center, Faculty of Health Science, Aarhus University, Aarhus, Denmark; [2]Institute of Clinical Medicine, Arhus University, Aarhus, Denmark; [3]Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Taegu, Korea.*

The aim of this study was to assess whether neutrophil gelatinase associated lipocalin (NGAL) could represent a novel marker of kidney function during the early stage of chronic kidney disease (CKD) in 5/6 nephrectomised rats.

CKD was induced by excision of ~ 2/3 of the left kidney and right 1/1 nephrectomy using the excision remnant kidney model. Sham operations were also performed.

Creatinine, urea and albumin were determined on 24 hr urine & serum samples. Direct ELISA was performed on 24 hr urine to detect NGAL.

Fourteen days after 5/6 nephrectomy, polyuria, polydipsia, azotaemia & proteinuria were observed in the rats with CKD. Urinary excretion of NGAL was increased in 5/6 nephrectomised rats.

| | Nephrectomy (n=10) | Sham (n=10) |
|---|---|---|
| **P-Creatinine(μmol/L)** | 71.64 ± 4.19* | 37.10 ± 1.23 |
| **P-Urea(mmol/L)** | 18.40 ± 1.58* | 6.06 ± 0.59 |
| **U-Albumin (g/L)** | 0.32 ± 0.13 | < 0.036 |
| **Creatinine clearance (ml/min)** | 0.72 ± 0.06* | 1.54 ± 0.12 |
| | Nephrectomy (n=25) | Sham (n=24) |
| **U-NGAL(Units/day)** | 18071.74 ± 1.16* | 5584.70 ± 1.13 |
| **Body weight (gram)** | 266.2 ± 6.0* | 300.46 ± 5.38 |
| **Urine output (μl/min/kg BW)** | 112.18 ± 8.79* | 36.46 ± 2.43 |
| **Water intake (μl/min/kg BW)** | 144.13 ± 6.26* | 61.87 ± 3.12 |

Values are calculated as means (±SE). * P < 0.05 compared to Sham

Pearsons correlation analysis (n=10) showed, as expected, that both plasma creatinine & urea correlated significantly with creatinine clearance (CLcr) (r=-0.66, p<0.05 & r=-0.84, p<0.05 resp.). Unexpectedly urinary NGAL concentrations did not correlate with plasma creatinine & urea (r=-0.55, p>0.05, r=-0.60, p>0.05 resp.), CLcr (r=0.41, p>0.05) & proteinuria (r=0.16, p> 0.05).

These data suggest that NGAL can serve as a novel urinary biomarker of CKD in rats exposed to 5/6 nephrectomy. However NGAL might not be able to assess the severity of CKD in this animal model, as opposed to conventional biomarkers of CKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB046**

**Hematological Parameters in Patients Treated with MIRCERA® after Conversion from rHuEPO** N. J. Vega, Gloria Antón Pérez, Raquel Santana Estupiñán, Patricia Pérez Borges, Roberto Gallego Samper, Fernando Henríquez Palop, Faina González Cabrera, Leocadia Palop Cubillo. *Department of Nephrology, Dr. Negrin University Hospital of Gran Canaria, Las Palmas de Gran Canaria, Spain.*

**Objectives**: To analyze whether conversion from weekly rHuEPO to monthly MIRCERA® is a safe option in patients with chronic kidney disease (CKD) on renal replacement therapy (hemodialysis (HD) and peritoneal dialysis (PD)) and those attending the advanced CKD (ACKD) unit (stages IV and V). **Methods:** 102 patients (39 HD, 37 PD, and 26 ACKD) were evaluated at baseline and after conversion during 6 months of follow-up regarding hematological parameters and anaemia treatment. Administration route was intravenous in HD and subcutaneous in PD and ACKD patients. **Results:** Mean age was 61.8±14.3 years and 59.8% of patients were men. The most frequent aetiology of CKD was diabetic nephropathy (45.1%), followed by CKD of unknown cause (15.7%) and chronic glomerulonephritis (12.7%). The 6.9% of patients were naive for erythropoiesis stimulating agent administration, 93.1% had received previously a rHuEPO. The results for hemoglobin (Hb) and the MIRCERA® dose over time are shown in the table below.

| Patients | Parameters | Baseline | 2 months | 4 months | 6 months |
|---|---|---|---|---|---|
| HD (n=39) | Hb (g/dL) | 11.51 ± 1.33 | 11.33 ± 1.13 | 11.28 ± 1.22 | 11.46 ± 1.18 |
| | MIRCERA (μg) | 157.69 ± 77.19 * | 157.69 ± 84.13* | 179.49 ± 84.25 | 179.48 ± 75.84 |
| PD (n=37) | Hb (g/dL) | 12.14 ± 1.32 | 11.52 ± 1.51 | 11.60±1.39 * | 11.94 ± 1.50 |
| | MIRCERA (μg) | 123.65 ± 55.88 * | 143.24 ± 50.17 * | 156.03±60.78 | 162.84 ± 76.06 |
| ACKD (n=26) | Hb (g/dL) | 12.29 ± 1.27 | 12.30 ± 1.56 | 12.62±1.63 | 12.64 ± 1.53 |
| | MIRCERA (μg) | 95.19 ± 38.09 ** | 119.23 ± 54.45 * | 126.92±56.97 | 133.65 ± 58.84 |

*p < 0.05; **p < 0.01

**Conclusions:** Conversion to MIRCERA® effectively and safely maintained the mean Hb level between 11 and 13 g/dL throughout the follow-up period. Upward dose adjustment of MIRCERA® was required over time in all patient subgroups, the largest dose increases being required in HD followed by PD and ACKD.

Disclosure of Financial Relationships: nothing to disclose

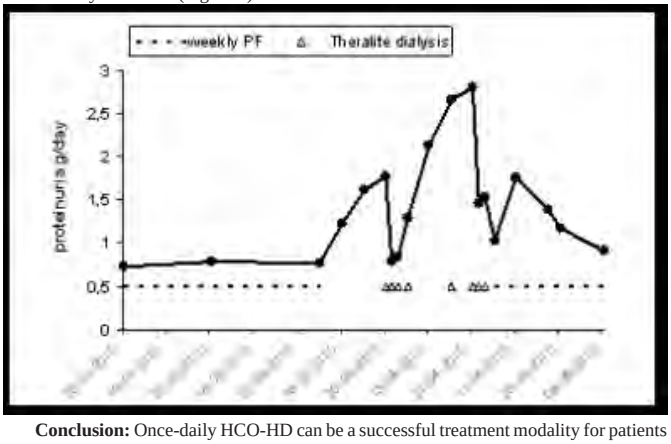
**PUB047**

**Reduction of Proteinuria in Recurrent Focal Segmental Glomerulosclerosis after Renal Transplantation with High Cut-Off Hemodialysis** Marike Wabbijn, Dennis Alexander Hesselink, Michiel G. H. Betjes. *Nephrology, Erasmus MC, Rotterdam, Netherlands.*

**Background:** Timely treatment with plasmapheresis (PP) may lead to remission of recurrent idiopathic focal segmental glomerulosclerosis (iFSGS). A circulating permeability factor of 30-50 Kd has been postulated to be present in iFSGS, but has never been fully characterized. Recently, high cut-off (HCO) dialyzers have become available that allow for removal of molecules with a Mw up to 100 Kd. HCO-dialyzers have been used successfully to remove light chain immunoglobulins in patients with myeloma cast nephropathy We tested whether HCO dialyzers can be effective in the treatment of recurrent iFSGS after renal transplantation.

**Patient case:** A 28-yr old male underwent a 2nd kidney transplantion from a living-related donor. Proteinuria developed shortly after the 2nd kidney transplantation and recurrence of iFSGS was diagnosed on renal biopsy. PP treatment was started and stable remission of proteinuria was achieved. For the past two years, he has remained in an excellent clinical condition with low grade proteinuria, however dependent on once-weekly PP.

Recently, we started to treat this patient with HCO-HD, hoping the proteinuria would remain <0.5 gr/day and frequency of treatment could be decreased. The protocol was started 2 weeks after the last PP, when his proteinuria increased from 0.5 gr to >2 gr/day. Initially, we treated with HCO-HD every other day which lead to a rapid remission of proteinuria. However, once-weekly treatment lead to increasing proteinuria. We repeated this treatment schedule and confirmed the results: rapid remission but reappearance of proteinuria with once-weekly treatment (Figure 1).




**Conclusion:** Once-daily HCO-HD can be a successful treatment modality for patients with recurrent iFSGS after renal transplantation, supporting the hypothesis of a circulating permeability factor.

Disclosure of Financial Relationships: nothing to disclose

**PUB048**

**Peroxisome Proliferator Activated Receptor Gamma Agonist Inhibits Lipopolysaccharide-Induced the Expression of Chemokines in Renal Tubular Epithelial Cells** Weiming Wang, Ying Lu, Xu Hao, Qiao Zhou, Cong Li, Nan Chen. *Ruijin Hospital.*

**Objective** The aim of this study is to investigate the anti-inflammatory actions of PPARgamma agonist in LPS-stimulated renal tubular epithelial cells and further to clarify the mechanisms. **Methods** HK-2 cells were divided into four groups: control, LPS (1μg/ml), 15d-$PGJ_2$( 5μM)**,** LPS (1μg/ml)+ 15d-$PGJ_2$(5μM). The expression of MCP-1 and IL-8 were measured using Real-time PCR and ELISA . To determine whether the inhibitory effects of 15d-$PGJ_2$ were PPARγ-dependent or not, RNAi experiments were performed. The location of NF-κB and the IκB phosphorylation was detected by immunofluorescence and western blot methods respectively. **Results** The IL-8 and MCP-1 protein expressions were increased by 1.6 and 2.3 times, respectively, in LPS-stimulated HK-2 cells ($p<0.05$), and the level of p-IκB in cytoplasm was significantly increased compared with control group. In 15d-$PGJ_2$-pretreated cells, IL-8 and MCP-1 were decreased by 74%, 79% in mRNA level and 69%, 49% in protein level compared with LPS group($p<0.05$). In 15d-$PGJ_2$ -pretreated PPARγ negtive cells, the levels of IL-8 and MCP-1 still was reduced by 59%, 44% in mRNA level and 47%, 39% in protein level compared with LPS group. 15d-$PGJ_2$ significantly reduced LPS-induced IκB phosphorylation and NF-κB nuclear translocation in PPARγ negative cells. **Conclusions** 15d-$PGJ_2$ could inhibit LPS-induced chemokines expression, and are not entirely dependent on PPARγ, which may be related to inhibition of IκB phosphorylation.

Disclosure of Financial Relationships: nothing to disclose

**PUB049**

**Effect of Aggregated IgA1 from IgA Nephropathy Patients on Podocin Expression in Podocytes** Cheng Wang,[1] Xun Liu,[1] Zengchun Ye,[1] Hui Peng,[1] Hua Tang,[1] Hui Zhang,[2] Tan-Qi Lou.[1] *[1]Deparment of Nephrology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China; [2]Department of Medicine, Thomas Jefferson University, Philadelphia, PA.*

Background: Abnormal-galactosylated immunoglobulin (Ig)A1 play a pivotal role in pathogenesis of IgA nephropathy, and podocin is an important marker in podocyte and function in maintaining the glomercular basement membrane.We used mouse podocytes as the experimental model to investigate the effect of aggregated IgA1 (aIgA1) isolated from IgA nephropathy (IgAN) patients on podocin expression in podocytes through different pathways.

Methods: Jacalin affinity chromatography and Sephacryl S-200 molecular sieve chromatography were used to isolate IgA1 from blood of IgAN patients which was therefore became aIgA1 by heating. Podocytes were incubated with aIgA1 or special mesangial medium, which was supernatant of mesangial cells cultured with aIgA1 from IgA patients or healthy control. Podocin expression in podocytes was measured by real-time polymerase chain reaction, immunofluorescence and western blot analysis.

Results:AIgA1 from IgAN patients or healthy controls didn't show any effect on podocin expression in podocytes at mRNA and protein levels when compared with podocytes incubated with control medium (RPMI-1640 with 0.5% foetal bovine serum) ($P>0.05$). While medium from mesangial cells incubated with aIgA1 from IgAN reduced podocin expression in podocytes at mRNA and protein levels when compared with podocytes incubated with medium from mesangial cells with aIgA1 from healthy controls ($P<0.05$), and these podocytes pretreated with enalaprilat or valsartan cann't restored podocin expression to the level as control.

Conclusion: Our findings implicate that aIgA1 from IgAN patients could inhibit podocin expression by cross-talk, and renin-angiotensin system inhibitor cann't improve podocin expression, these mechanisms remain to be clarified.

Disclosure of Financial Relationships: nothing to disclose

**PUB050**

**Mesangial Medium with IgA1 from IgA Nephropathy Inhibit Podocytes Adhesive Capacity through Local Renin Angiotensin System** Cheng Wang,[1] Zengchun Ye,[1] Hui Peng,[1] Xun Liu,[1] Hui Zhang,[2] Tan-Qi Lou.[1] *[1]Department of Nephrology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China; [2]Department of Medicine, Thomas Jefferson University, Philadephia, PA.*

Background: Podocytes numbers in urine is closely related with severity of IgA nephropathy, and some of them in urine were viable, which suppose detachment of podocytes may be a major cause of podocytopenia. We used mouse podocytes to investigate the effect of aggregated IgA1 (aIgA1) isolated from IgA nephropathy (IgAN) patients on podocytes adhesive capacity .

Methods:Jacalin affinity chromatography and Sephacryl S-200 molecular sieve chromatography were used to isolate IgA1 from blood of IgAN patients which was therefore became aIgA1 by heating. Podocytes were incubated with mediumof mesangial cells co-incubated with aIgA1 which was isolated from IgAN patients, and enalaprilat(10–5 M) and valsartan(10–5 M) and separately. Adhesive capacity of podocytes was assessed by cell counting manually and hexosaminidase assay.

Results: The level of angiotensinogen and angiotensin-converting enzyme mRNAs in podocytes, as well as angiotensinII, was also increased by mesangial medium of mesangial cells co-incubated with aIgA1 from IgAN patients($P<0.05$). The special mesangial medium induced more detachment of podocytes and enhancd ILK expression when compared with podocytes incubated with medium from mesangial cells with aIgA1 from healthy controls ($P<0.05$). Enalaprilat or valsartan partly improved adhesive capacity of podocytes and lower ILK expression when compared with podocytes exposed to the special medium ($P<0.05$), while Enalaprilat plus valsartan can restore adhesive capacity and ILK expression of podocytes to the level of podocytes exposed to medium of mesangial cells stimulated by aIgA1 from healthy control ($P>0.05$).

Conclusion: Our findings implicate that local renin angiotensin system activation in podocytes involved in down-regulation of adhesive capacity by mesangial medium in IgA nephropathy, which help to understand the pathogenesis of IgA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**PUB051**

**Role of Non-Glucose Based Solution in Lipopolysaccharide (LPS)-Induced Epithelial Mesenchymal Transformation in Human Peritoneal Mesothelial Cell** Tae-Hyun Yoo, Bo Young Nam, Jwa-Kyung Kim, Jung Tak Park, Shin-Wook Kang. *Brain Korea 21 Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.*

Background: Peritoneal fibrosis is one of the most common complications of peritonitis which often leads to changes in peritoneal characteristics. Moreover, high glucose conditions are known to be associated with poor outcome in infection. Therefore, it is assumed that the glucose based peritoneal dialysis (PD) solution itself could aggravate the complications of peritonitis. Recently, non-glucose PD solutions have been proved to improve metabolic derangements and have been shown to preserve residual renal functions better than glucose based solutions. However, it is not clear whether non glucose solutions have a protective role against peritoneal fibrosis after peritonitis in CAPD patients. The aim of this study was to evaluate the effect of high glucose or non-glucose based PD solutions on lipo-polysaccharide (LPS) induced human peritoneal mesothelial cell (HPMC) epithelial-mesenchymal transformation (EMT).

Methods: HPMCs were isolated from a piece of human omentum and were exposed to 5.6 mmol/L glucose (NG), NG+1.5% and 4.25% Dianeal, or Extraneal PD solution with or without 2μg/L LPS. E-cadherin/α-SMA and fibronectin mRNA and protein were determined by real-time PCR and western blot.

Results: α-SMA and fibronectin mRNA and protein expression were significantly increased in LPS-treated group compared to NG group. E-cadherin mRNA and protein expression were significantly decreased in LPS group compared to NG group. 4.25% PD solutions were more increased in the α-SMA/E-cadherin mRNA and protein expression ratio. These high glucose associated changes were significantly lower in Extraneal+LPS group. EMT associated markers were similar between 1.5%+LPS and Extraneal+LPS. Morphological changes as EMT were seen in LPS+NG group and were more prominent in 4.25%+LPS group compared to 1.5%+LPS or Extraneal+LPS groups.

Conclusion: This study suggests that non-glucose based PD solution may be beneficial to prevent EMT in acute peritoneal inflammatory states such as CAPD peritonitis.

Disclosure of Financial Relationships: nothing to disclose

**PUB052**

**β3-Integrin Inhibit Skeletal Muscle Fibrosis during Muscle Regeneration through Suppress TGF-β Signaling Pathway** Liping Zhang,[1] Jie Du,[1] Xiaonan H. Wang,[2] William E. Mitch.[1] *[1]Internal Medicine, Baylor College of Medicine, Houston, TX; [2]Internal Medicine, Emory University, Atlanta, GA.*

CKD impairs the actions of muscle progenitors/Satellite cells, interfering with the maintenance of muscle mass. These cells control skeletal muscle regeneration through their activation, proliferation and fusion. Mechanisms leading to satellite cell dysfunctions are unknown. Satellite cells are in intimate contact with the specialized component of the extracellular matrix (ECM) composing the basement membrane of myofibers. Integrin are cell surface adhesion molecules that bind a large array of ECM ligands including collagens, fibronectin, tenascin and laminin; these cells play a central role in translating mechanical and structure cues into intracellular molecular signals that affect satellite cell behavior and function. For example, the α1 and β3 subfamily of integrins reportedly play a critical role in muscle cell differentiation and β3 could be a trigger of myogenesis since αvβ3 interacts with fibronectin, laminin and collagens I and IV. These proteins are major constituents of the ECM for skeletal muscle development. In this study, we demonstrated that satellite cells express β3 integrin, and its expression is significantly increased in injured muscles. In a mouse model of β3 integrin knockout (KO), muscle regeneration was decreased and this was accompanied by reduced expression of myogenic genes including MyoD, Myf5 and Myogenin. There also was significantly increased expression of TGF-β1 in injured muscles of β3 integrin KO mice. On these mice, there was stimulation of TGF-β1 signaling, including an increase in Smad2 and Smad3 phosphorylation in both intact and injured muscle. In isolated satellite cells, TGF-β1 treatment or β3 KO suppressed satellite cell differentiation. In an effort to identifying the mechanism for these response,we found that β3 integrin associated with a latent form of TGF-β1 by immunoprecipitation . When TGF-β1 was suppressed, there was reduced fibrosis formation and improved muscle regeneration in injured muscle of β3 integrin KO mice. These results identified a novel mechanism by which β3 integrin influence muscle regeneration.

Disclosure of Financial Relationships: nothing to disclose

**PUB053**

**Inhibitory Effect of Vascular Endothelial Growth Factor on Tubulointerstitial Fibrosis and Epithelial-Mesenchymal Transition in UUO Mice** Falei Zheng, Yaoguo Lian. *Div. of Nephrol, Peking Union Medical College Hospital, Beijing, China.*

**Aim:** Epithelial-mesenchymal transition (EMT) is an important event in renal interstitial fibrosis (RIF). This study is designed to determine the effect of vascular endothelial growth factor (VEGF) on RIF and EMT in unilateral ureteral obstruction (UUO) mice and on the expressions of TGF-β1, CTGF and BMP-7.

**Methods:** 36 male mice were divided into three groups: sham group(A) ,UUO group(B), and UUO+VEGF group(C ). The mice of group C were subjected to left ureteral ligation and received $VEGF_{121}$ (100 μg/kg/d, subcutaneously) from the first day after operation. Mice were sacrificed at day 3, 7 and 14 after operation, respectively. The histologic degree of RIF was determined by pathologic techniques. The expressions of α-smooth muscle actin (α-SMA), E-cadherin, TGF-β1, CTGF and BMP-7 in the kidneys were determined by Western blot and RT-PCR.

**Results:** Morphology changes were distinguished after UUO operation: accumulation of extracellular matrix in tubular-interstitial areas, atrophy of tubules. RIF in UUO mice was markedly distinct compared with that in sham mice, and significantly aggravated from day 3 to day 14 after operation. The degree of RIF, the expressions of α-SMA, TGF-β1, and CTGF increased significantly in UUO group, while E-cadherin and BMP-7 expressions decreased significantly in UUO group, compared with those in sham group at day 3, 7 and 14, respectively ($P$<0.05). VEGF treatment reduced RIF, expressions of α-SMA, TGF-β1 and CTGF, but increased the expressions of E-cadherin and BMP-7, compared with UUO mice at day 3 and 7, respectively ($P$<0.05). No differences in the expressions of these markers were found between the VEGF-treated and UUO mice at day 14 after operation except for CTGF expression.

**Conclusions:** VEGF administration may ameliorate RIF at the early stage in UUO mice. This effect may be probably related with inhibition of EMT by VEGF.

Disclosure of Financial Relationships: nothing to disclose

**PUB054**

**Ets-1 Is a Regulator of LOX Expression in Hyperuricemia Nephrosis** Yang Zhou, Ruoyun Tan, Junwei Yang. *Center for Kidney Disease, the 2nd Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China.*

Hyperuricemia is reported to induce renal fibrosis and progressive renal disease. Lysyl oxidase (LOX), which plays pivotal roles in extra cellular matrix (ECM) maturation, is a critical mediator of hyperuricemia induced renal sclerosis. However, the molecular events underlying LOX expression in response to uric acid treatment are poorly understood. The transcription factor Ets-1 is rapidly induced both in kidneys of hyperuricemia mice and in proximal tubule cells (NRK-52E) incubated with uric acid. As compared with control, ECM expression and deposition after uric acid treatment are significantly diminished in NRK-52E cells transfected with Ets-1 siRNA. We have identified LOX as a novel target for Ets-1. Expression of LOX is similarly reduced in NRK-52E cells transfected with Ets-1 siRNA compared with control after uric acid treatment, which results in significantly diminished deposition of fibronectin in vitro. In summary, our results support a critical role for Ets-1 as a transcriptional regulator of LOX expression in hyperuricemia renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB055**

**Fatty Acid-Binding Protein 3 in db/db Mouse Kidney: A New Insight for the Treatment of Diabetic Nephropathy** Hui-Mei Chen, Qing Gao, Wen-Wen Shen, Ming-Chao Zhang, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China.*

Previous studies from our laboratory showed that Chinese herbs were suggested to be the new drugs for diabetic nephropathy (DN), including Triptolide and Rhein. The kidney metabolizes actively lipophilic molecules, and our previous gene chip screening identified a novel DN-associated lipid-binding protein, fatty acid-binding protein 3 (FABP3). In this study, we determined the expression of FABP3 in db/db mice and the association with therapeutic effects. db/db mice with DN were administrated with Triptolide or Rhein. After 4, 8 and 12 weeks of treatment, 24-h urine albumin level, blood lipid parameters and podocyte changes were measured. FABP3 was quantitatively evaluated using immunohistochemistry and image analysis. We found that protein expression of FABP3 significantly increased in the glomeruli of db/db mice, when compared with that of db/m mice. It further increased with age in db/db mice, accompanied with albuminuria, hyperlipidemia and podocyte lesions. Immunohistochemistry revealed that FABP3 was mainly present in the podocyte cells. After either Triptolide or Rhein treatment, urinary albumin excretion was reduced and plasma levels of cholesterol, triglyceride, and low density lipoprotein cholesterol were decreased, and podocyte density and foot process were improved. Moreover, the positive expression area of FABP3 was markedly increased in glomeruli. The efficacy increased with the prolonging of the both treatment. In conclusion, the Chinese herbs, Triptolide or Rhein, may improve DN in db/db mice due to alleviated podocyte injury, while the lipid protein in plasma and FABP3 in glomeruli might mediate such process. Our results expand the understanding of the new approaches in the treatment of DN, but underlying mechanisms await further investigation.

Disclosure of Financial Relationships: nothing to disclose

**PUB056**

**Effects of Irbesartan on the Expression of Integrin-Linked Kinase and Its Relationship with Podocyte Injury in Rats with Diabetic Nephropathy** Hou-Yong Dai,[1] Rining Tang,[1] Kun Ling Ma,[1] Jie Ni,[1] Bi-Cheng Liu.[1] *[1]Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China; [2]China.*

**Objective:** Recent studies suggested that integrin-linked kinase (ILK) expression might play a role in the podocyte injury and production of albuminuria in diabetic nephropathy, which renin angiotensin system has been involved. In this study, we investigated the influence of irbesartan, an angiotensin II receptor antagonist, on ILK expression and podocyte injury in experimental DN.

**Methods:** After induction of diabetes by combination of high-sucrose, high-fat diet and intraperitoneal injection of low dose of streptozotocin (35 mg/kg) in spontaneously hypertensive rats (SHRs), rats were randomly divided into two groups: diabetic SHRs (DN, $n$=8) and diabetic SHRs treated with irbesartan (50 $mg.kg^{-1}.day^{-1}$ by gavage, DN+Irb, $n$=9) for 8 weeks. Non-diabetic normotensive Wistar-Kyoto rats (WKYs) were used as controls (controls, $n$=11). The expression of ILK was determined via immunohistochemistry, RT-PCR and western blot. The typcial changes of podocyte injury were shown by light microscopy and electronic microscopy.

**Results:** Compared to the controls, the rats in DN were associated with hyperglycaemia, hypertension, hyperlipidaemia, insulin resistance, and albuminuria, which were similar to the presentation of human T2DN. In addition, these rats were presented with expansion of mesangial matrix, loss of podocyte and podocyte injury. More importantly, both mRNA and protein expression of ILK in DN were upregulated, which could be significantly prevented by the treatment with irbesartan, along with the decrease of albuminuria, reduction of blood pressure. Furthermore, the morphological study showed irbesartan treatment could significantly prevent the podocyte injury and inhibition of mesangial matrix expansion.

**Conclusion:** Irbesartan could downregulate the overexpression of ILK in diabetic kidney, which could be related with its renoprotective effect via prevention of podocyte injury.

Disclosure of Financial Relationships: nothing to disclose

**PUB057**

**Angiotensin II Receptor Antagonist Ameliorates the Phenotypic Alterations of Podocytes in Early Diabetic Nephropathy** Hou-Yong Dai, Rining Tang, Kun Ling Ma, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China.*

**Objective**: Diabetic nephropathy (DN) as the leading cause of end-stage renal disease, the precise pathogenesis is still unclear. Emerging evidence has indicated that podocyte injury is a crucial event in the early stage of diabetic nephropathy, which angiotensin II has been involved. In this study, we firstly investigated the influence of irbesartan on the phenotypic alterations of podocyte in experimental DN model.

**Methods:** Experimental DN was induced by high-scrouse, high-fat diet (HSFD) and intraperitoneal injection of low dose of streptozotocin (35 mg/kg) in spontaneously hypertensive rats (SHR). This special model presented with hyperglycemia, hypertension, hyperlipidemia and insulin resitance. Diabetic rats were either treated with irbesartan (50mg/kg/day by gavage for 8 weeks) or vehicle. The pathological changes were investigated by light and electron microscope. Expression of nephrin and desmin were detected by real time RT-PCR and western blot.

**Results:** It was found that diabetic rats presented with a significantly loss of podocytes, effacement of foot process, thickening of the glomerular basement membrane (ECM) as well as mesangial matrix deposition. Furthermore, the expression of nephrin was significantly reduced while expression of desmin was significantly increased compared to that in control. More interestingly, it was demonstrated that irbesartan treatment not only lowered blood pressure and proteinuria, but also significantly attenuated the phenotypic changes of podocytes in this animal model.

**Conclusion**: This study provided novel data that inhibiting the early phenotypic change of podoctye might be an important mechanism of irbesartan to prevent the development of diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**PUB058**

**Treatment of Liver Abnormalities in Obesity/Diabetes** Jesus H. Dominguez,[1,2] Katherine J. Kelly.[1] *[1]Medicine/Nephrology, Indiana University School of Medicine, Indianapolis, IN; [2]Medicine/Nephrology, Roudebush VA Medical Center, Indianapolis, IN.*

Diabetic nephropathy is accompanied by cardiovascular and hepatic complications that increase morbidity and mortality. We have found abnormalities in liver function in obese/diabetic ZS rats with nephropathy. For example, serum aspartate aminotransferase was 219 ± 22 units/L in obese/diabetic Zs rats vs 113 ± 10 in lean littermates (p<0.02). This was accompanied by hepatic fibrosis in the obese group. We hypothesized that the oxidized low density lipoprotein (LDL) receptor LOX-1 is critical in these abnormalities as LOX-1 functions to internalize oxidized lipids, which are increased in diabetes and the metabolic syndrome. We further postulated that systemic blockade of LOX-1 with specific antibody would reduce hepatic toxicity. We have demonstrated renal protection with LOX-1 blockade (AJP 294:F110, 2008). We studied 4 groups of ZS rats 6 to 21 weeks of age: lean controls (L), obese/diabetic (O), obese/diabetic rats injected weekly with normal rabbit IgG (OS) or obese/diabetic rats injected weekly with anti-LOX-1 IgG (OLOX). The three obese/diabetic groups were equally obese, hyperglycemic and dyslipidemic throughout

the study. Hepatic LOX-1 expression was markedly increased in the obese/diabetic rats by 21 weeks of age and could be blocked with anti-LOX-1 treatment. Liver triglycerides (mg/20mg tissue) were 414±104 in L, 1008 ±89 in O, 1046 ± 35 in OS, and 850 ± 35 in OLOX ($p < 0.03$, OLOX vs O and OS). In the same groups, mean reduced glutathione level (umol/mg tissue) were 49 ± 5; 29 ± 4; 27 ± 2, and 41 ± 2 respectively ($p < 0.001$ OLOX vs O and OS). We conclude that early hepatic lipotoxicity in rats with diabetic nephropathy is extensive, manifested by lipid loading, abnormal liver function tests, fibrosis and decreases in glutathione levels which may have profound effects on drug metabolism. Blockade of the proinflammatory receptor, LOX1 blockade is remarkably protective.

Disclosure of Financial Relationships: nothing to disclose

**PUB059**

**Evaluation of Podocin as an Early Biomarker of Diabetic Nephropathy in db/db Mice** Anette E. Ericsson, Annett Ferm, Anne-Cristine Carlsson, Camilla Johansson, Gerhard Bottcher. *Bioscience and Safety Assessment, AstraZeneca R&D Molndal, Sweden.*

High glucose levels may injure the podocyte by altering the expression of structural proteins such as podocin. To investigate if podocin is a useful biomarker for early development of diabetic nephropathy we characterized podocin by immunohistochemistry in the kidneys of male non-diabetic db/m mice (n=10), diabetic db/db mice (n=15) and PPARγ agonist (rosiglitazone, n=15) treated db/db mice. At 21 weeks of age db/db mice exhibited marked albuminuria and hyperglycemia compared with lean db/m mice (plasma glucose 41.6±0.9 mM versus 9.4±0.3 mM). Plasma cystatin C and creatinine were significantly lower in db/db mice (308±20ng/ml and 0.23±0.01 mg/dl, respectively) as compared to lean controls (507±30 ng/ml and 0.27±0.01mg/dl) indicating renal hyperfiltration. In the group treated with rosiglitazone from 8-21 weeks of age, plasma glucose was 23.3±1.4 mM i.e. a clear anti-hyperglycemic effect. In addition, albuminuria was lower (159±29 mg/mg creatinine) as compared to the untreated db/db mice (374±41 mg/mg creatinine). Despite glucose lowering effect of rosiglitazone no effect on plasma creatinine or cystatin C was found compared to db/db controls. Podocin was a sensitive marker for structural podocyte rearrangement and glomerular mesangial expansion, but semi-quantitative analysis of podocin staining intensity did not reveal any clear differences between db/db and lean db/m mice, indicating that neither high plasma glucose levels nor albuminuria might alter podocin in this early phase of diabetic nephropathy. However, rosiglitazone treatment slightly increased podocin staining, which may suggest a role in restoring or protecting glomerular integrity, and the structural podocin pattern and glomerular morphology was similar to that of db/m controls.

In conclusion, in contrast to reported *in vitro* data, podocin *in vivo* in the kidney appeared to be unaltered by plasma glucose level and albuminuria. Therefore, altered podocin may not be amongst the earliest events in the pathogenesis of diabetic nephropathy. Interestingly, anti-hyperglycemic treatment with a PPARγ agonist seems to restore the podocin staining pattern and morphology.

Disclosure of Financial Relationships: nothing to disclose

**PUB060**

**Cannabinoid Receptor1 Antagonist Attenuates Insulin Resistance and Diabetic Nephropathy in Type 2 Diabetic Mice** Young Sun Kang,[1] Young Youl Hyun,[1] Jinjoo Cha,[1] Ji Eun Lee,[2] Hyunwook Kim,[2] Kum Hyun Han,[3] Hyoung-Kyu Kim,[1] Dae R. Cha.[1] *[1]Nephrology, Korea University, Ansan, Republic of Korea; [2]Nephrology, Wonkwang University, Gunpo, Republic of Korea; [3]Nephrology, Inje University, Goyang, Republic of Korea.*

The cannabinoid 1(CB1) receptor has an important role in the pathogenesis of obesity and insulin resistance. Recent studies suggest that the glomerular expression of CB1 receptor is increased in obese zucker rats, and associated with abnormal renal lipid metabolism. However, the exact mechanism for the effect of CB1 receptor inhibition on type 2 diabetic nephropathy is uncertain. We investigated the effect of CB1 receptor antagonist (CB1RA, SR141716: 10mg/kg/day in drinking water) on insulin resistance and diabetic nephropathy in type 2 diabetic db/db mice. Interestingly, CB1RA treatment significantly decreased food intake. Although FPG did not show significant difference, intraperitoneal insulin tolerance/oral glucose tolerance test were significantly improved. Plasma insulin levels and HOMA-IR were also improved by CB1RA treatment. In accordance with these metabolic improvements, plasma cholesterol, triglyceride and isoprostane levels were markedly decreased by CB1RA. In addition, its treatment decreased epididymal fat mass and induced the phenotypic change of adipocyte, and decreased hepatic steatosis. Simultaneously, LPO levels in adipose tissue and liver also showed significantly lower values in treated mice than that in control mice. Furthermore, CB1RA improved renal hypertrophy and decreased urinary albumin excretion by only 1 month of treatment, and showed significantly decreased serum creatinine level. Interestingly, CB1RA markedly suppressed urinary isoprostane, LPO levels, cholesterol and triglyceride contents of kidney, with an improvement of renal glomerulosclerosis in diabetic mice. From these results, we suggest that CB1RA improves insulin resistance and oxidative stress in adipose and hepatic tissues, and improves renal function through both metabolic and direct effect in the kidney, finally leading to the protective effect for the progression of diabetic nephropathy in type 2 diabetic mice.

Disclosure of Financial Relationships: nothing to disclose

**PUB061**

**Gamma Linolenic Acid Can Prevent Extracellular Matrix Accumulation Via Anti-Inflammatory Effects in Diabetic Nephropathy** Hye-Young Kang, Jwa-Kyung Kim, Jung Tak Park, Shin-Wook Kang, Tae-Hyun Yoo. *Brain Korea 21 Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.*

Recent studies suggest that inflammatory mechanism may contribute to the development and progression of diabetic nephropathy. Gamma linolenic acid (GLA), one of polyunsaturated fatty acids (PUFAs), have a positive effect on health by generating modulatory molecules for inflammatory responses. Previous studies have shown that GLA have protective properties in diabetic neuropathy, but the effect of GLA on diabetic nephropathy has been largely unexplored. This study was undertaken to investigate the effect of GLA on inflammation and ECM accumulation in experimental diabetic nephropathy. In vitro, rat mesangial cells (RMCs) and NRK-52E cells were exposed to media containing 5.6 mM glucose (NG), NG+24.4mM mannitol (NG+M), and 30mM glucose (HG) with or without GLA (10 or 100 μM). In vivo, 24 Sprague-Dawley rats were injected either with diluent (n=8, C) or streptozotocin intraperitoneally (n=16, DM), and 8 rats from diabetic group were treated with evening primrose oil (EPO) by gavage (450 mg/kg/day) for 3 months. RT-PCR and Western blot were performed for monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1) and fibronectin (FN) mRNA and protein expression, respectively, and immunohistochemical staining (IHC) for ICAM-1, and FN and Masson's trichrome staining with renal tissue was also performed. GLA significantly inhibited the increase in ICAM-1 mRNA expression and protein levels under diabetic conditions both in vitro and in vivo. MCP-1 and FN expression showed a similar pattern to the expression of ICAM-1. Twenty four hour urinary albumin excretion was significantly increased in DM rats and GLA treatment significantly reduced albuminuria ($p<0.05$). On Masson's trichrome staining, the extent of tubulointerstitial fibrosis was significantly higher in DM compared to C kidney ($p<0.005$), and this increase was significantly attenuated by EPO treatment ($p<0.01$). In conclusion, GLA prevents not only inflammation via inhibition of enhanced MCP-1 and ICAM-1 expression, but also ECM accumulation in diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**PUB062**

**Advanced Oxidation Protein Products Inhibite Insulin Secretion Via Activating of Renin-Angiotensin System in Rat Pancreatic Islet** Min Liang, Lei Yang, Fan Fan Hou. *Nephrology, Nanfang Hospital, Guangzhou, Guangzhou, Guangdong, China.*

Diabetes mellitus (DM) is a common endocrine metabolic disease, which is increasingly concerned in the medical field. Decreased insulin secretion due to islet cells injury is one of the most important reasons in the development of diabetes, however, the exact pathogenesis of dysfunction of islet remains unclear. Advanced oxidation protein products (AOPPs), which were found to be elevated in the plasma of DM, chronic kidney disease (CKD), obesity and hypertension patients, are relative with oxidative stress and inflammation. In this study, the effects of AOPPs on islet insulin secretion and its pathogenic mechanisms were evaluated. Insulin release index of rat islet cells decreased after stimulating with AOPPs modified rat serum albumin (AOPPs-RSA). Upreglulation of expression of angiotensin receptor 1 (AT1R) mRNA and protein, angiotensingen (AGT) mRNA and angiotensin converting enzyme (ACE) mRNA, and increase of angiotension II (Ang II) concentration of islet cells were observed after AOPPs-RSA stimulation. AOPPs-RSA also induced increase of intracellular reactive oxygen species (ROS) production detected by a flow cytometry of fluorescence intensity of 2',7'-dichlorodrofluorescein diacetate. Pre-incubation of islet with cytoplasmic super oxide dismutase (C-SOD) and the NAD(P)H oxidase inhibitors apocynin inhibited upexpression of AT1R, AGT, ACE and Ang II. Renin–angiotensin system inhibitor (RASI), lisinopril and telmisartan, and C-SOD and apocynin can improve insulin secretion dysfunction of islet induced by AOPPs-RSA. These results documented that AOPPs decrease rat pancreatic islet cells insulin release through activating local RAS mediated by oxidative stress.

Disclosure of Financial Relationships: nothing to disclose

**PUB063**

**Dysregulation of Apoptosis and Angiogenic Growth Factors in a Podocyte Model of Diabetes and High Protein Diet** Rick L. Meek,[1] Robert J. Anderberg,[1] Sheryl K. Cooney,[1] Katherine R. Tuttle.[1,2] *[1]Research Department, Providence Medical Research Center, Sacred Heart Medical Center, Spokane, WA; [2]Division of Nephrology, Department of Medicine, University of Washington School of Medicine, Spokane and Seattle, WA.*

Advanced glycation end products (AGE) are pro-inflammatory metabolites that incite cellular injury leading to diabetic complications. High dietary protein, a rich source of AGE and their precursors, can induce or exacerbate albuminuria. In response to injury, glomerular podocytes decrease in number and reduce production of angiogenic growth factors, both mechanisms for albuminuria. The aim of this study was to determine if conditions modeling diabetes and high protein diet lead to production of AGE as well as podocyte apoptosis and reduction in angiogenic growth factor expression. Conditionally immortalized mouse podocytes were exposed for up to 2 weeks to an amino acid mixture designed to mimic high protein diet (AA), high glucose (30mM, HG), the combination (AA/HG), or AGE-bovine serum albumin (AGE-BSA). Podocyte phenotype was confirmed by Wilms Tumor antigen-1 and synaptopodin mRNA (RT-PCR) and protein (immunocytochemistry)

expression. Carboxymethyllysine (CML), a prominent AGE, increased in conditioned media from AA, HG, AA/HG, and AGE-BSA conditions. Podocyte number decreased by 2 weeks of culture in the experimental conditions together with increased indicators of apoptosis including: TUNEL staining, caspase 3/7 activity, and caspase 4 mRNA (gene expression array confirmed by RT-PCR). Podocyte expression (RT-PCR) of connective tissue growth factor (CTGF) mRNA declined in AA, AA/HG, and AGE-BSA conditions, while vascular endothelial growth factor-A (VEGF-A) mRNA decreased in AA/HG and AGE-BSA conditions. Conclusions: Conditions characteristic of diabetes and high dietary protein lead to increased AGE exposure along with podocyte apoptosis and reduced angiogenic growth factor expression.

Disclosure of Financial Relationships: nothing to disclose

**PUB064**

**Differential Expression of Cyclin-Dependent Kinase Inhibitors (CKIs) and Apoptosis-Related Molecules in Diabetic Kidney Disease** Bo Young Nam, Hye-Young Kang, Tae-Hyun Yoo, Shin-Wook Kang. *Brain Korea 21 Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.*

Kidney size is increased in diabetes primarily due to glomerular and tubular hypertrophy, principally mediated by CKIs. Additionally, CKIs also play an important part in the development of apoptosis, which has been considered one of the underlying causes of mesangial cell and podocyte loss in diabetic nephropathy (DN). We have previously shown that the nephrin expression was different between relatively small and large glomeruli isolated from early diabetic rats. Moreover, prior studies have suggested that glomerular hypertrophy in diabetes does not develop in all glomeruli concurrently. Based on these findings, it is supposed that apoptosis may also occur differentially in diabetic glomeruli. In this study, we hypothesized that more hypertrophied glomeruli rather than less hypertrophied glomeruli may undergo apoptosis. Rats were injected either with diluent (n=15, C) or STZ IP (n=15, DM). Glomeruli were isolated using sieves with pore sizes of 250, 150, 125, and 75μm after 3 months, and classified into large glomeruli (on 125μm sieve, LG) and small glomeruli (on 75μm sieve, SG) groups. The ratio of Bax/Bcl-2 protein expression and active caspase-3 protein expression were significantly increased in DM-LG compared to DM-SG and C-SG ($p<0.01$). In contrast, Akt activity was significantly increased in DM-SG, while it was significantly decreased in DM-LG ($p<0.05$). p27 and p21 expression were significantly increased in DM-SG compared to DM-LG and C-SG ($p<0.05$), but there was no difference in p27 and p21 expression between DM-LG and C-SG. The numbers of total glomerular cells in DM-SG and C-LG were significantly higher than that in C-SG ($p<0.05$). In contrast, there were significantly less total glomerular cells in DM-LG compared to the other groups ($p<0.05$). Apoptosis-related molecules and CKIs were differentially expressed according to glomerular size in DN, suggesting that hypertrophic process is ongoing only in less hypertrophied glomeruli and once they become large enough, the hypertrophic mechanism seems to be replaced by apoptosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB065**

**Simvastatin Ameliorates High Glucose Induced Glomerular Endothelial Cells Hyperpermeability and Tight Junction Disruption by Inhibiting RhoA Activation** Hui Peng,[1] Pengli Luo,[1] Zengchun Ye,[1] Yan-Ru Chen,[1] Cheng Wang,[1] Canming Li,[1] Farhad R. Danesh,[2] Tan-Qi Lou.[1] *[1]Department of Nephrology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, GD, China; [2]Department of Medicine, Baylor College of Medicine, Houston, TX.*

High glucose has been showed to increase endothelial cells monolayer permeability in diabetes mellitus. We hypothesis high glucose may induce endothelial cells hyperpermeability by disrupting tight junctions, and statin could inhibit this effect through inhibiting RhoA activity. For this purpose, rat glomerular endothelial cells (rGEnCs) were cultured for 12h, 24h, and 48h in control medium (5mM D-glucose) or high glucose medium (30mM D-glucose). Mannitol was used as high osmotic control. The permeability of rGEnCs was evaluated by trans-endothelial electrical resistance (TEER). TEER was significantly decreased in high glucose compared with control or mannitol group from 2h. It reached the lowest level at 24h ($P<0.01$). Tight junction proteins was evaluated by Western blot. It showed that occludin was significantly decreased while claudin-5 increased after exposed to high glucose for 24h and 48h. However, ZO-1 and JAM-A did not change in high glucose. Pre-incubated with simvastatin(2.5μM) could partly reverse the hyperpermeability effect of high glucose($P<0.05$) as well as the change of proteins. But when cells co-incubated with simvastatin and mevalonate, simvastatin did not reverse the effect of high glucose. Pull-down assay indicated RhoA was significantly activated in high glucose for 24h compared with control group. Simvastatin could inhibit high glucose induced RhoA activation, while mevalonate reversed this effect of simvastatin. Taken together, these data suggest that high glucose may induce glomerular endothelial hyperpermeabilty by injuring tight junction integrity, and simvastatin could reverse this effect to protect endothelial permeability. Protection effect of simvastatin may be partly mediated through inhibition of mevalonate and subsequent inhibiting RhoA activated. The result implied a new mechanism of hyperglycemia damages glomerulis and a new protection mechanism of statins in diabetic mellitus.

Disclosure of Financial Relationships: nothing to disclose

**PUB066**

**The Effect and Mechanism of Norcantharidin on Tubulointerstitial Fibrosis of Diabetic Nephropathy** Youming Peng, Qiong Chen, Ying Li, Guanghui Ling, Fu-You Liu. *Nephrology, Second Xiangya Hospital, Center of Kidney Disease and Dialysis in Hunan Province, Central South University, Changsha, Hunan, China.*

Our studies previously found that NCTD inhibited the tubulointerstitial lesions in protein-overload nephropathy and NCTD could attenuate the tubulointerstitial fibrosis and inhibit tubular cell EMT (AJKD). However if NCTD could inhibits tubulointerstitial fibrosis in diabetic nephropathy (DN) is unknown. To investigate the effect of NCTD in tubulointersitial firosis of DN and its related mechanism, the type I of DN model was established by STZ injection and treated by NCTD for 8 weeks. Results shown that the ECM deposition and tubulointerstitial fibrosis and increased CaN expression were observed in diabetic kidney, while the pathological changes were inhibited in treated by NCTD ( n=20, $P<0.05$).,which was consistent with the decreased in the mRNA and protein expressions of FN, collagen IV and TGF-β1 and CaN ($P<0.05$). In vitro study, the mRNA and protein expression of FN, collagen IV, TGF-β1 and CaN increased in HK-2 cells exposed to with 30mM D-glucose in a time-depedent manner, but reduced in treated by 5μg/ml of NCTD ($P<0.05$). To verify the mechanisms of which factors involved this process, the CaN and NFATc expression was determined. HK-2 cells treated by NCTD (5μg/ml) a exhibit decreased in NFATc nuclear translocation induced by 30 mM D-glucose was seen compared to control($p<0.01$),Moreover, CaN-siRNA increased the protein and mRNA level of FN , collagen IV and TGF-β1 induced by HG, whereas significantly decreased in that of treated by NCTD ($P<0.05$). These data indicated that NCTD may have a positive effect in attenuating tubulointerstitial fibrogenesis in the early stage of DN rats. However it is not mediated through down-regulating CaN/NFATc pathway.

Disclosure of Financial Relationships: nothing to disclose

**PUB067**

**Sucrose Induces Fatty Liver and Type II Diabetes in Rats Independent of Energy Intake** Carlos Alberto Roncal-Jimenez,[1] Miguel A. Lanaspa,[1] Christopher J. Rivard,[1] Takahiko Nakagawa,[1,2] L. Gabriela Sanchez-Lozada,[1,3] Diana I. Jalal,[1] Ana Andres-Hernando,[1] Katsuyuki Tanabe,[1] Magdalena Madero,[3] Yuri Y. Sautin,[2] Richard J. Johnson.[1] *[1]Medicine-Division of Renal Diseases and Hypertension, University of Colorado, Aurora, CO; [2]Medicine-Division of Nephrology Hypertension, University of Florida, Gainesville, FL; [3]Medicina, Instituto Nacional de Cardiologia Ignacio Chavez, Mexico City, Mexico.*

Recent studies have implicated a role for fructose-containing sugars, such as sucrose and high fructose corn syrup, in the epidemic of obesity and diabetes. However, experimental studies are often criticized for using only purified fructose, often at clinically irrelevant concentrations, or for failing to control for energy intake. We therefore tested the hypothesis that a diet of 40% sucrose might induce metabolic abnormalities independent of energy intake. Rats were pair fed 40% sucrose or 40% starch for 4 months. Sucrose fed rats developed features of metabolic syndrome, with postprandial elevations in blood pressure, hypertriglyceridemia, and insulin resistance. Sucrose fed rats developed fatty liver with evidence for increased fat synthesis and decreased fat oxidation. Sucrose fed rats also developed variable hyalinization of pancreatic islets with low grade inflammation and a reduction in insulin levels and the development of frank type II diabetes. Finally, sucrose feeding was associated with increased expression of the fructose transporter, Glut5, in the jejunum and with increased expression of fructokinase in the jejunum and liver. In conclusion, sucrose, at concentrations ingested by a subset of Americans, can induce metabolic syndrome, fatty liver and type 2 diabetes, and this is independent energy intake. These data support a role for fructose-containing sugars in the epidemic of obesity, fatty liver, and metabolic syndrome.

Disclosure of Financial Relationships: nothing to disclose

**PUB068**

**Adrenocorticotropic Hormone Attenuates Diabetic Nephropathy in Zucker Diabetic Fatty Rats Via Inhibition of Oxidative and Inflammatory Responses** Haiping Wang, Zhanjun Jia, Gang Liu, Tianxin Yang. *Internal Medicine, University of Utah, Salt Lake City, UT.*

Clinical evidence suggests a potent anti-proteinuric effect of adrenocorticotropic hormone (ACTH) in patients with various chronic kidney diseases including diabetic nephropathy. The objective of this study was to evaluate the therapeutic potential and safety profile of a natural source $ACTH_{1-39}$ peptide formulation, Acthar Gel (Acthar, Questcor Pharmaceuticals, Inc.) in Zucker diabetic fatty (ZDF) rats. Three cohorts (n=8-9 per group) of ZDF rats were created: 1 vehicle-treated and 2 Acthar-treated (5 or 20 units/kg IM, 3 times/ week, for 3 months). Vehicle-treated lean rats (n=4) served as controls. Physiologic and morphological measures were performed immediately after the 3 month Acthar treatment. There was a trend of reduced urine albumin and total urine protein for both doses of Acthar ($p=0.06$-$0.08$); the effect reached statistical significance ($p<0.05$) when the Acthar data were pooled. Histological analysis revealed evidence of renal fibrosis, protein casts, and infiltration of inflammatory cells in the diabetic kidney, which were significantly attenuated with either dose of Acthar ($p<0.05$). Compared to lean rats, there was a dose-dependent effect on renal concentration of thiobarbituric acid-reactive substances (TBARs) and suppression of NOX-1, TNF-α, and TGF-β renal mRNA level, with complete normalization with the high dose of Acthar. Both Acthar doses significantly reduced kidney hypertrophy ($p<0.05$). Acthar resulted in reduction of hypertrophy in other organs (including liver, heart, spleen),

as well as plasma triglyceride and plasma free fatty acid levels at the trend level. Acthar did not affect food and water intake nor urine volume. Plasma glucose and K+ also remained stable in Acthar treated rats. Together, these data suggest that Acthar protects against diabetic nephropathy via inhibition of oxidative and inflammatory responses and also provides an additional beneficial effect on the metabolic profile.

Disclosure of Financial Relationships: nothing to disclose

**PUB069**

**Sulodexide Reduces Proteinuria and Abnormal Matrix Protein Accumulation in C57BL/6 Mice with Streptozotocin-Induced Diabetes** Susan Yung, Mel Chau, Qing Zhang, Owen Chan, Daniel Tak Mao Chan. *Department of Medicine, University of Hong Kong, Hong Kong.*

Diabetic nephropathy (DN) manifests with proteinuria and histopathologic abnormalities characterized by abnormal matrix deposition. Sulodexide is a mixture of purified glycosaminoglycans. It has been reported to reduce proteinuria in patients with diabetic nephropathy, but the mechanism of action remains unclear. We investigated the effect of sulodexide on proteinuria and renal histology in an experimental model of type I diabetic nephropathy.

C57BL/6 mice were rendered diabetic by 5 consecutive intraperitoneal injections of streptozotocin (50mg/kg). Blood glucose levels and proteinuria were monitored weekly. Once proteinuria (>100mg/dl) was established, mice were randomized to receive either saline or sulodexide (1mg/kg/day) treatment by oral gavage for selective time periods up to 12 week (n=6 for each time point), after which time mice were sacrificed, and urine, blood and kidney samples collected for analysis.

Sulodexide had no effect on blood glucose level, but significantly decreased urine albumin-to-creatinine ratio after 12 weeks. (8.96±4.10 vs 105.30±51.47g/μmol, with vs without sulodexide treatment, $P<0.001$). Diabetic mice developed glomerular hypertrophy, thickening of the GBM and Bowman's capsule, PKC-α activation, increased glomerular expression of TGF-β1, fibronectin, collagen type I, and decreased perlecan expression. Sulodexide treatment resulted in less glomerular hypertrophy, a progressive reduction in glomerular expression of matrix and fibrous tissue components or mediators with increasing treatment duration, and restoration of perlecan expression.

Our data demonstrate that sulodexide treatment improves phenotypic and histopathologic manifestations of nephropathy in diabetic mice, which could be mediated in part through increased renal perlecan expression.

Disclosure of Financial Relationships: nothing to disclose

**PUB070**

**Measuring Carnosinase Activity in Diabetic Nephropathy** Ana Zutinic, Antien Mooyaart, Jan A. Bruijn, Emile De Heer, Hans J. Baelde. *Department of Pathology, Leiden University Medical Centre, Leiden, Netherlands.*

A relationship has been shown between carnosinase activity and the amount of leucine repeats in exon 2 of the *CNDP1* gene. A relationship between higher amounts of leucine repeats in *CNDP1* and increased risk of developing diabetic nephropathy has also been shown. In our study we investigated the optimal assay method for determining carnosinase activity in serum and heparin plasma, with the aim of accurately investigating the direct relationship of carnosinase activity with diabetic nephropathy.

The carnosinase assay method was adapted from Schoen *et al*. and Verena Peters. Samples were diluted in reaction buffer containing carnosine and the histidine release was quantified. In order to determine optimal incubation time, multiple carnosinase assays were carried out at: 0, 10, 20, 30 and 40 minutes of healthy serum incubation with carnosine at 37°C. Additionally, to test the optimal serum or heparin plasma concentration, eight different concentrations were tested ranging from sample:buffer ratio of 2:1 to 1:12. A serum:buffer ratio of 4:1 was used in previous studies. To compare carnosinase activity in heparin plasma and serum, both kinds of samples from healthy volunteers were tested for carnosinase activity.

In both serum and heparin plasma, carnosinase activity was linear from 0 to 10 minutes of sample incubation with reaction buffer. The optimal concentration to determine carnosinase activity accurately was a 1:1 ratio of sample:buffer . This means less material can be used for this assay than used in previous studies. The intra-assay variation was 6% and inter-assay variation was 16%. Carnosinase activity in serum and heparin plasma was comparable ($R^2$=0.9112). This means that both serum and heparin plasma can be used to accurately determine carnosinase activity of a subject. The described optimalisation of the carnosinase assay can lead to clarifying the relation between carnosinase activity and diabetic nephropathy due to being able to accurately measure carnosinase activity.

Disclosure of Financial Relationships: nothing to disclose

**PUB071**

**A Method To Control the Rate of Administration of Venofer®** Mary M. Showers,[1] Jose A. Diaz-Buxo.[2] *[1]Renal Solutions Inc.; [2]Fresenius Medical Care-NA.*

**Background** Administration of Venofer® (iron sucrose) is an effective treatment for iron deficiency anemia.

**Method** We conducted usability testing of a novel Venofer Pump Module (VP), developed by Renal Solutions, Inc. in a simulated chronic hemodialysis use environment The module was used with the Fresenius 2008 T dialysis machine. Following visual prompts on the screen of the Venofer Pump Module, tubing made specifically for the Venofer Pump was snapped into place, threaded past the fluid detector through the pump, and leur locked to the port of the venous chamber of the dialysis blood tubing. A 100mg vial of Venofer® was inverted and attached to the tubing by pushing the spike through the rubber stopper of the vial. The Venofer Pump Module is capable of delivering 100mg, 75mg, or 50mg set doses of Venofer® at a rate of 34mg/min. Users were trained in the procedure and then asked to give 3 set doses of the Venofer®.

**Results** In-vitro testing completed with 34 end users, including 18 registered nurses, showed successful delivery of 100mg. 75mg. and 50mg. doses of Venofer® at 34mg/minute via the VP. Over 90% of the tasks associated with Venofer® delivery were successfully completed by the registered nurses and patient care technicians. 100% of users were able to self-correct any error that could result in an unacceptable risk, according to the VP Risk Analysis.

**Conclusion :**. The study showed adequate automated in-vitro delivery of Venofer® by all users. The VP may diminish human factors from the delivery of Venofer®

*Venofer Pump Module is pending FDA approval.

Disclosure of Financial Relationships: nothing to disclose

**PUB072**

**"Unifying Vasculogenic Hypothesis" for Kidney and Solid Organ Regeneration** Leon G. Fine. *Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA.*

All examples of chronic solid organ fibrosis with loss of function show progressive loss of the interstitial microvasculature. In the kidney the initial insult to the organ usually leads to local areas of hypoxia. Hypoxia, per se, is a profibrogenic stimulus which leads to scar formation. Scars, in turn, obliterate microvessels, leading to a downward spiral of decreased function. Substantial evidence has accrued to show that the "Chronic Hypoxia Hypothesis" has been substantiated for chronic renal diseases (Fine LG and Norman JT, *Kidney International, 74:867-872, 2008*).

Attempts to regenerate chronically-diseased solid organs, by the transfer of progenitor cells which differentiate into the parenchymal phenotypes of the organ, are likely to fail in the long-term, since these cells will not survive the hypoxic environment. Resident progenitor cells suffer the same fate. Furthermore, even if transferred parenchymal cells were to survive, they would be unlikely to be able to recreate the complicated internal architecture of an organ (e.g. new nephrons, each containing up to 30 different phenotypes, cannot be formed in the adult kidney).

The Unifying Vasculogenic Hypothesis for Solid Organ Regeneration states: "Regeneration of solid organs after chronic injury and scarring, can be achieved solely by restoring the microvasculature. This can be achieved by adoptively transferring endothelial progenitor cells into the organ. The ensuing improvement in microvasculature, with relief of hypoxia, will stimulate resident progenitor cells to reconstitute the vascular network of the organ. Resident parenchymal progenitor cells and differentiated cells will consequently be restored to function, which will regenerate the parenchyma of the organ, including partial remodelling of scarred areas, within its existing architectural structure."

In summary, to restore solid organ function after chronic injury, it is hypothesized that all that is required to initiate the process, is a restored microvascular circulation. There should be no need to generate and transfer stem/progenitor cells of the specialized phenotype of each organ. This would apply to organs such as kidney, liver, lungs and heart. This hypothesis should be tested.

Disclosure of Financial Relationships: nothing to disclose

**PUB073**

**A Markov Model Study on the Hierarchical Prognosis and Risk Factors in Patients with Chronic Kidney Disease** Xun Liu, Cheng Wang, Chenggang Shi, Cailian Cheng, Hua Tang, Zhujiang Chen, Tan-Qi Lou. *Division of Nephrology, Department of Internal Medicine, The Third Affiliated Hospital of Sun Yet-Sun University, Guangzhou, Guangdong, China.*

Background: Chronic kidney disease (CKD) has been identified as a public health problem. The hierarchical prognosis and risk factors in patients with CKD is, however, less well studied. Method: 272 outpatient CKD patients were investigated. Mean serum creatinine level of enrolled patients was 1.33 mg/dL (range 0.34-6.62). Mean GFR was 87.0 ml•min-1•(1.73m2)-1 (range 6.8-342.0). Markov model including six states [CKD1 stage, CKD2 stage, CKD3 stage, CKD4 stage, CKD5 stage as well as death/ end-stage renal disease (ESRD) stage] was established. Results: The mean following period was 2.0 years. 16 (5.9%) patients were lost at follow-up. 14 patients reached composite endpoint (death/ ESRD stage). The causes of death included severe pneumonia (N =2) and septic shock (N =1). Transition rate from CKD1 stage to CKD2 stage, from CKD2 stage to CKD3 stage, from CKD3 stage to CKD4 stage, from CKD4 stage to CKD5 stage and from CKD5 stage to death/ESRD stage were 9.2%/year, 10.9%/year, 13.2%/year, 16.1%/year and 47.1%/year respectively. The mean duration in CKD1 stage, CKD2 stage, CKD3 stage, CKD4 stage and CKD5 stage were 8.4 years, 5.8 years, 4.0 years, 1.8 years and 0.8 years respectively. Mean renal survival time or dialysis free period was 20.8 years. Multivariates analysis of Markov model showed the impact factors of prognosis. Old age, high diastolic blood pressure and hyperuricemia were the independent prognostic factors for the transition from CKD1 stage to CKD2 stage. Smoking and low hemoglobulin level were the independent prognostic factors of the transition from CKD2 stage to CKD3 stage. History of cardio-cerebral-vascular disease and low hemoglobulin level were the independent prognostic factors of the transition from CKD3 stage to CKD4 stage. RAS blockade therapy was the independent prognostic factors of the transition from CKD4 stage to CKD5 stage. Conclusions: Evaluation of severity and the treatment of CKD patients should be made according to the prognosis and influencing factors of different stage in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB074**

**Computational Systems Biology in the European Union Research Project on Chronic Kidney Disease – SysKid** Paul Perco,[1,2] Irmgard Muehlberger,[1] Raul Fechete,[1] Gil Stelzer,[3] Doron Lancet,[3] Gert J. Mayer,[4] Rainer Oberbauer,[2,5] Bernd Mayer.[1] *[1]Emergentec Biodevelopment GmbH; [2]Medical University of Vienna; [3]Weizmann Institute of Science; [4]Medical University of Innsbruck; [5]KH Elisabethinen Linz.*

We present a computational Systems Biology framework for integrating clinical data, Omics data, as well as results from functional studies aimed at characterizing molecular processes associated with diabetic nephropathy (DN).

SysKid (Systems Biology towards novel chronic kidney disease diagnostics and treatment) is a large-scale integrating research project funded by the European Union within the 7th framework programme. The consortium encompasses clinical, molecular and epidemiological research forming the basis for integrative approaches as epitomizing Systems Biology.

Major aims of SysKid are defined as (i) identifying of persons at risk of developing chronic kidney disease (CKD), (ii) deciphering of molecular processes leading to CKD and the identification of associated biomarkers, and based on these results (iii) developing of novel diagnostic and therapeutic strategies for prevention or control of disease progression. To accomplish these aims, broad Omics screening covering transcriptomics, proteomics, metabolomics, GWAS, and microRNAs, utilizing clinically well-defined samples from patients with DN along with functional experiments in cell culture and animal models is driven. For enabling an integrated analysis of such heterogeneous data sets, a dedicated Systems Biology pipeline has been implemented.

Sample specifications, study plan documents, Omics data files, as well as analysis reports from functional studies are managed in an object-centered record management system combined with a molecular feature database holding full human genome/proteome annotation. Explicit as well as automated inference is implemented for allowing persistent linkage of clinical data categories and molecular pathways ascribable to the pathophysiology of DN.

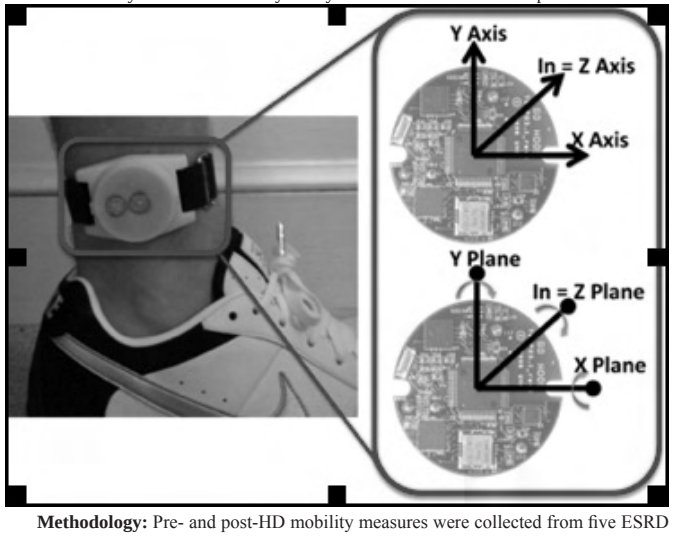
As implementation of the individual Omics regimes for significant sample numbers has become feasible concepts for data management, annotation and integration are needed, as exemplified by SysKid for chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**PUB075**

**Identifing Mechanisms of Falls in ESRD Patients on HD Using Non-Invasive Portable Device** Emaad M. Abdel-Rahman,[1] John Lach,[2] Rasheed A. Balogun.[1] *[1]Nephrology, UVA, Charlottesville, VA; [2]Electrical and Computer Engineering, UVA, Charlottesville, VA.*

**Introduction:** Falls are highly prevalent in patients with ESRD on HD and are associated with poor outcomes. To prevent falls, mobility mechanisms contributing to falls must be understood. The TEMPO (Technology-Enabled Medical Precision Observation) is a wireless body sensor network platform that provides precise human motion and orientation data continuously and non-invasively in any location over an extended period of time.




**Methodology:** Pre- and post-HD mobility measures were collected from five ESRD patients on HD over the course of 5 months. To assess the effects of post-HD-fatigue on fall risk , participants were asked to perform the same 4 tasks both before and after their HD treatment: 2 minutes of walking, 3 Get-Up & Go tests, 3 Postural Locomotion and Manual (PLM) control tests, and 3 tests of foot strength using a custom made measurement device. Additionally, the effects of inter-HD periods (two days vs. three days) were investigated. **Results:** While HD treatment influenced strength and mobility (i.e., weaker and slower after the dialysis) , there was no significant differences in dynamic stability between the pre- and post-HD sessions. Furthermore, mobility was better after the three-day interdialytic period than the two-day interdialytic period. **Conclusion:** Problem of fall-risk in HD patients is unique, with post-HD fatigue playing a role in falls in this population increasing the susceptibility to falls while returning home. Furthermore, the inter-dialytic period influenced pre-HD profiles increasing the susceptibility to falls before they come in for a HD treatment with the two-day interdialytic period suggesting that the residual effects of post-HD fatigue was still evident. Further study is planned with more participants and with longer data collections.

Disclosure of Financial Relationships: nothing to disclose

**PUB076**

**Bioengineering of Bioactive, Hierarchical Kidney Membranes** Patricia Y. W. Dankers. *Institute for Complex Molecular Systems, and Laboratory of Chemical Biology, Eindhoven University of Technology, Eindhoven, Netherlands.*

Novel strategies to treat patients with kidney failure include the use of a bioartificial kidney set-up applied in conjunct with a conventional dialysis system. This bioartificial kidney should be composed of renal cells maintained on a membrane. The cells are proposed to additionally clear the blood from waste products. However, conventional membranes do not provide the organotypical environment the cells need, to be phenotypically preserved, and to properly function.

Here, we disclose the development of novel bioactive, supramolecular membranes that are proposed to be eminently suitable for this purpose. Especially, because they can be prepared as two-layered constructs with epithelial cells at one side, and endothelial cells or a non-fouling surface at the other side.

We developed these supramolecular membranes in order to mimic the natural basement membrane and its underlying extracellular matrix in a hierarchical fashion. The membranes are composed of supramolecular polymers based on hydrogen bonding moieties coupled to short oligomers. They self-assemble into nanofibers that can be processed into microfibers, resulting in hierarchical fibrous membranes. Bioactivity was introduced into these nanofibers by intercalation of bioactives modified with similar hydrogen bonding units. In addition, we developed new chemical strategies to post-modify these nanofibers with the bioactives.

Furthermore, our supramolecular strategy allows us to easily incorporate epithelial-specific bioactives at one side, and endothelial-specific bioactives or non-fouling agents at the other side. In this way we envision specific interactions of either epithelial or endothelial cells with the membranes. The non-fouling surface would be imperative in preventing unwanted interactions of blood constituents with the membrane, when no endothelial cells are applied at the blood side. We were able to prepare these different membranes, and we showed increase in function of renal epithelial cells, and adhesion of endothelial cells. Currently, we are studying the performance of different cell types on the novel membranes in a newly developed culture system in which we can monitor cell function.

Disclosure of Financial Relationships: nothing to disclose

**PUB077**

**Pediatric Urolithiasis and Obesity: Are They Related?** Uri S. Alon,[1] Bryn C. Dekosky,[1] Ashley K. Sherman.[2] *[1]Pediatric Nephrology, Children's Mercy Hospital and Clinics, University of Missori at Kansas City School of Medicine, Kansas City, MO; [2]Medical Research, Children's Mercy Hospital and Clinics, University of Missori at Kansas City School of Medicine, Kansas City, MO.*

*Introduction:* There is evidence that in adults, obesity promotes stone formation. There is also evidence of increased incidence of urolithiasis in children concomitantly with increasing incidence of obesity. It is unclear if the latter two are related by cause and effect.

*Objective:* The aim of the study was to examine if there is an association between the incidence of kidney stones and that of obesity in children.

*Methods:* We retrospectively reviewed the data on children presented to the Renal Clinic due to urolithiasis between 5/12/2004 to 5/18/2009. Data collected included patients' gender, age and BMI, and when available stone chemistry analysis. Data were compared with three recent local and national studies on the incidence of pediatric obesity.

*Results:* Total number of patients of 139 which included 74 females with mean age of 11.3 and 65 males with mean age of 10.6 years (NS).

table 1. Obesity incidence in children with urolithiasis

| Study | | | Controls | | |
|---|---|---|---|---|---|
| | Percent | CI | | Percent | CI |
| BMI>85 | | | BMI>85 Locally | | |
| Both Sexes | 30.22 | 22.58 - 37.85 | | | |
| Female (n=74) | 27.03 | 16.91 - 37.15 | Female | 38 | No data |
| Male (n=65) | 33.85 | 22.34 - 45.35 | Male | 40 | No data |
| BMI>95 | | | BMI>95 Locally | | |
| Both sexes | 16.55 | 10.37 - 22.72 | Both Sexes | 17 | No data |
| BMI>95 | | | BMI>95 Nationally | | |
| Both Sexes | 17.99 | 11.60 - 24.37 | Both Sexes | 16.9 | 14.1 - 19.6 |
| Female (n=74) | 10.81 | 3.73 - 17.89 | Female | 15.9 | 12.6 - 19.1 |
| Male (n=65) | 26.16 | 15.47 - 36.84 | Male | 17.8 | 14.7 - 20.8 |

Of the 38 stones analyzed, the composition was: calcium-based 34, uric acid 2, Struvite 1 and cystine 1.

*Conclusions:* In our population, children afflicted by urolithiasis show the same trends towards obesity as the general population; thus it seems that obesity *per se* does not promote pediatric urolithiasis. This may be explained by the fact that in our patients the vast majority of stones continue to be calcium-based which are not known to be caused by obesity.

Disclosure of Financial Relationships: nothing to disclose

**PUB078**

**Calcium-Phosphorus Product and Blood Pressure in CKD** Ziad Maurice Ashkar. *Acadiana Renal Physicians, Lafayette, LA.*

Hypertension is very common in chronic kidney disease(CKD) with prevalence increasing with severity of renal failure. Disorders of mineral metabolism and increased calcium-phosphorus product have been associated with increased mean arterial pressures (MAP) in dialysis patients. There are scarce data looking into the association of calcium-phosphorus product with blood pressure in CKD.

85 patients with CKD stages 2,3 and 4 were included in a cross-sectional study over a two-year period. Linear regression analysis was done between the averages of calcium-phosphorus product(caxph) and blood pressures. There was no association detected between MAP and caxph (p=0.223) or between pulse pressures(PP) and caxph(p=0.924).

Multi-linear regression analysis was then done between caxph and blood pressures adjusting for age,sex,race, erythropoesis stimulating agents (ESA) use, diabetic status(Dm),PTH levels and average blood pressure meds per patient.

No association was found between blood pressures and caxph.

MAP was positively associated with PTH level(p=0.015,R squared=0.249), and male sex(p=0.01). PP was significantly associated with age(p<0.0001) and Dm(p=0.01).

Multilinear regression between MAP and caxph

| | p value | coefficient |
|---|---|---|
| caxph | 0.319 | -0.22 |
| PTH | 0.015* | 0.059 |
| age | 0.2 | -.0115 |
| male sex | 0.01* | 5.83 |
| race | 0.179 | -3.07 |
| Dm | 0.633 | -1.09 |
| BPmeds | 0.664 | 0.368 |
| ESA | 0.707 | 0.98 |

*statistically significant

The study findings suggest no association between calcium-phosphorus product and blood pressure in CKD.

The significant association of MAP with PTH in CKD suggest an early role of PTH in hemodynamic vascular effects, before caxph and phosphorus roles are manifested in end stage renal disease.

Disclosure of Financial Relationships: nothing to disclose

**PUB079**

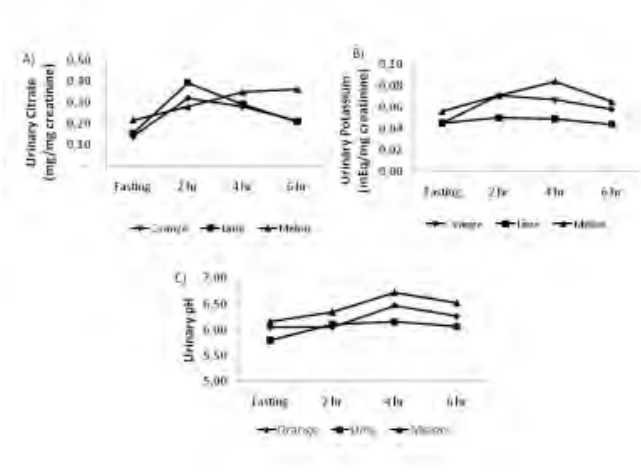
**Acute Effects of Fruits upon Citrate and Potassium Excretion in Hypocitraturic Stone Formers** Leandro Cunha Baia, Alessandra Calábria Baxmann, Ita Pfeferman Heilberg. *Nephrology Division, Federal University of São Paulo, São Paulo, Brazil.*

Numerous studies have reported increases in 24-hour urinary citrate excretion induced by orange or lemon juices, but it has been observed that because citrate in orange is complexed mainly by potassium, the resultant alkali-load provides better citraturic effects. The aim of the present study was to compare the acute effects of dietary potassium supply alone, from non-citrus sources (melon), with the effect provided by citric (orange, lime) natural juices. METHODS: Hypocitraturic stone-forming patients collected two-hours urine samples after an overnight fast (baseline), 2, 4 and 6 hours after consumption of either a fresh melon juice (n=10, 3M/7F, 48±13yrs), freshly squeezed orange juice (n=10, 1M/9F, 48±11yrs) or freshly squeezed lime juice (n=10, 4M/6F, 49±12 yrs), for determination of urinary parameters. Results are shown in the table and figure below:

Dietary contents and urinary parameters

| | | Melon | Orange | Lime |
|---|---|---|---|---|
| Juice Citrate Content (mEq) | | - | 50 | 50 |
| Juice Potassium Content (mEq) | | 19.7 | 19.7 | 2.5 |
| Juice pH | | 5.71 | 3.78 | 2.46 |
| Urinary Citrate | AUC | 0.32 | 0.27 | 0.30 |
| | Δ | 159* | 200* | 193* |
| Urinary Potassium | AUC | 0.08 | 0.06 | 0.05 |
| | Δ | 133* | 140* | 10 |
| Urinary pH | AUC | 6.46 | 6.23 | 6.08 |
| | Δ | 9.8* | 6.9* | 6.4 |

AUC (mg/mg creatinine); (* p<0.05) *vs* baseline




A significant percentual (%) increment (Δ) of urinary citrate was observed for melon, orange and lime after 4 hrs *versus* baseline, while Δ for potassium and pH were statistically significant only for melon and orange. However, the mean AUC (area under curve) did not differ between groups. CONCLUSION: Dietary potassium from non-citrus sources may provide equivalent amounts of increase in urinary citrate compared to citric sources due to its alkalinizing effects.

Disclosure of Financial Relationships: nothing to disclose

**PUB080**

**Effect of Cystone® over One Year on Urine Cystine and Stone Formation** Stephen B. Erickson,[1] Terri J. Vrtiska,[2] Vincent J. Canzanello,[1] John C. Lieske.[1,3] *[1]Division of Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [2]Department of Diagnostic Radiology, Mayo Clinic, Rochester, MN; [3]Department of Laboratory Medicine & Pathology, Mayo Clinic, Rochester, MN.*

Cystine kidney stones frequently recur because inadequate prevention exists. We recruited 10 documented recurrent cystine kidney stone formers into a 2 phased study to assess safety and effectiveness of Cystone®, an herbal treatment for prevention of kidney stones. The first phase was a randomized double-blinded 12 week cross over study assessing the effect of Cystone® vs. placebo on urinary chemistries. The second phase was an open label one year study of Cystone® to determine if renal stone burden decreased, as assessed by quantitative and subjective assessment of CT. Results revealed no statistically significant effect of Cystone® on urinary composition short (6 weeks) or long (52 weeks) term. Average renal stone burden increased rather than decreased on Cystone®. Therefore, this study does not support the efficacy of Cystone® to treat cystine stone formers.

Disclosure of Financial Relationships: nothing to disclose

**PUB081**

**Increased Sensitivity to 1,25(OH)$_2$Vitamin D$_3$ in Genetic Hypercalciuric Stone-Forming Rats** Kevin K. Frick,[1] John R. Asplin,[2] Murray J. Favus,[2] Christopher David Culbertson,[1] Stephanie Yee,[1] Nancy Krieger,[1] David A. Bushinsky.[1] *[1]University of Rochester School of Medicine; [2]University of Chicago.*

Genetic hypercalciuric stone-forming (GHS) rats have been selectively bred to excrete more urine (U) calcium (Ca) than Sprague-Dawley (SD) controls. GHS rats uniformly form kidney stones and express an increased number of vitamin D receptors (VDR) in kidney, intestine and bone. To assess the biological activity of the GHS VDR, we injected 25(OH)D$_3$ replete GHS or control Sprague-Dawley (SD) rats, fed a Ca replete (1.2% Ca) diet, with either 25 ng/d 1,25(OH)$_2$D$_3$ (Vit D) or vehicle (Veh) for 7d. Administration of Vit D to both SD and GHS rats increased serum Ca equally (increase of ~1.5 mg/dl) and suppressed PTH to undetectable levels in each. UCa in GHS+Veh (10.5 mg/d) was elevated compared to SD+Veh (1.7 mg/d); UCa was increased in SD+Vit D (24.4 mg/d), and further increased in GHS+Vit D (41.9 mg/d; all groups n=8, all comparisons p<0.01). To examine gene expression important for renal tubular Ca reabsorption, total kidney RNA was isolated and analyzed by QRT-PCR. VDR was increased in SD+Vit D and GHS+Vit D relative to respective Veh. Expression of the distal luminal renal Ca transporter TRPV5, Ca binding protein calbindin D$_{28K}$ and the basolateral Na/Ca exchanger NCX were all similarly increased in SD+Vit D and GHS+Vit D. Expression of klotho, which activates TRPV5, was decreased only in GHS+Vit D. The Ca transporter TRPV6 was increased in SD+Vit D and further increased in GHS+Vit D. Levels of the basolateral Ca ATPase PMCA were inhibited in GHS+Vit D but not in SD+Vit D. There were no differences in expression of thick ascending limb paracellular protein claudin 16, Na/K/2Cl transporter NKCC2 and K channel ROMK between the SD+Vit D and GHS+Vit D groups. Thus the greater increase in UCa with Vit D in GHS compared to SD indicates that the enhanced number of VDR in GHS amplifies the biological response to administered Vit D. The mechanism of the

increased UCa in GHS+Vit D may involve a reduction in klotho, which activates TRPV5, and/or a reduction in PMCA, resulting in less renal tubular Ca reabsorption and greater hypercalciuria in GHS+Vit D as compared to SD+Vit D.

Disclosure of Financial Relationships: nothing to disclose

**PUB082**

**Factors Involved in Changes of Serum Magnesium (sMg) Levels in Hemodialysis (HD) Patients and the Impact of Treatment of Secondary Hyperparathyroidism (SHP) in Magnesium Levels** Emilio E. Gonzalez-Parra, Beatriz Fernández, Catalina Martin Cleary, Pablo Justo Avila, Jesus Egido. *Nephrology, Fundacion Jimenez Diaz, Madrid, Spain.*

Magnesium (sMg)is increased in HD patients. It has some similar homeostatic mechanisms to calcium and is influenced by treatments like cinacalcet and paricalcitol are not well known. ¿That these treatments may influence sMg?.

**OBJECTIVES**

Determine sMg in different techniques of HD

Influence of treatment with phosphate binders in serum sMg

Influence of treatment for SHP with Cinacalcet, paricalcitol and 25 OH vitamin D in sMg .

**MATERIAL AND METHODS**

59 patients on HD (23 w, 36 m), mean age 70.3. 13 in HDFonline and 46 in high flow conventional HD (CHD).sMg in the dyalisate was 0.5 mmol / L. 27 of these patients were diabetic.

Paricalcitol was held in 15 cases and 5 cases were treated with cinacalcet. 18 patients were treated with oral calcidiol (0,266 mg / week). The paricalcitol mean dose of 3.2 mGR / week, cinacalcet daily dose of 60 mg / day.

**RESULTS**

Mean sMg was 2.066 ± 0.038 mg / dl. sMg did not correlate with sCa (r 0,075), p 0.482.

sMg was lower in CHD than in olHD ; 2.02 ± 0.25 vs 2.2 ± 0.304, p <.032.

No correlation with phosphate binders, neither its doses. sMg not correlated with sPTH or with 25 OH vit D. Calcidiol correlated with Ca and P, p <.05, sMg has not the same behavior to similar cations.

No differences between sMg in diabetics and non-diabetic patients; 2107 ± 0.3 vs 2023 ± 0.24 (ns) .

sMg quartiles, higher level of sMg, increased level of calcidiol, but that was not significant. Higher sMg quartile corresponded to higher PTH than lower sMg (0-1.9 mg / dl), but non-significant.

No significant differences in sMg between patients treated or not with paricalcitol (2.15 ± 033 vs 2.02 ± 0.24 mg/dl); calcidiol (2.11 ± 0.27 vs 2.04 ±0.30 mg/dl) and cinacalcet (2.3 ± 0.25 vs. 2.04 ± 0.27 mg/dl).

**CONCLUSIONS**

1. - Magnesium behaves differently to calcium, there is no correlation with PTH, 25 OH vitamin D, calcium and phosphate.

2. – No influence of Paricacitol or Cinacalcet treatment on sMg was observed

3. - In olHD sMg is higher than in CHD.

4. – Calcidiol level is higher on patients with higher sMg but this is non-significant.

Disclosure of Financial Relationships: nothing to disclose

**PUB083**

**A New Model of Quantifying Phosphate Removal in Conventional Hemodialysis Therapy** Chuan-Ming Hao. *Huashan Hospital, Fudan University.*

**Background** Hyperphosphatemia is closely associated with mortality in patients on hemodialysis. We report a simple formula to estimate phosphate removal by each hemodialysis session to develop therapeutic strategies and analyze the factors that may affect removal. **Methods** 167 MHD patients were enrolled in this study. They had received the following dialysis treatment:4-hr each session, 3 times per week, Polysulfone 1.2$m^2$ or Triacetate 1.3$m^2$,Dialysate flow was 500ml/min. The out-flow dialysate samples were collected every 15min during 4 hours to measure the concentration of phosphate. Total amount of phosphate removal was estimated by area under the curve of dialysis volume (x) and dialysate phosphate concentration (y). This estimation was very close to the actual phosphate removal. The levels of serum phosphate, Hct, PTH, $TCO_2$ (all before treatment)and kt/v(urea), ultrafiltration rate, dry weight, blood flow were also recorded. 137 data sets were randomly selected to generate a model and the remaining 30 data sets were used to validate. **Results** The total amount of phosphate clearance by four-hours dialysis treatment was mostly between 600-900mg. By backward stepwise regression, the best model to describe the removal of phosphate per 4h dialysis as a function of treatment variables was: total amount

=79.6´T45(mmol/L)-0.023´age(years)+0.065´Weight(kg)-0.12´TCO2+0.05´Clearance(ml/min)-3.44. T45 represented phosphate concentration of dialysate after 45min of treatment; Clearance represented the phosphate clearance of dialyser when blood flow was 200ml/min. The validation results showed that the residual were all within 3mmol, indicating that the error would not exceed 10% of the real value. MSE and MSPR were very close suggesting the model was highly accurate. **Conclusions** This study derived a new approach to quantify phosphate removal of conventional hemodialysis by a simple formula, which may be helpful for clinicians to evaluate the phosphate balance in MHD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB084**

**Not All 25 OH Vitamin D Defiency Is True Vitamin D Deficiency: Syndrome of Excess Endogenous 1,25 Vitamin D: Nephrolithiasis, Nephrocalcinosis, Osteopenia, Growth Retardation, Hypoparathryoidism** Jessica G. Lucas, Barbara A. Clark. *Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

Clinicians are increasingly aware of the ubiquitous nature of vitamin D deficiency in the general population and measurement of 25 OH Vitamin D levels are becoming more routine. This may present a clinical dilema in patients with calcium nephrolithiasis with hypercalciuria. Idiopathic hypercalciuria has been, in some cases, attributed to excess endogenous 1,25 vitamin D. However, this is not widely appreciated, levels are not routinely measured and the extreme clinical manifestations of this disorder are even more under appreciated. A 17 yo male with a history of recent nephrolithiasis was referred by his endocrinologist to determine the risk/benefit of vitamin D therapy for his newly diagnosed osteopenia, growth retardation (bone age of 14) and low 25 OH Vit D levels, prior to initiating growth hormone therapy. Testing revealed nephrocalcinosis, serum calcium of 9.9 mg/dl. phosphorus of 3.8 mg/dl, HCO3 27 mmol/L, creatinine 0.7 mg/dl, 25 OH Vit D level 14ng/ml (nl 20-100), intact PTH < 3.0 pg/ml (nl 9-69), urine calcium 457 mg/d (normal <300), phosphorus 492 mg/gm creatinine (nl 210-750), citrate 1938 umol/d, oxalate 14 mg/d , urine pH 6. A 1,25 Vit D level returned at 160 pg/ml (nl 15-60). Family history was notable for nephrolithiasis in father and paternal GF. The patient was treated with HCTZ, high fluid intake, low salt intake and somatropin injections (for his growth retardation). Vitamin D therapy was not recommended. Repeat urine calcium/creatinine ratios were 135-238 mg/gm, Dexa scan showed improved bone mineralization and the patient grew 2 inches over 8 months. The hypercalciuria and marked suppression of PTH secretion were attributed to the excess 1,25 Vit D levels and the bone demineralization was attributed to the renal calcium loss. In summary, low 25 OH vitamin D levels should not lead to automatic repletion of exogenous vitamin D in calcium stone formers. Increased vigilance for idiopathic hypercalciuria with endogenous 1,25 Vitamin D excess, especially with a positive family history, is indicated.

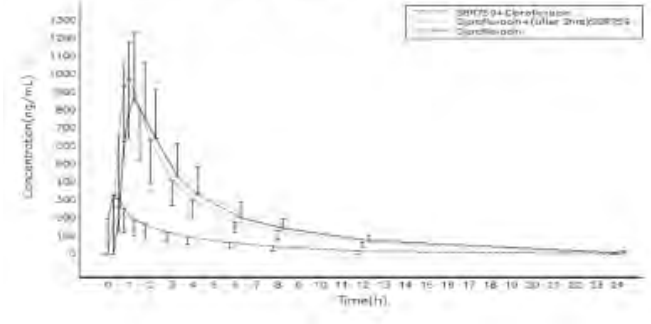
Disclosure of Financial Relationships: nothing to disclose

**PUB085**

**SBR759 Drug Interaction with Ciprofloxacin: Separating Administration by 2 Hours May Avoid Need for Therapeutic Substitution** Alan J. Slade,[1] Sayaka Shimada,[1] Stephan De la Motte,[2] Robert L. Schmouder.[1] *[1]Novartis Institute for Biomedical Research; [2]Harrison Clinical Research.*

SBR759 is a novel, calcium-free, polymeric, iron (III)-based phosphate binder being developed for the treatment of hyperphosphatemia in patients with chronic kidney disease. SBR759 is an orally-dosed, powder that effectively binds phosphorus in the GI tract. As expected, due to the class interaction between fluoroquinolone antibiotics and di- and tri-valent metal cations, an interaction between SBR759 and ciprofloxacin was detected during *in vitro* testing. This Phase I study was conducted in healthy volunteers to characterize the pharmacokinetics (PK) of ciprofloxacin when administered with or 2 hours before SBR759. **METHODS:** An open-label, 3-period, single-dose, cross-over study design was utilized. Volunteers received 250 mg ciprofloxacin alone, 2 hours before and in combination with 5 g SBR759. Ciprofloxacin PK samples were collected through 24 hours post dose. **RESULTS:** 36 volunteers were enrolled in the study. The most common AEs were discolored stool (11/36), diarrhea (8/36) and flatulence (8/36). The PK time-concentration curves for the three treatment arms are shown below.

Figure 1: SBR759 Drug Interaction: Ciprofloxacin time-concentration profile (arithmetic mean (SD)(*n=36*)




When SBR759 was administered with ciprofloxacin (dashed line), there was a 68% decrease in the ciprofloxacin geometric mean ratio of Cmax (0.32) and AUC0-inf (0.31). Alternately, when ciprofloxacin was administered 2 hours prior to SBR759 (dotted line) there was no discernible interaction (Geo. mean ratio - Cmax: 1.04 and AUC0-inf: 0.96). **CONCLUSIONS:** Simultaneous administration of ciprofloxacin and SBR759 leads to a significant PK interaction. Administration of ciprofloxacin 2 hours prior to SBR759 allows adequate time for absorption thus no interaction was measured in healthy volunteers. These data suggest that with care and counseling, clinicians can use ciprofloxacin in patients receiving SBR759.

Disclosure of Financial Relationships: Employer: I am an employee of Novartis Pharmaceuticals.

**PUB086**

**Improved Management of Mineral Metabolism in Hemodialysis Patients; Sevelamer-Carbonate (SC) Versus Sevelamer-Hydrochloride (SH)** Brett W. Stephens,[1] Donna Roy,[2] Donald A. Molony.[1] *[1]Renal Diseases and Hypertension, University of Texas Houston Medical School, Houston, TX; [2]DaVita PDI South Dialysis, Houston, TX.*

Successful management of hyperphosphatemia amongst dialysis patients remains an important but incompletely achieved goal. Recent evidence supports the view that the concurrent achievement of KDOQI targets for $PO_4$, Ca, iPTH is associated with the best patient survival. An important barrier to successful management is the need to take $PO_4$ binders. As part of the quality improvement process, we evaluated the achievement of KDOQI mineral metabolism goals in a single dialysis center, for the six months before initiation of and after full conversion of all patients from SH (Renagel®) to SC (Renvela®) as their phosphate binders. All other aspects of the dialysis care remained unchanged. The table lists the data from these two periods.

| | % of patients with PO4 of >3.5 to < 5.5 mEq/L | % of patients with iPTH of 150 – 300 pg/ml | % of patients with Ca of > 8.2 to <9.5 mEq/L | % of patients with all three parameters within the KDOQI target range |
|---|---|---|---|---|
| 7/1/2007 to 12/31/2007 | 69.4 ± 8.7 | 60.6 ± 8.5 | 81.3 ± 7.3 | 46.3 ± 2.8 |
| 1/1/2009 to 6/30/2009 | 76.8 + 5.6± | 61.3 ± 6.4 | 95.5 ± 3.0 | 54.2± 7.3 |
| p | NS | NS | p = 0.07 | p < 0.05 |

86% and 98% of patients were treated with SH and SC as their primary binder in the first and second time periods, respectively. Significantly, more patients achieved the 3-KDOQI goals during period two. Although changes in either $PO_4$ or Ca did not quite reach statistical significance, improvements in either or both likely accounted for the observed improvement in the number of patients achieving the 3-KDOQI goals concurrently. The possibility of temporal trends accounting for the observed improvement can only be excluded by a randomized controlled clinical trial. These observations, however, support the view that phosphate binder type may influence importantly whether clinical goals are achieved. In this single practice, SC appeared to offer an advantage over SH for achieving KDOQI mineral metabolism goals.

Disclosure of Financial Relationships: nothing to disclose

**PUB087**

**Effect of Diet Sunkist Orange Soda on Urinary Lithogenicity** Nicola T. Sumorok,[1] Brian H. Eisner,[4] Marshall L. Stoller,[2] John R. Asplin,[3] David S. Goldfarb.[1] *[1]Nephrology, New York University School of Medicine, New York, NY; [2]Urology, University of California-San Francisco, San Francisco, CA; [3]Litholink Corporation, Chicago, IL; [4]Urology, Massachusetts General Hospital, Harvard Medical School, Boston, MA.*

**Purpose:** Studies have shown that certain beverages decrease urinary lithogenicity by increasing urine citrate excretion. Citrate and total alkali concentrations in 12 diet sodas showed that Diet Sunkist Orange soda has the highest concentration of potential base. We studied the effect of Diet Sunkist Orange soda consumption on urinary chemistry.

**Methods:** 9 healthy men and women ages 26-54 completed the study. During the control period, subjects drank 32 oz. of water for 3 days in addition to their own, ad lib diet and recorded a food diary. During the study period, the subjects drank 3-,12-oz cans of Diet Sunkist Orange soda a day instead of water, and replicated their diets from the control period. In each period, the subjects performed 24-hour urine collections on days 2 and 3. Urine chemical analysis was performed, including urinary citrate levels and pH.

**Results:** Diet Sunkist Orange soda increased urinary citrate excretion by 24.8 mg/day, which was not statistically significant (95% CI –71.5 to 121.1, p-value 0.57). There was no significant change in pH from the control period to the study period (pH 6.27 to 6.22; 95% CI –0.111 to 0.202, p=0.52). Urine volumes and creatinine excretions were not significantly different between the control and study periods.

**Conclusions:** Despite the relatively high citrate and total alkali content of Diet Sunkist Orange soda, there is not enough citrate content to affect the urinary chemistry in healthy subjects who have normocitraturia at baseline. The positive effect that was seen in a prior study looking at Performance, a sports drink, can be attributed to the higher content of citrate in an alkaline form and the higher pH of Performance. The effect of Diet Sunkist Orange soda on the urinary chemistry in patients with hypocitraturia and nephrolithiasis is not likely to have a clinically significant effect to prevent calcium or uric acid stones.

Disclosure of Financial Relationships: nothing to disclose

**PUB088**

**Assessment of Bone Mineral Density (BMD) and Structure in Children with Idiopathic Hypercalciuria (HC)** Shamir Tuchman,[1] Babette Zemel,[2] Mary B. Leonard.[2] *[1]Pediatric Nephrology, Children's National Medical Center, Washington, DC; [2]The Children's Hospital of Philadelphia, Philadelphia, PA.*

DXA studies have reported BMD deficits in children with HC. Peripheral quantitative CT(pQCT) provides discrete measures of trabecular(Trab) and cortical(Cort) volumetric BMD and cortical dimensions. The purpose of this pilot study was to use pQCT/DXA to characterize differences in BMD in HC differentiated based on a calcium restricted diet. 13 subjects were categorized as absorptive HC (AH; n=7) or renal HC (RH; n=6) based on urinary changes after a 300mg/day, 3-week calcium restricted diet (AH urine calcium decreased to < 4 mg/kg/d). Tibia QCT measures included Trab_BMD(distal metaphysis) and Cort_BMD and Section Modulus($Z_p$; summary measure of cortical dimensions and strength in torsion)(mid-diaphysis). DXA measures include AP_spine_BMD, whole body(WB)_BMD and width-adjusted spine BMD(WA_BMD; estimates vertebral volumetric BMD using AP and lateral scans excluding spinous processes). Z-scores were generated using reference data in over 600 controls. The results are summarized below. The overall results and results in AH and RH subjects did not differ significantly from controls or between each group (p>0.05). However, the data demonstrated (1) a pattern of greater Trab_BMD, WB_BMD, and WA_BMD in AH subjects, and (2) a pattern of lower Trab_BMD, WB_BMD, and spine_BMD in RH subjects. These data suggest that assessment of bone health in children with HC should consider distinct differences in measures of BMD between AH and RH. Larger studies are needed to determine if this results in clinically significant alterations in bone health.

| Z-score | Overall (n=13) | Renal HC (n=7) | Absorptive HC (n=6) |
|---|---|---|---|
| pQCT | | | |
| Trab_BMD | -0.04+/-1.02 | -0.46+/-0.94 | 0.38+/-1.00 |
| Cort_BMD | -0.25+/-0.83 | 0.05+/-0.73 | -0.56+/-0.87 |
| Zp | -0.08+/-0.87 | -0.12+/-0.66 | -0.04+/-1.11 |
| DXA | | | |
| WB_BMD | 0.13+/-1.20 | -0.35+/-1.26 | 0.61+/-1.02 |
| AP_spine_BMD | -0.06+/-1.18 | -0.33+/-1.38 | 0.20+/-0.99 |
| WA_BMD | 0.09+/-0.96 | -0.27+/-0.92 | 0.46+/-0.93 |

Disclosure of Financial Relationships: nothing to disclose

**PUB089**

**Hormonal and Dietary Determinants of Urinary Phosphate Excretion in Healthy Volunteers and Patients with Chronic Kidney Disease Stage 1-4** Liesbeth Viaene, Bjorn K. I. Meijers, Vanrenterghem Yves, Pieter Evenepoel. *Nephrology, University Hospital Gasthuisberg, Leuven, Belgium.*

Introduction: Phosphate homeostasis is disrupted in chronic kidney disease (CKD). In steady-state, 24-hour urinary phosphate excretion (24h-$Ur_{Phos}$) corresponds to net daily intestinal absorption. We aimed to identify hormonal and dietary determinants of 24h-$Ur_{Phos}$ and 24 hour urinary fractional excretion of phosphate ($FE_{phos}$). Methods: 20 healthy volunteers (HV) (age 34 ±10 year) and 170 stable CKD stage 1-4 patients (age 59±15 year) were studied. Fasting blood and 24-hour urine samples were analyzed for parameters of mineral metabolism including calcidiol, calcitriol, FGF-23 and PTH. Dietary data were available in all HV and in 72 CKD patients. Results: Table1 summarizes relevant dietary and biochemistry data.

Relevant biochemistry data

| | HV | >60 ml/min/m² | 30-60 ml/min/m² | 15-30 ml/min/m² | p-value |
|---|---|---|---|---|---|
| Phosphate (mg/dl) | 3.5 | 3.1 | 3.3 | 3.2 | 0.06 |
| Biointact PTH (ng/l) | 17 | 10 | 18 | 29 | <0.0001 |
| Calcitriol (ng/l) | 59 | 64 | 41 | 38 | <0.0001 |
| FGF-23 (ng/l) | 28 | 33 | 64 | 69 | <0.0001 |
| FEphos (%) | 16 | 20 | 30 | 36 | <0.0001 |
| 24h-UrPhos (mg/day) | 895 | 791 | 792 | 726 | 0.27 |
| Dietary phosphate intake (mg/day) | 1513 | 1315 | 1191 | 1247 | 0.11 |

Median values are shown.

Only dietary phosphate intake was independently associated with 24h-$Ur_{Phos}$ ($R^2$=0.20, p<0.0001). 24-Hour $FE_{phos}$significantly increased along the progression of CKD. In multivariate analysis, high 24h-$Ur_{Phos}$, high PTH, high FGF-23, low serum phosphate, low calciuria and low eGFR were independently associated with high $FE_{phos}$ ($R^2$= 0.60; p<0.0001). Conclusions: Our data confirm and extend previous experimental and clinical findings. The inverse association between calciuria and $FE_{phos}$supports the notion of regulation of proximal tubule phosphate transport by a calcium-sensing receptor. 24h-$Ur_{Phos}$ and $FE_{phos}$ are readily available and inexpensive measures of dietary phosphate exposure and renal phosphate handling. They may prove to be of added value for monitoring phosphate homeostasis in CKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB090**

**A Multi-Center Randomized Double-Blind Placebo-Controlled Trial on Chewing Effect of a Chitosan Loaded Gum HS219 for the Control of Serum Phosphorous in Maintenance Hemodialysis (HD) Patients** Tadao Akizawa,[1] Yoshinari Tsuruta,[2] Yoichi Okada,[3] Yoshihiro Miyauchi,[4] Akio Suda,[5] Hirosi Kasahara,[6] Nobuhiro Sasaki,[7] Yoshitaka Maeda,[8] Takako Suzuki,[9] Noriaki Matsui,[10] Jun Niwayama,[11] Toshiaki Suzuki,[12] Hideaki Hara,[13] Yasushi Asano,[7] Masafumi Fukagawa.[14] *[1]Showa University School of Medicine; [2]Meiyo Clinic; [3]Maruko General Hospital; [4]Asahi General Hospital; [5]Suda Clinic; [6]Japanese Red Cross Suwa Hospital; [7]Japanese Red Cross Koga Hospital; [8]Toride Kyodo General Hospital; [9]Komagome Kyouritsu Clinic; [10]Tsuchiura Kyodo General Hospital; [11]Sumiyoshi Clinic Hospital; [12]Asagaya Suzuki Clinic; [13]Gifu Pharmaceutical University; [14]Tokai University School of Medicine, Japan.*

Background: Control of serum phosphate level is a critical step in management of CKD-MBD. HS219 is a chewing gum designed to trap salivary phosphorous, which is an alternate source of phosphorous with higher concentration than serum. Aim: To evaluate the efficacy and safety of HS219 when given three times a day for 3 weeks to the HD patients with hyperphosphatemia. Method: 68 HD patients maintained on calcium carbonate (33 subjects) or sevelamer (35 subjects) were enrolled. The major inclusion criteria required serum phosphorous of >5.5 and < 9.0 mg/dL. The primary end point was the changes from baseline in serum phosphorus. Secondary end points included change from baseline in salivary phosphorous, serum calcium, PTH and FGF23. Results: 63 patients chewed at least one gum: 35 HS219 and 28 placebo. HS219 was well tolerated and safe. No significant

effects of HS219 on reduction of serum and salivary phosphorous were observed. There was no correlation between salivary and serum phosphorous. However, statistically significant difference was observed in change from baseline to end of chewing for iPTH (P=0.018), particularly in sevelamer group (P=0.001). Effect on iPTH was correlated to serum phosphorous reduction whilst not changing serum calcium. Conclusion: HS219 has no effects on serum and salivary phosphorous, but reduces PTH in sevelamer group. Further exploratory study is needed to evaluate its efficacy.

Disclosure of Financial Relationships: Consultancy: Chugai, Kirin,AbbottResearch Funding: Chugai, Kirin.

**PUB091**

**A Bioimpedance Study on Factors That Influence Bone Health in a Haemodialysis Population** Julie E. Browne, Caroline McConnell, Charlotte Griffin, Ying Kuan. *Renal Unit, Altnagelvin Hospital, Londonderry, Northern Ireland, United Kingdom.*

**Introduction:** Patients with end stage renal failure have altered bone metabolism and are at increased risk of spontaneous fracture compared to the general population. Dual energy X-ray absorptiometry (DXA) is commonly used to evaluate bone health but involves radiation exposure. Multifrequency bioimpedance analysis (MF-BIA) measurements can provide a non-invasive method for assessing body composition that has been found to correlate closely with values measured by DXA. Bone Mineral Content (BMC), measured by BIA, may provide an indication of bone health. This study assesses the factors that influence BMC in a Hemodialysis (HD) population, and the possible utility that this measurement may have as a screening tool.

**Method:** BMC was measured using a MF-BIA analyzer (InBody S20, Biospace) in 50 HD patients in a single renal unit (Altnagelvin Hospital, Londonderry, UK). Date of test, body weight (kg), BMI and BMC (kg) was recorded in addition to sex and length of time on renal replacement therapy (RRT). Maximum values of PTH (PTHmax) in the 12 months preceding the study were also recorded as surrogate biochemical measure of metabolic bone health. The results were analysed using Medcalc (MedCalc Software, Inc, Mariakerke, Belgium), by correlating BMC, as percentage of body weight (%BMC), with age, gender, time on RRT and PTHmax.

**Results:** 30 males and 20 females were included in the study. Mean time on dialysis was 4.8 years (range 0.2-31.5 years). Other results are shown in Table 1. Multivariate analysis suggests that %BMC correlates negatively with time on dialysis ($p<0.05$).

Table 1.

| Variable | Mean | ± 1SD* |
|---|---|---|
| Age (years) | 61 | 16 |
| Weight (kg) | 74.5 | 13.2 |
| PTH max (ng/l) | 646 | 392 |
| BMC (kg) | 3.6 | 0.8 |
| %BMC | 4.8 | 1.0 |

*SD=standard deviation

**Conclusion:** MF-BIA offers a non-invasive measurement of body composition, yielding important information, including nutritional status in the HD population. BMC estimated in our study suggests a detrimental impact of long term RRT on bone health. This finding is similar to that found from other studies with DXA, and suggests that further studies on MF-BIA as a screening tool in this population should be pursued.

Disclosure of Financial Relationships: nothing to disclose

**PUB092**

**Vertebral Trabecular Bone Attenuation, Mineral Metabolism and Coronary Artery Calcification in Patients with Normal Renal Function** Ana L. E. Cancela,[1] Raul D. Santos Filho,[2] Silvia Titan,[1] Carlos Eduardo Rochitte,[2] Russell Gotschall,[3] Brian S. Kelley,[3] Susan Schiavi,[3] Vanda Jorgetti,[1] Rosa Ma Moyses.[1] *[1]Nephrology, Universidade de São Paulo, Brazil; [2]Instituto do Coração, Universidade de São Paulo, Brazil; [3]Genzyme Co.*

This study explores the relationships among vertebral trabecular bone attenuation (TBA), coronary artery calcification (CAC) and mineral metabolism markers in 271 patients with suspected coronary artery disease (CAD) and normal renal function (MDRD >60 ml/min/1.73 $m^2$) submitted to multislice CT (MSCT) to assess CAC. Agatston Scores (AS) were assigned. CT images were analyzed to measure thoracic TBA (mean of 3 measures), which was obtained in Housfield Units (HU). We also analyzed serum P, ionized Ca (iCa), lipid profile, alkaline phosphatase (AP), PTH, 25-vitamin D [25(OH)D] and intact FGF-23. Patients were considered to have CAC when AS was >10 (CAC group; $n$=155) and when AS was ≤ 10 they were assigned to the non-CAC group ($n$=116). Mean age was 58.17± 9.4 years, 57.2% were male, 65.3% white, 81.1% were hypertensive and 35.8% diabetic. Mean serum P was 3.57±0.54 mg/dl, median FGF-23 and Creat clearance were 59.3 pg/ml and 92.4 ml/min/1.73 $m^2$. Median TBA and AS were 200.9 HU (interquartile range:163.9-241.7) and 40.0 HU (interquartile range: 0-275.0). TBA was lower in women (p= 0.046) and white patients (p=0.01).There were negative correlations between TBA and AS ($r$=-0.211; $p$<0.0001) and TBA and age ($r$=-0.419; $p$<0.0001). There was a trend towards a correlation between TBA and FGF23 ($r$=-0.10; $p$=0.10) and between TBA and PTH ($r$=-0.111; $p$=0.069). We found no correlation between TBA and iCa, AP, P or 25(OH) D. Compared to the non-CAC group, CAC group had lower TBA (median 184.1 vs 211.0 HU, $p$=0.004). Multivariate analysis revealed that TBA was dependent on age ($p$<0.0001, CI -3.42-1.52) and FGF-23 ($p$=0.04, CI -0.053-0.001). In conclusion, we found that TBA was negatively correlated to coronary calcification, age and serum FGF-23 in a population with normal renal function and suspected CAD. Interactions between vascular and bone physiology and common pathogenetic factors for bone loss and CAC may be responsible for these associations.

Disclosure of Financial Relationships: nothing to disclose

**PUB093**

**Variability of Serum Phosphate in Patients on In-Center or Home Hemodialysis** Katie E. Cardone,[1] George R. Bailie,[1] Rachid Daoui,[2] Shari A. Meola,[2] Christopher D. Hoy.[2] *[1]ANephRx, Albany College of Pharmacy and Health Sciences, Albany, NY; [2]Hortense and Louis Rubin Dialysis Centers, Saratoga Springs, NY.*

Background: Limited data exist regarding extent of variability in serum phosphorus concentrations ([P]) amongst those on different dialysis modalities. Despite this, clinicians frequently rely on these values to make clinical decisions in patients on dialysis. The purpose of this study was to retrospectively examine the extent and magnitude of variability in monthly, routine estimates of [P] over a 1 yr period in a cadre of patients at an outpatient dialysis facility.

Methodology: A retrospective chart review of [P] was conducted amongst patients with end stage kidney disease receiving renal replacement therapy. All adult patients who received treatment at the facility between 1/1/2009 and 12/31/2009 whose records were available for review, & who had ≥2 [P] lab values were included. Patients may have received either in-center intermittent hemodialysis (HD) or frequent home hemodialysis (≥5 days/wk) (FHH).

Results: A total of 153 patients were included (HD: n=118; FHH: n=35).

| | HD (n=118) | FHH (n=35) |
|---|---|---|
| DEMOGRAPHIC | | |
| Mean±SD Age (yr) | 65.5±16.4 | 57.8±11.6 |
| % Caucasian | 80 | 88.6 |
| % Male | 53 | 63 |
| LABORATORY DATA | | |
| Mean±SD number of laboratory orders | 12.9±7.3 | 15.7±7.0 |
| Geometric Mean±SD [P] (mg/dL) | 5.0±1.2 | 5.1±1.2 |
| Mean±SD Intrapatient [P] Range | 3.2±1.7 | 3.7±1.4 |
| Mean±SD Intrapatient [P]: | | |
| % of values ≥6.5 mg/dL | 17.2±25.6 | 19.0±27.3 |
| % of values >5.5 mg/dL | 31.9±32.2 | 34.5±31.5 |
| % of values 3.5-5.5 mg/dL | 54.9±28.2 | 51.6±25.5 |
| % of values <3.5 mg/dL | 13.2±19.6 | 13.9±19.6 |
| % of values ≤2.5 mg/dL | 3.3±10.1 | 3.1±6.7 |

*p<0.05

Conclusion: Intrapatient variation in [P] is not different amongst those on HD or on FHH. This may be due to differences in management of [P], including diet, extent of phosphorus restriction, and medication use. For the average patient, [P] remains within the range of 3.5-5.5 more than half the time.

Disclosure of Financial Relationships: Research Funding: Merck & Co.

**PUB094**

**Calcification in Abdominal Aorta and in Coronary Arteries in Hemodialysis Patients Is Not Directly Related to Plasma Concentration of Fibroblast Growth Factor 23** Jerzy Chudek,[1,2] Marcin Adamczak,[1] Andrzej Wiecek,[1] Przemyslaw Pencak,[3] Joanna Witkowicz,[1] Beata Czerwienska.[1] *[1]Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland; [2]Department of Pathophysiology, Medical University of Silesia, Katowice, Poland; [3]Division of Radiology, SPSK-M, Katowice, Poland.*

**Introduction:** Fibroblast growth factor 23 (FGF-23) is recognized as a circulating phosphaturic hormone which suppresses the proximal tubular Na/Pi-2a and Na/Pi-2c cotransporters. FGF-23 deficient mice develops severe vascular calcification, however the role of FGF-23 in the development and progression of vascular calcifications in chronic renal failure (CKD stage 5) remains unknown. The aim of this cross-sectional study was to analyse the interrelation between plasma concentration of FGF-23 and calcifications in abdominal aorta and coronary arteries in hemodialysis patients applying multislice computer tomography.

**Patients and methods:** Seventy five patients (37 M, 38 F; aged 56, range 23-83 years) hemodialysed three times a week for 2-228 months (mean±SD 36±46) were enrolled into this study. In all patients coronary calcium score (CCS) and calcium score of abdominal aorta (AoCS) using multislice computer tomography were performed. Plasma intact FGF-23 concentrations were measured by ELISA (Immutopics, CA, USA). Data are presented as means and 95% CI.

**Results:** Coronary arteries and abdominal aorta calcifications were detected in 57 (76%) and 61 patients (81.3%), respectively. The mean CCS and AoCS were 1014 (640-1388) and 2704 (1906-3502), respectively. There was a strong correlation between CCS and AoCS (R=0.721, p<0.001) and between CCs or AoCS and time of dialysis therapy (R=0.256, p=0.03 and R=0.281, p=0.02, respectively). There was no correlation between CCS or AoCS and plasma intact FGF-23 concentration (also after logarithmic transformation). There was a strong correlation between serum phosphate and intact FGF-23 concentration (R=0.513; p<0.001)

**Conclusion:** Plasma FGF-23 is not related to the intensity of vascular calcifications in hemodialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB095**

**Comparative Effects of Phosphate Binders in Experimental Adynamic Bone Disease** Juliana C. Ferreira, Guaraciaba O. Ferrari, Raquel T. Cavallari, Cássia M. Puci, Wagner V. Dominguez, Luciene M. dos Reis, Fabiana G. Graciolli, Rosa Ma Moyses, Katia R. Neves, Vanda Jorgetti. *Nephrology Division, Universidade de São Paulo, Brazil.*

Despite the increasing prevalence of adynamic bone disease (ABD), there are no studies of the effects of P binders on ABD. Rats submitted to 5/6 Nx+PTx were divided into: Ca ($CaCO_3$); Sev (Sevelamer $CO_3$); CKD (untreated); and control (sham). After 8 wks, we performed biochemical and histomorphometric (BHist) analyses, as well as von Kossa staining in aortic sections. All Nx rats showed higher creatinine and P and lower Ca than did control rats. Serum P was similar in all Nx groups, but fractional excretion of P (FeP) was significantly lower in the treated groups. Calciuria was higher in the Ca group than in the Sev group. A non-significant decrease in serum FGF-23 was seen in Sev group. None of the rats developed VC. BHist revealed ABD in all Nx groups, as confirmed by lower bone formation rates, as well as decreases in osteoid volume, osteoblast surface and osteoclast surface and absence of fibrosis. We found no differences among the study groups in terms of BHist parameters, except for greater eroded surface in the Ca group.

| | Ca (n=7) | Sev (n=5) | CKD (n=7) | Control (n=5) |
|---|---|---|---|---|
| Creatinine (mg/dl) | 1.4±0.5 | 1.5±0.4 | 1.4±0.2 | 0.6±0.1[a] |
| ionized Ca (mmol/L) | 0.5±0.1 | 0.5±0.3 | 0.6±0.2 | 1.1±0.0[a] |
| P (mg/dl) | 11.5±2.2 | 10.6±3.5 | 11.3±3.0 | 5.2±0.2[a] |
| FeP (%) | 1.8±1.7[a] | 23.1±12.1[a] | 53.8±23.3[a] | 6.1±3.0[a] |
| FGF-23 (pg/ml) | 173±81 | 85±72 | 124±92 | 213±69 |
| Bone Volume (%) | 29.1±6.4 | 25.1±7.5 | 24.0±6.1 | 22.3±5.7 |
| Osteoid S (%) | 2.7±2.3 | 5.7±6.3 | 2.1±0.8 | 17.6±8.3[a] |
| Eroded S (%) | 10.4±2.8[c,d] | 6.9±1.3 | 5.7±2.9 | 19.1±3.0[a] |
| Osteoblast S (%) | 2.4±2.0 | 4.1±4.3 | 1.7±0.7 | 14.7±7.2[a] |
| Osteoclast S (%) | 2.4±1.1 | 0.9±0.4 | 1.0±0.8 | 4.3±1.8[a] |
| Bone Formation Rate $\mu^3/\mu^2$/day | 0.01±0.01 | 0.03±0.03 | 0.02±0.01 | 0.08±0.04[a] |

S=surface; [a]p<0.05 vs. all; [b]p<0.05 vs. Ca; [c]p<0.05 vs. Sev; [d]p<0.05 vs. CKD

This experimental model could be useful for the evaluation of ABD in future studies.

Disclosure of Financial Relationships: nothing to disclose

**PUB096**

**Paricalcitol (Pc) Therapy Upregulates Bone Fibroblast Growth Factor 23 (FGF23) and Improves the Mineralization Defect in Experimental Uremia** Michael Freundlich,[1] Ezequiel R. Bellorin-Font,[2] R. C. Pereira,[3] Evelyn Alonzo,[2] Yasmir Quiroz,[4] Janaury Bravo,[4] Eudocia Rojas,[2] Orlando Suniaga,[2] Isidro B. Salusky,[3] Bernardo Rodriguez-Iturbe,[4] Jose R. Weisinger.[2] *[1]Pediatric Nephrology, University of Miami, Miami, FL; [2]Nephrology and Renal Transplant, Hospital Universitario, Caracas, Venezuela; [3]Pediatric Nephrology, UCLA, Los Angeles, CA; [4]Renal Service, Hospital Universitario, Maracaibo, Venezuela.*

FGF23 may directly impact skeletal mineralization independent of systemic phosphate (P) homeostasis and $1,25(OH)_2D_3$ stimulates osteoblast and osteocyte FGF23 production, but little is known on the skeletal response of both hormones in CKD. We evaluated bone expression of FGF23 by immunohistochemistry and bone histomorphometry in 5/6-nephrectomy rats. Treatment (Rx) 8 weeks with Pc or enalapril (E) similarly atenuated renal insufficiency vs. vehicle-treated controls (U) (creatinine 0.76±0.1 and 0.76±0.06 vs. 1.67±0.75 mg/dl, respectively, p<0.05). Plasma Ca was similar in all groups and P remained unchanged (Pc 6.46±1.83, En 6.34±1.7, U 7.31±1.89 mg/dl). Osteocyte FGF23 expression was↑in U vs. sham-operated rats (S) and even more prominently following Pc Rx. Bone histomorphometry (Table) revealed indices of defective mineralization, markedly attenuated with Pc. In addition, Pc improved significantly eroded surfaces (ES/BS). In contrast, E displayed↓bone FGF23 expression with less attenuated %OS/BS and %ES/BS (p<0.05 vs.Pc). In summary, in experimental uremia a mineralization defect is highly prevalent and associated with↑bone FGF23 expression despite normal P levels; Pc Rx upregulated bone FGF23 expression and partially improved the mineralization defect and bone resorption. The effects of the different vitamin D sterols on osteocyte function remain to be defined in CKD.

Bone histomorphometric parameters in rats with renal ablation

| | Sham (n=10) | Uremic controls (n=8) | Uremic Enalapril (n=5) | Uremic Paricalcitol (n=7) |
|---|---|---|---|---|
| OV/TV % | 1.78±0.35 | 3.5±1.9 | 2.0±0.2 | 2.65±2.3 |
| OS/BS % | 6.36 ±0.57 | 15.4±8.1• | 8.5±2.8 | 7.8±3.8 |
| ES/BS % | 13.1±2.0† | 22.1±7.9 | 24±2.6‡ | 16±4 |

• p <0.01 vs.sham and p<0.05 vs.Pc; †p<0.01 vs.U and vs.En; ‡p<0.05 vs.Pc;

Disclosure of Financial Relationships: nothing to disclose

**PUB097**

**Metabolic Syndrome Is a Risk Factor for Coronary Artery Calcification Severity in Stage 3-5 CKD Patients** Jocelyn S. Garland,[1,2] Rachel M. Holden,[1,2] Wilma M. Hopman,[3] Robert Louis Nolan,[4] Alexander R. Morton.[1,2] *[1]Medicine, Queen's University, Kingston, ON, Canada; [2]Queen's University Vascular Calcification Investigators; [3]Community Health and Epidemiology, Kingston General Hospital; [4]Radiology, Queen's University.*

Few studies have investigated chronic kidney disease patients (CKD) for the presence of metabolic syndrome, and its relationship to coronary artery calcification (CAC). The primary research objective was to determine the cross-sectional associations of metabolic syndrome (defined as per NCEP ATP III 2004 criteria), insulin resistance (assessed by HOMA IR) and CAC (quantified by MSCT) risk factors in 95 stage 3-5 CKD patients. Biochemical risk factors for CAC including FGF-23 and osteoprotegerin (OPG) were measured. Mean age was 64 years, 40% had diabetes, 57 % male, mean BMI 31.8 kg/m$^2$ and mean eGFR 25 ml/min/1.73m$^2$. Metabolic syndrome (3 of 5 criteria) was identified in 80%. Median CAC was higher in those with metabolic syndrome (239 *v* 10; P=0.001). By multi-nomial logistic regression, with CAC categories as the dependent variable and CAC = 0 as the reference group, an increased risk in CAC severity was demonstrated in those with metabolic syndrome. This relationship remained when adjusted for age and eGFR. By linear regression with all five metabolic syndrome criteria as covariates, hyperglycemia, hypertriglycidemia and low HDL were the most important risk factors in predicting logCAC. Considering insulin resistance, HOMA IR was measured in 72 of 95 patients who were not receiving insulin therapy. Median HOMA IR was higher in those with metabolic syndrome (2.5 *v* 1.1; P=0.001), and was positively correlated with log FGF 23 (r = 0.25; p = 0.04). HOMA IR inversely correlated with 25-OHD level (r = 0.22; p = 0.06), OPG (r = -0.20; p = 0.08) and OPG corrected for BMI (r = -.47; p < 0.001). In conclusion, metabolic syndrome was found to be highly prevalent in this cohort of stage 3-5 CKD patients, and there was an increased risk of CAC severity in metabolic syndrome patients. Insulin resistance was associated with risk factors for CAC. Studies are needed to identify whether treatment of metabolic syndrome improves CAC severity and progression.

Disclosure of Financial Relationships: nothing to disclose

**PUB098**

**Intravascular Treatment to Symptomatic ADPK(L)D** Rikako Hiramatsu, Yoshifumi Ubara, Junichi Hoshino, Takafumi Toyohara. *Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

(Introduction) Since kidney and liver in ADPKD patients are usually supplied by well-developed arteries, we have attempted to contract enlarged kidney and liver in ADPKD patients by transcatheter arterial embolization (TAE) method using intravascular coils.

(Method)From 1996 to 2010, 785 patients with ADPKD were treated for abdominal distension due to renal enlargement and hepatomegaly. (Results)Kidney-dominant enlargement was present in 69% of ADPKD patients. The kidney and liver were similarly enlarged in 18%. Liver-dominant enlargement was present in 13 %. We have tried renal TAE in 653 patients with intractable enlarged kidneys. After TAE till today 587 patients showed a favorable clinical course. On 66 patients concomitant complications of other organs, such as cancer and cardio-cerebral vascular diseases became the cause of death. After renal TAE, 18 patients underwent renal transplantation without nephrectomy. Next, we have tried hepatic TAE in 282 patients with intractable symptomatic polycystic liver. 247 out of 282 patients have had a favorable clinical course. However, we could not cure 35 patients with severe hepatic failure and peritonitis with massive ascites, and hepatic cystic infection.(Conclusion)No major complications related to the TAE procedure have been encountered for either the kidney or the liver. Our treatment thus far may be less effective in reducing total liver volume than surgical hepatectomy. On the otherhands, our treatment should be safe even when performed repeatedly or after a relapse following surgical therapy. We are preparing for further TAE in patients with a poor initial result. TAE also may be an option for treatment of patients with symptomatic polycystic liver as well as kidneys in poor general medical condition who are not candidates.

Disclosure of Financial Relationships: nothing to disclose

**PUB099**

**Histomorphometrical Analysis of Ectopic Ossification in a Hemodyalisis Patient** Rikako Hiramatsu, Yoshifumi Ubara, Junichi Hoshino, Takafumi Toyohara. *Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

A 55-year-old Japanese woman receiving hemodialysis during 5-years was admitted to our hospital for the evaluation of hypercalcemia and the ectopic calcinotic lesion in June 2009. Radiography revealed massive metal-density deposition on soft tissue near the bilateral hips, bilateral shoulders and left forearm. Because histology of ectopic calcinotic lesion showed bone tissue, histomorphometric examination using undecalcified thin sections was performed on iliac bone as well as ectopic bone on outer side of the iliac bone, and was compared with each other. Both specimens showed osteitis fibrosa, but bone formation rate was accelerated in ectopic bone than in iliac bone by using dLS/BS (39.9/17.2=2.3) and BFR/BV (798.5/214.0=3.7). The rate of bone formation to bone resorption was accelerated in ectopic bone (81.6/14.6=5.59) than in iliac bone (53.8/22.8=2.35) by using the rate of OS/BS to ES/BS. Woven bone formation was more prominent in ectopic bone. Trabecular bone structure was revealed to continue to layers of unmineralized osteoid lesion including mineralized island bones independently on areas different from calcification(Fig).

The mechanism how bone arises from multipotential mesenchymal cells in soft tissue could not yet be proved. However, once bone formation is initiated, bone formation would be accelerated due to this driving force of hyperparathyroidism, resulting in an increase of bone volume.

Disclosure of Financial Relationships: nothing to disclose

**PUB100**

**Bone Mineral Density Can Predict Bone Loss after a Well-Functioning Kidney Transplantation** Carolina Lara Neves, Luciene M. dos Reis, Katia R. Neves, Rosa Ma Moyses, Vanda Jorgetti. *Nephrology, University of São Paulo, São Paulo, São Paulo, Brazil.*

**Introduction:**Post-transplant bone disease is a complex disorder that extends beyond simple alterations BMD. Low BMD had not enough predictive value of fracture and bone histology.The KDIGO suggest that DXA screening should only be done in individuals with a well-functioning allograft (CKD stages 1-3T).**Objectives:** Evaluate BMD after stable kidney transplantation (KT) and determine factors associated to bone loss. Ensure the predictive value of BMD on bone histology.**Methods:** Patients were 36.4 ± 8.9 years and CKD 1-3T.They were receiving low prednisone dosis and the same immunosuppressive therapy since KT. We excluded patients with gonadal disfunction, parathyroidectomy or with calcium, vitamin D or bisphosphonates treatment. They were submitted to clinical and biochemical evaluation, DXA and transiliac bone biopsy with histomorphometric analysis. We classified patients with bone loss through DXA T score < -1,0 SD, on femur or lumbar.**Results:** Hypercalcemia was observed in 40.7%, hypophosphatemia in 26%. Users of tacrolimus had lower phosphorus than users of cyclosporine(p=0.019). PTH > 65 pg/ml in 30% of them. 85% and 95% presented elevation of osteoprotegerin and deoxypyridinoline, respectively. 63% of our patients had 25(OH) < 30ng/mL. 57% of the patients had osteopenia or osteoporosis by DXA. Mean BV/TV was not decreased, but 30% presented low bone volume. Low BFR was seen in 60% of the patients, 46% presented enlarged MLT. All patients with 25 (OH) D insufficiency/deficiency had enlarged MLT. PTH was the only predictor of serum phosphorus (p=0.042), lumbar BMD (p=0.044) and osteoid volume (p=0.001). Patients with bone loss according to DXA presented lower BMI (p=0.02), BV/TV (p=0.03) and TbN (p=0.01), and higher TbSp(p=0.009).**Conclusion:** After stable and well-functioning KT, patients still presented abnormalities of mineral metabolism and bone histology. The predominance of mixed bone disease are related to immunosuppressive drugs, hypophosphatemia, persistence of hyperparathyroidism and 25 (OH) D insufficiency. BMD through DXA evaluation showed a good predictive value to structural and statics parameters of bone histology.

Disclosure of Financial Relationships: nothing to disclose

**PUB101**

**Efficacy of Weekly (QW) or Bi-Weekly (BIW) IV Vitamin D Analog Therapy in Hemodialysis Patients** Neeta O'Mara,[1] Amelia Gajary,[1] Toros Kapoian,[2] Lois E. Lamanna,[1] Diane M. Brink,[1] Christine L. Velsor.[1] *[1]Dialysis Clinic, Inc (DCI), North Brunswick, NJ; [2]Med/Neph, UMDNJ-RWJ Med School, New Brunswick, NJ.*

**BACKGROUND**: Secondary hyperparathyroidism is a common complication in dialysis patients. Management includes methods to reduce serum phosphate and vitamin D analog therapy such as calcitriol, doxercalciferol, or paricalcitol. However, these agents are supplied in a limited number of dosage strengths in single-dose vials. **METHODS**: In order to minimize waste, we designed a protocol to align the dose and dosing interval with vitamin D analog vial size. This resulted in a number of patients receiving QW or BIW IV vitamin D rather than the usual thrice weekly (TIW) dosing. Less frequent vitamin D dosing has previously been reported to be effective in a limited number of dialysis patients. **RESULTS**: Twelve patients (mean age 66 years; mean dry weight 80.4 kg; 8 M, 4 F; 7 black, 5 white; 5 diabetes) received QW (n=10) or BIW (n=2) IV vitamin D analog therapy. Nine patients received doxercalciferol (mean dose 3.9 mcg/week; range 1.5-8 mcg/week), and 3 received paricalcitol (1 mcg/week). Results provided in table below. We defined responders as those patients who either had an elevated PTH (>300) which declined with therapy, or who had a PTH in target (150-300) that remained in target.

| | Baseline | 3 months |
|---|---|---|
| | mean (±SD) | mean (±SD) |
| **iPTH (pg/mL) all** | 370 (254) | 492 (311) |
| iPTH (pg/mL) responders | 324 (223) | 303 (158) |
| iPTH (pg/mL) non-responders | 433 (331) | 755 (279) |
| **cCa (mg/dL)** | 9.4 (0.45) | 9.0 (0.55) |
| **PO4 (mg/dL)** | 5.5 (1.6) | 5.1 (1.4) |

Of the 12 patients, 5 patients demonstrated significant increase in PTH value (non-responders) and were subsequently changed back to TIW dosing. The need for more frequent dosing did not appear to be dependent on initial PTH level, specific vitamin D analog, or dose of vitamin D used. **CONCLUSIONS**: Less frequent IV (BIW or QW) vitamin D analog dosing appears to be an acceptable option in some patients. However, more information is needed to better prospectively identify patients in whom this regimen will be effective.

Disclosure of Financial Relationships: nothing to disclose

**PUB102**

**CKD Is Associated with Increased Serum Levels of Energy-Regulating Hormones** Rodrigo B. Oliveira,[1] Luciene M. dos Reis,[1] Fabiana G. Graciolli,[1] Ana L. E. Cancela,[1] Maria Eugenia F. Canziani,[2] Aluizio B. Carvalho,[1] Lilian Cuppari,[2] Yves Sabbagh,[3] Vanda Jorgetti,[1] Susan Schiavi,[3] Rosa Ma Moyses.[1] *[1]Nephrology, University of Sao Paulo, Brazil; [2]Nephrology, Federal University of Sao Paulo, Brazil; [3]Genzyme Co.*

Recent studies have shown that bone and energy metabolism are balanced with each other and are likely co-regulated. Serum proteins, such as Leptin (Lep), Adiponectin (Adip) and Serotonin (Ser) regulate energy metabolism, as well as bone mass. Previous studies have shown an increase of Lep and Adi in CKD, but there is no data regarding Ser. We evaluated serum levels of Lep, Adip and Ser in 40 predialysis CKD patients [50 ± 11 yrs., creatinine clearance (CrCl) = 35 ± 16 ml/min] that were randomized to receive calcium acetate (Ca) or sevelamer hydrochloride (Sev) during 6 weeks. At baseline, median serum Lep was 19.7 ng/ml (5.6-54.1) and correlated significantly with BMI (R=0.55), serum phosphate (R=0.38) and 25 vitamin D (R = -0.49). Median serum Adip was 11.0 mg/ml (8.1-13.9. Serum Ser was elevated (220 ± 102 ng/ml), and correlated with BMI (R = -0.39) and CrCl (R = -0.42). After 6 wks on P binder therapy, we observed a significant decrease of serum Lep in the entire study population (19.7 vs. 14.9; $p < 0.05$). However, although presenting non-different baseline values, a significant decrease was observed only in Sev-treated patients (9.8 vs. 7.4 ng/ml; $p < 0.05$), whereas this was not found in Ca-treated patients (26.5 vs. 26 ng/ml, ns). We found no differences between the intra-group changes in serum Lep. No significant changes were seen in serum Ser after P binder therapy. Our results confirm that hormones that regulate energetic metabolism are dysregulated in CKD. The correlation between Lep and phosphate, and the apparent reduction of serum Lep after P binder therapy suggest that pharmacological inhibition could be achieved in CKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB103**

**Phosphate Binding by Sevelamer: Effects of HCl, Carbonate, Tablet, Powder & pH** Edward A. Ross,[1] Jennifer Paugh-Miller,[1] Chris Batich,[2] Sam Popwell.[2] *[1]Division of Nephrology, Univ. of Florida; [2]Dept of Materials Science & Engineering, Univ. of Florida, Gainesville, FL.*

**Background & Aims:** The amount of phosphate (P) bound by sevelamer is dependent on 1) uptake of aqueous P-containing solution into the expanding hydrogel, and 2) its cationic charge due to protonation during manufacture and that resulting from the milieu's acidity. With the importance of pH and physical properties of gel-expansion, we questioned whether there would be differences in P binding *in vitro* between sevelamer HCl and carbonate, and whether they were in tablet or powdered formulations.

**Methods:** We incubated sevelamer HCl (tablets or laboratory crushed) and carbonate (tablets or factory-powdered) for 1 hr at pHs 1, 2.3 or 7 (simulating degrees of gastric acidity), stirred at 37° C. They were then stirred at pH 7 for 3 hrs in a 20 mM P solution (simulating small bowel conditions). Atomic absorption quantified [P] for calculating P bound per gm binder.

**Results:** The sevelamer HCl *v.* carbonate compounds had statistically ($p>0.8$) similar P binding for every experimental condition. For tablets there was greater P binding with increasing levels of incubation acidity: for pH 7, 2.3 and 1 there was, respectively, 1.42, 2.97, and 4.22 meq P bound/g sevelamer HCl tablet; compared to 1.61, 2.26, and 4.61 meq/g sevelamer carbonate tablet ($p<0.001$), representing up to approx 200% greater binding between pH 1 and 7. While there was no difference in P binding powder *v.* tablet at pH 7, acidity had less of an effect on powders: 1.36, 1.78, and 2.73 meq/g sevelamer HCl powder *v.* 1.30, 1.51, and 3.29 meq/g sevelamer carbonate powder. Longer acid incubations at 3 hrs had no greater effect than that at 1 hr (p=NS).

**Conclusions:** Despite differences in the synthesis of sevelamer (HCl *v.* carbonate) and its formulation (tablet *v.* powder) there is similar P binding that has a significant dependency on pH in incubation conditions that we believe simulate exposure to gastric acidity in vivo. These *in vitro* findings support clinical P-binding equivalency between sevelamer HCl and carbonate; however, we believe hydrogel diffusin kinetics affect the pH dependency of P binding by tablets *v.* powdered formulations.

Disclosure of Financial Relationships: Other Relationship: Consultancy and honoraria from Genzyme and Shire Corps.

**PUB104**

**Sevelamer Improves Serum Electrolyte Profile, Metabolic and Cardiovascular Markers and Survival in ESRD Patients on Hemodialysis Treatment** Siren Sezer, Sebnem Karakan, Nurhan Ozdemir. *Nephrology, Baskent University Hospital, Ankara, Turkey.*

**Background:** This study prospectively evaluated the 2 years impact of sevelamer hydrochloride and calcium acetate on the metabolic indices in HD patients.

**Methods:** 126 HD patients were included between 2007 to 2009. HD patients with serum phosphate levels more than 5.5 mg/dl were randomly assigned to sevelamer hydrochloride (group 1, n=63) or calcium acetate (group 2, n=63). Baseline laboratory and demographic features of the patient groups were similar.

**Results: P**atients in group 1 exhibited lower levels of serum potassium, total cholesterol, serum low-density lipoprotein and homocysteine and higher HDL and vitamin B12 levels. All cause of mortality was 8% in sevelamer and 11% in calcium acetate group (log-rank $p<0.05$).

Comparison between groups

| Parameter (serum) | GROUP 1 | | GROUP 2 | |
|---|---|---|---|---|
| | Baseline-24 month | p | Baseline-24 month | p |
| Phosphorus (mg/dL) | 8.0 ±3.2-6.1 ± 1.6 | <0.05 | 7.4± 1.5-5.7±1 | <0.05 |
| Alkaline phosphatase (UI) | 227±69-195±81 | <0.05 | 275 ±130-198 ±78 | <0.05 |
| Calcium (mg/dL) | 9.3±0.1-9,1±0.2 | <0.05 | 9.2± 0,8-9,7 ± 0,4 | <0.05 |
| iPTH (pg/mL) | 367.5±306.2 -385.5±298.4 | <0.05 | 492.6±384.2 -321.5 ± 199.7 | <0.05 |
| Ca x P product | 74,4±12.88 -55,51±11.8 | <0.05 | 68.08± 14,56 -55.25 ±10,9 | <0.05 |
| K (mmol/dL) | 4,95±0,65 -3,86±0,78 | <0.05 | 5,29±0,80- 5.14 ±0,73 | non significant |
| Total cholesterol (mg/dl) | 196.8±57.8 -157.8±44.9 | <0.05 | 181,8±59,0 -187 ±39.4 | non significant |
| LDL ( mg/dL) | 99,3±48,0 -84,3±27.5 | <0.05 | 120.5±42,1- 112 ±32.4 | non significant |
| HDL ( mg/dL) | 43.0±5.4-49.0±7.3 | <0.05 | 33,1±6,5-30,6 ±4.9 | non significant |
| Vitamin B12 (IU/L) | 472.8±308.72 -458.8±258.1 | <0.05 | 335,96±191,79 -398,96 ±162,45 | <0.05 |
| Homocysteine | 17,7±10,1-14,5±7.9 | <0.05 | 16,0±11,3-15,6±11,3 | non significant |

**Conclusion:** Sevelamer improves serum lipid profile, vitamin B12 and homocysteine levels indicating that sevelamer has the potential to significantly improve cardiovascular risk. All-cause mortality is decreased in sevelamer group.

Disclosure of Financial Relationships: nothing to disclose

## PUB105

**Efficacy and Tolerability of Lanthanum Carbonate in Clinical Practice. Retrospective Multicenter Study** Jose-Vicente Torregrosa,[1] M. T. Gonzalez,[2] Jorge B. Cannata-Andia,[3] Dolores Arenas,[4] Jesus Montenegro,[5] Jose Mora-Macia,[6] Emilio E. Gonzalez-Parra,[7] F. Rios.[8] *[1]H Clinic; [2]H Asturias; [3]H P Socorro; [4]H Galdakao; [5]FMC Granollers; [6]FJ Díaz; [7]FMC S Luciano; [8]H Belvitge.*

Introduction: Lanthanum Carbonate (LC) is an effective and well tolerated noncalcium-based phosphate binder to treat hyperphosphatemia in CKD patients. The efficacy and tolerability of LC therapy was assessed in daily clinical practice

Methods: Retrospective multicenter study recording data of patients starting LC and a follow-up 12 months from 65 Spanish HD units. Blood levels of P, Ca, PTH, AST, ALT, GGT were collected before starting LC and at 1,3,6,9 and 12 months. Adverse events (AEs) were recorded.

Results: 622 (406 male-216 female) were analyzed, mean age 61(22-94) years, time on dialysis 77±68,3 months, 23% diabetics. Evolution of P and Ca are shown in the Table 1. There was a statistically significant decrease in levels of P between basal visit and the follow-up analyzed visits ($p<0.01$)* with a total reduction of 25.2% from basal to final visit. No significant changes in PTH levels. Mean AST, ALT and GGT blood at starting and after 12 months were respectively (U/l): 17.1±10.3 vs 16.1±10.6, 16.5±11.8 vs 15.5±8.9 and 35.8±51.4 vs 41.4±83.8 (p=ns in all of them). 124 (19.3%) patients reported AEs. The most frequent AEs (%) were: nausea (16), flatulence (14), dyspepsia (13), constipation (13), abdominal pain (13), diarrhea (6), vomiting (5) and anorexia (5). None was of seriously intensity. The mean dose of CL was progressively increased during the first 6 months but not significantly.

| Visit (Months) | CL Average Dosage(mg) Mean±SD | P Levels ( mg/dl) Mean±SD | Ca Levels ( mg/dl) Mean±SD |
|---|---|---|---|
| Basal | 1906.9±603 | 6.47±1.4 | 9.00±1.29 |
| 1 | 2008.5±639 | 5.48*±1.5 | 9.00±0.9 |
| 3 | 2189.4±671 | 5.36*±1.5 | 8.98±1.24 |
| 6 | 2297.0±749 | 5.03*±1.4 | 8.99±1.24 |
| 9 | 2318.6±821 | 5.04*±1.3 | 9.00±0.89 |
| 12 | 2290.6±783 | 4.84*±1.3 | 8.90±0.91 |

Conclusion: In clinical practice, LC was significantly effective since the first month, showing a progressively reduction of P levels. LC showed acceptable tolerance and safety profile.

Disclosure of Financial Relationships: nothing to disclose

## PUB106

**Non-Invasive Assessment of Renal Osteodystrophy: Comparison of Bone Histology with Quantitative Computed Tomography (QCT)** Katherine Wesseling-Perry,[1] Harald Jueppner,[2] Vicente Gilsanz,[3] Kevin V. Lemley,[3] Joshua Zaritsky,[1] Barbara Gales,[1] Isidro B. Salusky.[1] *[1]Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Endocrine Unit, Harvard Medical School/Mass General Hospital, Boston, MA; [3]Children's Hospital Los Angeles, Los Angeles, CA.*

The need for non-invasive assessment of bone has been emphasized by KDIGO. Thus, femoral shaft and lumbar vertebral structure were characterized by QCT in 28 CKD pts. (19M/9F, 23 CKD5/5 CKD2-4, age 16±2 yrs). Values were compared to healthy children of similar age and size. Biochemical values (SD) were: Ca: 8.9±0.9 mg/dl; P:5.8±1.3 mg/dl; Alk P'tase: 267±190 IU/L; tCO2: 22±4 mEq/L; PTH: 549±324 pg/ml. Both lumbar and femoral bone density were greater in patients than in normal controls (cancellous bone Z: 1.7±2.1(SD) ($p<0.05$ from control), cortical bone Z: 0.9±1.4 ($p<0.05$ from control)). 16 subjects underwent BBx within 2 wks of imaging and histomorphometric parameters were compared to QCT measures [table 1]. Femoral CBD was inversely correlated with trabecular separation but directly related to osteoid accumulation. Femoral CBD was inversely correlated with Alk P-tase (r= - 0.38, z=1.91). Thus, defects in bone structure and mineralization may be detectable by QCT; further studies are warranted.

Correlation between Bone Histology and QCT

| QCT | Bone Histology | | | | |
|---|---|---|---|---|---|
| | Bone volume (BV/TV) (%) | Trabecular number (Tb.N) (n) | Trabecular Separation (Tb. Sp) (um) | Osteoid volume (OV/BV) (%) | Osteoid thickness (O.Th) (um) |
| Vertebral cancellous bone density | r=0.24, NS | r=0.27, NS | r= - 0.33, NS | r=0.30, NS | r=0.27, NS |
| Vertebral cross sectional area | r= - 0.12, NS | r= - 0.04, NS | r= -0.11, NS | r=0.10, NS | r=0.17, NS |
| Femoral cortical bone density | r=0.07, NS | r=0.43, p=0.09 | r= - 0.21, NS | r= - 0.01, NS | r= - 0.04, NS |
| Femoral cross sectional area | r=0.24, NS | r=0.39, p=0.13 | r= - 0.62, p=0.01 | r=0.24, NS | r=0.42, p=0.10 |
| Femoral cortical bone area | r=0.35, NS | r=0.41, p=0.12 | r= - 0.67, p<0.01 | r=0.35, NS | r=0.40, p=0.12 |

Disclosure of Financial Relationships: Honoraria: Genzyme.

## PUB107

**Cinacalcet Hydrochloride with Very Low Doses of Vitamin D Does Not Reduce the Poorly Mineralized Bone Areas in Patients with Secondary Hyperparathyroidism** Aiji Yajima,[1] Masaaki Inaba,[2] Shigeru Otsubo,[3] Kosaku Nitta,[4] Tadao Akizawa.[5] *[1]Nephrology, Towa Hospital, Tokyo, Japan; [2]Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan; [3]Nephrology, Sangenjaya Hospital, Tokyo, Japan; [4]Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan; [5]Nephrology, Showa University, School of Medicine, Tokyo, Japan.*

Purpose; The increase of poorly mineralized bone areas will lead to high fracture rate. Direct mineralization around lacunae was seen and the poorly mineralized areas were significantly reduced after parathyroidectomy (Renal Week 2009, JBMR in press). We investigated changes in poorly mineralized areas after the treatment with cinacalcet hydrochloride (HCl).

Methods; Seven hemodialysis (HD) patients received 12.5-50 mg/day of cinacalcet HCl with 0.5-1.5 µg/day of oral alfacalcidol for 1 year (Group I) to reduce serum PTH levels. In addition, three other HD patients received cinacalcet HCl with 2.5-5.0 µg/day of Maxacalcitol intravenously for 1 year (Group II). Transiliac bone biopsy specimens were obtained before and after the treatment to measure the poorly mineralized area in basic multicellular unit (PM.BV/BV(BMU)) and bone structural unit (PM.BV/BV(BSU)).

Results; PM.BV/BV(BMU) before and after the treatment were 15.6 ± 11.2 and 15.0 ± 14.6 % in Group I, but were reduced from 4.5 to 1.3 %, from 0.9 to 0.5 %, and from 0.7 to 0.5 %, in the 3 Group II patients, respectively. PM.BV/BV(BSU) before and after the treatment were 5.0 ± 12.7 and 7.9 ± 4.6 % in Group I, but were reduced from 11.3 to 1.6 %, from 6.4 to 1.1 %, and from 5.5 to 2.4 %, in the 3 Group II patients.

Conclusions; Plasma $1,25(OH)_2D_3$ levels measured from 7 to 10 days after the initial administration of 2.0 µg/day of alfacalcidol were below the upper limit of the normal range (20 to 60 pg/mL) (AJKD 2003). Oral administration of 0.5-1.5 µg/day of alfacalcidol may not be effective to mineralize the uncalcified matrix and poorly mineralized areas could not be reduced after the treatment with cinacalcet HCl, though 2.5µg/day or more of Maxacalcitriol is sufficient to reduce the poorly mineralized areas in patients receiving cinacalcet HCl.

Disclosure of Financial Relationships: nothing to disclose

## PUB108

**Severe Hypercalcemia on Osteoporosis Prevention with Calcium-Vitamin D Supplements in Elderly Patients with Chronic Kidney Disease** Yisfalem W. Alamdew, Hugo J. Villanueva, Lin N. Lwin, Jinil Yoo. *Nephrology, Montefiore Medical Center North Division, Bronx, NY.*

One of the most prevalent and disabling medical conditions in elderly women is osteoporosis for which daily supplement of cacium-vitamin D (Ca-Vit D) is recommended. In a sub group of elderly women with chronic kidney disese(CKD), severe symptomatic hypercalcemia is observed without a clear etiology even after extensive diagnostic work-ups for malignancies, hyperparathyroidism, and granulomatous diseases, but with clinical evidences of low bone turnover and on daily dose of Ca-Vit D.

We illustrate 2 cases who were on Ca-Vit D for osteoporosis. Case I who was on metolazone for fluid overload presented initially with hypercalcemia (18.5mg/dL), and metabolic alkalosis ($HCO_3$ 31 mEq/l and PH=7.51), suggestive of milk-alkali syndrome. Case II had several episodes of symptomatic hypercalcemia of undetermined etiology and underwent a bone biopsy, diagnostic of adynamic bone disease. The following is a summary of clinical features.

| | Case I | Case II |
|---|---|---|
| Age/Gender | 79/Female | 76/Female |
| CKD stage/etiology | III to IV/type II DM | IV to V/hypertension |
| Serum calcium (mg/dL) (8.5-10.5) | 18.5 | 13.3 |
| Phosphate (mg/dL) (2.5-4.5) | 3.5 | 5.8 |
| Albumin (g/dL) (3.2-4.8) | 4.1 | 3.3 |
| Intact PTH * (pg/dL) (10-65) | 4.7 | 12.3 |
| 25(OH) Vit-D (ng/dL) (10-55)** | 38 | 16 |
| 1,25(OH)$_2$ Vit-D (pg/dL) (20-76) | 14 | 16 |

* Parathyroid hormone ** The Laboratory's reference range

The hypercalcemic episodes were resolved quickly with treatment and not recurred by avoiding Ca-Vit D supplements. The PTH rose gradually above normal levels. A subgroup of elderly patients with CKD have low to normal levels of PTH, decreased urinary calcium excretion because of decreased GFR but normal intestinal calcium absorption in presence of normal 25-OH Vit D and/or 1,25(OH)$_2$ Vit D, especially on Ca-Vit D supplements. In these cases, a low bone turnover induced by relatively suppressed PTH, can cause hypercalcemia because of its decreased calcium buffering capacity.

We present our cases to heighten awareness of the disorder, "hypercalcemia and low bone turnover in CKD" on elderly women with Ca-Vit D supplements for osteoporosis.

Disclosure of Financial Relationships: nothing to disclose

## PUB109

**Paricalcitol Use Allows Greater Exposure to Vitamin D Receptor Activation Compared to Doxercalciferol in Hemodialysis Patients** Utpaul Audhya, Samina Khan, Beverly A. Johns, Steven E. Marx. *Abbott, Abbott Park, IL.*

INTRODUCTION: Vitamin D receptor (VDR) activators have a suppressive effect on the RAAS, as well as anti-inflammatory and anti-fibrotic effects, which most likely accounts for their overall mortality benefit. Improved survival with higher dose of VDR activators has previously been established. The objective of this study is to compare VDR activation by using dose exposure between paricalcitol and doxercalciferol in adult hemodialysis patients.

METHODS: A cohort analysis from 2004 through 2009 was conducted in a large dialysis organization in adult hemodialysis patients. Cohorts were based on adult patients receiving a minimum of 10-consecutuive doses of paricalcitol or doxercalciferol. Consecutive was defined as simultaneous dialysis session. The index date was the first dose of paricalcitol or doxercalciferol. Generalized linear models with a log link Poisson regression, adjusting for age, gender, race, diabetes status, study entry period, and iPTH was conducted. A propensity matched (age, gender, race, diabetes status, iPTH) analysis was conducted as described above. An additional subanalysis was conducted including baseline albumin in the propensity match.

RESULTS: Multivariate analysis of paricalcitol-treated patients (n=39,476) vs. doxercalciferol-treated (n=2,681) demonstrated statistically significant greater number of doses administered with paricalcitol: 1.12, p<0.0001. Propensity matched analysis (n=2,671 per cohort) demonstrated similar results: 1.10, p<0.0001 more doses administered in the paricalcitol cohort compared to doxercalciferol cohort. The subanalysis including baseline albumin demonstrated the same results: 1.10, p<0.0001.

CONCLUSION: This study demonstrated that in both a multivariate and a propensity matched analysis patients received more doses in paricalcitol treated cohort compared to the doxercalciferol cohort. More VDR activator doses administered may lead to greater vitamin D receptor activation, which may improve clinical outcome. Further research is needed to confirm these results.

Disclosure of Financial Relationships: Employer: Abbott Laboratories; Ownership: I have Abbott stock.

## PUB110

**Anemia and Vitamin D Deficit in Chronic Kidney Disease Stage 2-5nd: A New Vitamin D Pathogenic Role?** Secundino Cigarran,[1] Emilio E. Gonzalez-Parra,[3] Guillermina Barril,[4] Francisco Coronel,[2] Montserrat Pousa.[1] *[1]Nephrology, Hospital Da Costa, Burela, Lugo, Spain; [2]Nephrology, Hospital Clinico San Carlos, Madrid, Spain; [3]Nephrology, Fundación Jimenez Díaz, Madrid, Spain; [4]Nephrology, Hospital Universitario de La Princesa, Madrid, Spain.*

Patients with chronic kidney disease (CKD) are at high risk for cardiovascular disease, and despite recent advances in hypertension control, anemia management, mortality remains high. Vitamin D deficiency is high at early stages of CKD and plays a biologic role, independiently associated to decrease glomerular filtration rate (GFR), as a pleitropic hormone. The aim of this, cross-sectional, study is to assess the vitamin D influence on anemia in CKD patients.

563 pts were enrolled,mean age 68-±13 years, mean GFR 46.67± 23.9 ml/min/1.73m$^2$, 38% female and 30% DM, mean Hb level 13.16 ± 1.68 gr/dl. Anemia defined as Hb level<12.5 gr/dl was met in 201 patiens (35.9%), Parameters analyzed were, Hb, eGFR, nutritional (albumin, prealbumin), inflammation (C reactive protein, fibrinogen) and cardiovascular (Uric acid, urinaryAlb/ creatinin index) parameters and mineral bone markers. Data were analyzed with SPSS 15 for Windows (SPSS, Chicago ILL. USA).

Compared with non anemic patients, anemic were older (71.17± 11.79 vs 66.03±13.7 years , P< 0.001), diabetic (35.6 vs 25.7% , P<0.001), lower GFR (36.54±17.9 vs 52.36 ± 25.0, p< 0.001).

T paired test

| Variable | Anemic ( N=201) | Non Anemic N=359 | P |
|---|---|---|---|
| Serum Albumin (gr/dl) | 4.18 ±0.41 | 4.34±.30 | .001 |
| Prealbumin (mg/dl) | 29.93±6.96 | 31.62±7.13 | .001 |
| Calcium (mg/dl) | 9.20±.53 | 9.38±.41 | .001 |
| Phophorous (mg/dl) | 3.76± .83 | 3.41±..61 | .001 |
| Parathormone (pg/ml) | 100±96.8 | 68±57.3 | .001 |
| CaxP (mg2/dl2) | 34.59±8.11 | 32.52±6.66 | .001 |
| 25 vit D (ng/ml) | 16.7±9.47 | 18.6±10.7 | .043 |
| 1, 25 D3 (pg/ml) | 34.6±14.24 | 40.85±16.43 | NS |
| Urinary Alb/Cr Index (mg/gr crea) | 541.63±1260 | 295.2±598 | .001 |

NS = Not Significance

Conclusion: Vit D has influence on anemia. Given the high prevalence of anemia and 25 D deficiency, further studies should explore the effects of 25D nutritional replacement on erythropoiesis in CKD patients.

Disclosure of Financial Relationships: nothing to disclose

## PUB111

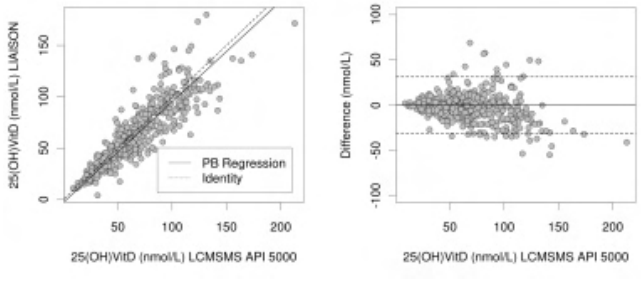
**Comparison of Diasorin LIAISON Vitamin D Assay Against LC-Tandem MS in a CKD Cohort from the CanPREDDICT Study** Daniel T. Holmes, Suzette M. Walsh, Hans Frykman, Ognjenka Djurdjev, Adeera Levin. *University of British Columbia.*

The purpose was to compare an automated 25(OH)VitaminD (VitD) method with a reference method in patients with CKD. A 416 pt cohort was taken from the Canadian Study of Prediction of Risk and Evolution to Dialysis, Death and Interim Cardiovascular Events Over Time (CanPREDDICT) study. Serum was collected at 25 Canadian sites and stored at -80°C until analysis. LCMSMS was performed on the Applied Biosystems API5000 using a modification of their iMethod. Automated VitD, Bone ALP (BAP), and 1-84PTH analysis was performed using the Diasorin LIAISON analyzer (kits provided by Diasorin). Passing Bablok regression was: LIAISON=0.980[CI 0.932-1.031]xLCMSMS-2.89[CI -5.45,-0.134]nM. Correlation between methods was:ρ=0.90 overall, 0.55 for definite deficiency (<25nM,n=27),0.77 for moderate deficiency (25-75nM,n=227), and 0.57 for VitD sufficient pts (>75nM,n=162). Interpretive concordance rates (CI) in these 3 categories were: 0.85(0.65-0.95), 0.88(0.83-0.92), and 0.80(0.73-0.86). Discrepancies did not appear related to eGFR. The Diasorin LIAISON VitD method produces more accurate results compared to LCMSMS than has been previously reported, with slightly lower ρ. Clinical concordance rates of LCMSMS and LIAISON VitD results were reasonable to good.

Patient Characteristics

| | **Median** | **IQR** | **Normal Range** |
|---|---|---|---|
| Age (y) | 66.8 | [59,76] | NA |
| eGFR (mL/min) | 25.9 | [20.0-33.0] | NA |
| Cre M (umol/L) | 220 | [176-281] | 60-100 |
| Cre F (umol/L) | 171 | [143-214] | 50-90 |
| Ca (mmol/L) | 2.30 | [2.2-2.4] | 2.18-2.58 |
| PO4 (mmol/L) | 1.18 | [1.00-1.33] | 0.80-1.60 |
| BAP (ug/L) | 14.1 | [9.8-18.4] | see below |
| 1-84PTH (pmol/L) | 4.63 | [2.96-7.80] | 0.70-3.90 |

Table 1: Relevant clinical and biochemical markers in the 202 F and 214 M subjects. BAP N Range, M: 6-30, F: 3-19 (premenopause); 6-26 (postmenopause) ug/L.




Disclosure of Financial Relationships: nothing to disclose

## PUB112

**Intraperitoneal Paricalcitol Therapy in CAPD Patients with Secondary Hyperparathyroidism** Ji-Min Jeon, Yong-Ki Park. *Divison of Nephrology/ Internal Medicine, DongRae BongSeng Hospital, Busan, Korea.*

Secondary hyperparathyroidism(2HPT) is a major complication in ESRD patients undergoing dialysis. In hemodialysis patients with secondary hyperparathyroidism, intravenous administration of paricalcitol became widely utilized. In CAPD patients, however, the intravenous administration of paricalcitol which requires frequent visits to the clinic is not practical. The purpose of this study was to determine the safety and effect of intraperitoneal paricalcitol therapy in CAPD patients. The subjects of this study were two CAPD patients with 2HPT . They had already received oral calcitriol pulse therapy for 3 months. and they had refused parathyroidectomy and intravenous paricalcitol which require frequent visit to the hospital. and It is not introduced paricalcitol capsule in korea yet. so, We have experimentally tried the intraperitoneal paricalcitol therapy. Two patients were taught to inject the paricalcitol(5μg) directly into the dialysate three times per week before bedtime. Blood samples for measurement of intact parathyroid hormone(iPTH),

serum ionized calcium, serum phosphate, serum total alkaline phosphatase levels were obtained at baseline and 1,2,3 months of treatment. If hypercalcemia(>10.5 mg/mL) and hyperphosphatemia(>6.5 mg/dL) developed, the patients were stopped from treatment. After intraperitoneal paricalcitol for 3 months, there was a significant drop of iPTH leve.

Table 1.Patients characteristics and serial change of intact parathyroid hormone

| | Age(years) | Sex | Duration of CAPD(months) | Baseline(pg/mL) | 1 month | 2 months | 3 months |
|---|---|---|---|---|---|---|---|
| Case 1 | 55 | Male | 98 | 924 | 684 | 621 | 598 |
| Case 2 | 70 | Male | 82 | 870 | 852 | 764 | 707 |

There were no definite hypercalcemia and hyperphosphatemia. In conclusion, Intraperitoneal paricalcitol therapy might be effective for suppressing iPTH in CAPD patients with 2HPT. A large-scale and longterm study must be conducted for safety and clinical effect

Disclosure of Financial Relationships: nothing to disclose

**PUB113**

**Racial Differences in Vitamin D, Parathyroid Hormone and Fibroblast Growth Factor-23 Levels in Chronic Dialysis Patients** Anna Jeanette Jovanovich,[1] Michel B. Chonchol,[1] Alfred K. Cheung,[2,4] James S. Kaufman,[3] Tom H. Greene,[4] William L. Roberts,[4] Gerard John Smits,[1] Jessica B. Kendrick.[1] *[1]University of Colorado Denver Health Sciences Center, Denver, CO; [2]VASLCHCS, Salt Lake City, UT; [3]VA Boston Healthcare System, Boston, MA; [4]University of Utah, Salt Lake City, UT.*

Purpose: Abnormalities of mineral metabolism have not been described across races in patients requiring dialysis.

Methods: Study was conducted among 654 dialysis patients who participated in the Homocysteine in Kidney and End Stage Renal Disease study. 25-hydroxyvitamin D [25(OH)D], calcitriol, intact parathyroid hormone (iPTH), and fibroblast growth factor (FGF-23) levels were measured in plasma samples. Multivariable regression analyses were performed to investigate the association between race and levels of vitamin D, iPTH, and FGF-23 levels.

Results: There were a total of 654 patients on chronic dialysis. 51% was non-Hispanic black (NHB), 38% was non-Hispanic white (NHW) and 11% were categorized as other races. NHB had the lowest 25(OH) D levels when compare to NHW and others (15.0±10.0 vs. 21±31 vs. 24±25 ng/mL respectively; p<0.0001). There was no significant difference in calcitriol levels among races. NHB had higher iPTH concentrations than NHW and others (326±299 vs. 232±263 vs. 275±246 pg/mL respectively; p<0.0001). The median [IQR] FGF-23 levels among NHB, NHW and those of other race were 3914 [1350-13682], 4036 [1393-12878] and 5658 [1916-16017] RU/mL, respectively (p=0.14). After adjustment for demographics and cardiovascular risk factors, NHB was independently associated with lower 25(OH)D (β= -0.12; p=0.009) and higher iPTH (β=0.12; p=0.005) levels than non-Blacks. NHB was independently associated with increased iPTH when further adjusted for calcium, phosphorus, 25(OH)D, calcitriol and FGF-23 suggesting other mechanism play a role in elevated iPTH levels in NHB on dialysis. There were no racial differences for FGF-23 levels in multivariable-adjusted models.

Conclusions: Disorders of mineral metabolism, particularly low 25(OH)D and elevated iPTH levels are more severe in NHB when compare to non-blacks requiring dialysis.

Disclosure of Financial Relationships: nothing to disclose

**PUB114**

**Role of Teriparatide in the Treatment of Hypocalcemia Related to Hypoparathyroidism Post-Renal Transplantation** Estela Nogueira, Alice Santana, José Guerra, Sonia Silva, Clara Mil-Homens, Antonio Gomes da Costa. *Nephrology, Hospital Santa Maria, CHLN, EPE, Lisbon, Portugal.*

The aim of this study was to retrospectively analize the efficacy and safety profile of teriparatide use, in patients with severe hypocalcemia post kidney transplant, due to previous parathyroidectomy. Teriparatite is a recombinant human PTH that was approved by U.S FDA for the treatment of osteoporosis with high risk of fracture. Additionally, some studies have suggested a role in the management of iatrogenic hypoparathyroidism post kidney tranplantation.

Efficacy was evaluated by calcium and phosphorus serum levels, calcium carbonate and calcitriol doses administrated and safety profile by serum creatinine levels and secondary effects pre and post therapy with teriparatide (Forteo®), at 20µg/day administred subcutaneously.

5 patients (3F and 2M), with 52±9,2 years (mean±SD) developed severe hypocalcemia (5,6±0,9mg/dl) immediately following renal transplantation, requiring high doses of calcium gluconate at 10% (4,4±1,5 vials/day). PTH levels were under the normal range (5,3±7,7pg/ml) and as hypocalcemia persisted, teriparatide was administred 33±11days post transplant. After therapy, calcium levels increased (7±1.2 vs 8.5±1.2mg/dl) and phosphorus levels declined (5,4±1.5 vs 4 ±0.9 mg/dl). Simultaneously, calcium carbonate (12,2±7,2 vs 6±2 g/day) and calcitriol (4±1.5 vs 2.5±1 cp/day) required doses decreased and intravenous calcium was suspended in all patients. Teriparatide therapy was maintained during 13.8±10,8 months. In 2 patients it was not possible to suspend it (at 17 and 30 months respectively) and another patient required it after a withdrawal period, due to persistent hypocalcemia. No secondary effects were reported and serum creatinine levels remained stable after teriparatide administration.

Our data suggest that teriparatide use was safe and efficient to stabilize calcium levels and decreased calcium and calcitriol requirements in patients with iatrogenic hypoparathyroidism post-kidney transplant.

Disclosure of Financial Relationships: nothing to disclose

**PUB115**

**Hypophosphatemia and Secondary Hyperparathyroidism Associated with Bisphosphonate Use** Uzoamaka T. Nwaogwugwu, Barbara A. Clark. *Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

Maintaining normal phosphorus is essential for cellular function. Hypophosphatemia may present as nonspecific symptoms of myalgias/ weakness. There are very few reports of acquired drug induced hypophosphatemia and hyperphosphaturia and most of these are attributed to direct proximal tubular toxicity. We report a case of symptomatic hypophosphatemia attributed to secondary hyperparathyroidism associated with chronic use of alendronate. A 55 yo woman with osteopenia maintained on alendronate for 4 years presented with several months of unexplained fatigue and myalgias. Serum phopsphorus levels were repeatedly less than 2.3 mg/dl over a 4 month period despite attempts to replete with oral phosphate. She was referred for further evaluation. She was vitamin D replete (on weekly vitamin D3 50,000 IU weekly for the past 4 years) (25 OH vitamin D levels 45-53 ng/ml; 1,25 vitamin D level 31 pg/ml). Urine phosphorus was 1500 mg/24h; urine calcium 408mg/24h, corrrected serum calcium 8.9-9.7 mg/dl and intact PTH levels were persistently mildly elevated (71-80 pg/ml). Alendronate was held for one month without any improvement in the lab values or symptoms. Work up for primary hyperparathyroidsim with neck CT scan, US and sestamibi scan were unrevealing. A trial of cinacalcet corrected the hypophosphatemia and hyperparathyroidism (phosphorus 2.9 mg/dl; calcium 8.6 mg/dl, iPTH 58 pg/ml) and the patient felt better. The cinacalcet was discontinued after 8 months and all parameters remained normal an additional 8 months later. This is consistent with the known delayed clearance of alenodrante from bone stores. We present a case of symptomatic hypophosphatemia occuring after long term bisphosphonate use that took months to resolve. The correction with cinacalcet confirms the mechanism as secondary hyper parathyroidism, but was not related to hypocalcemia or vitamin D deficiency, as our patient had neither. We recommend periodic screening of serum phosphorus and PTH with chronic bisphosphonante use, especially if there are complaints of muscle pain or weakness. The mechanism for the hyperparathyroidism remains to be elucidated but can occur in the absence of hypocalcemia.

Disclosure of Financial Relationships: nothing to disclose

**PUB116**

**Is Pamidronate More Effective Than Alendronate in Adult Patients for the Prevention of Bone Loss after Kidney Transplantation?** Rasika A. Sirsat, Vipla S. Puri, Sandeep Dasharath Holkar, Alan F. Almeida, Amit Chandrakant Langote, Jatin Piyush Kothari, Rajaram R. Jagdale. *Nephrology, P D Hinduja Hospital, Mumbai, Maharashtra, India.*

Purpose: A randomized non blinded study comparing safety and efficacy of Pamidronate versus Alendronate for the prevention of bone loss after renal transplantation in adult patients.

Methods: A total of 41 patients were randomly assigned to receive either Alendronate (70 mg/wk for 1 year) or Pamidronate (60 mg IV injection at baseline and at 6months post transplantation. All patients were also given oral calcium 1 g and 500 IU vitamin D supplements daily. Estimates of BMD by DEXA , Sr Calcium, Sr Phosphorus, Sr albumin, Sr Alkaline phosphatase, PTH , vitamin D, Osteocalcin, Urine crosslaps were done at baseline and at one year.

Results: Baseline characteristics in both groups were similar with respect to immunosuppressive protocols, calcineurin exposure, steroid use, age, calcium, phosphorous and Vitamin D levels. In both Alendronate and Pamidronate groups, a mean rise in BMD of femur and spine, osteocalcin and a decrease in serum PTH was seen but failed to reach statistical significance. Parameters such as albumin, Calcium, Phosphorus and Vitamin D showed significant increase from baseline among both the groups .Other parameters such as Sr Calcium, Alkaline phosphatase, Osteocalcin, PTH, Urinary crosslaps were comparable but the difference was not statistically significant. There was statistically significant increase in mean femur T score of 20.2% in Alendronate group which was statistically significant( P< 0.05 ). None of the patients had to discontinue bisphosphonates due to any adverse effects during the study period.

Conclusions : Our study confirms that bisphosphonate use is safe and efficacious for the prevention of bone loss in renal transplant patients. Alendronate group showed statistically significant increase in femur T score as compared to Pamidronate group and needs further study as to whether additional doses of pamidronate need to be given.

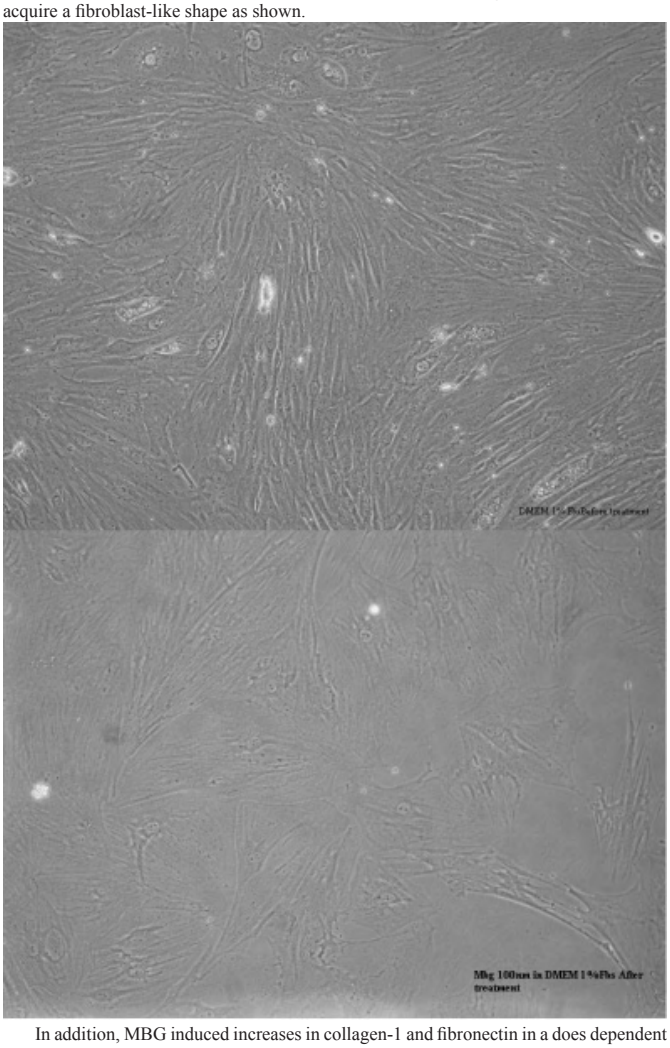
Disclosure of Financial Relationships: nothing to disclose

**PUB117**

**Rat Bone Marrow Derived Mesenchymal Stem Cells Differentiate into Fibroblast-Like Shaped Cells in the Presence of Marinobufagenin** George Budny,[1] Sankaridrug Periyasamy,[1] Alexei Bagrov,[2] Olga Fedorova,[2] Christopher J. Cooper,[1] Nader Abraham,[1] Joseph I. Shapiro.[1] *[1]Medicine and Physiology/ Pharmacology, University of Toledo Medical Center, Toledo, OH; [2]National Institute of Aging, Baltimore, MD.*

We recently demonstrated that the cardiotonic steroid marinobufagenin (MBG) induces cardiac and renal fibrosis in vivo as well as directly stimulates collagen type I secretion by isolated fibroblasts. However, it is unclear how much fibrosis is due to the activation of resident fibroblasts in tissues as opposed to other processes. In this study, the effect of MBG on differentiation of bone marrow derived mesenchymal stem cells in rats was investigated. Mesenchymal stem cells (MSCs) are multipotent stem cells that can differentiate into a variety of cell types including renal tubular epithelium and fibroblasts. To examine whether MBG alone can trigger MSC differentiation into fibroblasts, we used

MSCs prepared in our lab from bone marrow which was extracted from Sprague-Dawley rat's tibia and femur bones. The cells were cultured and subcultured in DMEM with 15%FBS until they reached confluence. We then treated the MSCs with MBG in different concentrations. Digital photographs were taken 24 hours after adding the MBG to the cell culture media (DMEM with 1%FBS). Cells were also collected and protein expression was measured with Western blot. MBG (100 nM) caused MSCs grown to confluence to acquire a fibroblast-like shape as shown.



In addition, MBG induced increases in collagen-1 and fibronectin in a does dependent fashion. Our data suggest that cardiotonic steroids such as MBG may be an important factor in inducing MSCs differentiation into fibroblasts and, through this mechanism, may play a role in progressive renal fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB118**

**Leptin Exerts a Pro-Apoptotic Effect on Rat Renal Tubular Cells Via Prostaglandin $E_2$ Aggregation** Yen Cheng Chen,[1] Yung-Ho Hsu,[2] Cheng-Hsien Chen,[1] Tso Hsiao Chen.[1] *[1]Division of Nephrology, Taipei Medical University-Wan Fang Medical Center, Taiwan; [2]Division of Nephrology, Taipei Medical University-Shuang Ho Hospital, Taiwan.*

Leptin, a circulating hormone secreted mainly from adipose tissues, is reported to act on kidney in pathophysiological states. However, the influence of leptin on renal tubular epithelial cell is still unclear. In the present study, we intended to investigate the influence of leptin on gentamicin-induced aoptosis in rat renal tubular cells. Gentamicin-induced apoptosis in renal tubular cells (NRK-52E) was examined using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling. We found that the 48-h treatment of leptin with a high dosage (from 50 to 250 ng/ml) exerted a pro-apoptotic effect on gentamicin-induced apoptosis in NRK-52E cells although an anti-apoptotic effect was found at 24 h. Leptin obviously reduced the expression of Bcl-$x_L$, and increased cleaved caspase-3 in 48-h treatment. In caspase-3 activity assay, the gentamicin-induced activity of caspase-3 was also reduced by leptin treatment at 24 h, but enhanced at 48 h. We also found that leptin induced the expression of cyclooxygenase-2, prostacyclin ($PGI_2$) and prostaglandin $E_2$ ($PGE_2$) in a dose-dependent manner. The siRNA transfection for $PGI_2$ synthetase diminished the anti-apoptotic effect of leptin at 24 h, and enhanced the pro-apoptotic effect at 48 h. Additionally, NS398 blocked $PGE_2$ augmentation and the pro-apoptotic effect of leptin at 48 h. Our results reveal that a long-term treatment of leptin exerts a pro-apoptotic effect on rat renal tubular cells through $PGE_2$ aggregation.

Disclosure of Financial Relationships: nothing to disclose

**PUB119**

**Hyperosmolal Microenvironments Induce Differentiation of Bladder Epithelial Cells in Culture** Bradley P. Dixon, Jeff Henry, John J. Bissler. *Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Urinary bladder epithelium is chronically exposed to a hyperosmolal microenvironment. Superficial urothelial cells adapt to this microenvironment through the accumulation of organic osmolytes and formation of the asymmetric unit membrane comprised of uroplakins on the apical cell surface. Failure to activate such differentiation in cells repairing inflammatory or surgically created defects in the bladder epithelium and exposed to hyperosmolality may lead to osmotic stress, an aberrant DNA damage response, and apoptosis.

We cultured a novel urothelial cell line, ULTI, under isoosmolal culture conditions as well as gradually adapted these cells to osmolalities of both 450 mOsm/kg and 600 mOsm/kg by addition of either sodium chloride or urea. Cell proliferation was measured by both trypsin detachment and counting with a hemocytometer, and by crystal violet DNA staining. Cells were lysed, RNA and protein were isolated, and RT-PCR was performed to detect markers of urothelial differentiation such as cytokeratin 18, cytokeratin 20 and uroplakin II. Western blot was used to measure activation of apoptosis through cleavage of PARP and caspase-3.

ULTI cells demonstrated robust proliferation under isoosmolal conditions, but arrested proliferation following adaptation to hyperosmolal conditions. Markers of apoptosis were not present in cells gradually adapted to hyperosmolality. Under both isoosmolal and hyperosmolal conditions, ULTI cells express cytokeratin 18 indicating their epithelial nature. However, only cells gradually adapted to hyperosmolal conditions demonstrated both cytokeratin 18 and 20 expression, indicative of an "umbrella cell" phenotype.

These results demonstrate that a hyperosmolal microenvironment can facilitate urothelial cell differentiation. Failure of cells to activate this differentiation program may lead to osmotic stress, aberrant DNA damage response, and ultimately apoptosis or carcinogenesis.

Disclosure of Financial Relationships: nothing to disclose

**PUB120**

**Protease-Activated Receptor-2 in Renal Cell Carcinoma** Glenda C. Gobe,[1] Retnagowri Rajandram,[1] Jacky Yung Suen,[2] David Fairlie,[2] David W. Johnson,[1] David A. Vesey.[1] *[1]Centre for Kidney Disease Research, The University of Queensland, Brisbane, Queensland, Australia; [2]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia.*

Protease Activated Receptor-2 (PAR2) is a G protein-coupled receptor that is activated by trypsin-like proteases. It is implicated in the progression of pancreatic, colon and stomach cancers where its expression is often enhanced and one of its natural ligands trypsin, is frequently over produced. Its role in clear cell renal carcinoma (ccRCC) has not been investigated. The aims of this study were to examine: 1. PAR2 and trypsin/trysinogen (TRY) expression in ccRCC tissue and 2. The effect of PAR2 activation on RCC cell proliferation.

A tissue microarray (TMA) consisting of 67 confirmed archived cases of ccRCC and paired uninvolved cortical renal tissue was constructed. Sections were stained for PAR2 and TRY. Cell surface PAR2 was examined in the RCC cell lines Caki-1, ACHN, SN12K1, 786-O A498 and primary cultures of human proximal tubule cells (PTC) by an intracellular $Ca^{2+}$ mobilization assay (fluo-3 fluorimetry). TRY expression was measured by immunoblotting and DNA synthesis by $^3$H-thymidine incorporation.

PAR2 expression was greater and more intense in the normal tissue, (proximal tubules), than ccRCC tissue (84.4% vs 59.7%). There was negligible staining in distal tubular cells (DTC). TRY staining was greater in the ccRCC tissue than the normal tissue (94% vs 85.7%). Both PTC and DTC showed cytoplasmic staining. Cultured PTC showed the highest level of PAR2 cell surface expression, similar in magnitude to the colon carcinoma cell line, HT29. The other RCC cells showed decreasing levels of cell-surface PAR2 (Caki-1>ACHN>786-O>A498>SN12K1). By immunoblotting all the cell lines showed some TRY expression including the PTC. Trypsin (5nM) but not the PAR2 activating peptide, 2f-LIGRLO-$NH_2$ (2μM), increased DNA synthesis Caki-1 cells.

Both PAR-2 and TRY are expressed in ccRCC tissue and may contribute to cancer progression. TRY induces DNA synthesis in Caki-1 cells by a PAR2 independent mechanism.

Disclosure of Financial Relationships: nothing to disclose

**PUB121**

**The Direct Effects of Hepcidin-25 on Human Proximal Tubular Epithelial Cells** Steven Michael Harwood, Elizabeth Miller, Martin J. Raftery, Magdi Yaqoob. *Translational Medicine and Therapeutics, Queen Mary, University of London, London, United Kingdom.*

Hepcidin is a peptide hormone primarily synthesized in the liver that is a key mediator of iron homeostasis. Hepcidin concentrations are elevated in CKD and may be suppressed in response to erythropoietin therapy. Recent work has suggested that hepcidin is localized

in other organs including the kidney and that proximal tubular epithelial cells (PTEC) can actively absorb hepcidin from the renal filtrate. We hypothesized that hepcidin might in addition to its recognized role have other directly mediated cell functions. To study these putative effects we incubated active hepcidin-25 peptide on cultures of human PTEC (HK-2) in the absence and presence of cellular injury. Hepcidin (0-200ng/mL) was found to cause a linear dose-response increase in cell cytotoxicity within 2h with a two-fold rise in cytotoxicity induced fluorescence found with 75ng/mL hepcidin (n=8, $p<0.05$). Cytotoxicity was determined using a CytoTox-Fluor cytotoxicity assay using the dead cell substrate bis-AAF-R110 with similar results obtained with LDH methods. No rise in caspase-3 activity was seen indicating that cell death was necrotic and not apoptotic in nature (n=5, P=NS). Proliferation (viability) was determined by MTS assay with the finding that low concentrations of hepcidin having a profound effect after just 2.5h (2-fold increase at 50ng/mL, n=8, $p<0.05$). However, the rise in MTS was not sustained at higher hepcidin concentrations. Further work then examined the effect of hepcidin on peroxide induced injury (1 and 2mM $H_2O_2$ for 2h) and ATP depletion injury (50μM antimycin A and 10μM deoxyglucose for 1h). Both injury models produced highly significant reductions in proliferation. However hepcidin at 100ng/mL at the time of insult was found to have no additive injurious effect. On the contrary, when cultures were pre-incubated with 100ng/mL hepcidin for 1h prior to ATP depletion injury, a highly significant cytoprotective effect was seen (n=6, $p<0.0002$). We conclude that hepcidin is likely to be more than just an iron regulator in patients with CKD and that the direct effects upon PTEC in the clinical setting may be both cytoprotective and cytotoxic.

Disclosure of Financial Relationships: nothing to disclose

**PUB122**

**Rapamycin Induced Nuclear Sequestration of mTOR May Control Downstream Signaling in Primary Vascular Smooth Muscle Cells** Yuxia He,[1] Christi M. Terry,[1] Mary Carlson,[1] Alfred K. Cheung.[2,1] *[1]Dept. of Medicine, Univ. of Utah, SLC, UT; [2]Medical Service, VA Healthcare System, SLC, UT.*

Dysregulated proliferation of vascular smooth muscle cells (VSMC) contributes to the failure of vascular stents and hemodialysis vascular accesses. Rapamycin (RAP) is used to inhibit restenosis after angioplasty and is under investigation for treatment of vascular access stenosis. RAP inhibits the phosphorylation of mammalian target of rapamycin (mTOR). Activated mTOR regulates VSMC proliferation through phosphorylation (and activation) of the cytoplasmic target P70S6 kinase. Previous studies in transformed cell lines or cancer cells showed that mTOR shuttled between the nucleus and cytoplasm and that nuclear sequestration may also be a cellular means to regulate mTOR. In this study, we showed by immunocytofluorescence (ICF) and western blotting that in proliferating cultured primary VSMC, mTOR was localized in both the cytoplasm and nucleus. RAP treatment (100 nM) inhibited the phosphorylation of mTOR and cell proliferation as expected, but it also promoted mTOR nuclear accumulation by 6h that was maintained up to 48h, as determined by western blotting of nuclear fractions. Further, exposure of VSMC for 24h to 20 ng/ml of leptomycin B (LMB), a specific inhibitor of nuclear export, resulted in enhanced mTOR nuclear sequestration, as determined by ICF, indicating that mTOR is actively shuttled between nucleus and cytoplasm. RAP exposure markedly reduced phosphorylation of Thr389 in the catalytic domain by 6h that was maintained up to 48h. RAP inhibited the phosphorylation of Thr421 in the P70S6 kinase auto-inhibitory domain at 1h, but in contrast to Thr389, phosphorylation levels of Thr421 returned to pre-treatment levels by 24h. Thus, phosphorylation of specific sites with different functions in the P70S6 kinase, is differentially affected by RAP. These data suggest that the intranuclear sequestration of mTOR induced by RAP in primary VSMC may be a means to separate mTOR from its cytoplasmic targets, such as P70S6 kinase, and this mechanism may play a role in RAP-induced inhibition of VSMC proliferation.

Disclosure of Financial Relationships: nothing to disclose

**PUB123**

**Reactive Oxygen Species Contribute to Hypertrophy and Vascular Thickening in Remaining Renal Mass after Unilateral Ischemia, but Not Uninephrectomy** Hee-Seong Jang,[1] Jinu Kim,[2] Jee In Kim,[1] Min Hyun Cho,[3] Kwon Moo Park.[1] *[1]Anatomy and BK21 Program, Kyungpook National University School of Medicine, Daegu, Republic of Korea; [2]Department of Anesthesiology, College of Physicians and Surgeons of Columbia University, New York, NY; [3]Department of Pediatrics, Kyungpook National University hospital, Daegu, Republic of Korea.*

Undesirable alterations of remaining renal mass following renal mass reductions induced by unilateral renal ischemia (UI) or unilateral nephrectomy (Ux) have been shown to induce long-term renal insufficiency. However, the molecular mechanisms underlying these alterations remain to be clearly elucidated. We then evaluated the involvement of reactive oxygen species (ROS) in alterations of the contralateral kidneys (CKs) after UI and Ux. Ux is relatively less pathologic than UI. Mice were subjected to either 30 min of UI or Ux. Some mice were treated with Mn(III) Tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP, a superoxide dismutase (SOD) mimetic) daily beginning 1 day after the operations. Ux and UI resulted in hypertrophy and medial thickening renal cortical artery of the CKs at 9 days after the operations. UI increased superoxide levels and reduced manganase SOD (MnSOD) expression in the CKs, but Ux did not. MnTMPyP-administration blocked kidney hypertrophy and UI-induced increases in the medial area of CK along with reductions in superoxide production, but it did not affect any of the Ux-induced phenomena. In conclusion, ROS contributes profoundly to changes in the CK after UI, but not to changes in the CK after Ux, thereby suggesting that long-term renal insufficiency occurring after renal mass reductions should be treated differently, depending on the cause of the insufficiency.

Disclosure of Financial Relationships: nothing to disclose

**PUB124**

**An Antiapoptotic Role of Cadmium in Human Renal Epithelial Cells** Nileshkumar Shah,[1] Iain Macphee,[2] Mark Edward Dockrell.[1] *[1]SW Thames Institute for Renal Research, London, United Kingdom; [2]Renal Medicine, St George's, University of London, London, United Kingdom.*

$Cd^{2+}$ is an important pollutant that poses a significant health risk currently ranking 7th in the Agency for Toxic Substances and Disease list of hazardous substances. The NHANES study (1999-2006)demonstrated high levels of serum $Cd^{2+}$ are associated with chronic kidney disease (CKD) and albuminuria. $Cd^{2+}$ has also has teratogenic and carcinogenic activities. Studies on renal proximal tubule epithelial cells(PTEC) have shown relatively specific effects on the cadherin(CDH)-dependent cell junctions. CDH are trans-membrane proteins which regulate epithelial integrity by the formation of extracellular complexes and modulation of intracellular signaling. CDH2 (N cadherin) is associated with migration and considered a hallmark of fibrosis and metastasis.

The aim of current study was to investigate the mechanism of cadmium toxicity on human PTEC with regard to cadherin expression and induction of apoptotis.

Transformed human PTEC (HKC-8) were treated with $CdCl_2$ (5-50 uM) for 1-24 h. Cell morphology was studied and CDH1 (E cadherin) and CDH2 were studied by immunoblotting along with mediators of apoptosis (Cleaved caspase 3, pERK5 and pAKT).

$Cd^{2+}$ caused concentration dependent morphological changes in PTECs; cells became round, detached from each other and at higher concentrations, from the basal surface .

In contrast to our hypothesis, Cd2+ caused a transient reduction in CDH1 at 6 h but not at 24 h with a persistent rise in CDH2.

Following a transient rise, cleaved caspase 3 was reduced in a dose dependent manner with a sustained rise in pERK5. There was a fugacious rise in pAKT.

$Cd^{2+}$ is an important pollutant associated with CKD and carcinogenesis, however the mechanism of cadmium toxicity is not fully understood. Surprisingly, in these experiments $Cd^{2+}$ was associated with a reduction in apoptosis at 24 h along with induction of CDH2, which may explain its carcinogenic potential. Although the concentrations used here are relatively high, chronic exposure to lower concentrations may have similar effects and therapeutic intervention to block pERK5 may allow apoptosis and reduce the carcinogenic potential of $Cd^{2+}$ in the kidney.

Disclosure of Financial Relationships: nothing to disclose

**PUB125**

**Effects of the *Echinacea purpurea* as Anti-Proliferative on Renal Adenocarcinoma Cells (CAKI-1) in Culture** Renata Cristina Tassetano, Marcelo Andery Naves, Maria Dalboni, Fernanda Teixeira Borges, Nestor Schor. *Medicine, University Federal of Sao Paulo, Sao Paulo, Brazil.*

Renal cell carcinoma (CR) is rare compared to other cancers. Angiogenesis is an important route of tumor growth which is regulated by numerous pathways, especially Ras and Akt, which are targets of many therapeutic agents. Due to the resistence of CR to conventional treatments is increasing interest in alternative treatments. The Ep is used as an alternative treatment for some cancers. The aim is analyze the effect of *E.p.* on the RAS and Akt signaling pathway in tumor angiogenesis.

**METHODS**: Sorafenib (Sor 3uM) and Ep150ug/ml were added to culture for analysis: apoptosis (f. cytometry), protein expression of Ras and Akt (W.blot), performed after 24, 48 and 72 hours of treatment. Analyzed by One Way ANOVA, $p<0.001$ vs CT.

**RESULTS:** We observed that both the Sor as Ep demonstrated significant anti-tumor activity with increased apoptotic cells and decrease Ras and Akt proteins in both treatments.

Table 1

| | Ep 24h | Ep 48h | Ep 72h | S 24h | S 48h | S72h |
|---|---|---|---|---|---|---|
| Apo C | 7.2 ± 0.8 | 7.5 ± 0.3 | 4.7 ± 0.4$& | 7.2 ± 0.8 | 7.5 ± 0.3 | 4.7± 0.4$& |
| Apo T | 9.3 ± 0.6* | 6.8 ± 0.4$ | 7.6±0.7*#$& | 10.1 ±1.2* | 5.6 ± 0.9*$ | 9.2±0.6*$& |
| RAS C | 0.484±0.05 | 0.208±0.01# | 0.117 ± 0.02#$ | 0.95±0.05 | 0.869±0.01# | 0.353±0.02#$ |
| RAS T | 0.289±0.02* | 0.27±0.01*# | 0.183±0.009*#$ | 0.423±0.02* | 0.672±0.01*# | 0.397±0.009*#$ |
| AKT C | 0.280 ± 0.05 | 0.343± 0.01# | 0.301 ± 0.02$ | 0.956 ± 0.05 | 0.350 ± 0.01# | 0.868 ± 0.02#$ |
| AKT T | 0.207± 0.02* | 0.223± 0.01* | 0.193 ± 0.009* | 0.964 ± 0.02 | 0.128 ± 0.01*# | 0.518±0.009*#$ |

*P=<0.001 vs CT, # vs Sorafenib, $ vs 24 h, & vs 48 h

So we conclude that the E.P. is a promising complementary treatment in the CR since activity has anti-proliferative and anti-angiogenesis in vitro.

Disclosure of Financial Relationships: nothing to disclose

**PUB126**

**Whether and How MDM2 (Murine Double Minute 2 Oncogene) Was Involved in Aldosterone-Induced Human Mesangial Cell Lines (HMCLs) Proliferation** Haiyun Wang, Limeng Chen, Xue-Wang Li, Xuemei Li. *Nephrology Department, Peking Union Medical College Hospital, Beijing, China.*

**Objective** To investigate whether and how MDM2, the p53 binding protein, a negative regulator of p53, was involved in HMCLs proliferation induced by aldosterone. **Methods** RT-PCR, immunoflourescence and Western blot were used to make sure the expression of MDM2 in HMCLs, and the relationship among time, dose and MDM2 expression. Proliferation of HMCLs was evaluated by flow cytometry. Small interference RNA of MDM2 was invocated to confirm the relationship among aldosterone, MDM2 expression and proliferation of HMCLs. Spironolactone, a mineralcorticoid receptor (MR) blocker was used to estimate the role of whether MR was involved in aldosterone inducing MDM2 elevation; cycloheximide, a protein synthesis inhibitor was inducted to estimate whether the

rapid nongenomic mechanisms regulate aldosterone inducing MDM2 expression directly. **Results** Both MR and 11β-HSD type 2 mRNAs were detected implied the reactive potency to aldosterone in HMCLs . MDM2 protein expression was detectable in both the nucleus and the cytoplasm. Aldosterone significantly increased MDM2 expression in a dose and time-dependent manner compared with control ones. The percentage of S phase increased in HMCLs stimulated with certain concentration of aldosterone at 24 hours ($P<0.05$). A reduction of MDM2 protein was dose-dependent confirmed by transfection of MDM2 siRNAs. Under the transfection of MDM2 siRNA, aldosterone did not promote the HMCLs proliferation. All these implied MDM2 participates in aldosterone inducing HMCLs proliferation. As aldosterone with spironolactone did not promote expression of MDM2 mRNA and protein, MR involved in this process was identified. Aldosterone with CHX did not increase expression of MDM2 protein indicated it is not directly regulated by the rapid nongenomic mechanisms. **Conclusion** MDM2 participates in aldosterone inducing HMCLs proliferation. MDM2 is significantly induced by aldosterone via MR and inhibited by spironolactone. The increase expression of MDM2 protein induced by aldosterone is not directly regulated by the rapid nongenomic mechanisms.

Disclosure of Financial Relationships: nothing to disclose

**PUB127**

**Mizoribine (MZR) Suppresses Proliferation of Rat Glomerular Epithelial Cells (GEC) in Culture and Inhibits Increase of MCP-1 and MIP-2 Stimulated by Thrombin** Hideaki Yamabe, Michiko Shimada, Reiichi Murakami, Yuko Shimaya, Takeshi Fujita, Norio Nakamura. *Department of Nephrology, Hirosaki University Hospital, Hirosaki, Japan.*

Glomerular crescents play an important role in progressive glomerular injury. The lesions consist of GEC, macrophages and fibrin deposition. MCP-1 is a chemoattractant of monocytes, which have a procoagulant activity. MIP-2 is a chemoattractant of neutrophils, which mediate acute necrotizing injury in crescentic glomerulonephritis. MZR is an immunosuppressive drug, which inhibits selectively inosine monophosphate dehydrogenase, has been used for organ transplantation. The aim of this study is to investigate the effects of MZR on GEC. Cultured rat GEC were used. GEC proliferation was determined by using MTT assay. MCP-1 and MIP-2 were quantified by ELISA in culture supernatants and their mRNA expressions were analyzed by real-time RT-PCR. NF-kappa B in GEC was examined by immunofluorescence study. The proliferation of GEC was suppressed by MZR in a dose-dependent manner in the range of 1.0-100.0 μg/ml. These concentrations of MZR had no toxic effect to GEC. Thrombin (0.5-5.0 U/ml) enhanced the production of MCP-1, MIP-2 and their mRNA expressions. The stimulatory effect of thrombin on the productions of MCP-1 and MIP-2 was inhibited by addition of MZR (MCP-1; control: 0.20±0.07 pg/μg cell protein, thrombin: 0.52±0.03 pg/μg, thrombin+MZR: 0.40±0.02 pg/μg, $p<0.01$ vs thrombin, MIP-2; control: 4.06±0.08 pg/μg, thrombin: 11.67±0.54 pg/μg, thrombin+MZR: 8.77±1.40 pg/μg, $p<0.001$ vs thrombin). The relative mRNA expressions of MCP-1 and MIP-2 stimulated by thrombin were also inhibited by addition of MZR (MCP-1; control:1.00 ±0.1, thrombin: 9.75±0.4, thrombin+MZR: 7.52±0.8, $p<0.01$ vs thrombin, MIP-2; control: 0.99±0.2, thrombin: 17.9±1.0, thrombin+MZR: 9.53±1.0, $p<0.001$ vs thrombin). MZR (10 μg/ml) suppressed the thrombin-induced translocation of NF-kappa B into the nucleus of GEC. These data demonstrated a suppressive effect of MZR on the proliferation of GEC and the production of MCP-1 and MIP-2 stimulated by thrombin possibly via inhibition of NF-kappa B. It is suggested that MZR may be useful for the treatment of crescentic glomerulonephritis.

Disclosure of Financial Relationships: nothing to disclose

**PUB128**

**Altered Exon 4 KIM-1 Gene Sequence in Renal Cell Carcinoma (RCC) and Concordant Expression of KIM-1 and CD68** Ping L. Zhang,[1] Venkata Sabbisetti,[2] Joseph V. Bonventre.[2] *[1]Department of Anatomic Pathology, William Beaumont Hospital, Royal Oak, MI; [2]Renal Division, Brigham and Women's Hospital, Boston, MA.*

KIM-1 is a type I transmembranous protein which is a sensitive and specific marker of proximal tubule (PT) injury. Our studies demonstrates that KIM-1 confers a phagocytic phenotype on injured PT cells. KIM-1 is also upregulated in human PT-derived renal cell carcinomas (RCC) including clear cell RCC and papillary RCC. The goals of this study were to determine whether human RCC had altered KIM-1 gene sequence and whether there was concurrent expression of KIM-1 and another phagocytic factor CD68, a transmembrane glycoprotein mainly present in macrophages. Genomic DNA was prepared from paraffin-embedded tumor tissue containing 3 clear cell RCC and 3 papillary RCC. Using the purified DNAs as the template, PCR was performed to amplify each of the nine major coding exons of the KIM-1 gene. Each PCR product then went through a cloning and sequencing (GENEWIZ) process. Vector NTI software was used to compare exon DNA sequence to the published sequence of KIM-1 (HAVCR-1;NM_012206). There were mutations in exon 4 in 4 of 6 patients (the mucin-like domain), but not in the remaining eight exons.

A tissue microarray containing 42 RCC derived from PTs and 48 tumors derived from distal nephron tubules were analyzed for KIM-1 (AKG7 antibody) and CD68 (KP1 clone) expression using immunohistochemistry. KIM-1 was expressed in 18/20 papillary RCC and 18/22 clear cell RCC, but not in distal nephron tubules derived renal tumors (0/25 chromophobe RCC and 0/23 oncocytoma). Expression of CD68 was similar to that of KIM-1 (positive in 17/20 of papillary RCC and 13/22 of clear cell RCC, but negative in chromophobe RCC (0/25) and oncocytomas (0/23)). There was a significant correlation between staining intensity (graded 0 to 3+) of KIM-1 and CD68 ($r = 0.56$ and $p = 0.001$).

In summary, gene sequence changes were found in exon 4 of human RCC derived from PTs. There was concurrent expression of KIM-1 and CD68 in PT-derived RCC. Both membrane proteins facilitate phagocytosis in RCC and may be important for immunological modulation that may enhance RCC resistance to therapy.

Disclosure of Financial Relationships: nothing to disclose

**PUB129**

**$GABA_B$ Receptor Mediated Chemotaxis and Cytoskeleton Rearrangement Are Dependent on Signaling Via PI3-K/Akt and Src Tyrosine Kinases** Michelle T. Barati, Janice Scherzer, Madhavi J. Rane, Jon B. Klein. *Kidney Disease Program, Univeristy of Louisville, Louisville, KY.*

Chemotaxis is essential to numerous biologic and pathophysiologic processes, including inflammation, neuronal development, and cancer cell metastasis. We have previously reported g-amino butyric acid (GABA) type B receptors ($GABA_BR$) to be present in and function as chemoattractant receptors in response to GABA agonists, in human neutrophils. The $GABA_BR$ is a metabotropic G protein-coupled receptor comprised of a heterodimer of $GABA_{B1}$ and $GABA_{B2}$ subunits interacting via leucine zipper motifs in the C-terminal domains. The goal of this study was to define signaling mechanisms regulating $GABA_BR$-mediated chemotaxis. We used rat basophilic leukemic cells (RBL-2H3 cells) stably transfected with human $GABA_{B1b}$ and $GABA_{B2}$ receptors. The $GABA_BR$ agonist baclofen induced chemotaxis that was dependent on $GABA_BR$. To define the cell signaling mechanisms regulating this chemotaxis, cells were treated with inhibitors to Phosphatidylinositol 3-Kinase (PI3-K), Akt, and Src kinases, prior to chemotaxis assays. Signaling via all three pathways regulated baclofen-induced chemotaxis. In addition, baclofen-induced Akt activation was dependent on both PI3-K and Src kinase-dependent signaling. Directed cell migration requires substantial rearrangement of the cytoskeleton. Cortical actin polymerization was increased suspended RBL cells subject to a baclofen gradient, and exposure of adherent RBL cells to baclofen caused cell spreading with increased actin polymerization and localization in membrane ruffles. Baclofen stimulation of RBL cells also increased formation of microtubules as determined by fractionation of cells and quantitation of soluble / free tubulin and insoluble microtubules. Treatment of RBL cells with either cytochalasin D or nocodazole to inhibit actin or tubulin polymerization, respectively, completely abolished baclofen-mediated chemotaxis. In conclusion, $GABA_B$ receptor stimulation causes cytoskeletal rearrangement and chemotaxis in RBL cells. These data suggest a role for $GABA_B$ receptor stimulation in cellular response to injury and disease.

Disclosure of Financial Relationships: nothing to disclose

**PUB130**

**Inhibition of PAI-1 Protects Glomerular Endothelial Cells Against Pro-Apoptotic Effects of Adipocyte-Derived Media** Bridgette Corsa,[1] Dan Gao,[1,2] Agnes B. Fogo,[1] Li-Jun Ma.[1] *[1]Department of Pathology, Vanderbilt University Medical Center, Nashville, TN; [2]Department of Nephrology, First Affiliated Hospital, Zhengzhou University, Zhengzhou, China.*

Background: Chronic kidney disease (CKD) is increased in obesity. A hallmark of CKD is progressive glomerulosclerosis with loss of capillary loops. We have shown that adipocyte-derived factors induce apoptosis of glomerular endothelial cells. Plasminogen activator inhibitor-1 (PAI-1) is a potent inducer of apoptosis. We hypothesized that targeting endogenous PAI-1 in glomerular endothelial cells (GENs) protects against the pro-apoptotic effects of the adipocyte-derived media.

Methods: Apoptosis was induced in mouse GEN either by adding recombinant human PAI-1 (rhPAI-1, 22.7nM, 48 hours) or by adding adipocyte-derived media to GEN with knockdown of PAI-1 or intact PAI-1. Endogenous PAI-1 in GENs was knocked down by transfecting with PAI-1 siRNA and compared to scrambled RNA transfected GENs. Cleaved caspase-3 and tight junction protein ZO-1 were assessed by Western blot. Data are expressed as mean±SE.

Results: Mouse GENs treated with exogenous rhPAI-1 showed a 2-fold increase in cleaved-caspase-3 expression when compared to untreated GENs. PAI-1 siRNA effectively knocked down PAI-1 expression by 90% in GENs. Adipocyte-conditioned media vs. normal media induced a 5.4-fold increase in cleaved caspase-3 expression in GENs treated with scrambled RNA. In contrast, GENs with knockdown of PAI-1 were protected against adipocyte-conditioned media effects, with only minimal induction of cleaved caspase-3 (only 9% vs. scrambled RNA treated GENs). ZO-1 expression was not different between groups.

Conclusions: Inhibition of endogenous PAI-1 protects glomerular endothelial cells against the pro-apoptotic effects of adipocyte-derived media. Our data suggests that modulation of endothelial PAI-1 may provide protection against obesity-induced chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**PUB131**

**Dorsal Root Ganglion Neurons Exibit Specific Firing Patterns Due to Different Electrophysiolgical Properties of Voltage Activated Ion Channels** Wolfgang Freisinger, Tilmann Ditting, Kristina Rodionova, Sonja Heinlein, Peter Linz, Karl F. Hilgers, Roland Veelken. *Nephrology, Friedrich-Alexander University Erlangen Nürnberg, Erlangen, Germany.*

PURPOSE: We could characterize recently a special group of medium-sized neurons with renal afferents (ARN) that exhibited significantly more frequently a tonic firing pattern upon stimulation than controls without projections from the kidney. We tested the hypothesis that a different firing pattern is related to changes in voltage activated sodium channels.

METHODS: 114 dorsal root ganglion (DRG) neurons from Th11-L2 (8 SD-rats) were investigated with whole cell patch clamp technique. ARN were identified by retrograde fluorescent labelling (DiI). All neurons were characterized by action potential generation due to current injection (40-12000 pA, 600 ms), tonic cells exhibited persisting AP, phasic 1to6/600ms. Static membrane properties were recorded; cells underwent voltage changes (-100 to 60 mV, 1 Step 10mV, 50ms) to investigate voltage activated channels.

RESULTS: We examined 114 DRG neurons, of which 76 stained positively for DiI. Cells with tonic firing pattern were found in 34% of DiI positive renal DRG neurons vs. 13% in non-renal DRG neurons. After depolarizing current injection (dDCI), over all 73% showed a phasic, 27% a tonic firing pattern. Voltage activated Na+-currents showed a significantly smaller inward current between -100mV to 20mV(e.g. at -40mV -7520±1988 pA vs. 20589±1162,6374, p<0,0001) in tonic firing cells. Furthermore, the voltage of half activation of the sodium channels was significantly higher in tonic vs. phasic cells (-40mV vs. -60 mV).

CONCLUSION: Cells with tonic firing pattern are found more frequently among ARN. All cells with tonic firing pattern after dDCI showed a significant change of voltage induced currents. In tonic firing neurons, Na+-channels exhibited lower inward currents being activated at higher voltage changes. As tonic neurons are more frequently found in renal afferents, we suppose that renal neurons may exhibit different subtypes of voltage dependent Na+-channels.

Disclosure of Financial Relationships: nothing to disclose

**PUB132**

**Fluvastatin Attenuates High Glucose-Induced Fibronectin Expression by Inhibiting the Signal Pathway of Rho-Kinase in HK-2 Cells** Jia Liu, Changying Xing. *Dept of Nephrology, First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China.*

Objective: To explore the effect of Rho-kinase signal pathway in renal interstitial fibrosis of diabetic nephropathy (DN) and the mechanism of fluvastatin in the prevention of renal interstitial fibrosis of DN. Methods: Human renal proximal tubular epithelial cells (HK-2 cells) were cultured in vitro. Rho-kinase activity was expressed as phosphorylation of myosin-phosphatase target 1 (p-MYPT1). The level of p-MYPT1 and fibronectin (FN) stimulated by high glucose was determined by Western blot at the time of 0h, 6h, 12h, 24h, 48h. Same marks were detected when high glucose cultured HK-2 cells treated with different concentrations (10-7, 10-6and 10-5 mol/L) of fluvastatin for 12h and when treated by lysophosphatidic acid (LPA) further. Result: High glucose enhance the expression of p-MYPT1 and FN at the time of 6h, 12h, 24, 48h compared with the time of 0h in culured HK-2s. The increase of FN expression stimulated by high glucose was in time-dependent fashion and the increased level of p-MYPT1 reached the peak at 12h. Fluvastatin decreased high glucose-mediated level of p-MYPT1 and FN in dose-dependent manner. The inhibitory effect of fluvastatin on up-regulation of p-MYPT1 and FN stimulated by high glucose was reversed by LPA. Conclusions: Rho-kinase may be one of the initiation signals of renal interstitial fibrosis of DN. Fluvastatin can prevent the development of renal interstitial fibrosis of DN by inhibiting Rho-kinase signaling pathway.

Disclosure of Financial Relationships: nothing to disclose

**PUB133**

**Constitutive Gene Expresion in Renal and Vascular Cells Is Regulated by Basal Levels of Reactive Oxygen Species** Gemma Olmos,[1] Marta Gonzalez,[1] Maria Del Nogal,[1] Susana Marquez,[1] Ines Mora,[1] Manuel Rodriguez-Puyol,[1] Diego Rodriguez-Puyol.[1-2] *[1]Physiology, Universidad de Alcala, Alcala de Henares, Madrid, Spain; [2]Nephrology Section and Research Unit, Hospital Principe de Asturias, Alcala de Henares, Madrid, Spain.*

Genes are considered as constitutive when they are detected in cells in basal conditions. Stimuli such as reactive species oxygen (ROS) may up-regulate the expression of these genes but the mechanisms involved in their continuous basal expression are unknown. As ROS concentrations are tightly maintained at intracellular level because the equilibrium between synthesis and degradation, we hypothesized that the basal levels of ROS could be responsible for the expression of these constitutive genes in renal and vascular cells. We analyzed the expression of some genes in cultured cells after decreasing intracellular ROS concentration and we explored the mechanisms involved.

In different human cell types (mesangial cells HMC; umbilical vein endothelial cells HUVEC; fibroblasts IF) exogenous catalase (CAT) addition produced an increased intracellular CAT activity and a decreased intracellular ROS concentration. Basal TGF-β1 and eNOS protein content decreased in HMC and HUVEC, respectively. In contrast, no changes were detected in other proteins:erythropoietin (IF), COX1 (HMC) or protein kinase G (HMC). As the presence of AP-1 responsive elements is one of the differential characteristic between the catalase-inhibited or non-inhibited genes, we analysed the relationship between the basal activity of AP-1 and ROS deprivation. Catalase decreased the whole TGF-β1 promoter activity and this effect disappeared when AP-1 binding site of TGF-β1 promoter was removed. Although the intracellular ROS concentration was inhibited to a similar level by diphenyleneiodonium (DPI, NADPH oxidase inhibitor), rotenone (R, mitochondrial complex I inhibitor) and allopurinol (xanthine dehydrogenase inhibitor) only DPI and R decreased TGF-β1 promoter activity.

That suggest low levels of ROS could be essential for the basal expression of constitutive AP1-dependent genes in kidney and vascular cells. Several systems involved in the synthesis of ROS could play different roles on this regulation.

Disclosure of Financial Relationships: nothing to disclose

**PUB134**

**Uric Acid Induces Lipid Accumulation in Liver Cells** L. Gabriela Sanchez-Lozada,[1,2] Miguel A. Lanaspa,[2] Carlos Alberto Roncal-Jimenez,[2] Richard J. Johnson.[2] *[1]Nephrology, INC Ignacio Chavez, Mexico City, DF, Mexico; [2]Renal Dis & Hypertens, U. of Colorado, Aurora, CO.*

Hyperuricemia has been associated with hepatic steatosis in several studies. In rats, the experimental elevation of serum uric acid was associated with increased deposition of intrahepatic lipids; these findings suggest a causative role of UA on the abnormal accumulation of fat within liver cells. To better understand the cellular mechanisms implicated in this outcome, we examined the effect of UA on fat accumulation as well as in specific enzymes involved in fat metabolism in human hepatoma cells (HepG2).

HepG2 cells (ATCC, USA) were cultured with uric acid (UA, 12 mg/dL) for 48 hr. Intracellular triglycerides (TG) were quantified by an enzymatic-coupled colorimetric assay. Fatty acid synthase (FAS) and enoyl-CoA-hydratase-2 (ECoAH) expressions were evaluated by western blot. In addition translocation of carbohydrate responsive element binding protein (ChREBP) was evaluated in nuclear and cytoplasm fractions.

UA induced a 20% increment in intracellular TG; this rise was similar in magnitude as the induced by fructose (5 mM), a known stimulator for TG synthesis in liver cells. Co-incubation of UA with fructose further increased TG synthesis. UA is a byproduct of intrahepatic fructose catabolism; as such the synergistic effect of UA and fructose suggests that UA derived from fructose contributed, at least partially, to further rise intracellular TG. In addition, UA dose dependently decreased the mitochondrial enzyme enoyl-CoA-hydratase-2 (ECoAH) an essential enzyme for b-oxidation. On the other hand UA increased the cytosolic enzyme fatty acid synthase (FAS), which is central in the pathway of the novo lipogenesis. ChREBP nuclear translocation is a strong regulator of FAS expression, as such we found that UA significantly increased the nuclear translocation of this transcription factor.

These findings supports that UA is acting to induce liver steatosis by at least two mechanisms: lowering the disposal of intracellular lipids by reducing the expression of ECoAH and through inducing the nuclear translocation of ChREBP with a secondary overexpression of FAS.

Disclosure of Financial Relationships: nothing to disclose

**PUB135**

**Overexpression of Nitric Oxide Synthase and Soluble Guanylate Cyclase and Inhibition of Urea Cycle in Platelets from Hemodialysis Patients** Sergio F. F. Santos,[1] Monique B. Moss,[2] Tatiana M. C. Brunini,[2] Mariana A. S. Siqueira,[2] Antônio Cláudio Mendes-Ribeiro.[2] *[1]Disciplina de Nefrologia, UERJ, Rio de Janeiro, Brazil; [2]Farmacologia, UERJ, Rio de Janeiro, Brazil.*

**Background:** Chronic renal failure (CRF) is characterized by the presence of endothelial dysfunction and platelet activation. Nitric oxide (NO), a potent inhibitor of platelet function, is synthesized from the amino acid L-arginine. Arginase competes with nitric oxide synthase (NOS) for the substrate L-arginine, modulating its activity. Our group has previously demonstrated an activation of L-arginine-NO pathway in blood cells from uraemic patients. The aim of this study was to investigate the effects of CRF in the activity and expression of arginase and expression of inducible (iNOS) and endothelial (eNOS) NOS and soluble guanylate cyclase (sGC) in human platelets.

**Methods:** In this study, 13 uraemic patients under hemodialysis and 12 healthy controls matched for sex and age were included. The expression of iNOS, eNOS, sGC and arginase I and II expressions was accessed by Western Blotting. Arginase activity was analysed through the conversion of [$C^{14}$]-L-arginine into [$C^{14}$]-urea. Mann Whitney test was used for statistical analysis.

**Results:** It was demonstrated an overexpression of both iNOS and eNOS in platelets from CRF patients. The expression of sGC was also increased in CRF patients compared to controls. In contrast, there were no alterations in the expression of both isoforms of arginase in patients with renal failure, although arginase activity was decreased (2.8±0.5 vs 1.3±0.1 pmol urea/ mg protein/2h) in these patients.

**Conclusion:** The present study suggested that the increased of NO synthesis previously observed in CRF may be explained by an overexpression of eNOS and iNOS in platelets. Moreover, the expression of sGC, was also increased in CRF, which could lead to generation of cGMP and inhibition of platelet function. Nevertheless, impairment of arginase activity may provide more substrate for NO production. In this context, the rise of NO bioavailability may contribute to the prolonged bleeding time and bleeding tendency in uraemic patients under hemodyalisis.

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Disclosure of Financial Relationships: nothing to disclose

**PUB136**

**Macrophage Migration Inhibitory Factor (MIF) Activates the PI3K/mTOR Signalling Pathway in Renal Tubular Epithelial Cells** Anisha Tanna, Simona Deplano, Frederick W. K. Tam. *Imperial College Kidney and Transplant Institute.*

**Purpose:**

The phosphoinositide-3-kinase/mTOR signalling pathway plays a crucial role in the cellular response to growth factors and is a key regulator of cell metabolism, proliferation and survival. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine which has been associated with chronic inflammatory diseases such as glomerulonephritis

and recently with tumorigenesis. However, there is limited understanding of the downstream signalling pathways. In this study we investigated the cell signalling cascade following stimulation with recombinant MIF in renal tubular epithelial cells.

**Methods:**

*(1). Cell culture and immunoblotting*

Rat tubular epithelial cells (NRK 52-E) were stimulated with recombinant rat MIF at different concentrations (50-100 ng/ml) and for various time periods (5'- 240') and changes in protein phosphorylation were examined by Western blotting.

*(2). Measurement of rat Monocyte Chemoattractant Protein-1 (MCP-1)*

Rat tubular epithelial cells were treated with different concentrations of recombinant rat MIF (1-50 µg/ml) for 24 hours in the presence or absence of the PI3 kinase inhibitor Wortmannin. MCP-1 production was measured by sandwich ELISA.

**Results:**

(1).At low concentrations, in the range of nanograms per mililitre, MIF induces an increased phosphorylation of Akt and S6 ribosomal protein in a time and dosage-dependent manner.

(2). At higher concentrations, in the range of micrograms per millilitre, MIF leads to significant secretion of MCP-1 in a dose-dependent manner. The presence of the PI3 kinase inhibitor Wortmannin does not appear to affect the production of MCP 1.

**Conclusion:**

Low concentration of MIF is able to upregulate the PI3K/mTOR signalling pathway in renal tubular epithelial cells and at higher concentrations also leads to the production of MCP-1 in renal tubular epithelial cells. This implicates MIFas a potential link between cell proliferation and inflammation and hence an important potential therapeutic target.

Disclosure of Financial Relationships: nothing to disclose

## PUB137

**Repression of Tumor Necrosis Factor alpha Promoter in Human Mesangial Cells by Peroxisome-Proliferator Activated Receptor gamma and Thyroid Hormone Receptor** Angélica Amorim Amato,[1] Flora A. Milton,[1] Juana B. Woitechumas,[1] Marie Togashi,[1] Luiz A. Simeoni,[1] Guilherme M. Santos,[2] Francisco R. Neves.[1] *[1]Pharmaceutical Sciences, University of Brasilia, Brasilia, Federal District, Brazil; [2]Laboratory of Molecular Biology, Medical Research Council, Cambridge, United Kingdom.*

Glomerular mesangial cells have been shown to play critical roles in renal physiology, such as regulation of glomerular filtration, clearance of immunocomplexes and extracellular matrix production. Histopathological findings frequently correlate mesangial cell proliferation and extracellular matrix accumulation to glomerulopathies. Among the etiologic factors of glomerulopathies, it is well known that grow factors and cytokines have a great importance, in particular tumor necrosis factor alpha (TNF-). Antidiabetic drugs such as thiazolidinediones (TZDs), which act as PPAR gamma agonists, have been recently shown to prevent glomerulopathy in animal models by repressing the transcription of many genes involved in renal damage. We investigated whether pioglitazone, a TZD, decreased the mesangial cell proliferation induced by TNF- and if this effect could be attributed by transcriptional repression by the receptor of pioglitazone on TNF- promoter. We also investigated if thyroid hormone influenced TNF- promoter activity. Our results showed that pioglitazone decreased mesangial cell proliferation induced by TNF-. Moreover, it was shown that in these cells pioglitazone repressed the activity of TNF- promoter. We identified that the site for this repression was between -125 and -82 upstream the TATA box and the IC50 was 1,7 x 10-7 M. This effect was not observed in another cell line, namely U937 promonocytes. These results suggest possible mechanisms to explain how TZDs may prevent glomerular injury.ααααααα

Disclosure of Financial Relationships: nothing to disclose

## PUB138

**Losartan Reverts the Mesangial Cell-Myofibroblast Transdifferentiation Induced by High Glucose and Angiotensin II** Mirian A. Boim, Carine Prisco Arnoni, Edgar Maquigussa, Luciana Guilhermino Pereira, Clévia Dos Santos Passos. *Medicine - Renal Division, Federal University of São Paulo, São Paulo, São Paulo, Brazil.*

Diabetic glomerulosclerosis is initiated by mesangial cell (MC) activation, resulting in mesangial matrix expansion and production of pro-fibrotic cytokines. This activation is accompanied by a cell transdifferentiation process, where the MC presents myofibroblastic characteristics, like the expression of smooth muscle α-actin (αSMA) and acquired migration capacity. Angiotensin II (AngII) AT1 receptor blocker, losartan has been shown to revert the renal fibrosis in diabetic animals. The aim of this study was to evaluate the effect of the losartan, on the regression of the MC to myofibroblast transdifferentiation, induced *in vitro* by high glucose environment (HG, 30 mM) and by AngII ($10^{-7}$M). MCs were stimulated with HG and AngII or glucose and AngII simultaneously during 4 days (4d G+AngII) or 6 days (6d G+AngII) to induce the cellular transdifferentiation. The expressions of the αSMA, collagens I and IV, fibronectin and growth factors, including transforming growth factor β1 (TGF-β) and connective tissue growth factor (CTGF) were evaluated by real time PCR and Western blot. Cells of the group 4d G+AngII presented myofibroblastic transdifferentiation characteristics that were even more evident in group 6d G+AngII with migration capacity more than 100% compared with the control cells. This group was used to evaluate the effect of growing doses of losartan ($10^{-4}$-$10^{-6}$M) during the last 48 hr of stimulation. All doses of losartan significantly reduced the expression of the mesangial matrix components, αSMA and cell migration but the highest dose of ($10^{-4}$M) was the more effective to induce reversion of MC to myofibroblast transdifferentiation in the present *in vitro* model. Results suggest that the renin angiotensin system inhibition can be a potential strategy, not only to avoid the progression of the diabetic glomerulosclerosis, but also to reverse the fibrogenic process typical of the diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## PUB139

**Importance of Podocyte B7-1 Expression in Hypoxic Injury and Its Salvage by siRNA Knockdown and Antioxidants** Jer-Ming Chang.[1,2,3] *[1]Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan; [2]Division of Nephrology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [3]Faculty of Renal Care, Kaohsiung Medical University, Kaohsiung, Taiwan.*

**Background/Aim** Hypoxia is a well-recognized factor for renal functional deterioration. We had shown that the hypoxia-induced damage was correlated with cytoskeletal disruption and B7-1 expression in podocytes. It's not clear if inhibition of B7-1 expression may prevent the hypoxia-induced damages and if antioxidants can relieve the injuries.

**Materials and methods** Podocyte cell line derived from H-2K$^b$-tsA58 transgenic mice was cultured. Hypoxic stimuli to podocytes were done by putting cells in the 1% $O_2$ incubator. Molecular cloning of B7-1 and hypoxia-inducible factor (HIF) into plasmids were done with standard method and protein interaction was proved by co-immnunoprecipitation. PCR/Western blot and immunohistochemistry were performed with proper methods. Stress fiber was evaluated by stainingwith rhodamine-phalloidine. RNA knockdown was done by introducing lentiviral plasmid into podocytes. Oxidative injuries were assessed by probing nitrotyrosine production. ADMA (asymmetric dimethylarginine) and aminoguanidine were used to reduce oxidative injuries by LPS-induced iNOS expression.

**Results** Our experiments showed that HIF was stably expressed in podocytes and was enhanced by hypoxic and LPS stimuli, along with the expression of B7-1 (both mRNA and protein). B7-1 and HIF interacts each other by co-IP and endogenous IP. Stress fibers of podocytes were disrupted by either LPS or hypoxic stimuli, demonstrated by merging B7-1 and HIF fluorescence images. iNOS and nitrotyrosine levels were both increased significantly by LPS treatment and moderately by hypoxia. The above changes in podocytes after hypoxic stimuli were prevented by lentiviral-B7-1 RNA interference. However, aminoguanidine and ADMA fail to relieve the oxidative injuries provoked by nitroprusside effectively.

**Conclusion** We found that hypoxia-induced podocyte injuries are B7-1-dependent. Effect of antioxidants has to be re-evaluated in our system. Animal studies concerning development of proteinuria will be our next step to verify our cellular finding.

Disclosure of Financial Relationships: nothing to disclose

## PUB140

**Ghrelin Suppresses Angiotensin II-Induced Renal Tubular Scenscence by Blocking p38-MAP Kinase Pathway** Keiko Fujimura, Shu Wakino, Koichi Hayashi. *Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan.*

(Objectives) Renal cell senescence is one of the main pathways for deterioration of renal function. Both in cellular and organ system, it is believed that environmental stress induces senescence. However, cellular mechanism(s) involved in this signaling have not been fully elucidated. Ghrelin is a growth hormone secretagogue which derived from stomach and has been suggested to exert tissue protective effects. In this study, we examined the mechanisms of angiotensin II (AngII)-induced renal tubular cell senescence and the effects of novel peptide, ghrelin on this cellular change. (Methods) Cultured human proximal cell line, HK-2 cells were utilized. 72 hours after cells were stimulated with 1 mM of AngII, cells were assayed with scenscence-associated b-Gal staining (SA-bGAL staining). Expressions and phosphorylation levels of several molecules involved in cellular senescence were analysed by Immunoblotting. The effects of Ghrelin on AngII-induced senescence were tested by 30 minutes pretreatment with Ghrelin. (Results) In HK-2 cells, the expression both Ghrelin and AngII receptors were expressed. SA-bGal assay demonstrated that AngII induced renal tubular cell senescence, which was attenuated by Ghrelin. AngII activated p38 MAP kinase and the expression of p21 and p53 were increased by Ang II. These molecular changes were also blocked by ghrelin. (Conclusions) Our data indicated that ghrelin suppressed AngII-induced renal tubular senescence by blocking p38-MAP kinase pathway. Ghrelin could be a novel therapeutic strategy against senescence-associated kidney damages including renal atherosclerosis or diabetic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

## PUB141

**Angiotensin II Stimulation of Aquaporin-2 Expression and Trafficking Involves cAMP, PKA, PKC, and Calmodulin-Dependent Signaling Pathways Via AT1 and V2 Receptor** Chunling Li, Weidong Wang, Christopher J. Rivard, Miguel A. Lanaspa, Robert W. Schrier. *Division of Renal Diseases and Hypertension, Univercity of Colorado Denver, Aurora, CO.*

Angiotensin II (ANG II) plays a major role in renal water and sodium regulation. In the immortalized mouse renal collecting duct principal cells ($mpkCCD_{cl4}$) cell line, we treated cells with ANGII and examined AQP2 protein expression, trafficking, and mRNA levels, by immunoblotting, immunofluorescence, and reversed transcription-polymerase chain reaction. After 24 hr incubation, ANGII-induced AQP2 protein expression was observed at the concentration of $10^{-10}$ M and increased in a dose-dependent manner. ANG II ($10^{-7}$ M) increased AQP2 protein expression and mRNA levels at ½, 1, 2, 6, 24 hrs.

Immunofluorescence studies showed that ANGII increased the apical membrane targeting of AQP2 from 30 min to 6 hr. Next, the signaling pathways underlying the ANGII-induced AQP2 expression were investigated. The PKC inhibitor RO 31-8220 ($5\times10^{-6}$ M) and the PKA inhibitor H89 ($10^{-5}$ M) blocked ANGII-induced AQP2 expression, respectively. Calmodulin inhibitor W-7 markedly reduced ANGII and/or dDAVP-stimulated AQP2 expression. ANGII ($10^{-9}$ M) and/or dDAVP ($10^{-10}$ M) stimulated AQP2 protein levels and cAMP accumulation, which was completely blocked by pretreatment with the vasopressin V2 receptor (V2R) antagonist SR121463B ($10^{-8}$ M). Pretreatment with angiotensin AT1 receptor (AT1R) antagonist losartan ($3\times10^{-6}$ M) blocked ANGII ($10^{-9}$ M) stimulated AQP2 protein expression and cAMP accumulation, and partially blocked dDAVP ($10^{-10}$ M) and dDAVP+ANGII induced AQP2 protein expression and cAMP accumulation. In conclusion, ANG II regulates AQP2 protein, trafficking and gene expression in renal collecting duct principal cells. ANGII-induced AQP2 expression involves cAMP, PKC, PKA, and calmodulin signaling pathways via V2 and AT1 receptors.

Disclosure of Financial Relationships: nothing to disclose

## PUB142

**The $EP_4$ Receptor Antagonist L-161 982 Reduces the Expression of $Na^+/K^+$-ATPase and Increases the Apoptotic Response in Hypertonic Mouse M-1 Cortical Collecting Duct Cells** Genevieve Paris, Rania Nasrallah, Richard L. Hebert. *Cellular and Molecular Medicine, Kidney Research Centre, University of Ottawa, Ottawa, ON, Canada.*

Prostaglandin (PG) $E_2$, is highly produced in the kidney and regulates sodium reabsorption, which is driven by the $Na^+/K^+$-ATPase (NKA) pump. It has been reported that high salt (HS) affects the activity of NKA in the cortical collecting duct. The current study addresses the role of $PGE_2$ in the modulation of NKA in a hypertonic environment for 24 hours. We demonstrated by immunoblotting a 2-fold increase in NKA expression and activity following hypertonic conditions (240mMol/L of NaCl, choline chloride, mannitol). Hypertonicity also stimulated cyclooxygenase (COX)-2 expression and $PGE_2$ production as measured by EIA. We detected by real-time PCR an increase in $EP_3$ receptor mRNA, but $EP_1$ and $EP_4$ were unaltered by HS. COX inhibition (indomethacin and ibuprofen) and EP antagonists (L-161 982 ($EP_4$), SC 51089 ($EP_1$)) did not reverse the increase in NKA expression or activity, suggesting that $PGE_2$ is not responsible for the high expression of NKA in HS. However, the $EP_4$ antagonist L-161 982 decreased the expression of NKA α-1 in HS. This effect might result from apoptosis of the cells in the presence of L-161 982, since we demonstrated that anisomycin decreases NKA α-1 expression. Also, we observed that L-161 982 in HS increases the expression of cleaved caspase-3 (2-fold), increases caspase activity (2-fold), decreases AKT phosphorylation (30%), and increases the number of apoptotic nuclei with Hoechst 33342. Additionally, $^3$H-leucine and $^3$H-thymidine incorporation were reduced by 50 % in HS conditions and with L-161 982. None of these apoptotic effects were reversed by $PGE_2$ treatment in HS. These data suggest that L-161 982 can affect cell growth and apoptosis independent of the $PGE_2/EP_4$ system. Further analysis is needed to determine how the $EP_4$ antagonist L-161 982 induces apoptosis in these cells. In summary, we demonstrated that the elevated $PGE_2$ production in HS does not regulate NKA expression and activity or apoptosis, future studies will determine the role of HS-induced $PGE_2$.

Disclosure of Financial Relationships: nothing to disclose

## PUB143

**Does Untreated Obstructive Sleep Apnea Speed-Up Progression of Chronic Kidney Disease in Patients Who Are Not on Dialysis?** Ashok K. Ananthasayanan,[1] Ahmad Kilani,[1] Arash Rashidi.[1,2] *[1]Dept of Internal Medicine, Fairview Hospital, A Cleveland Clinic Hospital, Cleveland, OH; [2]Nephrology & Hypertension, Ohio Medical Group, Westlake, OH.*

Sleep apnea (SA) is common in general population. SA correlates with diseases such as coronary heart disease, arrhythmias, pulmonary hypertension, cor pulmonale, and systemic hypertension. SA has a highly prevalent in patients with end stage renal disease (ESRD), but it is not clear whether SA is a risk factor for progression of chronic kidney disease. We are reporting a case on untreated SA with progressive worsening of kidney function, who responded to SA treatment. A 50 year old Caucasian female with hypertension, diabetes, SA, hypothyroidism, hepatitis C and morbid obesity admitted with heart rate of 35/min and was in renal failure on admission with BUN of 39 and creatinine of 2.18. Her baseline creatinine level prior to this admission was 0.99 to 1.58. She had been diagnosed to have obstructive sleep apnea based on polysomnography. Detailed nephrology workup and investigation pertaining to renal failure was negative, except strong evidence of ischemic kidney. Renal biopsy not done as there was no clear indication and additional benefit. She was given intravenous fluid initially, which did not show any improvement in her renal function. Kidney function continued getting worse and eventually she was started on hemodialysis as her renal function deteriorated. Once she was started on oxygen and BiPAP, her renal function started improving and her dialysis gradually weaned and stopped. Her renal function improved to its baseline and remained stable without a need for dialysis after treating her SA. We like to stress the importance of untreated SA in CKD patients as it can cause ischemic kidney and kidney failure with combination of other factors such as bradycardia, which potentially can diminish oxygen delivery to kidney.

Disclosure of Financial Relationships: nothing to disclose

## PUB144

**Mortality Prediction for Patients with APACHE IV System for ICU Patients Requiring Emergent Hemodialysis** Vijaya Surekha Bhamidipati,[1] Winston Lee,[2] Sheldon Greenberg.[3] *[1]Internal Medicine, Christiana Care Health System, Newark, DE; [2]Nephrology, Maimonides Medical Center, Brooklyn, NY; [3]Nephrology, Maimonides Medical Center, Brooklyn, NY.*

**PURPOSE**

To determine outcome correlation with APACHE IV (Acute Physiology & Chronic Health Evaluation) scores at admission & 24 hours prior to emergent Renal Replacement Therapy (RRT) in patients with Acute Renal Failure in ICU (Intensive Care Unit).

**METHODS**

• Retrospective chart review of single center Medical ICU database of all patients who underwent emergent RRT over 18 months.

• Data was collected to calculate the APACHE IV score on admission to the ICU and 24 hours prior to RRT.

• Correlation between the 2 APACHE IV scores and the outcomes of these patients (death or survival to discharge) was determined.

• Exclusions: ESRD patients, palliative care patients receiving RRT, subsequent ICU admissions.

**RESULTS**

· A total of 70 patient charts were reviewed. 43% (n=30) of the patients died during hospitalization after receiving RRT and 57% (n=40) of patients survived.

· Cox regression analysis was performed with both scores and the APACHE scores 24 hrs prior to RRT correlated better with outcome compared to APACHE score at ICU admission.

· The p value was 0.045 for the analysis with scores at ICU admission and was 0.003 for analysis with scores 24 hrs prior to RRT.

· The hazard ratio increases by 2.1% for every 1 point increase in APACHE I score at ICU admission and increases by 3.2% for every 1 point increase in APACHE score 24 hrs prior to RRT.

**CONCLUSIONS**

· The patients in medical ICU with ARF requiring emergent RRT continue to have very high mortality rates. We have previously submitted results showing that APACHE scores at ICU admission correlate strongly with outcomes after RRT in this subset of patients.

· Calculating scores at admission is standard of care in ICUs, however determining the APACHE IV score 24hrs prior to RRT may be a better guide for predicting outcomes in this group of patients. Further studies are needed with larger patient populations to propose a scoring system that can be used for this group of patients and APACHE IV may be a useful tool for such studies.

Disclosure of Financial Relationships: nothing to disclose

## PUB145

**Comparison of 4 Severity Scoring Systems for Predicting Outcomes in ICU Patients with Acute Renal Failure** Vijaya Surekha Bhamidipati,[1] Winston Lee,[2] Sheldon Greenberg.[3] *[1]Internal Medicine, Christiana Care Health System, Newark, DE; [2]Nephrology, Maimonides Medical Center, Brooklyn, NY; [3]Nephrology, Maimonides Medical Center, Brooklyn, NY.*

**PURPOSE**:

To compare APACHE IV(Acute Physiology & Chronic Health Evaluation), SOFA(Sequential Organ Failure Assessment), MODS (Multi Organ Dysfunction Score ) and SAPS II ( Simplified Acute Physiology Score) scores to predict outcomes in patients in the ICU(Intensive care Unit) with acute renal failure needing emergent Renal replacement therapy (RRT).

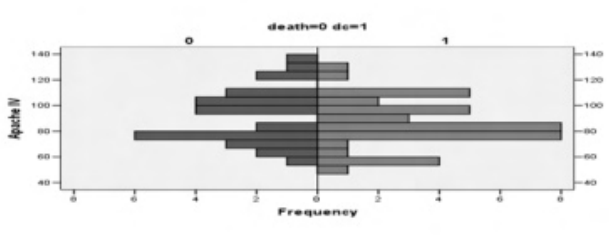
**METHODS**:

· Retrospective chart review of single center Medical ICU database of patients who underwent emergent RRT over 18 months.

· Data was collected to calculate the 4 scores on ICU admission.

· Correlation between the above mentioned scores and the outcomes of patients (death or survival to discharge) was determined after RRT.

Exclusions: ESRD, palliative RRT, subsequent ICU transfers.




**RESULTS**:

· 70 charts reviewed.

· Group 0(Death) =30; Group 1(Survival to discharge)=40

· APACHE IV: Mean in Group 0=92; Group 1= 86.6

· MODS: Mean in Group 0 =9.53; Group 1=8.93( Nonsignificant)

· SAPS II: Mean in Group 0=53.76; Group 1=58.39

· SOFA: Mean in Group 0=10; Group 1=10.44 (Nonsignificant)

· Cox Regression analysis was performed with all 4 scores and APACHE IV score correlated highest with mortality.

**CONCLUSIONS**:

· We have previously presented the correlation between APACHE IV score with outcomes and compared MODS and SAPS II scores with APCAHE IV which was found to have the strongest correlation. We now have similar results with another scoring system, SOFA. We think that the complexity of this system accounts for the strongest correlation.

· Further studies are needed with larger patient populations to propose a scoring system that can be used for this group of patients and APACHE IV may be a useful tool for such studies.

Disclosure of Financial Relationships: nothing to disclose

**PUB146**

**B-Type Natriuretic Peptide and Acute Kidney Injury in Critically Ill Patients** Massimo De Cal,[1] Paolo Lentini,[1] Grazia Maria Virzì,[2] Dinna N. Cruz,[2] Claudio Ronco.[2] *[1]Nephrology, University of Padua, Padua, Italy; [2]Nephrology, San Bortolo Hospital, Vicenza, Italy.*

AKI is a common clinical problem in ICU patients and independently predicts poor outcome. AKI can affect the heart through several pathways, whose hierarchy is not yet established. BNP has diagnostic and prognostic utility in patients with acute decompensated heart failure, and it is an independent predictor for cardiovascular events and overall mortality in various patients group including those with chronic kidney disease.

Our study aimed at investigating whether BNP levels in the first 48 hours may be useful in detecting and/or predicting AKI. We studied a cohort of 26 consecutive patients admitted to ICU of our Hospital. Primary outcome was presence of AKI during admission or development of AKI during ICU stay. Plasma BNP was measured with fluorescence-based immunoassay with Triage point-of-care analyzer (Biosite Inc., USA).

In patients with AKI on admission we found a higher SOFA score (10.0±2.4 vs 6.1±2.1; p=.002) and, as expected, higher creatinine levels (1.85 vs 0.82; p=.001) compared to No-AKI patients. Moreover, AKI patients had higher BNP values on admission compared to No-AKI patients: 510 vs 197 pg/mL, respectively. Plasma BNP levels were also statistically significantly higher for AKI patients at 24 and 48 hours after admission compared to No-AKI patients (p<.05). Moreover, increase in BNP of AKI patients during 48 hours was significant (p=.012).

We also analyzed levels of creatinine and BNP in all patients (9) developing AKI at any point during 48 hours.

For those patients, the difference in BNP versus Non-AKI patients at admission was even more pronounced (p=.05). Moreover, for these patients, creatinine and BNP levels at baseline, and at 24 and 48 hours were significantly higher compared to No-AKI patients (p<.05).

This is the first investigation about BNP levels in AKI or Non-AKI ICU patients. We have shown that patients with AKI have high levels of Creatinine and BNP. Moreover, we found a significant increase of BNP levels in patients with AKI at admission during 48 hours. High levels of plasma BNP may help identify patients with elevated risk of AKI in the ICU setting.

Disclosure of Financial Relationships: nothing to disclose

**PUB147**

**Renal Safety of Ibandronate 6 mg Infused over 15 Minutes Versus 60 Minutes in Breast Cancer Patients with Bone Metastases: A Randomized Open-Label Equivalence Trial** Luc Frimat,[1] Alain Lortholary,[2] Sophie Abadie Lacourtoisie,[3] Fawzia Mefti,[4] Eric Pujade Lauraine,[5] Pascal Bleuzen,[6] Philippe Morvan,[6] Sandrine Kraemer,[6] Xavier Pivot.[7] *[1]Nancy University, France; [2]Catherine de Sienne, Nantes, France; [3]Paul Papin, Angers, France; [4]René Huguenin, Saint-Cloud, France; [5]Hôtel Dieu, Paris, France; [6]Roche, Neuilly-sur-Seine, France; [7]Jean Minjoz, Besançon, France.*

Ibandronate (IB) is indicated to prevent skeletal complications in patients (pts) with breast cancer and bone metastases. A short term infusion could help to overcome time and cost health care issues. The aim of the study is to demonstrate the renal safety equivalence of IB 6 mg infused over 15 min versus 60 min. Pts were women having breast cancer with bone metastasis. Exclusion criteria were renal failure (Cockcroft-Gault < 30mL/min), tooth/jaw disorder or uncontrolled severe diseases. Eligible pts were randomly assigned to receive nine IB 6 mg i.v. infusions over either 15 min or 60 min every 3 weeks for 24 weeks. The primary criterion was creatinine clearance (CC), 28 days after the last infusion. Clinical equivalence was demonstrated if 95% confidence interval (95CI) is in the range [-8,8] mL/min. Out of 334 randomized pts (165 in 15-min group vs 169 in 60-min group), 325 (159 vs. 166) and 312 (151 vs. 161) were respectively included in intent to treat (ITT) and in per protocol (PP) analysis. The difference of CC (95CI) between groups were respectively -2.91 [-7.99, 2.16] and -3.00 [-8.18, 2.18] in ITT and PP, whereas after post-hoc adjustment according to age, the CC are equivalent in both PP and ITT populations. Death and serious adverse event rates did not differ between groups. Three serious adverse events were considered related to IB: an osteonecrosis of the jaw (15-min group), a pain in jaw and an enamel cracking (60-min group). Two renal failures, reported in the 60-min group, were not considered related to IB. None occurred in the 15-min group. In conclusion, IB may be infused over 15 min for 6 months without clinically significant consequence on renal function.

Disclosure of Financial Relationships: nothing to disclose

**PUB148**

**Eculizumab for Atypical Hemolytic-Uremic Syndrome (aHUS) in an Infant Reverses Renal Failure and Need for Dialysis** Christian Hanna, Randa Razzouk, Scott Schurman, James Listman. *Department of Pediatrics, State University of New York, Upstate Medical University, Syracuse, NY.*

Objectives: The aHUS is a clinical triad of microangiopathic hemolytic anemia, thrombocytopenia, and renal impairment. aHUS is a rare and life-threatening disorder associated with congenital or acquired uncontrolled activation of the complement system that leads to thrombotic microangiopathy and acute renal failure. Plasma infusions have variable efficacy, and end-stage renal disease often develops in treatment failure. Eculizumab (Ecu) is a humanized monoclonal antibody that binds to C5 and inhibits terminal complement activation. We describe here the youngest reported case of aHUS who responded to treatment with Ecu. The patients coures and serologic monitoring of Ecu therapy will be discussed.

Case design: A 10-month old infant girl was hospitalized with severe relapsing aHUS. Plasma infusion therapy initially seemed effective during the first 2 months of her presenting diagnosis. However, she subsequently became more recalcitrant to therapy with rapid deterioration necessitating initiation of hemodialysis as well as switch from plasma infusion to plasma exchange. After start of Ecu intervention, she showed rapid response in clinical and serologic parameters. As of two months of Ecu treatment, she continues with sustained response that includes normalization of renal and hematological parameters without the aid of burdensome plasma therapy or dialysis.

Result: Complete control of a plasma-resistant form of aHUS was achieved over a few weeks and sustained following initiation of chronic treatment with Ecu.

Conclusions: Ecu intervention appears to be more effective and simpler treatment option for aHUS than plasma-based approaches, including affecting reversal of renal failure.

Disclosure of Financial Relationships: nothing to disclose

**PUB149**

**Extramedullary Hematopoiesis: A Rare Cause of Acute Kidney Injury** Jay S. Hochman,[1] Stephen D. Vaughan,[1] Suchin Worawichawong,[2] James C. Gough,[2] Hallgrimur Benediktsson,[2] Farshad Sepandj.[1] *[1]Internal Medicine, University of Calgary, Calgary, AB, Canada; [2]Pathology, University of Calgary, Calgary, AB, Canada.*

Extramedullary hematopoiesis (EMH) is a recognized complication of adults with myeloproliferative disease, but renal impairment is rare. We present two cases of biopsy-proven renal EMH, which presented with acute kidney injury (AKI).

The first case involved a 73 year old woman with a known history of myelodysplastic syndrome (MDS) who presented with subacute renal failure. Her history, exam and biochemical investigations did not reveal a diagnosis. She therefore underwent renal biopsy, which showed renal EMH and acute tubular injury. To our knowledge this is the first reported case of renal EMH in a patient with MDS. As no ischemic or toxic insult could be indentified, we hypothesize that renal EMH may lead to acute tubular injury. Prior to the biopsy results, this patient received an empiric course of high-dose steroids and had a biochemical improvement in renal function. As there is little evidence to suggest a beneficial effect of steroids in EMH we hypothesize that steroids may have had a non-specific anti-inflammatory effect, which halted the renal tubular injury.

The second case involved a 62 year old female with myelofibrosis and subacute renal failure. Similar to the first case, this patient's renal biopsy demonstrated EMH and acute tubular injury. In review of her history, there were several factors that may have contributed to her renal failure including a lower GI Bleed. However, we suspect that renal EMH contributed to her tubular injury adding supportive evidence to our interpretation of the first case.

In conclusion, renal EMH is a rare finding that may cause or contribute to development of acute tubular injury in patients with certain hematologic disorders.

Disclosure of Financial Relationships: nothing to disclose

**PUB150**

**The Profile of Acute Kidney Injury in Patients with Acute Viral Hepatitis A on Recent Endemic Outbreaks: A Single Center Experience in Korea** Moon-Jae Kim, Woo Chul Joo, Joon Ho Song, Seoung Woo Lee. *Division of Nephrology & Hypertension, Inha University Hospital, Incheon City, Korea.*

**Background:**

Flu-like outbreaks of viral hepatitis A(VHA) were occured in South Korea for recent years. The existence of VHA-related renal failure as a distinct disease entity is controversial. The purpose of this study was to analyze the clinical features of acute renal injury(AKI) in adults with hepatitis A.

**Methods:**

Clinical and laboratory data of patients with AHA from January 2005 to December 2009 were analysed in our hospital. The diagnosis of AKI was based on appropriate laboratory investigations and RIFLE criteria.

**Results:**

Total 795 patients with VHA were admitted during 5 years and the incidence of AHA was explosively increased since 2008. AKI was noted in 39 cases in those hepatitis A patients(4.9%). Mean ages of AKI patients were in the middle of 4th decade, male was 33 (82.5%). In AKI group serum creatinine levels were peak (6.3 ± 5.7 mg/dl) at 3rd hospital day. Serum AST, ALT and total bilirubin were 2353 ± 2547 IU/l, 2887 ± 2052 IU/I, and 9.3 ± 5.4 mg/dl respectively at that time. The serum creatinine(11.4 ± 5.5 vs 3.1 ± 1.8 and total bilirubin(8.7± 4.6 vs 4.5 ± 2.1mg/dl) were significantly higher in patients of AKI

group than non-AKI group. Oliguria was seen in 85 % of AKI group, and three patients died with fulminant hepatic failure. Mean 4.3 ± 2.3 sessions of HD were performed in oliguric AKI with high level of bilirubin, renal function was completely recovered early than liver function.

**Conclusion:**

AKI is not rare complication of viral hepatitis A. The renal function and mortality were associated by the liver dysfunction in hepatitis A, and the high level of total bilirubin seems to be related to severity of AKI. But any renal injury was not obesrved.

Disclosure of Financial Relationships: nothing to disclose

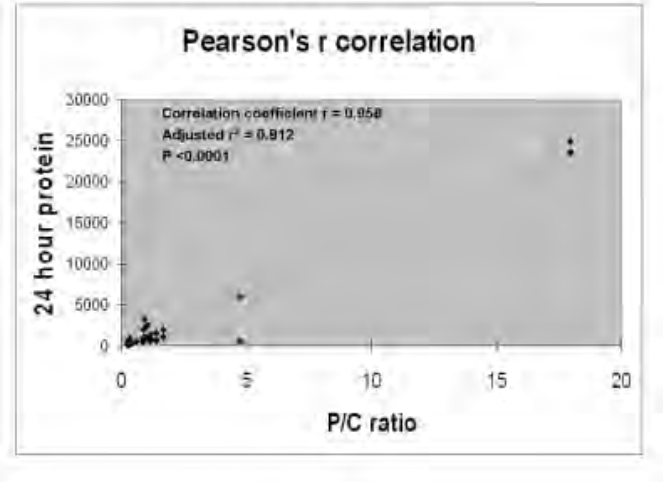
**PUB151**

**Correlation of Spot Urine Protein/Creatinine (P/C) Ratio to 24-Hour Urinary Protein in Patients with Acute Kidney Injury (AKI)** Ayman Layka,[1] Mihail Ion Soare,[1] Mauro Braun,[1] Beth L. Fromkin,[1] Rute C. Paixao,[1] Dianne T. Sandy,[1] Gian M. Novaro.[2] *[1]Department of Nephrology, Cleveland Clinic Florida; [2]Department of Cardiology, Cleveland Clinic Florida, Weston, FL.*

Objective: To determine the correlation of random single voided P/C ratio to measurement of protein in 24 hour urine collection in group of inpatients with acutely compromised glomerular filtration Rate (GFR).

Methods: Spot urine P/C ratio and 24-hour urinary protein were measured by standard methods in group of inpatients with non-oliguric AKI not undergoing renal replacement therapy. AKI is defined as an abrupt (within 48 hours) absolute increase in the serum creatinine concentration (Scr) of ≥0.3 mg/dL from baseline. The correlation between the two methods of measurement of proteinuria was calculated by using Pearson correlation coefficient (r).

Results: A total of 17 patients were available for assessment; 13 males and 4 females with total mean age of 66 ± 14 years. 14 patients had hypertension, and 7 patients had diabetes. Mean baseline Scr was 1.5 ± 0.9 and mean Scr on day of urine collection was 3.7 ± 1.9. Mean GFR estimated by MDRD formula was 24 ± 14 mL/min/1.73m² and mean creatinine clearance calculated from 24 hour urine collection was 36 ± 29 ml/min. Mean 24 hour protein was 2490 ± 5832 mg/24 h and mean P/C ratio was 2.1 ± 4.2 mg/g. The bivariate comparison between the the P/C ratio and 24 hour protein collection, using Pearson's r correlation, demonstrated highly positive correlation, which was statistically significant [adjusted r2 = 0.958; p < (2-tailed) 0.0001].




Conclusion: The correlation between spot urine P/C ratio and 24-hour urinary protein is maintained when GFR is abruptly decreased, and this ratio in random single voided urine may serve as reliable surrogate of a timed protein excretion in patients with AKI.

Disclosure of Financial Relationships: nothing to disclose

**PUB152**

**64 Year Old Woman with Shortness of Breath, Weight Loss, Fatigue and New Onset of Proteinuria, Hematuria and Renal Failure** Snezana H. Mijovic-Das. *Division of Nephrology, Albany Medical College, Albany, NY.*

Hepatitis C (HCV) infection is by far the most common cause of Mixed Cryoglobulinemia (MC). Cryoglobulinemic glomerulopathy is well described in patients with known Hepatitis C infection. By contrast, renal involvement in patients with Mixed Cryoglobulinemia ( MC ) not associated with HCV is less known and it has been poorly described with only a few cases reported.

We were consulted on a 64 year old, African American woman, treated with Methotrexate and Imuran for Polymyositis for more than ten years, for increased shortness of breath, fatigue and weight loss of 80 lbs, new onset of microscopic hematuria , subnephrotic range proteinuria with acute rise in creatinine to 1.2 ( base line 0.6). The serology revealed anemia, increased creatinine to 1.2, ANA > 5000, and >300 anti ds ab, low complements, elevated anti SSa ab and anti RNP ab ; positive cryoglobulins, but negative for Hepatis C. Both IgM and IgG were elevated and SPEP revealed an IgM kappa paraprotein. Renal biopsy was done and all features of cryoglobulinemic glomerulopathy were present. Neither renal biopsy nor bone marrow revealed presence of lymphoma. We treated the patient with Rituximab with obvious improvement of renal function ( Cr 0.6) and decreased proteinuria.

We concluded that the patient had mixed cryoglobulinemic glomerulopathy likely associated with presence of lupus. We believe that mixed cryoglobulinemia in patients without Hepatitis C is of importance because number of patients with Type II Mixed Cryoglobulinemia and IgM kappa component as a marker of B cell proliferation almost exclusively associated with renal involvement is high mostly in regions where the prevalence of HCV infection is relatively low. Occurrence of overt B cell lymphoma later on in the course of disease is well known and repetitive clinical evaluation of the patients is important.

Disclosure of Financial Relationships: nothing to disclose

**PUB153**

**Anti-GBM Disease with Normal Renal Function – 6 Cases Report** Lijun Mou, Limeng Chen, Yan Qin, Yubing Wen, Mingxi Li, Hang Li, Xuemei Li, Xue-Wang Li. *Nephrology Department, Peking Union Medical College Hospital, Beijing, China.*

**Objective** To investigate the clinical and pathologic characteristics of anti-GBM disease with normal renal function. **Methods** In the past 11 years, clinical and pathology data of 35 hospitalized patients with anti-GBM disease in Peking Union Medical College Hospital were reviewed retrospectively. The circulating anti-GBM antibodies were detected by enzyme-linked immunoassay (ELISA). All the patients received the pulmonary CT scan and kidney biopsy. **Results**: 6 of 35 (17.1%) patients, who kept normal renal function for 26m (8m to 129m) were enrolled in this study. The average age of the 4 male and 2 female patients were 32.50±8.12 years old. All of the 4 male had pulmonary hemorrhage, with smoking history. 5 patients were accompanied with renal impairment, microhematuria, proteinuria or nephrotic syndrome. One patient had pulmonary hemorrhage only. The antineutrophil cytoplasmic antibody (ANCA) serology was negative of all. Renal biopsies from 5 patients revealed linear deposition of IgG along the GBM, 1 patient showed linear deposition of IgG along the tubular basement membrane accompanied by granular mesangial C3 deposits, by immunofluorescence. Mild mesangial proliferation and crescents formation in 3 patients , normal lightmicroscopy in two patients and endothelial proliferative glomerulonephritis in one patient were observed. Immunosuppression and/or Plasmapheresis were given to 6 patients. The follow-up of patients (8m-129m) revealed a good prognosis, 4 patients achieved complete remission, 2 had mild proteinuria and microhematuria. Compared with the other 30 anti-GBM disease with renal dysfunction patients, the titers of anit-GBM antibody, ratio of percentage of crescents and anemia of the patients with normal renal function were less severe, prognosis is better. **Conclusion** Anti-GBM disease with normal renal function is not uncommon in younger patients. Under the aggressive treatment, a good prognosis of maintaining normal renal function can be expected, in spite of mild proteinuria or hematuria may leave in some patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB154**

**Clinical Analysis of 35 Cases with Anti-Glomerular Basement Membrane Disease** Lijun Mou, Limeng Chen, Yubing Wen, Yan Qin, Wenling Ye, Jianling Tao, Wei Ye, Hang Li, Xuemei Li, Xue-Wang Li. *Nephrology Department, Peking Union Medical College Hospital & Chinese Academy of Medical Science, Beijing, China.*

**Objective** To investigate the clinical features, treatment and prognosis of anti-glomerular basement membrane (GBM) disease, examine the efficiency and safety of double filtration plasmapheresis (DFPP). **Methods** A total of 35 hospitalized patients diagnosed as anti-GBM disease were recruited in our center. All patients were divided into 3 groups as the manifestation at admission. Group I: patients with severe pulmonary hemorrhage or RPGN were treated with pulse methylprednisone with or without DFPP, then followed by prednisone and CTX (n=24). Group II: patients without severe pulmonary hemorrhage and RPGN were treated with prednisone and CTX (n=5). Group III: 5 ESRD patients and 1 normal renal function patient (n=6) were not received immunosuppression thearapy. Anti-GBM antibody titers of pre and post-DFPP in 4 patients were measured consectively, removal rates were calculated. **Results** The mean age of all patients was 41.1±16.6 y. 45.7% presented with pulmonary-renal syndrome. 20% patients had positive P-ANCA serology. 54.2% crescentic glomerulonephritis and 8 with other types of glomerulonephritis were revealed by kidney biopy (n=24). Patients in Groups I showed more severe manifestation at admission: higher concentration of serum creatinine, peak titers of anit-GBM antibody, percentage of crescents, with the follow-up 7 died, but 50% patients avoided death and/or dialysis-dependent end-stage renal disease. No patient died in group II and III. The older age, anemia, higher Scr(>300μmol/L), oligouria or anuria and immediate HD required at admission, more glomerular sclerosis, but not the percentage of crescent and therapy strategy, predicted poor prognosis. The anti-GBM antibody was negative after 4-6 times of DFPP , the median removal rate was 55%. During total 94 DFPP sessions, there were no unacceptable morbidity. **Conclusions** Different thearapy strategy is necessary for anti-GBM disease with different clinical manisfestation. DFPP is an effective and safe way to clear the anti-GBM antibody.

Disclosure of Financial Relationships: nothing to disclose

**PUB155**

**Anuric Acute Kidney Injury Induced by Acute Mountain Sickness Prophylaxis with Acetazolamide** Javier Neyra,[1] James Castle,[3] James E. Novak.[2] *[1]Department of Internal Medicine, Henry Ford Health System, Detroit, MI; [2]Division of Nephrology and Hypertension, Henry Ford Health System, Detroit, MI; [3]Division of Nephrology and Hypertension, British American Hospital, Lima, Peru.*

Acetazolamide (ACZ) is a carbonic anhydrase inhibitor used as a diuretic, anticonvulsant, and antiglaucoma agent. ACZ is also used as prophylaxis for acute mountain sickness before ascent to high altitude. A 55-year-old healthy man with a history of incidentally-detected kidney stones traveled to Peru. He took 2 doses of ACZ 250 mg before ascent to Ancash, Peru (4500 meters above sea level) and 3 doses of 250 mg while at peak ascent, all at intervals of 12 h. He had taken similar prophylaxis without incident on previous trips. 24 h after peak ascent, he developed headache, nausea, bilateral low back pain, and oliguria. Renal ultrasound showed uncomplicated right-sided nephrolithiasis without obstruction and an empty bladder, confirmed by bladder catheterization. Creatinine and BUN were 9.5 mg/dL and 94 mg/dL, respectively. Blood tests showed mild leukocytosis, high anion gap metabolic acidosis, and hyperphosphatemia. The patient received normal saline with minimal response and subsequently required 2 sessions of hemodialysis for anuric acute kidney injury (AKI). Renal Doppler ultrasound, serum complement, ASO, CPK, and myoglobin were normal. UA showed pH 6, urine protein:creatinine ratio 0.24, and fractional excretion of sodium 22%. Urine microscopy showed isomorphic RBCs (12-15/hpf) but no eosinophils, casts, or crystals. Renal function returned to baseline after 96 h of supportive care and the patient was discharged. 24-h urine collection after recovery revealed normal excretion of electrolytes, creatinine, and uric acid. The pathogenesis of AKI was attributed to ACZ crystalluria causing tubular obstruction. This classic presentation of anuria and renal colic has been described previously in patients receiving ACZ for glaucoma and epilepsy. However, ours is the first report of AKI after exposure to prophylactic doses of ACZ to prevent acute mountain sickness. This case highlights the risk of adverse outcomes even in previously healthy individuals and suggests that increased fluid intake may be advisable in travelers taking ACZ prophylaxis.

Disclosure of Financial Relationships: nothing to disclose

**PUB156**

**Non-Dilated Obstructive Uropathy – An Unrecognized Cause of Acute Renal Failure in Hospitalized US Patients** Macaulay A. Onuigbo,[1,2] Kayode C. Lawrence,[3] Nnonyelum T. Onuigbo.[4] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Midelfort Clinic, Eau Claire, WI; [3]Nephrology, Elmhurst Hospital Center, New York, NY; [4]IT, NT Systems, Eau Claire, WI.*

Background/Objective

The syndrome of non-dilated obstructive uropathy (NDOU) and ARF is well reported. However, the literature suggests that this syndrome is rare, accounting for less than 5% of cases of urinary obstruction. Our recent experience with three cases of NDOU seen within a space of months suggests otherwise.

Methods

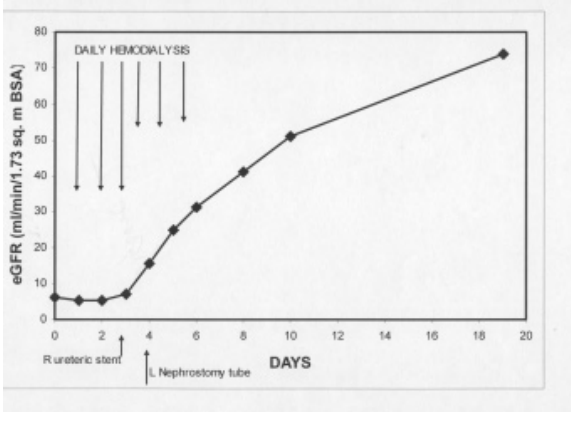
Between March 2009 and October 2009, in a small Mid-western American town Nephrology practice, we successfully managed three cases of NDOU. They all presented with newly symptomatic ARF.

Results

Renal imaging revealed no dilatation in both kidneys in one, unilateral dilatation only in the second, and dilatation was absent in a single functioning kidney in the third. Two males, three females, mean age 61 years. Peak creatinine, 320–880 umol/L. Despite absence of dilatation on renal imaging, strong suspicion for NDOU led to decompression procedures with prompt recovery of kidney function in all three patients – two required percutaneuous nephrostomy tube placements and/or ureteric stents and one responded to simple Foley catheter drainage. One required temporary hemodialysis (Table 1, Figure 1).

Conclusion

We submit that NDOU may be more common than previously speculated. A high index of suspicion is warranted as significant renal salvage can often be achieved by timely decompression procedures.




Charateristics of 3 patients with NDOU

| AGE/SEX | Peak S Cr (mg/dL) | New S Cr (mg/dL) | Cause of Non-dilatation | Hemodialysis | Treatment |
|---|---|---|---|---|---|
| 56/M | 10.3 | 1.2 | Metastatic bladder cancer | YES | R Ureteric stent + L perc Nephrostomy |
| 59/F | 4.4 | 1.08 | Metastatic uterine cancer | NO | L perc Nephrostomy |
| 67/M | 4.0 | 1.8 | BPH, short duration | NO | Foley Catheter |

Disclosure of Financial Relationships: nothing to disclose

**PUB157**

**N-acetyl Cysteine in the Management of Carboplatin- and Ifosfamide-Induced Nephrotoxicity** Rasmi Palassery, Osvaldo Regueira, Curtis W. Turner, Tetyana L. Vasylyeva. *Department of Pediatrics, Texas Tech University Health Sciences Center, Amarillo, TX.*

**Background**: Carboplatin and ifosfamide are alkylating chemotherapeutics used to treat solid tumors. N-acetyl cysteine (NAC) was protective in ifosfamide-induced nephrotoxicity *in vitro*, but its clinical use for carboplatin nephrotoxicity has not been described. We report the case of NAC treatment for acute renal injury in an adolescent who received ifosfamide, carboplatin, and paclitaxel. **Case Report:** A 15-year-old male received chemotherapy for abdominal recurrence of a mixed germ cell tumor of the testis. The primary tumor was treated with cisplatin, etoposide, and bleomycin eight-months earlier. Serum creatinine after the first chemotherapy cycle ranged between 1.05 and 1.61 mg/dL. The day before initiating the second course of chemotherapy, the patient's serum creatinine was 1.23 mg/dL. After hydration with IV fluids, he received paclitaxel (257 mg), carboplatin (653 mg), and ifosfamide (3400 mg) admixed with mesna (684 mg) on day 1. He received ifosfamide (3420 mg/day) admixed with mesna (684 mg), with appropriate hydration for 4 subsequent days. During the week, urinary output decreased and serum creatinine steadily increased to 2.06 mg/dL by day 7. NAC (600 mg PO BID) was initiated. Creatinine rose to 2.25 mg/dL the next day and then began to fall. NAC was discontinued on day 11 and the patient was discharged on day 15 when his creatinine was 1.12 mg/dL. **Conclusion:** NAC ameliorates chemotherapy-induced nephrotoxicity. The observed effect may be due to alterations in mitochondrial apoptotic pathways and/or glutathione replenishment. NAC's antioxidant properties are known to reverse free-radical-mediated cell damage and mitochondrial injury, both possible pathways of carboplatin and ifosfamide renal injury. Prophylactic or concomitant NAC treatment with carboplatin and ifosfamide should be considered to prevent nephrotoxicity.

Disclosure of Financial Relationships: nothing to disclose

**PUB158**

**Acute Tubular Necrosis Associated with Vancomycin Alone: Is It so Uncommom?** Mohammad A. Quasem. *Internal Medicine, United Health Services Hospitals, Binghamton, NY.*

***Introduction:*** Vancomycin is nephrotoxic. Nephrotoxicity was reported in 0-5% in 1980s. However recent reports indicate higher incidence of nephrotoxicity. It is more nephrotoxic in combination with other nephrotoxic medications particularly aminoglycosides. According to recent publications, nephrotoxicity of vancomycin alone as high as 19% and as high as 28% in combination with aminoglycosides.. The most common type of toxicity is acute interstitial nephritis (AIN). Acute tubular necrosis (ATN) from vancomycin alone is very rare. Some earlier literatures report vancomycin alone never causes ATN.

***Case:*** We present a biopsy-proven case of ATN from vancomycin alone. Our patient, a 63 yr old female admitted with left hip cellulitis. She was put on iv vancomycin. After 5 days, she was discharged home with administration of iv vancomycin at home by home care nurse. Vancomycin (random) level was 84 mcg/ml after 10 days and she developed acute kidney injury(AKI) with serum creatinine of 4.6 mg/dl (baseline 0.8 mg/dl). She was re-admitted to hospital and kidney biopsy showed tubular injury without any inflammation to suggest AIN. Vancomycin was stopped and the patient had full recovery of renal function after 4 weeks.

***Conclusion:*** Vancomycin associated ATN probably more common than anticipated as biopsies are rarely done.

Disclosure of Financial Relationships: Ownership: Pfizer, Astra-Zaneca; Honoraria: Novartis Pharmaceutical.

**PUB159**

**Unusually Slow Recovery from Acute Tubular Necrosis Due to Zoledronic Acid. A Case Report** Frederic F. Rahbari-Oskoui,[1] Odicie O. Fielder,[3] Nima Ghasemzadeh,[1] Randolph A. Hennigar.[2] *[1]Medicine, Emory University, Atlanta, GA; [2]Pathology, Emory University, Atlanta, GA; [3]Medicine, Kaiser Permanente, Atlanta, GA.*

**Background:** Dose dependant acute tubular necrosis (ATN) due to Zoledronic acid (Zometa) has been reported. Most cases have involved prostate cancer and multiple myeloma, where majority of patients required chronic hemodialysis. All reported cases of recovery, if present, occurred within 4 months of acute insult.

**The case:** A 59 year old white female with diabetes mellitus, hypertension and metastatic breast cancer and normal kidney function was treated with 15 monthly injections of Zometa for prevention of hypercalcemia and had developed two episodes of mild acute kidney injury (AKI) with serum creatinine levels of < 2 mg/dl, which had resolved by withholding the treatment. After the 16th injection, she developed severe AKI

due to ATN based on urine microscopy. Zometa was the only possible offender. Serum creatinine stabilized around 8.0 mg/dl without hyperkalemia, hypervolemia or uremic symptoms, therefore initiation of dialysis wasn't deemed necessary. But due to lack of significant improvement of kidney function, worsening of subnephrotic range proteinuria and appearance of eosinophiluria over the next 3 months, a kidney biopsy was performed to rule out other possible etiologies. Diagnosis of pure ATN was confirmed. The course was remarkable for an unusually slow and progressive recovery over more than 15 months, with stabilization of serum creatinine around 3.0 mg/dl without any need for dialysis. This slow pattern of recovery is extremely unusual for any cause of ATN.

**Conclusion:** Repeated injections of Zometa may result in severe AKI even if previous reversible episodes of mild AKI occurred. Presence of such episodes may represent a warning sign for development of severe ATN by cumulative dose toxicity and indicate the need for discontinuation of treatment. Even though most reported cases of ATN needed immediate initiation of dialysis without any reported recovery, in the absence of absolute indications, dialysis may not be necessary and progressive recovery of renal function may occur up to 15 months after the original insult.

Disclosure of Financial Relationships: nothing to disclose

## PUB160

**Granulomatous Interstitial Nephritis: A Rare Presentation of Crohn's Disease** Manish K. Saha, Tarek Hamieh, Vishal Sagar. *Department of Medicine, Regions hospital, University of Minnesota, Saint Paul, MN.*

Case Presentation: A 17-year-old male with Attention Deficit Hyperactivity Disorder was admitted with generalized weakness and malaise. Work-up revealed a creatinine of 4.0 mg/dL. Urinalysis was negative for RBC, cast or significant proteinuria. Hepatitis panel was nonreactive. A percutaneous kidney biopsy revealed noncaseating granulomas, acute on chronic tubulointerstitial nephritis (TIN) with normal glomeruli . A diagnosis of granulomatous interstitial nephritis (GIN) was made and prednisone was started. Two weeks later, creatinine decreased to 2.8 mg/dL.However patient developed diarrhea when prednisone was tapered. Colonoscopy was consistent with Crohn's disease (CD). He was restarted on prednisone with improvement of his GI symptoms and stable creatinine. He did not tolerated higher doses of prednisone and attempts at tapering prednisone resulted in worsening of kidney function. Subsequently he also failed azathioprine. Infliximab was started with improvement in his creatinine to 2.6mg/dl and resolution of diarrhea.

Discussion: We present this case of granulomatous interstitial nephritis (GIN) as the initial manifestation of Crohn's disease (CD). Historically, most cases of TIN associated with CD have been strongly attributed to aminosalicylates (5-ASA) exposure. Our case was unique in that the initial manifestation of CD was renal insufficiency secondary to GIN. The GIN found on kidney biopsy subsequently raised the suspicion for inflammatory bowel disease (IBD) in our patient when he presented with diarrhea. Additionally, this case highlights the need to consider TNF-alpha inhibitors before pursuing further aggressive measures such as renal replacement therapy, transplant, or proctocolectomy. Since it may be the same immune mechanism causing intestinal inflammation and interstitial nephritis, infliximab may play a role in renal insufficiency in patients refractory or intolerable to corticosteroids/aminosalicylates. Infliximab may also be better at controlling diarrhea than other immunomodulators thus decreasing the prerenal effects of hypovolemia on existing CKD.

Disclosure of Financial Relationships: nothing to disclose

## PUB161

**Biopsy-Proven Acute Tubular Necrosis in High Vancomycin Level Associated Acute Kidney Injury: Case Series** Ganesh B. Shidham,[1] Ravish Shah,[1] Anil K. Agarwal,[1] Tibor Nadasdy.[2] *[1]Internal Medicine, Ohio State University Medical Center, Columbus, OH; [2]Pathology, Ohio State University Medical Center, Columbus, OH.*

**Purpose of study:** To our knowledge this is the first case series reporting biopsy-proven acute tubular necrosis (ATN) associated with high levels of vancomycin. Recently there has been a 10- to 20-fold increase in vancomycin use. Outpatient vancomycin therapy, higher trough levels, longer treatment duration, concomitant nephrotoxic agents, and chronic kidney disease are among the risk factors for nephrotoxicity. Although allergic interstitial nephritis is a well-known complication, vancomycin induces oxidative stress, resulting in ATN as shown in animal studies. Human cases with biopsy-proven vancomycin-associated ATN have been rarely reported in the literature. **Methods:** A retrospective chart review was performed on patients who underwent a kidney biopsy for acute renal failure (ARF) and high levels of vancomycin with clinical suspicion of vancomycin-associated ARF. Fifteen cases were identified from Nov 2008 to Dec 2009.

**Results:**

| Number of biopsies | Age (years) | Creatinine baseline (mg/dl) | Creatinine peak (mg/dl) | Creatinine at recovery (mg/dl) | Vancomycin Level* (mcg/ml) | Biopsy Findings | |
|---|---|---|---|---|---|---|---|
| | mean: 58.4 | mean: 1.04 | mean: 6.5 | mean: 1.5 | mean: 48.8 | ATN | ATN + TIN |
| 15 | range: 22-83 | range: 0.5 to 2 | range: 3.8-14 | range: 0.8 to 2.2 | range: 22 to 149 | 5 | 10 |

TIN: Tubulointerstial nephritis. * Vancomycin therapeutic level 15-20 mcg/ml

**Conclusion:** Our retrospective review of kidney biopsies in patients with ARF and high levels of vancomycin shows universal evidence of ATN and in some cases mild to moderate tubulointerstitial nephritis. Though a few patients needed renal replacement therapy, prognosis of vancomycin-associated ARF is uniformly good with improvement of creatinine to baseline. We recommend close monitoring of trough levels of vancomycin, particularly in those with risk factors such as chronic kidney disease, advanced age, liver disease, and after exposure to contrast agent to prevent vancomycin-associated ARF.

Disclosure of Financial Relationships: nothing to disclose

## PUB162

**How Safe Is Exenatide in Type2 Diabetics with Renal Impairment?** Rajesh Shrivastava, Ashraf I. Mikhail, Sadananda V. Aithal. *Morriston Hospital, United Kingdom.*

The management of type 2 diabetes mellitus in patients with renal impairment is complicated by weight gain and hypoglycaemic events.Incretin-mimetics hoped to address these issues. Exenatide is licenced for use in type2 diabetes mellitus in patients with mild renal impairment(crcl 50-80ml/min) and with caution in moderate renal impairment (crcl 30-50ml/min).

We report the effects of exenatide in a 63year old male with poorly controlled type2 diabetes, obesity (Wt 105.5 Kgs, BMI 34.4),hypertension,hyperlipidaemia and stable chronic kidney disease(creatinine 138µmols/l,eGFR 45mls/min/1.73m2) with microabuminuria. He commenced Exenatide 5µg twice daily, increased to 10µg in four weeks. He developed nausea and intermittent vomiting with the increased dose. His glycaemic control improved and weight dropped to 99.6Kgs. He presented 2months later with a creatinine of 1393µmol/L. He appeared euvolaemic and maintained a urine output of 2l/day. His BP was 145/70. Urinalysis was normal. He commenced haemodialysis 48hrs later.

Renal biopsy showed mild diffuse mesangial sclerosis.The striking feature was proximal tubular dilatation with hydropic vacuolation and features of acute tubular necrosis(ATN).

Exenatide, ramipril, metformin, frusemide and rosuvastatin were discontinued. His renal function improved as shown and he was off dialysis after 3weeks.

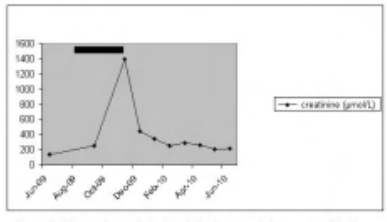



Figure 1. Change in creatinine in relation to exenatide exposure (black rectangle)

The potential natriuretic action of exenatide along with diuresis and ACE inhibition may have compounded the effects of subclinical volume depletion in causing ATN in our case.This highlights the need to exercise extreme caution with the use of exenatide even in patients with stable early CKD in the presence of other risk factors for renal injury. Exenatide induced renal injury requiring dialysis has been reported to FDA. However there are no published reports describing the natural course of patients needing dialysis following acute kidney injury post exenatide use.

Disclosure of Financial Relationships: nothing to disclose

## PUB163

**Coexisting IgA Nephropathy and Antiglomerular Basement Membrane (Anti-GBM) Disease** Vishwanath Siddini, Sudarshan H. Ballal. *Nephrology, Manipal Hospital, Rustom Bagh, Bangalore, Karnataka, India.*

BACKGROUND: Antiglomerular basement membrane (GBM) disease is characteristically described with linear deposition of IgG along glomerular basement membrane. Concurrence of IgA nephropathy and anti-GBM disease is very rare. Here is a case report of a patient who was diagnosed to have concurrent anti-GBM disease and IgA nephropathy

CASE: A 31 yr old female with no previous comorbidities presented with history of hematuria, oligoanuria and nausea and vomiting. On examination she had features of volume overload. Investigations showed serum creatinine of 7mg/dl and Hemoglobin of 8.4 gm/dl. Urinalysis showed 3+ protein with 10-15 RBCs. Ultrasound abdomen showed normal sized kidneys. Serum C3, ANA and ANCA was negative and serum Anti-GBM antibody was positive. She underwent renal biopsy after initiation of hemodialysis. LM showed enlarged glomeruli with cellular and fibrocellular crescents, mesangial proliferation and mild irregular basement membrane thickening. IF revealed linear IgG deposits and significant mesangial IgA deposits. Serum Anti-GBM antibody was positive. She was diagnosed to have antiGBM disease with IgA nephropathy. She received total 3 gms of iv methyl prednisolone followed by oral steroids. She also received 8 sessions of plasmapheresis, intermittent hemodialysis and 500 mg of IV cyclophosphamide. She continued to be anuric. Currently, she is on maintenance hemodialysis and the oral steroid has been stopped.

CONCLUSIONS: Concurrence of anti-GBM disease with other IgA nephropathy is very rare. After extensive search we could find only 2 case reports of coexisting IgA nephropathy and anti-GBM disease. To our knowledge this is the first case report of IgA nephropathy with anti-GBM disease from India.

Disclosure of Financial Relationships: nothing to disclose

**PUB164**

**Plasmapheresis in Non-Diarrhoeal HUS Patients** Vishwanath Siddini, Sudarshan H. Ballal. *Nephrology, Manipal Hospital, Bangalore, Karnataka, India.*

**AIM**: To study clinical profile and role of Plasmapheresis in atypical HUS.

**MATERIALS AND METHODS:** 11 patients between 4 and 15 years of age with non-diarrhoeal HUS were studied. The clinical profile and severity of lesions on renal biopsy were studied. Plasmapheresis (single volume) was instituted soon after diagnosis and continued till hematological parameters were near normal and stable. Efficacy of PP was assessed based on duration and severity of illness, number of sittings required and time for normalization of hematological and renal parameters.

**RESULTS:** There were 8 males and 3 females with mean age of 9.4 years (range 4 to 15). Patient's parameters are in the table.

Table 1

| Age (years) | Duration of Illness | Urine Output | Hb% | Platelet Count | LDH | Peak S. Cr. (mg/dL) | Renal Biopsy |
|---|---|---|---|---|---|---|---|
| 5 | 1 month | Oliguric | 6.5 | 50,000 | 1754 | 5.2 | Glomerular |
| 11 | 1 month | anuric | 7.6 | 41,000 | 1007 | 6.8 | Arteriolar |
| 8 | 3 days | non-ologuric | 5.7 | 20,000 | 2691 | 3.8 | Glomerular |
| 4 | 1 week | Oliguric | 6.2 | 30,000 | 2340 | 4.5 | Glomerular |
| 6 | 10 days | oliguric | 7.5 | 55,000 | 1608 | 2.4 | not done |
| 9 | 1 week | non-oliguric | 8.5 | 20,000 | 1998 | 9.8 | Glomerular |
| 11 | 2 days | Oliguric | 9.3 | 83,000 | 5623 | 4.9 | not done |
| 13 | 1 week | non-oliguric | 6.6 | 67,000 | 1156 | 3 | glomerular |
| 11 | 2 days | Oliguric | 5.3 | 65,000 | 2718 | 16.8 | Arteriolar |
| 12 | 1 week | Oliguric | 4.3 | 92,000 | 3084 | 5 | not done |
| 15 | 6 weeks | Oliguric | 7.3 | 18,000 | 1898 | 7.1 | Arteriolar |

All patients except 2 required dialytic support. Patients required a mean of 11.4 sittings of PP to normalize and stabilize their haematologic parameters. One patient had recurrence of renal and haematologic abnormalities after 10 days and received another sitting of 5 cycles of PP. Another patient had recurrence after 5 days requiring 6 sittings of PP. Renal parameters took longer (> 4 weeks) to recover and all except 3 have normal renal functions on follow up. One patient expired. Two patients are still dialysis dependent.

**CONCLUSIONS**: PP is an effective form of therapy in atypical HUS and we found it to be useful even with a long duration of disease and severe lesions (arteriolar) on biopsy.

Disclosure of Financial Relationships: nothing to disclose

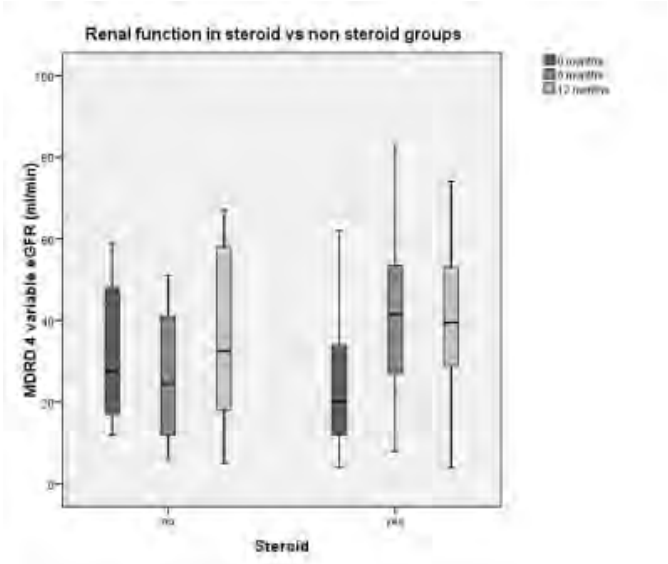
**PUB165**

**Long Term Outcome in the Largest Single Centre Study of the Effect of Steroids in Acute Idiopathic Interstitial Nephritis** Anisha Tanna, Jenny Rayner, Alan D. Salama, Frederick W. K. Tam, David Taube, Seema Singh, Charles D. Pusey, H. Terence Cook, Neill D. Duncan. *Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom.*

There are no prospective randomised control trials of steroid treatment for Acute Idiopathic Interstitial Nephritis [AIIN], and retrospective studies to date are very limited. This pathology is relatively common, AIIN was found in 271/2855 native renal biopsies at our centre 2000-2010.

**Methods:** This is a retrospective longitudinal study of all patients with biopsy proven acute interstitial infiltration in the absence of additional renal pathology. Patients on maintenance steroid treatment for proven vasculitis or other chronic inflammatory condition were excluded. Treated patients received oral Prednisolone ≥0.5mg/kg, an intention to treat analysis was performed.

**Results:** Eighty one patients were treated with steroids: 48 male, 51±17yrs, follow-up 32±26 months. Sixteen patients were not treated with steroids: 13 male, 55±17yrs, follow-up 37±26 months. Compared to eGFR at initiation of treatment, there was a significant difference in the steroid group at 6 and 12 months (paired t-test, p<0.001). In the non-treated group there was no significant difference in GFR at 6 or 12 months.




The mean change in EGFR to six months was plus 16.3 ml/min/month for the steroid group and minus 14.0 ml/min/month for the non-treated group (unpaired ttest, p<0.05). There was no significant difference in the proportion of patients on RRT at last follow between the two groups (Chi squared P=0.86)

**Conclusion:** Corticosteroids were beneficial in the treatment of AIIN, however despite the size of this study there are limitations of retrospective analysis, and treatment bias must be taken into account. A prospective randomised controlled trial into the use of corticosteroids in this condition is urgently needed.

Disclosure of Financial Relationships: nothing to disclose

**PUB166**

**A Case of Acute Renal Failure with Rapidly Enlarging Kidneys** Lakshmi Turlapati, Tejas P. Desai, Karlene O. Hewan-Lowe. *Department of Nephrology, East Carolina University, Greenville, NC.*

**Case Information:** A 52-year-old obese female admitted for pancreatitis was treated empirically with vancomycin and piperacillin-tazobactam. Her renal function at admission was normal. A week into her hospitalization, she developed progressively worsening renal failure and generalized macular blanching rash. She became oliguric and required dialysis support. Her urine microscopy revealed many monomorphic red blood cells. She had proteinuria (1 gram/24 hours). Her serological work up was significant for low complements. Serial non-contrast CT scans showed rapidly increasing kidney size from 12.6 cm to 16 cm bilaterally within 8 days. Subsequently she had renal biopsy which showed dispersed interstitial mononuclear inflammatory infiltrate with rare eosinophils and tubular damage. She was diagnosed as having acute interstitial nephritis secondary to piperacillin which was discontinued. She was treated with steroids resulting in clinical improvement.

**Discussion:** Acute interstitial nephritis is a known reversible cause of acute renal failure. Major secondary causes of acute interstitial nephritis include drugs, infections and autoimmune disorders. Most cases are idiopathic. Symptoms of AIN usually start approximately 3 weeks after the drug exposure but may range from 1 day to 2 months. Clinical manifestations may vary from asymptomatic elevation of BUN/creatinine or abnormal urinary sediment to generalized hypersensitivity syndrome with fever, rash, eosinophilia and oliguric renal failure. Our patient had fever, rash and oliguric renal failure but did not have peripheral eosinophilia or eosinophiluria. It has been reported that kidneys may be normal or enlarged in size. In our case we report a significant acute enlargement in kidney size on serial scans which could increase the positive predictive value for acute inflammatory processes affecting the kidneys. Supportive treatment with withdrawal of the causative agent usually results in improvement of renal failure. The role of steroids still needs to be defined.

Disclosure of Financial Relationships: nothing to disclose

**PUB167**

**Acute Tubulo-Interstitial Nephritis in a Patient with Hepatitis B on Tenofovir** Hima Bindu Yalamanchili,[1] Sandeep Ravi,[1] Yue Shen,[1] Gilbert W. Moeckel,[2] George Abdelsayed,[1] James P. Gavin.[1] *[1]Internal Medicine/ Nephrology, Bridgeport Hospital/Yale University, Bridgeport, CT; [2]Department of Pathology, Yale University School of Medicine, New Haven, CT.*

Introduction: Acute Interstitial Nephritis (AIN) has been reported in Human Immunodeficiency Virus patients on atazanavir and tenofovir. We present a patient with Hepatitis B on tenofovir monotherapy presenting with acute renal failure secondary to acute tubulo-interstitial nephritis.

Case: A 67-year-old African American male with past medical history of chronic active Hepatitis B infection treated with tenofovir and hypertension was admitted with generalized weakness,nausea for one week. His medications were atorvastatin, hydrochlorothiazide, and atenolol. There were neither any new medications nor any dosage changes prior to

current presentation. Initial serum chemistry revealed Blood urea nitrogen of 173 mg/dl, Creatinine 18.6 mg/dl, bicarbonate 22 meq, Phosphorus level 12.5 mg/dl, (a baseline creatinine 1.23) and Creatinine phosphokinase of 741 IU/l. Urine analysis demonstrated specific gravity 1.014, no casts, negative for urinary glucose with rare urine eosinophils. 24 hr urinary protein was 830 mg/day. Investigations for HIV and cryoglobulins were negative. Renal ultrasound demonstrated normal sized kidneys. Biopsy demonstrated acute tubulo-interstitial nephritis with focal glomerulosclerosis. His renal function gradually improved with transient hemodialysis(10 days),Tenofovir discontinuation and 3-day course of pulsed methylprednisolone followed by prednisone taper.

Unique features: To our knowledge acute tubulo-interstitial nephritis was not previously described on tenofovir monotherapy. Few previous case reports of AIN were reported in patients with HIV on combination of tenofovir and atazanavir. Tenofovir alone was associated with Fanconi's syndrome and proximal tubular dysfunction. Due to the temporal association and improvement of renal function off tenofovir, tenofovir is the most likely cause of acute renal failure in this patient.

Objective: This case highlights the importance of awareness that tenofovir might be a rare cause of acute tubulo-interstitial nephritis.

Disclosure of Financial Relationships: nothing to disclose

**PUB168**

**Stage 3 Chronic Kidney Disease (CKD): Aliskiren and Proteinuria** Marcos Luis Álvarez Alejandre,[1] Consolación Rosado Rubio,[1] Raquel López de la Fuente,[2] Jose L. Lerma,[1] Cyntia Gonzalez Álvarez,[1] José Luis Rodríguez Commes.[1] *[1]Servicio de Nefrología, Hospital Clínico de Salamanca, Salamanca, Spain; [2]Centro de Salud Santa Elena, Zamora, Zamora, Spain.*

Introduction:

Block early proteinuria is an effective mechanism to slow the progression of renal damage and reduce cardiovascular morbidity, greatly increased in the ERC. One of the fundamentals is to inhibit angiotensin Rennin axis. However, the dual blockade with ACEI + ARB, can impair kidney function and induce hyperkalemia, especially in the elderly.

Recently it has aliskiren, direct renin blocker, but is unknown effect on proteinuria and progression of CKD in the elderly when added to ACEI / ARB.

Objectives:

1. To assess the antiproteinuric effects of aliskiren for 6 months.
2. To determine the impact on renal function
3. Establish clinical and metabolic side effects

Methods:

We studied prospectively a population of 44 patients (mean age 70 +/- 5) CKD stage III treated with ACEI and/or ARBs 150-mg/dia Aliskiren was added. Each patient was in control of himself and used a control group of 12 patients with similar characteristics treated with ARB (olmesartan) was determined microalbuminuria, serum K, serum Cr. Ccr (MDRD) The statistical analysis was conducted using as variance using the Student t test or Kruskal-Wallis test, depending on variables.

Results:

There was a significant reduction from 1012 to 875 microalbuminuria ($p < 0.002$)

The average concentration of serum K was 4.5 mg / dl, unchanged throughout the study. ($p = 0.86$)

There was a significant reduction in serum Cr from 1.98 to 1.94 mg / dl ($p < 0.003$) and Ccr increased from 36.61 to 37.8 ml/min ($p < 0.013$).

Conclusions:

1. Microalbuminuria Aliskiren significantly reduced in elderly patients with CKD stage III, previously treated with ACE inhibitors and / or ARB.

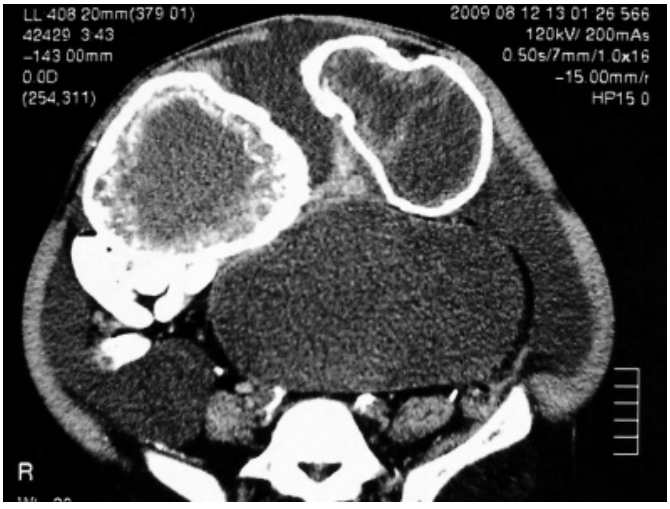
2. The good tolerability of aliskiren, the lack of metabolic adverse effects (hyperkalemia) and the moderate increase in glomerular filtration allow its use in this population with close monitoring, but requires long-term randomized studies to verify these results.

Disclosure of Financial Relationships: nothing to disclose

**PUB169**

**Unusual Calcified Mesenteric Pseudocysts in a Chronic Hemodialysis Patient** Heino R. Anto,[1,2] Suk Chul Kim,[1] Anatole Hounnou,[2] Richard Steinberg.[2] *[1]Nephrology, Saint John's Episcopal Hospital, New York, NY; [2]Nephrology, Peninsula Hospital Center, New York, NY.*

A 32 y/o male on hemodialysis for 8 years and whose renal replacement was exclusively hemodialysis, developed nephrogenic ascites over a 7 year period. During his dialysis tenure, the patient was poorly compliant in keeping his dialysis sessions, following his diet, or taking his phosphate binders. His serum phosphorous was frequently over 5.5 mg/dl, and serum intact PTH over 1000 pg/ml. A 2009 CT of his abdomen revealed calcified mesenteric pseudocysts which were not present on a 2003 CT scan.




We believe that the structure of these pseudocysts are extremely unusual, and have never been reported in the medical literature. In conclusion, we postulate that these pseudocysts are the result of ectopic calcification, possibly related to a high calcium x phosphorous product, fetuin-A , and undercarboxylated Gla-protein deficiencies.

Disclosure of Financial Relationships: nothing to disclose

**PUB170**

**Pitfalls in Managing Cardiovascular Risk of Young Patients with Chronic Kidney Disease Stages III and IV** Rammohan Sripad Bhat,[1] Ajay Prabhakar Dhaygude,[2] Christopher Federick Wong,[1] Christopher John Goldsmith,[1] Edmond O'Riordan,[4] Poonam Batra,[2] Prasad Rajendran,[1] Anindya Banerjee.[3] *[1]Nephrology, University Hospital Aintree, Liverpool, Cheshire, United Kingdom; [2]Nephrology, Royal Preston Hospital, Preston, United Kingdom; [3]Nephrology, Arrowe Park Hospital, Wirral, United Kingdom; [4]Nephrology, Salford Royal Hospitals NHS Trust, Manchester, United Kingdom.*

Purpose of the study was to assess the prevalence and management of cardiovascular risk in young adult population with Chronic Kidney Disease stages III and IV

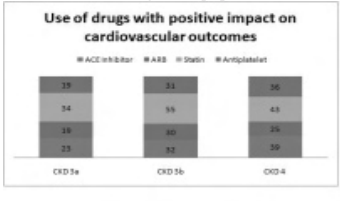
We collected demographic, clinical and lab data on patients aged 65 years or less with CKD stage III and IV attending nephrology outpatients at 4 different hospitals across the North-West of England. Results were compared with Renal Association standards.

Data was collected on 217 patients of whom 60 belonged to CKD stage IIIa, 77 to stage IIIb and 80 to stage IV. Males constituted 36% of the study population. We found that significant proportion of patients were still hypertensive and obese, whilst smoking habit and cholesterol level were not checked or documented in medical notes in substantial number of patients.

Prevalence of poorly addressed cardiovascular risk factors in study population

| Risk Factor | No of Patients (%) | Data not recorded in medical notes (%) |
|---|---|---|
| Diabetes (HbA1c > 7.5%) | 70 (32.2%) | 0 |
| Hypertension (BP > 130/80) | 164 (75.5%) | 17 (7.8%) |
| Obesity (BMI > 25.0) | 78 (35.9%) | 22 (56.2%) |
| Smoking | 35 (16.12%) | 22 (56.22%) |
| Cholesterol > 4.0mmol/L | 80 (36.86%) | 97 (44.7%) |
| Protenuria (ACR > 3.5 or PCR > 15) | 106 (48.84%) | 31 (14.28%) |

Figure 1 shows use of medications which can positively impact on cardiovascular outcomes of this high risk population.




Whilst various guidelines recommend that we address cardiovascular risk factors during every outpatient contact, we found less than acceptable attention to them.

Disclosure of Financial Relationships: nothing to disclose

**PUB171**

**A Prospective Observational Multicenter Study To Evaluate the Effectiveness and Safety of Methoxy Polyethylene Glycol-Epoetin Beta (Mircera®) in Patients with Anemia Secondary to Chronic Kidney Disease. MINERVA Study** Aleix Cases,[1] J. M. Portolés,[2] J. Calls,[3] Alberto M. Martinez-Castelao.[4] *[1]Hospital Clinic, Barcelona, Spain; [2]Hospital Fundación Alcorcón, Spain; [3]Hospital de Manacor, Mallorca, Spain; [4]Hospital de Bellvitge, Barcelona, Spain.*

**Background:** Mircera® is a continuous erythropoietin receptor activator approved for the treatment of anaemia associated with chronic kidney disease (CKD) in patients on dialysis and patients not on dialysis with once-monthly maintenance dosing.

**Objective:** This study examined the effectiveness and safety of Mircera® when administered in patients with anemia secondary to CKD in routine clinical practice.

**Methods:** A prospective observational multicenter study. Information about patients on haemodialysis and conservative therapy were collected. The results of a preliminary analysis of the 3-month follow-up are presented.

**Results:** A total of 240 patients were evaluated (56.3% male): mean age: 70.8±14.7years. Diabetic nephropathy was the most common etiology (23.3%). 60.8% of patients were in IV-V stage, 27.7% were naïve and 33.1% converted patients. Of patients on haemodialysis (39.2%), 11.4% were naïve and 27.7% converted patients Among converted patients, 64.5% previously received epoetin beta and 32.9% darbepoetin alpha; mean weekly doses 7,122±6,660.3IU and 38.9±37.8µg, respectively. Median doses of Mircera® at baseline and 3 month were 87.5(75-150)µg/month and 112.5(75-162.5)µg/month in naïve patients, and 100(75-150)µg/month and 100(75-175)µg/month in converted patients, without significant differences between groups during this period. Mean Hb levels in converted patients were 11.4±1.3g/dL at the time of conversion and 11.4±0.7g/dL after 3 months. No differences between groups in Hb levels were detected during the evaluation period. 68% of converted patients at baseline and 72.2% in month 3 presented Hb levels ≥11mg/dL. Only a non-drug related adverse event was reported.

**Conclusions:** The preliminary results appear to indicate that Mircera® is an effective and safe election for correct and maintenance of stable Hb levels. Further analyses will be needed to confirm these data.

Disclosure of Financial Relationships: nothing to disclose

**PUB172**

**Is It Possible To Control Dyslipidemia Using Atorvastatin in Patients with Chronic Kidney Disease?** Eleni Chelioti, Athanasios Georgiou, Antonios Zagorianos, Prokopis Papazafeiris, Sotiris Mikrow, Gabriel Papadakis. *Dept. of Nephrology and Renal Unit, General Hospital of Piraeus, "Tzaneio", Athens, Greece.*

Purpose: Dyslipidemia is proposed to be a secondary cause of renal damage. Using of statins ameliorates renal function and reduces the cardiovascular risk in patients with stage 3 and 4 chronic kidney disease (CKD). This analysis looked at lipid profile in patients with stage 3 and 4 CKD who were receiving atorvastatin and which of them had lipid levels according to published guidelines.

Method: A comparative study was performed in Outpatient Clinic. Participants were 109 (50 female/ 59 male, age range 55-85 years) patients with CKD (clearance creatinine range 30-60 ml/min/1.73m2). The group of patients who were received atorvastatin, had a dose range 10-40mg. Laboratory parameters were evaluated for total cholesterol (tChol), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). The CKD was identified using the MDRD equation. Statistical analysis based on percentage evaluation.

Results: The patients using atorvastatin had a mean eGFR 48 ml/min/1.73m2 and them without statins had mean eGFR 34 ml/min/1.73m2 ($p<0.05$). Fifty nine percent of patients not used statins and 41% were receiving atorvastatin with a mean dose 20±10mg. The percentage of patients without statins had tChol <200mg/dl, TG <150mg/dl, LDL <100mg/dl, and HDL >35mg/dl was 45%, 35%, 37% and 39% respectively and patients using atorvastatin had 64% of tChol <200mg/dl ($p<0.001$) , 60% TG <150mg/dl ($p<0.001$), 56% LDL<100mg/dl ($p<0.001$) and 44 % HDL >35mg/dl (p=NS).

Conclusion: It is difficult to control dyslipidemia in patients with stage 3 and 4 CKD despite the fact that more half of apparently controlled patients using atorvastatin had lipid levels below targets in compare to patients without statins. Although, the patients who receiving atorvastatin had a better renal function. These observations may help to render plausible a strategy for a strength use of statins.

Disclosure of Financial Relationships: nothing to disclose

**PUB173**

**Primary Physicians Rarely Detect Chronic Kidney Disease in Type 2 Diabetic Patients** Laura Cortes Sanabria, Héctor R. Martínez Ramírez, Alfonso M. Cueto-Manzano, Enrique Rojas-Campos. *Unidad de Investigación Médica en Enfermedades Renales, UMAE, HE, IMSS, Guadalajara, Jalisco, Mexico.*

Type 2 diabetes mellitus (DM2) is the most common cause of end-stage renal disease (ESRD) in many parts of the world. A large number of individuals at early stages of chronic kidney disease (CKD) are largely undiagnosed and untreated; consequently, ESRD is continuously increasing. Primary physicians (PP) are the most indicated to perform the measures for preventing, diagnosing and treating CKD at early stages.

***Aim:*** To determine the frequency with which PP identify risk factors and establish the diagnosis of CKD in DM2 patients.

***Methods:*** Six hundred twenty seven patients were randomly selected from the total DM2 population ≥18 years attending to 11 primary health-care units. Clinical charts were reviewed searching for clear evidence of CKD diagnosis, classification and risk factor identification. CKD was defined according to K/DOQI criteria.

***Results:*** Patients were 63±13 years, 56% female, and had 11±7 years of DM2 vintage. Age >60 years was present in 63%, smoking in 38%, obesity in 59%, dyslipidemia in 48%, hypertension in 58%, use of nonsteroidal anti-inflammatory drugs in 24%, and urinary tract infection in 10%. Inadequate control was observed for blood glucose in 53%, for hypertension in 71%, and for serum lipids in 21%. None of the latter variable was identified as risk factor for CKD. Serum creatinine was measured in 354 patients (56%) within the previous 18 months; from these, only 11 patients had an estimated GFR. Using clinical record data, CKD stages 3-5 was present in 21% of the sample; however, in none of the cases CKD diagnosis and classification were established by PP.

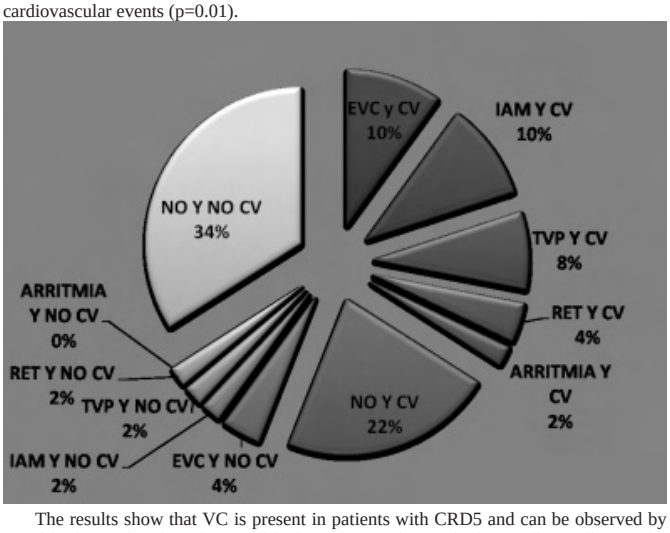
***Conclusions:*** CKD was neither diagnosed nor classified in this sample of patients (in spite of being present in a 21% of them). A high frequency of CKD risk factors was present, but in no case they were clearly identified in the clinical records. Despite PP were taking care of DM2 patients at high risk for CKD, they rarely requested-calculated indicators of renal function. Efforts have to be done to increase the clinical competence of PP for diagnosing CKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB174**

**Survey of Vascular Calcification and Atherosclerosis in Patients with Chronic Renal Disease in Dialysis by Carotid Echography** Luis Gerardo D'Marco, Dayana J. Olmos, Lurlinys Flores, Liana Brito, Lisbeth Martinez. *Nephrology, Ruiz y Paez University Hospital, Bolivar, Venezuela.*

Clinic and epidemiological studies have shown that cardiovascular disease is related to an increase in the mortality rate of patients with chronic renal disease (CRD). Vascular complications are mainly secondary to calcification and atherosclerosis. CV does not only involve passive calcium and phosphorus deposits in atherosclerotic blood vessels but also active 'ossification' of vascular structures. High phosphate levels and the increased Ca x P play an important role in this process, and seem to be associated with an increased mortality. **OBJECTIVE**: The purpose of this study was to determine the presence of VC and atherosclerosis by means of carotid echography in 50 patients with CRD5 in dialysis. **METHODS**: Fisher Test was used to determine whether or not the differences were significant, which was decided based on p–value obtained in the test (<0.05). **RESULTS**: The age of patients averaged 45.34 years and most of patients were female (56%). The predominant dialysis method was hemodialysis (52%) for an average period of 23.38 months (SD: ± 29.52). The average laboratory test values were: hemoglobin (9.8 g/%), urea (141.3 mg/dl), creatinine (7.4 mg/dl), uric acid (6.9 mg/dl) (p=0.050), cholesterol (188.2 mg/dl), triglycerides (206.5 mg/dl), albumin (3.47 mg/dl), calcium (8.69 mg/dl), phosphorus (6.5 mg/dl), and Ca x P (55.4). VC was observed in 56% of patients; 46% had echographic criteria for atherosclerosis (intima-media thickness ≥ 0.9 mm) with an overall average of 0.89 mm (SD: ±0.28) (p=0.0006). The group with VC had more adverse cardiovascular events (p=0.01).




The results show that VC is present in patients with CRD5 and can be observed by carotid echography; thus, the use of the latter as a routine method to predict cardiovascular events in this group.

Disclosure of Financial Relationships: nothing to disclose

**PUB175**

**Association between Hemoglobin (Hb) Stability and Clinical Outcomes in Patients Receiving Erythropoiesis-Stimulating Agents (ESAs)** Angel Luis M. De Francisco. *Servicio de Nefrologia, Hospital Universitario Marques de Valdecilla, Santander, Spain.*

**Purpose:** To investigate the specific effect of Hb variability on cardiac and other safety outcomes.

**Methods:** Data from Phase III trials with methoxy polyethylene glycol-epoetin beta (a continuous erythropoietin receptor activator [C.E.R.A.]) were used to assess associations between Hb variability and clinical outcomes. A total of 1879 patients (pts) on dialysis and not on dialysis receiving C.E.R.A. every 2 weeks (Q2W) or once monthly (Q4W), or comparator ESA treatments (epoetin alfa or beta, darbepoetin alfa) were included in the analysis. Hb levels were to be maintained between 10.5-13 g/dL. Logistic regression was used to analyze the association between Hb values and adverse event (AE) occurrence.

**Results:** During the studies, 25% of pts had ≤47.2% of Hb values within ±1 g/dL of baseline (pre-treatment), 25% had >47.2% and ≤62.8%, 25% had >62.8% and ≤77.3%, and 25% had >77.3%. A total of 783 pts had 1136 AEs: vascular access thrombosis (n=220), arrhythmia (n=168), congestive heart failure (n=92), sepsis (n=84), cerebrovascular accident (n=53), and cardiac arrest (n=50). There was a significant association between Hb stability and a reduced incidence of several categories of AEs.

| Adverse events | Odds ratio (95% confidence interval) |
|---|---|
| Arrhythmia | |
| Q2 | 0.98 (0.64, 1.49) |
| Q3 | 0.75 (0.48, 1.16) |
| Q4 | 0.62 (0.39, 0.99) |
| Cardiac arrest | |
| Q2 | 0.69 (0.35, 1.36) |
| Q3 | 0.40 (0.18, 0.89) |
| Q4 | 0.23 (0.09, 0.63) |
| Cerebrovascular accident | |
| Q2 | 0.54 (0.27, 1.04) |
| Q3 | 0.22 (0.09, 0.55) |
| Q4 | 0.31 (0.14, 0.70) |
| Congestive heart failure | |
| Q2 | 0.82 (0.48, 1.39) |
| Q3 | 0.52 (0.29, 0.95) |
| Q4 | 0.46 (0.25, 0.86) |
| Sepsis | |
| Q2 | 0.62 (0.38, 1.06) |
| Q3 | 0.30 (0.16, 0.57) |
| Q4 | 0.15 (0.08, 0.35) |
| Vascular access thrombosis | |
| Q2 | 0.88 (0.61, 1.27) |
| Q3 | 0.74 (0.50, 1.08) |
| Q4 | 0.50 (0.33, 0.76) |

Odds ratio reflects the comparison to the lowest quartile (Q1: ≤47.2% of baseline ±1 g/dL)

Q2=% Hb ≤62.8, n=473

Q3=% Hb ≤77.3, n=481

Q4=% Hb >77.3, n=456

Q Quartile of percentage of Hb values within ±1 g/dL of baseline

Pts who had ≤47.2% of Hb levels within ±1 g/dL of baseline had the highest risk of these AEs.

**Conclusion:** These preliminary analyses suggest that there is a potential association between Hb stability and improved cardiac and other clinical outcomes.

Disclosure of Financial Relationships: Consultancy: Shire, GambroResearch Funding: Amgen, Roche.

## PUB176

**Widespread Pain, Depression, Sleep Disturbance, and Quality of Life in Dialysis Patients** Ma. Clarissa H. Del Rosario, Afton L. Hassett, Malathi Chamarthi, John A. Walker, Naomi Schlesinger. *Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ.*

**Background:** End Stage Renal Disease (ESRD) is often accompanied by multiple co-morbidities, including sleep disturbances, depression, chronic pain, and fatigue. Few studies have evaluated whether fibromyalgia (FM) occurs concomitantly with these symptoms.

**Objective:** To evaluate the prevalence of depression and sleep disturbance in ESRD patients requiring renal replacement therapy (RRT) and whether these are associated with concomitant FM.

**Methods:** Outpatients at two dialysis facilities were invited to participate in this study. Patients who did not speak English or who were severely demented were not invited to participate. Of those invited, 48 patients gave informed consent and completed the Quick Inventory of Depressive Symptomatology, the Pittsburgh Sleep Quality Index, and the SF-36. All were evaluated for FM using American College of Rheumatology criteria with tender point examination.

**Results:** Of the 48 patients who completed the study protocol, 26 (56.5%) were men. Mean patient age was 62.1 years (SD±15.6) and mean years of education was 14.3 (SD±3.2). None of the participants met criteria for FM although one patient had widespread pain. Depression was common amongst patients; 22 (45.8%) earned scores consistent with at least mild depression. Most patients (53.1%) also earned scores consistent with likely sleep disturbance. Those with at least mild depression were more likely to have sleep disturbances than those without depression (t= 3.961, P>0.001). Health related quality of life was within the mean expected for patients with medical illness (Physical Component Scale score=47.7[SD±19.3] and Mental Component Scale score= 59.1[SD±18.5]).

**Conclusions:** In this study, patients receiving RRT commonly suffered from depressive symptoms. Sleep disturbances also were common and associated with the presence of depression. Despite this, patients' health related quality of life was similar to that of other medical patient populations. Our findings suggest that routine screening for depression and sleep disturbance should be an important part of the management of patients receiving RRT.

Disclosure of Financial Relationships: nothing to disclose

## PUB177

**Influence of Serum Magnesium Levels on the Prevalence and Progression of Vascular Calcification in CKD 4 and Dialysis Patients** Philip D. Evans,[1] Mhairi K. Sigrist,[2] Chris W. McIntyre.[1,3] *[1]Royal Derby Hospital, Derby, United Kingdom; [2]University of British Columbia, Vancouver, Canada; [3]School of Graduate Entry Medicine, University of Nottingham, Derby, United Kingdom.*

Magnesium (Mg) has been implicated as a factor associated with reduced levels of vascular calcification (VC). The aim of this study was to investigate if serum Mg levels characteristically seen in a CKD population treated with non-Mg based phosphate binders and conventional lower Mg concentration dialysate was associated with the prevalence, or development, of progressive VC.

We studied 134 patients (60 on hemodialysis [HD], 28 on peritoneal dialysis [PD], and 46 with stage 4 CKD). Predialysis serum Mg levels were measured and VC assessed at baseline, 12 and 24 months. A calcification score (CaSc) was generated by quantifying the calcification in a standardized section of the superficial femoral vein from multislice CT. A standard diasylate magnesium concentration of 0.5mmol/L was used for both HD and PD patients, no oral Mg salts were used in this population.

The groups were well matched for age, gender, diabetic status, smoking status, body mass index and dialysis vintage (where appropriate). There was no significant difference in mean serum Mg (HD[±SD]: 1.04±0.16mmol/L; PD: 0.9±0.15mmol/L; CKD4: 0.93±0.12mmol/L; p=NS).There was a statistically significant difference between baseline prevalent CaSc (HD [median +IQR]: 121 [0 to 610]; CKD4: 2 [0 to 197]; p=<0.05; PD: 21 [0 to 343]; p=NS) and no difference in mean CaSc progression (HD[±SD]: 123.8±213.5; PD: 101.6±194.1; CKD4: 121.1±250.1; p=NS). There was no association of serum Mg as a continuous or categorical (converted into tertiles) variable with either prevalent or progressive CaSC.

We were unable to demonstrate an association with serum pre-dialysis Mg levels and any measure of prevalent or progressive VC in CKD patients characterised by Mg levels largely within the normal range.

Disclosure of Financial Relationships: nothing to disclose

## PUB178

**The Usefulness of Serum Cystatin C for Prediction of Microvascular Complications in Type 2 Diabetic Patients** Sung-Kyu Ha,[1] Sung Jin Moon,[1] Sung Chang Bae,[1] Jwa-Kyung Kim,[1] Sang Hun Lee,[1] Jung Eun Lee,[2] Hyeong Cheon Park.[1] *[1]Internal Medicine, Gangnam Severance Hospital, Seoul, Korea; [2]Internal Medicine, Yongin Severance Hospital, Yongin, Korea.*

Introduction: The objective of this study was to evaluate the prognostic value of cystatin C for macro- and micro-vascular complications in type 2 diabetic patients.

Methods: A total of 300 patients (200 men, mean age 62.9±8.7 years) with type 2 diabetes at Gangnam severance hospital were included. Receiver operator characteristics (ROC) analyses of cystatin C to detect major adverse cardiac events (MACE), retinopathy, and microalbuminuria were conducted.

Results: ROC curve analyses demonstrated cystatin C 1.285 and 0.96 as the cutoff levels to predict retinopathy (AUC: 0.694) and microalbuminuria (AUC: 0.684), respectively. On multivariate logistic regression analysis adjusted by age, sex, DM duration, and HbA1C, cystatin C levels ≥ 1.285 (OR: 3.419, 95% CI 1.741-6.716, p-value <0.001) and ≥ 0.96 (OR: 2.464, 95% CI 1.082-5.610, p-value =0.032) were independent predictors for retinopathy and microalbuminuria, respectively. However, cystatin C could not detect the MACE.

Colclusion: This study demonstrates that cystatin c may predict the retinopathy and microalbuminuria in type 2 diabetic patients.

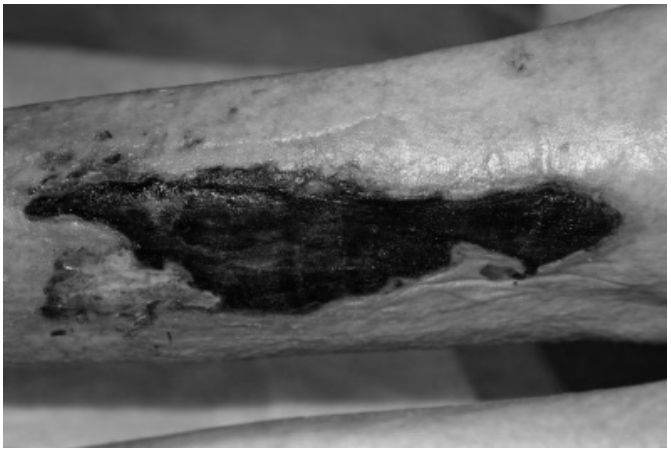
Disclosure of Financial Relationships: nothing to disclose

## PUB179

**Presenting an Unusual Case of Calciphylaxis in a 64 Year Old Female Hemodialysis Patient Despite Normal Clinical Markers and Negative Skin Biopsy** Hooman Hajian. *Division of Nephrology, University of Washington, Seattle, WA.*

Calciphylaxis is an extra-osseous vascular calcification which can occur in end-stage renal disease (ESRD). It is a rare but serious condition due to systemic medial calcification of the arterioles leading to ischemia and subcutaneous necrosis. Microscopically, there is small vessel mural calcification with or without endovascular fibrosis, extravascular calcification, and thrombotic occlusion. It most commonly occurs in ESRD patients on Hemodialysis (HD) or after kidney transplant, although it may also happen without ESRD. It manifests as areas of painful ischemic necrosis usually over the abdomen, buttock, and thigh. Some of the risk factors for Calciphylaxis in ESRD are female sex, obesity, elevated phosphorus, co-administration of certain medications, autoimmune disorders, and hypercoagulable states.

We present a case of a 64 year old Caucasian female with hypertension, Diabetes Mellitus type 2, DVTs, hypothyroidism, CVA, myocardial infarction, paroxysmal atrial fibrillation on no anticoagulation, and ESRD who presented with multiple ulcers on bilateral upper and lower extremities (LE) and right hip. The LE ulcers were well demarcated with thick black eschar and painful.



A LE skin biopsy a week earlier did not show Calciphylaxis. Serum calcium and phosphorus levels were 7.7 and 3.7 mg/dL, respectively. Intact PTH was 139 pg/mL (normal 9-73). Cryoglobulin, Cryofibrinogen, and ANCA screen were negative. Given the clinical suspicion, a repeat skin biopsy was done which confirmed Calciphylaxis and proper management was initiated.

Given its low incidence and high mortality, timely diagnosis of Calciphylaxis is crucial. While the importance of skin biopsy to establish diagnosis is uncertain, it is commonly used to confirm the diagnosis. However, it should not over-rule or replace a clinician's clinical assessment or suspicion.

Disclosure of Financial Relationships: nothing to disclose

**PUB180**

**Antihypertensive Therapy Modulates Markers of Cardiovascular Disease Risk in Older People with Chronic Kidney Disease** Stephen G. John,[1] Paul J. Owen,[1] Jane H. Youde,[1] Chris W. McIntyre.[1,2] *[1]Royal Derby Hospital, Derby, United Kingdom; [2]University of Nottingham, Derby, United Kingdom.*

Chronic kidney disease (CKD) is highly prevalent in older people and is associated with changes in cardiovascular (CV) function and elevated CV risk. Whilst aggressive blood pressure (BP) control is the cornerstone of CKD management, doubt exists concerning the current optimal BP targets in this group, primarily due to falls risk. As part of a prospective study of CV function and falls in response to the introduction and escalation of antihypertensive therapy (AHT) we assessed systemic markers of CV health in older (>70 years) non-diabetic patients with CKD 3/4.

We recruited 61 subjects (including non-CKD controls). AHT was fully withdrawn for 2 weeks before initial assessments of skin Advanced Glycation End-products (AGE) as a measure of cumulative metabolic stress, routine bloods and proteinuria (UPCR). AHT was restarted to a target BP of 130/80mmHg. Assessments were repeated at four weeks after full titration of medication (AHTr), and after a further 12 months follow-up (FU).

Mean age was 76±4yrs, mean eGFR in the CKD group was 42±14ml/min/1.73m$^2$. AHT used was in line with current guidelines and predominantly based on RAAS inhibition (mean achieved BP 128/69 mmHg). Significant proteinuria was present in 18%. Skin AGE, haemoglobin, albumin and proteinuria did not change. Serum sodium fell with AHTr (median 141±3 to 140±3mmol/l; p<0.001), but rose over one year (140±3mmol/l; p=0.017). Whilst CKD did not progress in the CKD group, serum creatinine rose at FU in non-CKD patients (76±19 to 84±25mmol/l; p=0.038). Increased serum corrected calcium at AHTr did not further change at FU (2.34; 2.38; 2.36mmol/l; p=0.005; p=ns). An initial rise in serum phosphate with AHT was normalised by one year (1.11; 1.17; 1.10mmol/l; p<0.001; p=0.004). 25-OH vitamin D fell over the study (58 to 42 ng/ml; p<0.001).

AHT introduction rapidly causes alteration of humoral markers of CV health, which are partially reversed over time. There is no appreciable change in tissue AGE levels as marker of cumulative metabolic stress over a one year period in either CKD patients or hypertensive subjects with normal renal function.

Disclosure of Financial Relationships: nothing to disclose

**PUB181**

**A Study of the Seasonal Change in Albuminuria** Koichi Kanozawa, Tokushi Nakajima, Hajime Hasegawa. *Division of Nephrology and Hypertension, Saitama Medical Center, Saitama Medical University, Kawagoe, Saitama, Japan.*

Background: Microalbuminuria is important not only for early diabetic nephropathy but the early diagnosis of CKD that it is the risk factor of a cardiovascular disease or end stage renal disease from the first. Glomerular hyperfiltration, some problems of blood vessel side including the the impaired endothelial function and the oxidative stress, and reabsorption in the renal tubules are suggested to the appearance mechanism of urinary albumin excretion (UAE), and also being associated with blood pressure (BP) is known. On the other hand, BP is influenced of temperature and a seasonal variation exists.

Aims: It is examined whether a seasonal change exists in UAE.

Methods: For the outpatient during hypertensive medical examination, we measured UAE, office and home BP for all seasons. We distributed them in each season, and analyzed. Furthermore, we evaluated seasonal variation in hemoglobin (Hb), HbA1c, estimate glomerular filtration rate (eGFR), and serum 8-hydroxy-2'deoxyguanosine (8-OHdg) level.

[Results] This study was performed in a medical center and a clinic of Saitama in Japan. There is no change in the dose of anti-hypertensive agents during observation, and UAE evaluated a total of 1286 measurement by 178 patients who had normo- or microalbuminuria (less than 300 mg/gCr). The UAE of the summer was significantly low in comparison with the UAE of other seasons (autumn; 43.1±58.8 mg/gCr, winter; 45.0±55.4 mg/gCr, spring; 38.0±55.1 mg/gCr, summer; 29.1±40.8 mg/gCr, respectively. This variation was accepted each in the non-diabetes-mellitus (DM) and in DM group. On the other hand, UAE correlated also with which each systolic and diastolic BP. The seasonal change existed in each BP. In the systolic home BP, the group which was more than an average accepted the seasonal change of UAE strongly compared with the group which was below an average. In Hb, HbA1c, eGFR, and serum 8-OHdg level, the seasonal variation was not accepted.

Conclusion: There is a seasonal change in UAE and it was suggested that it was influenced of the seasonal variation of BP. In evaluation of the UAE, seasonal variation should be considered.

Disclosure of Financial Relationships: nothing to disclose

**PUB182**

**Effects of IV Iron Use in Non Dialysis CKD** Margaret Knight,[1] Hongyan Du,[2] L. Tammy Ho,[1] Stuart M. Sprague.[1] *[1]Division of Nephrology and HTN, Northshore University HealthSystems, Evanston, IL; [2]Center on Outcomes, Northshore University HealthSystems, Evanston, IL.*

The use of intravenous iron (IVfe) is a cornerstone of anemia management in the dialysis population. IVfe is not well studied in nondialysis CKD (NDCKD) patients (pts). Limited studies have shown that IVfe can raise iron saturation (%fe) and improve hgb, however effects of longer term use in NDCKD pts receiving EPO is not known.

Methods: 112 pts with NDCKD receiving IVfe were retrospectively studied using a system wide electronic medical record, available since 2006 for both inpatient and outpatient care. Utilizing billing records, pts receiving IVfe were identified from 2006-2009. Pts with estimated GFR (eGFR) above 60 ml/min were excluded, as well as pts identified with active malignancy and GI bleeding. Frequency of IVfe, effect on hemoglobin (hgb), %fe, BP and eGFR were determined before/after IVfe. Effects of IVfe on EPO dosing over a mean followup period of 8.55 (1.5-40)months were determined. Effects of individual fe formulations were also assessed.

Results: 49 % of pts were male with mean eGFR 32 ± 21 ml/min. About 25% of pts receiving IVfe were not followed in a CKD clinic. These pts were more likely to receive IVfe as an inpt p<0.0001, to receive a transfusion for anemia p<0.027, had less hgb correction, and received less IVfe, p<0.0007. The remaining pts, part of a CKD clinic, receiving EPO, were given total mean fe dose of 1.18 g per course. Mean hgb increase(↑) was 1.1 ±1.2 g/dl, p<0.0001. After IVfe, there were significant ↑'s in %fe, ferritin and eGFR, p<0.0001. Change of %fe positively correlated with hgb, ferritin and negatively correlated to serum PTH p<0.048. No changes in BP were noted before/after dosing. No differences between fe formulations were noted. In NDCKD receiving EPO, mean interval of dosing of IVfe was 235.3 ± 137.7 days, with ↓ EPO dosing at 0-6 weeks and 3-6 months following IVfe, p<0.1, 0.02. At 3-6 months, EPO dosing decreased by 2000 units per week.

Use of IVfe appears effective in ↑ %fe and ↓ epo requirements in NDCKD for a mean interval period of almost 8 months. Effective managment of IVfe, anemia in CKD appears best in a CKD clinic setting.

Disclosure of Financial Relationships: nothing to disclose

**PUB183**

**Trial Announcement: Vitamin K2 To Slow Vascular Calcification in Hemodialysis Patients, "VitaVasK"** Thilo Krueger,[1] Ralf Westenfeld,[9] Georg Schlieper,[1] Mario Cozzolino,[2] Johannes Jacobi,[3] Michel Y. Jadoul,[4] Markus Ketteler,[5] Lars C. Rump,[6] Peter Stenvinkel,[7] Andrzej Wiecek,[8] Karel M. Leunissen,[10] Ralf-Dieter Hilgers,[12] Leon J. Schurgers,[11] Jurgen Floege.[1] *[1]Nephrology, University of Aachen, Aachen, Germany; [2]Renal Division, S. Paolo Hospital, Milan, Italy; [3]Nephrology, University of Erlangen-Nuremberg, Erlangen, Germany; [4]Nephrology, University of Louvain, Brussels, Belgium; [5]Nephrology, Klinikum Coburg, Coburg, Germany; [6]Nephrology, University of Duesseldorf, Duesseldorf, Germany; [7]Nephrology, Karolinska Institute Stockholm, Stockholm, Sweden; [8]Nephroloy, University of Katowice, Katowice, Poland; [9]Cardiology, University of Duesseldorf, Duesseldorf, Germany; [10]Nephrology, University of Maastricht, Maastricht, Netherlands; [11]CARIM, University of Maastricht, Maastricht, Netherlands; [12]Biostatistics, University of Aachen, Aachen, Germany.*

Patients on hemodialysis (HD) exhibit an increased cardiovascular mortality associated with vascular calcification (VC). Matrix Gla protein (MGP) is a powerful vascular wall-based inhibitor of VC. MGP needs activation by vitamin K-dependent carboxylation.

The ERA/EDTA sponsored "VitaVasK" study will be the first clinical trial in HD patients to target the progression of VC using vitamin K-containing supplements. VitaVasK is a randomized, double-blind, placebo-controlled trial with parallel groups. Participants are recruited from nine European nephrology centers. Multislice spiral computed tomography (MSCT) will be used to screen for coronary artery calcification (CAC). Stable HD patients with CAC scores >100 will be randomized to a daily oral supplementation of vitamin K2 (n=178) or placebo (n=178) for 18 months. Primary outcomes will be attenuation of progression of coronary and aortic calcification at 12 and 18 months using volume scores determined by MSCT. Secondary outcomes will be regression of VC, attenuation of

progression of aortic and mitral valve calcification, major adverse cardiovascular events, mortality, and change in plasma levels of undercarboxylated and carboxylated MGP. Patients will be followed beyond the primary study duration to determine the mortality at three and five years.

Disclosure of Financial Relationships: nothing to disclose

**PUB184**

**QM C.E.R.A. Is as Effective as QW or Q2W Darbepoetin Alfa (DA) in Correcting Hemoglobin (Hb) Levels in Chronic Kidney Disease (CKD) Patients Not on Dialysis, Regardless of Etiology of CKD** Maurice Laville. *Claude Bernard University, Lyon, France.*

**Purpose:** This post hoc analysis assessed the impact of the etiology of CKD on anemia correction in CORDATUS, a randomized, multicenter study, which demonstrated the non-inferiority of once-monthly (QM) methoxy polyethylene glycol-epoetin beta (a continuous erythropoietin receptor activator [C.E.R.A.]) vs once-weekly (QW) or once every 2-weekly (Q2W) DA in the correction of anemia in CKD patients not on dialysis.

**Methods:**

Patients with CKD (n=307) were randomized to receive QM C.E.R.A. or DA QW/Q2W for 20 weeks followed by an 8-week evaluation period. Primary efficacy analyses (Hb response defined as increase in Hb ≥1 g/dL from baseline and a concentration >10.5 g/dL; non-inferiority (NI) test for Hb change from baseline in group comparison) were repeated in the 4 most common etiologies of CKD: diabetes (DM); hypertension/large vessel disease (HTN); glomerulonephritis (GN); interstitial nephritis/pyelonephritis (IN/PN).

**Results:** Response rates were similar for QM C.E.R.A. vs QW/Q2W DA regardless of the etiology of CKD. The NI test for Hb change from baseline was also significant for each etiology, with the exception of GN, where the response rate was 100% in both groups but the p-value for NI test was not significant, primarily because twice as many Hb values in patients treated with DA were >12 g/dL.

| Etiology of CKD | Hb response rate (%) | | NI test for the difference between groups in Hb change from baseline to evaluation | |
|---|---|---|---|---|
| | C.E.R.A. | DA | Difference in mean Hb (g/dL) | p-value |
| DM | 94.3 | 94.2 | 0.001 | <0.0001 |
| No DM | 94.0 | 92.9 | -0.052 | <0.0001 |
| HTN | 94.7 | 92.1 | -0.044 | <0.0001 |
| No HTN | 93.6 | 94.5 | -0.051 | <0.0001 |
| GN | 100.0 | 100.0 | -0.579 | 0.2299 |
| No GN | 93.3 | 92.8 | 0.009 | <0.0001 |
| IN/PN | 95.0 | 76.9 | 0.048 | 0.0281 |
| No IN/PN | 94.0 | 95.0 | -0.037 | <0.0001 |
| Any of the above | 94.2 | 92.9 | -0.075 | <0.0001 |

**Conclusion:** Regardless of the etiology of CKD, QM C.E.R.A. provides similar efficacy in correcting Hb levels when compared with QW/Q2W DA in CKD patients not on dialysis.

Disclosure of Financial Relationships: nothing to disclose

**PUB185**

**Revisiting Guidelines on Screening and Monitoring of Calcium, Phosphate and Haemoglobin Abnormalities in Chronic Kidney Disease Stage 3** Ping Tyug Loh, Katrina Koh, Vathsala Anantharaman. *Division of Nephrology, Department of Medicine, National University Hospital, Singapore, Singapore.*

**Objective**: We aim to examine the incidence and prevalence of Chronic Kidney Disease (CKD) complications, specifically serum calcium and phosphate abnormalities as well as anaemia in patients with moderate CKD (CKD stage 3).

**Methods**: 226 patients with CKD stage 3 (Age 66±13, eGFR 40±8, Male 65%, Chinese 70%) based on estimated Glomerular Filtration Rate (eGFR) by Modification of Diet in Renal Disease (MDRD) formula who were referred to National University Hospital Renal Division in 2009 were studied. Patients were further categorized into CKD3a (eGFR45-59mL/min) and CKD3b (eGFR30-44mL/min). Serum calcium, phosphate, and haemoglobin levels from their first visit until May 2010 were recorded.

**Result:** Result is as shown in Table 1. 2% of these patients did not have data on serum calcium, phosphate, or haemoglobin within the evaluation period.

Incidence and Prevalence of Hypocalcaemia, Hyperphosphataemia, and Anaemia in CKD Stage 3.

| | CKD3a | | CKD3b | |
|---|---|---|---|---|
| | Incidence (per 100 patient months) | Prevalence (%) | Incidence (per 100 patient months) | Prevalence (%) |
| Hypocalcaemia (<2.15mmol/L) | 2 | 4.8 | 2 | 4.3 |
| Hyperphosphataemia (≥1.45mmol/L) | 2 | 3.2 | 3 | 4.9 |
| Anaemia (<10.5g/dL) | 2 | 6.3 | 5 | 16.0 |

**Conclusion**: Hypocalcaemia and hyperphosphataemia are rare in CKD3a and CKD3b. Hence, screening and monitoring for hypocalcaemia and hyperphosphataemia at CKD stage 3 may not be cost effective. Similarly, anaemia is also rare at CKD3a. Screening and monitoring for anaemia may not be necessary until CKD3b, when it is more common.

Disclosure of Financial Relationships: nothing to disclose

**PUB186**

**A Multiple-Intervention Model May Preserve Better the Renal Function of Patients with Type 2 Diabetes Mellitus and Early Nephropathy Compared to Conventional Health-Care Model** Héctor R. Martínez Ramírez, Laura Cortes Sanabria, Ivan E. Perales Rodriguez, Erika Gomez Garcia, Alfonso M. Cueto-Manzano. *Unidad de Investigación Médica en Enfermedades Renales, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico.*

To determine the effect on renal function of patients with type 2 diabetes mellitus (DM2) and early nephropathy (EN), a Primary Care Unit with a multiple-intervention (MI) model *vs* another with conventional health-care model were compared. All FP received training on EN (40h). Patients in MI model received an educative intervention (2 h/wk) by multidisciplinary team over 4 wks, including the following sessions: emotional management (social worker), nutritional counselling (dietitian), exercise (physical trainer), health-related problems (FP).

One-hundred six patients have been included; 25 in MI and 35 in conventional model have concluded 6 months of follow-up. Main results are shown in the table. No difference in medical treatment was observed between groups.

Comparison of delta results between groups at the end of follow-up.

| Lifestyle Questionnaire§ | Multiple-Intervention (N 25) | Conventional (N 35) |
|---|---|---|
| Knowledge of disease | 2.4±2.3†* | 0.9±2.8 |
| Adherence to treatment | 1.4±5.5 | 0.7±4.9 |
| Emotion management | 3.3±3.6†* | 0.2±3.2 |
| Exercise | 0.1±4.0 | 0.6±3.2 |
| Tobacco Consumption | 0.2±0.9 | 0.3±1.0 |
| Alcohol Consumption | 0.3±2.7 | 0.6±2.0 |
| Diet | 3.8±4.2* | 2.2±4.6* |
| Total | 8.5±10* | 6.5±11.7* |
| Clinical/Biochemical Variables | | |
| Body mass index (Kg/m2) | -14±15* | -17±16* |
| Waist circumference (cm) | -5±11 | -8±10* |
| Systolic BP (mmHg) | -0.7±1.4* | -0.2±0.9 |
| Diastolic BP (mmHg) | -1.6±4.2 † | -0.5±4.5 |
| HbA1c (%) | -0.6±1.6 | 0.2±2.0 |
| Triglycerides (mg/dl) | -51±161*† | -32±137* |
| LDL-cholesterol (mg/dl) | -0.11±20 | 2.48±27 |
| eGFR (ml/min/1.73m2) | 0.09±0.19 | 0.02±0.18 |
| Albuminuria (mg/day) | -7.3±106* | 18±236 |

§Total maximal score is 100, the higher the score the better lifestyle; *p<0.05 vs baseline of the same group;†p<0.05 vs conventional model.

A MI model may positively influence on the lifestyle habits of patients with DM2 and EN, and subsequently preserve renal function.

Disclosure of Financial Relationships: nothing to disclose

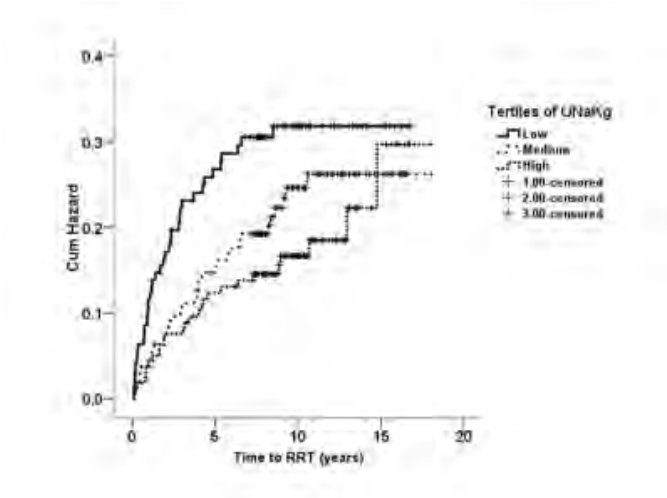
**PUB187**

**Low Urinary Sodium Predicts Progression to RRT in Patients with CKD** Emily P. Mcquarrie,[1] Patrick Barry Mark,[1] Jamie P. Traynor,[2] Jonathan Fox,[3] Alan G. Jardine.[1] *[1]University of Glasgow, Glasgow, United Kingdom; [2]Monklands Hospital, Airdrie, United Kingdom; [3]Glasgow Royal Infirmary, Glasgow.*

Aim: To establish whether 24h urinary sodium excretion (UNa), a marker of dietary sodium intake, determines long-term risk of requiring renal replacement therapy (RRT).

Methods: Adult CKD patients with at least one UNa measurement were identified. Patients were excluded if: receiving RRT at time of measurement; no simultaneous eGFR measurement and at least one further reading; decline in eGFR >10ml/min/yr or UNa <70mmol. UNa was adjusted for weight (UNaKg) due to the high correlation between measures (r=0.384, p<0.001). Kaplan Meier survival analysis was performed comparing time to RRT by tertile of UNaKg. Binary logistic regression was performed to determine independent predictors of requirement for RRT.

Results: 488 patients were identified with median 8.6y(IQR 4.2-11.4) follow-up. 50.4% were male, mean age 51.8y(SD 16.7), mean weight 78.0Kg(18.0), baseline eGFR 48.4ml/min(25.6), SBP 139mmHg(24), DBP 79(13), UNa 159.3mmol(63.7). 103 patients required RRT at a median of 9.3y. UNa correlated significantly with weight, SBP, DBP and eGFR. Male gender and requirement for RRT were also significantly associated. No correlation seen with diuretic therapy. Using Kaplan Meier analysis, patients in the lowest tertile of UNaKg were significantly more likely to require RRT than those in the highest tertile (p=0.024).




[Hazard plot of time to RRT by tertile of UNaKg], independent of sex. On univariate analysis UNaKg was a significant predictor of requiring RRT (ExpB = 0.678 (95% CI 0.5-0.92) p=0.011) however on multivariate analysis only eGFR remained a significant predictor.

Conclusions: Low urinary sodium excretion is a significant predictor of requirement for RRT but not independent of renal function. High dietary sodium does not have a detrimental effect on renal outcome.

Disclosure of Financial Relationships: nothing to disclose

**PUB188**

**Salmonella Empyema in End Stage Renal Disease Patient** Penchala S. Mittadodla, Robert S. Gayner, Nicole Denise Gray, Swapnil Khare, Fabio Dorville. *St Luke's Hospital, Bethlehem, PA.*

Pulmonary infections account for up to 25% of infections in end stage renal disease (ESRD) patients and confer a 14-16 fold higher mortality compared to general population. We report a rare case of pleural involvement of group B salmonella in an ESRD patient receiving hemodialysis, which has not been previously reported in ESRD patients.

A 49-year old Caucasian male was admitted to the intensive care unit with respiratory distress requiring BIPAP. He had a one-week history of worsening dyspnea, cough and fever. One month ago, he had self-limiting diarrhea lasting 3 days. His past medical history included end stage renal disease, on hemodialysis, hypertension, diabetes mellitus, coronary artery disease and asthma. He was not on any immunosupressant medications.

His vital signs were notable for T-102.2 F, HR-115/min, BP-91/64 mm Hg, RR-24/min and initial O2 sats-83% on room air. Physical examination revealed crackles with reduced air entry in right base and bilateral wheezing. He also had bilateral lower extremity edema. Workup revealed elevated segmented neutrophils, elevated BUN 44 mg/dl and creatinine 6.7 mg/dl. Arterial blood gas analysis showed a mild mixed respiratory and metabolic acidosis. Chest x-ray and a CT scan of chest revealed right lower lobe consolidation with pleural effusion. Thoracocentesis revealed exudative pleural fluid, which grew group B salmonella. A chest tube was placed to drain the empyema and a 14-day course of ceftriaxone was completed.

According to the literature, up to 33% of patients with extraintestinal salmonella infections have a recent history of gastrointestinal symptoms like our patient. Salmonella can spread hematogenously from the gastrointestinal tract or may remain dormant in the reticuloendothelial system, later seeding the pleural space. Immunocompromised conditions like HIV, malignancy, steroid use, sickle cell disease and diabetes mellitus have increased risk for salmonella infections. Prompt administration of antimicrobials and drainage is recommended.

Disclosure of Financial Relationships: nothing to disclose

**PUB189**

**Retinal Vessel Narrowing Is Not Reversed with Renal Transplantation** Qi-Lun Ooi,[1] Foong Kien Newk-Fon Hey Tow,[1] Mohd Afzal Alias,[1] Rajeev Deva,[1] Ryo Kawasaki,[2] Tien Y. Wong,[2,3] Deb J. Colville,[1] Anastasia F. Hutchinson,[1] Judith A. Savige.[1] *[1]The University of Melbourne, Northern Health, Melbourne, VIC, Australia; [2]Centre for Eye Research Australia, The University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, VIC, Australia; [3]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore.*

**Background and Objectives:** Small vessel disease contributes to stroke, and possibly vascular dementia, and diastolic dysfunction. Retinal arteriole and venule calibre reflect systemic small vessel changes, and these vessels are narrowed in hypertension and chronic kidney disease (CKD). This study examined whether renal transplantation reversed the narrowing seen in CKD5.

**Design, setting, participants and measurements:** This was a cross-sectional study of 100 patients who had a renal transplant for a median of 6.4 years (range 0.8 - 28.8) and 70 patients with CKD5. Nineteen (19%) transplant patients had normal renal function, 75 (75%) had CKD 3 or 4 and 5 (5%) had CKD5. In addition, 5 patients were studied pre- and 18 months post-transplant. Retinal vessel diameters were measured from digital fundus images (Canon CR5-45NM non-mydriatic camera) by a trained grader using a computer-assisted grading method (Univeristy of Wisconsin, Maddison, WI, USA) and Knudtson's formula. These were summarized [TW1] as the central retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE) and arteriole-to-venule ratio (AVR).

**Results:** Patients with a transplant were younger than patients with CKD5, and were just as likely to have hypertension (85% versus 84.3%, OR 1.06, CI 0.45 - 2.46, p=1.000), but had less diabetes (21% versus 39%, OR 0.42, 0.21 – 0.84, p=0.015). The mean CRAE in patients with a transplant was 134.8 $\pm$7.6 um versus 137.9 $\pm$ 14.9 um in patients with CKD5 (p = 0.086). Mean CRVE was 198.5 $\pm$17.8 um versus 202.4 $\pm$ 27.8 um (p=0.265). CRAE and CRVE did not change significantly in the 5 individual patients studied post-transplant.

**Conclusions:** The retinal microvascular narrowing seen in CKD5 persists after renal transplantation. These results suggest systemic microvascular disease is not modified by renal transplantation.

Disclosure of Financial Relationships: nothing to disclose

**PUB190**

**Once-Monthly C.E.R.A. Administration Maintains Stable Hemoglobin Concentrations in Dialysis Patients with Varying C-Reactive Protein** Nicolas D. Papagalanis. *(on Behalf of the ML20952 Study Group) Nephrology Department, Red Cross Hospital, Athens, Greece.*

**INTRODUCTION AND AIMS:** Continuous erythropoietin receptor activator (C.E.R.A.) is a novel agent with unique receptor activity, allowing once-monthly (Q4W) administration for effective maintenance of stable hemoglobin (Hb) in patients with chronic kidney disease (CKD). We examined the effect of C-reactive protein (CRP) levels and dialysis conditions on the maintenance of Hb concentrations, and the relationship between CRP levels and C.E.R.A.dose.

**METHODS:** This prospective, multicentre, single arm, open label study assessed the efficacy, safety and tolerability of once-monthly administration of C.E.R.A. for the maintenance of Hb levels in dialysis patients with chronic renal anemia. Patients (n=188) in Greece (≥18y) receiving adequate dialysis and intravenous epoetin or darbepoetin with stable baseline Hb (10.5-12.5 g/dL) entered a 16-week C.E.R.A. dose titration phase (DTP) followed by an 8-week efficacy evaluation period (EEP). At DTP (week 16) and EEP (week 24) the efficacy parameters and exposure were analyzed by baseline CRP (≤6.0 mg/L, >6.0 mg/L), using an ANOVA test.

**RESULTS**: Mean baseline, week 16 and week 24 Hb concentrations for patients on C.E.R.A Q4W with baseline CRP levels ≤6.0 mg/L was 11.6 (SD=0.57) g/dL, 11.9 (0.97) g/dL and 11.6 (1.09) g/dL and for patients with CRP levels >6.0 mg/L were 11.5 (0.55) g/dL, 11.6 (0.97) g/dL and 11.4 (1.14) g/dL, respectively. The table below shows that patients with CRP levels >6.0 mg/L were exposed to a higher C.E.R.A. dose than patients with CRP ≤6.0 mg/L (DTP *p=0.028*, EEP *p=0.085*).

| CRP | N (DTP) | DTP mean (SD) dose (µg) | N (EEP) | EEP mean (SD) dose (µg) |
|---|---|---|---|---|
| ≤6.0 mg/L | 119 | 152 (63.4) | 103 | 139 (95.5) |
| >6.0 mg/L | 58 | 176 (80.3) | 50 | 170 (124.3) |

**CONCLUSIONS:** Dialysis patients with elevated CRP levels are exposed to more C.E.R.A than to patients with CRP ≤6.0 mg/L. However once-monthly C.E.R.A. administration effectively maintains stable Hb levels in dialysis patients with CKD, despite CRP levels.

Disclosure of Financial Relationships: nothing to disclose

**PUB191**

**Vegetarian Low Protein Diets Supplemented with Chetoanalogues. Is It Feasible in the Routine Clinical Practice?** Giorgina B. Piccoli,[1] Valentina Consiglio,[1] Maria Chiara Deagostini,[1] Stefania Scognamiglio,[1] Rossella Attini.[2] *[1]SS Nefrologia, ASOU San Luigi, Orbassano, Turin, Italy; [2]Maternofoetal Unit, OIRM S Anna University of Torino, Torino, Italy.*

**Background.**

Vegetarian supplemented diets are cited as promising tolls for slowing CKD progression. The opinion that they are scarcely feasible in the clinical practice limits their use.

**Aim** of the study was to assess the feasibility of the diet in a new Nephrology Unit, where it was routinely prescribed to stage 3-5 CKD patients.

**Methods**

Prospective analysis: December 2007-April 2010. Diet composition: Vegan diet with Protein content 0.6 g/Kg/day; supplementation: Ketosteril 1 every 10 Kg; 1-3 free meals/week, monthly follow-up, simplified schema based upon " allowed " and "forbidden" food. Side effects and compliance were recorded at the visits. Progression was evaluated as loss of GFR (Cockroft formula) in patients with at least 6 months of follow-up. Logistic regression was performed on SPSS.

**Results**

Out of over 1500 patients followed since the start of the Unit (December 2007), 84 non pregnant adult patients started the supplemented low protein diet(median age 67 years, 38% diabetics, multiple comorbidities in 42%, educational level >12th grade 18%). Median data at start: proteinuria 1.3 g/day, GFR 20 mL/min, creatinine 3.5 mg/dL.

At April 2010, 42 patients were continuing the diet, 10 discontinued it (5 for personal preferences, 5 for side effects), 2 died, started dialysis, 2 dropped out from follow-up, 3 improved and discontinued the diet, 25 started dialysis (planned in 19; acute cardiovascular or infectious problens led to emergency start in 6 cases). The main side effect was poor

gastrointestinal tolerance; severe oedema was recorded in 1. No baseline data correlated with "succesful follow-up", defined as at least 6 months on the diet, in uni and multivariate analysis. In the subset of cases followed for at least 6 months, median GFR decrease was -2 ml/min/year.

**Conclusion**

Vegetarian, supplemented low protein diets are routine feasible in non selected CKD patients. A trial period may help identifying patients who can benefit from this regimen.

Disclosure of Financial Relationships: nothing to disclose

## PUB192

**Cancer History among Persons with Chronic Kidney Disease in the United States** Laura C. Plantinga,[1] Chi-Yuan Hsu,[1] Vahakn B. Shahinian,[2] Rajiv Saran,[2] Meda E. Pavkov,[3] Sharon Saydah,[4] Neil R. Powe.[1] *[1]University of California, San Francisco, CA; [2]University of Michigan, Ann Arbor, MI; [3]Centers for Disease Control and Prevention, Atlanta, GA; [4]Centers for Disease Control and Prevention, Hyattsville, MD.*

Certain cancers are more common in ESRD patients, but less is known about its association with earlier stages of CKD. In the 1999-2006 National Health and Nutrition Examination Surveys, 22,540 adult participants (≥20 years) were surveyed regarding their history of cancer, including type(s) and age(s) at diagnosis. CKD was defined as a urinary albumin:creatinine ratio of ≥30 mg/g (stages 1 and 2) or MDRD-estimated GFR of 15-59 ml/min/1.73 m$^2$ (stages 3 and 4). Adjusted prevalence of cancer history was calculated using multivariable logistic regression with U.S. population-based weighting. Among those with no CKD, CKD stages 1 and 2, and CKD stages 3 and 4, the unadjusted prevalence of self-reported history of any cancer was 6.7%, 10.5%, and 21.3%, respectively ($P$<0.001). With adjustment for current age, however, there was no significant association of history of any cancer with current CKD status (8.1%, 7.8%, and 8.5%, respectively; P=0.66). Among those with CKD stages 3 and 4 and a history of cancer, the age-adjusted prevalence of kidney or bladder cancer was 4.8% among those with CKD, compared to 2.3% of those with no CKD ($P$=0.02). Similarly, with age adjustment, 9.1% (CKD stages 3 and 4) vs. 6.1% (no CKD) reported having had gastrointestinal cancer ($P$=0.05). Skin cancer was less commonly reported in those with CKD stages 1 and 2 (29.3%) compared to those with no CKD (38.8%; P=0.04), but no other specific type of cancer examined differed significantly by CKD status with age adjustment. Additional adjustment for gender, race/ethnicity, and smoking did not alter the results. While the unadjusted prevalence of self-reported cancer is >3 times higher in those with moderate to severe CKD compared to no CKD, much of this observed association appears to be due to older age, a risk factor for both conditions. CKD may, however, be associated with greater prevalence of specific types of cancer, including kidney, bladder, and gastrointestinal cancers.

Disclosure of Financial Relationships: nothing to disclose

## PUB193

**Effects of Plasma Calcium and Phosphorus Levels outside Target Ranges as Proposed by KDOQI and KDIGO in Pre-Dialysis Patients** Iris Postmus,[1] Dinanda J. De Jager,[1] Nynke Halbesma,[1] Elisabeth W. Boeschoten,[2] Friedo W. Dekker,[1] Diana C. Grootendorst.[1] *[1]Clinical Epidemiology, LUMC, Leiden, Netherlands; [2]Hans Mak Institute, Naarden, Netherlands.*

In dialysis patients disturbed mineral metabolism has been associated with poor outcome. Aim of this study was to assess the effect of calcium (Ca) and phosphorus (P) levels outside the targets as proposed by KDOQI and KDIGO, on time to initiation of dialysis in pre-dialysis patients.

*Methods*

237 Incident adult pre-dialysis patients (71% M, 65±15 yrs, P 4.46±1.05 mg/dl, Ca 9.39±0.63 mg/dl) from the prospective Dutch PREPARE-2 cohort were included. The effects of P and Ca levels below, within, and above target ranges on time to dialysis initiation were assessed using Cox regression.

*Results*

Patients achieving target ranges and the associated incidence rates and hazard ratios (HRs) for initiation of dialysis treatment

| | KDOQI | | | KDIGO | | |
|---|---|---|---|---|---|---|
| **P**[a] | Below | Within | Above | Below | Within | Above |
| | | | | | | |
| n(%) | 12(4.7) | 164(69.8) | 60(25.5) | 4(1.7) | 128(54) | 104(44.3) |
| Incidence (/100py) | 29.9(0.6;59.3) | 29.1 (21.6;36.5) | 67.1 (44.3;90.0) | 0 | 21.9 (14.8;28.9) | 63.9(47.5;80.4) |
| Crude HR (95%CI) | 1.21(0.4;3.4) | 1 | 2.15(1.4;3.4) | n.a. | 1 | 2.71(1.8;4.2) |
| Adj. HR (95% CI)[b] | 0.97(0.3;2.9) | 1 | 1.36(0.9;2.2) | n.a. | 1 | 1.48(0.9;2.4) |
| **Ca**[c] | | | | | | |
| n(%) | 19(8.1) | 201(85.5) | 16(6.4) | 21(8.9) | 186(79.1) | 29(11.9) |
| Incidence (/100py) | 40.6(12.5;68.7) | 36.6 (28.7;44.6) | 28.1 (5.6;50.5) | 39.6 (13.7;65.4) | 35.2 (27.1;43.2) | 40.7(18.6;62.8) |
| Crude HR (95% CI) | 1.15(0.6;2.4) | 1 | 0.82(0.4;1.9) | 1.16(0,6;2.3) | 1 | 1.30(0.7;2.4) |
| Adj. HR (95% CI)[d] | 1.39(0.6;3.1) | 1 | 0.61(0.2;1.5) | 1.06(0.5;2.5) | 1 | 1.21(0.6;2.4) |

a. Target: KDOQI: CKD3-4: 2.7-4.6 mg/dl, CKD5: 3.5-5.5 mg/dl, KDIGO: 2.5-4.5 mg/dl; b. Adj. for age, sex, primary kidney disease, eGFR, SBP and Hb; c. Target: KDOQI: 8.4-10.2 mg/dl, KDIGO: 8.5-10.0 mg/dl; d. same as b. + P

*Conclusion*

P levels above both the KDOQI and KDIGO target ranges are associated with a higher risk of dialysis initiation. No association was found between Ca and the risk of dialysis initiation. Therefore, successful maintenance of P levels might be beneficial in postponing dialysis.

Disclosure of Financial Relationships: nothing to disclose

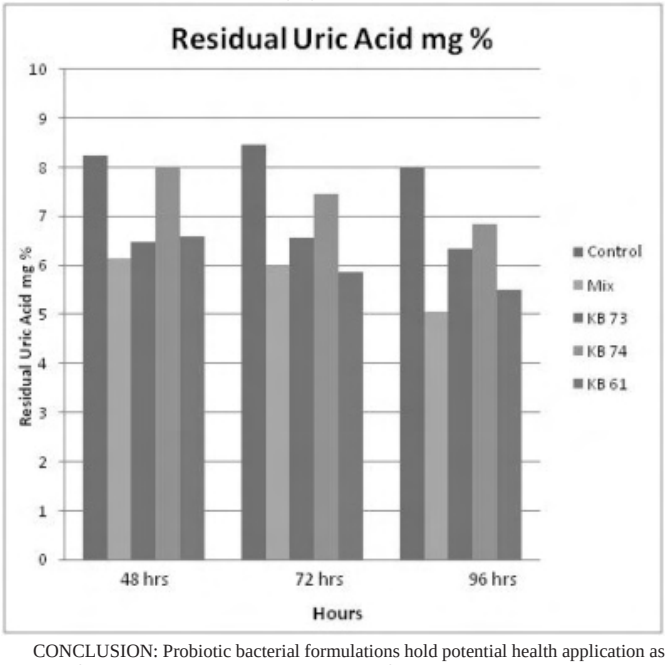
## PUB194

**A Novel Approach of Uric Acid Removal with a Probiotics Dietary Supplement Product Formulation (In-Vitro Studies)** Natarajan Ranganathan,[1] Pari Ranganathan,[1] Usha N. Vyas,[1] Eli A. Friedman.[2] *[1]Kibow Biotech Inc, Newtown Square, PA; [2]Down State Med Ctr - SUNY, Brooklyn, NY.*

PURPOSE: Over 5 years have been devoted to continuous in vitro design and testing of novel probiotic product formulations based on the concept that substituting a usual bowel function for reduced or absent kidney function (with various generally accepted biomarkers) may preempt the clinical consequences of chronic kidney disease (CKD). The present report recounts our exploratory studies aimed at reducing plasma uric acid biomarker levels as a means of preventing symptomatic hyperuricemia and/or gout in patients with CKD.

METHODS: An Artificial Intestinal Fluid (AIF) was prepared according to US pharmacopeia standards. To this, uric acid was added as a potassium or sodium urate salt at concentrations of 8.34 mg%. Over 50 different well characterized probiotic strains - both aerobes and anaerobes - with a known concentration of Colony Forming Units (CFU's) in multiple of billions were added individually and collectively to a 100 ml aliquot of AIF. Each text mixture was individually incubated in a shaker at 37$^0$C and 50 rpm. Control volumes without added probiotic bacteria were also incubated in the same manner. 1.0 ml samples were withdrawn every 24 hours for a period of 96 hours. These samples were then centrifuged for 10 minutes at 8000 rpm to settle the biomass. Residual uric acid in the supernatant was quantified using a Quantichrom Uric Acid kit from Bio-assay System, CA.

RESULTS: Of the bacteria studied, three Lactobacillis strains (KB 61, KB 73 and KB 74) individually and together demonstrated specific utilization of Uric acid as biomarker with varied and different affinities ranging from 15% to 40%.




CONCLUSION: Probiotic bacterial formulations hold potential health application as a means of reducing intra-intestinal concentration of uric acid.

Disclosure of Financial Relationships: Employer: Kibow Biotech, Inc; Ownership: holds substantial stock holdings in Kibow Biotech Inc; Scientific Advisor: Board of Director at the International Probiotic Association, Board of visitor, College of Science and Technology, Temple University; Other Relationship: Co-founder and director of Kibow Biotech Inc.

## PUB195

**Elderly Patients with Stage 3 Chronic Kidney Disease (CKD): Eplerenone. Drug Effectivity and Safety** Consolación Rosado Rubio,[1] Marcos Luis Álvarez Alejandre,[1] Raquel López de la Fuente,[2] Jose L. Lerma,[1] Cyntia Gonzalez Álvarez,[1] José Luis Rodríguez Commes.[1] *[1]Servicio de Nefrología, Hospital Clínico de Salamanca, Salamanca, Spain; [2]Centro de Salud Santa Elena, Zamora, Zamora, Spain.*

Introduction:

An early blocking of proteinuria is an effective mechanism to slow the progression of renal damage. However, as evidenced by the ONTARGET study, a dual blockade that combines ACEI and ARB is not without risks and can result in further deterioration of the

renal function and hyperkalemia, especially in the elderly. Direct blockers of aldosterone and eplerone are antifibronegic and beneficial for the cardiovascular system. Theoretically, they lack hormonal or metabolic adverse effects. However, their effect on proteinuria and CKD progression in the elderly is still unknown.

Objectives:

1.To assess the antiproteinuric effect of eplerenone

2.To identify its influence on renal function

3.To establish its clinical and metabolic side effects

Material/Methods:

We performed a prospective study of 46 patients (age 69 +/-5) with stage 3 CKD under treatment of ACEI and/or ARBs who started taking 25 mg/day of eplerenone. Each patient served as own control. We assessed proteinuria, serum K and serum Cr. The statistical study was performed with analysis of variance with Student's t-test or Kruskal-Wallis test, depending on the variables.

Results:

74% of the patients showed a reduction of proteinuria from 1.53 to 1.12 ($p<0.02$).

The average concentration of serum K was 4.4 mg/dl, and the concentration at the end of the follow-up was 4.5 mg/dl. This difference was not statistically significant ($p=0.83$). No cases of severe hyperkalemia were observed.

The initial mean value of serum Cr in the sample is 1.6 mg/dl, and the final value is 1.8 mg/dl. This deterioration was not statistically significant ($p=0.52$).

Conclusions:

- Eplerenone reduced proteinuria significantly in elderly patients with stage 3 CKD who had been previously treated with ACE inhibitors and/or ARB.

- The good tolerance of eplerenona, the absence of hyperkalemia and the moderate increase in glomerular filtration support the conclusion that it can be used with close monitoring in these patients, although randomized studies are required.

Disclosure of Financial Relationships: nothing to disclose

## PUB196

**Urinalysis as a Predictor of Microalbuminuria in Stage 3 CKD** Shayan Shirazian, Jai Radhakrishnan, Herbert S. Chase. *Nephrology, Columbia University Medical Center, New York, NY.*

Purpose: NKF guidelines recommend quantitative urine protein (QUP) assessment in patients with Stage 3 CKD. The purpose of this study was to determine the proportion of Stage 3 CKD patients with urine protein measurement and to test the effectiveness of urinalysis as a screening test for microalbuminuria.

Methods: Patients enrolled in this study were ambulatory clinic patients followed consistently over a four year period (registered > 10 times at the Columbia University Medical Center clinic from 2004-2008). Demographic information, diabetic and hypertensive status, serum creatinine values and urine protein values were extracted from the clinical data warehouse. A minimum of 4 creatinine values separated by at least one year were required for study inclusion. Stage 3 CKD status was assigned if eGFR was between 30 and 60 for at least 3 months. eGFR was calculated using the MDRD formula. Urinalysis (UA) and urine microalbumin-creatinine ratio (UACR) results were compared using UACR as the 'gold standard' for microalbuminuria.

Results: 1160 adult patients were classified as Stage 3 CKD (69% female, 61% diabetic, 98% hypertensive, 24% Black or Hispanic). Of these, 1020 had UA or QUP measurement, 741 (65%) had QUP measurement, 538 had UACR, 459 had spot urine protein and 28 had 24hr urine protein or albumin measurement. 140 patients (12%) did not have qualitative or quantitative urine protein testing. 466 patients had both UA and UACR testing. UA testing correlated poorly with UACR in evaluating for microalbuminuria in both diabetics (Sensitivity=0.71, Specificity=0.76) and non-diabetics (Sensitivity=0.6, Specificity=0.83). Patients with true positive UA results had a significantly faster rate of GFR decline than those with false positive UA results (-2.95 vs. -1.48 ml/min/year, $p<0.05$).

Conclusions: A large percentage of patients with Stage 3 CKD, followed consistently in a tertiary care ambulatory clinic, lack quantitative urine protein testing. UA does not accurately predict microalbuminuria in Stage 3 CKD and and has limited utility in determining the risk of GFR decline.

Disclosure of Financial Relationships: nothing to disclose

## PUB197

**Involvement of Serum Indoxyl Sulfate in Development of Anemia in Non-Dialysis CKD Patients: A Retrospective Cohort Study** Tatsuya Shoji,[1] Naohisa Tomosugi,[2] Takuya Uehata,[3] Yusuke Sakaguchi,[1] Akira Suzuki,[1] Yoshiharu Tsubakihara.[1] *[1]Department of Kidney Disease and Hypertension, Osaka General Medical Center, Osaka, Japan; [2]Division of Advanced Medicine, Medical Research Institute, Kanazawa Medical University, Kahoku, Ishikawa, Japan; [3]Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

**Background:** Role of uremic toxins in exacerbating renal anaemia is still unclear. In CKD patients, serum indoxyl sulfate, a uremic toxin, is markedly accumulated and was shown to accelerate the progression of renal dysfunction and vascular disease. This retrospective observational study examines whether serum indoxyl sulfate is involved in development of anaemia in non-dialysis CKD patients.

**Methods:** Study design: Retrospective cohort study. Setting & Participants: Consecutive 357 non-dialysis and erythropoietin-naive CKD patients were recruited from single outpatient nephrology clinic at referral hospital. Predictor: serum indoxyl sulfate. Outcomes: Primary end point was initiation of erythropoietin treatment.

**Results:** Participants were predominantly man (56.6%), and non-diabetic (82.1%); participant mean age was 61.9 years [SD, 14.9]. Mean eGFR was 47.4 ml/min/1.73m$^2$ [SD, 25.0], mean hemoglobin was 12.7 g/dL [SD, 1.9], and median indoxyl sulfate was 0.24 mg/dL [interquartile range, 0.12-0.46]. 25 events occurred in each gender over a mean follow-up of 22.9 months. One of the covariates, gender, violated the proportional hazard assumption of Cox's model, so we constructed Cox's model in each gender separately. The limited number of events did not allow us to include more than three variables for each model. The table shows hazard ratios (HR) associated with serum indoxyl sulfate (mg/dL) in each gender model.

HR associated with serum indoxyl sulfate (mg/dL)

| | HR (95% CI) | |
|---|---|---|
| | Male | Female |
| Unadjusted | 10.0 (5.4-18.5) | 3.0 (1.9-4.6) |
| Adjusted for age | 10.0 (5.4-18.6) | 2.5 (1.9-4.6) |
| Adjusted for estimated GFR | 4.4 (1.9-9.9) | 0.7 (0.4-1.4) |

**Conclusion:** These results suggested that serum indoxyl sulfate was involved in development of anemia in non-dialysis CKD patients, at least in male.

Disclosure of Financial Relationships: nothing to disclose

## PUB198

**Once-Monthly C.E.R.A. Administration Maintains Stable Hemoglobin Concentrations in Dialysis Patients Regardless of Age, Gender or Diabetes** Kostas I. Sombolos. *(on Behalf of the ML20952 Study Group) Renal Unit G.H. "G. Papanikolaou", Thessaloniki, Greece.*

**INTRODUCTION AND AIMS:** Continuous erythropoietin receptor activator (C.E.R.A) is a novel agent with unique receptor activity, allowing once-monthly administration for effective maintenance of stable hemoglobin (Hb) in patients with chronic kidney disease (CKD). Since males, patients >65 years (y) and patients with diabetes are an expanding CKD population, we examined the effect of age, gender and diabetes on the maintenance of Hb concentrations with C.E.R.A. over time.

**METHODS:** This prospective, multicentre, single arm, open label study assessed the efficacy, safety and tolerability of once-monthly administration of C.E.R.A. for the maintenance of Hb levels in dialysis patients with chronic renal anemia. Patients (n=188) in Greece (≥18y) receiving adequate dialysis and intravenous epoetin or darbepoetin with stable baseline Hb (10.5-12.5 g/dL) entered a 16-week C.E.R.A. dose titration phase (DTP) followed by an 8-week efficacy evaluation period (EEP). At DTP (week 16) and EEP (week 24) the efficacy parameters were analyzed by age (<65y, 65-75y, >75y), gender and diabetes (yes/no), using an ANOVA test.

**RESULTS**: Mean Hb concentration at baseline, week 16 and week 24, for patients <65y, 65-75y, >75y, male (M) and female (F), diabetic or non-diabetic are shown in the table below. No statistically significant differences were found between the sub-populations or study phases.

| | N | Baseline mean (SD) (g/dL) | Hb Week 16 Hb mean (SD) (g/dL) | Week 24 Hb mean (SD) (g/dL) |
|---|---|---|---|---|
| Age y | | | | |
| <65 | 89 | 11.7 (0.58) | 11.8 (0.92) | 11.5 (1.11) |
| 65-75 | 65 | 11.5 (0.51) | 11.8 (1.06) | 11.6 (1.14) |
| >75 | 34 | 11.6 (0.63) | 11.7 (1.01) | 11.7 (1.14) |
| Gender | | | | |
| M | 115 | 11.6 (0.58) | 11.9 (0.89) | 11.6 (1.13) |
| F | 73 | 11.6 (0.58) | 11.6 (1.10) | 11.5 (1.12) |
| Diabetes | | | | |
| Yes | 35 | 11.7 (0.51) | 11.6 (1.10) | 11.4 (1.34) |
| No | 153 | 11.6 (0.58) | 11.8 (0.95) | 11.6 (1.07) |

**CONCLUSIONS:** Regardless of age, gender, or diabetes, once-monthly C.E.R.A. administration in dialysis patients can effectively maintain Hb stability.

Disclosure of Financial Relationships: Ownership: RocheResearch Funding: Roche.

## PUB199

**Low Glomerular Filtration Rate in Normoalbuminuric TypeII Diabetic Patients Is Related with Aging Kidney** Oonishi Takahiro. *Nephrology, Yamada Red Cross Hospital, Ise, Mie, Japan.*

Some diabetic patients have low GFR with normoalbuminuria. The aim of this study was to identify a group of normoalbuminric type 2 diabetic patients with low GFR and compare them with normoalbuminuric patients with normal GFR.Altogether,147 normoalbuminuric type 2 diabetic patients with average 10 years of diabetes duration that patients were divided these patients into elderly group (E group, average age 68.9 years old) and the non-elderly group under 65 years old(N group, average age 51.1 years old) and examined blood pressure, HbA1c, creatinine clearance (Ccr),estimated GFR and pulse wave velocity(PWV), LV mass index and relative wall thickness (RWT) by echocardiogram.

Result: Ccr of E group deteriorated significantly than N group(69.2 +- 26.0 ml/min vs 86.2 +- 25.0 ml/min,$p<0.01$), and E group was higher than N group in comparison of blood pressure and PWV.In addition,RWT of E group was significantly lager than N group. Conclusion: Some of the elderly diabetic patients with normoalbuminuria reduced the GFR. It was related that the atherosclerosis and left ventricule hypertrophy. It might be that GFR in elderly diabetic patients dose not show only renal function but also cardiovascular disease.

Disclosure of Financial Relationships: nothing to disclose

**PUB200**

**Pulmonary Hypertension in Patients with Chronic Kidney Disease** Eranga S. Wijewickrama,[1] Wasantha Kapuwatte,[2] Hasantha Ranawaka,[2] Thamal Vithanage,[3] Mihirini Abeywickrama,[3] S. Naranthiran,[2] Anuja Abeyadeera,[4] Godwin Constantine,[3] Rushika D. Lanarolle.[3] *[1]University Medical Unit, National Hospital of Sri Lanka, Colombo, Sri Lanka; [2]Institute of Cardiology, National Hospital of Sri Lanka, Colombo, Sri Lanka; [3]Department of Clinical Medicine, Faculty of Medicine, Colombo, Sri Lanka; [4]Department of Surgery, Faculty of Medicine, Colombo, Sri Lanka.*

Background

High prevalence of pulmonary hypertension had been observed in patients with end stage renal failure undergoing chronic haemodialysis. The objective of this study was to evaluate the prevalence of pulmonary hypertension (PH) among the pre-dialytic chronic kidney disease (CKD) patients.

Methods

The prevalence of PH was evaluated in 32 randomly selected patients with CKD who were attending an outpatient renal clinic at the National Hospital of Sri Lanka. Pulmonary artery pressures were evaluated by two cardiologists using a single Doppler echocardiography machine. All patients with PH were screened for secondary causes.

Results

Six patients (18.8%) had PH as defined by pulmonary artery systolic pressure (PAP) >35mmHg. PAP was >45mmHg in 4 of these patients which constituted 12.5% of all patients. None of these patients were having functioning arterio-venous fistulae. Three of these patients had left ventricular diastolic dysfunction but the remaining 3 patients had no clinical or echocardiographic evidence of a cause for PH. The mean serum creatinine concentration in the study population was 3.8mg/dl and the mean haemoglobin concentration was 9.6g/dl. The aetiology of CKD, the degree of renal dysfunction and the levels of haemoglobin, calcium and phosphate were no different in the group of patients with PH compared to patients without PH.

Conclusion

There is a high prevalence of PH even among patients in the pre-dialytic stages of CKD. Further studies need to be carried out to identify factors which predispose these individuals to development of PH.

Disclosure of Financial Relationships: nothing to disclose

**PUB201**

**Once-Monthly C.E.R.A. Is Non-Inferior to Darbepoetin alfa for Correcting Anemia in Patients (pts) with Chronic Kidney Disease (CKD) Regardless of Age, Gender, Diabetic Status, Hypertension, or Presence of Hyperlipidemia** Rainer Woitas. *Department of Internal Medicine I, University of Bonn, Germany.*

**Purpose:** The CORDATUS study showed that the continuous erythropoietin receptor activator (C.E.R.A.) effectively corrected anemia when administered once monthly (Q4W) to pts with CKD not on dialysis. A subgroup analysis was performed to establish whether these data were dependent on age, gender, or presence of diabetes, hypertension, or hyperlipidemia.

**Methods:** Adult pts with stage 3/4 CKD not on dialysis received either subcutaneous C.E.R.A. Q4W with a starting dose of 1.2 „g/kg or darbepoetin alfa (DA) once weekly (QW) or once every 2 weeks (Q2W) according to local labeling. The primary efficacy analyses (Hb response, defined as increase in Hb ≥1 g/dL from baseline and a concentration ≥10.5 g/dL; non-inferiority (NI) test for Hb change from baseline in each group) were repeated for the subgroups defined according to gender, age, diabetic status, hypertension, or presence of hyperlipidemia.

**Results:** Hb response rates were similar for Q4W C.E.R.A. and QW/Q2W DA, irrespective of gender, age, or the presence of diabetes, hypertension, or hyperlipidemia. The NI test for Hb change from baseline was significant for each category analyzed, with the exception of the 'no hypertension' group, which included only 10 patients.

| Variable | Hb response rate (%) | | Non-inferiority for the difference between groups in Hb change from baseline to evaluation | |
|---|---|---|---|---|
| | C.E.R.A. | Darbepoetin alfa | Difference in mean Hb (g/dL) | p-value |
| Male | 95.5 | 94.0 | 0.174 | <0.0001 |
| Female | 93.0 | 93.1 | -0.182 | 0.0002 |
| Age ≤65 years | 93.2 | 95.9 | 0.062 | <0.0001 |
| Age >65 years | 94.7 | 92.4 | -0.049 | <0.0001 |
| Diabetes | 94.2 | 95.1 | 0.010 | <0.0001 |
| No diabetes | 94.0 | 91.8 | -0.078 | <0.0001 |
| Hypertension | 94.0 | 93.9 | -0.015 | <0.0001 |
| No hypertension | 100.0 | 85.7 | -0.941 | 0.0003 |
| Hyperlipidemia | 94.3 | 93.5 | -0.021 | <0.0001 |
| No hyperlipidemia | 93.6 | 93.6 | -0.090 | 0.0026 |

**Conclusion:** In this study, C.E.R.A. Q4W was statistically non-inferior to DA for correcting anemia in pts with CKD, regardless of age, gender, diabetic status, hypertension, or presence of hyperlipidemia.

Disclosure of Financial Relationships: Consultancy: Roche.

**PUB202**

**The Effect of Cardiac Resynchronization Therapy on Cardio-Renal Anemia Syndrome** Takeshi Yokoyama, Katsuomi Matsui, Yugo Shibagaki, Takashi Yasuda, Kenjiro Kimura. *Nephrology and Hypertension, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan.*

Background: Chronic kidney insufficiency and anemia are often present in the severe heart failure (HF) patient. Cardio-renal anemia (CRA) syndrome is medically intractable and develop into end stage renal failure. Although Cardiac resynchronization therapy (CRT) is known to improve cardiac functional status and symptom in severe HF patients with ventricular dyssynchrony, there is a paucity of data whether CRT have an salutary effect for CRA syndrome.

Purpose: To determine if CRT improves renal function and anemia in patients with chronic kidney disease (CKD) and severe HF.

Methods: All the patients who were treated with CRT in our hospital were reviewed retrospectively and 22 patients with CKD stage 3 or above were selected. Renal function and anemia, cardiac function before and after CRT were compared.

Results: Of the 22 subject, there are 15 patients were with CKD stage 3 (stage 3 group), and 7 patients with CKD stage 4 (stage 4 group) pre-CRT. HF symptom of both groups improved significantly. Estimated GFR (eGFR) did not significantly improved in stage 3 group, but in stage 4 group, it improved significantly ($p < 0.05$). Slope of approximate line of time to eGFR tended to increase in stage 3 group and it increased significantly in stage 4 group. Slope of aooroximate line of time to hemoglobin tended to increase in both groups.

Conclusion: CRT may cotribute to amelioration of CRA syndrome in patients with advanced CKD. Especially, hemodynamic improvement by CRT may contribute to amerioration of renal function.

Disclosure of Financial Relationships: nothing to disclose

**PUB203**

**Awareness of Kidney Disease among Black Africans Living in the UK** Adekunle Bamidele Adesina,[1] Samuel Abiamuwe,[2] Gillian Olumide.[2] *[1]Renal Unit, Morriston Hospital, ABM University Health Board, Swansea, United Kingdom; [2]School of Health Sciences, University of Wales, Swansea, United Kingdom; [3]School of Health Sciences, University of Wales, Swansea, United Kingdom.*

BACKGROUND: Chronic kidney disease leads to end stage renal failure (ESRF) and it is a global growing epidemic. ESRF leads to death unless treated by dialysis or kidney transplant. In the UK, Black Africans have a fourfold incidence of ESRF compared to Whites due to their higher rates of diabetes and hypertension.

PURPOSE : To investigate the knowledge and perceptions of kidney health among people of Black African origin living in the city of Swansea.

METHODS : The study adopted a qualitative design which used semi-structured individual interview as the tool for data collection.

RESULTS :The sample comprised of a balanced number of eight individuals (four men and four women) aged between 23 to 58 years, drawn from various locations within the city and county of Swansea. Data were analyzed using thematic content analysis. Overall, we found that current level of knowledge of kidney health and related issues of renal diseases including its primary risk factors was generally very low. Perceived risk of predisposition to kidney disease was also poor. These findings hold for both men and women. Reason such as lack of information and education regarding kidney disease, access to health services and mistrust in the health professionals and value of treatment were frequently cited.

CONCLUSIONS :These findings underscore the need for increased public health measures to raise awareness, stimulate early detection among blacks and manage risk factors in this community.

Disclosure of Financial Relationships: nothing to disclose

**PUB204**

**A Five-Factor Behavioural Intervention Study of Risk Modification on Kidney Disease Prevention in Black British Populations – Rationale and Design** Adekunle Bamidele Adesina,[1] Sally Altree,[2] George Karani,[3] Fope Adesina,[4] Kofi Obuobie.[5] *[1]Renal Unit, Morriston Hospital, Swansea, United Kingdom; [2]Renal Dietetics, University Hospital of Wales (UHW), Cardiff, United Kingdom; [3]Cardiff School of Health Sciences, University of Wales Institute (UWIC), Cardiff, United Kingdom.*

**RATIONALE** There is a well recognised excess prevalence of kidney disease amongst UK ethnic minorities notably black African-Caribbean. Although studies such as the AASK have addressed specific risk factor modification amongst American blacks, there is relative paucity of similar data among Black British populations.

**Purpose of study**

To examine the impact of modifying multiple risk factors for kidney disease including salt, but also sugar/calorie intake, smoking, stress/social isolation and a sedentary lifestyle.

**DESIGN**

Prospective cohort study of >/=5000 subjects in community adults (18-65 years), of black-African and African-Caribbean descent residing g in five cities of South Wales and West of England. duration 5 years.

**Interventions**

Salt Restriction (≤5g/day)

Sugar & alcohol reduction, accoding to UK standards

Structured (planned) >/=30 min./day and 'opportunistic' exercises at least 5 days of the week

· Brisk walking, dancing or jogging

Social support through enhanced contacts with relatives and friends.

Smoking cessation through enhanced access to existing programmes of local UK agencies

**Evaluations**

Questionnaire survey on personal medical/drug history and family history of kidney and related chronic health conditions, awareness of dietary risk factors, stress and social well-being, Weight, Height, Eating habits, Urinalysis (dipstick and spot urine) on two samples over a three month interval, Blood pressure, fasting serum creatinine, lipids and glycaemia on two measurements over a two to three-month interval 24-hour urinary measurements of sodium and potassium at baseline and three monthly intervals.

The analyses would focus on time-trends of incidence and prevalence of kidney disease, high blood pressure, and weight in all subjects and by sub-groups' pre-defined levels of compliance with each of the five components of the behavioural intervention.

Disclosure of Financial Relationships: nothing to disclose

## PUB205

**MPO-ANCA Associated Vasculitis with Pulmonary Fibrosis** Nishkantha Arulkumaran,[1] Naomi Periselneris,[1] Nicola H. Strickland,[3] Philip W. Ind,[2] Charles D. Pusey,[1] Alan D. Salama.[1] *[1]Department of Nephrology, Imperial College Kidney and Transplant Institute, London, United Kingdom; [2]Department of Respiratory Medicine, Imperial College, London, United Kingdom; [3]Department of Radiology, Imperial College, London, United Kingdom.*

Purpose: The occurrence of Anti-neutrophil cytoplasm antibody (ANCA) associated vasculitis (AAV) and pulmonary fibrosis (PF) in the same patient is rare. We ascertained characteristics and clinical outcomes of patients with AAV and PF.

Methods: A retrospective observational cohort study of 500 patients (142 with microscopic polyangiitis; MPA) attending our nephrology clinic from 1974-2009

Results: 15 AAV patients had PF. Eleven were male and 4 were female- all with MPA. Those tested were MPO-ANCA positive (n=14). Mean age was 66.5 (52-86) years. Two were smokers, 8 ex-smokers. PF diagnosis was concurrent with AAV in 9, preceded AAV in 2 and followed AAV in 4 patients. Ten patients had a renal biopsy that showed FSGN, with glomerular tuft necrosis in 7. Mean serum creatinine on presentation was 5.85mg/dL (median 4.35mg/dL). Induction was with steroids and cyclophosphamide (CyP) in 9. In addition to this, 4 had plasma exchange, and 1 had rituximab. One patient had steroids alone. Respiratory complications were the commonest cause of mortality (5 out of 10). There was no significant difference in survival between patients with MPA with PF and those without PF (p=0.127).

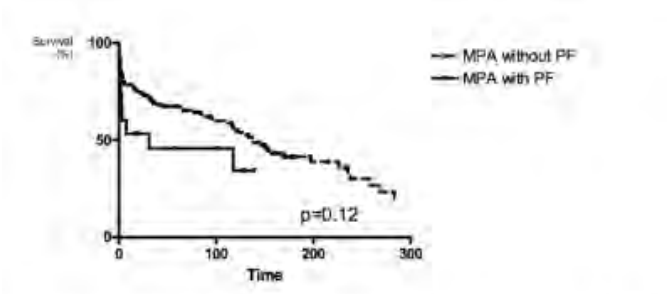



Figure 1: Kaplan Meier survival curve comparing the cohort of patients with MPA and PF and patients with MPA alone

Survival was worse in MPA patients with serum creatinine >5mg/dL at presentation compared to those with creatinine <5mg/dL (p<0.0001). Mean creatinine at follow up in 11 patients who achieved remission was 2.55mg/dL (median 2.15mg/dL).

Conclusion: AAV and PF may occur concurrently. Degree of renal impairment at presentation is a stronger predictor of survival than presence of PF, though morbidity from PF is significant. Optimum treatment of PF in MPA needs to be determined.

Disclosure of Financial Relationships: nothing to disclose

## PUB206

**Spectrum of Renal Diseases in a Tertiary Care Hospital of Pakistan** Syed Rizwan Bokhari, Hafiz I. Ahmad, Muhammad Awais. *Nephrology Department, Allama Iqbal Medical College, Lahore, Pakistan.*

Patients with renal diseases make a large group among patients presenting to our tertiary care hospital in Pakistan. We aimed at studying the spectrum and etiology of renal diseases referred for Nephrology consultation from inpatient floors and emergency department. PLACE AND DURATION OF STUDY: The study was conducted in the Department of Nephrology over a period of three and half months (Feb to May, 2010). DESIGN: A single center based prospective observational study RESULTS: A total of 202 patients were studied. Of these 100 were males and 102 females. Mean age of the patients in this series was 32.7 years with a range of 14 to 85 years. Of these 202 patients 59(29.2%) had acute renal failure (ARF) / Acute Kidney Injury (AKI), 141(69.8%) had chronic kidney disease (CKD) and 2 (1%) had transplant dysfunction.

Out of 59 ARF/AKI patients, etiologies included peri-partum ARF 20 (34%), sepsis 19(32%), nephrotic syndrome 10 (17%), non nephrotic protienuria 4 (7%), post surgical ATN 7 (12%), hypovolemia 5 (8.4%), drug induced 4(7%), acute Glomerulonephritis 4(7%), hepetorenal 4 (7%) and obstructive uropathy 2 (3.3%).

Out of 141(69.8%) patients with CKD18 (13%) had CKD 4, 74 (52%) had CKD 5 and 49(35%) had ESRD. Causative factors for CKD included HTN 108 (77%), DM 48 (34.5%), combined HTN and DM 33(24%), obstructive uropathy 12(8.6%), nephrotic syndrome 2 (1.4%) and Lupus Nephritis 2 (1.4%).

CONCLUSION: This study highlighted two important issues. Peri-partum renal failure makes the largest group of ARF patients and a large number of patients present with advanced CKD (stages 4 &5) as their first presentation of renal disease. These findings underscore the need for improved obstetric care and launching of intensive efforts for early screening strategies and risk factor management for diagnosis and treatment of Chronic Kidney Disease.

Disclosure of Financial Relationships: nothing to disclose

## PUB207

**Hepatitis B Virus Infection Is Associated with Lower Renal Function in Chinese Adults of Beijing** Jianfang Cai, Xiaohong Fan, Hang Li, Xuemei Li, Xue-Wang Li. *Nephrology, Peking Union Medical College Hospital, Beijing, China.*

**AIM:** To explore the association between Hepatitis B virus (HBV) infection and renal function and urinary albumin excretion.

**DESIGN AND METHODS:**In this cross-sectional study, a random sample of 6856 Chinese rural adults aged 30 to 75 years were selected in Beijing during 2008 to 2009. HBV infection was defined as serum HBsAg positivity. Estimated glomerular filtration rate (eGFR) was calculated with the estimating equation developed by CKD-EPI. The estimated marginal means (EMMs) and standard error of means (SEMs) of eGFR, the logarithm of serum creatinine (Cr) and the logarithm of urinary albumin-creatinine ratio (ACR) by HBV infection status were calculated and compared with generalized linear models, adjusted for age, gender and other confounding factors (e.g. hypertension, diabetes, dyslipidemia, obesity, smoking, nephrolithiasis). All statistical analyses were conducted with SPSS14.0 and results were expressed as EMMs±SEMs.

**RESULTS:** HBV infection had an overall prevalence of 4.90% in the study population. The unadjusted EMM of eGFR was lower (92.39±0.85 vs. 94.62±0.19 ml/min/1.73m2, p=0.010) and that of logarithm of Cr (1.905±0.004 vs. 1.893±0.001, p=0.002) was higher in persons with than in those without HBV infection. So were multivariate-adjusted EMM of eGFR (89.52±0.95 vs. 91.78±0.71 ml/min/1.73m2, p=0.001) and that of logarithm of Cr (1.917±0.005 vs. 1.907±0.004, p=0.002), after adjusted for age, gender, and other confounding factors (e.g. hypertension, diabetes, dyslipidemia, obesity, smoking, nephrolithiasis).However, there was no difference in the multivariate-adjusted EMMs of logarithm of ACR between persons with and those without HBV infection.

**CONCLUSIONS:** HBV infection is associated with lower renal function, but not with urinary albumin excretion in Chinese general population of Beijing.

Disclosure of Financial Relationships: nothing to disclose

## PUB208

**The Prevalence of Type 2 Cardio-Renal Syndrome in Inpatients with Chronic Heart Disease** Xi Chen, Zhaohui Ni, Shan Mou, Qin Wang. *Renal Division, Renji Hospital Shanghai Jiaotong University School of Medicine, Shanghai, China.*

***Instruction*:** Recent studies have showed that patients with chronic heart disease (CHD) are at high risk of chronic kidney disease, which is defined as type 2 cardio-renal syndrome (type 2 CRS). The high prevalence and early diagnosis of type 2 CRS in CHD population don't gain enough attention of clinicians. In this study, we aim to investigate the prevalence of type 2 CRS in CHD patients and find out the related factors of type 2 CRS.

***Methods:*** A retrospective, cross-sectional analysis of 1275 CHD inpatients of Cardiology Department from July 2008 to July 2009 was conducted. Estimated glomerular filtration rate (GFR) was calculated using the simplified Modification of Diet in Renal Disease (MDRD) equation. Baseline characteristics including age, gender, lifestyle, medical history, physical examination, laboratory variables were collected and analyzed.

***Results:*** Of all the 1275 CHD inpatients, the prevalence of type 2 CRS was 18.4% (235/1275). Patients with type 2 CRS were older than patients without CRS. Compared to study patients without CRS, type 2 CRS patients were more likely to have medical history of diabetes mellitus (28.9% vs 21.8%, $P$ <0.05), hyperuricemia (41.3% vs 26.4%, $P$ <0.001) and cerebrovascular disease (9.8% vs 5.7%, $P$ <0.001). Univariate analysis showed the significant correlation between type 2 CRS and lactate dehydrogenase(LDH) ($r$= 0.127, $P$=0.042), type B natriuretic peptide(BNP)($r$= 0.182, $P$<0.001), blood urea nitrogen(BUN)($r$= 0.298, $P$<0.001), serum creatinine(SCr)($r$= 0.301, $P$<0.001), uric acid(UA)($r$= 0.142, $P$<0.001), eGFR($r$= -0.334, $P$<0.001). Moreover, age (odds ratio [OR] 1.038, 95% confidence interval [CI] 1.025-1.051) and hyperuricemia (OR 1.855, 95% CI 1.380-2.494) were risk factors of type 2 CRS.

***Conclusion:*** This study suggested that the prevalence of type 2 CRS was high in CHD patients and some cardio-renal related markers like LDH, BNP, SCr, BUN, UA and eGFR were associated with type 2 CRS. Especially for CHD patients with advanced age and hyperuricemia, early diagnosis and treatment of type 2 CRS were needed.

Disclosure of Financial Relationships: nothing to disclose

**PUB209**

**Comparison of Three Methods of Estimation of Glomerular Filtration Rate with Creatinine Clearance in Kidney Transplant Recipients** Lukasz B. Chrobak,[1] Alicja Debska-Slizien,[1] Andrey Petranyuk,[2] Magdalena Maria Jankowska,[1] Boleslaw Rutkowski.[1] *[1]Nephrology, Transplantation and Internal Medicine, Medical University of Gdansk, Gdansk, Poland; [2]English Division of Medical University School, Medical University of Gdansk, Gdansk, Poland.*

Background:

Estimation of glomerular filtration rate (GFR) after renal transplantation is performed with the use of methods which are standardized for population of non-transplant patients with chronic kidney disease. Results of estimation of GFR may be different in population of kidney recipients due to comorbidities, altered nutritional status, residual clearance and donor specific factors affecting graft function. So far there are very few studies comparing creatinine based GFR estimation methods with creatinine clearance or isotope based methods in this population.

Aim:

The aim of the study was to compare creatinine clearance with three GFR estimation methods in group of kidney transplant recipients.

Patients and methods:

We calculated eGFR with 4-variable Modification of Diet in Renal Disease (MDRD) formula, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and Cockroft-Gault (C-G) formula in 226 consecutive kidney graft recipients transplanted in Medical University of Gdansk in years 2007-2009. These results were analyzed against creatinine clearance. Patients with unstable creatinine concentration were excluded from the study.

Results:

We have noticed significantly different results of assessment of GFR depending on calculation method. The median values were: 56.6 ml/min, SD=17.6 for creatinine clearance; 48.6 ml/min/1.73/m(2), SD=19,9 for MDRD eGFR; 42,3 ml/min/1.73/m(2), SD=18.9 for CKD-EPI equation and 55.3 ml/min, SD=18.6 for C-G formula. Pearson's correlation analysis showed that C-G formula correlates best with creatinine clearance in our group of patients (r=0.67).

Conclusions:

Our results confirm that C-G formula correlates best with creatinine clearance in our cohort of kidney transplant recipients. Use of appropriate formula for estimation of kidney function in this group of patients is essential for proper drug dosage and decision-making.

Disclosure of Financial Relationships: nothing to disclose

**PUB210**

**Study of Early Screening of Chronic Kidney Disease (CKD) in the Population of Palmas/Tocantins-Brazil: An Appliance of the Scored Compared to the Conventional Methods** Itágores Hoffman II Coutinho,[2] Joao Egidio Romao, Jr.,[1] Manuel C. Castro,[1] Ibsen S. Trindade,[2] Balduino F. Andrade.[2] *[1]University of São Paulo, São Paulo; [2]Federal University of Tocantins, Palmas, Brazil.*

The determination of the risk of development and the early diagnosis of CKD offer opportunities of treatments that block the progression of the nephropathy and reduce its morbidity and mortality. Objectives: To make an early screening of the CKD in adult population of Palmas/Tocantins-Brazil through the Scored questionnaire, and compare it to conventional diagnosis methods. Methods: The Scored provides 20% of CKD's hidden chance when ≥4. Randomly 707 participants of general population who lived or worked in the raffled domicile were selected. A questionnaire with the 11 questions of the Scored, and others important risk factors for CKD, weight, height, abdominal circumference, blood pressure, capillary glucose, fasting glucose, serum creatinine and urinalysis were evaluated. CKD was defined as estimated creatinine clearance (eGFR - Cockcroft-Gault equation) <60 mL/min/1.73 m². The frequency of all variables were determined and the bivariated analyses, through the crossing of the independent variables with the denouement Scored and CKD, determining the odds ratio (OR). Results: Mean age was 40.1±15.6 (range 18-87) yr and 63% were female. Scored was positive in 27.7% of the participants and CKD diagnosis was established in 3.4%. In group with eGFR <60 mL/min/1.73 m², mean eGFR was 42.2±12.7 (range: 18.4 to 59.6). Comparing the results of the Scored with the CKD, we observed OR=20.3 (p <0.00001). Analyzing the Scored with the age ≥65 yr, diabetes mellitus (DM) and hypertension (HTN), we observed OR of 28.8 (p <0.00001), 5.1 (p <0.00001) and 3.4 (p <0.00001), respectively. In comparison of CKD with the age ≥65 yr, DM and HTN, we found OR of 42.5 (p <0.00001), 21.2 (p <0.00001) and 3.2 (p =0.004), respectively. Conclusion: The Scored shown strong relationship with CKD and its risk factors, being useful on CKD's screening of the general population. Due its easy applicability, it could be implemented in public health politics, and the physicians should be encouraged to use this tool as initial screening.

Disclosure of Financial Relationships: nothing to disclose

**PUB211**

**Comparison of the CKD-EPI and the MDRD Equations for Estimating GFR and the Prevalence of CKD in Icelandic Adults** Sverrir I. Gunnarsson,[1] Runolfur Palsson,[1,3] Gunnar Sigurdsson,[2,3] Olafur S. Indridason.[1] *[1]Division of Nephrology; [2]Division of Endocrinology, Landspitali University Hospital; [3]University of Iceland, Reykjavik, Iceland.*

The CKD-EPI equation has been proposed as a replacement for the MDRD equation to generate the estimated glomerular filtration rate (eGFR) from serum creatinine. In this study, we examined the performance of these two equations in a large cohort of community-dwelling adults.

We examined data from a large cross-sectional study on bone health conducted in 2001-2003, that included IDMS-standardized serum creatinine values for 1628 randomly recruited Caucasian subjects from the Reykjavik area. The eGFR was calculated by the MDRD and the CKD-EPI equations and the relative performance of the equations examined. The prevalence of CKD (eGFR <60 mL/min/1.73 m²) obtained by the two equations was compared.

The mean age of the subjects was 59.7 ± 14.8 years and 63.8% were women. The median (range) eGFR by the CKD-EPI equation was higher than by the MDRD equation, 97.0 (21.2-136.1) vs. 95.9 (22.6-213.9) mL/min/1.73 m² (p<0.001), respectively. Correlation between the CKD-EPI and MDRD equations was strong (r=0.871; p<0.01) and excellent for those with CKD (r=0.987; p<0.01). The median of the difference in eGFR obtained with the two equations (CKD-EPI eGFR minus MDRD eGFR) was 0.32 (-98.2–18.6) mL/min/1.73 m². For individuals with eGFR <45, 45-59, 60-74, 75-90 and >90 mL/min/1.73 m², the median and percent difference in eGFR by the two equations (CKD-EPI minus MDRD) was -0.79 (-2.24%), 0.37 (0.81%), 2.82 (3.98%), 5.24 (6.32%) and -4.64 (-4.40%) mL/min/1.73 m², respectively (p<0.001). The CKD-EPI equation yielded a lower prevalence of CKD for women than the MDRD equation (5.6% vs. 6.2%; p<0.001) but for men the prevalence was equal (3.9%). Agreement between the equations was good with only 8% of the patients diagnosed as having CKD by the MDRD equation having eGFR >60 by the CKD-EPI equation.

In healthy subjects, the correlation between the CKD-EPI and MDRD equations is very good. The difference in eGFR by the two equations varies by kidney function and is minimal at lower levels of eGFR. The CKD-EPI equation may be less prone to overestimate CKD in women.

Disclosure of Financial Relationships: nothing to disclose

**PUB212**

**Percutaneous US-Guided Renal Biopsy Experience from a Single-Center in China: A Retrospective Study Comparing Two Different Nurses' Method** Xuelin He, Huijuan Ye, Heng Li, Jianghua Chen. *Kidney Disease Center, The First Affiliated Hospital of Medical College, Zhejiang University, Hangzhou, Zhejiang, China.*

Renal biopsy is essential for the diagnosis of kidney diseases, but complications and the degree of comfort, remain problematic. To improve the comfort and acceptability we upgraded our nurses' method, and we conducted a retrospective observational study on the rate of major and minor complications in our kidney disease center to assess the safety and comfort profile of the two different nurses' method.

2220 biopsies performed in our kidney diseases center from Aug 2006 to Oct 2009 was undertaken. The patients were divided in two groups: group A(Aug 2006 to Sep 2007, 702 biopsies) was treated by abdominal bandage with sandbag pressure dressing in 6 hours and kept strictly in prostration position 24 hours; group B(Oct 2007 to Oct 2009, 1518 biopsies) was treated by abdominal bandage without sandbag pressure dressing in 6 hours and only kept strictly in prostration position 6 hours. The major complications (perinephric hematoma, gross hematuria) and minor complications (postural hypotension due to vasovagal response, urinary retention, back pain, insomnia, and gastrointestinal symptoms such as nausea, vomiting and abdominal distention ) were observed in two groups.

There was no significant difference on gross hematuria (group A 3.1% vs group B 3.5%, P>0.05) and perinephric hematoma (group A 11.2% vs group B 10.9%, P>0.05) in two groups. Compared with group A, group B had lower rate of postural hypotension (group A 10.8% vs group B 7.2%,P<0.05), urinary retention needed urinary catheter (group A 20.8% vs group B 9.3%, P<0.01), back pain (group A 26.1% vs group B 11.5%, P<0.01), gastrointestinal symptoms (group A 39.5% vs group B 16.8%,P<0.01), and insomnia(group A 31.6% vs group B 21.1%,P<0.01).

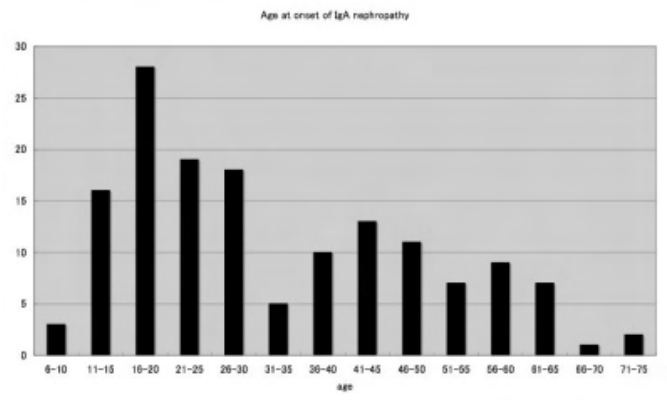
The rate of minor complications was decreased, without increase the rate of major complications in percutaneous renal biopsy after nurses' method improvement. However, the degree of comfort and acceptability was increased.

Disclosure of Financial Relationships: nothing to disclose

**PUB213**

**Clinical and Histological Differences by the Age-at-Onset of Primary IgA Nephropathy in a Single Center Japanese Population** Makoto Inoue, Shin-Ichi Takeda, Shigeaki Muto, Eiji Kusano. *Nephrology, Jichi Medical University, Shimotsuke, Tochigi, Japan.*

PURPOSE: IgA nephropathy is the most common primary glomerulonephritis in Japan and the patients may present at any age. When we retrospectively reviewed 149 patients with primary IgA nephropathy who had been admitted to Jichi Medical University hospital from January 2005 to November 2009, there were two peak incidence in 16-20 and 41-45 years.




The aim of this study was to define the clinical and histological differences in IgA nephropathy between the two groups; young patients group (YG) and non-young group (NYG).

METHODS: Data were obtained on 89 YG patients under 35 years (mean age 21 years, 8-35) and 60 NYG patients over age 35 (50 years, 36-75).

RESULTS: The NYG more frequently had hypertension ($p<0.0001$). Compared with YG, NYG patients had higher blood pressure (BP); mean systoric BP was 138 mmHg vs 121 mmHg ($p<0.0001$), mean diastoric BP was 81 mmHg vs 69 mmHg ($p<0.0001$), serum creatinine was 85.8 µmol/L vs 62.8 µmol/L ($p<0.0001$), serum IgA was 344 mg/dl vs 311 mg/dl ($P<0.05$) and urinary protein: creatinine ratio was 137.9 mg/mmol vs 87.0 mg/mmol ($p<0.05$). Renal pathological investigation showed the chronic lesions were dominated in NYG. YG had significantly less glomerular sclerosis ($P<0.005$), renal tubule atrophy ($P<0.05$), and arteriolosclerosis ($P<0.0001$).

CONCLUSION: IgA nephropathy patients were more likely to suffer from hypertension, renal insufficiency and chronic pathologic lesions depend on the age, which might be the risk factors for the patient's unfavorable prognosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB214**

**Nicaragua Revisited: Prevalence of CKD in a High Altitude, Coffee Growing Village** Timothy S. Laux,[1] Philip Bert,[2] Gerardo Barreto,[2] Mark L. Unruh,[1] Aurora Aragon,[2] Cecilia Torres.[2,3] *[1]Renal-Electrolyte, University of Pittsburgh Medical Center, Pittsburgh, PA; [2]Center for Research in Health, Work, and Environment, National Autonomous University of Nicaragua at Leon, Leon, Nicaragua; [3]Occupational and Environmental Medicine Sciences, Uppsala Universitet, Uppsala, Sweden.*

Purpose/Background: Chronic kidney disease (CKD) has been documented as widespread in parts of Central America, but knowledge gaps remain concerning CKD's prevalence in regions located at higher altitudes where coffee is grown.

**Methods**

Study Design: Cross-sectional study using an interviewer administered questionnaire covering demographic information, lifestyle, diseases, medications and measures quantifying serum creatinine (SCr) values >1.2 mg/dL for men and >0.9 mg/dL for women, estimated glomerular filtration rate <60 mL/min/1.73 $m^2$, dipstick proteinuria stratified as microalbuminuria (30-300 mg/dL) and macroalbuminuria (300+ mg/dL), hypertension, and BMI.

Setting and Participants: Total population between the ages of 20-60 years in one coffee growing village in Nicaragua located at 1,000 meters elevation. 267 participants (120 men, 147 women, 82% response).

Predictor or Factor: Participant sex, age, occupation; conventional CKD risk factors and factors suggested by previous surveys in Central America.

Results: Prevalence of abnormal SCr levels was low: 1 male (0.4%) and 4 females (1.5%). Prevalence of estimated GFR < 60 mL/min/1.73 $m^2$ was 0 (0%) of men and 2 (1.4%) of women. Overall proteinuria rate among men was 27.5% (33 individuals), with 27 having low (81.8%) and six having high range (18.2%). The overall rate among women was 21.4% (32 individuals), all having low range. There was a difference between proteinuria distributions and sex ($p=0.019$) as well as age ($p=0.042$).

Conclusions: The GFR rates and CKD prevalence in this village are comparable to a previously studied Nicaraguan coffee farming region and different than either the 1988-1994 or 1999-2004 NHANES study. This data can be used to guide Nicaraguan CKD screening criteria and further refine hypotheses about the process(es) behind the high rates of CKD seen in other regions of the nation.

Disclosure of Financial Relationships: nothing to disclose

**PUB215**

**A Comparison of Prediction Equations for Estimating Glomerular Filtration Rate in Chinese Patients with Chronic Kidney Disease** Xun Liu, Cheng Wang, Hua Tang, Chenggang Shi, Cailian Cheng, Zhujiang Chen, Tan-Qi Lou. *Division of Nephrology, Department of Internal Medicine, The Third Affiliated Hospital of Sun Yet-Sun University, Guangzhou, Guangdong, China.*

Background Accurate measurement of renal function is important for the detection of chronic kidney disease (CKD). We sought to evaluate the applicability of formulas based on serum creatinine (SC) levels in Chinese patients with CKD.

Materials and methods 327 CKD patients who had undergone 99mTc-DTPA-glomerular filtration rate (GFR) estimation were enrolled. Average sGFR measured by 99mTc-DTPA GFR estimation was 44.5±26.9 ml/min/1.73 m2. The Cockcroft-Gault-equation, MDRD1-equation, abbreviated MDRD-equation, Jelliffe-1973-equation, Mawer-equation, Hull-equation, Jelliffe-1971-equation, SC-reciprocal-equation, Gate-equation and Bjornsson-equation were tested. Using the 99mTc-DTPA-GFR as the reference standard GFR (sGFR), the accuracy of estimated GFR (eGFR) was compared with sGFR in various stages of CKD.

Results Bland-Altman analysis demonstrated that Cockcroft-Gault-equation, Bjornsson-equation and Hull-equation were better than the other equations. However, the agreement limits of all the equations exceeded the prior acceptable tolerances defined as 60 ml/min/1.73 m2. Linear regressions showed that the slopes of Jelliffe-1973-equation and Cockcroft-Gault-equation were closer to the identical line. The median of difference of Jelliffe-1973-equation, Bjornsson-equation and abbreviated MDRD-equation were smaller. The median % absolute difference of MDRD1-equation, Bjornsson-equation and Cockcroft-Gault-equation were smaller. Accuracy of Bjornsson-equation, Jelliffe-1973-equation and Cockcroft-Gault-equation were higher than those of the other equations. But 30% accuracies of all the equations were less than 70%. When compared the bias as well as accuracy of eGFR with sGFR in different stages of CKD, GFR estimated by Bjornsson-equation, Cockcroft-Gault-equation and Jelliffe-1973-equation showed good results.

Conclusion When SC was measured by enzymatic method, GFR estimation equations may show great bias in Chinese CKD patients. Further improved formulas are needed to assess GFR in Chinese CKD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB216**

**Rigorous Derivation of a Formula for Estimation of Average Creatinine Clearance (CCA) in the Non-Steady State** Douglas L. Somers. *Internal Medicine, University of Iowa, Iowa City, IA.*

In clinical care or research, it is sometimes useful to estimate intrinsic renal function while serum creatinine (sCr) is changing, for instance in the interdialytic period in End Stage Renal Disease, or in Acute Kidney Injury. In the face of changing sCr, an estimator sometimes used for CCA over the time of urine sample collection is : CCA = (Urine creatinine excretion/sample collection duration)/( sCr at start of collection + sCr at end of collection)/2). To understand the limitations of this equation, it was rigorously derived from a differential equation describing non-steady state accumulation and excretion of body creatinine. This derivation requires the following assumptions: 1)Single pool kinetics, 2)No extrarenal losses, 3)The patient's volume is approximately constant, 4) Creatinine Generation Rate is approximately constant over the collection time, 5)Volume of Distribution of Creatinine= Body water, 6)Serum Creatinine increases or decreases in a monotonic manner, and , 7)The mass of creatinine in the urine collection is small, (compared to twice the time-averaged mass of creatinine in the body). The final assumption means that the estimator is in error when the renal function is improving, and the remaining assumptions suggest other limitations. Nonetheless, comparison to numeric solutions of the original differential equation shows that the simple estimator of CCA is usually within a few percent of the true CCA, when sCr is rising.

Disclosure of Financial Relationships: nothing to disclose

**PUB217**

**Kidney Function in Patients with Abdominal Multivisceral/Small Bowel Transplantation** Michiko Suzuki, Muhammad Ahmad Mujtaba, Asif A. Sharfuddin, Muhammad S. Yaqub, Dennis P. Mishler, Tim E. Taber. *Nephrology, Indiana University/ Clarian Transplant Institute, Indianapolis, IN.*

**Background:** Renal dysfunction is a recognized complication seen after transplantation of gastrointestinal organs, which may require renal replacement therapy. In multivisceral transplantation (MVT), etiologies of acute kidney injury (AKI) include hypovolemia, drug-induced nephrotoxicity, acute tubular necrosis, portomesenteric thrombosis, and hyperbilirubinemia.

**Methods:** Renal function at the two different time points, pre-transplant and one year post-transplant, was assessed by estimated Creatinine Clearance (ClCr) calculated by Cockcroft-Gault formula and Schwartz formula for adult and pediatric subjects, respectively. One year post-transplant kidney function was analyzed with demographic and clinical variables, including pre-transplant risk factors for kidney disease and post-transplant sequential laboratory data. AKI was defined as serum Cr greater than 2.0-fold increase from baseline.

**Results:** The final cohort included 48 patients, 25 multivisceral (liver, pancreas, and small bowel), 15 isolated small bowel, and 8 modified multivisceral (small bowel combined with liver or pancreas) transplants. The average age of the study subjects was 33.7±22.6 years old. The mean level of pre-transplant serum Cr (sCr) was 0.7±0.7 mg/dl with 69% (n=33) of patients having ClCr greater than 80ml/min. One year after transplant, 58% of

patients (n=28) had ClCr less than 80ml/min, 46% of patients (n=22) had ClCr less than 60ml/min. In linear regression analysis, age at transplant, baseline sCr, pre-transplant ClCr, sCr at 1 months, 3months, 6 months and 9 months after transplant, mean Tacrolimus level at post operative day (POD) 7, and AST at POD180 showed significant correlation with post-transplant ClCr. Pre-existing hypertension and AKI after transplant were also correlated with post-transplant ClCr (P<0.05).

**Conclusion:** In MVT/ small bowel transplant patients, risk factors for post-transplant chronic kidney disease include older age, pre-existing kidney dysfunction, pre-existing hypertension, and AKI after transplant.

Disclosure of Financial Relationships: nothing to disclose

**PUB218**

**SDMA Outperforms CKD-EPI and MDRD Derived eGFR for the Assessment of Renal Function in Patients with Adult Congenital Heart Disease** Oktay Tutarel,[1] Agnieszka Denecke,[1,2] Stefanie M. Bode-Böger,[3] Jens Martens-Lobenhoffer,[3] Mechthild Westhoff-Bleck,[1] Jan T. Kielstein.[2] *[1]Cardiology, Hannover Medical School, Hannover, Germany; [2]Nephrology, Hannover Medical School; [3]Clinical Pharmacology, Otto-von-Guericke University, Magdeburg, Germany.*

*Purpose*

Adults with congenital heart disease exhibit a 3-fold higher mortality in the presence of moderate or severe chronic kidney disease. However formulas for the estimation of glomerular filtration rate (GFR) have not been evaluated in this special patient population. The aim of this study was to compare different markers and equations for the estimation of renal function in adults with congenital heart disease.

*Methods*

Renal function was assessed in 102 patients using the MDRD equation, the simplified MDRD equation, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, the Cockcroft-Gault formula and the Larsson equation. Additionally, symmetrical dimethylarginine (SDMA) was measured.

*Results*

GFR estimated by the original MDRD (Pearson r = 0.465, p<0.001) and the CKD-EPI equation (r = 0.462, p<0.001) showed a similar strong correlation with the eGFR based on a Cystatin C based equation. While GFR according to the simplified MDRD equation showed a weaker correlation (r = 0.439, p<0.001). Creatine clearance according to the Cockcroft-Gault formula showed no correlation at all to the Cystatin C based eGFR (r = 0.144, p = 0.17). The strongest correlation was observed for SDMA and Cystatin C based eGFR (r = -0.552, p<0.001).

*Conclusion*

GFR in adults with congenital heart disease should be estimated using the original MRDR or the CKD-EPI formula. SDMA seems to be a promising marker of renal function for this patient group and should be further evaluated in the future.

Disclosure of Financial Relationships: nothing to disclose

**PUB219**

**Is eGFR Representative for the Retention of Other Uremic Solutes in CKD before Start of Dialysis?** Raymond C. Vanholder,[1] Eva Schepers,[1] Daniela Veit Barreto,[2] Fellype Barreto,[2] Griet Lrl Glorieux,[1] Ziad Massy,[2] Sunny Eloot.[1] *[1]Department of Internal Medicine - Nephrology, Ghent University Hospital, Gent, Belgium; [2]Clinical Pharmacology, Amiens University Hospital, Amiens, France.*

The severity of chronic kidney disease (CKD) can be expressed in terms of Glomerular Filtration Rate (GFR), which can be determined directly or calculated/estimated according to different formulae based on creatinine and/or cystatin C (eGFR). The aim of our study was to investigate whether the currently used eGFR-values are representative for the progressive increase of concentration of uremic retention solutes with different degrees of renal dysfunction.

The association with eGFR was evaluated for a broad set of uremic solutes [creatinine (Crea), uric acid (UA), symmetric dimethyl arginine (SDMA), asymmetric dimethyl arginine (ADMA), and free and total fractions of hippuric acid (HA), 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), indoxyl sulfate (IS), indole acetic acid (IAA), and p-cresylsulfate (pCrS)] in a group of patients with different degrees of kidney failure, i.e. CKD stage 2 to 5, not on dialysis. Concentration was correlated with eGFR calculated with formulae based on creatinine (MDRD), cystatin C (Le Bricon et al, Filler et al, Rule et al), and the combination of both (epi-GFR).

Creatinine showed the best fit (MDRD $R^2$=0.833) followed by total IS ($R^2$=0.416), SDMA ($R^2$=0.237), free pCrS ($R^2$=0.150), and free IAA ($R^2$=0.123). There was a substantial disparity in significance of fits among solutes. There was a substantial disparity in significance of fits among solutes. The formulae only based on cystatin C did not show different correlations versus the epi-GFR or the MDRD formula, even for solutes other than creatinine. Free fractions of protein bound solutes showed better correlations compared to the total fractions, except for indoxyl sulfate.

Hence, eGFR is only partially representative for the progressive increase of concentration with worsening kidney function for all studied retention solutes, with borderline significance for UA and even no significance for CMPF.

Disclosure of Financial Relationships: nothing to disclose

**PUB220**

**The Chronic Disease Nephropathy Prevention Clinic in Siksika Nation: A Quality Improvement Project** David Ward,[1] Ellen M. Novak,[1] Sony Brar,[2] Brenda Hemmelgarn.[1] *[1]Department of Medicine, University of Calgary, Calgary, AB, Canada; [2]Alberta Kidney Disease Network, AB, Canada.*

**Background:** The prevalence of ESRD is higher amongst the Aboriginal population. Diabetes, access to health care services, and limited ongoing assessment and follow-up may contribute to this increased risk.

**Objective:** To determine whether a community-based clinic led by a nurse practitioner will improve the management of Aboriginal People at risk of developing kidney disease.

**Methods:** The study population included Aboriginal patients from Siksika Nation with diabetes or difficult to control blood pressure (BP). Patients were assessed by a nurse practitioner who provided ongoing management of BP, blood sugar, and lipid control; according to national clinical practice guidelines. Outcome variables of interest were reduction in haemoglobin A1C (HbA1C), BP, and LDL, as well as the proportion of patients initiated on medications indicated (ACE-I or ARB, statins and ASA). Differences between groups were analyzed using paired T-test and Chi-square as appropriate.

**Results:** A total of 78 patients were followed regularly in the clinic (62% female; mean age 56 years). Amongst those above target at baseline, there was a significant reduction in mean HbA1C (0.96%; p<0.01), LDL (0.62mmol/L; p<0.01), systolic BP (15.9 mmHg; p<0.01), and diastolic BP (9.7 mmHg; p<0.01). Additionally, there was a significant increase in the proportion of subjects initiated on an ACE-I or ARB (42.4%; p<0.01), statin (35.9%; p<0.01), and ASA (35.9%; p<0.01).

**Conclusions:** Adequate management of patients at high risk of developing kidney disease has been shown to reduce progression of kidney disease and development of ESRD. Although limited by lack of a comparison group, our data suggest that a community-based, nurse-practitioner led clinic can significantly improve management in many clinically relevant areas. The impact of these interventions has been shown in other studies to reduce the risk of death and cardiovascular events; the effect in the Aboriginal population on these hard clinical endpoints remains to be determined.

Disclosure of Financial Relationships: nothing to disclose

**PUB221**

**Estimation of Glomerular Filtration Rate Based on Serum Cystatin C Concentration in Old and Very Old Subjects – PolSenior Study** Andrzej Wiecek,[1] Jerzy Chudek,[1,2] Marcin Adamczak.[1] *[1]Department of Nephrology Endocrinology and Metabolic Diseases, Medical University of Silesia in Katowice, Katowice, Poland; [2]Department of Pathophysiology, Medical University of Silesia in Katowice, Katowice, Poland.*

**Introduction:** Cystatin C-based Hoek' equation, applicable for estimation of glomerular filtration rate (eGFR) does not include age, unlikely those routinely used, creatinine-based (Cocroft-Gault, MDRD and CKD-EPI equations). Thus the estimation of eGFR by this method is not influenced by age and could be more accurate in very old people. The assessment of eGFR based on cystatin C equations has not been performed in very old population yet.

Aim: The estimation of frequency of chronic kidney disease - CKD (stage 3-5) based on Hoek' equation in older than 65 years.

**Patients and methods:** Study was performed in 113 subjects in age 55-60y. and 565 in age 65-101y. from the PolSenior study population. Subjects in age 65-101y. were divided into three subgroups (65-75; 76-85 and over 85y.). Serum cystatin C concentration was assessed by enzyme-linked immunoabsorbent method. eGFR was estimated with cystatin C-based Hoek' equation.

**Results:** Serum cystatin C concentration was increasing in subsequent age subgroups (mean±SD) (0.78±0.17mg/ml in 55-60y. subgroup, 0.94±0.30mg/ml in 65-75y. subgroup, 1.06±0.26mg/ml in 76-85y. subgroup and 1.22±0.35mg/ml in older than 85y. subgroup). The frequency of subjects with eGFR<60 ml/min (cystatin C-based equation) was increasing with age, from 0.9% in 55-60y. subgroup through 8.5% in 65-75y. subgroup and 23.0% in 76-85y. subgroup to 41.1% in over 85y. subgroup. In the oldest subgroup the prevalence of subjects with eGFR<60ml/min estimated with cystatin C-based equation was significantly higher (p<0.01) than estimated in subjects with MDRD formula (41.1% vs. 35.5%).

There was a strong correlation between serum cystatin C concentration and age (r=0.480, p<0.001) in the whole group of analyzed subjects. Serum cystatin C concentration was not related to BMI (r=0.026 p=0.51).

**Conclusion:** Cystatin C-based Hoek' equation for estimation of glomerular filtration rate seems to be very useful and accurate for diagnosis of chronic kidney disease in old and very old subjects.

Disclosure of Financial Relationships: nothing to disclose

**PUB222**

**Kidney Disease Is Independently Associated with Increased Risk of Hospitalization and Death in the US Veteran Population** Ziyad Al-Aly, Michael I. Rauchman, Tarek M. Elachkar. *Division of Nephrology, Saint Louis Veterans Affairs Medical Center, Saint Louis, MO.*

We wanted to examine the effect of kidney disease on the risk of hospitalization and death among United States veterans. Using the Department of Veterans Affairs national databases, we built a cohort of 28,980 patients who have at least 2 eGFR measurements that are at least 3 months apart in fiscal year 2000. Patients were followed until the end of fiscal year 2008. Patients were categorized into the non-CKD group if the 2 eGFRs were above 60 ml/min, and into CKD stage 3a, 3b, 4, and 5 if these eGFRs were between

45-59, 30-44, 15-29, and <15 ml/min; respectively. We then built separate Cox survival models to examine the effect of kidney disease on the risks of hospitalization and death. Compared to patients without kidney disease, patients with kidney disease had significantly increased risk of hospitalization; HR=1.190 (CI=1.140-1.242), HR=1.412 (CI=1.343-1.484), HR=1.705 (CI=1.592-1.826) and HR=2.201 (CI=2.000-2.424), for CKD stage 3a, 3b, 4, and 5; respectively. We then examined the effect of kidney disease on the risk of death and found that declining kidney function is associated with increased risk of death; HR=1.540 (CI=1.453-1.633), HR=2.460 (CI=2.314-2.615), HR=3.508 (CI=3.250-3.786) and HR=3.673 (CI=3.297-4.092), for CKD stage 3a, 3b, 4, and 5; respectively. The results show a differential risk of hospitalization and death among patients with CKD stage 3a and 3b; suggesting perhaps that CKD stage 3 is too broad and should be subdivided into stage 3a and 3b. Furthermore, the results indicate that there is an independent and graded association between kidney function and the risks of hospitalization and death in US veterans.

Disclosure of Financial Relationships: nothing to disclose

## PUB223

**Peripheral Vascular Disease in Chronic Kidney Disease Patients** Behnoud Beroukhim, Jose Jesus Perez, Ojas Naik, Venkataraman Ramanathan. *Nephrology, Michael E DeBakey VA Medical Center, Baylor College of Medicine, Houston, TX.*

INTRODUCTION: Peripheral vascular disease (PVD) coexists in patients with chronic kidney disease (CKD) and is associated with significant morbidity and mortality. However, disparities exist in the diagnosis and management of symptomatic PVD in this population.

METHODS: In this retrospective study, we reviewed the charts of consecutive veterans who underwent vascular ultrasound evaluation in a 6-month period for symptoms suggestive of PVD. Patients with prior history of PVD including those who underwent amputation or percutaneous or surgical revascularization were excluded. We measured ABI, segmental pressures in high and low thigh, calf and ankle, digital pressures and qualitative waveform in each extremity. MDRD GFR was estimated and patients were stratified into 2 groups: Group A - eGFR <60 ml/min and Group B - eGFR >60 ml/min. .

RESULTS: A total of 187 patients underwent vascular evaluation for symptoms suggestive of PVD and significant PVD was noted in 114 veterans (61%). As shown in the table, proportion of veterans who had significant PVD was higher in Group B.

| | Group A (eGFR<60) | Group B (eGFR>60) | p value |
|---|---|---|---|
| n | 57 | 130 | |
| Significant PVD (doppler) | 28 (49%) | 86 (66%) | 0.03 |
| Surgery consult | 23 (82%) | 51 (59%) | 0.04 |
| Revascularization | 12 (43%) | 51 (59%) | ns |
| Amputation | 6 (21%) | 4 (5%) | 0.01 |
| Re-hospitalization | 19 (68%) | 8 (9%) | <0.0001 |
| 1-yr mortality | 14% | 8% | ns |

Surgery consult was requested more frequently in Group A patients, but revascularization procedures were performed less often in this group. During follow up, the rate of atraumatic lower extremity amputation was significantly higher in patients with eGFR <60 ml/min. Similarly, re-hospitalization for PVD symptoms were significantly higher in this group. One-year mortality rate was also higher in Group A, but it failed to reach statistical significance.

CONCLUSIONS: Among veterans with symptomatic PVD, eGFR <60 ml/min is associated with higher rate of amputations and re-hospitalizations.

Disclosure of Financial Relationships: nothing to disclose

## PUB224

**Anemia Management with Methoxy Polyethylene Glycol-Epoetin Beta in Routine Clinical Practice: Preliminary Results of the OCEANE Study in Patients with Chronic Kidney Disease (CKD) Not on Dialysis** Gabriel Choukroun,[1] Christopher R. Mariat,[2] Marc C. Froissart,[3] Paul Landais,[4] Luc Frimat.[5] *[1]Nephrology, CHU Amiens; [2]Nephrology, CHU Saint-Etienne; [3]Nephrology, HEGP Paris; [4]DIM, Necker; [5]Nephrology, Nancy.*

Methoxy polyethylene glycol-epoetin beta (MPGE) is a new Erythropoiesis Stimulating Agent (ESA) licensed in Europe since 2007. We aim to describe anemia management with MPGE in routine practice and its accordance with European recommendations (EMEA 2007).

**Methods:** OCEANE is a one-year french multicentre prospective observational study. 605 patients (515 CKD patients not on dialysis, 90 transplanted patients) initiating MPGE were included. The primary endpoint is the proportion of patients with hemoglobin (Hb) level within 10-12 g/dL at 6 months. Here are the first results for the 515 CKD patients not on dialisis.

**Results:** The population included 53% men, with a mean age of 72±14 years and a CKD stage 2 (1%), 3 (29%), 4 (55%) and 5 (15%). Main etiologies of CKD were vascular (47%) and diabetic nephropathies (27%). Estimated GFR was 25±12 mL/min/1.73m$^2$, transferrin saturation was 24%, serum ferritin 218 ng/mL and 73% of the patients had a CRP<10 mg/L. Iron therapy was ongoing in 34% of the patients. At baseline, 248 patients (48%) were ESA-naive, 126 patients (25%) were previously treated with darbepoetin alfa, 103 patients (20%) with epoetin beta, and 31 patients (6%) with epoetin alfa. In ESA-naive patients, mean Hb level was 10.0±0.9 g/dL and the median starting dose of MPGE was 50 $\mu$g per injection (range: 30-150). In non ESA-naive patients, mean Hb level was 11.3±1.4 g/dL and the median starting dose of MPGE was 100 $\mu$g per injection (range: 30-360). The administration frequency was monthly in 46% of ESA-naive patients and 84% of non-ESA naive patients. The administration type was self-injection (14%), injection by a relative (4%) or a nurse at home (82%).

**Conclusion:** In current practice, MPGE is used in ESA-naive and non ESA-naive CKD patients regardless of the previous ESA treatment. At initiation, lower doses seem to be used rather than theorical ones, and a monthly dosing seems to be used immediatly in almost 50% of ESA-naive patients.

Disclosure of Financial Relationships: nothing to disclose

## PUB225

**Quality Assessment and Performance Improvement (QAPI) in a Pediatric Dialysis Unit: Taking Control of Phosphorus** Sheila Jones Coakley, Marie Y. Saint-Vil, Myerly Kertis, Alexandrina Pop, Gaston E. Zilleruelo, Carolyn L. Abitbol. *Pediatric Dialysis, University of Miami/Holtz Children's Hospital, Miami, FL.*

Quality Assessment/Performance Improvement (QAPI) projects are required by the Conditions of Coverage issued by the Center for Medicaid and Medicare Services (CMS) for all dialysis facilities in the U.S. In pediatric dialysis patients, phosphorus (P) targets have been one of the most difficult clinical measures to achieve. At the Holtz Children's Hospital pediatric dialysis unit, located in a multiethnic urban community, hyperphosphatemia was identified as a major deficiency of quality control compared to adult units in Florida Network 7. Our objective was to develop a QAPI multidisciplinary program focused on improving and sustaining serum P levels within target for the majority of pediatric patients on hemodialysis. A unit based assessment and improvement program was developed with the nursing manager and staff, social, dietary and physician services. Attention focused on education and adherence to diet and P binder medications. Incentive tools included individual dietary counseling, puzzles and games, weekly and monthly report cards, and friendly competition among patients. Rewards included meal vouchers with a grand prize of an Itunes card. P levels were monitored weekly with review of diet and medications. During a period of 1 year from May 2009 through May 2010, 33 patients (15 Males) with an average age of 16.9±3.8 years were hemodialyzed 3 times weekly. Progressive tactics were employed from October 2009 through May 2010. For the 3 years prior to the QAPI, only 18 % of the Holtz patients achieved the target P goal compared to 52% of Network patients. With the introduction of QAPI strategies, the quarterly percentage of patients within the P target increased from 25% to 88% with an average of 33% per month. In conclusion, significant improvement in P levels can be achieved in a pediatric dialysis unit, although sustainability is difficult. Patient education and motivational strategies that are ongoing and age appropriate are required.

Disclosure of Financial Relationships: nothing to disclose

## PUB226

**'Reverse Epidemiology' of Blood Pressure in Elderly Pre-Dialysis Patients** Moniek C. M. de Goeij,[1] Nynke Halbesma,[1] Dinanda J. De Jager,[1] Nora Voormolen,[2] Diana C. Grootendorst,[1] Elisabeth W. Boeschoten,[3] Friedo W. Dekker.[1] *[1]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands; [2]Department of Clinical Radiology, Leiden University Medical Center, Leiden, Netherlands; [3]Hans Mak Institute, Naarden, Netherlands.*

In dialysis patients and in the elderly population reverse epidemiology has been found, where low blood pressure is associated with higher mortality and high blood pressure has a survival advantage. This study aimed to investigate whether reverse epidemiology is already present before the start of dialysis, by analyzing the association between systolic (SBP) and diastolic (DBP) blood pressure and time to renal replacement therapy (RRT) in elderly (≥65 years) pre-dialysis patients.

Methods:

In the retrospective PREPARE cohort 547 incident pre-dialysis patients were included when referred to pre-dialysis outpatient clinics between 1999 and 2001. The clinical status of the patients was followed through medical charts until start of RRT, mortality, or end of study (1 January 2008). Patients were stratified for age into <65 years and ≥65 years. Cox regression for time to RRT was used (a) during first year, (b) conditional on not starting RRT the first year on pre-dialysis, and (c) total follow-up.

Results:

Time to RRT was decreased in elderly patients (n=240) with SBP <140 or ≥160 mmHg compared to patients with 140-159 mmHg, resulting in a U-shaped association; for first year, after first year, and entire follow-up. For DBP, only the patients with 70-79 mmHg had a decreased time to RRT compared to patients with 80-89 mmHg during the first year of follow-up; adjusted HR (95% CI) of 1.85 (0.99-3.46). After the first year of follow-up, patients with DBP <70 mmHg or DBP ≥100 mmHg had a faster start of RRT compared to patients with 80-89 mmHg; adjusted HR (95% CI) of 4.92 (1.75-13.86) and 4.30 (1.28-14.42) respectively.

Conclusion:

Indications of reverse epidemiology are already present in elderly pre-dialysis patients. In this patient group both low and high SBP and DBP decreased the time to RRT. Future research is necessary to find a biological explanation.

Disclosure of Financial Relationships: nothing to disclose

## PUB227

**Systematic Evaluation of Loss to Analysis in Randomized Controlled Trials in Chronic Kidney Disease** Aneet J. Deo,[1] Christopher H. Schmid,[2] Katrin Uhlig.[1] *[1]Division of Nephrology, Tufts Medical Center, Boston, MA; [2]Institute for Clinical Research and Health Policy Studies, Tufts Medical Center, Boston, MA.*

**Objective:** To systematically evaluate the treatment of missing outcome data for primary outcome analysis in randomized controlled trials (RCTs) of patients with chronic kidney disease (CKD) by assessing the amount and reporting of loss to analysis, evaluate the use of intention to treat (ITT) analysis and report the methods used to handle missing outcome data.

**Methods:** MEDLINE search for English-language reports of RCTs of adults with CKD (including dialysis and transplantation) published from January 1st, 2007 through December 31st, 2008. Loss to analysis was present if the number of participants included in the main pre-specified analysis of the primary outcome was less than the number randomized. ITT use was judged to be complete if the study included all randomized participants by their originally allocated groups in the analysis of the primary outcome

**Results:** We found 196 eligible RCTs. Twenty seven percent of these studies did not clearly describe a primary outcome. Sixty-seven (61%) of 110 studies with complete data on outcomes had loss to analysis, among which the median number of patients lost was 9%. Only 50% of the trials that claimed primary outcome analysis by ITT actually included all randomized participants in outcome analysis. The method of imputation for missing outcome data was only described in 4 (4%) of 110 studies.

**Conclusion:** The quality of reporting of trials in patients with CKD remains sub-optimal, especially with respect to reporting of missing outcome data, use of ITT analysis and outcome data imputation. This impedes critical appraisal of study quality. Explicit description of reasons for loss of outcomes data and of how this is handled is needed.

Disclosure of Financial Relationships: nothing to disclose

## PUB228

**Is Red Cell Distribution Width (RDW) Associated with Renal Function or Renal Anemia?** Mireille E. Emans,[1] Wouter W. van Solinge,[4] Karlijn L. van Rooijen,[4] Maarten Jan M. Cramer,[1] Pieter Doevendans,[1] Branko Braam,[3] Carlo A. Gaillard,[2] Johanneke L. Breeijen Den.[4] *[1]Cardiology, University Medical Center Utrecht, Utrecht, Netherlands; [2]Nephrology, VU University Medical Center, Amsterdam, Netherlands; [3]Division of Nephrology and Immunology, University of Alberta, Edmonton, Canada; [4]Clinical Chemistry and Hematology, University Medical Center Utrecht, Utrecht, Netherlands.*

RDW is associated with mortality in a community based cohort (PMID19307522) and in heart failure (HF). In HF, chronic kidney disease (CKD) and anemia correlate with RDW (PMID19781428). We studied determinants of RDW, focussed on renal function and renal anemia.

Data from the Utrecht Patient Oriented Database, an infrastructure of relational databases, were used. Of all adult patients of whom RDW was performed in 2008 as part of the hematology measurement, we ascertained data about renal function (MDRD), haemoglobin (Hb), immature reticulocyte fraction (IRF), reticulocyte count, leucocytes and C-reactive protein (CRP). Subpopulations were defined as: CKD (MDRD<60ml/min/m2), anemia (Hb<12.6 g/dL men, Hb<11.9 g/dL women) and both. Multiple linear regression models for RDW were generated using a stepwise selection process.

RDW was higher in anemia, CKD and both. Only in non-anemic patients MDRD was a weak predictor for RDW. Remarkably, IRF was the strongest predictor for RDW.

Markers for the degree of red cell turn over (IRF) rather than renal function (red cell production) predict RDW. These data may elucidate some of the mechanisms causing the association between RDW and risk.

Multilinear regression analysis for RDW

| | **all patients (n=5960)** | **CKD (n=1396)** | **anemia (n=835)** | **anemia and CKD (n=359)** |
|---|---|---|---|---|
| RDW (%, ± SD) | 12.5±1.53 | 12.9±1.67 | 13.9±2.07 | 13.9±2.30 |
| | **variable, $R^2$** | | | |
| Step no. | 1. IRF, 0.22 | 1. IRF, 0.22 | 1. IRF, 0.15 | 1. IRF, 0.16 |
| | 2. Hb, 0.29 | 2. Hb, 0.28 | 2. Hb, 0.28 | 2. MCV, 0.23 |
| | 3. MCV, 0.31 | 3. MCV, 0.30 | 3. MCV, 0.25 | 3. reticulocytes, 0.26 |
| | 4. age, 0.33 | 4. MDRD, 0.31 | 4. reticulocytes, 0.28 | 4. Hb, 0.30 |
| | 5. gender, 0.34 | 5. reticulocytes, 0.33 | 5. age, 0.29 | |
| | 6. MDRD, 0.35 | 6. age, 0.34 | 6. leucocytes, 0.31 | |

Disclosure of Financial Relationships: nothing to disclose

## PUB229

**Age and Gender Comparisons between 1,971 Patients Reaching Stage 5 CKD and 2,122 Patients Commencing RRT at 7 UK Renal Centers** Daniel Ford,[1,3] Dirk J. Van Schalkwyk,[1] David Ansell,[1] Charles Tomson,[1,2] Yoav Ben-Shlomo,[3] Damian G. Fogarty.[1,4] *[1]UK Renal Registry, Bristol, United Kingdom; [2]Southmead Hospital, Bristol, United Kingdom; [3]University of Bristol, Bristol, United Kingdom; [4]City Hospital, Belfast, United Kingdom.*

**Introduction** The UK Renal Registry (UKRR) has shown that the male to female ratio starting RRT is 1.6:1. Other studies have shown that this ratio is reversed (1:1.85) for patients with an eGFR <30. The aim of this study was to compare the gender and age differences of patients with CKD stage 5 (not on RRT) with those commencing RRT.

**Methods** All adult patients reaching CKD stage 5 (two eGFR results <15ml/min/1.73m², >90 days apart with no intervening result ≥15) during 2006-7 at 7 UK renal centers were included. Baseline data including: age, gender, ethnicity, renal disease and socioeconomic deprivation were extracted direct from the centers' IT systems. Results were compared with UKRR data from patients commencing RRT at the same centers during 2007-8.

**Results** 1,971 adult patients reached CKD stage 5 during 2006-7. 2,122 patients commenced RRT during 2007-8. The M:F ratio of patients reaching CKD5 was 1.29:1. This was lower than the ratio of patients starting RRT (1.62:1, p<0.001) (Figure 1).

The population reaching CKD5 was significantly older than the incident RRT population (median age 69.7 vs. 64.9 years, p<0.001) (Figure 2).

**Conclusion** These results show that there are both gender and age differences between patients reaching CKD stage 5 and those accepted on to RRT. There are a number of possible explanations for these differences including: differing rates of kidney function decline, starting RRT at different levels of function, differing mortality rates, differing conservative care policies and differing RRT acceptance policies and access. The reasons for these differences are under investigation.

Figure 1. Numbers of male and female patients in incident CKD5 and RRT cohorts

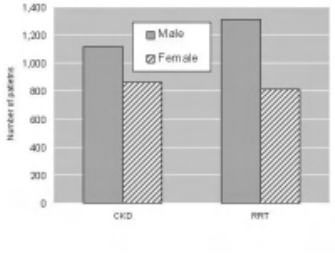
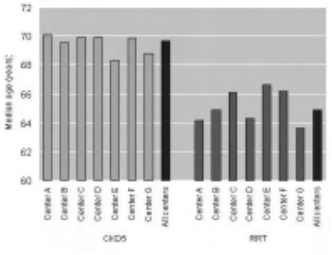



Figure 2. Median age of CKD5 and RRT patients by renal center




Disclosure of Financial Relationships: nothing to disclose

## PUB230

**Extent of Abdominal Aortic Calcifacation on X-Ray Predicts the Presence of Significant Coronary Artery Disease in Dialysis Patients** Arien Gaasbeek,[1] Mihaly K. De Bie,[2] Joanne D. Schuijf,[2] Ton J. Rabelink,[1] Martin J. Schalij,[2] Jeroen J. Bax,[2] J. Wouter Jukema.[2] *[1]Nephrology, Leiden University Medical Center; [2]Cardiology, Leiden University Medical Center.*

**Introduction:**

Coronary Artery Disease (CAD) is highly prevalent in asymptomatic dialysis patients. Given their poor prognosis identifying these patients is relevant. The extent calcified lesions in the aorta may correlate with the extent of atherosclerosis in the coronary arteries. The aim of this study was to evaluate the association of the extent of abdominal aortic calcification (AAC) as assessed on abdominal X-ray and the presence of significant CAD in dialysis patients.

**Methods:**

A total of 43 consecutive dialysis patients (23 male, avg. age 66 ± 8yrs), currently participating in the ICD2 trial with no history of CABG or PCI were included. The presence of significant CAD (≥50%luminal narrowing one of the major epicardial vessels) was assessed with CT-Angiography. The extent of calcification of the abdominal aorta was assessed on lateral X-ray and was graded using a previously validated grading system, resulting in a score ranging from 0-24 points.

**Results:**

Two patients were excluded from due to poor scan quality. Significant CAD was present in 15 (36.8%) patients. The extent of calcification in the abdominal aorta, assessed on X-ray, was significantly higher in patients with significant CAD (AAC-score 12.4 ± 4,9 vs. 5.4 ± 3.9 p<0,001). Using binary logistic regression controlling for age and gender the association of the AAC score and the presence significant CAD was assessed: every point increase in the AAC score was associated with an 1.4 (95% CI 1,1 - 1,8) fold higher chance of having significant CAD. ROC analysis of the AAC score resulted in an area under the curve of 0.87.

**Conclusions:**

The extent of calcification of the abdominal aorta is associated with the presence of significant coronary artery disease in dialysis patients. A simple lateral X-ray of the abdomen, on which the extent of calcification can be quantified, may therefore be a useful tool to identify dialysis patients at high risk for having significant coronary artery disease.

Disclosure of Financial Relationships: nothing to disclose

**PUB231**

**CKD-3 and CKD-4 Patients Have More Probability To Initiate Dialysis Than Die. MERENA Study (Morbimortality in Chronic Kidney Disease Patients). Final Results after Five Year Follow-Up** Jose L. Gorriz,[1] Alberto M. Martinez-Castelao,[2] J. M. Portolés,[3] Aleix Cases,[4] Jose Luno,[5] Juan F. Navarro,[7] Fernando Dealvaro-Moreno.[6] *[1]Servicio de Nefrologia, Hospital Universitario Dr Peset, Valencia, Spain; [2]Servicio de Nefrologia, Hospital Bellvitge, Barcelona, Spain; [3]Servicio de Nefrologia, Fundacion Hospital Alcorcon, Alcorcon, Madrid, Spain; [4]Servicio de Nefrologia, Hospital Clinic, Barcelona, Spain; [5]Servicio de Nefrologia, Hospital Gregorio Marañon, Madrid, Spain; [6]Servicio de Nefrologia, Hospital Infanta Sofia, Madrid, Spain; [7]Servicio de Nefrologia, Hospital Nra Sra de la Candelaria, Tenerife, Canary Islands, Spain.*

The MERENA study, is a 5-year follow-up prospective, observational, multicenter study aimed to assess renal disease and morbi-mortality in CKD patients stages 3 or 4.

**Patients** & **method**: Inclusion criteria : CKD-stage 3 or 4, life expectancy >1 year after inclusion. We included 1129 consecutive patients visited in the outpatient clinic of 54 Spanish Nephrology centres.

**Results:** After five year follow-up, 542 patients (37.9 %) reached the primary end–point of the study (need for renal replacement therapy or death) (16.1 % and 21.9 respectively). 182 patients dead (16.1%), 195 initiated hemodialysis (17.3 %), 44 peritoneal dialysis (3.9%), and 8 received an preemptive kidney transplant (0.7%). 22 patients were transferred to other unit (1.9%) and 91 were lost for follow-up (8.1%).

In non-diabetic CKD-3 patients mortality rate was 7.5 %, in diabetic CKD-3, 12.7 %, in non-diabetic CKD-4 22.2%, and in diabetic CKD-4, 24 % ($p<0.01$, log rank test). KM survival at five years was 90 %, 80%, 67% and 61% respectively in those groups.

Causes of death: cardio-vascular (47.2%), neoplasms (16.7%), infections (14.3%), hepatic or gastrointestinal diseases (3.8%) or other and unknown (18%).

**In conclusion**: After 5-year follow up, the MERENA study shows that CKD patients have high mortality rates, especially in diabetics CKD-4. In our patients, contrary to other studies, the probability of initiating renal replacement therapy was more frequent than the risk of dying.

Disclosure of Financial Relationships: nothing to disclose

**PUB232**

**Race/Ethnicity, Poverty, and the Association between Periodontal Disease and CKD** Vanessa Grubbs,[1] Deidra C. Crews,[5] Kirsten Bibbins-Domingo,[1] Elizabeth Hedgeman,[3] Rajiv Saran,[3] Priti Patel,[4] Kristina L. Ernst,[4] Neil R. Powe.[2] *[1]University of California, San Francisco, CA; [2]San Francisco General Hospital, CA; [3]University of Michigan, Ann Arbor, MI; [4]Centers for Disease Control and Prevention, Atlanta, GA; [5]Johns Hopkins University, Baltimore, MD.*

Recent studies suggest an overall association between chronic kidney disease (CKD) and periodontal disease, but it is unknown whether this association varies by race/ ethnicity or poverty status. We sought to examine this using 1999-2004 National Health and Nutrition Examination Survey data, which included11,400 dentate, adult participants (aged 21-75) with periodontal exams. CKD was defined as urinary albumin:creatinine ratio of >30 mg/g or estimated glomerular filtration rate of 15-59 ml/min/1.73 m2. Adjusted odds ratios (AORs) were calculated using multivariable logistic regression with U.S. population-based weighting. The estimated prevalence of moderate/severe periodontal disease was 4.6% among whites, 8.6% among blacks, and 5.5% among Mexican-Americans ($p<0.001$). CKD prevalence was 10.8% among whites, 12.6% among blacks, and 9.4% among Mexican-Americans ($p<0.001$). Periodontal disease was independently associated with CKD (AOR 1.51, 1.20-1.91) after adjusting for race/ethnicity, age, gender, hypertension, diabetes, education, and poverty index ratio (PIR). Although there were no statistically significant interactions between periodontal disease and race/ethnicity or poverty, subgroup analyses showed the most profound association of periodontal disease and CKD among the very poor (PIR<=1; AOR 2.38, 1.23-4.58) and, particularly, among very poor whites (AOR 3.15, 1.27-7.82). Further study is needed to understand whether periodontal disease is a CKD risk factor and, if so, whether targeted treatment could improve disparate CKD outcomes.

Disclosure of Financial Relationships: nothing to disclose

**PUB233**

**Low Dose Losartan/Benazepril Alone or Combination in Primary Glomerulonephritis with Proteinuria but Intolerance to Large or Regular Dose Renin-Angiotensin Antagonists** Qiang He, Lina Shao, Xiayu Li, Jianghua Chen. *Kidney Disease Center, the First Affiliated Hospital, Medical College, Zhejiang University, Hangzhou, Zhejiang, China.*

To observe the efficacy and safety of low-dose losartan / benazepril alone or combination in primary glomerulonephritis patients with proteinuria but intolerance to large or regular dose rennin-angiotensin antagonists. 27 patients were recruited during January 2007 to February 2009 in our hospital. All diagnoses were confirmed by renal biopsy, including 21 cases of IgA nephropathy, 6 cases of non-IgA mesangial proliferative glomerulonephritis. All patients were normal blood pressure, proteinuria <2.0g / d, eGFR> = 60ml/min, without the history of immunosuppressants application. All patients could not tolerate even though a single dose of ACEI or ARB before recruit. The patients were divided into 3 groups: A group :16 cases, as low dose losartan group (12.5-25mg / d); B group : 7 cases, as low doses benazepril group (2.5mg / d); C group: 4 cases, as low dose of losartan combined with low dose of benazepril group (losartan 12.5mg / d + Benazepril 2.5mg / d). All Patients were followed 6 months, The 24-hour urine protein, β2-microglobulin (MG), eGFR, mean arterial pressure (MAP) and symptom dizziness were evaluated each month. We found that 27 patients could well tolerate low dose losartan / benazepril alone or in combination therapy, After 6 months, Urinary protein were all significantly decreased: A group: 43.5% ($p = 0.01$), B Group: 36% ($p = 0.012$), C group: 56.3% ($p = 0.001$); 6 cases in A group of, 2 cases in B group and 2 cases in C group showed complete remission. The urinary β2- MG were also significantly decreased in all groups, A group: 68% ($p = 0.001$), B group: 63.7% ($p = 0.01$), C group: 75.2% ($p = 0.001$). The decline of urinary protein and β2-MG were not significant different among the 3 groups ($p> 0.05$; ANOVA). And the eGFR, MAP were not significant different during the follow-up. We made the conclusion that: The normotensive patients with primary glomerulonephritis who not tolerate high-dose or conventional dose ACEI / ARB , may be still effective and well tolerance by low-dose losartan / benazepril alone or combination therapy.

Disclosure of Financial Relationships: nothing to disclose

**PUB234**

**Effect of KDOQI Guidelines on Patient Awareness of CKD** Tarundeep Kaur,[1] Ajay Singh Behl.[2] *[1]Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN; [2]HealthPartners Research Foundation, HealthPartners, Minneapolis, MN.*

**Purpose of study:** To test awareness of CKD among patients after introduction of the KDOQI guidelines.

**Background:** End Stage Renal Disease (ESRD) is the most public form of Chronic Kidney Disease (CKD). Studies suggest that clinical practice guidelines of the National Kidney Foundation Disease Outcomes Quality Initiative (NKF KDOQI) ™ formed in 1997 and the relation of CKD with Cardio-Vascular Disease (CVD) have increased the recognition of CKD amongst primary physicians. Two studies based on the National Health and Nutrition Examination Survey (NHANES) reported low awareness amongst patients regarding their CKD status.

**Significance and Hypothesis:** The studies based on NHANES were done using the 1999-2000 dataset, which was soon after the KDOQI guidelines were issued for the first time. We believe that awareness among patients is representative of the communication by the physician and the patient. We test this by analyzing the self-reported awareness amongst patients regarding their Chronic Kidney Disease (failing or weak kidneys) in the NHANES 2005-2006 dataset.

**Methods:** NHANES 2005-2006 data is used. We use complex survey design based multivariate logit regression with the awareness levels as the dependent variable and lab values and socioeconomic variables as the explanatory variables.

**Results**: There were 4965 respondents who replied either a yes or a no to the question regarding awareness about their having failing or weak kidneys. 136 (2.7%) of them reported being told that they had failing or weak kidneys. Of the total respondents 383 (93.73%) had CKD stage 3, 24 (4.26%) had CKD stage 4 and 11 (2.01%) had stage 5. Of these, 354/383, 13/24 and 1/11 were unaware about their CKD. Compared to the findings from the 1999-2000 datasets, the awareness did not increase.

**Conclusions**: We need to rethink and improve the communication and dissemination techniques regarding clinical guidelines for CKD management. Unless the patients know about their kidney disease, the guidlines will not be helpful in imparting education and taking measures to decrease the progression of the disease at the primary care level.

Disclosure of Financial Relationships: nothing to disclose

**PUB235**

**Distance-Counselling on Drug Dosage Adjustments (DDA) in Patients with Renal Insufficiency: The 10-Year Experience of "Service ICAR" in France** Vincent Launay-Vacher, Sarah Zimner-Rapuch, Nicolas Janus, Sabine Amet, Gilbert Deray. *Service ICAR Department of Nephrology, Pitie Salpetriere Hospital, Paris, France.*

**Background**: Drug Dosage Adjustment (DDA) in renal insufficiency (RI) is crucial to avoid overdosage from non-adjustment or underdosage from empirical dose reduction. All drugs may require dosage adjustment, even if the main elimination pathway is non-renal. Several studies indeed reported the need for DDA for drugs that are hepatically metabolized, due to reduced metabolism from interactions with uremic toxins and enzyme activity or hepatocyte transport. The "Service ICAR", founded in 1999, provides physicians with advices on DDA, based on evidence from the international literature. A dedicated team of physicians and pharmacists (5 full-time) prospectively searches and analyzes the literature to elaborate recommendations. Responses include specific drug pharmacokinetic considerations, subsequent DDA and schedule, adverse effects, specific monitoring in case of overdosage and data on potential drug transfer through the dialysis membrane and consequent optimal time of administration.

**Results**: From March 1999 to December 2009, 17'847 requests have been carried out, on 970 different drugs. Questions were asked by 3'398 physicians, mainly nephrologists, but also infectiologists, hematologists, and oncologists, for 8'758 patients. Patients were on hemodialysis in 56.3%, peritoneal dialysis in 4.2%, non dialyzed in 37.9% and on continuous veno-venous hemodialysis in 1.6%. Anti-infectious agents (43%) and anti-cancer agents (29%) were the drugs most frequently involved.

**Conclusion**: The activity of our Service ICAR shows that drugs' handling in RI patients is a critical clinical problem, especially in dialysed patients. From these data, it is suspected that in the general population, where fine renal function evaluation is usually not performed, the risk for drug inappropriate dosage and the need for DDA is also critical. Our Service ICAR is currently developing a website on which clear data will be available, and access restricted to health care professionals, physicians and pharmacists.

The Service ICAR receives unrestricted educational grants from Roche and Gilead.

Disclosure of Financial Relationships: nothing to disclose

## PUB236

### C.E.R.A. Efficiently Corrects and Controls HB in Chronic Kidney Disease Patients Not on Dialysis Independent from Pre-Treatment and Specified Administration Aspects – Analysis of the Non-Interventional Study SUPRA

Frank Leistikow. *Nierenzentrum Mannheim, Mannheim-Käfertal, Germany.*

**INTRODUCTION AND AIMS:** The SUPRA trial was designed to investigate the use of once-monthly (QM) C.E.R.A. in every day clinical practice in anemic chronic kidney disease (CKD) patients (pts) not on dialysis without the constraints of an interventional protocol. The study investigated if C.E.R.A. corrects and maintains Hb in defined targets.

**METHODS:** This non-interventional study (NIS) was conducted in 46 German centers. Data of 335 pre-dialysis pts treated with QM C.E.R.A. were collected over a 9-month-treatment period. Analysis of the collected data were performed in a descriptive way.

**RESULTS:** 335 pts were treated and formed the safety population. We present data from 259 (77.3%) pts from whom at least one Hb measurement was available in month 7-9 (evaluation period) . Pts who had been previously switched (n=124) from frequent-applicable ESA to C.E.R.A., and pts who had been started *de novo* on C.E.R.A. (n=125) were included. Main reason for CKD was hypertensive nephrosclerosis (38.6%). Baseline Hb was 10.2 ±0,9 g/dL in ESA naive pts and 11.3±1.0 g/dL in ESA pretreated pts. At study end Hb had been 11,3 ±0,7 g/dL. In month 7-9 43,6% and 68,6% of ESA-pretreated and 45,9% and 69,6% of ESA naive pts had all measured Hb values in the ranges of 10-12/10-13 g/dL. 76 pts had been treated with C.E.R.A. with a frequency > QM with Hb values in the above mentioned ranges of 43,4% and 67,11%. From the 147 at home treated pts 45,6% and 70% reached the described Hb-targets. In month 7-9 the hemoglobin fluctuation < = 1g/dl had been 91,9% in ESA pre-treated pts and 92,61% in ESA naive pts. 28,2%, 32%, 21,6%, 3,5%, 10%, 2,3% and 2,3% required <= 50, <= 75, <= 100, <= 120, < = 150, <=200 and >200 µg C.E.R.A. per month during month 7-9. There were 13 pts experiencing adverse drug reactions during the study.

**CONCLUSIONS:** Data collected within this NIS on CKD pts exposed to C.E.R.A. therapy indicate good efficacy on a once monthly and > once monthly schedule, administered at practice and at home, in ESA naive and in ESA treated pts and exhibit a low incidence of drug-related events.

Disclosure of Financial Relationships: nothing to disclose

## PUB237

### Renin-Angiotensin System Blockade Contributed to Severe Hyponatremia in the Outpatients without Diuretics

Jun Matsuda,[1] Ryohei Yamamoto,[2] Daisuke Ito,[1] Daisuke Mori,[1] Hiroyuki Kadoya,[1] Hisako Murata,[1] Masanobu Takeji,[1] Yasuyuki Nagasawa,[2] Yoshitaka Isaka,[2] Hiromi Rakugi,[2] Atsushi Yamauchi.[1] *[1]Nephrology, Osaka Rosai Hospital, Sakai, Osaka, Japan; [2]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Hyponatremia (hypoNa) is one of the most common electrolyte disorders in clinical practice. However, little information on the contributors of hypoNa is available except older age and use of diuretics, which were exclusively assessed in the hospitalized patients. The aim of the present study was to identify the contributors to hypoNa in the outpatients without diuretics.

Design: A single-center cross-sectional study. Patients: Medical records of 20964 outpatients who visited Department of Internal Medicine, Osaka Rosai Hospital between April 2007 and November 2009 were assessed. Among a total of 114 patients with severe hypoNa (sNa≤125 mEq/L), 61 patients received no diuretics at the onset of hypoNa (hypoNa group), who were compared with 61 age- and sex-matched patients without diuretics during the study period (normal group). Outcome: HypoNa (sNa≤125 mEq/L). Independent variables: BMI, BP, eGFR, serum albumin, use of renin-angiotensin system (RAS) blockade and calcium channel blockade, and past histories of diabetes (DM), cardiovascular diseases (CVD), and hepatic diseases. Statistics: multivariate conditional logistic regression models

Results: Clinical characteristics between hypoNa and normal group were as follows; age 71±13 vs. 70±12 yr (mean±SD), male 65.6 vs. 65.6%,BMI 20.2±3.8 vs. 23.1±2.7 kg/m2, serum albumin 3.6±0.7 vs. 4.2±0.4 g/dL, systolic BP 130±17 vs. 135±14 mmHg, eGFR 81 (61-109) vs. 69 (57-82) mL/min/1.73m2 (median (interquartile range)), DM 23.0 vs. 27.9%, CVD 16.4 vs. 23.0%, hepatic disease 9.8 vs. 4.9%. Multivariate analyses revealed that RAS blockade was significantly associated with hypoNa (odds ratio 8.93 [95%CI 1.38 - 57.9], P = 0.022), along with BMI (0.67 [0.51 - 0.88], P = 0.004), serum albumin (0.03 [0.00 - 0.29], P = 0.002), and past history of CVD (9.53 [1.03 - 87.9], P = 0.047).

Conclusion: RAS blockade contributed to hypoNa, besides BMI, serum albumin, and past history of CVD.

Disclosure of Financial Relationships: nothing to disclose

## PUB238

### CKD in Primary Care: Baseline Data from the Renal Risk in Derby ($R^2$ID) Study

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**INTRODUCTION:** Most patients with CKD3 are managed in primary care but there are few data regarding their characteristics. Previous studies have largely excluded patients >75yrs, who make up a large proportion. We undertook a study of these patients with the long-term goal to define risk of cardiovascular disease (CVD) and CKD progression.

**METHODS**: Participants with KDOQI defined CKD stage 3 were recruited from 33 Primary Care Practices between July 2008 and March 2010. Adult patients of any age were invited. Medical history and demographic data were obtained as well as a clinical assessment including urine and serum biochemistry tests.

**RESULTS**: 1741 participants were recruited: mean age of 73(±9)yrs; 60%(n=1052) female; 98%(n=1698) white; 17%(n=294) had diabetes;17%(n=293) had microalbuminuria; 76%(n=1329) had CKD3a; 65%(n=1123) used ACEi/ARB; 22%(n=387) had a history of CVD. Baseline data and analysis of clinically relevant subgroups are shown below.

Baseline data

| | Total n=1741 | No Diabetes n=1447 | Diabetes n=294 | <75yrs n=917 | ≥75yrs n=824 |
|---|---|---|---|---|---|
| BMI (± SD) | 29 ±5 | 28.6 ±5 | 31.1 ±6* | 29.7 ±6 | 28.3±5* |
| SBP (mmHg; ± SD) | 134 ±18 | 134 ±18 | 135 ±19 | 131 ±17 | 137 ±19* |
| DBP(mmHg; ± SD) | 73 ±11 | 74 ±11 | 68 ±10* | 75 ±11 | 71 ±11* |
| PWV (m/sec; mean ± SD) | 9.9 ±2.0 | 9.8 ±2.0 | 10.3 ±2.0* | 9.2 ±1.7 | 10.6±2.1* |
| UACR (mean ± SD) | 4.6 ±33 | 3.2 ±25 | 11.6 ±57* | 5.7 ±44 | 3.5 ±13 |
| eGFR (mL/min/1.73m)² | 53 ±10 | 53 ±10 | 49 ±10* | 55±10 | 50 ±10* |
| Hb (g/dL) | 13.2±1.5 | 13.3±1.4 | 12.6±1.5* | 13.4±1.5 | 13 ±1.4* |

ΔAlbuminuria >microalbuminuria threshold. *p=<0.05 vs comparitor group.

**CONCLUSION**: Patients with CKD in primary care, in Derbyshire, are mainly elderly, female and overweight or obese. The majority fall into CKD stage 3a and significant albuminuria uncommon. BP was well controlled but PWV was elevated. Sub-group analysis revealed those with CKD 3b, diabetes and those >75 yrs had a higher risk profile. 10 yr follow-up will afford a unique opportunity to study CKD 3 and its consequences in patients often excluded from other studies, and will provide an important comparison with CKD cohorts recruited from secondary care, including the CRIC study.

Disclosure of Financial Relationships: Research Funding: Research funding from Roche; Honoraria: Chairing honoraria from LINC medical.

## PUB239

### Renal Granulomatous Sarcoidosis: A Long Term Follow Up of 33 Patients

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Sarcoidosis is a systemic inflammatory disease of unknown pathophysiology. Renal failure can occur by several mechanisms, but the most typical is granulomatous interstitial nephritis.

We present a retrospective series of 33 patients (pts, 23 men), mean age 60+/-13 y, with histological proven granulomatous interstitial nephritis due to sarcoidosis. We studied clinical and biological parameters at presentation and during follow-up (mean 69 ± 54 months) and compared long-term renal outcomes in 2 groups of pts: 17 pts treated with methylprednisolone pulses (MP) followed by oral corticosteroids (CS) and 16 pts on oral CS alone. Adverse effects of steroids were also recorded.

At presentation, mean eGFR was 19±14 mL/min/1.73m², mean proteinuria 1.1g/d, microhematuria and leucocyturia were found in 50% pts. Ten pts had hypercalcemia. Transient hemodialysis was necessary in 3 cases. Extra-renal manifestations were: fever 20%, chest abnormalities 79%, cholestasis 36%, lymphopenia 90%, and elevated ACE 61%. All pts received oral prednisone 0.5-1.0 mg/kg/d for 2 months, then progressively tapered. Renal function improved with a mean eGFR increase of 19.5ml/min/1.73m2 with maximal response at the end of the first month of treatment. At the end of follow up, mean eGFR was 41±16 mL/min/1.73m². Pts who were treated with MP + oral CS had a better renal outcome compared to those treated by oral CS without MP (Δ eGFR: +27±15 mL/min/1.73m² *vs* Δ eGFR: +14±7 mL/min/1.73m²; p=0.04). Twenty four percent of pts had a renal relapse, either during CS tapering or after their withdrawal. CS side-effects were observed in 45% pts: hypertension 21%, diabetes 24%, cardiovascular events 15% (3 deaths), infection 18% and osteoporosis 18%.

Renal sarcoidosis is characterized by severe renal failure and systemic manifestations. Better long-term renal outcomes are obtained with MP followed by oral CS than with oral CS alone. The high incidence of steroid-adverse events emphasizes the need for steroid-sparing regimens.

Disclosure of Financial Relationships: nothing to disclose

**PUB240**

**Effects of Mono- and Dual Blockade of RAS on Escape Phenomenum** Takanori Nagai,[1] Kahori Hori,[1] Yushi Nakayama,[2] Yukiko Hasuike,[1] Yuichiro Izumi,[3] Masayoshi Nanami,[1] Takahiro Kuragano,[1] Yoshinaga Otaki,[1] Hiroshi Nonoguchi,[1] Takeshi Nakanishi.[1] *[1]Division of Kidney and Dialysis, Dept of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [2]Nephrology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan; [3]LKEM, NHLBI/NIH, Bethesda, MD.*

The renin-angiotensin-aldosterone system (RAS) blockers have renoprotective effects in proteinuric CKD patients. However, rebound increase of proteiuria after 3-4 years' treatment with ACE inhibitors (ACE-I) is known as an escape phenomenunon. We retospectively examined whether monotherapy with ARB or dual blockade with ARB plus ACE inhibitor cause rebound increase in proteiuria in CKD patients. 54 CKD patients (mean age of 58 years old, 32 IgA nephropathy, 17 diabetic nephropathy, 5 non-IgA nephropathy) with initial decrease in proteinuria (larger than 50 %) by the treatment with RAS blckers were selected and the presence/absence of rebound increase in proteiuria (larger than initial level of proteinuria) was examined. Basal proteinuria was 1.4 and 1.9 g/day in ARB (n=23) and ARB/ACE-I (n=31) treated patients, respectively. Peak reduction of proteinuria was observed at 18 and 23 months after the start of the treatment in patients treated with ARB alone and dual blockade, respectively. Proteinuria was reduced to 22±10 (mean±SD) and 15±12% of the basal level by ARB and ARB/ACE-I, respectively. Rebound increase in proteinuria was observed in 7 (30%) patients with 16 months after the start of the treatments in ARB treated patients. In contrast, rebound increase in proteinuria was seen in 4 (13% ) with 26 months after the start of the treatment in dual blockade with ACE-I and ARB. In remaining patients, rebound increase in proteinuria was not oberved during 51 and 49 months follow-up in ARB and ARB/ACE-I, respectively. These data show that 1) rebound increase in proteinuria was observed in patients treated with ARB or ARB plus ACE-I, and 2) dual blckade with ARB and ACE-I should be considered to reduce the risk of rebound increase in proteinuria in patients with CKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB241**

**Urine Cotinine in Adolescents with Chronic Kidney Disease: A Report from the Chronic Kidney Disease in Children (CKiD) Cohort Study** Abiodun A. Omoloja,[1] Judith Jerry-Fluker,[2] Alison G. Abraham,[2] Derek Ng,[2] Bradley A. Warady,[2] Susan L. Furth,[2] Mark Mitsnefes.[3] *[1]Pediatrics, Wright State University, Dayton, OH; [2]The CKiD Study Investigators; [3]Cincinnati Children's Hospital Meidcal Center, Cincinnati, OH.*

Second hand smoking (SHS) has been associated with cardiac disease. The goal of this study was to determine the relationship between exposure to SHS and clinical and laboratory parameters of adolescents at baseline in CKiD, an observational cohort study of 586 children (aged 1-16 yrs) with Schwartz estimated GFR of 30-90 ml/min/1.73m$^2$. Urine cotinine concentration [Uc] was used to determine exposure to SHS.

Of the 239 adolescents who had self-reported data on smoking and SHS exposure, 149 had Uc data available. Seven subjects were excluded because they had Uc levels ≥ 75 ng/ mL consistent with active tobacco use. In the remaining 142 subjects, Uc ≥ 1 ng/ mL was considered as evidence of exposure to SHS. Analysis was performed using Fisher's Exact Test for categorical variables and Wilcoxon Rank Sum scores for continuous variables.

There were 39 subjects (27%) exposed to SHS and 103 who were not. There was no racial, age, or gender differences between both groups. Baseline wide range C reactive protein, lipid profile, iohexol GFR and hemoglobin were not statistically different between groups. The group exposed to SHS had a higher median SBP percentile (70%) compared to unexposed group (50%) [P < 0.01]. Those exposed to SHS had a higher prevalence (29%) of left ventricular hypertrophy compared to 10% in those not exposed [P = 0.04]. Nephrotic range proteinuria (Up/c ≥ 2.0) was more prevalent in children exposed to SHS (34%) in comparison to 10% in those not exposed [P < 0.01].

These results suggest that in non-smoking adolescents with CKD, SHS exposure, as measured by Uc, may be an unrecognized variable that may adversely affect CKD progression and cardiovascular outcome.

Disclosure of Financial Relationships: nothing to disclose

**PUB242**

**Is Vitamin D Deficient in the Sunshine State of Florida?** Umabala Pasupala, Rute C. Paixao, Mauro Braun, Dianne T. Sandy, Beth L. Fromkin, Noelle Barrera. *Nephrology and Hypertension, Cleveland Clinic Florida, Weston, FL.*

Vitamin D deficiency has been increasingly recognized as an important public health problem and has been associated with multiple extra skeletal functions. One of the major risk factors for vitamin D deficiency is Chronic Kidney Disease (CKD). Recent studies have been challenging the assumption that vitamin D deficiency may be less prevalent in people living in sunny climate; however limited data is available so far.

To verify the prevalence of Vitamin D deficiency in sunny areas and its association with other co-morbidities, we conducted a cross sectional descriptive study at Cleveland Clinic Florida on patients with CKD stage III to V from spring 2008 to winter 2009. Patients with malignancy-related hypercalcemia, secondary hyperparathyroidism, multiple myeloma, Sarcoidosis, or Paget's disease were excluded.

2010 patients admitted to Internal Medicine and its subspecialties were screened, from those 97 with eGFR < 60 (MDRD equation) met the inclusion criteria, but 11 were excluded due to acute renal failure, and 2 due to unavailable Vit D levels. The remaining 84 patients were checked for 25-hydroxy vitamin D, 1,25-dihydroxy vitamin D, and intact parathyroid hormone levels. According to KDOQI guidelines, 25-OH vitamin D levels below 30 ng/ ml were considered to be insufficient, and below 15 ng/ml as deficient.

Hypovitaminosis D was prevalent in 70% of the study group (52% vitamin D insufficient and 18% vitamin D deficient). The mean 25-OH Vit D level was 24.7 ng/ml. The majority of patients were CKD stage 3, and 75% of those were vitamin D deficient. Also hypovitaminosis D showed significant association with Diabetes Mellitus (p= 0.03), and some association with anemia (p=0.437), and with cancer (p= 0.221).

In conclusion, Vitamin D deficiency is highly prevalent in CKD patients admitted to a South Florida Hospital and is significantly correlated with Diabetes Mellitus. Routine monitoring of vitamin D levels in early stages CKD and in diabetes can be beneficial even in sunny areas. Larger multi-center population-based studies are needed to verify the prevalence and outcomes of vitamin D deficiency.

Disclosure of Financial Relationships: nothing to disclose

**PUB243**

**Association of CHF and LVH with Mortality in Men with Moderate-to-Advanced Non-Dialysis Dependent Chronic Kidney Disease** Jason J. Payne,[1,2] Smriti I. Sharma,[1,2] Dexter G. De Leon,[1,2] Jun Ling Lu,[3] Fregenet A. Alemu,[2] Rasheed A. Balogun,[4] Sandra M. Malakauskas,[2] Kamyar Kalantar-Zadeh,[5] Csaba P. Kovesdy.[2,4] *[1]Carilion Clinic, Roanoke, VA; [2]Salem VA Medical Center, Salem, VA; [3]Salem Research Institute, Salem, VA; [4]University of Virginia, Charlottesville, VA; [5]Harbor-UCLA, Torrance, CA.*

The interrelationship of left ventricular hypertrophy (LVH) with congestive heart failure (CHF) and their impact on mortality in non-dialysis dependent CKD (NDD-CKD) is unclear.

We examined associations of echocardiographycally measured ejection fraction (EF) and LVH with all-cause mortality in a historic cohort of 650 men with moderate-to-advanced NDD-CKD (mean eGFR: 37±21 ml/min/1.73m2). EF and LVH were examined separately and after categorizing patients by combining their EF and presence/absence of LVH. Associations with mortality were examined in Cox models with adjustments for potential confounders.

EF <50% was associated with higher all-cause mortality (hazard ratio, 95%CI: 1.61 (1.21-2.15), p=0.001) even after adjustment for age, race, comorbidities (including coronary artery disease), smoking status, blood pressure, eGFR, albumin, bicarbonate, cholesterol, calcium, phosphorus, hemoglobin, white blood cell count and percent lymphocytes in white blood cell count, proteinuria and medication use. LVH in itself was not associated with mortality (0.95 [0.72-1.25], p=0.7), but the presence of LVH combined with an EF<50% was associated with a 2.4-fold greater death risk after multivariable adjustments (Table).

Mortality associated with CHF and LVH

| | EF>50%, No LVH Reference Group | EF>50%,LVH | EF<50%, No LVH | EF<50%, LVH |
|---|---|---|---|---|
| Unadjusted | 1 | 0.92 (0.73-1.17) | 1.38 (1.08-1.75) | 1.33 (0.96-1.85) |
| Case-mix adjusted | 1 | 0.91 (0.67-1.22) | 1.32 (0.97-1.79) | 1.24 (0.83-1.89) |
| Case-mix + labs + BP adjusted | 1 | 0.97 (0.86-1.39) | 1.33 (1.0-2.06) | 2.38 (1.41-4.02) |

HR, 95%CI

Low EF is associated with higher mortality in patients with NDD-CKD. In the presence of a low EF, LVH is also associated with higher mortality. Clinical trials are needed to determine if interventions targeting patients with low EF and LVH can lower mortality in NDD-CKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB244**

**The Clinical and Imaging Presentation of Acute "Non-Complicated" Pyelonephritis** Giorgina B. Piccoli, Valentina Consiglio, Stefania Scognamiglio, Rossella Attini, Maria Chiara Deagostini. *SS Nephrology, ASOU San Luigi, Torino, Italy.*

**Background.** Acute pyelonephritis (APN) is differently defined according to imaging data (CT scan or Magnetic resonance) or clinical criteria (tetrad of fever, costovertebral pain, lower urinary tract symptoms, positive urinary cultures). In adults updated information on the relationship between imaging and clinical data is lacking.

**Aim**: to analyze the relationship between the clinical and imaging presentation of APN, defined according to imaging criteria (CT or Nuclear Magnetic Imaging), in a single setting where patients undergo routine imaging studies.

**Methods.** All consecutive patients hospitalized for "non-complicated" (primary, with the exclusion of local or systemic predisposing factors) APN in our institution in June 2005-December 2009 were considered. Clinical, biochemical and imaging data at hospitalization were analyzed by univariate and logistic regression analysis (SPSS vers 17.0). The main outcome variable considered was the presence of abscessed lesions.

**Results**. There were 119 patients, all females, median age 32 years (15-72). Non typical presentations were common. At hospitalization, fever was absent in 6.7%, pain in 17.8% and lower urinary tract infection symptoms in 52.9%; 42% had taken antibiotics in the previous 3 days; the median interval before referral was 3 days. Inflammatory markers were elevated (CRP median: 12.1 mg/dL (0.2-39); WBC mean 12528±4127). Urinary and blood cultures were positive in a minority of cases (23.5%; 12.6%). Lesions were bilateral in 12.6%, multiple in 79.8%; abscesses were present in 39.5%. Renal scars were present at diagnosis in 18 patients (15.1%), prosumably sign of previous APN. APN was however undiagnosed in 15/18. No presenting sign/symptom discriminated between small lesions, abscesses or multifocal involvement, with the exception of positive cultures, correlated with multiple infectious foci (multivariate OR 4.2; CI 1.139-15.515).

**Conclusions.**
The presentation of APN is protean, with high prevalence of non typical oligosymptomatic presentations. In the absence of clinical or biochemical markers, imaging studies are required to assess the severity of kidney involvement.
Disclosure of Financial Relationships: nothing to disclose

**PUB245**

**Adherence to K/DOQI Practice Guidelines for Bone Metabolism and Disease in Patients with CKD in an Academic Internal Medicine Clinic** Milap Pokharel. *Department of Internal Medicine, Saint Joseph Mercy Hospital, Ann Arbor, MI.*

Chronic kidney disease (CKD) is associated with a variety of bone disorders and disorders of calcium and phosphorus metabolism. Elevated plasma parathyroid hormone (PTH) levels have been linked to adverse clinical outcomes which can be prevented through early detection and treatment. Guidelines recommend checking serum calcium, phosphorus and intact PTH in all patients with CKD stage 3 and higher plus vitamin D levels in patients with elevated PTH. The aim of our study was to assess the adherence to above guidelines in an Academic Internal Medicine (AIM) clinic.

Among all patients 18 years or older that visited AIM clinic between September 1, 2006 and August 31, 2008, a random sample of 400 eligible patients was included in the study based on a sample size calculation. A retrospective chart review was done and specific variables such as age, sex, calcium levels, phosphorus levels, PTH, vitamin D were collected and analyzed.

51% were women, 56% were white and the mean age was 43.7 years. 315(78.75%) had a glomerular filtration rate (GFR) evaluation. 169 of the 315 patients who were tested had a previous GFR measurement. 31(8%) patients of the total sample had a GFR below 60. Of the 31 patients, 5 (1.58%) did not have a previous test result reported, 14 (4.44%) had an abnormal GFR on both measurements and 12 (3.8%) of the patients with abnormal GFR did not have a GFR below 60 on the previous measurement.

Of 31 patients with an abnormal GFR on the index visit, 29 (94%) had serum calcium evaluated and 20 (65%) had a serum PO4 reported. 4 (13%) had both PTH and 25OH-vitamin D level reported. Of the 14 with confirmed CKD by definition, all had corrected serum calcium, 9 (64%) had a PO4, and 3 (21%) had both PTH and 25OH-vitamin D levels reported.

Our study shows that adherence to guidelines related to bone metabolism is not adequate in the AIM clinic. This study will increase physicians awareness regarding the management of bone metabolism in patients with CKD, and remind them that current practice falls short of meeting the guidelines.
Disclosure of Financial Relationships: nothing to disclose

**PUB246**

**The Slight Increase in Urine Albumin and beta 2-Microglobulin Are Independently Related with Blood Pressure in General Japanese Population: The Takahata Study** Kazuko Suzuki, Ami Ikeda, Yusuke Mashima, Kazunobu Ichikawa, Satoshi Takasaki, Tsuneo Konta, Isao Kubota. *Department of Cardiology, Pulmonology, and Nephrology, Yamagata University School of Medicine, Yamagata, Japan.*

**Background:** Essential hypertension is a multifactorial disorder and a risk factor for renal failure and cardiovascular diseases. Recently it is advocated that subtle acquired renal injury such as renal microvascular and tubulointerstitial damage evokes salt-sensitive hypertension. The objective of this study is to examine the relation between blood pressure and these renal abnormalities in general population.

**Methods:** The participants of this community-based, cross-sectional study were 1,965 subjects over 40 years old, without antihypertensive medication or renal insufficiency. Urine albumin-creatinine ratio (UACR) and beta 2 microglobulin-creatinine ratio (UBCR) obtained from single spot urine were used as markers of renal microvascular and tubulointerstitial damage, respectively.

**Results:** Multiple linear regression analysis showed a significant positive correlation between systolic blood pressure and UACR, and UBCR, but not estimated glomerular filtration rate. In multiple logistic regression analysis the increase in UACR and UBCR levels were independently related with hypertension after adjustment with possible confounders and slight increases of UACR (5-9 mg/g) and UBCR (100-199 μg/g) showed a significantly higher risk for hypertension, compared with UACR <5mg/g and UBCR <100μg/g, respectively. Furthermore the positive relation between urine sodium excretion and blood pressure was observed in subjects with low UACR and all UBCR levels, but not high UACR levels (≥5 mg/g).

**Conclusions:** This study revealed that the slight increases in urine albumin and beta 2-microglobulin were independently related with blood pressure in general Japanese population. These renal abnormalities might differently affect the development of hypertension.
Disclosure of Financial Relationships: nothing to disclose

**PUB247**

**Lupus Nephritis in the Elderly** Tanyarat Teerapornlertratt. *Department of Medicine, Faculty of Medicine, Siriraj Hospital, Bangkok, Thailand.*

**Background** The patients are now getting older as well as SLE patients. We describe the prevalence, clinical manifestations and prognosis in lupus nephritis patients of ≥ 50 years.

**Material and Methods** Thirty lupus nephritis patients of ≥ 50 years were analyzed retrospectively for 17 years.

**Results** Of 30 patients (male 7, female 23) the average age was 56.6 ± 4 years (range 50-72 years). Follow up period was 25.8 months, the major extrarenal manifestations were anemia (39.3%) and musculoskeletal (33.3%). Renal manifestations were hypertension 66.7%, nephrotic range proteinuria 62.9%, azotemia 41.3%. Renal biopsy revealed LN WHO type 4 for 63.3%, the overall probability of one year survival was 94.1%, 3 years for 68.6%. The cause of death was infection.

**Conclusion** The elderly LN patients who presented with arthritis, anemia and renal involvement were not uncommon in ≥ 50 years. The prompt diagnosis and treatment will rescue and prolong good quality of life in these patients.
Disclosure of Financial Relationships: nothing to disclose

**PUB248**

**Management of CKD and Clinical Guidelines in France: Results of PREPARE Study** Malik Touam,[1] Paul Stroumza,[2] Eric Daugas,[3] Bertrand Dussol,[4] Laurent Juillard,[5] Patrick Henri.[7] *[1]AURA, Paris, France; [2]Résidence du Parc, Marseille, France; [3]Hôpital Bichat, Paris, France; [4]Hôpital de la Conception, Marseille, France; [5]Hôpital E.Herriot, Lyon, France; [6]Hôpital Clemenceau, Caen, France.*

In this transversal study we analysed the quality of CKD management before dialysis or transplantation in France. During the week 23- 27 nov. 2009, 2190 CKD pts (GFR <60 ml/min/1,73 m², without history of dialysis or transplantion) were included by 301 nephrologysts. The characteristics of 2089 pts by CKD stage (119 excluded, missing datas or GFR>60) are:

| | st III | st IV | st V | Total+st II |
|---|---|---|---|---|
| N-median age,yrs | 688-73 | 746-73.5 | 314-69 | 2089-72 |
| Sexe F-Diabetes,% | 39.2-35.2 | 40.9-41.6 | 42.7-36 | 40.6-36.9 |
| IC,HF,St*,% | 38.1 | 46.5 | 37.9 | 39.3 |
| CKD vintage-Nephrol referral, months | 40-16 | 44-19 | 61-22 | 43-18 |
| Hypertension>130/80 mm Hg,% | 39.9 | 46.9 | 49.9 | 62.2 |
| HbA1c>8%,% | 17.6 | 19.5 | 16.9 | 18.9 |
| RAS blockers-Statines,% | 73.1-56.4 | 70.6-57 | 65.9-51.6 | 70-55.2 |
| Vit D-Ca salts-P binders, % | 28-9.6-1 | 39-24.5-6 | 55-42.4-20 | 35-19.6-5.6 |
| ESA,% | 12.9 | 34.6 | 55.1 | 26.1 |
| Hbs vaccine-AVF creation,% | - | 31.3-17.4 | 46.1-35.8 | - |

*IC ischemic cardiopathy, HF heart failure, St stroke

Hypertension was found in 87.7% and treated in 84.9% of cases. The dyslipidemia was present in 53.2% of cases, with a median of LDL> 2.70 mmol/l (st III to V). At st V, iPTH was <150 (25%),>300 (37.9%) and >600 pg/ml (7%). At st IV-V, Hb target [10-12g/dl] was found in 52.8% of pts. The nephrology referral is a late at st IV-V. At st V the monitoring was monthly (71.7%) and quarterly (25.3%). Age and cardiovascular morbidity were lower at st V, suggesting that the setting on dialysis was early, or/and the mortality was higher. ESRD was expected in 1398 pts with a orientation to hemodialysis-peritoneal dialysis in 84% -16% of cases.The Hbs vaccine and the creation of AVF were performed in a small percentage of pts, while the hemodialysis treatment is planned for a majority of pts.
Disclosure of Financial Relationships: nothing to disclose

**PUB249**

**Profile of Patients Oriented Exclusively to Conservative Treatment of ESRD** Malik Touam,[1] Eric Daugas,[3] Bertrand Dussol,[4] Patrick Henri,[5] Laurent Juillard,[6] Paul Stroumza.[7] *[1]AURA, Paris, France; [2]Hôpital Bichat, Paris, France; [3]Hôpital de la Conception, Marseille, France; [4]Hôpital Clemenceau, Caen, France; [5]Hôpital E. Herriot, Lyon, France; [6]Clinique de la Résidence du Parc, Marseille, France.*

***Background:*** The characteristics of pts with CKD progressing to ESRD and oriented exclusively to conservative treatment (CT) are poorly understood.

***Methods:*** As part of an french study PREPARE, one day in november 2009, 301 nephrologists have described the characteristics and their intent to treat 2089 pts with a GFR <60 ml/min/1,73 m2 (not on dialysis or transplantation).

***Results:*** The ESRD was expected in 1382/2089 (68.5%) pts, and CT were the preferred orientation in 7% (n = 97/1382) of cases. This option was selected by 23.2% of nephrologists (n = 70/301) while the CKD was at stage 5 (15.5%), 4 (51.5%) or 3 (33%). The main reasons for this choice were the great age (67%), cardiovascular comorbidity (21.6%), neoplasia (16.5%), psychiatric disorders (11.3%), or for other reasons whose refusal of dialysis by pts(24.7%).

The comparative characteristics of pts referred to dialysis (without transplantation, n = 863) and those to CT are:

| | CT n=97 | Dialysis n=863 | p |
|---|---|---|---|
| Age, years | 82,4 ± 9.1 | 74.4 ± 10 | < 0.0001* |
| Denutrition,% | 31,5 | 6.1 | < 0.0001* |
| BMI, kg/m2 | 24.8 ± 5.2 | 27.9 ± 5.7 | < 0.0001 |
| Nephrol. monitoring, years | 1.7 ± 3.7 | 1.3 ± 3.6 | 0.31 |
| IC, HF, St **,% | 62.9 | 47.2 | 0.003 |
| Solid Tumor,% | 20.6 | 12.4 | 0.02 |
| Diabetes,% | 28.9 | 41.5 | 0.02 |
| Charlson score modified | 7.2±2.3 | 6.5 ± 1.9 | 0.004 |

*Univariate and multivariate analysis ** IC ischemic cardiopathy , HF heart failure, St stroke

The distinctive parameters of pts oriented to CT are a great age and a poorer nutritional status, regardless of duration of follow-up and co-morbidities. Nearly 25% of nephrologists give other non-physical reasons for this choice. This suggests that factors such as social isolation or rejection of dialysis treatment, involved in a crucial decision. Further studies are needed to clarify this.

Disclosure of Financial Relationships: nothing to disclose

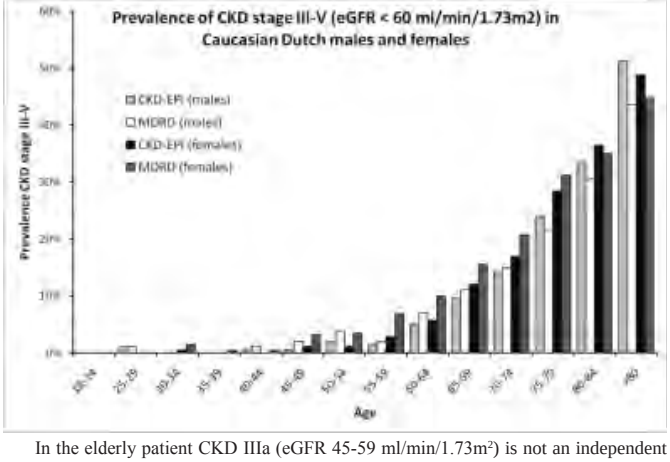
**PUB250**

**The Effect of the CKD-EPI Equation To Estimate Glomerular Filtration Rate: Does the Patient Benefit?** Jan A. J. G. van den Brand, Jack F. Wetzels. *Department of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

A reduced glomerular filtration rate (GFR) below 60 ml/min/1.73m$^2$, is sufficient to diagnose chronic kidney disease (CKD) stages III-V. Recently the CKD-EPI equation to estimate GFR was developed. We assessed the consequences of its introduction on CKD prevalence in our population.

We calculated $eGFR_{CKD\text{-}EPI}$ and $eGFR_{MDRD4}$ for 2 823 and 3 272 age stratified randomly selected Caucasian males and females. We obtained information on health status and medication use through a questionnaire. Serum creatinine was measured with a Jaffé assay calibrated versus isotope diluted mass spectrometry.

The CKD-EPI equation generally gave higher estimates of GFR than the $MDRD_4$ formula did. Change in prevalence of CKD III-V was dependent on age and gender (see figure). Thus, introduction of the CKD-EPI formula resulted in a lower prevalence in younger persons, but a higher prevalence in the elderly. In the general population the overall result is a reduction in the number of persons identified with CKD III-V. However the benefit for the individual patient can be questioned.




In the elderly patient CKD IIIa (eGFR 45-59 ml/min/1.73m$^2$) is not an independent risk factor, rather an indicator of cardiovascular risk profile. Use of the CKD-EPI formula would thus unnecessarily increase the number of patients referred for nephrology care. In contrast, for young patients CKD IIIa is an independent risk factor, eGFR being well below the fifth percentile of normal kidney function. The CKD-EPI equation would result in classifying these young persons upward, thus lead to under diagnosis of CKD.

In conclusion, introduction of the CKD-EPI formula leads to higher estimates of GFR on a population level. However, redefining CKD stages is needed to improve risk stratification and care for the individual patient.

Disclosure of Financial Relationships: nothing to disclose

**PUB251**

**Rural-Urban Disparities in Health Related Quality of Life (HRQoL) in Chronic Kidney Disease (CKD): The Study of Treatment for Renal Insufficiency: Data and Evaluation (STRIDE)** Tiffany C. Veinot,[1] Anca Tilea,[1] Brenda W. Gillespie,[1] Margaret A. Kiser,[2] Fredric O. Finkelstein,[3] George Eisele,[4] Peter Kotanko,[5] Rajiv Saran.[1] *[1]U. of Mich., Ann Arbor, MI; [2]U. of N. Carolina, Chapel Hill, NC; [3]Hospital of St. Raphael Yale U., New Haven, CT; [4]Med. College of Albany, Albany, NY; [5]RRI, New York, NY.*

Research in HRQoL among CKD patients by rural versus urban residence has been limited. We examined differences in HRQoL among urban and rural residents in a national CKD cohort. The STRIDE is a prospective cohort study at 79 US renal clinics (n=1,502). Patients were categorized as rural (n=234) or urban (n=1,268) using the zipcode-based Rural-Urban Commuting Area (RUCA) classification. The two groups' baseline Kidney Disease Quality of Life Short Form-36 (KDQoL) scores were compared (see Table) using 2-sample t-tests (unadjusted) and linear regression (adjusted for age, gender, race, education, diabetes, hypertension, hemoglobin and eGFR).

Rural and urban patients were similar, with a mean age of 63±12, and a mean eGFR (MDRD) of 24±10 ml/min/1.73m$^2$, but there were significantly more rural whites (80% *vs.* 66%, p<0.0001).

Rural patients had statistically significant lower scores for:energy/fatigue, physical component summary, general health and burden of kidney disease. There were no statistically significant differences in other KDQoL scores. After adjustment, rural patients had significantly worse scores for: energy/fatigue, the kidney disease component summary and burden of kidney disease. Rural patients reported significantly better cognitive functioning.

| KDQoL-SF36 Measure | Urban* (n = 1,268) | Non-Urban* (n = 234) | P-value Unadjusted | P-value Adjusted |
|---|---|---|---|---|
| Mental Component Summary | 50±10 | 50±10 | 0.90 | 0.77 |
| Energy/Fatigue | 48±24 | 43±24 | 0.01 | 0.005 |
| Physical Component Summary | 38±12 | 36±12 | 0.02 | 0.07 |
| General Health | 47±19 | 44±17 | 0.02 | 0.11 |
| Kidney Disease Component Summary | 57±10 | 56±11 | 0.36 | 0.04 |
| Burden of Kidney Disease | 61±26 | 56±26 | 0.01 | 0.04 |
| Cognitive Functioning | 52±38 | 56±37 | 0.14 | 0.04 |

*Urban defined as RUCA 1-3, non-urban defined as RUCA 4-10

Rural residence may confer HRQoL challenges for CKD patients related to access to care or other factors. Given known associations between HRQoL, morbidity and mortality, further research is needed in this area.

Disclosure of Financial Relationships: nothing to disclose

**PUB252**

**Chronic Kidney Disease in Queensland (CKD.QLD)** Sree Krishna Venuthurupalli,[1] Robert G. Fassett,[2, 3] Tracy A. Lowis,[2] Wendy E. Hoy.[2] *[1]Renal Medicine, Rockhampton Hospital, Rockhampton, Queensland, Australia; [2]Centre for Chronic Disease, University of Queensland, Brisbane, Queensland, Australia; [3]Renal Medicine, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia.*

Aim: To establish a practice network, registry and research platform for chronic kidney disease (CKD) in Queensland (QLD) Australia.

Background: CKD is a leading cause of morbidity and mortality in Australia with huge economic implications. However, definitions, staging and predictions of course are all imperfect. Varying models of CKD service delivery are evolving but are not yet rigorously evaluated. CKD.QLD is a multidisciplinary CKD research group, established in September 2009 in QLD, a state with a multiethnic population of 4.5 million people, and a single public health provider (Queensland Health).

Methods: The first phase consisted of a cross-sectional profile of CKD activity in all renal practices in QLD, both hospital based and community-based, conducted between December 2009 and January 2010.

Results: Sixteen separate public renal practices were surveyed, where 10,469 CKD patients were identified as attending. All services collected patient data but usually in a service designed excel spreadsheet. Models of CKD care varied greatly from nurse-practitioner-led multidisciplinary community clinics to hospital based renal clinics. Specialist clinics in hospitals focussed mainly on stages 4-5 CKD but community clinics included management of earlier stages. Stages 1-2 CKD were frequently referred back to primary care. Estimates suggested equal or greater numbers of patients in private practice as in the public setting.

Conclusions: CKD is a common disease and many cases are followed within Queensland renal clinics. However, stage of CKD patients seen and models of care vary considerably across the sites. We plan more detailed review of services, establishment of a registry, sampling of CKD patients in other practice settings, including primary care, and development of a research agenda, which will include outcomes research, biomarkers, clinical trials, and models of care.

Disclosure of Financial Relationships: nothing to disclose

**PUB253**

**The Epidemiology of Metabolic Syndrome in Sichuan and Relationship between Chronic Kidney Disease and Metabolic Syndrome** Li Wang. *Department of Nephrology, Sichuan Provincial Hospital, Chengdu, Sichuan, China.*

Objective: To investigate the epidemiology of metabolic sydrom an it's relationship and chronic kidney diseaes.Method:3024 people older than 18 years were enrolled by cluster random sampling.This reseach included a questionnaire,physical exminations and laboratory examinations . CKD was diagnosed by decreased eGFR(<60ml/min/1.73m$^2$) or presentation of microalbuminuria, macroalbuminuria, proteinuria or hematuria. MS was diagnosed according to IDF standard . Result:The prevalence and age-and-sex-standardized prevalence of hypertension was 16.5% and 11.9% respectively with a higher prevalence in Guanghan than in Chengdu(22.0% vs 11.0%, p<0.001). The prevalence and age-and-sex-standardized prevalence of diabetes was 6.6% and 5.1% respectively with a higher prevalence in Guanghan than in Chengdu(9.2% vs 4.0%, p<0.001), The prevalence and age-and-sex-standardized prevalence of hyperlipidemia was 53.4% and 50.76% respectively with a higher prevalence in Guanghan than in Chengdu(57.5% vs 49.3%, p<0.05), The prevalence and age-and-sex-standardized prevalence of obesity was 60.0% and 65.30% respectively with a higher prevalence in Chengdu than in Guanghan (65.8% vs 54.1%,

p<0.001), The prevalence and age-and-sex-standardized prevalence of hyperuricemia was 14.4% and 15.13% respectively with a higher prevalence in Chengdu than in Guanghan (18.3 vs 10.4%, p<0.001), The prevalence and age-and-sex-standardized prevalence of metabolic syndrome was 10.0% and 8.57% respectively without difference in Chengdu and Guanghan (9.9% vs 10.0%, p>0.05).the prevalence of CKD was higher in MS people than non-MS people(26.2% vs 18.4, p<0.001). Low HDL-c[OR0.524(0.432~0.636)], high FPG[OR3.499(2.838-4.312], hypertension[OR2.138(1.771-2.582], Centralobesity [OR1.502(1.234-1.828] are risk factors. Based on central obesity, prevalence of CKD increases as the number of abnormal metabolic components increases. When the number increases to 1,2,3 or 4, the OR1.737(1.105~2.731),2.236(1.542~3.509),2.093(1.263~3.466 )or2.700(1.279~5.698)respectively.Conclusion:The prevalence of metabolic syndrome and its components is high in Sichuan Province. Prevalence of CKD are higher in MS patients than in general population in Sichuan.

Disclosure of Financial Relationships: nothing to disclose

**PUB254**

**Once Monthly C.E.R.A. Provides Stable Hb-Values in CKD-Patients; Results from a Metaanalysis of 3 Studies** Thomas Weinreich,[1] Danilo Fliser,[2] Stefan N. Heidenreich.[3] *[1]Dialysezentrum, Villingen-Schwenningen, Germany; [2]Innere Medizin IV, Universitätsklinikum des Saarlandes, Homburg/Saar, Germany; [3]KfH Dialyse, Aachen, Germany.*

INTRODUCTION AND AIMS: CERA (Mircera®) was approved as a long acting ESA for patients (pts.) suffering from renal anemia. The studies SESAM, SUPRA and MIRACEL haven been conducted in CKD pts of stages IV and V in germany. Given the large no. of pt. data and similar time schedules of these studies, the pooled data offered the chance for subgroup analyses.

METHODS: Data from all 3 studies were pooled in a common database and analyzed with respect to individual Hb-fluctuation, reaching of target Hb-range and number of dose adaptations needed. Separate analyses were performed for the following subgroups:

Route of admin. (i.v./s.c.)
Age (<=65/>65)
Gender (male/female)
Previous ESA treatment (Epoetin/Darpeoetin/CERA)
Diabetic pts (yes/no)

RESULTS: Study population consisted of 1343 hemodialysis pts (MIRACEL study 424 pts; SESAM 919 pts.) and 335 CKD 4 and 5 pts (SUPRA). When comparing the various subgroups the following results were observed:

Table 1: Results of the subgroup analysis (% pts)

| | Hb-Fluctuation from individual mean | | | Patients in target range | | Dose adaptations | |
|---|---|---|---|---|---|---|---|
| | <= 1 g/dl | 1-2 g/dl | > 2 g/dl | 10-12 g/dl | 10-13 g/dl | <=2 | >2 |
| Route of admin (i.v./s.c.) | 84.8 / 84.8 | 13.7 / 13.8 | 1.5 / 1.4 | 37.3 / 44.1 | 66.4 / 68.3 | 66.1 / 59.5 | 33.9 / 40.5 |
| Age (<=65/>65) | 82.1 / 86.6 | 16.7 / 11.7 | 1.1 / 1.7 | 34.9 / 42.2 | 64.9 / 68.4 | 63.8 / 64.1 | 36.2 / 35.9 |
| Gender (f/m) | 83.9 / 85.5 | 13.8 / 13.7 | 2.3 / 0.8 | 39.8 / 38.9 | 63.4 / 69.8 | 63.1 / 64.7 | 36.9 / 35.3 |
| Previous ESA trt (Epoetin/Darbepoetin /CERA) | 86.1 / 82.6 / 83.0 | 12.7 / 15.1 / 14.3 | 1.2 / 2.3 / 2.7 | 36.9 / 44.2 / 36.7 | 68.4 / 66.4 / 63.3 | 65.1 / 63.8 / 56.7 | 34.9 / 36.2 / 43.3 |
| Diabetic pts (no/yes) | 83.7 / 85.7 | 14.2 / 13.4 | 2.0 / 1.0 | 37.4 / 40.9 | 63.2 / 70.1 | 63.2 / 64.6 | 36.8 / 35.4 |

Evaluation Phase (V7-V9)

CONCLUSIONS: Once monthly CERA trt maintained stable Hb-values with most pts having only a minimal Hb-fluctuation. These results could be confirmed for various subgroups, thus demonstrating that once monthly CERA is a suitable trt for pts suffering from CKD related anemia whith only few dose-changes needed.

Disclosure of Financial Relationships: nothing to disclose

**PUB255**

**Bioimpedance Measurement for Volume in Patients with Chronic Kidney Disease Participating in the CanPREDDICT Study: Design of a Prospective Study and Results of a Single Centre Pilot** Trevor J. Wilkieson,[1] Mukesh Khandelwal,[1] Cathy Z. Kotsamanes,[1] Adeera Levin,[2] Azim S. Gangji,[1] Catherine M. Clase.[1] *[1]Medicine, McMaster University, Hamilton, ON, Canada; [2]Medicine, University of British Columbia, Vancouver, BC, Canada.*

**Background.** Volume status is difficult to assess clinically. Hypervolaemia may predispose patients to congestive heart failure (CHF) in the short term and in the longer term cause arterial changes and cardiac remodelling that lead to myocardial infarction (MI), stroke, and sudden death. Data on volume status in patients with chronic kidney disease (CKD) not on dialysis are sparse.

**Methods.** Nested in the multi-centre CanPREDDICT study, we conducted a single-centre pilot to examine the feasibility of conducting a substudy measuring bioimpedance in prevalent patients with CKD who were already participating in CanPREDDICT. We measured bioimpedance in triplicate on a single occasion. We analyzed the data according to the method of Piccoli, classifying patients based on the resistance (R) – reactance (Xc) graph. Primary outcomes for the pilot were the proportion of CanPREDDICT participants who were eligible and consenting to the substudy, and the proportion of substudy participants who were volume overloaded. For the main study, we plan to recruit 500 of 1136 CanPREDDICT participants in 7 to 9 centres and examine whether volume overload, assessed by bioimpedance predicts a primary outcome composite of stroke, MI, amputation for peripheral vascular disease, CHF or vascular death, with power of 0.7 to detect hazard ratios of 1.6 or greater.

**Results.** From 2009 December 09 through 2010 June 03, 57 consecutive CanPREDDICT participants were approached at their first return visit. Of these, 46 participants were eligible, 36 (78%) participated and 10 declined. Participants were 39% women, mean age 67 years, 44% were volume overloaded by RXc graph.

**Conclusions.** The study appears feasible; volume overload by RXc graph is prevalent.

Acknowledgement: CanPREDDICT is sponsored by an unrestricted research grant from Janssen-Ortho Inc.

Disclosure of Financial Relationships: Employer: Astellas Pharma Canada Inc. Research Funding: Bodystat Ltd.

**PUB256**

**Association of Microalbuminuria with Family History of Renal Disease in Pediatric Nephrology Patients** Robert Woroniecki,[12] Karolina I. Woroniecka.[23] *[1]Pediatric Nephrology, Children's Hospital at Montefiore, Bronx, NY; [2]Center For Pediatric Kidney Diseases and Hypertension, Flushing, NY; [3]Undergraduate Studies, Brown University, Providence, RI.*

Microalbuminuria (MA) is a biomarker of subclinical cardiovascular disease, endothelial dysfunction and is a prognostic marker of kidney disease in patients (PTs) with diabetes mellitus (DM) and hypertension (HTN). MA has been associated with glomerular hyperfiltration (GHF) found in DM, metabolic syndrome (SX), sickle cell disease (SCD), and reported in children with chronic kidney disease, HTN, vesicoureteral reflux (VUR), single kidney, etc. However, it is unclear if MA in children is a marker of genetic predisposition to kidney disease, i.e. is associated with family history of renal disease/HTN. We examined the records of 35 PTs who were tested for MA due to following clinical indications: HTN (n=11), aplasia/hypoplasia (n=4), obstruction/dysplasia/reflux/pyelonephritis (n=10), cystic disease (n=6), and GHF (n=4) in SX, SCD and DM.

| | MA (n=14) | No MA (n=21) | P value |
|---|---|---|---|
| Age (years) | 12.9± 4.4 | 13.7±4.6 | 0.61t |
| Female (%) | 7 (50) | 9 (43) | 0.74f |
| Height percentile | 72.6±29.2 | 69.4±32.5 | 0.71t |
| MAP (mmHg) | 78.7±10.5 | 79.8±14.3 | 0.80t |
| SCr (mg/dl) | 0.65±0.21 | 0.71±0.21 | 0.39t |
| Birth weight (kg) | 3.27±0.59 | 3.45±0.37 | 0.47t |
| Positive FX (%) | 11 (78.6) | 9 (42.8) | 0.046f |
| Positive FX-HTN (%) | 9 (64.3) | 3 (14.3) | 0.004f |

MAP-mean arterial pressure, SCr-serum creatinine, FX: family history of dialysis, renal stones, cystic disease, and HTN, FX-HTN: family history of renal disease excluding idiopathic HTN, t-test, f-Fisher's exact, ± standard deviation.

We found an association between MA and FX, and no association with other tested variables. The association of MA with FX was stronger if idiopathic HTN was excluded from FX (table). PTs with FX had odds ratio (OR) of 4.9 (CI 1.05-22.84) of having MA, while there was no association of MA with PTs renal diagnosis, p=0.74. OR for PTs with FX-HTN having MA was 10.8 (CI 2.1-55.7). PTs with FX-HTN may be at risk of GHF and may benefit from early screening for MA.

Disclosure of Financial Relationships: nothing to disclose

**PUB257**

**Wide Range Overt Proteinuria in Chronic Kidney Disease Patients with Diabetes Has Different Predictive Value for Renal and Cardiovascular Outcome** Keiko Yasuda, Koichi Sasaki, Masaki Hatanaka, Terumasa Hayashi. *Nephrplogy, Izumisano Municipal Hospital, Rinku General Medical Center, Izumisano, Osaka, Japan.*

Purpose: Recently, significance of proteinuria in chronic kidney disease (CKD) has been reevaluated since estimated glomerular filtration rate was adopted as the definition of CKD. Although CKD patients with diabetes mellitus (DM) have wide range overt proteinuria, its predictive value for renal and cardiovascular, has not been fully clarified.

Methods: 221 diabetic patients with CKD stage 2-5 not on dialysis were prospectively enrolled as a hospital cohort since August 2004. Exclusion criteria was age over 90, malignancy, active infection, low cardiac ejection fraction (<40%). Primary outcome was combined renal outcome of serum creatinine (Cr) doubling or end stage renal disease requiring dialysis. Cardiovascular events was also defined as secondary outcome. Valuables such as proteinuria and other established predictive factors were incorporated into Cox proportional hazards analysis to determine the independent predictors for renal and cardiovascular outcome.

Results: Mean follow-up period was 3.4±1.5 years. Mean age was 63.8±11.1 and 66% were men. Mean serum Cr and proteinuria level were 2.27±1.35mg/dl and 3.99±3.74g/day. The amount of proteinuria was significantly greater among 133 patients who reached the combined renal endpoint compared with 88 patients who did not (5.33±3.95g/day vs 1.95±2.19g/day, P<0.001). After adjustment with other established predictive factors, proteinuria (HR 1.190, 95%CI 1.103-1.284) and serum Cr (HR 1.466, 95%CI 1.178-1.824) were strongly and independently associated with renal outcome. In contrast, there was no significant difference in proteinuria level between patients with (4.20±4.01g/day, N=72) and without secondary outcome (3.88±3.61g/day, N=149). Cox univariate proportional hazard model revealed that proteinuria (HR 0.910, 95%CI 0.766-1.081) tended to be associated with secondary outcome.After adjustment, proteinuria (HR 0.937, 95%CI 0.781-1.123) was independently associated with secondary outcome.

Conclusion: Wide range overt proteinuria in CKD patients with DM has different predictive value for renal and cardiovascular outcome.

Disclosure of Financial Relationships: nothing to disclose

**PUB258**

**Clinical and Pathological Analysis in the Serum HBV Antigen Negative and Antibody Positive Patients with Kidney Injury** Shengqiang Yu, Yuxian Zhu. *Institute of Nephrology, Shanghai Changzheng Hospital, Shanghai, China.*

**Objective:** To investigate the clinical and pathological characteristics in the serum HBV antigen negative and antibody positive patients with kidney injury. **Methods:** A total of 21 serum HBV antigen negative and antibody positive patients with kidney injury were included into this study. All of them were accepted routine laboratory analyses and renal biopsy. HBV-antigens (HBsAg and HBcAg) in renal tissues were detected by indirect immunofluorescence assay. **Results:** 7 of the patients revealed severe proteinuria which was diagnosed as nephrotic syndrome, 14 of them revealed non-nephrotic proteinuria. 3 of the patients were only serum HBcAb positive, 10 of them were serum HBsAb+HBcAb positive, 7 of them were HBsAb+HBeAb+HBcAb positive and 1 of them was HBeAb+HBcAb positive. All the patients' HBV-DNA copies were lower than 500 copies/ml. According to the renal biopsy, there were 7 patients diagnosed as HBV associated glomerulonephritis. **Conclusions:** The results suggested that renal biopsy, especially the HBV-antigen detection in renal tissue were very important to the patients with kidney injury whose serum HBV antigen negative and antibody positive.

Disclosure of Financial Relationships: nothing to disclose

**PUB259**

**Do the Costs Born by the Nephrology Department in the Hospital Justify Its Transfer to a Health Establishment with a Short Stay Unit?** Juan Abascal Ruiz. *Nephrology, "Lozano Blesa" Universitary Hospital, Zaragoza, Spain.*

**OBJECTIVES:** The work developed by our department and its assistance complexity do not require all the necessary processes for the optimal functioning of a modern hospital. On the other hand, the work legislation makes the costs of specialized staff be larger if the work is done within a hospital or if it is done in another Health establishment.

**MATERIAL AND METHODS:** Throughout the years 2000-2008 we have studied the whole health activity of our department and the costs it generates. We have done the same with the Hospital general costs and their fraction assigned to our department. The established method has been: For Human Resources the distribution of work loads measured in hours considering the hour as the unit of technical value. For the health assistance we have applied microeconomics techniques (analytical management) and established the doctrine of homogeneous functional group.

**RESULTS:** They are exposed for each homogeneous functional Group (hospitalization, consultations and its areas, interventionist Nephrology, hypertension maps, intravenous iron, haemodialysis and peritoneal haemodialysis and intra-hospital and emergencies. The cost of staff in our department is 47.61%; The functioning costs are 31.32%; The costs of using other services are 17,15% and the structure costs of other services are 3.65% of our budget.

**DISCUSSION:** Considering another alternative in the use of public financing in Health is simple possible with a more rational use of resources and looking for the achievement of its cost-effectiveness with the application of technical criteria and social management mechanisms of the resources disregarding its finalist pre-assignment.

**SUMMING UP:** The ability to negotiate is possible in the 83.85% of our Department ´s budget considered as final cost unit. The possibility of negotiating, with existing work legislation can be done above the 47.61%. The saving percentage of 10 points is possible on the 10.90% of present budget.

Disclosure of Financial Relationships: nothing to disclose

**PUB260**

**Australian Home Dialysis Uptakes Vary Markedly, State by State and within States. Better High-to-Low Home-Achiever Protocol Sharing Is Required** John W. M. Agar, Carmel M. Hawley. *Home Dialysis Advisory Group, Kidney Health Australia, Melbourne, Victoria, Australia.*

**Introduction:** Australian home-based dialysis therapy (HBDT) uptake varies markedly, state by state. Individual renal unit home haemodialysis (HHD) utilisation ranges 1% to 22% while peritoneal dialysis (PD) ranges 15-46% while the national mean is 10% (HHD) and 22% (PD). Despite this known interstate data, intra-state unit vs unit HBDT utilization has not been documented.

**Aim:** To document unit-by-unit HBDT uptake for all major Australian dialysis services and to determine if HBDT utilisation is uniform within each state or if it also varies widely.

**Method:** Using ANZDATA (December 2008), we compared the % of facility-based HD (FHD) with the % of HBDT (both HHD and PD) for all 32 Australian units with >100 total all-modality dialysis patients, both inter-state across all states and territories and intra-state within each state.

**Results:** While some states were uniformly low HBDT achievers – in part explained by geographic and demographic differences – within states with the highest HBDT achieving units were other units which ranked among the lowest HBDT achievers. While the unit with the highest HBDT [one of the largest units in Australia] recorded 20% HHD and 46% PD (66% HBDT) and only 34% FHD, several large units recorded total HBDT rates of <20%. A unit-by-unit breakdown of HBDT uptake within each state confirmed similar marked intra-state variability with some renal units within the same state achieving HBDT penetration rates of 2-3 times those of other like renal units within that state. This variable high-to-low HBDT utilisation pattern recurred across most states.

**Conclusion:** State vs state variability in HBDT uptake may be due to state-specific demographics and geography or to differing state funding models or unit/patient-directed incentive programs. However, inter-unit variations within individual states are more likely the result of local, unit-specific medical and/or nursing expertise or bias, home training logistics or HBDT emphasis during pre-dialysis education. We believe that HBDT uptake may be improved by high to low achiever inter-unit protocol-sharing and staff education.

Disclosure of Financial Relationships: Consultancy: Medical Advisory Board: Renal Solutions Inc. Warrendale, PA; Honoraria: Travel Assistance, Amgen Australia Travel Assistance, Fresenius Australia.

**PUB261**

**Lessons from Implementing the Advanced CKD Patient Management Toolkit (ACPMT)** Jameta N. Barlow,[1] William E. Haley,[2] Uptal D. Patel.[1] *[1]Duke Univ, Durham, NC; [2]Mayo Clinic, Jacksonville, FL.*

**Background:** Management of advanced CKD can be facilitated by improvements in care processes; however, successful implementation of quality improvement (QI) interventions is challenging. During a QI study of the Renal Physicians Association's ACPMT (consisting of 16 management tools) in 10 community nephrology practices, we sought to assess lessons learned by practices from the implementation experience.

**Methods:** After completion of the 6 month intervention, we conducted 13 semi-structured interviews of key clinical or managerial personnel (medical/physician assistant, nurse, nephrologist, office manager, and social worker) from 8 study sites (2 pending). Interviews were recorded, transcribed, and qualitatively analyzed using the constant comparative method to assess practices' experience with implementing the ACPMT.

**Results:** Interviewees' responses revealed 3 lessons related to their implementation experience. Facilitators that optimized the practice to the QI intervention included ensuring ongoing engagement across all staff, defining and measuring local goals, and customizing QI processes and components of the ACPMT. Barriers to implementing the QI intervention included limited ability to use ACPMT components with electronic medical records, low perceived value of some tools by physicians, exigencies of clinical work flow, and limited patient interest in patient-specific tools. Finally, the dedicated QI effort prompted increased team spirit surrounding the management of advanced CKD care. Consequently, despite expressed disappointment upon finding that there were limited improvements in performance measures across all study sites, strong endorsement for the QI experience remained with all sites stating that they have continued using the ACPMT.

**Conclusion:** A diverse sample of community nephrology practices was able to implement the ACPMT after customizing some components of the intervention. The barriers and facilitators encountered were common to QI implementation. Despite minimal impact on improving performance measures, this QI experience has catalyzed ongoing continuous QI efforts within participating practices.

Disclosure of Financial Relationships: nothing to disclose

**PUB262**

**Captopril Mitigates Chronic Renal Failure after Bone Marrow Transplant: Extended Follow-Up of a Randomized Trial** Manpreet Bedi,[2] John E. Moulder,[2] Eric P. Cohen.[1] *[1]Medicine, Medical College of Wisconsin, Milwaukee, WI; [2]Radiation Oncology, Medical College of Wisconsin, Milwaukee, WI.*

We have tested captopril compared to placebo in adults and children undergoing radiation-based bone marrow transplant (BMT, hematopoietic stem cell transplant). There is better kidney function and better patient survival in subjects on captopril (n=28) compared to placebo (n=27) (Int J Radiat Biol Oncol Phys, 2008). Use of captopril was as a mitigator, not as a radioprotector, i.e. the drug was started one month after irradiation and continued for up to one year thereafter. We have extended this study by longer follow-up and by addition to it of contemporaneous subjects eligible for the study but who did not enroll (n=74). This non-study cohort is added to the placebo group to create a larger no-drug group for this analysis. Endpoints are BMT nephropathy/HUS and patient survival. The captopril group had only one case of BMT nephropathy, while there are 11 in the combined no-drug group. Patient survival is better in the subjects on captopril compared to the no-drug subjects (p=0.07 for the actuarial curves). Median days of patient survival are 309 in the combined no-drug group, and 724 days for those on captopril. Safety analysis showed no difference in adverse event rates between the captopril and the placebo groups, and there was no difference in cancer relapse rates. We conclude that captopril is an effective and safe mitigator of chronic renal failure after radiation-based BMT.

Disclosure of Financial Relationships: nothing to disclose

**PUB263**

**The Experiences of Close Persons of ESKD Patients on Maximum Conservative Management: A Narrative Study** Aine Burns,[1] Joe T. Low,[2] Jason Myers,[2] Sheila J. Johnston,[1] Louise Jones.[2] *[1]Centre for Nephrology, University College London, London, United Kingdom; [2]Marie Curie Palliative Care Research Unit, University College London, London, United Kingdom.*

Background: Close persons play an important role in the care of elderly renal patients, though few studies have looked at the impact on those caring for patients who have elected not to dialyze and are following a maximum conservative management (MCM) care pathway. Such patients do not undergo dialysis, but are offered other medical support to prolong life and alleviate symptoms. Aims: To explore (i) the impact of end stage kidney

disease (ESKD) on the main close persons of patients with chronic kidney disease stage 5 who opt for MCM and (ii) how support for close persons can be improved. Methodology: Qualitative interviews were carried out with 26 purposefully sampled close persons across five London renal centers, using a narrative approach. Findings: The following key themes were identified: the specific diagnosis of ESKD had little impact on close persons as patients already had other chronic health problems; close persons understood patients' decisions to not dialyze, but had little influence in the final decision; close persons only had a vague understanding of the nature and implications of MCM; the quality and depth of the relationship between the close person and patient determined what care was provided with other family members and friends playing supporting roles; close persons appreciated the support provided by the renal teams, though they identified areas for improvement such as out of hours advice; there was less satisfaction with the continuity in the general provision of health care; close persons experienced uncertainty about the long term future, and only a few discussed end of life issues with patients. Conclusion: Close persons were satisfied with care provided by renal services, though areas for improvement included "out-of hours" support, better continuity of care across medical specialties, handling of crisis situations and uncertainty about the future. This unique examination of close person experiences provides evidence for the development of interventions to improve the lives of this important group.

Disclosure of Financial Relationships: nothing to disclose

## PUB264

**A Narrative Study of the Experiences of Patients with Kidney Disease on a Maximum Conservative Management (MCM) Programme** Aine Burns,[1] Joe T. Low,[2] Sarah Davis,[2] Henry W. L. Llewellyn,[2] Louise Jones,[2] Sheila J. Johnston.[1] *[1]Centre for Nephrology, UCL, London, United Kingdom; [2]Dept of Mental Health Science, UCL, London, United Kingdom.*

Dialysis treatments are widely available, but for elderly patients with multiple co-morbidities, they may have no benefit to quality of life or survival. For these patients, Maximum Conservative Management (MCM) is becoming more widely available across the UK, wherein renal teams have responsibility for providing a generalist palliative approach with input from palliative care specialists. Little is known about the experiences of patients on MCM and about how they make and cope with the decision not to have dialysis. This qualitative study explores narratives of patients on MCM programmes about their illness and experiences of the care they receive. Semi-structured interviews aim to elicit patients' narratives on their illness, coping styles, decisions around care (especially with reference to their decision not to have dialysis) and their experiences of MCM itself. Verbatim transcripts are analysed thematically using a constant comparative approach. Recruitment is ongoing with 10 patients of 25 recruited. Emerging themes reveal the complexity of perceived clinical, psychological and social factors in decisions regarding care, and the pervasiveness of typically negative second-hand experiences of dialysis. Equanimity around death in this population may explain their rejection of life-prolonging treatment in favour of less invasive care. However, most patients do not consider their decision to be final, even though dialysis would no longer be clinically appropriate for some in the very late stages of disease. Patients consider the decision to be theirs, though welcome guidance from their clinical teams and families. Despite some vagueness in patients' definition and description of the MCM programme, they feel well-supported by the approach's holistic structure. This study thus confirms the viability of MCM and underscores the importance that clinicians are aware of the weight and complexity of decisions regarding care.

Disclosure of Financial Relationships: nothing to disclose

## PUB265

**Carotid Atherosclerosis (CA) and Body Composition Assessment by Bioelectrical Vectorial Impedance (BIVA) in Chronic Kidney Disease (CKD) Patients Stage 2 – 5nd** Secundino Cigarran,[1] Francisco Coronel,[3] Montserrat Pousa,[1] Ignacio Docal,[1] Guillermina Barril,[4] Emilio E. Gonzalez-Parra.[2] *[1]Nephrology, Hospital Da Costa, Burela, Lugo, Spain; [2]Nephrology, Fundación Jimenez Díaz, Madrid, Spain; [3]Nephrology, Hospital Clinico Universitario de San Carlos, Madrid, Spain; [4]Nephrology, Hospital Universitario de la Princesa, Madrid, Spain.*

Evidence suggests atherosclerotic vascular diseases are a major cause of morbi-mortality in CKD patients. The prevalence of CA by ultrasonoghraphy was significant higher in CKD than general population. The aim of corss-sectional study is to assess the relationship of CA with body composition markers. 227 pts with CKD stage 2-5, were examined by high-resolution B-mode ultrasonography (USBM) with a 7.5MHZ linear array probe ( LogiQ PRO, GEE . USA). Body composition assessment was performed by whole tetrapolar bioelectrical vectorial impedance analysis (BIVA) ( EFG, Akern Firenza Italy). Data derived derived from BIVA were Na-K exchange, Phase angle, body cell mass /ecw ratio, PA standarized (PA and PA± std derived from healthy population).Data were analyzed by SPSS15.0. 58 pts had not CA. 168 (74.4%) had CA and compared with non CA were older (68.66±10.1 vs 54.34 ±10.5 years, P<0.001), Diabetic (36.1 vs 17.3% , p<0.001).

T-Paired test

| Variable | Non CA (N=58) | CA (N=169) | P |
|---|---|---|---|
| Na-K exchange | 0.96±.14 | 1.05±.19 | .001 |
| Body Cell Mass (%) | 50.91±5.48 | 49.64±6.22 | .001 |
| ECW (%) | 46.96±4.62 | 48.96±4.68 | .001 |
| ICW (%) | 53.03±4.62 | 51.04±4.69 | .001 |
| Resting Energy Expenditure (cal/day) | 1510.1±254.8 | 1430±272.3 | .046 |
| BCM/ECW (Kg/L) | 1.46±.30 | 1.32±.31 | .001 |
| Phase Angle(º) | 5.81±.9 | 5.42±.92 | .001 |
| PA standarized | -.62±.82 | -.98±.83 | .001 |
| Hb (gr/L) | 13.70±1.49 | 13.14±1.54 | .001 |
| Albumin (gr/dl) | 4.45±.29 | 4.30±.31 | .001 |

CA= Carotid Atehosclerosis. ECW extracellular water. ICW intracellular water.

No other significance was met.

CA is associated to older, lower GFR , diabetic and cellular damage expresed by increased Na-K exchange, lower PA, PA std, and BCM/ECW ratio. Further clinical trials are required to explain this biologic significance.

Disclosure of Financial Relationships: nothing to disclose

## PUB266

**Cost-Effectiveness of Adding Aliskiren to Losartan and Optimal Antihypertensive Therapy in Patients with Hypertension, Type 2 Diabetes, and Nephropathy with Residual Proteinuria** Thomas E. Delea,[1] Aaron Moynahan,[1] Charu Taneja,[1] Helen Lau,[2] Jean Lian,[2] Hans-Henrik Parving,[3] Sean D. Sullivan.[4] *[1]PAI (Policy Analysis Inc.), Brookline, MA; [2]Novartis Pharmaceuticals Corp., East Hanover, NJ; [3]Rigshospitalet, University Hospital of Copenhagen, Copenhagen, Denmark; [4]University of Washington, Seattle, WA.*

**Background**: AVOID was a randomized, double-blind, 6-mo. study of adding aliskiren to losartan and optimal antihypertensive therapy (AHT) in patients with hypertension, type 2 diabetes, and nephropathy with residual proteinuria (urinary albumin creatinine ratio [UACR]>100 mg/g) after ≥3 mo. of 100 mg/d losartan. Aliskiren reduced mean UACR by 20% vs placebo (P=.009).

**Methods**: A Markov model was used to project lifetime incidence of myocardial infarction (MI), stroke, heart failure (HF) and end-stage renal disease (ESRD), life years (LYs), quality-adjusted life years (QALYs), and expected lifetime costs (US healthcare system perspective) with losartan + AHT with or without aliskiren. Albuminuria progression by treatment was projected by generalized linear model regression equations fit to UACR data from AVOID. Probabilities of CVD/ESRD by UACR and death without CVD/ESRD by age were obtained by calibrating the model to yield results for losartan and placebo in the RENAAL study. Other probabilities, costs, and utilities were based on published sources.

**Results**: If effects of aliskiren on progression of UACR observed in AVOID are maintained while on treatment, adding aliskiren is projected to reduce lifetime incidence of ESRD by 5% and MI/stroke/HF by 4% vs. losartan and AHT only. LYs are increased by 0.17; QALYs by 0.09. Savings from averted MI/stroke/HF ($5400) and ESRD ($11,800) more than offset additional cost associated with aliskiren treatment. Savings in total lifetime costs with aliskiren are $7500. Findings are sensitive to the assumed duration of aliskiren benefit.

**Conclusion**: Adding aliskiren to losartan + AHT in patients with hypertension, type 2 diabetes, nephropathy and residual proteinuria is projected to reduce costs and increase QALYs and may be cost-effective from a US healthcare system perspective.

Disclosure of Financial Relationships: Consultancy: Novartis Pharmaceuticals Corp. Research Funding: Novartis Pharmaceuticals Corp.

## PUB267

**Supporting Pathways to Palliative Care for People Diagnosed with Chronic Kidney Disease** Robert G. Fassett,[1,2] Helen G. Healy,[1] Iain Robertson,[3] Loren M. Youl,[4] Sarah Anne Challenor,[5] Rose Mace,[5] Rosalind M. Bull.[4] *[1]Renal Medicine, Royal Brisbane Hospital, Brisbane, Queensland, Australia; [2]Medicine, University of Queensland, Brisbane, Queensland, Australia; [3]School of Human Life Sciences, University of Tasmania, Launceston, Tasmania, Australia; [4]Nursing and Midwifery, University of Tasmania, Launceston, Tasmania, Australia; [5]Renal Unit, Launceston General Hospital, Launceston, Tasmania, Australia.*

Aims;To identify current practice of offering palliative care and compare with evidence-based best practice and determine associations between initiation of palliative care and predictors of uptake of conservative care.

Background: The increasing acceptance of elderly onto dialysis programs has heightened interest in and study of the process of end-of-life decision-making in ESKD, and the role of palliative care in the later stages of treatment. A chart review was conducted as part of a wider research program to describe current clinical practice.

Methods: A chart review of the 45 CKD and dialysis patients who died in 2006-2008 in North Tasmania aimed to determine the associations between patient or family request, or actual withdrawal of RRT and/or referral for palliative care, and recorded potential predictors of withdrawal in the last 12 months of life. Qualitative and quantitative analysis was performed.

Results: The presence of, advanced health care directives, patients wish to die, and stroke were associated with family request for withdrawal. The loss of will to live, behavioural changes, severe pain, loss of ADLs were associated with patient request for

withdrawal. Expressed need to die, behavioural changes, loss of ADLs and appetite were associated with actual withdrawal. There was a cycle of ambiguity as patient and families change their minds about treatment withdrawal. Who controls this process fluctuates from time to time. A limited range of language is used to express the recognition of the need to die.

Conclusion: Loss of function, particularly from stroke, and severe pain are interpreted as representing levels of suffering which would justify the need to withdraw. The influence of patient, family and clinicians on this decision involves negotiation and equivocation.

Disclosure of Financial Relationships: nothing to disclose

**PUB268**

**Measure of Satisfaction with Body Image of Patients Undergoing Chronic Hemodialysis** Simone Adriana Guaraldo,[2] Geison Stein Meirelles Ramos,[1] Clarissa B. B. Uezima,[1] Bárbara Margareth Menardi Biavo,[2] José Adeirton Bezerra da Silva,[1] Everton Aparecido Teodoro,[1] Mary E. C. Costa,[1] Camila Machado de Barros,[2] Elzo R. Junior,[2] João Paulo L. B. Martins,[1] Elvino Barros,[3] Carmen B. Tzanno-Martins.[1] *[1]Integrated Center of Nephrology, Guarulhos, São Paulo, Brazil; [2]Home Dialysis Center, São Paulo, São Paulo, Brazil; [3]Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.*

**Objective**: To evaluate satisfaction with body image concerning the general characteristics of this population.

**Methodology:** Cross-sectional study with 73 patients undergoing chronic hemodialysis, with preserved cognition, confirmed by the Modified Mini-Mental (3MS) test application. 39 of them were male (53.4%) and 34 female (46.6%). Demographic data (gender, age, dialysis time), body mass index (BMI) and silhouette scale were evaluated in order to assess body image (current silhouette, ideal silhouette and one year ago silhouette - Sunkard and Sorensen, 1993).

**Results:** The mean age was 52.18 ± 14.87 years, hemodialysis time was 25.64 ± 25.30 months, mean BMI was 24.5 ± 5.18 kg / m². 39 patients (53.4 %) were well nourished. Most male (74.4%) and female (73.5%) were dissatisfied with their body image. Patients dissatisfied with their body image were those in the beginning of hemodialysis and those with more than 2 years of treatment. The group that showed higher satisfaction were the eutrophic ones. However 64% of them were dissatisfied. All groups showed a high percentage of dissatisfaction regardless of the compared variables.

**Conclusion:** Most patients undergoing chronic hemodialysis are dissatisfied with their body image, regardless of the compared variable. Thus, we suggest that aesthetic, nutritional and physical education interventions are implemented in order to bring benefits to this population.

Disclosure of Financial Relationships: nothing to disclose

**PUB269**

**Participate in Activities of Daily Living Scale and Nutritional Assessment of the Elderly** Yukihiro Nishimoto. *Dept. of Nephrology and Dialysis, Chukyo-Kosei Clinic, Nagoya, Aichi, Japan.*

Activities of daily living scale (ADL) deterioration in the elderly patients has influence on an action in eating, transference of walking or wheelchair. However, its utility in the relation with biochemical parameter are not elucidated clearly for circumstances, respectavily. In this study it was to investigate the relation of nutritious state to eating condition, transference, age, nursing level and biochemical parameters.

ADL was evaluated at three classified eating actions, six classified transfer conditions, five classified nursing levels. Albumin, pre-albumin, retinol binding protein, cholinesterase and transferrin were measured (n=84; 25male and 59female)

Both eating actions and transfer ability level decreased, albumin level (3.9+/-0.1 vs. 3.6+/-0.1 g/dl, p<0.01), pre-albumin level (21.8+/-0.8 vs. 18.0+/-1.4 mg/dl, p<0.05), cholinesterase level (256+/-9.4 vs. 185+/-14.3 U/l, p<0.01) and transferrin level (221+/-6.0 vs. 179+/-9.5 mg/dl, p<0.01) decreased significantly. There were no significant differences between age and biochemical analysis. Pre-albumin, retinol binding protein, cholinesterase along with transferrin and albumin in particular decreased significantly in relation to higher required levels of nursing support. Data from hemodialysis patients is still under analysis. ADL, albumin and transferrin levels were quantitative correlation. In addition, long half-time assay were significant differences. These results suggested that long half-time studies are relatively adequate and valuable in the index.

Disclosure of Financial Relationships: nothing to disclose

**PUB270**

**Examination of Understanding of Prognosis and Disease Progression in Elderly Patients with Advanced Kidney Disease** Jane O. Schell,[1] Uptal D. Patel,[1] James Tulsky.[2] *[1]Nephrology, Duke University, Durham, NC; [2]Center of Palliative Care, Duke University, Durham, NC.*

CKD is a common condition in an aging population. Elderly patients who progress to kidney failure suffer increased disability, morbidity, and mortality. Yet, little is known about how well these patients understand their diagnosis and its prognosis in order to plan for the future. Our goal was to describe, through focus groups and in-depth interviews, how elderly patients at different CKD stages understand their disease course.

**Methods:** From one academic and one community nephrology practice, we recruited 23 subjects over age 65 who were receiving dialysis or had documented CKD. Patients participated in either an in-depth interview (n=11) or focus group (n=22 in four groups). All interviews were audio-recorded and transcribed. We used qualitative content analytic methods with open and axial coding to identify common and recurrent themes describing how disease progression is explained, understood and experienced by the patient.

**Results:** Patients reported that kidney disease was frequently explained to them using medical terminology and blood chemistry levels with considerably less emphasis on prognosis. Patients described their experience with CKD and impending dialysis as one of fear and worry. Many of them had thought little about the future, either because they were unaware of how ill they were, or simply chose not to. When they did, their thoughts about the future were dominated by uncertainty and lack of knowledge of what the future might hold.

**Conclusions:** Because elderly CKD patients are at high-risk for increased disease burden and disability, understanding of disease progression may assist in goals of care planning and preparation for the future. This study highlights limitations in how patients understand and are prepared for their kidney disease course. Improved communication techniques may enhance elderly CKD patient understanding and experience.

Disclosure of Financial Relationships: nothing to disclose

**PUB271**

**How Do Nephrologists Discuss Prognosis and Disease Progression with Chronic Kidney Disease Patients?** Jane O. Schell,[1] Uptal D. Patel,[1] James Tulsky.[2] *[1]Nephrology, Duke University, Durham, NC; [2]Center of Palliative Care, Duke University, Durham, NC.*

End stage renal disease (ESRD) is a chronic life-limiting illness that impacts patient survival, quality of life and functional status. Despite the tremendous effect ESRD has on patients' lives, we know little about how nephrologists discuss prognosis and disease progression with patients. Our goal was to document from nephrologist descriptions, through focus groups and in-depth interviews, how they communicate prognosis and disease progression to their patients.

**Methods:** We conducted focus group and in-depth interviews with 12 nephrologists from two nephrology practices (one academic and one community). All interviews were audio-recorded and transcribed. We used qualitative content analytic methods with open and axial coding to identify common and recurrent themes of how nephrologists discuss prognosis and disease progression with patients.

**Results:** Discussions of disease progression depended upon patient factors, including comorbidities and age, and clinical factors, such as rate of renal function decline. Discussions of prognosis were noted to be infrequent, especially within the advanced CKD population. When these conversations did occur, they were generally prompted by the patient or a decline in clinical status. All nephrologists expressed that it was their role to have these discussions with their patients. Nevertheless, nephrologists described perceived barriers to engaging in these conversations including insufficient time to spend with patients, the potential negative impact on patient hope, and the lack of privacy for conducting discussions with ESRD patients in dialysis units.

**Conclusions:** Discussions of disease progression and prognosis in patients with chronic life-limiting disease such as ESRD can be challenging. How nephrologists engage in these conversations may impact patients' experience and preparation. This study describes nephrologists' perceptions about discussions of disease progression and prognosis. By studying how nephrologists talk to patients, future communication techniques can be developed to improve patient care.

Disclosure of Financial Relationships: nothing to disclose

**PUB272**

**How Many Courses of Steroid Pulse Therapy Are Necessary for the Induction of Remission in IgA Nephropathy? A Retrospective Study on Tonsillectomy Plus Steroid Pulse Therapy** Takahiro Uchida,[1] Takashi Oda,[1] Keishi Higashi,[1] Kojiro Yamamoto,[1] Taketoshi Kushiyama,[1] Toshitake Hyodo,[1] Naoki Oshima,[1] Soichiro Miura,[2] Hiroo Kumagai.[1] *[1]Department of Nephrology, National Defense Medical College; [2]Department of Internal Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan.*

Recently, tonsillectomy plus steroid pulse therapy has been reported to be quite effective for the treatment of IgA nephropathy, however, no data is available regarding the optimal number of courses of steroid pulse therapy in the protocol. We therefore conducted a retrospective study as to the relation between the number of courses of steroid pulse therapy and the therapeutic effects. Between 2002 and 2009, we identified 159 patients as IgA nephropathy by renal biopsy in our renal department and 34 of them received tonsillectomy plus steroid pulse therapy. Twenty-three patients received 3 courses of steroid pulse therapy, 1 patient received 2 courses, and 10 patients received 1 course. We compared therapeutic effects between those patients received 3 courses and 1 course. No significant basal difference was found between the two groups in terms of gender, age, urinary protein, hematuria, serum creatinine or estimated GFR. In 23 patients treated with 3 courses of steroid pulse therapy, 13 patients obtained clinical remission (complete disappearance of urinary abnormalities, including proteinuria and hematuria) and 18 patients experienced disappearance of hematuria. On the other hand, in 10 patients treated with 1 course of steroid pulse therapy, 4 patients obtained clinical remission and 9 patients experienced disappearance of hematuria. No statistical difference was found in clinical remission or in disappearance of hematuria between the two groups ($p$=0.25, and $p$>0.99, respectively). These data suggest that only one course of steroid pulse therapy may be necessary and sufficient for the induction of remission in IgA nephropathy treated with tonsillectomy plus steroid pulse therapy.

Disclosure of Financial Relationships: nothing to disclose

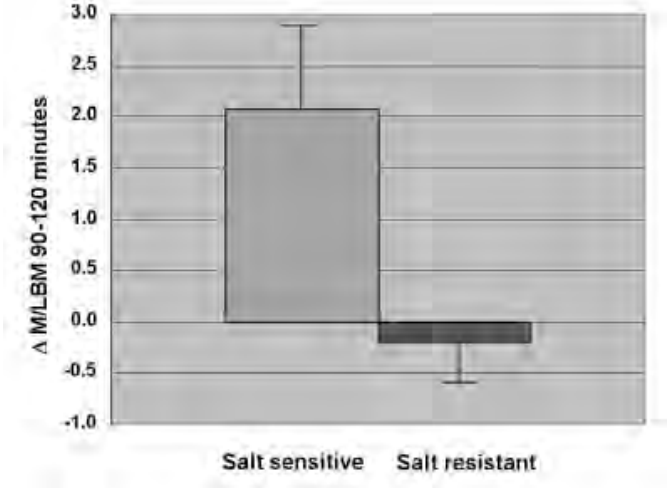
**PUB273**

**Type 2 Diabetics (T2D) with Salt-Sensitivity (SS) Substantially Improve Lipid Control and Insulin Sensitivity on a Paleolithic-Type Diet** Lynda A. Frassetto, Shelley L. Mccoy, Anthony Sebastian. *University of California San Francisco.*

**Background:** Dietary salt can induce insulin resistance in rats and men. In soleus muscle, salt loading induced significant insulin resistance in the Dahl-SS, but not Dahl-SR (salt resistant) rats. In normal weight men on a 200 mmoL salt diet for 5 days, insulin sensitivity [mean insulin mediated glucose disposal (M values)] measured by euglycemic hyperinsulinemic clamp (EHC) decreased 15%.

**Methods:** T2D patients on their usual diets were randomized to a lower salt diet, either a "Paleo"-diet (excludes dairy, legumes and grains, n=9) or an ADA diet (n=6) for 2 weeks. Subjects had one EHC and flow mediated dilation of the brachial artery (BAR, a measure of elasticity), and repeat fasting lipid, glucose and BP measures on the last 3 days of their usual and study diet. BPs were measured after 5 minutes sitting, in triplicate. Subjects were considered SS if BP decreased ≥ 5 mmHg. All measures were averaged, results reported as mean±SD.

**Results:** Both study diets improved glucose and lipid values. The Paleo diet induced *greater improvements* in glucose (p=0.04) and total cholesterol (TC, p=0.006) compared to the ADA diet, as well as in MAP (p=0.05). BP did not fall with the ADA diet (=SR). BP fell with the Paleo-diet in 7 subjects (=SS) and not in 2 (=SR) (MAP -5±3 vs +4±2 mmHg, p=0.003). With the Paleo-diet, SS compared to SR subjects had lower TC (-39±30 vs -18±15 mg/dL), LDL-chol (-25±20 vs -6±2 mg/dL), glucose (-27±30 mg/dL vs -20±33 mg/dL) and fructosamine (-37±33 vs -15±35 mmol/L). SS T2Ds had greater improvement in insulin sensitivity (M value +2.1±2.8 vs -0.2±0.6) and a 19 % improvement in BAR vs 10 % in the SR group (SR, 5.1 ±0.3 to 5.7±0.6%; SS, 5.2±0.7 to 6.2 ±1.2%).




**Conclusions:** A Paleo-diet is superior to an ADA diet in improving metabolic abnormalities in T2D; SS T2Ds particularly benefit from a low salt Paleo-diet.

Disclosure of Financial Relationships: nothing to disclose

**PUB274**

**Comorbidities and Survival of Patients with Type 1 Diabetes on Renal Replacement Therapy** Jaakko Helve,[1] Mikko Haapio,[2] Per-Henrik Groop,[3] Carola Gronhagen-Riska,[2] Patrik Finne.[1] *[1]Finnish Registry for Kidney Diseases, Helsinki, Finland; [2]Division of Nephrology, Helsinki University Central Hospital, Helsinki, Finland; [3]Folkhälsan Research Center Biomedicum Helsinki, Folkhälsan Institute of Genetics, Helsinki, Finland.*

**Objective:** To estimate effect of comorbidities on survival of patients with type 1 diabetes on renal replacement therapy.

**Research design and methods:** An incident cohort of all patients with type 1 diabetes entering chronic renal replacement therapy (n=656) in Finland between 2000 and 2008 was followed until death or the end of follow-up on 31 December 2008. All data were obtained from the Finnish Registry for Kidney Diseases, which collects information on comorbidities at the start of renal replacement therapy. Main outcome measure was relative risk of death according to comorbidities.

**Results:** At start of renal replacement therapy, 22% of patients had coronary artery disease, 18% peripheral vascular disease, 10% cerebrovascular disease, 33% left ventricular hypertrophy, and 7% heart failure. All these comorbidities were significant predictors of death in univariate analysis (RR 1.6-4.9). The 5-year survival probability of patients without comorbidities was 74%, while it was 56% and 37%, respectively, for those with one or more than one comorbidity. When the comorbidities were studied in a multivariate model, adjusting for age and gender, peripheral vascular disease (RR 1.9), left ventricular hypertrophy (RR 1.7) and heart failure (RR 2.5) remained independent risk factors of death. One third of deaths in the study population could be attributed to comorbidities.

**Conclusions:** Among patients with type 1 diabetes entering renal replacement therapy, comorbidities are common and strong predictors of death. Therefore, it is essential to identify and adequately treat comorbidities.

Disclosure of Financial Relationships: nothing to disclose

**PUB275**

**Clinical Significance of Urinary Fibrin/Fibrinogen Degradation Product (u-FDP) in Diabetic Nephropathy: Correlation with Proteinuria, and Effectiveness of Anticoagulant Therapy** Takehiko Kawaguchi, Yasuhiro Ohtsuka, Keiji Horike, Daijo Inaguma, Asami Takeda, Kunio Morozumi. *Department of Nephrology, Japanese Red Cross Nagoya Daini Hospital, Nagoya, Japan.*

Background: Coagulation/fibrinolysis system is involved in intraglomerular inflammation of kidney diseases. It is assumed that urinary fibrin/fibrinogen degradation products(u-FDP) be associated with the pathogenesis and activity of diabetic nephropathy(DMN), and that anticoagulant therapy be effective for the reduction of proteinuria.

Method: Data on 18 DMN patients with nephrotic syndrome, who anticoagulant therapy with heparin and the sebsequent warfarin were newly initiated for, were analyzed. All the patients had already taken angiotensin-converting enzyme inhibitors(ACEI) or angiotensin II receptor blocker(ARB) for renoprotection before the anticoagulation. The treatment target was to prolong the activated partial thromboplastin time(aPTT) with unfractionated heparin to 2 times the mean of the control value, and to prolong prothrombin time international normalized ratio(PT-INR) with warfarin to 2.0. Pearson's correlation coefficient(r) was used to identify cross-sectionally the relationship between u-FDP and urinary protein(u-Pro). Paired t-test was also used to examine the difference in u-FDP and u-Pro between means in patinets before and after the anticoagulation.

Results: The mean age of the patients was 62±11 years, and 56% were female. The mean values of mean blood pressure, serum creatinine, eGFR, serum albumin, u-Pro and u-FDP were 95±10mmHg, 2.8±1.4mg/dl, 25±17ml/min/1.73m$^2$, 2.8±0.6g/dl, 6.5±2.8g/gCr, 18,000±25,000ng/ml, respectively. A significant positive correlation was observed between u-FDP and u-Pro both at baseline(r=0.77, p=0.0005) and one month after the treatment(r=0.84 , p<0.0001). The mean u-FDP and u-Pro after the treatment was both significantly reduced from baseline(uFDP: 12,100±20,200ng/ml, p=0.02, u-Pro: 4.6±2.3g/dl, p=0.001).

Conclusion: This preliminary analysis on DMN suggested that u-FDP was highly correlated with u-Pro, and that anticoagulant therapy could have an impact on renoprotection. Further studies are needed to confirm the effectiveness of anticoagulation for DMN.

Disclosure of Financial Relationships: nothing to disclose

**PUB276**

**The Remedial Effect of Strict Diet Treatment and Exercise Therapy Using Information Technology (IT) on Metabolic Syndrome** Hideyasu Kiyomoto,[1, 5] Tadashi Sofue,[1] Daisuke Nakano,[2] Kumiko Moriwaki,[3] Atsuhiro Ichihara,[4] Akira Nishiyama.[2, 5] *[1]Division of Nephrology and Dialysis, Department of CardioRenal and CerebroVascular Medicine, Faculty of Medicine, Kagawa University, Kagawa, Japan; [2]Department of Pharmacology, Faculty of Medicine, Kagawa University, Kagawa, Japan; [3]Division of Nephrology & Hypertension, University of Miami, Leonard M. Miller School of Medicine, Miami, FL; [4]Department of Endocrinology & Anti-Aging Medicine, Keio University School of Medicine, Tokyo, Japan; [5]Team Kagawa, Consortium to Conquer Diabetes, Kagawa, Japan.*

[Background] The number of metabolic syndrome (MS) patients is increasing exponentially in the modern society. MS causes chronic kidney disease, but early aggressive intervention prevents it from progressing to renal failure. In this prospective study we examined if the supporting system of strict diet treatment and exercise therapy using IT is effective in treating metabolic syndrome. [Method] We divided 60 untreated MS patients into 4 groups of exercise therapy, diet treatment, combination therapy of them, and their own effort by the lot. We obliged them to carry pedometers managed on the internet and report their meals by signing up for accounts on the website, and conducted the anti-obesity program under strict direction of trainers and nutritionists for 6 weeks. Insulin resistance of every subject was estimated by tolerance to meal before and after the program to reduce their weight for 6 weeks. [Results] The remedial effects on MS such as significant reduction in BMI, leptin, MDA-LDL, glucagon, and insulin resistance were observed in every group except the one of their own effort. There was no change in renal function, urinary albumin excretion, plasma renin activity, and serum aldosterone level, however a positive correlation between improvement of BMI and decrement of systolic blood pressure was observed. In 6 weeks of short-term program, sole therapies of exercise or diet treatment were less effective, but the combination therapy of them showed the prominent additive effect. [Conclusion] Strict correction of lifestyle using IT is useful in treating MS.

Disclosure of Financial Relationships: nothing to disclose

**PUB277**

**The Pattern of Clinical Practice Affects on the Outcome in Diabetes Mellitus** Ho Seok Koo,[1] Sewon Oh,[3] Yunjung Oh,[1] Dong Ki Kim,[1] Ho Jun Chin,[3] Kook-Hwan Oh,[1] Ki Young Na,[2] Kwon Wook Joo,[1] Chun-Soo Lim,[4] Yon Su Kim,[1] Dong Wan Chae,[3] Curie Ahn,[1] Jin Suk Han,[1] Suhnggwon Kim.[1] *[1]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea; [2]Department of Internal Medicine, Catholic University College of Medicine, Seoul, Republic of Korea; [3]Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul, Republic of Korea; [4]Department of Internal Medicine, Seoul National University Boramae Hospital, Seoul, Republic of Korea.*

Background:The diabetic nephropathy is the leading cause of ESRD.Many guidelines are published for DM,CKD but they are not always followed by clinicians.We analyzed the adherence to guidelines and patients' outcome.Methods:We searched all patients with DM who visited to single center in 1yr and checked whether patients were tested and was prescribed medication as in guidelnies.Results:Among5,623 DM patients,physicians tested HbA1c in4,524paitents(80.5 %),total cholesterol,83.4%,LDL,44.5 %,serum creatinine,79.2 %,urine protein(dipstick),66.9 %,UACR,31.6 %.The rate of appropriate control of BP was 27.3%,HbA1c(<7%); 45.4%, LDL(<100 mg/dL);49.1%.The ACEI(ARB) was prescribed in63.9%,statin,31.1%,EPO,66.2% among a recommended group as in guidelines.During follow-up,602 patients were dead and 93patients were progressed to ESRD.674 patients reached to the composite outcome (12.1%/5567 patients).The univariate risk factors to outcome were gender,level of cholesterol, HbA1c,presence of (cholesterol,HbA1c,UACRor Cr) data, and use of ACEI(ARB),antiplatelet agent.With Cox's hazard proportional model, appropriate control of cholesterol(<200 mg/dL) reduced outcome risk to81.5%(68.4-97.1%),HbA1c<7% reduced the risk to 80.9% (67.4-97.1%), usage of antiplatelet agent reduced the risk to83.1%(69.5-99.5%), and presence both of renal functional data reduced the risk to71.7%(59.0-87.1%).The patients with the data of renal function had taken the medicine of ACEI,ARB,statin more frequently.Conclusion:Testing HbA1c and renal function in DM patients was related to better prognosis in death rate and ESRD rate and might be important to improve the prognosis of renal function and mortality.

Disclosure of Financial Relationships: nothing to disclose

**PUB278**

**Discovery and Early Development of Bardoxolone Methyl (BARD), an Antioxidant Inflammation Modulator (AIM) Targeting the Keap1-Nrf2 Pathway** Colin Meyer,[1] Christian Wigley,[1] Deborah A. Ferguson,[1] James Warren Huff,[1] Robin Kral,[1] Karen Liby,[2] Michael B. Sporn.[2] *[1]Reata Pharmaceuticals, Inc., Irving, TX; [2]Dartmouth Medical School, Hanover, NH.*

Bardoxolone methyl (BARD) and related analogs in the AIM class are the most potent known inducers of the Keap1-Nrf2 pathway. AIMs were discovered through a medicinal chemistry campaign designed to identify novel molecules to inhibit inflammation-induced carcinogenesis. Oleanolic acid, a triterpenoid natural product, was selected as the initial scaffold due to its weak anti-inflammatory and anti-carcinogenic activity. Synthetic derivatives were evaluated by measuring suppression of NO production in activated macrophages; BARD was one of the most potent analogs ($IC_{50}$=0.11 nM). Biochemical assays subsequently established that BARD directly interacts with regulatory cysteine residues on Keap1, which play a key role in regulating inflammation and oxidative stress. Activation of Keap1 promotes accumulation of Nrf2 in the nucleus, inducing transcription of genes that increase antioxidant capacity, induce glutathione synthesis, and conjugate and export potentially harmful molecules from the cell. In addition to inducing Nrf2, activation of Keap1 appears to suppress the transcriptional activity of NF-κB resulting in reduction of inflammation. The BARD structure and activity profiles resemble that of the endogenous activators of Nrf2 which play an important role in the resolution of inflammation. BARD protects against pro-inflammatory stimuli *in vitro* and *in vivo* in an Nrf2-dependent manner. BARD and related analogs have also been shown to improve endothelial dysfunction, suppress mesangial cell contraction, and increase inulin clearance in preclinical studies. Clinical trials with BARD were initiated in oncology patients; substantial improvements in measures of kidney function occurred in 80% of patients and were sustained in patients treated for six months. In a subsequent Phase 2 clinical trial, BARD significantly increased glomerular filtration rate and improved other renal function markers (BUN, Uric Acid, Phosphorus) in patients with Stage 3b/4 chronic kidney disease. A 12-month pivotal study of BARD is underway in this same patient population.

Disclosure of Financial Relationships: nothing to disclose

**PUB279**

**HOMA Test in Patients with Simultaneous Kidney Pancreas Transplant** George A. Osuchukwu, Adeel Ahmad, Tina Kochar. *Nephrology and Hypertension, University of Texas Medical Branch, Galveston, TX.*

Objective

The aim of this study is to evaluate the usefulness of the HOMA (homeostasis model of assessment) to determine insulin secretion and insulin resistance in diabetic patients with a functioning kidney-pancreas transplant.

Research Design and Methods

43 Kidney-Pancrease transplants done in our center in 2 years were followed over a period of time 2 – 18 months. Serial fasting glucose, insulin and C. peptide levels were obtained for Estimation of the Beta cell function HOMA-B and insulin resistance HOMA-%S using the HOMA2 calculator. These values were compared to the normal expected values for the general population.

Results

HbA1C and fasting glucose levels were within normal limits for all subjects. Beta cell function was higher than 100% the value usually reported in the normal population. Insulin resistance was persistent but did worsen over the period of follow up.

Conclusion

This study shows that the HOMA score can be a valuable tool in the assessment of metabolic function in patients with a functioning pancreas transplant. Due to the systemic delivery of insulin there is an over estimation of beta cell activity.Insulin resistance was high and persisted throughout the period of follow up. More research will be needed to assess its application to predict impending graft failure by worsening insulin resistance or decreasing beta cell activity.

Clinical Characteristics and Metabolic Parameters

| | |
|---|---|
| Number | 43 |
| Age | 37.7 ± 8.9 |
| BMI | 25 ± 3 |
| follow up Time | 11 ± 6.7 |
| HbA1C | 5.8 ± 1.1 |
| HOMA B% | 197 ± 89 |
| HOMA IR% | 2.9 ± 1.3 |
| HOMA S% | 53 ± 27 |
| Basal Glycemia | 86 ± 7 |

Table 1

Disclosure of Financial Relationships: nothing to disclose

**PUB280**

**BNP as a Novel Cardiovascular Risk Factor in Stage 3 Chronic Renal Patients with Diabetes Mellitus Type 2** Ana Paula Silva, Ana Cabrita, Anabela Malho, Ana Pinho, Pedro Neves. *Nephrology, Hospital de Faro, Faro, Portugal.*

The diabetic cardiomyopathy is a myocardial disease caused by diabetes mellitus (DM) unrelated to vascular and valvular pathology or systemic arterial hypertension.

The introduction of novel molecular biology techniques offers new diagnostic approaches. The most promising application is the analysis of the B-type natriuretic peptide (BNP).

The BNP is released by the cardiac ventricles in response to myocardial stretch, and the clinical effects of this peptide are mainly vasodilatation and increase of diuresis. The BNP values reflect the baseline myocardial conditions and the degree of neurohormonal activation.

The aim of this study was to examine the influence of inflammation, oxidative stress, and renal function on BNP levels in patients with diabetes mellitus type 2.

This observational cross-sectional study included 25 patients (w=11, m=14) from our diabetic nephropathy outpatient clinic, with a eGFR (MDRD) of 55 ml/min/1.73 $m^2$ and a mean age of 66.2 years.

The exclusion criteria were: antioxidant therapy during the previous 6 months, clinical cardiovascular disease and uncontrolled hypertension (pressure values ≥140/90 mmHg)

Several laboratorial parameters were evaluated, including serum BNP, high-sensitive C reactive protein (hsCRP), oxidative stress (malondialdehyde) and glomerular filtration rate (eGFR)

We found that BNP levels were influenced, in a single regression model, by malondialdehyde (r=0,505 p=0,001), CRP (r=0,966 p=0,003) and eGFR (r=0,505 p=0,014). In a multiple regression model ($R^2$= 0,932 p=0,001) we found that only malondialdehyde (β=0,643 p=0,001) and CRP (β=0,293 p=0,033) independently influenced the BNP levels.

In this study with stage 3 chronic renal patients with diabetes mellitus type 2, inflammation and oxidative stress were associated with higher BNP values. Further studies, with more patients, are needed to evaluate the potential role of BNP, as an early biomarker of cardiovascular disease in chronic renal patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB281**

**Vitamin D Levels and Microalbuminuria-to-Creatinine Ratio in Type 2 Diabetic Patients** Ana Paula Silva, Ana Cabrita, Ana Pinho, Pedro Neves. *Nephrology, Hospital de Faro, Faro, Portugal.*

The active vitamin D (aVD) has many important functions including immunomodulation, anti-proliferation and potentially renoprotective functions.

Earlier studies have demonstrated that glomerular haemodynamic changes, podocytes abnormalities and mesangial activation are associated with proteinuria in diabetic patients. The growing amount of evidence of the beneficial effect of aVD on glomerular structures, is reinforced by clinical studies showing that aVD decrease proteinuria in renal patients.

The aim of this study was to examine the association between active vitamin D blood levels and microalbuminuria-to-creatinuria ratio in patients with type 2 diabetes mellitus.

This observational cross-sectional study included 50 type 2 diabetic patients, from our renal outpatient clinic (f=18, m=32), mean age = 66.9 years and a mean eGFR (MDRD) of 50 ml/min/1.73 $m^2$.

The exclusion criteria were: Hg A1c ≥7,5 %, BMI > 27, vitamin D therapy during the previous 6 months, uncontrolled hypertension (pressure values ≥140/90 mmHg), previous physical activity, and infection, neoplasic or psychiatric disease.

Several laboratorial parameters were evaluated: microalbuminuria-to creatininuria ratio in the stop morning sample, active vitamin D levels and the glomerular filtration rate (eGFR).

We found a strong direct correlation between the microalbuminuria-to creatininuria ratio and the vitamin D blood levels (r= - 0,786 p=0,001).

In our study, the vitamin D blood levels were predictive of increased proteinuria in type 2 diabetic patients. Further studies, with greater number of patients and with therapeutic intervention with vitamin D analogues should be carried, to confirm this beneficial role of aVD.

Disclosure of Financial Relationships: nothing to disclose

**PUB282**

**Acute Renal Failure in Diabetes: Etiology and Outcome** Saubhik Sural, Sandip K. Bhattacharya. *Nephrology, Peerless Hospital & B K Roy Research Center, Kolkata, West Bengal, India.*

**Purpose:**Some studies have shown that diabetic patients are more prone to develop ARF. An analysis was done in diabetic patients who developed ARF to determine the etiological spectrum, to find out various variables affecting the outcome and compare it with ARF in non diabetics.

**Methods:**All diabetic patients who suffered from ARF during last 3 years at the institute were studied. The patients who presented with multiorgan failure, severe shock, or expired within 24 hours of admission were excluded from the study. The study sample was divided into two groups- group1- patients with overt proteinuria (stage IV) and group2- patients without overt proteinuria. The demographic profile, cause of ARF, need for dialysis, duration of oliguria, time of recovery, degree of recovery of renal function , other complications , mortality and other variables affecting the outcome were noted. All these factors were statistically compared between group1 and group 2, and also compared with non diabetic ARF.

**Result:**Of 88 patients suffering from diabetic ARF 57 patients had overt proteinuria(group1 ) and 31 were in group2. The various causes of ARF were urosepsis (15 in group1&9 in group2), other sepsis (9 in group1 and 6 in group2, fungal infection (5 in group1 and 2 in group2), obstructive uropathy including papillary necrosis (7 in group1 and 4 in group2), drugs (9 in group 1 and 5 in group2), contrast (4 in group1 and 1 in group2), vasculitis (3 in group1 and 1in group2), other toxins like consumption of raw fish gall bladder(5 in group1 and 3 in group2). On statistical analysis papillary necrosis, Non Steroidal Anti Inflammatory Drugs(NSAIDS) and conrtast were found to be significantly more common in group1. Overall mortality was 11%. Sepsis was only significant factor predicting mortality. There was no difference in outcome between group2 and non diabetic ARF. Delayed recovery and partial recovery were more common in group1.

**Conclusion:**Diabetic patients with overt proteinuria are more prone to develop ARF, most significantly from NSAIDS compared to non diabetics and diabetics without overt proteinuria. Delayed and partial recovery is more common in this group. Mortality depends on underlying condition like sepsis.

Disclosure of Financial Relationships: nothing to disclose

**PUB283**

**Liver Enzymes Are Associated with Diabetic Nephropathy and Kidney Function in Patients with Diabetes** Dorien M. Zelle,[1] Alaa Alkhalaf,[1] Nicole Deetman,[1] Eva Corpeleijn,[2] Reinold O. B. Gans,[3] Ronald Stolk,[2] Gerjan Navis,[1] Stephan J. L. Bakker.[1] *[1]Kidney Centre, Unversity Medical Centre Groningen, Netherlands; [2]Epidemiology, Unversity Medical Centre Groningen, Netherlands; [3]Internal Medicine, Unversity Medical Centre Groningen, Netherlands.*

**Background**

Diabetes is associated with many metabolic disturbances, including fatty liver, which may lead to development of diabetic nephropathy (DN). To assess the influence of metabolic abnormalities regarding the liver, we studied the liver enzymes alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (AP) and bilirubin (BIL) in type 2 diabetic patients (DMT2) with and without DN.

**Methods**

In this case-control study, cases with DN were matched for age, sex and duration of diabetes with controls without DN. Diabetes was defined according to WHO criteria. Liver enzymes and BIL were measured by routine assays. GFR was estimated by MDRD.

**Results**

We included 32 cases and 32 controls. Median [interquartile range] duration of diabetes was 15 [10-20] , age 64±9 years and 54% male. Estimated GFR was 65±23 mL/min/1.73m² in cases vs 84±15 mL/min/1.73m² in controls (p<0.001). Urinary albumin concentration was 752[239-1074] mg/l in cases vs 3[3-6] mg/l in controls (p<0.001). In logistic regression analyses, risk for DN increased with each doubling of GGT (OR=1.85[1.0-3.4], P=0.045) and AP (OR=6.9[1.5-31.6],P=0.01), but not ALT(OR=0.9[0.4- 2.0],P=0.9). BIL (OR=0.25[0.1-0.8],P=0.03) was inversely associated with DN. This was independent of age, sex, BMI, HDL-cholesterol and blood pressure. In linear regression analyses GGT and AP were inversely associated with eGFR (GGT:$R^2$=0.33, $\beta_{STD}$=-4.8,P=0.01 ; AP:$R^2$=0.33, $\beta_{STD}$=-12.6,P=0.009), whereas BIL was positively associated with eGFR ($R^2$=0.34 $\beta_{STD}$= 9.2,P= 0.009).

**Conclusions**

GGT and AP, but not ALT, were positively associated with DN and inversely associated with kidney function. BIL was negatively associated with DN and positively associated with kidney function. Further research is needed to elucidate the mechanisms behind the protective association of endogenous BIL and the relationship between liver enzymes and DN.

Disclosure of Financial Relationships: nothing to disclose

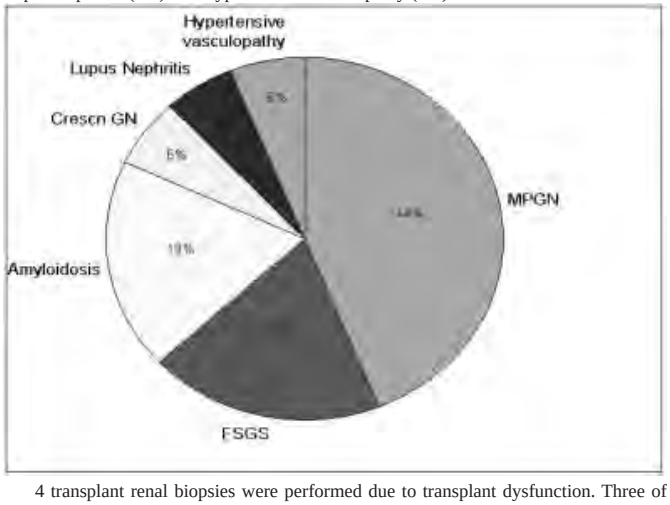
**PUB284**

**Spectrum of Renal Diseases Observed Through Renal Biopsy** Hafiz I. Ahmad, Syed Rizwan Bokhari, Muhammad Awais. *Department of Nephrology, Allama Iqbal Medical College, Lahore, Pakistan.*

We analyzed 19 serial Renal Biopsies carried out at Division of Nephrology Jinnah Hospital, Lahore over a period of 1 year from March 2009 to May 2010.Due to resource limitations only light microscopy was employed to evaluate the biopsy specimens.

Out of 19 patients 14 were male and 5 female with average age 23.9 (range 16-58). The male to female ratio was 2.8:1. Serum Creatinine ranged from 1.1 to 13.9 mg/dl (mean 3.65). Proteinuria in Nephrotic syndrome patients ranged from 3.5 to 12.7gm/24hrs (mean 5.12). All kidney biopsies were done by experienced nephrologist with automated 18 gauge biopsy needle (Bard- Monopty) using ultrasound guidance under aseptic conditions.

Out of 19 procedures 15 were performed on native kidneys and 4 on transplanted ones. The most common indication in native kidneys was Nephrotic Syndrome (80%). Three others were Nephritic Syndrome , RPGN & Lupus Nephritis; 6.6% each .The histological diagnoses were MPGN (44%), FSGS (19%), Crescentic GN (19% ), Amyloidosis (6%), Lupus Nephritis (6%) and Hypertensive Vasculopathy (6%).




4 transplant renal biopsies were performed due to transplant dysfunction. Three of them showed acute cellular rejection and one was cyclosporine toxicity.

We compared our biopsy findings with studies done in South Asian, Mid Eastern and European countries. The spectrum of our findings was most consistent with that of Nepal, i.e. membranoproliferative glomerulonephritis (MPGN) being the commonest lesion in nephrotic syndrome patients.

Our study provides an insight in the pattern of various glomerulonephritides in this country and underscores the need for more extensive data collection for optimizing management strategies.

Disclosure of Financial Relationships: nothing to disclose

**PUB285**

**Collapsing FSGS: A Clinicopathological Study** Mohammed A. Al-Ghonaim,[1] Hala M. Kfoury,[1] Abdulkareem Alsuwaida.[1] *[1]King Saud University; [2]King Saud University; [3]King Saud University.*

Background:

Collapsing FSGS is one of the recognized type of FSGS it is tend to be related to HIV infection and or IV drug use. However, no HIV related collapsing FSGS rate is increasing.It is characterized clinically by marked proteinuria and renal insufficiency and histologically by global or segmental wrinkling of glomerular basement membrane, glomerular capillary collapse, severe podocyte injury, and glomerular epithelial cell proliferation.

Method:

We reviewed renal biopsy records between 2005 and 2010 in two tertiary hospitals in Riyadh, Saudi Arabia, and identified 13 cases of Collapsing FSGS. Patients' demographic, clinicopathologic data, treatment and outcome were collected from medical records. Renal biopsies were studied by light, immunofluorescence, and electron microscopy, The histological selection criteria for Collapsing FSGS were performed according to the Columbia classification.

Result:

All the patient were young (mean age of 28 +/- 15 years), there were 7 male. All presented with nephrotic range proteinuria (24-h urinary protein: 3.5 +/- 4.9 gm/day) and the serum creatinine on presentation 171+/- 124 µmol/l. Mean follow up duration is 2.3 years. 4 patients developed end stage renal disease (ESRD). The mean serum creatinine in patient who did not develop ESRD is 132+/- 73 µmol/l.

Conclusion: the incidence of collapsing form of FSGS is increasing, clinical course tend to be variable with worst renal survival in patient presenting with high serum creatinine.

Disclosure of Financial Relationships: Employer: King Saud University.

**PUB286**

**Recovery of Renal Function in ANCA Vasculitis Continues throughout the 1st Year of Immunosuppressive Therapy** Isabelle Chapdelaine, Remi Goupil, Josee Bouchard, Clement Deziel, Stephan Troyanov. *Medicine, Hôpital du Sacré-Coeur de Montréal, Montreal, QC, Canada.*

The time to maximal renal recovery in ANCA-associated vasculitis (AAV) is uncertain. Prolonged immunosuppression can lead to severe complications while abandoning prematurely such treatments can deny patients vital renal function. We sought to determine the time course of renal recovery in patients presenting with an eGFR below 30 ml/min/1.73m2 at onset.

We identified AAV patients by reviewing all ELISA ANCA measurements performed since February 2004. Demographics, clinical and laboratory assessment at diagnosis and during follow-up were collected from medical records.

Of the 39 patients identified, 22 had an eGFR below 30 ml/min/1.73m2 at onset. Their eGFR was 13 ± 8 ml/min/1.73m2 with an age of 58 ± 16 years. The median follow-up was 36 months. All patients received oral corticosteroids, 19 pulse methylprednisolone, 20 cyclophosphamide and 12 plasmapheresis. Nineteen patients had a renal biopsy showing, on median, moderate interstitial fibrosis, 20 and 7 percent of glomeruli with crescents and global glomerulosclerosis, respectively. Over time, the eGFR slowly increased from 13 ± 9 at onset to 19 ± 11, 21 ± 13, 25 ± 15, 25 ± 15 and 28 ± 16 ml/min/1.73m2 at 1, 3, 6, 12 and 24 months, respectively ($p<0.001$ for within-subject effect) with every post-hoc pairwise comparison (by LSD method) significant except for 6 vs. 12, 6 vs. 24 and 12 vs. 24 months ($p>0.1$, 0.06 and 0.08, respectively). Ten required dialysis, 3 of which were able to exit at 5, 7 and 14 months while 4 died within the first year.

This small cohort of AAV patients with severe renal insufficiency but relatively preserved renal parenchyma displayed slow but progressive and significant eGFR improvement over at least the first year. Prudence and patience is warranted in assessing renal response to therapy.

Disclosure of Financial Relationships: nothing to disclose

**PUB287**

**Type I Membranoproliferative Glomerulonephritis as the Initial Presentation of Waldenstrom Macroglobulinemia** Emanuela Didita, Qamar Iqbal, Mirela A. Dobre, Andrew Lazar. *Internal Medicine, Huron Hospital, East Cleveland, OH.*

Introduction

Nephrotic range proteinuria is rare in patients with Waldenström macroglobulinemia. When present, it is usually due to amyloid light-chain deposition. We present the first case, to our knowledge, of type I membranoproliferative glomerulonephritis as the initial presentation of Waldenstrom macroglobulinemia.

Case report

A 61 year old female with hypertension for 10 months presented with proteinuria and the progressive deterioration of her kidney function. She had 30 pound weight loss over two years, but no gross hematuria, foamy urine, chest pain, shorthness of breath, skin changes, hemoptysis, joint pain, or headache. She was taking triameterene/hydroclorothiazide 37.5/25 mg daily, omega-3 fatty acids 1000 mg three times daily, and multiple vitamins and mineral supplements daily. Physical examination showed only trace edema in the legs. Laboratory data revealed creatinine 1.95 mg/dL, urine protein 4.38 g/24 hours, cholesterol 331 mg/dL, low density lipoprotein 232 mg/dL. Urinalyis revealed proteinuria and hematuria without casts. Cryoglobulins, rheumatoid factor, hepatitis C, antinuclear, anti-DNA and anti-neutrophil cytoplasmic antibodies, were negative. The complement levels were normal: C3 70 mg/dL and C4 14 mg/dL. Serum protein immunelectrophoresis revealed a IgM/Kappa monoclonal protein. Kappa/Lambda ration was 2.29. The kidney biopsy revealed type I membranoproliferative glomerulonephritis. The bone marrow biopsy was diagnostic of Waldenstrom macroglobulinemia. The patiend was treated with the chemotherapeutic agents Rituximab, Bendamustine and the kidney function much improved.

Conclusion

Waldenstrom macroglobulinemia should be included in the diferential diagnosis of type I membranoproliferative glomerulonephritis with negative cryoglobulins. Prompt diagnosis and treatment of macroglobulinemia is essential for the prognostic of membranoprolipherative glomerulonephritis type I, as the patients left untreated will rapidly progress to end stage renal disease.

Disclosure of Financial Relationships: nothing to disclose

**PUB288**

**Cyclosporine Therapeutic Drug Monitoring in Nephrotic Syndrome: Which Time Point To Use for a Generic Product?** Xiaoli Du,[1] Haiyun Wang,[2] Qiang Fu,[1] Limeng Chen,[2] Zhu Zhu,[1] Xuemei Li.[2] *[1]Pharmacy Department, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China; [2]Department of Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China.*

***Background*** Although C2 monitoring has been accepted as the optimal TDM strategy for cyclosporine (CsA) in organ transplantation, but there is limited information in nephrotic syndrome (NS). In China, most NS patients were prescribed generic product of CsA, and monitored by C0, without data supporting this practice. ***Objective*** To investigate the appropriate monitoring method for generic CsA product in NS patients. ***Methods*** An open-label, prospective study was carried out at Peking Union Medical College Hospital in NS patients receiving generic CsA product, Tianke®. The dose was 3-4 mg/kg/d given in two divided doses before meal. Blood samples were collected prior to (t=0), and at 1, 2, 3, and 4 h following a steady-state morning dose. CsA concentrations were assayed by HPLC. $AUC_{0-4}$ was calculated using trapezoidal method. Limited sampling strategies (LSSs) for $AUC_{0-4}$ were determined using multiple regression analysis. ***Results*** 16 patients were enrolled in this study. The C0, C1, C2, C3 and C4 were 76.6±40.0, 791.0±408.0, 574.2±284.7, 342.4±151.3, and 236.9±111.4 ng/ml, respectively. The $AUC_{0-4}$ was 1828.4±775.4 ng·h/ml. The $t_{max}$ was 1.3±0.5 h, shorter than that reported for Neoral®. C0 had the worst correlation with $AUC_{0-4}$ ($r^2$= 0.38, 0.64, 0.68, 0.76, and 0.72 for C0, C1, C2, C3, and C4, respectively). For the prediction of $AUC_{0-4}$, the LSS incorporating C1 and C2 gave the best result among the LSSs including two blood samples ($r^2$=0.99). Among the 5 time-points, only C2 showed significant difference between the patients with normal and abnormal BUN or Ccr levels ($P$<0.05). ***Conclusion*** For the investigated generic CsA product, C2 was the best single time-point sample for the prediction of CsA exposure and renal toxicity. To achieve more accurate prediction of $AUC_{0-4}$, the LSS incorporation C1 and C2 could be adopted. It is suggested that LSSs specific to brand be developed in different countries.

Disclosure of Financial Relationships: nothing to disclose

**PUB289**

**Renal Limited "Lupus-Like" Glomerulonephritis** Ana Huerta,[1] Vassilios Liakopoulos,[2] Amudha Palanisamy,[1] Andrew S. Bomback,[1] Jai Radhakrishnan,[1] Glen S. Markowitz,[1] Gerald B. Appel.[1] *[1]Columbia Univ. College of Physicians and Surgeons, NY; [2]Univ. of Thessalonika.*

***Bacgkround:*** The histopathology of proliferative lupus nephritis (LN) may be confused with other immune complex (IC)-mediated glomerulonephritides (GNs). In general, though, proliferative LN is marked by the following histolopathologic findings: 1) a "full house" IF pattern; 2) extraglomerular deposits (EGDs); 3) electron dense deposits (EDDs) at mesangial, subendothelial, and subepithelial locations; and 4) tubuloreticular inclusions (TRIs). It remains unclear whether a LN diagnosis should be given to pts with inconclusive SLE serologies and no clinical SLE symptoms when a renal biopsy shows the classical patterns of LN.

***Methods:*** We reviewed the courses of 3 pts with "lupus-like" GN. All pts had at least 3 of the above 4 histopathologic criteria for LN. Their course and serologies, however, never confirmed the diagnosis of SLE despite long-term follow.

***Results:*** Pts were all female and aged 19, 37, and 50 yrs at presentation. Biopsies were read as IC-mediated diffuse proliferative GN with dominant IgG glomerular immune deposits and variable codeposits of IgA, IgM, C3 and C1q. All biopsies showed EDD at mesangial, subendothelial and subepithelial locations, and TRIs; 2 pts' biopsies also displayed a "full house" IF pattern and/or extraglomerular deposits. All of the pts maintained negative/inconclusive serologic SLE tests and no extrarenal symptoms of SLE over mean follow-up of 3.4 yrs. All 3 pts were treated with steroids; 2 pts also received MMF or CTX. Despite therapy, all pts had a progressive decline in renal function leading to ESRD in 2 pts and CKD stage 4 in 1.

Biopsy findings

| Case | IF staining | EGDs´ location | mesangial,subendothelial & subepithelial EDD | TRIs |
|---|---|---|---|---|
| 1 | IgG,C3,C1,κ,λ | TBM & interstitial | + | + |
| 2 | IgG,IgA,IgM,C3,C1,κ,λ | - | + | + |
| 3 | IgG,IgA,IgM,C3,C1,κ,λ | TBM | + | + |

***Conclusion:*** Pts with biopsy patterns resembling LN but without extrarenal SLE manifestations or SLE-consistent serologies at diagnosis and during follow-up may constitute a unique group. These pts may be less responsive to therapy than typical LN pts. Awareness of "lupus like" GN will clarify the incidence and significance of this population.

Disclosure of Financial Relationships: nothing to disclose

**PUB290**

**Plasmapheresis in the Hemodialysis Unit: Single Center Experience** Rumeyza Kazancioglu, Meltem Gursu, Serhat Karadag, Zeki Aydin, Savas Ozturk, Sami Uzun, Emel Tatli. *Nephrology, HaseKi Training and Research Hospital, Istanbul, Turkey.*

**INTRODUCTION:** Theurapeutic plasma exchange (TPE; plasmapheresis) is a mode of extracorporeal therapies applied by membrane separation or centrifugation method; the first method more commonly used. We present the analysis of plasmapheresis results of our unit.

**METHODS:** Patients who had plasmapheresis within 2009 were included. Age, gender, primary disease, number of series and sessions, replacement fluid, complications and the outcome were recorded.

**RESULTS:** 41 patients had plasmapheresis with mean age and male/female ratio of 45.17±16.49years and 18/31, respectively. The indication for plasmapheresis was crescentic glomerulonephritis(GN) in 8, post-transplant GN in 6, Wegener's granulomatosis(WG)/microscopic polyangiitis(MPA) in 6, pre-transplant PRA(panel reactive antibody) positivity in 5, HUS(Hemolytic uremic syndrome)/TTP(Thrombotic thrombocytopenic purpura) in 5, neurological demyelinating diseases in 3, multiple myeloma(MM) in 3, systemic lupus erythematosus(SLE) in 1, FSGS(focal segmental glomerulosclerosis) resistant to conventional treatments in 2 patients and FSGS prior to transplantation in 1 patient. 35 patients had 1 (5.9±3.3 sessions), 5 patients had 2 series(5.5±4.5 sessions) and 1 patient had 10 series(4.2±2.3 sesions) of plasmapheresis. The replacement fluid was fresh frozen plasma in 34 and human albumin in 7 patients. The outcomes are presented in Table 1.

Table-1

| | Complete response | Partial response | No response |
|---|---|---|---|
| Crescentic GN | 1 | 3 | 4 |
| Post-transplant GN | 0 | 3 | 3 |
| WG/MPA | 0 | 5 | 1 |
| Pre-transplant PRA positivity | 0 | 3 | 2 |
| HUS/TTP | 2 | 1 | 2 |
| MM | 1 | 2 | 0 |
| FSGS resistant to other treatments | 0 | 2 | 0 |
| FSGS prior to transplantation | 1” | 1 | 0 |
| Neurological demyelinating disease | 2 | 0 | 1 |
| SLE | 1 | 0 | 0 |

Outcome of the primary disease

**DISCUSSION:** Although plasmapheresis has been used mostly for non-nephrological diseases; it is a procedure convenient to use in hemodialysis units due to similarities regarding technique and complications. In cities where there is no unit specialized for extracorporeal treatments as in our city; HD units may improve their practice about TPE and provide early treatment of mentioned diseases.

Disclosure of Financial Relationships: nothing to disclose

## PUB291

**Clinical Characteristics of Patients with MPO-ANCA-Positive Interstitial Pneumonia with or without Glomerulonephritis** Kiyoki Kitagawa,[#1] Akinori Hara,[#1] Kengo Furuichi,[#1] Takashi Wada.[#2] *[1]Department of Disease Control and Homeostasis, Kanazawa University, Kanazawa, Japan; [2]Department of Laboratory Medicine, Kanazawa University, Kanazawa, Japan.*

Lung and kidney are two major target organs in patients with MPO-ANCA related vasculitis. In this study, we investigated the clinical characteristics of MPO-ANCA-positive interstitial pneumonia (IP) Japanese patients with or without glomerulonephritis (GN). A total of 7 patients who were diagnosed as MPO-ANCA-positive IP in Kanazawa University Hospital was examined in this study. The patients were followed based on the diagnosis as IP to onset of GN or May 31, 2010 (mean period 1541±470 days). Three patients (43%) developed GN during the follow-up period (GN group: mean age 57.0±9.4, female 2, male 1). The others never showed renal involvement until May 31, 2010 (non-GN group: mean age 59.0±3.4, female 3, male 1). A mean period from diagnosis of IP to onset of GN was 1927±1025 days. The levels of MPO-ANCA during follow-up period were higher in GN group (at diagnosis of IP : 178±48 EU vs. 34±15 EU, p=0.02, at end point : 295±94 EU vs. 75±49 EU, p=0.07). However, at the time of the diagnosis of IP, none of urinary findings, renal function, serum CRP, respiratory function tests, Birmingham vasculitis activity score and vasculitis damage index had statistical difference between GN group and non-GN group. At the end point of this study, neither respiratory function tests nor doses of prednisolone differed between both groups.

In conclusion, the level of MPO-ANCA may be a predictive factor for subsequent onset of GN in patients with MPO-ANCA-positive IP.

Disclosure of Financial Relationships: nothing to disclose

## PUB292

**Characteristics of Patients Seen in Combined Renal-Rheumatology Clinic at Royal Brisbane and Women’s Hospital, Brisbane, Australia** Thaminda Liyanage, Helen G. Healy, Dwarakanathan Ranganathan. *Department of Renal Medicine, Royal Brisbane and Women’s Hospital, Brisbane, Queensland, Australia.*

Background

Patients with connective tissue diseases with multisystem involvement require long term follow up in different sub speciality clinics simultaneously. Royal Brisbane and Women’s Hospital combined Renal-Rheumatology clinic was implemented in July 2009 in an attempt to improve patient care and satisfaction, reduce burden of attending too many clinics, improve quality of clinical training and trainee satisfaction.

Aim

This descriptive study looked at patient characteristics in renal-rheumatology clinic from July 2009 to January 2010.

Method

Case notes of all patients followed up at Renal-Rheumatology clinic up to January 2010 were reviewed. Patients’ demographics, diagnosis at referral, dependency on maintenance dialysis were recorded. Descriptive statistics included number of subjects (n), age range, mean and standard deviation for age.

A patient satisfaction survey conducted with anonymous questioner.

Results

46 patients included- 29 females and 17 males; 30 had Systemic Lupus Erythematosus (SLE), 6 had other connective tissue disorders, 7 had vasculitis and diagnosis was uncertain in 3 patients. 20/30 (66%) of SLE patients were females. Only 7 (15%) required maintenance dialysis and most of them had SLE (5/7).

Overall age ranged from 18-82 years with mean age of 47.89 years (SD 17.00 years). Age of females ranged from 21-77 years with mean age of 47.96 years. Age of males ranged from 18-82 years with mean age of 47.76 years.

26/46 took part in patient satisfaction survey.

Patient satisfaction survey results

| Question | Mean score |
|---|---|
| Provides better care | 3.10 |
| Saves time | 3.86 |
| Saves money | 4.10 |
| Gets quick clinical decisions | 2.90 |
| Better than attending 2 different clinics | 4.40 |

Table 1

Conclusion

Patients and trainees expressed satisfaction in the combined clinic. As this is a monthly clinic most patients were seen only once or twice hence too early to assess clinical improvement. Patients have to be followed up for a longer period before one can establish whether combined clinic improves quality of care and clinical outcomes.

Disclosure of Financial Relationships: nothing to disclose

## PUB293

**Membranous Lupus Nephritis: A Single Center Experience** Smaragdi Marinaki,[1] Chrysanthi Skalioti,[1] Irene Synodinou,[1] Argyrios Georgalis,[1] Lydia Nakopoulou,[2] Charalambos Moutsopoulos,[3] John Boletis.[1] *[1]Nephrology and Transplantation Unit, Laiko Hospital, Athens, Greece; [2]Pathology Department, University of Athens, Athens, Greece; [3]Pathophysiology Department, University of Athens, Athens, Greece.*

Purpose: To evaluate the clinical characteristics, the therapy and the outcome of patients with membranous lupus nephritis.

From a single center cohort of 195 patients with biopsy proven lupus nephritis performed between 2000 and 2009, 29(15%) patients had pure membranous lupus nephritis (class V). We retrospectively analyzed the data that were available in 14(13 women,1 man) of them. Mean follow-up was 28.4 months, mean age was 39±9 years and mean SLE duration 55±51 months. In 10/14 patients membranous was the first manifestation of lupus nephritis, whereas in 4/14 patients it was conversion from another class. All patients but two (86%), had normal renal function at presentation. Nine patients (64%) presented either with nephrotic syndrome or with nephrotic range proteinuria while 5/14 had proteinuria <3g/24h at diagnosis. Complement levels were normal. Three patients underwent a second renal biopsy, one because of ongoing activity and two because of relapse after complete remission. There was no conversion to other classes of lupus nephritis. Eleven patients were treated with an ACE inhibitor. A total of seventeen episodes were treated in 14 patients. Most of them (12/17,71%) with MPA (2-3mg/day) and corticosteroids (0.5-1mg/Kg/day for the 1st month). In 5/12, the anti CD-20 mAb Rituximab was added. Two were treated with iv monthly pulses of cyclophosphamide and corticosteroids, 1/17 with corticosteroids only, one with Rituximab and corticosteroids, while one patient received no immunosuppression. Partial remission was achieved in 4/14(28.6%) patients after 4.5 months and complete remission in 10/14(71%) after 12 months. Complete remission was sustained in 9/10 patients. No episode of infection that needed hospitalization occurred. In conclusion, in our cohort, we observed the same rate of membranous lupus nephritis (15%)as reported in other series. MPA based therapy seems to be safe and effective with a complete remission rate of 71%.

Disclosure of Financial Relationships: nothing to disclose

## PUB294

**Increased Activity of (pro) Renin – Angiotensin System in Patients with Primary Chronic Glomerulonephritis** Magdalena Mostowska, Elzbieta Pawliczak, Andrzej P. Oko, Ilona Piechocka, Stanislaw Czekalski. *Department of Nephrology, Transplantology and Internal Medicine, Poznan University of Medical Sciences, Poznan, Poland.*

Introduction and aims:

Abnormal activation of (pro) renin – angiotensin system ((P)RAS) is involved in kidney damage in patient with primary chronic glomerulonephritis (GN). Not only angiotensin II (Ang II), but also prorenin and renin as agonists of prorenin - renin receptor can induce glomerulosclerosis, interstitial fibrosis and pathological proliferation. Till now, elevated plasma level of prorenin was detected in patients with diabetes mellitus and it was considered as an early indicator of development of diabetic kidney disease. The aim of the study was an evaluation of plasma (P)RAS components and to determine their relationship with largenes of proteinuria in primary chronic GN.

Material adh methods:

Fifty five (31 woman, 24 men) untreated patients (without immunosupressive and antihypertensive drugs) aged 39.9 ± 14.8 years with primary chronic GN in stages 1 – 3 of chronic kidney disease and 20 healthy persons (10 woman, 10 man) aged 36.8 ± 9.3 years participated in the study. Prorenin and Ag II levels in plasma were measured by ELISA while renin plasma concentration was measured by radioimminological method. In all patients 24 – hour urinary protein excretion was also evaluated.

Results:

Significantly higher plasma prorenin and Ang II levels ($p = 0.0003$ and $p = 0.002$, respectively) in patients with primary GN when compared to healthy controls were found. In contrast there was no difference in renin concentration in plasma between both groups. In patients with primary GN, plasma prorenin concentration was significantly correlated with 24 – hour urinary protein excretion ($r = 0.406$, $p = 0.002$).

Conclusions

In patients with primary chronic GN plasma concentrations of prorenin and Ang II are increased. Positive correlation between plasma prorenin concentration and proteinuria may suggest the role of this prohormone in glomerular damage.

Disclosure of Financial Relationships: nothing to disclose

**PUB295**

**A Case of Membranous Nephropathy Due to Antiphospholipid Antibody Syndrome** Saurabh A. Pande,[1] Khurram Mumtaz,[1] Kanwal Raghav,[2] Eric J. Bloom,[1] Rasib Raja.[1] *[1]Nephrology, Albert Einstein Medical Center, Philadelphia, PA; [2]Internal Medicine, Albert Einstein Medical Center, Philadelphia, PA.*

Introduction: Membranous nephropathy (MN), the most common cause of nephrotic syndrome in adults is classified as either primary or secondary. We report a case of secondary MN due to a rare etiology.

Case: 48-yr-old African American man presented with headache and blurry vision for a day. He also reported lower extremity swelling, foamy urine and chronic back pain for which he was using ibuprofen for the past year. Labs were consistent with nephrotic syndrome with a serum creatinine of 1.4mg/dL, serum albumin of 1.7g/dL and spot urine protein and creatinine ratio of 9mg/g. MRI of brain showed multiple acute posterior circulation infarcts. CT of abdomen revealed left renal vein thrombosis. Anticoagulation was started and a work-up for a hypercoagulable state was ordered.

Renal biopsy showed membranous nephropathy. Serologies for hepatitis B and C, HIV, syphilis, cryoglobulinemia and lupus were negative. Anti-phospholipid antibody titer was elevated. In the absence of other secondary etiologies, he was diagnosed as a case of membranous nephropathy (Grade 2 by Ehrenreich and Churgs classification) secondary to anti-phospholipid antibodies or NSAIDs use. The former is likely since the proteinuria did not resolve after cessation of NSAIDs use.

Discussion: MN has an identifiable cause in only 20% of cases. Minimal change glomerulopathy is often associated with NSAIDS use but association between NSAIDS and MN is uncommon. Several cases of NSAIDs induced MN have been reported, but most of them revealed a clear temporal association between NSAIDs cessation and resolution of proteinuria, which did not happen in our case. Fakhouri et al. reviewed kidney biopsies of 29 patients with primary antiphospholipid syndrome in the absence of associated systemic diseases, mainly SLE. Of these, 20 showed typical features of vascular APS nephropathy while the 9 showed predominant pathological features distinct from the vaso-occlusive disease characteristic of common vascular APS nephropathy. These atypical biopsies included 3 cases of membranous nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**PUB296**

**Cyclosporin A Therapy for Henoch-Schönlein Nephritis with Nephrotic-Range Proteinuria** Jee Min Park,[1] Ki Soo Pai,[1] Jae Il Shin.[2] *[1]Department of Pediatrics, Ajou University School of Medicine, Suwon, Republic of Korea; [2]Department of Pediatrics, Yonsei University College of Medicine, Seoul, Republic of Korea.*

To evaluate the therapeutic role of cyclosporin A (CyA) for the treatment of Henoch-Schönlein nephritis (HSN), twenty nine patients (18 boys, 11 girls) with nephrotic-range proteinuria were analyzed retrospectively. Mean age was 8.6 years (range 2.0-15.5 years) at diagnosis of Henoch-Schönlein purpura (HSP). All patients had developed the nephrotic-range proteinuria at a mean interval of 4.4 months (range 0-50.7 months) after the diagnosis of HSP. Mean duration of CyA treatment were 12.3 months (range 2.6-55.0 months). Mean follow-up times were 3.7 years (range 1.2-12.9 years) from the beginning of the CyA treatment. Steroids had tapered and stopped gradually after initiation of CyA. All patients responded to the CyA treatment within a mean of 1.8 months (range 1 weeks to 3.5 months). Twenty three patients achieved a stable remission with mean follow-up duration of 3.2 years and 6 patients seemed to become CyA dependent, since they developed proteinuria when the treatment was stopped. Renal function was preserved in all patients but one who developed to end stage renal disease because of poor compliance of CyA. We conclude that CyA treatment for HSN showing nephrotic-range proteinuria is effective and safe although some patients seem to develop CyA dependency.

Disclosure of Financial Relationships: nothing to disclose

**PUB297**

**Renal Outcome in Minimal Change Disease Associated with Mysthenia Gravis: A Case Series Report** Lawand A. Saadulla, Navin Verma. *Nephrology, PSU/ Hershey Medical Center, Hershey, PA.*

Minimal change disease (MCD) is the most common lesion observed in patients with thymoma-associated autoimmune disorders, such as myasthenia gravis (MG). It can also present few years after removal of thymomas. Long-term renal prognosis and outcome following immunosuppressive therapy are largely unknown. We present two cases of MCD and MG with varying response to immunosuppressive agents and renal outcome.

The first patient is a 63-year-old male with three year history of MG admitted to our hospital with acute renal failure (ARF) and anasarca. His urine protein to creatinine (UPC) ratio was 13g/day. Renal biopsy revealed MCD, and he was treated with various immunosuppressive regimen, including corticosteroids, calcineurin inhibitors and rituximab. Unfortunately, his renal function deteriorated, and he required hemodialysis and has been dialysis dependent since then.

The second patient is a 68-year-old male with MG for two years presented with ARF, nephrotic syndrome and anasarca. His UPC ratio was 14g/day. Renal histology revealed features of MCD. He was treated successfully with high dose corticosteroid and mycophenolate mofetil (MMF). He achieved complete remission in a few weeks and remains in remission for nine months now.

To our knowledge, the literature contains only a few other published cases of glomerulopathy associated with thymic-disease. This association is most likely underdiagnosed. The role of T-cell immune disorder and circulating cytokines has been postulated as a possible pathogenesis of selective proteinuria associated with MCD. The beneficial response to immunosuppressive therapy in one of our patients supports this theory. Various immunosuppressive regimens-other than MMF- have been used. However, a definitive conclusion cannot be drawn regarding the effectiveness of one treatment over another in improving the renal outcome due to the limited numbers of identified cases in the literature. Our report is the first to suggest the combination of MMF and prednisone as a viable treatment option in treating patients with MCD associated with MG.

Disclosure of Financial Relationships: nothing to disclose

**PUB298**

**Immunotactoid Glomerulopathy (Chronic Lymphocytic Leukaemia Associated): Response to Treatment with Rituximab-Cyclophosphamide, Vincristine and Prednisolone** Vikas Srivastava. *Renal Unit, Flinders Medical Centre, Adelaide, SA, Australia.*

Background – Immunotactoid (or fibrillary) glomerulopathy is a very rare cause of glomerulonephritis, with deposition of fibrillar material, renal impairment and/or proteinuria. It can be associated with lymphoproliferative disorders, but optimal treatment strategies have not been defined. We describe a case in which treatment was associated with remission of proteinuria and stabilisation of renal function.

Case report- A 66 year old female presented with renal impairment (Creatinine 154 µmol/L, eGFR 29ml/min), hypertension (160/100), nephrotic range proteinuria (5.5g /24 hours), anaemia and lymphocytosis. Renal biopsy revealed an immunotactoid glomerulopathy with mesangiocapillary glomerulonephritis on light microscopy and fibrils visualised on electron microscopy of 20-30 nm in diameter, some with a microtubular appearance. Bone marrow biopsy showed cellular infiltrates, 90% consisting of mature lymphocytes consistent with B-cell CLL. Our patient received four cycles of Rituximab/ Cyclophosphamide, Vincristine and Prednisolone at three weekly intervals with anti-hypertensives. With this treatment her proteinuria has reduced significantly to 0.19 g/24 hours, she has normal normal blood pressure and renal function (creatinine 100 µmol/L), and a recent bone marrow biopsy confirmed marked depletion in B cells (<1% by flow cytometry).

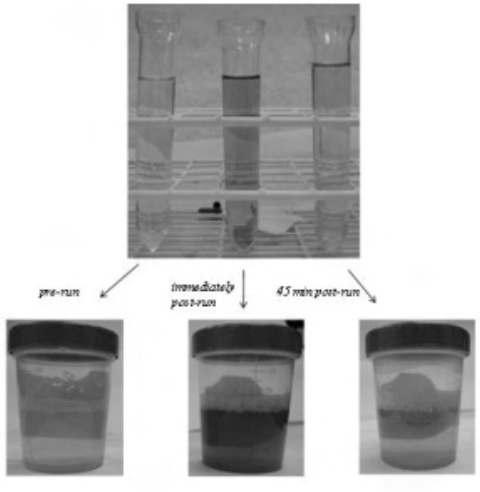
Conclusion - Whilst most cases of fibrillary glomerulonephritis and immunotactoid glomerulopathy are idiopathic, some cases are associated with chronic lymphocytic leukaemia (CLL) or other B-cell lymphomas. In such patients, therapy directed at the underlying disorder may be effective and despite the striking fibrillary deposition can be associated with marked improvements in renal disease. This case illustrates an excellent outcome of a treatment strategy in a disease whose rarity precludes large scale randomised trials and concurs with other reports of benefit of Rituximab in this condition (Collins et al, Am J Kidney Dis. 2008 Dec;52(6):1158-62).

Disclosure of Financial Relationships: nothing to disclose

**PUB299**

**Operation Iraqi Freedom Amputee with Running Induced Pigmenturia** Pallavi Belur,[1] Meagan M. Rizzo.[2] *[1]Department of Medicine, Walter Reed Army Medical Center (WRAMC); [2]Nephrology Service, WRAMC, Washington, DC.*

Reports of exercise induced concomitant hemoglobinuria and myoglobinuria are rare. Furthermore there are no reports of it in amputees in the literature. A healthy 29-year-old Operation Iraqi Freedom (OIF) soldier had a left below the knee amputation (BKA) in January 2005. In September 2005 he developed transient red urine after running with his prosthesis for ≥ 20 minutes. In 2010 he presented to Nephrology with persistent symptoms. His urine was initially unremarkable but post-run was grossly red and positive for blood and protein on dipstick; microscopy showed granular casts.




After centrifugation the supernatant remained red leading to the diagnosis of pigmenturia. Urine myoglobin and hemoglobin were markedly elevated post run. Urinary findings improved after 45 minutes. He was never anemic and had normal renal

function.

Laboratory Data

| Urine | Pre-run | Post-run | 45 min post run | Reference |
|---|---|---|---|---|
| Myoglobin | <27 | 1220 | 806 | <28mcg/L |
| Hemoglobin | 1.1 | 8.6 | 2.2 | < 1mg/dL |
| Spot protein/creatinine | 0 | 1.116 | 0.481 | <0.300 (ratio) |
| Microscopy | no casts | moderate granular casts | few granular casts | |

In conclusion, this soldier developed myoglobinuria, hemoglobinuria, protienuria and granular casts after a short run with his prosthesis. After reviewing the limited literature available, we recommended obtaining a new prosthetic with more shock absorbing ability and encouraged non-erect exercises (i.e. biking or swimming). We also advised decreasing running or running on softer surfaces such as a treadmill or on the grass to reduce the local trauma to the stump. Recognition of this process is important given the number of soldiers returning from deployments with amputations after improvised explosive device (IED) blast injuries. Questions still remain about the long term renal prognosis for such patients with transient, recurrent pigmenturia.

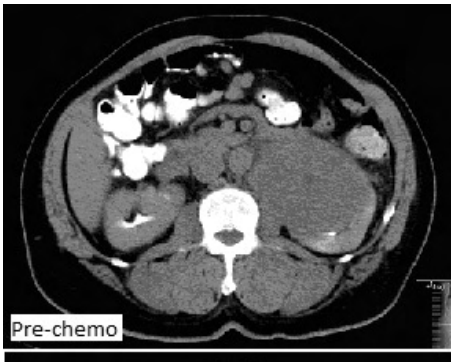
Disclosure of Financial Relationships: nothing to disclose

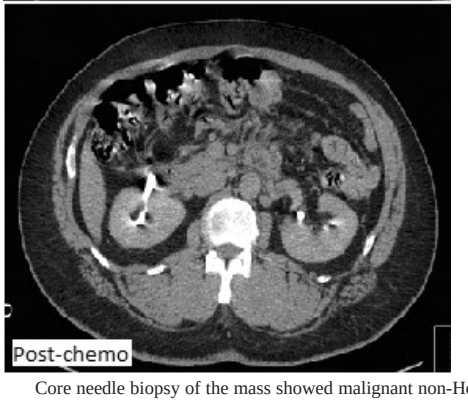
**PUB300**

**A Rare Cause of a Large Unilateral Renal Mass** Amarpali Brar, Moro O. Salifu, Syed I. Shah, Getinet Ayalew, Gurinder S. Sidhu. *Medicine, SUNY Downstate, Brooklyn, NY.*

Renal masses are a frequent incidental radiological finding. Renal cell carcinoma (RCC) is the most common primary renal malignancy, however primary renal lymphoma (PRL) is rare.

We report a case of a 73-year-old man who presented with left flank pain. He had no hematuria, and other than 20 lb weight loss, had no other complaints. On examination, he had no palpable flank mass, no temporal wasting and no palpable lymphadenopathy. CT scan revealed a large soft tissue mass measuring 11 cm in the upper pole of the left kidney. The mass abutted the aorta and surrounded the left renal vessels.




Core needle biopsy of the mass showed malignant non-Hodgkin lymphoma, large B cell type. The malignant cells stained positive for lymphocyte common antigen and CD-20; and negative for CD-3. Bone marrow biopsy didn't show any involvement by lymphoma. Positron Emission Tomography (PET) showed intense uptake in the left kidney (SUV-26). He was started on multi-agent chemotherapy with rituximab, cyclophosphamide, vincristine, doxorubicin and prednisone (R-CHOP). Soon after starting chemotherapy, patient's pain improved. Patient tolerated chemotherapy well, except for mild sensory peripheral neuropathy. He had complete resolution of the renal mass on CT scan and repeat PET scan 6 months later showed physiologic distribution of radiotracer. Two years after initial presentation he continues to do well and remains disease free.

PRL is a rare tumor of the kidney. The prognosis of PRL is poor in the cases reported in the literature. Our case demonstrates the effectiveness of R-CHOP in treatment of PRL, and that prognosis can be good after completion of chemotherapy. Chemotherapy can be safely administered despite large tumor involving the kidney. This case also demonstrates the usefulness of PET scan in assessing initial disease staging and then the response to chemotherapy.

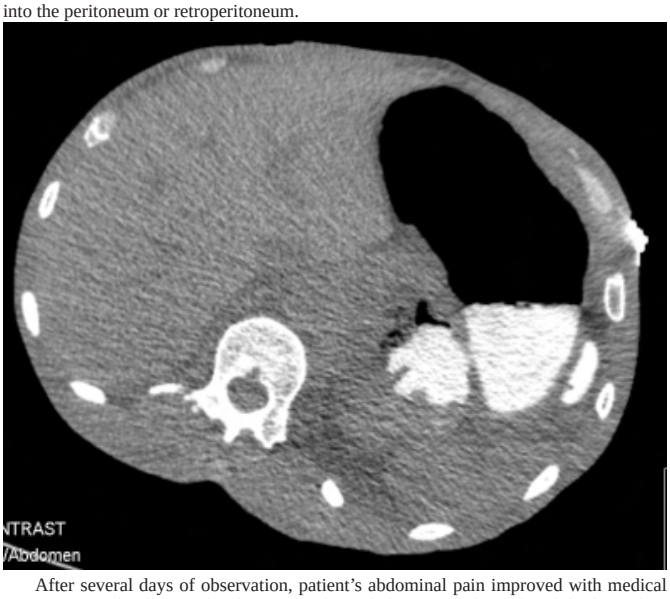
Disclosure of Financial Relationships: nothing to disclose

**PUB301**

**A Rare Complication Following Treatment of Left Sided Renal Cell Carcinoma (RCC)** Amarpali Brar, Ratesh Khillan, Getinet Ayalew, Gurinder S. Sidhu. *Medicine, SUNY Downstate Medical center, Brooklyn, NY.*

RCC is the most common cancer of the kidney. Radical nephrectomy (RN) can be curative for localized RCC and may improve survival in metastatic disease.

We report a case of a 52-year-old woman who presented with left flank pain and 20 lb weight loss. Her physical exam was unremarkable except for bitemporal wasting. CT scan revealed a large left upper pole renal mass. The mass was 7 cm, inseparable from the adrenal, abutting the pancreas and left renal vessels; with retroperitoneal lymphadenopathy. Percutaneous biopsy showed RCC, clear cell type. She was found inoperable at the time of surgery; and was started on sorafenib with control for several months; and then sunitinib. Upon increase in the mass, she was switched to temsirolimus. After having had temsirolimus for 12 weeks, she returned with abdominal pain and nausea. Physical exam was unremarkable but her performance status was poor. CT scan showed that the renal mass was smaller than before, had central necrosis and had eroded into the posterior wall of the stomach; and no other distant metastases. The oral contrast was seen in the stomach with direct flow into the necrotic center of the mass; with no extravasation of the contrast into the peritoneum or retroperitoneum.




After several days of observation, patient's abdominal pain improved with medical management; she did not develop any peritoneal signs and tolerated oral feeds. Patient chose non-surgical management and was transferred to a hospice.

This case illustrates the local problems that may result from an unresected RCC, despite absence of distant metastasis. The stomach invasion could be due to tumor necrosis as a result of temsirolimus or direct tumor extension. RCC can have severe local complications even in the absence of metastases; RN at initial presentation should be considered to prevent local complications.

Disclosure of Financial Relationships: nothing to disclose

**PUB302**

**Mycophenolate (Myfortic®) in Autoimmune Diseases: Effect on Clinical Course and Chemical Parameters** Helga Frank,[1] Sabine Seibel,[1] Martin Wittner,[2] Uwe Heemann.[1] *[1]Nephrology, Technical University Munich, Munich, Germany; [2]Dialyse Ebersberg, KfH, Ebersberg, Germany.*

Background:

The enteric-coated formulation of mycophenolate sodium (EC-MPS; Myfortic®), a reversible inhibitor of inosine monophosphate dehydrogenase with delayed release of MPS, is used to prevent rejection of renal allografts.

The objective of our prospective non-randomized open pilot phase III longitudinal study was to evaluate the effect of EC-MPS in antibody-associated autoimmune diseases with respect to the function of the affected organs, and immunserological course of antibodies.

Methods

Twenty-five patients (10 males; mean age: 49.3±12 years) with new-onset or relapsed autoimmune disease were consecutively enrolled. Underlying diseases were: ANCA-associated vasculits (n=7; c-ANCA: n=5, p-ANCA: n=2), IgA-nephritis (n=7), systemic lupus erythematodes/SLE (n=4), sclerodermia (n=2), membranous glomerulonephritis (n=2), dermatomyositis (1), sharp syndrome (1), focal glomerulosclosis with psoriasis (1). Patients received EC-MPS with a target daily dose of 2 x 720 mg/day combined with orally corticosteroids. Primary endpoints were function of the affected organ, course of chemical and immunserological results (ANA; ANCA; c3, c4) and self-assessment by questionary (SF 36) 3 (A) and 12 months (B) after treatment start.

Results:

After a mean follow-up of 45±12 weeks, creatinin-clearance (CCl) rose in patients with renal affection (n=11) from 87±50(baseline) to 92±50 (A) and 113±41 ml/min (B). Proteinuria decreased from 1.95±1.57 to 1.4±0.9 (A) and 1.24±0.7 g/gCrea (B). In patients

with extrarenal affections(n=19), symptoms (arthralgia, scleritis, cutaneous/muscular/neurological manifestations) improved in n=17 patients. ANA/ANCA (initially positive in n=14/7) improved in n=6/5. During the study period, 6 patients were hospitalized due to infections (urinary/respiratory tract: n=3/3). Two patients with cANCA-vasculitis and 1 with sclerodermia progressed and treatment was extended to cyclophosphamide. One patient with SLE died from cardiovascular disease. EC-MPS was well tolerated in terms of GI side effects.

Conclusion

EC-MPS can be a useful and steroid-sparing immunosuppressant in various autoimmune diseases.

Disclosure of Financial Relationships: nothing to disclose

**PUB303**

**The Trend of Anti-Hypertensive Agents of Incident Dialysis Patients Prior to Regular Hemodialysis** Yen Ni Hung,[1] Yee-Yung Ng,[2] Shiao Chi Wu.[1] [1]*Institute of Health and Welfare Policy, National Yang Ming University, Taipei, Taiwan;* [2]*Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan.*

Background

The ACEI and ARB have been reported to be beneficial to slow down the deterioration of renal function even in the patients with renal failure. The condition of administration of these agents in incident patient prior to regular dialysis is seldom investigated.

Methods

We retrospectively enrolled 23907 adult incident patients who began regular dialysis between January 1 2004 and December 31, 2006. All incident patients with first payment code for ACEI and ARB before 3 years of beginning regular dialysis were identified to analyze the condition of ACEI and ARB administration.

Results

In this study, we found that there is a trend of reducing administration of these agents for the incident dialysis patients closing to the regular dialysis.

The 3-years antihypertensive agents of incident patients before regular dialysis

| | Total | year 2004 | year 2005 | year 2006 |
|---|---|---|---|---|
| | number (%) | number (%) | number (%) | number (%) |
| The 1st year of anti-hypertensive agents prior to HD | | | | |
| ACEI inhibitors (%) | 465 (1.95) | 156 (2.03) | 164 (1.93) | 145 (1.87) |
| CCBs (%) | 2929 (12.25) | 910 (11.86) | 993 (11.7) | 1026 (13.24) |
| ARB (%) | 1156 (4.84) | 378 (4.93) | 398 (4.69) | 380 (4.9) |
| The 2nd year of anti-hypertensive agents prior to HD | | | | |
| ACEI inhibitors (%) | 847 (3.54) | 282 (3.67) | 314 (3.7) | 251 (3.24) |
| CCBs (%) | 3759 (15.7) | 1235 (16.09) | 1283 (15.12) | 1241 (16.02) |
| ARB (%) | 1777 (7.43) | 522 (6.8) | 644 (7.09) | 611 (7.89) |
| The 3rd year of anti-hypertensive agents prior to HD (%) | | | | |
| ACEI inhibitors (%) | 1156 (4.84) | 383 (4.99) | 439 (5.17) | 334 (4.31) |
| CCBs (%) | 4043 (16.91) | 1356 (17.67) | 1450 (17.09) | 1237 (15.97) |
| ARB (%) | 1864 (7.8) | 501 (6.53) | 695 (8.19) | 668 (8.62) |
| Total | 23907 | 7674 | 8485 | 7748 |

In conclusion, the reason of decreasing trend of ACEI and ARB administration in incident dialysis patients prior to regular dialysis should be further investigated in order to keep thses agents for slowing down the deterioration of renal function, and then delay the dialysis.

Disclosure of Financial Relationships: nothing to disclose

**PUB304**

**Acute Diabetes Insipidus in Tramatic Brain Injury** Kyung Soo Kim, Min Ji Lee, Bernice Kim, Eun Jin Kwon, Sung Zoon Shin. *Division of Nephrology, Department of Medicine, Dongguk University Ilsan Hospital, Goyang City, Kyunggi-do, Korea.*

**Aim**

Although Central Diabetes Insipidus (CDI) is a well-known complication, which is caused by a lack of the vasopressin, and induces severe dehydration, electrolyte imbalance, and cardiovascular collapse, almost all of research papers have been reported without making any mention of a contribution of nephrologist care or it's effect on the outcome.

The aim of this study was to evaluate the incidence and risk factors for acute CDI in traumatic brain injury, and assess the effect of nephrologist care on the morbidity and mortality.

**Method**

We reviewed medical data, retrospectively, of patients with CDI complicated in traumatic brain injury from Jan 2007 to Dec 2009. CDI was diagnosed if plasma sodium was > 150 mmol/L in the presence of polyuria of > 3 L/24 hours in the acute clinical setting, or if, after an overnight water deprivation test or an 8-hour observed water deprivation test, urine osmolality was > 600mOsmol/kg, and desmopressin acetate was used.

**Results**

CDI occurred in 34 (11%) patients among 300 patients who diagnosed of traumatic brain injury. The mean age(±SD) was 51.5(±15.8) years old, and the number of male gender was 20 (58.8%) in CDI patients. Independent risk factors for CDI in traumatic brain injury were cerebral edema, low Glasgow coma scale, and hypotension needed more than 2 inotropics. On 34 CDI patients, the 8 were under nephrologist care with other specialties including neurologists or neurosurgeons, and they demonstrated better survival rate (15 deaths in 26 CDI patients without nephrologist care (58%) vs. 3 deaths in 8 with nephrologist care (38%)), but not a statistically significant.

**Conclusion**

The CDI in traumatic head injury is not an unusual complication. Cerebral edema, low Glasgow coma scale, and severe hypotension were associated with development of CDI among the patients with traumatic brain injury.

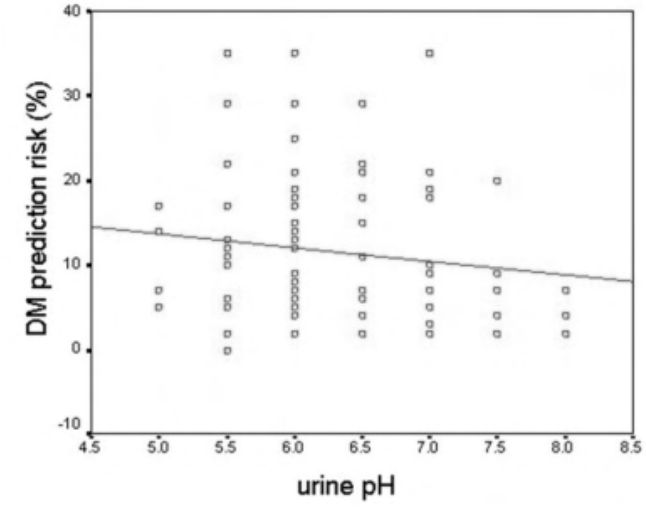
Although CDI is associated significantly with increased morbidity and mortality, nephrologist care might have a beneficial effect on the outcome. It will be needed of nephrologists to take an active part for the proper management of CDI patients in these critically ill settings.

Disclosure of Financial Relationships: nothing to disclose

**PUB305**

**Urine pH as a Predictor of Developing Type 2 Diabetes** Omar H. Maarouf, Majed Samarneh, Layla Kamal, Isabelle Ayoub, Rabih Nasr, Samer El Zarif, Marie Abdallah, Suzanne E. El Sayegh. *Medicine, Staten Island University Hospital, Staten Island, NY.*

The role of urine pH in predicting the risk for developing diabetes mellitus remains unclear. We hypothesize that as urine pH increases, the risk of developing diabetes mellitus decreases. We performed a retrospective cohort study of patients admitted to Staten Island University Hospital during the period from June 2008 to June 2009. Patients with conditions that are known to influence the urine pH were excluded: CKD (defined as GFR< 60 for over 3 months), urinary tract infection, acute kidney injury, and chronic indwelling bladder catheter. The final study cohort included 92 subjects who had an appropriate database to use the Atlanta diabetes risk prediction model for estimation of the diabetes risk score. Urine pH was inversely correlated with the diabetes risk prediction score but this was not statistically significant (r= -0.14, p=0.20). Univariate analysis showed that BMI ≥ 30 kg/m$^2$ was associated with a lower urine pH but this was not statistically significant. There was no association between urine pH and hypertension, low HDL or elevated TG. Our study had a few limitations. First, it is a retrospective cohort without a long term follow-up. Another limitation is the limited number of participants in the final cohort. Moreover, we have not validated the accuracy of a urine dip in calculating the urine pH for this study. Our study is the first to examine the relationship between urine pH as a marker of insulin resistance and the risk of developing diabetes. We found an inverse relationship between urine pH and the risk of developing diabetes although not statistically significant.




Urine pH is a promising marker of insulin resistance. If validated, urine pH has the potential to become one of the screening tools for diabetes in the primary care setting.

Disclosure of Financial Relationships: nothing to disclose

**PUB306**

**Non-Embolic Renal Infarction: Association with Fibromuscular Dysplasia: 16 Year Experience with 22 Patients, 1994-2010** Lionel U. Mailloux,[1] Susana Hong,[1] Craig Greben,[2] Eric John Gandras.[2] [1]*Department of Medicine/Nephrology, North Shore University Hospital, Port Washington, NY;* [2]*Department of Interventional Radiology, North Shore University Hospital, Manhasset, NY.*

Renal infarction (RI) has been seen in 22 patients with renal artery stenosis. Fibromuscular dysplasia (FMD) was present in 18 patients. Demographics: 16 female, 21 Caucasians, ages 31-81, median age 54. These patients presented to the Emergency Department with excruciating flank pain. Imaging was performed: CT studies in all patients were suspicious for RI. Diagnostic angiography was then performed with a diagnosis of FMD in 18 patients in addition to confirming the RI in all patients.

At the time of angiography, angioplasty and stent deployment were performed when fibromuscular dysplasia with any significant obstruction of blood flow was seen. The patients have been followed up for at least one year and there have been no further renal infarcts;

some patients have been followed for as long as 10 years. Three patients had previously developed total occlusion of the renal arteries, but prior to the time that the current infarcts were diagnosed. These 3 patients had FMD in their non-infarcted kidney. Three patients had intimal dissections of their renal arteries diagnosed at the time of angiography. All patients undergoing angioplasty and/or stent deployment were treated with clodiprogel for one year and either an ACE inhibitor or ARB for their blood pressure. Sequential follow up by renal Doppler ultrasound has been performed to determine the patency of the stent, and renal function is being monitored.

**Conclusion:** Although renal infarction is not rare, its association with the presence of fibromuscular dysplasia of the renal artery has previously not been reported in a series of patients. This is a series of cases in both men and women who have now been followed for a long period of time and who have subsequently done well after intervention. No one has developed progressive renal disease during the follow up period.

Disclosure of Financial Relationships: nothing to disclose

## PUB307

**A Retrospective Study of the Renal Biopsies Realized in Native Kidneys at the Cleveland Clinic Florida from 2000 to 2010** Julianne M. Parente, Mauro Braun, Ayman Layka, Rute C. Paixao, Dianne T. Sandy, Beth L. Fromkin. *Nephrology Department, Cleveland Clinic Florida, Weston, FL.*

**Introduction**: Renal biopsy is an invaluable tool in the diagnosis, prognosis and management of patients with kidney disease. The main indications for this procedure include establishment of the exact diagnosis, determine the nature of therapy, decide when treatment is futile or evaluate for potentially reversible changes.

**Objectives:** This study was conducted to determine the results of percutaneous renal biopsies realized in native kidneys during the last 10 years by the Nephrology group of Cleveland Clinic Florida. Investigate the characteristics of the population referred for biopsies in terms of age, sex distribution, presence of renal failure, presence of proteinuria and its range (nephrotic vs non-nephrotic) and diagnosed pathologies. Obtain epidemiologic data and compare to similar studies available on the literature.

**Material and Methods:** Results of 184 renal biopsies with definitive diagnosis realized in Cleveland Clinic Florida from 2000 to 2010 were retrospectively analyzed for age, sex distribution and diagnosis using Epi Info software.

**Results:** Among the 184 patients analyzed, the mean age was 53 ±17 years (range between 16 and 91) and 47.8% (88 patients) were male. Renal failure was present in 79.3% (146) of the patients and 95.7% (173) had proteinuria. Among those, 44.9% (79 patients) had nephrotic range proteinuria. The most frequent diagnoses encountered were Focal Segmental Glomerulosclerosis (FSGS) (35 patients, 19%); Lupus Nephritis (21 patients, 21.4%) and Acute Interstitial Nephritis (AIN) (20 patients, 10.9%). The gender distribution showed that Lupus Nephritis was the most common diagnosis in females (16.7%, 16 patients) and FSGS was the most common diagnosis in males (25%, 22 patients).

**Conclusion:** In our results, FSGS, Lupus Nephritis and AIN were the most frequent biopsy-proven renal diseases. In comparison to previous studies reported on the literature that showed IgA nephropathy as the most common diagnosis, our epidemiology seems to differ. We suggest the establishment of a national US registry database for future epidemiological analysis.

Disclosure of Financial Relationships: nothing to disclose

## PUB308

**Clinical Effects of CCB and RAAS-I on ΔeGFR in ADPKD Patients Was Assessed by Analysis of Covariance (ANCOVA)** Ken Tsuchiya, Michihiro Mitobe, Takumi Yoshida, Hidekazu Sugiura, Shunji Shiohira, Kosaku Nitta. *Department of Medicine IV, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan.*

Intracellular Ca level is likely to play critical roles in the cyst formation in the ADPKD patients. Although *in vitro* and *in vivo* study using an animal model of ADPKD showed that CCB might accelerate the cyst progression, the possibly unfavorable effects of CCB on the pathophysiological status of PKD have not been examined using a clinical approach. Previously we investigated about this issue using conventional statistical method, in this study, analysis of covariance (ANCOVA) was used to evaluate the influence of CCB and renin- angiotensin-aldosterone system inhibitors (RAAS-I; including ACEI and ang II receptor blocker) with respect to the decrease of the eGFR. This model has included following confounding factors, baseline eGFR, mean SBP, and mean DBP. The effects of profile, linearity, interaction, and co linearity in ANCOVA were examined using regression diagnostic analysis. Two-tailed P-values of less than 0.05 were considered to be significant. Periods during which the antihypertensive drug prescriptions for CCB and/or RAAS-I had not been changed for at least one year were selected from among the clinical histories of the 32 outpatients. The mean values were; treatment duration,2.4 years; age, 49.5 years; SBP/DBP, 125 and 78 mmHg, respectively. The mean baseline eGFR was 34.4 mL/min/1.73 $m^2$ and the mean change in eGFR per year (ΔeGFR) was -2.7 mL/min/1.73 $m^2$/year. Only CCB significantly contributed to a reduction in ΔeGFR in both a univariable ANCOVA (crude coefficient: -2.00, 95%CI: -3.29 to -0.71, p=0.004) and a multivariable ANCOVA (crude coefficient: -1.79, 95%CI: -3.28 to -0.30, p=0.020). In contrast, none of the other confounding factors, RAAS-I, the baseline eGFR, SBP, DBP, contributed to reductions in ΔeGFR in both a univariable and multivariable ANCOVA. There has been no evidence that CCBs directly influence on the polycystin protein, nevertheless CCB might have a unfavorable effect on renal function in ADPKD patients, to whom RAAS-I should be the first choice. To establish these results further randomized studies with larger sample sizes are required.

Disclosure of Financial Relationships: nothing to disclose

## PUB309

**Long Term Follow Up of Renal Function in Patients with Cystic Fibrosis** Marius C. Florescu,[1] Elizabeth Lyden,[1] Diana F. Florescu,[1] Jennifer A. Fillaus.[2] *[1]University of Nebraska Medical Center, Omaha; [2]Sioux Fall.*

**Background**

Cystic fibrosis (CF) patients have numerous infections requiring prolonged antibiotic treatments, some of them nephrotoxic. Inhaled antibiotics can reach detectable levels. The aim of our study was to determine the impact of long term antibiotics use on the kidney function in CF population.

**Methods**

Data was retrospectively collected for 113 adults over 8 years. Spearman correlation coefficients were used to look at associations of continuous variables and Wilcoxon rank sum test to compare kidney function with various covariates.

**Results**

57(50.4%) patients were males and 56(49.5%) females, [mean age 31.7 years (SD 9.68)] of which 31% had DM, 9.7% HTN and 8.8% renal stones.

Over 8 years follow-up there were no significant changes in BUN (p0.92) or creatinine (p0.2); the presence of DM or contrast use was not associated with changes in renal function.

22% of the group had ≥ 1 episodes of ARF. The presence of ARF was associated with increased BUN (p0.002) and creatinine (p0.056) at 8 years.

Use of intravenous colistin (COL), gentamicin (Gm) or tobramycin (Tob) did not correlate with increased BUN (p0.64; p0.49; p0.51) or creatinine (p0.43; p0.49; p0.17). Documentation of elevated Tob peak and through levels did not correlate with increased BUN or creatinine. The use of intravenous vancomycin did not significantly increase BUN (p0.47) or creatinine (p0.2).

Inhaled COL and Gm correlated with increased BUN (p0.009; p0.02) but not creatinine (p0.45;p0.46). Inhaled Tob did not correlate with increased BUN (p0.17) or creatinine (p0.58). Only inhaled COL correlated with episodes of ARF (p0.03).

Episodes of ARF and renal function did not correlate with none of the major CF genetic mutations.

**Conclusion**

This is the first study to assess the impact of antibiotic use on renal function in CF population. The use of nephrotoxic antibiotics in CF patients is not associated with significant renal toxicity over 8 years. Intravenous COL, Gm, Tob and vancomycin were not associated with elevated BUN and creatinine. Only inhaled COL and Gm were associated with increased BUN, but not creatinine.

Disclosure of Financial Relationships: nothing to disclose

## PUB310

**New Markers of Inflammation-Induced Renal Injury Subside When Endotoxin Tolerance Develops in Humans as Measured by Urine Proteomics** Suzanne Heemskerk,[1,2] Annelies Draisma,[1] Martijn P. W. J. M. Bouw,[1] Coby M. M. Laarakkers,[3] Johannes G. van der Hoeven,[1] Rosalinde Masereeuw,[2] Peter Pickkers.[1] *[1]Intensive Care Medicine, Radboud University Medical Center Nijmegen, Nijmegen, Netherlands; [2]Pharmacology and Toxicology, Radboud University Medical Center Nijmegen, Nijmegen, Netherlands; [3]Clinical Chemistry, Radboud University Medical Center Nijmegen, Nijmegen, Netherlands.*

**Background**

Sepsis has been identified as the most common cause of renal injury in the ICU. No large clinical studies are available that show an improvement of renal function in patients with sepsis and this may be related to the lack of early diagnostic tests that indicate the onset of renal injury.

**Objective**

The aim of the current study was to search for potential new early markers of renal injury during acute endotoxemia and to investigate whether renal injury can be ameliorated by the induction of lipopolysaccharide (LPS) tolerance.

**Methods**

Healthy males (n=5) received iv bolus injections of 2 ng/kg/day *E. coli* LPS for 5 consecutive days. We used Surface enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (Seldi-TOF MS).

**Results**

Repeated LPS administrations induced a diminished GFR of 33±7% (p=0.02) on day 2 and an increase in serum creatinine of 11±3% (p=0.002) on day 3, which was associated with the appearance of 15 peak intensities in the urinary protein profile including an increase in β2-microglobuline levels (p=0.04) 6 hours after the first LPS administration. Four of the 15 peak intensities on day 1 correlated with serum creatinine levels on day 3; 3950, 4445, 6723 and 7735m/z (p=0.03; 0.01; 0.02 and 0.05 respectively). With the development of LPS tolerance, renal function restored, reflected by a decrease in serum creatinine and β2-microglobuline levels to baseline (p=0.2 and 0.4 respectively, between day 1 and 5), and by attenuated peak intensities in the urinary protein profile (p<0.0001 for all 15 peak intensities).

**Conclusion**

In conclusion, renal injury occurs during repeated endotoxemia and can be predicted by new urinary markers using proteome research. The inflammation-induced renal injury subsided when LPS tolerance developed after 5 consecutive days of LPS administrations.

Disclosure of Financial Relationships: nothing to disclose

**PUB311**

**The Effects of Different Solvents on the Amount and the Efficiency of Glycoprotein Enrichment Using Concanavalin A** Xuejiao Liu,[1] Mingxi Li,[1] Bixia Gao,[1] Xiaohong Fan,[1] Xuemei Li,[1] Youhe Gao,[2] Xue-Wang Li.[1] *[1]Nephrology, Peking Union Medical College Hospital, Beijing, China; [2]Peking Union Medical College, Beijing, China.*

**Objective:** Glycoprotein is one of the most important post-translational modification proteins in urine. This study evaluates the effects of different solvents on the amount and the efficiency of glycoproteins enrichment using Concanavalin A (Con A) in urinary glycoproteome. **Methods:** The morning urine samples of six healthy donors (3M/3F, 20-30 year old) were collected and pooled together. The proteins were precipitated by acetone. The samples were divided into 4 groups according to different solvents: [circ1]25mM $NH_4HCO_3$,[circ2]Lysis buffer (7M Urea, 2M Thiourea, 1%DTE and 5%Tris-base), [circ3] Lysis buffer diluted five times, [circ4]Buffer of ConA-agarose. The same amount (1300mg) of proteins solved by different solvents were loaded on ConA-agarose and incubated at 4°C overnight. The sample of control was solved by 25mM $NH_4HCO_3$, loaded on Agrose and incubated at 4°C overnight. Bradford method was used to measure the concentration of proteins. The 1D-SDS-PAGE electrophoresis was used to compare the inter-group differences in glycoprotein bands. **Results**: Lysis buffer solved the most amount of proteins (2621mg) compared to that solved by 25mM $NH_4HCO_3$ (1987mg) and ConA-agarose (1536mg). Lysis buffer diluted five times(G3) captured the most amount of glycoproteins (75mg) among solvents (50mg in G1, 45mg in G2 and 56mg in G4). Agarose did not enrich any glycoproteins. The 1D-SDS-PAGE electrophoresis showed the glycoprotein bands of G2 and 3 were more than that of G 1 and 2.

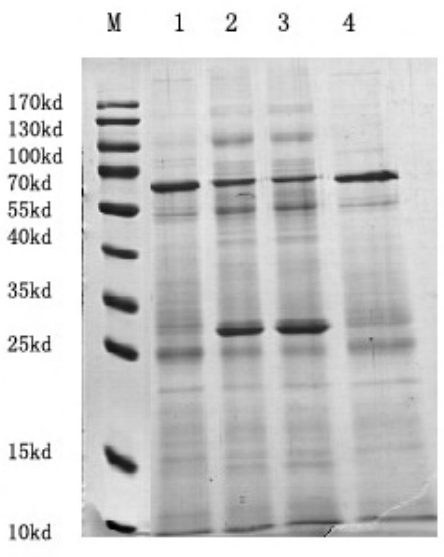



Figure 1:Glycoprotein captured by ConA in Group 1, 2, 3, 4 respectively (M:marker)

**Conclusion:**Among these solvents, lysis buffer solves the most amount of urinary proteins, and lysis buffer diluted five times captures the most amount of glycoproteins. This solvent also improves the efficiency of glycoproteins enrichment by Con A in glycoproteome study.

Disclosure of Financial Relationships: nothing to disclose

**PUB313**

**Dermal Hyperplasia in a Renin Secreting Transgenic Mouse: A Potential Animal Model for Nephrogenic Systemic Fibrosis (NSF)** Mandip Panesar,[1] Sandra Buitrago,[2] George Hajduczok,[2] Craig A. Jones,[2] Kenneth W. Gross.[1,2] *[1]Medicine, Physiology, State University of New York at Buffalo, Buffalo, NY; [2]Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY.*

NSF is a systemic fibrosing disorder occurring in renal failure patients and may be triggered by exposure to gadolinium-based contrast agents (GBCA). Given the ethical dilemma of administering GBCA to renal failure patients, an animal model is needed to better understand NSF. The purpose of this study was to determine whether an established transgenic mouse is a suitable animal model of NSF. We previously developed a transgenic (RenTag) mouse model in which the control region of the renin gene is used to promote expression of a viral oncogene. The mouse was created by fusing 4.6Kb of the 5'-flanking region of the mouse Ren2 gene to the SV40 T antigen structural gene. We previously reported a dramatic and spontaneous restructuring of the renal vascular bed consisting of hyperplasia of medial smooth muscle cells within the vascular wall leading to occlusion of the lumen as evinced by paleness consistent with low renal perfusion and was clearly evident by 12 weeks. The pathology progressed to encompass intimal lesions, inflammatory infiltrates in interstitial tissue, focal obstruction of collecting tubules, dilatation and degeneration of proximal tubules and hypercellular glomeruli with features of renal failure. We now report the skin of these transgenic animals to be non-elastic, tight, and thick. Histological analysis revealed thickened dermis with densely staining collagen bundles at 12 weeks. BUN was consistently elevated at 6, 8, 10 and 12 weeks. Collectively, these findings are suggestive of NSF in humans which is characterized by thickening and hardening of the skin. We also determined whether the dermal pathology was an outgrowth of impaired renal function. RenTag mice (6-10 weeks) with impaired kidney function were exposed to gadodiamide (0.3 mmol/kg, iv) to determine whether gadolinium exacerbated the NSF characteristics similar to the human clinical condition. This animal model may provide insights into the biological mechanisms underlying NSF.

Disclosure of Financial Relationships: nothing to disclose

**PUB314**

**Report of a Male Italian Imerslund-Grasbeck Patient with an Effective Null Mutation of the *CUBN* Gene** Tina Storm,[1] Francesco Emma,[2] Pierre J. Verroust,[1] Jens Michael Hertz,[3] Rikke Nielsen,[1] Erik I. Christensen.[1] *[1]Anatomy, Aarhus University, Denmark; [2]Nephrology and Urology, Ospedale Bambino Gesú, Italy; [3]Clinical Genetics, Aarhus University, Denmark.*

**Background:** Imerslund-Gräsbeck syndrome is a rare autosomal recessive disorder characterized by selective, intestinal, intrinsic factor-vitamin $B_{12}$ malabsorption. An additional proteinuria is frequently observed in these patients. The disease can be caused by mutations in the *CUBN* and *AMN* genes encoding the cubam receptor partners cubilin and amnionless. **Aim:** The aim of this study was to describe the functional renal phenotype of an Imerslund-Gräsbeck Syndrome patient. **Methods:** Mutation detection was performed through direct sequencing of the *CUBN* gene of a male Imerslund-Gräsbeck patient born from consanguineous parents. Immunohistochemistry of a renal biopsy collected from the patient, together with immunoblotting analyses of collected urine was used for phenotypic determination. **Results:** We identified a homozygous guanine for thymine exchange in the conserved donor splice site of exon 23 of the *CUBN* gene. No cubilin was detected in the renal biopsy suggesting an effective null mutation. Amnionless displayed an abnormal cytoplasmic localization in the proximal tubule cells demonstrating the interdependent relationship of the two receptor proteins, for the first time, in man. The patient´s urinary excretion pattern furthermore indicated a selective tubular proteinuria. **Conclusions:** We established direct correlations between the immunohistochemical data and the urinary protein excretion in an Imerslund-Gräsbeck patient hereby demonstrating the role of cubilin in handling of filtered proteins in the human kidney. Furthermore, no developmental abnormalities or physical defects of the patient suggest that cubilin is not essential for human embryonic development.

Disclosure of Financial Relationships: nothing to disclose

**PUB315**

**Effects of Uremic Toxins on Gene Expression in Human Renal Tubular Cells** Daniel M. Torres, Francisco Ramirez-Valle, Hania Kassem, Jiri Zavadil, Jerome Lowenstein. *Medicine, New York University Langone Medical Center, New York, NY.*

To test the hypothesis that uremic toxins are excreted by active renal tubular transport, and that the genes controlling these putative transporters might be upregulated in uremia (as a compensatory mechanism), we compared gene expression (using Affymetrix HG-U133 Plus 2.0 arrays) in cultured normal human renal tubular cells exposed for 24 or 48 hours to plasma from normal donors (N, n=2) or plasma obtained pre- and post-dialysis in patients with stable end-stage renal disease (ESRD, n=3).

Results: 527 genes were detected as significantly modulated in cultured cells incubated in N and in ESRD plasma. Comparing the gene expression in N and ESRD plasma, among the array of genes encoding putative transporters, we identified 16 genes whose expression were differentially effected by uremic plasma. Of these 6 were upregulated. Among these were genes encoding for a K conductance calcium channel (SLC23A2) and a calcitonin receptor (CALCA). A gene encoding the organic anion transporter SLC22A6 was downregulated.

178 genes believed to be associated with inflammatory responses were identified. Of these, these several genes related to TGF-beta signaling and its downstream profibrotic and promigratory effects (LTBP2, LAMC2, MMP9, SERPINE1, TGFBI) and other genes related to inflammation ,(CXCL2, CXCR7, IL6ST, TNFRSF19, TNFSF15) were upregulated on exposure to uremic plasma. The overexpression of the TGF beta signature was normalized following 3-3.5 hour hemodialysis.

Conclusions This pilot project appears consistent with our hypothesis that substances that are not completely removed by hemodialysis accumulate in the plasma of uremic patients and may play a role in the regulation of selected transport proteins serving as a compensation for the reduced nephron mass. Further, the increase in proinflammatory and profibrotic signaling may be implicated in the ongoing inflammation that characterizes the uremic state; normalization of the expression of these genes suggests that this increase may be attenuated at the molecular level by hemodialysis.

Disclosure of Financial Relationships: nothing to disclose

**PUB316**

**Renal Cyst Number Measured on MR Images in ADPKD Kidneys: Inter-Reader Variability and the Total Renal Cyst Number Estimated from the Cyst Number on a Single Mid-Slice Image** Kyong Tae Bae,[1] Cheng Tao,[1] Amol A. Patil,[1] Arlene B. Chapman,[2] Jared J. Grantham,[3] Vicente E. Torres,[4] Lisa M. Guay-Woodford,[5] Peter C. Harris,[4] Marva M. Moxey-Mims,[6] William M. Bennett,[7] Jin Hong Wang,[1] Diana Kaya,[1] James E. Bost.[1] *[1]U Pittsburgh; [2]Emory U; [3]U Kansas; [4]Mayo Clinic; [5]UAB; [6]NIDDK; [7]Legacy Good Samaritan.*

In ADPKD, renal cyst number is likely associated with the genotype, renal volume progression and ultimate decline in GFR. The purpose of the study is to evaluate inter-reader variability of measuring renal cyst number and to estimate the total renal cyst number from a single mid-slice MR image.

Two radiologists counted cysts with diameters ≥ 2 mm in each kidney on a mid-section of coronal T2 MR images (12- 65 sections) in 221 ADPKD CRISP subjects. Inter-reader reliability was assessed with intraclass correlation coefficient (ICC). Differences in total cyst count, averaged over the two readers, between gender and genotype (PKD1, PKD2) were assessed with t-tests. Pearson correlation coefficient of reader bias with total kidney volume was also determined. Furthermore, in 16 of 221 subjects, the two readers reviewed the entire set of images of each kidney and counted the total number of cysts (TNC). Association between TNC and the product of the mid-section cyst count (MNC) and the number of sections (NS) in each kidney was evaluated with correlation coefficients.

The inter-reader reliability of measuring MNC was extremely high (ICC=0.99). Reproducibility was within ±10% for 94% of the cases. Overall bias and variability (mean ± SD), as measured by the difference in cyst number between the two readers, was negligible at -0.26 ± 3.79. Cyst numbers were significantly higher in subjects with PKD1 than PKD2 (mean 63±28.4 vs 37±22.4; p< 0.001) but no difference was noted by gender. Bias was not significantly affected by genotype (p=0.664) or kidney volume (p=0.433). TNC correlated strongly ($r^2$=0.50) with MNCxNS and may be estimated from a regression relationship (TNC = 0.127xMNCxNS).

Cyst numbers measured in a mid-section of MR images are highly reliable and reproducible and may be used to estimate the total cyst number within the kidney.

Disclosure of Financial Relationships: Consultancy: Otsuka.

**PUB317**

**Vitamin A Deficiency with Decreased Serum Retinol Binding Protein in Dent's Disease** Rachel Becker-Cohen, Choni Rinat, Efrat Ben Shalom, Sofia Feinstein, Yaacov Frishberg. *Division of Pediatric Nephrology, Shaare Zedek Medical Center, Jerusalem, Israel.*

Dent's disease is an X-linked renal proximal tubular disorder manifesting as low molecular weight proteinuria, hypercalciuria, nephrocalcinosis and progressive renal impairment. Three patients with Dent's disease presented with complaints of impaired night vision, and xerophthalmia. Laboratory evaluation showed severely decreased serum vitamin A concentrations. Retinol binding protein (RBP) is a transport protein for vitamin A, delivering it from the liver to target organs. The retinol–RBP complex is filtered at the glomerulus and normally undergoes reabsorption at the proximal tubule. Our hypothesis was that renal losses of RBP in Dent's disease could be responsible for decreased serum retinol. We investigated vitamin A status and RBP in serum and urine of 8 patients with genetically confirmed Dent's disease. We found that in addition to the 3 boys with clinical vitamin A deficiency, 3 others had asymptomatic deficiency with a normal ophthalmologic examination, all had normal renal function. Two young men with Dent's disease and impaired renal function had normal serum vitamin A concentrations. Serum RBP concentrations were low in patients with vitamin A deficiency, and were correlated with vitamin A levels. Urinary RBP concentrations were increased in all patients (2000 fold), regardless of vitamin A status. We compared these results to patients with nephrotic range proteinuria due to glomerular disease and patients with advanced nephropathic cystinosis, another proximal tubulopathy. Patients with glomerular proteinuria had only mildly increased urinary RBP with normal serum RBP and vitamin A. Patients with cystinosis and impaired renal function had massive urinary RBP losses, but without a decrease in serum RBP or vitamin A levels, similarly to the adult patients with Dent's disease and low GFR. Treatment with moderate dose vitamin A supplements in 5 patients with vitamin A deficiency resulted in rapid resolution of ocular symptoms and an increase in serum vitamin A concentrations. We recommend screening patients with Dent's disease for vitamin A deficiency, and treating before the appearance of visual symptoms.

Disclosure of Financial Relationships: nothing to disclose

**PUB318**

**Endothelial Cell Proliferation after Cysteamine Exposure** Martine T. Besouw,[1,3] Lambertus V. Heuvel,[1,3] Mieke Dewerchin,[2] Elena N. Levtchenko.[1,3] *[1]Dept of Pediatric Nephrology, University Hospitals Leuven, Leuven, Belgium; [2]Vesalius Research Center, VIB-KULeuven, Leuven, Belgium; [3]Laboratory of Pediatrics, KULeuven, Leuven, Belgium.*

**Background**

Cysteamine is used to treat cystinosis, an autosomal recessive disease caused by intralysosomal cystine accumulation due to mutations in the *CTNS* gene. Recently 8 patients with cystinosis were reported with muscular-skeletal weakness, skin striae and bruising-like lesions on elbows after administration of high doses of cysteamine. Skin biopsies of the elbow lesions showed vascular endothelial cell proliferation called angioendotheliomatosis and a variability of collagen fiber caliber on electron microscopy. We aimed to study the mechanisms of these side effects *in vitro*.

**Methods**

Human microvascular endothelial cells (HMVEC) and human fibroblasts were incubated with a range of cysteamine concentration (0-10 mM) during 6 or 24 hours. Medium and cysteamine were refreshed every 6 hours. Cell viability was measured using WST-1, which represents cleavage of the tetrazolium salt WST-1 to a formazan-class dye by viable mitochondria. Next, we measured cell proliferation by BrdU, which is incorporated in the DNA of dividing cells.

**Results**

After 6 hours, cell viability did not differ significantly between specific cysteamine concentrations or cell types; cell proliferation increased significantly in HMVEC after exposure to concentrations between 0.03-3.0 mM. After 24 hours, a significant rise in WST-1 signal was found in both cell types and a significant rise in BrdU incorporation was found in HMVEC at concentrations between 0.03-1.0 mM. A concentration of 10 mM was toxic in all cell types.

**Conclusion**

Exposure to cysteamine at concentrations found in patient's plasma can increase proliferation of HMVEC, both measured by cell viability and proliferation assays. This is in line with the *in* vivo finding of cysteamine induced angioendotheliomatosis in cystinosis patients. The rise in WST-1 signal without a rise in BrdU signal seen in fibroblasts could be due by cysteamine increasing mitochondrial activity, rather than stimulating cell proliferation. These results are the first step in unraveling the mechanisms of newly reported cysteamine toxicity.

Disclosure of Financial Relationships: nothing to disclose

**PUB319**

**Novel Renin Gene Mutation Causes Autosomal Dominant Chronic Kidney Failure and Anemia and Responds to Fludrocortisone Treatment in Childhood** Anthony J. Bleyer,[2] Martina Zivna,[1] Katerina Hodanova,[1] Petr Vyletal,[1] Helena Hulkova,[1] Milan Elleder,[1] Stanislav Kmoch.[1] *[1]Charles University in Prague, Prague, Czech Republic; [2]Wake Forest University School of Medicine, Winston-Salem, NC; [3]National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.*

Background: A novel mutation (c.58T>C) resulting in the amino acid substitution of cysteine for arginine in the signal peptide of renin was identified in a father and daughter with chronic kidney disease. Methods: Clinical laboratory studies were performed before and after administration of fludrocortisone, 0.1 mg orally each day. Laboratory investigations were performed. Results: The daughter presented at age 6 years with a persistently elevated serum creatinine (1.2 mg/dl). The blood pressure was 82/52 mm Hg, with serum Na 140 mEq/l, K 5.5 mEq/l, urate 6.3 mg/dl with urate fractional excretion 5.2% (normal 6-20%), hemoglobin 10.5 g/dl. The plasma renin activity level was <0.5 ng/ml/h (normal 0.5-5.9 ng/ml/hr) with corresponding low aldosterone level. At age 10 years, the patient was started empirically on fludrocortisone, 0.1 mg orally each day. There was an increase in the baseline eGFR from 45 ml/min to 75 ml/min, which was sustained after four months of chronic treatment. The father suffered from more advanced chronic kidney disease, and his eGFR (35 ml/min) did not increase with fludrocortisone. Laboratory Investigations: Wild type (WT REN) and mutant (C20R REN) eukaryotic expression vectors were transfected into HEK 293 cells. In cell lysates, WT REN produced the fully glycosylated prorenin and successfully completed ER translocation and underwent cleavage of its signal sequence, whereas C20R REN produced only the non-glycosylated preprorenin. Immunofluorescence analysis demonstrated that the C20R REN protein did not form cytoplasmic granules and instead had intense diffuse cytoplasmic staining. CONCLUSION: Mutations affecting the renin signal peptide alter ER processing of preprorenin and result in hyporeninemia, anemia, hyperuricemia and CKD. Fludrocortisone treatment started in childhood improves eGFR.

Disclosure of Financial Relationships: Honoraria: Takeda Pharmaceutical Genzyme; Patent: #7,781,164 Method to diagnose inherited kidney disease.; Scientific Advisor: Editorial Board--JASN, ACKD.

**PUB320**

**Prevalence of Vitamin D Deficiency in Children and Young Adults with Autosomal Dominant Polycystic Kidney Disease** Melissa A. Cadnapaphornchai, Berenice Y. Gitomer, Michel B. Chonchol, Kim Mcfann, Wei Wang, Robert W. Schrier. *Department of Medicine, University of Colorado Denver, Aurora, CO.*

**Purpose:** Autosomal dominant polycystic kidney disease (ADPKD) is known to occur in childhood and to affect 1 in 400 live births. Borderline hypertension (75-95 percentile) and hypertension (>95 percentile) in children with ADPKD have been associated with larger kidney volumes and increased left ventricular mass index (LVMI) when compared to normotensive ADPKD children. Inhibition of the renin-angiotensin-aldosterone system has been shown to improve renal function and prevent the rise in LVMI in children with ADPKD compared to untreated children. Since active vitamin D is one of the most potent hormones for down-regulating renin expression in the kidney, we determined vitamin D status in young subjects with ADPKD.

**Methods:** We measured baseline serum 25(OH)D levels total in 105 (60 female and 45 male) young subjects with ADPKD aged 8-24 years. 25(OH)D was measured by ELISA assay.

**Results:** Subjects mean (SD) age and creatinine clearance were 16 (4) years and 129 (34) ml/min/1.73m² respectively. The study group comprised 94 Caucasian, 9 Hispanic and 2 African American subjects. The mean (SD) serum 25(OH)D level was 31.9(17.9) ng/ml. 53.4 % of these children had serum 25(OH)D levels < 30 ng/ml, and 25% of patients had vitamin D deficiency defined as a serum 25(OH)D level < 20 ng/ml. In these ADPKD children renal function was normal [mean (SD); 129(27) ml/min/1.73m²]. Mean (SD) 25(OH)D level was highest in 31 children whose samples were collected during the summer months, 46 (22) ng/ml, as compared to those patients whose samples were collected during winter months , 23 (13) ng/ml, p < 0.001.

**Conclusion:** In summary, vitamin D deficiency/insufficiency is prevalent among young patients with ADPKD despite normal kidney function. Vitamin D supplementation may be beneficial in patients with ADPKD however, future studies will be necessary to further address this issue.

Disclosure of Financial Relationships: nothing to disclose

**PUB321**

**Coexistence of a Novel *GLA* Mutation and *COL4A3* Variant in Two Chinese Females** Zhi-Yong Cai,[1,3] Su-Xia Wang,[1] You-Kang Zhang,[1] Yu-Qing Chen,[1] Lin-Chang Liu,[1] Yu Huang,[2] Hong Zhang.[1] *[1]Renal Division, Peking University First Hospital; [2]Department of Medical Genetics, Peking University Health Science Centre; [3]Department of Internal Medicine, Beijing Gongan Hospital.*

Fabry disease(FD) is an X-linked lysosomal storage disease caused by mutations of *GLA* gene. Thin basement membrane nephropathy (TBMN) is usually characterized by hematuria with uniformly thinned glomerular basement membrane (GBM).Mutations of *COL4A3* or *COL4A4* were identified in some TBMN patients. We observed a family with both phenotypes. The proband(II-1) was a 41-year-old Chinese female with proteinuria(0.75g/24hr), microscopic hematuria, Scr 81umol/l, and hypertention(BP 160/100mmHg).She also presented angiokeratomas,hypohidrosis and tinnitus.Her renal biopsy revealed vacuolization of podocytes and 7/35 glomeruli with segmental or global sclerosis.Electron microscopy indicated concentric lamellated inclusions in podocytes, parietal epithelial and distal tubular epithelial cells. Diffuse thinning of GBM with a mean thickness of [sup]216[/sup]±[sup]31nm[/sup] was identified and staining for α3(IV) and α5(IV) were positive. Her daughter(III-1) showed hematuria, neuropathic pain, hypohidrosis, and TIA. One of her sister(II-3) only presented with hematuria without signs of FD. The α-galactosidase A activity of II-1 and III-1 were 33 and 75 unit respectively, lower than normal range(100-500 unit). A novel mutation of 1208 ins 21bp in *GLA* gene was detected in II-1 and III-1. And a new variant of *COL4A3* gene (M1209I) was also confirmed in II-1 and III-1. However in II-3,with hematuria only, *COL4A3* M1209I was identified.The *COL4A3* M1209I polymorphism may be associate with TBMN. The co-occurrence of *GLA* mutation and *COL4A3* variant in II-1 and III-1 contributed to both FD and TBMN. The outcome for the coexistence of FD and TBMN need longer period of follow-up.

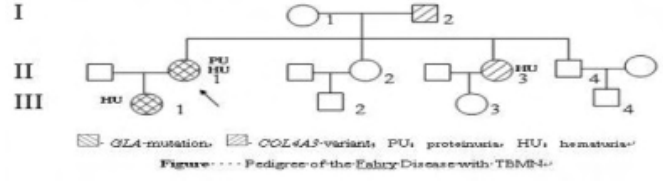


Figure · · · Pedigree of the Fabry Disease with TBMN

Disclosure of Financial Relationships: nothing to disclose

**PUB322**

**PKD1 and PKD2 Gene Variants in Italian Patients Affected by Autosomal Dominant Polycystic Kidney Disease (ADPKD)** Paola Carrera,[1] Francesca Rigo,[1] Cristina Montrasio,[1] Silvia Calzavara,[1] Lucia Palmieri,[3] Piergiorgio Messa,[2] Alberto Edefonti,[2] Francesco Scolari,[4] Paolo Manunta,[1] Alessandra Boletta,[1] Maurizio Ferrari.[1] *[1]San Raffaele Scientific Institute, Milano, Italy; [2]IRCCS Policlinico, Milano, Italy; [3]University of Modena, Italy; [4]Spedali Civili, Montichiari (BS), Italy.*

ADPKD is the most common genetic nephro-pathology in humans (about 1/1000). Aim of our work was to identify variants of *PKD1* and *PKD2* genes in Italian ADPKD patients. Analysis of gene variation would allow to: confirm the diagnosis in clinically uncertain/atypical cases; offer genetic counseling in at risk families; exclude mutation in related donors for kidney transplantation; define molecular spectrum of *PKD1* and *PKD2* in Italian patients.

A semi-automated Sanger direct sequencing protocol has been developed for detection of variants in exons and flanking regions. Specific amplification of PKD1 was achieved by previously published and by new protocols.

We analyzed 124 unrelated patients belonging to 108 families, 42 relatives and 16 patients with no reported familiarity. In the majority of patients (115/124) variants were found: 94(81%) PKD1; 11(10%) PKD2; 10(9%) both genes. In 2 familial and 7 sporadic cases no variants were found.

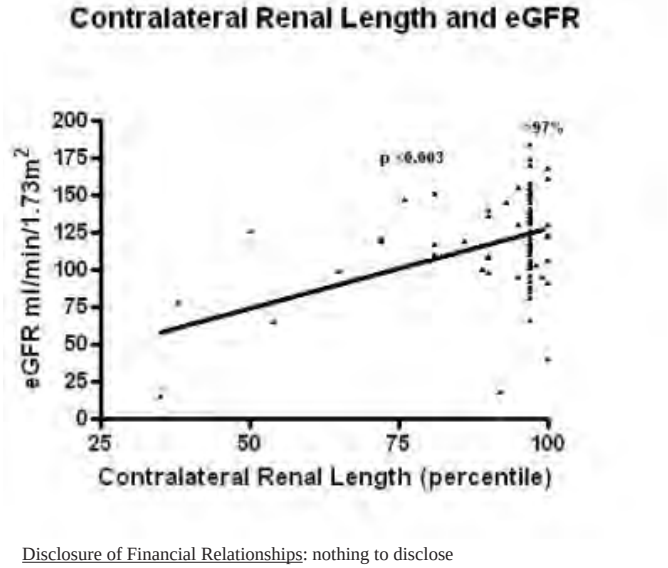
In 98% (106/108) of familial cases variants were found in PKD1 and/or PKD2. In sporadic cases, 53% (8/15) were positive. Based on the ADPKD Mutation Database, the majority of variants were not described (163/189). By combining results for truncating and known variants we identified pathogenic mutations in 73/115 (63%) patients. For variants not yet determined with respect to their pathogenicity a classification was not always feasible. We were able to assign as likely pathogenic 9 variants (7.8%) reaching a total 71% of affected alleles. When applicable, intra-familial segregation, frequency analysis in 200 DNA from >56 years health individuals, evaluation of type of substitution, conservation among orthologous, in-silico evaluation of intronic variants helped interpretation, nevertheless this issue remains cumbersome. Harmonization of criteria for interpretation of unclassified variants will improve counselling to patients and their relatives.

Disclosure of Financial Relationships: nothing to disclose

**PUB323**

**Long-Term Outcome in Children with Unilateral Multicystic Renal Dysplasia. Is the Contralateral Kidney Normal?** Jayanthi Chandar,[1] Carolyn L. Abitbol,[1] Maria Matilde Rodriguez,[2] Wacharee Seeherunvong,[1] Michael Freundlich,[1] Gaston E. Zilleruelo.[1] *[1]Department of Pediatrics, Division of Pediatric Nephrology, University of Miami, Miller School of Medicine, Miami, FL; [2]Department of Pathology, Division of Pediatric Pathology, University of Miami, Miller School of Medicine, Miami, FL.*

The clinical spectrum of renal dysplasia includes the non-functioning kidney with multicystic dysplasia (MCDK). We report our experience of the outcome of unilateral MCDK and its contralateral kidney in 101 children with the diagnosis of MCDK from 1985 to 2009. Demographics and data collected included urine protein/creatinine ratio, estimated GFR (eGFR), blood pressure, surgical intervention, renal length and abnormalities of the contralateral kidney, and involution rate. There was a predominance of left sided MCDK. Diagnosis was made prenatally in 86.7%. Contralateral abnormalities included vesicoureteral reflux (14%), UPJ obstruction (4.1%) and megaureter (2.4%). Complete involution of MCDK occurred within 5 years in 57%. Compensatory hypertrophy of the contralateral kidney to >97% occurred in 74.1%. Nephrectomy was performed in 19.8%. Chronic kidney disease (CKD) stage ≥2 was observed in 9.7%, proteinuria in 17% and hypertension in 6.9%. Hyperfiltration with mean eGFR of 149±13 ml/min/1.73m² was seen in 37.8%. There was a significant correlation between proteinuria and decreased eGFR, and hyperfiltration and increased contralateral kidney size (p<0.0001 and 0.003 respectively). Those with CKD ≥2 had contralateral abnormalities either at birth, or later.In conclusion, there is an increased risk of CKD in children with unilateral MCDK associated with hyperfiltration and proteinuria. Thus long-term follow up is recommended.




Disclosure of Financial Relationships: nothing to disclose

**PUB324**

**Progession of ADPKD: Importance of Follow-Up** Valentina Corradi,[1,2] Fiorella Gastaldon,[1] Anthi Panagiotou,[1,2] Grazia Maria Virzì,[1,2] Dinna N. Cruz,[1,2] Claudio Ronco.[1,2] *[1]Nephrology, San Bortolo Hospital, Vicenza, Italy; [2]IRRIV, International Renal Research Institute Vicenza, Vicenza, Italy.*

**Background**:ADPKD is the common genetic renal disorder and it is an important cause of CKD stage V. In dialysis population in Vicenza prevalence of ADPKD accounts for 13.4% versus 8.2% in Italy. In our previous epidemiological study we identified the presence of three different haplotypes to support the hypothesis of a founder effect in our region, suggesting the presence of a strong linkage-specific gene.The purpose of this study was to evaluate the progression of the disease in the pre-dialysis ADPKD out-patients (pts) in terms of the estimated GFR (eGFR/yr).

**Materials and Methods**:Three years follow-up was conducted (N=45). Pts were evaluated with Magnetic Resonance (MR) to measure the total kidney volume (TKV), eGFR (ml/min/1,73m²) was measured by the MDRD equation. Creatinine and blood pressure were registered on the first investigation (t0) and following control (t1). We calculated the difference of eGFR (ml/min/1,73m²)for year, between t0 and t1(Δmedian eGFR/1yr). Categorical data were expressed as percentages and continuous variables by median and interquartile range (IR) compared by Sign-test.

**Results**:Characteristics of ADPKD patients at t0, belong to 31 families, are shown on the table.

| Demographic Data | Median (IR) or N (%) |
|---|---|
| Age at first investigationl, years | 51 (43-57) |
| Age of diagnosis, years | 34 (26-45) |
| Male (%) | 24 (53,3%) |
| **Comorbidities** (%) | |
| Hypertension | 34 (75,5%) |
| Renal Stones | 11 (24,4%) |
| Diabetes Mellitus | 3 (6,7%) |
| **Kidney function parameters** | |
| CKD at first investigation | 26 (57,8%) |
| CKD stage 5 at interview (%) | 2 (4,4%) |
| Crea (mg/dL) t0 | 1,48 (1,04-2,10) |
| Crea (mg/dL) t1 | 1,69 (1,02 to 3,10) |
| eGFR (ml/min per 1,73m²) t0 | 48 (28-73) |
| eGFR (ml/min per 1,73m²) t1 | 45 (20-66) |
| max cysts diameter in right kidney (cm) | 4,0 (3,3-5) |
| max cysts diameter in left kidney (cm) | 4,0 (3-5,4) |

We calculated the Δ median eGFR/1yr (ml/min per 1,73m²):-2,0 (-5,5 – 0)and the difference for this period was found statistically significant (p=0.0009*).

**Conclusions**:ADPKD patients were younger than CKD pts. They had modestly enlarged kidneys in terms of max cysts diameter and moderate progression of CKD (expressed as a decrease of eGFR). These data suggest the importance of life style and diet behavior, control of blood pressure and a frequent follow-up in ADPKD patients, in order to preserve kidney function.

Disclosure of Financial Relationships: nothing to disclose

## PUB325

**Neuroimaging Findings of Children with Autosomal Dominant Polycystic Kidney Disease** John F. S. Crocker, Philip D. Acott. *Pediatrics, IWK Health Center, Halifax, NS, Canada.*

We previously reported autosomal dominant polycystic kidney disease (ADPKD) first presentation characteristics in a cohort of 55 children showing a significant prevalence of modifiable risk factors (hypertension = 22%; proteinuria > 150 mg/day = 7%; hyperlipidemia = 54%) despite normal renal function in 98% at diagnosis (AJKD 2004: 43-2, 296-303). This study evaluates the neuroimaging findings of a subset of ADPKD children presenting with headaches and/or family history of cerebral aneurysms from our expanded cohort.

63 ADPKD children (age 1 – 20 years; 25% < 1 year) are followed based on family history, ultrasound confirmation of cysts, and/or linkage analysis. Twenty ADPKD children (31.7%) age 3-20 years had central nervous system (CNS) investigation with CAT or MRI angiography after presentation with headaches. No child had focal neurological deficits, cognitive impairment, or other medical diseases or syndromes with the exception of one child with spina bifida and a ventriculo-peritoneal (VP) shunt.

No cerebral vascular aneurysms were found in the 20 children with CNS imaging (MRI angiography, n = 17; CAT scans, n = 3). Five (25%) ADPKD children (3 ♀; 2 ♂) showed a variety of CNS abnormalities including: a) areas of cerebral hemisphere white matter demyelination (n = 1), b) focal frontal cortex white matter demyelination (n = 1), c) cerebral and cerebellar atrophy with partial atrophy of posterior corpus callosum (n = 1), d) a 3 mm periventricular cyst (n = 1), and CNS lesions typical of known spina bifida with VP shunt placement (n=1). All children were from different families.

This pediatric ADPKD patient database is the first to document CNS neuroimaging anomalies before adulthood in 4 children (exclusive of expected findings of VP shunted child). These findings are likely developmental and are not in a vascular pattern. It is noted that ADPKD during early development has abnormal cilary function and primary cilia are critical to neural stem cell development as demonstrated by mice with Kif3A or Smo protein anomalies who demonstrate failure of radial astrocytes responsible for adult neurogenesis. Screening large groups of children will define the extent of the CNS lesions in ADPKD.

Disclosure of Financial Relationships: nothing to disclose

## PUB326

**Temporal Retinal Thinning Is Common in Alport Syndrome** Martin C. Gregory,[1] Faisal Ahmed,[2] Kandon K. Kamae,[2] Paul S. Bernstein.[2] *[1]Division of Nephrology, University of Utah Health Sciences Center, Salt Lake City, UT; [2]Department of Ophthalmology, University of Utah Health Sciences Center, Salt Lake City, UT.*

X-linked Alport Syndrome (XLAS) is an inherited nephropathy that commonly causes hearing loss and less commonly includes pathognomonic ocular findings such as anterior lenticonus and perimacular retinal flecks. We performed detailed ophthalmological examination including high-definition spectral-domain optical coherence tomography in 29 male and female patients with XLAS. Temporal retinal thinning was assessed as the proportional reduction of temporal compared to nasal retinal thickness using normative data from Grover et al (2009). Although lenticonus was present in only 2 eyes and retinal flecks in only 10 eyes, severe (> 2 SD) temporal retinal thinning was found in 37/55 (67%) and moderate (1-2 SD) temporal retinal thinning was found in 6/55 (11%) of eyes satisfactorily examined. Retinal thinning appeared similar in degree with mutations causing juvenile forms of XLAS as in those causing adult onset forms of XLAS (C1564S and L1649R).

Opthalmic manifestations with different COL4A5 Mutations

| Mutation | # of eyes | Nasal retinal thickness μ OD/OS | Temporal retinal thickness μ OD/OS | Anterior lenticonus | Retinal dots or flecks |
|---|---|---|---|---|---|
| Del ex2 | 2 | 308/321 | 265/265 | 0/2 | 0/2 |
| 4887del4 | 4 | 319±49/324/45 | 288.5±25/291±23 | 0/4 | 0/4 |
| 3528+T | 4 | 350±9/345±5 | 287±14/286±8 | 0/4 | 2/4 |
| L1097X | 4 | 323±9/311±9 | 284±20/271±33 | 0/4 | 2/4 |
| G96A | 2 | 311/317 | 297/290 | 0/2 | 0/2 |
| G567S | 4 | 352±3/353±2 | 308±3/309±6 | 0/4 | 0/4 |
| G635D | 2 | -/191 | -/221 | 1/2 | 0/2 |
| G1060S | 2 | 321/311 | 286/383 | 0/2 | 0/2 |
| C1564S | 6 | 292±49/296±52 | 270±37/269±36 | 0/6 | 0/6 |
| L1649R | 22 | 323±22/320±23 | 296±16/292±16 | 0/22 | 2/22 |
| Unknown | 6 | 331±25/320±23 | 289±11/289±9 | 2/6 | 2/6 |

OCT is increasingly available and is a rapid, inexpensive, and noninvasive method that merits further study as a diagnostic marker for XLAS in patients who lack other ophthalmologic features.

Reference: Grover S et al. Normative Data for Macular Thickness by High-Definition Spectral-Domain Optical Coherence Tomography (Spectralis). Am J Ophthalmol. 2009; 148:266

Disclosure of Financial Relationships: nothing to disclose

## PUB327

**Study of P53 Codon 72 Polymorphism & Clinico-Radiological Correlations in ADPKD Patients** Krishan L. Gupta,[1] Swarnjeet Kaur,[1] Rajindra Prasad,[2] Anupam Lal,[3] Vinay Sakhuja.[1] *[1]Deparment of Nephrology and; [2]Biochemistry; [3]Radio-Diagnosis, Postgraduate Institute of Medical Education and Research, Chandigarh, India.*

Although much is known about molecular genetics of ADPKD, recently a tumour suppressor gene, P53 has been identified as an endogenous negative regulator of PKD1. Present study analysed the clinical profile of 50 ADPKD patients, correlated the kidney volumes assessed on non contrast computerized tomography with creatinine clearance (CCl), estimated by Cockroft and Gault formula, and studied polymorphism at codon 72 of P53 gene. Mean age of diagnosis was 40 12.4years, clinical features were flank pain (80%), UTIs (34%), nephrolithiasis (32%), gross haematuria (30%), and proteinuria (56%) of patients. 64% had liver cysts, and 2% of subjects had pancreatic cysts and intracranial aneurysms. Hypertension was seen in 94%, chronic renal failure in 56%, 42, 85% of whom had ESRD. Mean estimated CCl, was 50.8537.38ml/min.

Mean total kidney volume on CT scan was 779.43409.98 ml. Kidney volumes had significantly negative correlation with CCl, (correlation coefficient-.718, significant at 0.01 level) even after adjustment for age, values were higher among males and subjects with proteinuria. Polymorphism at codon 72 of P53 gene was studied using polymerase chain reaction. 76% and 20% of patients were homozygote for pro/pro and arg/arg variant respectively, 4% heterozygote for arg/pro. Arg/arg variants had non significant trend towards higher kidney volumes. Conclusions: Renal volumes estimated by non contrast CT is a surrogate marker for disease progression in ADPKD. Arg/arg/ variant may be a risk factor for higher kidney volumes, underlying mechanism colud be higher rate of cellular proliferation in this variant. Larger studies however needed to confirm these results.

Disclosure of Financial Relationships: nothing to disclose

## PUB328

**Renal Manifestations of Patients with *MYH9*-Related Disorders** Kyoung Hee Han,[1] Hyun Kyung Lee,[1] Yun Hye Jung,[1] Hee Gyung Kang,[1] Il-Soo Ha,[1] Hae Il Cheong.[1,2] *[1]Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Republic of Korea; [2]Research Center for Rare Diseases, Seoul National University Hospital, Seoul, Republic of Korea.*

*MYH9*-related disorders are a group of autosomal dominantly inherited disorders caused by mutations of *MYH9*, which encodes the non-muscle myosin heavy chain IIA (NMMHC-IIA). May-Hegglin anomaly and Sebastian, Fechtner and Epstein syndromes belong to this group. While megathrombocytopenia is the common characteristic finding of *MYH9*-related disorders, basophilic cytoplasmic inclusion bodies in leukocytes (Döhle-like body), deafness, cataract and nephritis are found only in some patients.

In this study, renal manifestations of MYH9-related disorders were analyzed in seven genetically confirmed, unrelated Korean patients.

Among the seven patients with *MYH9* mutations, four cases had renal manifestation; one with positive family history (Patient 1) and three without (sporadic cases). All the three sporadic cases had severe renal involvement with rapid progression to end-stage renal disease (ESRD) except Patient 4, who is still young (now 7 years old), and their mutations were in the motor domain of NMMHC-IIA. The familial case (Patient 1) had milder renal involvement without progression to ESRD by his 30s, and his mutation was in the tail domain of the protein.

Clinical features of 4 patients with renal involvement

| Patient | FHx | Age at Dx | Kidney involvement | | Other Organs | | Mutation |
|---|---|---|---|---|---|---|---|
| | | | Kidney Bx / GBM change | ESRD | WBC | Deaf / Eye | (exon, domain) |
| 1 | (+) | 30 yrs | FSGS / (-) | (-) | (+) | (-) / (-) | D1424N (30, tail) |
| 2 | (-) | 22 yrs | FSGS / (+) | (+) | (-) | (+) / (+) | S96L (1, motor) |
| 3 | (-) | 12 yrs | Not done | (+) | (-) | (+) / (-) | S96L (1, motor) |
| 4 | (-) | 14 mos | Mesangial expansion / (+) | (-) | (+) | (-) / (-) | R718W (16, motor) |

The remaining three cases with *MYH9*-related disorders did not have renal manifestation, or ear and eye involvement. All of them had positive family history and their mutations were in the tail domain of NMMHC-IIA (E1841K, R1165C, and K373N, respectively).

In summary, renal involvement was found in 4 of 7 cases with *MYH9*-related disorders, and the patients with a mutation in the motor domain of NMMHC-IIA had more severe renal manifestation.

Disclosure of Financial Relationships: nothing to disclose

## PUB330

**Evaluation for the Effectiveness of Proteinuria to the Progression of Renal Dysfunction for Female Fabry Patients** Kazushige Hanaoka,[1] Mahiro Kurashige,[1] Toya Ohashi,[2] Hiroshi Kobayashi,[2] Yoshikatsu Eto,[2] Hiroyuki Ida,[2] Tatsuo Hosoya.[1] *[1]Devision of Kidney and Hypertension, Department of Internal Medicine, The Jikei University, School of Medicine, Minato, Tokyo, Japan; [2]Department of Pediatrics, The Jikei University, School of Medicine, Minato, Tokyo, Japan.*

Purpose: Nephropathy is one of the major and life-threatening complications of Fabry disease. The main signs of Fabry nephropathy are reduction of glomerular filtration rate (GFR) and proteinuria. The major signs of Fabry nephropathy are reduction of glomerular filtration rate (GFR) and proteinuria. Renal function progressively decreases when proteinuria is overt for male Fabry patients. However, correlation between proteinuria and renal function is still unclear for female Fabry patients. This study aimed to evaluate the effectiveness of proteinuria to the progression of renal dysfunction for female Fabry patients.

Methods: This was single center, retrospective study. The data were collected from 15 female patients treated by Enzyme replacement therapy (ERT) at least 30 months. Recombinant α-galactosidase B was administered in a dosage of 1 mg/kg body weight every other week. The estimated GFR (eGFR) was used for evaluating renal function. Proteinuria measured by urine collection and described protein/creatinine ratio.

Results: A mean age of 15 patients was 48±15 years. Mean observation time was 47±14 months. eGFR was declined from 87±22 to 79±22 ml/min/1.73m$^2$ (p=0.39). Reduction rate of eGFR was -1.9±2.2 ml/min/1.73m$^2$/year. Proteinuria was 204±110 mg/gCr. None of 15 patients had urine abnormalities except proteinuria. There was no correlation between proteinuria and eGFR (rs= -0.19). Neither reduction rate of eGFR correlated to proteinuria (rs=0.29).

Conclusion: Our results indicated that, unlike male patients, proteinuria did not correlate with progression of renal dysfunction in female Fabry patients.

Disclosure of Financial Relationships: nothing to disclose

## PUB331

**Natural History and Immunohistochemical Study of Japanese Male X-Linked Alport Syndrome** Yuya Hashimura,[1] Kandai Nozu,[1] Hiromi Otsubo,[1] Fusako Hashimoto,[1] Shingo Ishimori,[1] Koichi Nakanishi,[2] Norishige Yoshikawa,[2] Hiroshi Kaito,[1] Kazumoto Iijima,[1] Masafumi Matsuo.[1] *[1]Pediatrics, Kobe Univ, Kobe, Hyogo, Japan; [2]Pediatrics, Wakayama Medical Univ, Wakayama, Japan.*

Introduction

X-linked (XL) AS is caused by mutations of COL4A5 encoding α5 chains of type IV collagen. It has been reported that clinical severity in male XLAS patients depends on mutation types. In USA, 64% of XLAS males showed missense mutations and 15% of patients showed Glycine-X-Y mutations. The median age of ESRD was 37 years for those with missense mutations and 25 years for those with truncating mutations. Immunohistochemically, 80% of XLAS males showed complete absence of α5 expression in renal tissues, and 20% of them showed α5 positive in several staining patterns. Our objective was to evaluate the natural history and the α5 expression pattern in renal tissues of Japanese male XLAS.

Method

Fifty-six male patients with genetically defined XLAS and 47 their male families with urinary abnormalities and/or ESRD were included in this study. Mutational analyses of COL4A5 were carried out by using (1)PCR and direct-sequencing of genomic DNA, (2) RT-PCR of mRNA and direct sequencing and (3) multiplex ligation-dependent probe amplification. Renal survival rates were analyzed by Kaplan-Meier method.

Result

Forty-five percent of XLAS males had missense mutations and 92% of the missense mutations were Glycine-X-Y mutations. The median age of ESRD was 35 years for those with missense mutations and 24 years for those with other mutations. 25% of XLAS males showed atypical α5 staining patterns and 81% of them had a missense mutation and others had an in-flame deletion. Interestingly, there were three pairs of patients showed different staining patterns in spite of the same mutations, and one of those cases was familial.

Conclusion

Japanese male XLAS patients showed different proportion of genotype, but similar renal survival rates compared to the previous study in USA. Immunohistochemical studies of α5 staining of renal tissues were useful tool for diagnosis of male XLAS, but we should consider some cases show atypical immunohistochemical patterns, which do not depend on solely their mutations.

Disclosure of Financial Relationships: nothing to disclose

## PUB333

**Genetic Aberrations in Proteins of the Alternative Complement Pathway in Patients with Atypical HUS** Lambertus V. Heuvel,[1,2] Dineke Westra,[1] Elena Volokhina,[1] Nicole Van De Kar,[1] Marleen Huigen.[3] *[1]Pediatric Nephrology, Radboud University Nijmegen Medical Centre, Netherlands; [2]Pediatrics, University Hospital Leuven, Belgium; [3]Laboratory Medicine, Radboud University Nijmegen Medical Centre, Netherlands.*

**Objectives:** Defective complement control on the surface of host cells is thought to have an important role in the pathogenesis of atypical hemolytic uremic syndrome (aHUS). The disease is associated with predisposing mutations in complement (regulating) proteins and with autoantibodies against CFH (αFH). The presence of mutations might be a prognostic factor for the outcome of renal transplantations and kidney donation.

**Methods:** Mutational screening, by means of PCR and DNA sequencing, is performed on the genes encoding complement C3 (*C3*), complement factor D (*CFD*), decay accelerating factor (*DAF*), and clusterin (*CLU*) in a group of 65 aHUS patients. In previous studies, a mutation in *CFH*, *IF*, *MCP*, or *FB*, or the presence of αFH was found in 36.9% (24/65) of these patients. Influence of mutations on protein structure is analyzed with respect to available structural data.

**Results:** Two heterozygous missense mutations (p.Lys65Gln; p.Glu1258Ala) were found in *C3* in four aHUS patients. Furthermore, one strongly predisposing SNP (p.Arg161Trp) was found heterozygously in twelve patients and in only three healthy controls (95% CI: 0.029-0.130). Analysis of available structural data indicates that the amino acids altered by the two missense mutations and the predisposing SNP might be located in the proximity of the C3b interface with CFH. Prediction models for interaction between CFH and C3 will be displayed. No genetic aberrations were found in *CFD*, *DAF*, or *CLU*.

**Conclusions:** Three novel potentially pathogenic alterations were found in *C3* in 24.2% (16/ 66) of the patients. These alterations might be located near the binding site of CFH, thereby influencing the interaction between this protein and C3b. This might lead to an increased activity of the alternative complement pathway. No mutations were found in *CFD*, *DAF*, and *CLU*, but the role of these genes in the pathogenesis of aHUS cannot be excluded yet, as a larger cohort needs to be screened.

Disclosure of Financial Relationships: nothing to disclose

## PUB334

**Accumulation of Glycosphingolipids in Cystic Epithelial Cells Affects Molecular Pathways of Cystogenesis** Herve Husson,[1] Thomas A. Natoli,[1] Ryan J. Russo,[1] Bing H. Wang,[2] Yeva Budman,[2] Steven R. Ledbetter,[1] John P. Leonard,[3] Oxana Beskrovnaya.[1] *[1]Cell Biology, Genzyme Corp., Framingham, MA; [2]Analytical Research & Development, Genzyme Corp., Waltham, MA; [3]Pharmacology, Genzyme Corp., Waltham, MA.*

Polycystic kidney disease (PKD) is characterized by the progressive growth of cysts in the kidney and other organs. Cyst formation is associated with increased rates of epithelial cell proliferation, apoptosis and dysregulation of multiple signaling pathways. Glycosphingolipids (GSL) and sphingolipids (SL) are emerging as major regulators in many of these cellular processes. GSL are also key components of membrane rafts, and can modulate the function of cell-surface receptors. Alterations of GSL metabolism have been previously documented in human ADPKD and the *cpk* mouse model of PKD, suggesting that these changes may play a role in disease progression. We set out to explore a link between cystogenesis and GSL. We found that glucosylceramide (GlcCer) and ganglioside GM3 levels are elevated in ADPKD kidneys and in kidneys from several mouse models of PKD, including *Pkd1* conditional knock out, *pcy* and *jck* animals. We also showed upregulation of GlcCer Synthase mRNA in human and mouse cystic epithelial cells by SAGE analysis and real time quantitative PCR analysis respectively. To examine a link between GSL levels and cystogenesis, we analyzed the effect of GSL modulation on cell cycle progression in cultured epithelial cells. Inhibition of GlcCer accumulation with either GlcCer Synthase siRNA or a small molecule inhibitor caused delay in the cell cycle progression. Furthermore, we showed that activated Akt/mTOR signaling pathway in cultured cystic epithelial cells is directly affected by inhibition of GlcCer Synthase upon IGF-1 stimulation. These data support a molecular link between elevated GSL levels and cystogenesis, thus suggesting GSL metabolism as a new target for PKD therapy.

Disclosure of Financial Relationships: nothing to disclose

**PUB335**

**Survey of Unruptured Intracranial Aneurysm in Autosomal Dominant Polycystic Kidney Disease Patients in Japan** Mahiro Kurashige,[1] Kazushige Hanaoka,[1] Yuichi Murayama,[2] Tatsuo Hosoya.[1] *[1]Division of Kidney and Hypertension, Department of Internal Medicine, The Jikei University, School of Medicine, Minato, Tokyo, Japan; [2]Department of Neurosurgery, The Jikei University, School of Medicine, Minato, Tokyo, Japan.*

Background: Intracranial aneurysm (ICA) is one of the most common complication of autosomal dominant polycystic kidney disease (ADPKD). Almost 10 percent of the ADPKD patients was affected. We investigated the characteristics of ICA and the correlation between frequency of ICA and renal function in ADPKD patients.

Methods: We performed a retrospective chart review of 152 ADPKD patients who visited the Jikei University hospital from April 2007 to September 2009. There were 79 male (52%) and 73 female (48%) with mean age of 49 yr. The information evaluated for the study was sex, age, presence of hypertension, renal function at the time of MRA. The estimated GFR (eGFR) was used for evaluating renal function. All patients were screened by magnetic resonance angiography (MRA) for ICA. The two-sample t test and $\chi^2$- test were used as statistical analysis.

Results: Thirty-one ICAs were detected in 24 of 152 ADPKD patients (15.8%). Four patients had multiple ICAs. The frequency of ICA was 13% in male (10 out of 79) and 19% (14 out of 73) in female, showing no statistical difference. The frequency of ICA was higher in hypertensive ADPKD patients; 19% in hypertension patients vs 9% in normotension patients. Eight ICAs were > 5mm in diameter. The localization of ICA was as follows; 19% in anterior cerebral artery, 16% in middle cerebral artery, 42% in internal carotid artery, 13% in basilar artery, and 10% in vertebral artery. Correlation between the frequency of ICA and renal function was listed in Table1. We found that the frequency of unraptured ICA was high in ADPKD patients in CKD stage1-2 than normal population, and increased, according to progression of CKD stage.

frequency of ICAs and CKD stage

| | aneurysm | no aneurysm | total |
|---|---|---|---|
| CKD stage 1-2 | 7 (9.8%) | 64 (90.2%) | 71 |
| CKD stage 3-4 | 6 (12.8%) | 41 (87.2%) | 47 |
| CKD stage 5-5D | 11 (32.4%) | 23 (67.6%) | 34 |

$\chi$-square P<0.01

Conclusion: The results indicated that ICA screening and follow-up study by MRA may be important for ADPKD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB336**

**Case of Accelerated ADPKD in a Lupus Nephritis End Stage Renal Disease Patient** Hemant Magoo, Roger F. Carbajal Mendoza, Donald I. Baumstein, Alf M. Tannenberg. *Nephrology, Metropolitan Hospital Center/New York Medical College, New York, NY.*

**Introduction:** Autosomal dominant polycystic kidney disease (ADPKD) is an inherited systemic disorder occuring in 1 in 800 live births and accounts for 7 to 10% of the dialysis population. There have been isolated case reports of nephrotic range proteinuria secondary to lupus nephritis in ADPKD patients with rapid progression to kidney failure. The converse of this has never been reported. We present a case of a chronic dialysis patient with end stage renal disease (ESRD) secondary to lupus nephritis that developed accelerated ADPKD.

**Case:** A 33 year female with ESRD secondary to lupus nephritis since 2001 was evaluated for epigastric pain and dyspepsia. She started chronic hemodialysis at age of 24 in 2001. Her past medical history was significant for hypertension, Class IV and Class V lupus nephritis with high chronicity index diagnosed on renal biopsy in 1999 and 2001. Renal cystic disease was absent at the time of biopsy in 2001 and a right upper quadrant ultrasound in 2004 revealed absence of any cysts in the right kidney. Work up for dyspepsia in December 2009 with computed tomography of the abdomen revealed bilateral kidneys of 14 cm length with multiple bilateral renal cysts compatible with ADPKD. Repeat ultrasound of the kidney 6 months later revealed classic ADPKD pattern with bilateral enlarged kidneys of 18 cm length and loss of corticomedullary differentiation.

**Discussion:** While lupus nephritis is an autoimune disease, ADPKD occurs due to a germ line mutation in a single gene. The nature of inherited mutation, genetic factors, and associated conditions such as hypertension and interstitial fibrosis influence the rate of progression of ADPKD. The link between lupus nephritis and ADPKD, if it exists, is not known. The accelerated progression of ADPKD in our patient raises the possibility of interstitial fibrosis secondary to lupus nephritis as a trigger factor hastening this progression.

Disclosure of Financial Relationships: nothing to disclose

**PUB337**

**Whole Gene Deletion of *ATP6V1B1*: A Novel Disease Mechanism for Autosomal Recessive Distal Renal Tubular Acidosis** Elizabeth Norgett,[1] Anthony Yii,[1] Katherine G. Blake-Palmer,[1] Ariana Kariminejad,[2] Fiona E. Karet.[1] *[1]Medical Genetics, University of Cambridge, Cambridge, United Kingdom; [2]Kariminejad-Najmabadi Path. & Genetics Center, Tehran, Islamic Republic of Iran.*

Distal Renal Tubular Acidosis (dRTA) is a disorder of acid-base regulation caused by functional failure of alpha-intercalated cells in the distal nephron. Where it is inherited in a recessive manner, it is usually severe and often associated with sensorineural hearing loss. To date, mutations that underlie recessive dRTA have been found in the two genes that encode protein subunits a4 and B1 of the alpha intercalated cell's apical $H^+$ATPase: *ATP6V0A4* and *ATP6V1B1*.

Here, we describe a consanguineous kindred in which dRTA was diagnosed in the proband at age 2 months following failure-to-thrive. Nephrocalcinosis was present in infancy, and he was noted to be deaf at 2y. The parents and a sibling are clinically normal. Linkage to *ATP6V0A4* was excluded, but attempts to PCR-amplify *ATP6V1B1* from the proband's genomic DNA failed for all exons. A complete deletion of the whole gene was identified. Gene dosage was halved in both parents.

Mapping of the deletion breakpoints revealed the 5' end to fall in intron 1 of the ventral anterior homeobox 2 gene *VAX2*, which lies immediately 5' of *ATP6V1B1*, while the 3' breakpoint lies within the intragenic region between *ATP6V1B1* and *ANKRD53*, the gene immediately 3' of *ATP6V1B1*. The 56.8 Kb genomic fragment represented by this deletion is replaced in this family by 2 nucleotides (GG). This appears to be a unique genetic phenomenon, and has not previously been reported in any INDEL mutation.

This is the first time a whole gene deletion has been described as a mechanism for dRTA. In addition to delineating this novel deletion, our findings suggest functional redundancy of *VAX2* in humans; the homeobox protein encoded by this 3-coding-exon gene is essential for establishing the dorsal-ventral axis of the eye in mouse (Barbieri et al, Development 129:805-13, 2002), but the child described here does not display any ocular phenotype despite missing most of the coding portion of the gene.

Disclosure of Financial Relationships: nothing to disclose

**PUB338**

**Mutational Analysis of *NPHS1* in a Worldwide Cohort of CNS Patients** Bugsu Ovunc,[1] Shazia Ashraf,[1] Virginia Vega-Warner,[1] Friedhelm Hildebrandt.[1,2] *[1]Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; [2]Howard Hughes Medical Institute, .*

Congenital nephrotic syndrome (CNS) is defined as nephrotic syndrome which manifests within the first 3 months of life. Mutations in *NPHS1* gene encoding nephrin, are a major cause for CNS. Currently, more than 170 different mutations of *NPHS1* causing CNS have been published, affecting most exons.

We performed mutation analysis of *NPHS1* in a worldwide cohort of children with CNS. All 29 exons were examined using direct sequencing. New mutations were confirmed by showing their absence in 96 healthy control individuals.

We were able to detect disease causing mutations in 9/31 families (29%). 7 of the families showed a homozygous mutation, while 2 were compound heterozygous. In another two families, single heterozygous *NPHS1* mutations were detected. Out of 13 mutations discovered, 3 of them were novel, consisting of 1 splice site mutation and 2 missense mutations. Novel mutations occurred in individuals in whom both recessive disease alleles were discovered.

Our data demonstrate that the spectrum of *NPHS1* mutations is still expanding, involving new exons, in patients from a diverse ethnic background.

Disclosure of Financial Relationships: nothing to disclose

**PUB339**

**Bronchiectasis and Pulmonary Function Test (PFT) Abnormality in ADPKD** Qi Qian, Ladan Zand, Robert Hartman, Teng Moua, Xiangling Wang, Tobias Peikert, Kaiser Lim. *Mayo Clinic, Rochester, MN.*

**Backgrounds:** ADPKD, characterized by polycystic kidney dysfunction, is a systemic disease with multiple extra-renal manifestations. ADPKD patients exhibit an increased prevalence of radiographic bronchiectasis. The functional significance of such abnormality has not been defined. We examined the occurrence of pulmonary function test (PFT) abnormalities in a cohort of ADPKD patients with and without radiographic bronchiectasis.

**Methods:** Clinical records of adult ADPKD patients seen at Mayo clinic with both chest CT scan and PFT from year 1998 to 2008 were reviewed. A total of 65 patients were enrolled after excluding those with acute pneumonia or other acute pulmonary infiltrative processes. CT criteria for diagnosis of bronchiectasis included enlarged internal bronchial diameter, failure of airway tapering for at least 2 cm beyond the last branch point, and airway wall thickening in the lung periphery. PFT abnormalities were classified to five patterns: obstructive, restrictive, mixed (obstructive and restrictive), non-specific, and isolated reduction of diffusion lung capacity for carbon monoxide (DLCO).

**Results:** Thirty-two of the 65 (49.2%) ADPKD patients exhibited radiographic bronchiectasis. PFT abnormalities were noted in 24 of the 32 (75.0%) patients with and 16 of 33 (48.5%) without radiographic bronchiectasis (P<0.03). Mean age and eGFR were not statistically different between patients with and without PFT abnormalities. Except for the isolated reduction in DLCO, the distribution of PFT abnormalities was similar: 11 of the 24 (with bronchiectasis) vs. 7 of the 16 (without bronchiectasis) had an obstructive pattern; 3 of the 24 vs. 4 of the 16, restrictive pattern; 1 of the 24 vs. 1 of the 16, mixed pattern; and, 3 of the 24 vs. 4 of the 16, nonspecific pattern. The isolated DLCO reduction was noted exclusively in those with bronchiectasis, 6 vs. 0 in those without bronchiectasis.

**Conclusion:** In this cohort, ADPKD patients with radiographic bronchiectasis had a higher occurrence of PFT abnormalities, suggesting a functional significance of radiographic bronchiectasis in ADPKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB340**

**The Primary Cilium Is Required for Vasopressin Mediated Aquaporin-2 Trafficking** Takamitsu Saigusa,[1] P. Darwin Bell,[1] Robert J. Kolb.[2] *[1]Division of Nephrology, Department of Medicine, Medical University of South Carolina, Charleston, SC; [2]Pediatrics, Medical University of South Carolina, Charleston, SC.*

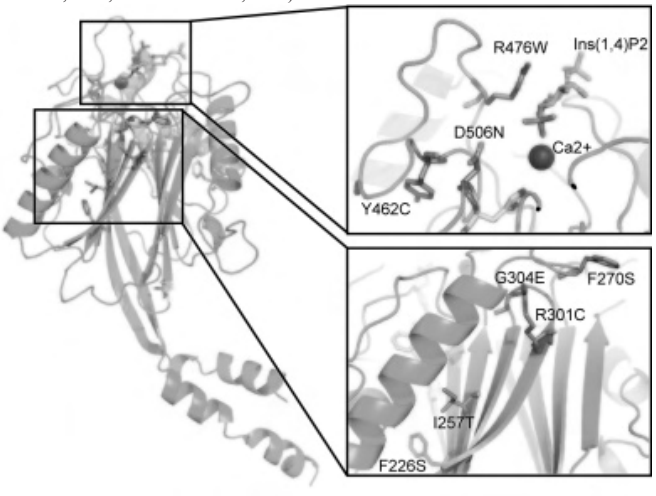
PCDNA (cilia -) and Bap2 (cilia +) are collecting duct cell lines derived from a PKD mouse model (orpk) and were used here to determine the role of the primary cilium in vasopressin (AVP) mediated aquaporin 2 (AQP2) trafficking and fluid absorption. Upon apical AVP addition, cilia (-) cells grown on impermeable supports had an increased net fluid absorption, as evident by the formation of epithelial domes, which were absent in cilia (+) cells. Immunofluorescence studies showed AQP2 localizes to cilia and in a subapical membrane compartment in cilia (+) cells. Upon AVP treatment, there was an increase in AQP2 at the apical membrane in cilia (-) but not in cilia (+) cells. Biotinylation and Western blot analysis confirmed this and also showed increased levels of a glycosylated form of AQP2 in cilia (-) vs. cilia (+) cells. Interestingly, in cilia (-) cells there was basolateral membrane insertion of AQP2 at a high level. In the absence of cilia, there appears to be an AVP mediated targeting of the V2 receptor to the apical membrane and enhanced AQP2 localization at both apical and basolateral membranes. We used electrophysiology to characterize V2R signaling and activation of ENaC by recording changes in amoliride/benzamil equivalent short-ciruit current (Isc). Addition of vasopressin to the apical membrane resulted in an increased equivalent Isc in cilia (-) cells; whereas only a minimal increase in Isc was found in the cilia (+) cells. To determine if apical V2R mediated water transport in cilia (-) cells also utilizes cAMP and PKA we pretreated cilia (-) cells with H89, an inhibitor of PKA. Treatment with H89 abolished changes in Isc in cilia (-) cells with addition of apical vasopressin. Thus, we propose AQP2 trafficking requires a normal cilium and when absent, the collecting duct cells becomes dedifferentiate leading to enhanced salt and water absorption which may contribute to the hypertension observed in PKD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB341**

**Structural Modeling of *OCRL1* Mutations in Patients with Dent 2 Disease** Steven J. Scheinman, Fosheng Hsu, Yuxin Mao.

The oculocerebrorenal syndrome of Lowe (LS) features proximal tubulopathy, cataracts, and mental developmental delay, with mutations in *OCRL1*. That gene is also mutated in 15% of patients with Dent disease (Dent 2), without eye or neurologic abnormalities. The majority of *OCRL1* missense mutations in both LS and Dent 2 occur in the phosphatase domain of the protein. We located 9 reported *OCRL1* missense mutations in patients with Dent 2 and 10 in LS on the computationally modeled 3-dimensional structure of the catalytic domain of the human OCRL1 protein based on the known structure from pombe yeast (Tsujishita, et al., Cell 105:379, 2001) and the C-terminal ASH-RhoGAP domains (Erdmann, et al., Dev Cell 13:377, 2007).




The figure shows 8 Dent 2 missense mutation on the catalytic domain of OCRL. These residues are shown in sticks and colored in grey. Two were located in loops close to the phosphatase catalytic site and predicted to alter substrate binding (Cat). Five were distant from the catalytic site in the protein interior and likely to alter protein stability (Stab); 2 of these had been reported to reduce protein abundance (Hoopes, et al., Am J Hum Genet 76:260, 2005). One (F270S) was located in an exposed region of a flexible loop; the functional consequences of this mutation are not clear. One was at the boundary of ASH-RhoGAP domains and expected to alter protein interactions (Int). Three of these were associated with cataracts: 1 Cat, 1 Stab and 1 Int. A similar spectrum was seen among the missense mutations in patients with LS, all of whom had cataracts and mental developmental delay. Differences in extrarenal phenotype between Dent 2 and LS may reflect variations in tissue-specific compensatory mechanisms rather than direct consequences of changes in protein structure by mutation.

Disclosure of Financial Relationships: nothing to disclose

**PUB342**

**Klotho Expression Is Reduced in Human Kidneys from Patients with Autosomal Dominant Polycystic Kidney Disease** Robert W. Schrier, Wei Wang, Michel B. Chonchol, Melissa A. Cadnapaphornchai, Kim Mcfann, Xiang-Dong Yan, Berenice Y. Gitomer. *Department of Medicine, University of Colorado Denver, Aurora, CO.*

**Purpose:** Autosomal dominant polycystic kidney disease (ADPKD) affects an estimated 600,000 patients within the U.S. Complete loss of renal function occurs in 50% of affected patients by age 60. The recently discovered FGF-23-Klotho axis is an important endocrine regulator of phosphate homeostasis and vitamin D levels. Klotho functions as an obligate co-receptor for FGF-23 binding in the kidney. Hence, deficiency of either FGF-23 or Klotho results in a similar phenotype characterized by premature aging and hyperphosphatemia. Significantly, angiotensin-II down-regulates renal expression of Klotho which may aggravate angiotensin-II induced renal injury. As angiotensin-II is up-regulated early in the course of ADPKD we hypothesized that expression of human Klotho would be reduced in human ADPKD kidneys and that the serum level may correlate with impaired renal function.

**Method:**[/bold] We measured expression of human membrane bound Klotho mRNA by quantitative PCR in 5 normal and 7 ADPKD kidneys. Serum Klotho level was measured in 40 ADPKD adults, comprising 17 women and 23 men [mean (SD) age: 30(12) years] by ELISA (Cubasio Biotech, Japan).

**Results**:[/bold] Mean(SD) expression of Klotho/GAPDH was significantly lower in ADPKD kidney compared to control kidney, ADPKD 0.13(0.10) vs. control 1.62(0.49), p =0.002. Mean (SD) serum level of Klotho was 28.6(30.3), however, no correlation between serum Klotho level and renal function over estimated glomerular filtration range 42-136 ml/min/$m^2$ was found.

**Conclusion:**[/bold] Klotho mRNA level is severely reduced in human ADPKD kidney and may exacerbate renal injury. The circulating form of human Klotho detected by ELISA assay does not correlate with renal function in ADPKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB343**

**Clinical Features in Patients with Autosomal Recessive Alport Syndrome** Vanessa Sivakumar, Mardhiah Binti Mohammad, Yanyan Wang, Hayat Dagher, Deb J. Colville, Judith A. Savige. *Medicine (Northern Health), The University of Melbourne, Melbourne, VIC, Australia.*

**Background and Objectives:** Most patients with Alport syndrome have X-linked disease, and the clinical features are well-described with this mode of inheritance. We have reviewed clinical features in our families with genetically-proven autosomal recessive disease who have presented over the past 20 years.

**Patients and Methods:** Alport syndrome was diagnosed on the basis of a lamellated GBM in a renal biopsy from the affected individual or a family member. Autosomal recessive inheritance was confirmed with the demonstration of *COL4A3* or *COL4A4* mutations or the exclusion of linkage to the *COL4A5* locus using haplotype analysis. Clinical features were noted and fundus photographs obtained in most cases.

**Results:** Of the 51 families with Alport syndrome we have seen, 8 (16%) had autosomal recessive inheritance demonstrated genetically. Two families (25%) were consanguineous. Fifteen affected individuals were identified, including 7 (47%) females. Three families (38%) had only one affected family member in a single generation. All 15 affected individuals developed endstage renal failure in adulthood at a median age of 28 years (range 23 - 38). Fourteen (93%) had hearing loss, 10 (10/13, 77%) had lenticonus, 9 (9/11, 82%) had a central retinopathy, and 9 (9/9, 100%) had a peripheral retinopathy. In contrast, only 2 females in the 42 families with X-linked Alport syndrome developed renal failure before 40 years of age. One of these had Alport syndrome, and the other was unaffected and had an unrelated disease.

**Conclusions:** Most individuals with autosomal recessive Alport syndrome develop renal failure in early adulthood together with hearing loss, lenticonus, and retinopathy. A female who presents with renal failure, hearing loss, lenticonus and retinopathy is very likely to have autosomal recessive Alport syndrome.

Disclosure of Financial Relationships: nothing to disclose

**PUB344**

**Inpatient Healthcare Utilization and Costs of Polycystic Kidney Disease** Cheryl L. Tran, Emily G. Herreshoff, Patrick Gipson. *University of Michigan, Ann Arbor, MI.*

Purpose: To investigate inpatient healthcare costs of polycystic kidney disease (PKD) in the United States.

Methods: Using the 2006 cohort from the Healthcare Cost and Utilization database, US Agency for Healthcare Research and Quality, we searched for all discharges with PKD as either a primary or secondary diagnosis. ICD 9 codes used were 753.12 (PKD NOS), 753.13 (Autosomal Dominant PKD), and 753.14 (Autosomal Recessive PKD). For discharges with PKD as the primary diagnosis, additional data were available on average hospital charges, aggregate (estimated national) charges, length of stay, and associated secondary diagnoses and procedures.

Results: In 2006, there were 20,441 hospital discharges with a diagnosis of PKD NOS, with 686 (3.4%) having this as the primary diagnosis; 8,230 with ADPKD, 1647(20%) as primary diagnosis; and 1,104 with ARPKD, 92 (9.1%) as primary diagnosis. The median charge per hospitalization for PKD NOS was $24,470, ADPKD was $13,520, and ARPKD was $27,179. However, when comparing the aggregate charges ("National Bill"),

ADPKD had the highest charges of $45,549,971. Charges for PKD NOS and ARPKD were $29,290,234 and $4,191,567, respectively. For ARPKD, the most common age of hospitalization was infants <1yr with 426 (55%), though 22.4% of hospitalizations were for patients ages 18-64yrs. Among pediatric hospitalizations for ARPKD or PKD NOS, 50 and 55% respectively were at Children's hospitals. Among pediatric hospitalizations for ADPKD, 80% were at Children's hospitals.

Conclusion: Most admissions for patients with PKD have a primary discharge diagnoses other than PKD. Seventy percent of admissions in patients with PKD have a discharge diagnosis of PKD NOS, not the more specific ADPKD or ARPKD. The cost of inpatient care for patients with PKD as the primary diagnosis is $79,000,000, but this accounts for only 8% of all inpatient hospital charges related to PKD. Children's hospitals care for the majority of inpatients under age 17 with PKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB345**

**Lipocalin 2 Is a Critical Mediator of Polycystic Kidney Disease Progression** Amandine Viau,[1] Khalil El Karoui,[1] Denise Laouari,[1] Clement Nguyen,[1] Martine Burtin,[1] Bertrand Knebelmann,[1] Jonathan M. Barasch,[2] Fabiola Terzi.[1] *[1]INSERM U845, Paris, France; [2]Medicine, Columbia University, NYC, NY.*

Polycystic cystic disease (PKD) is characterized by the development and growth of cysts that progressively lead to the destruction of the surrounding normal parenchyma and end stage renal failure (ESRF). Although the majority of PKD are hereditary, multiple renal cysts may develop as a result of developmental abnormalities, aging, drug or dialysis. The mechanisms of PKD progression are poorly understood. It has been suggested that cell proliferation may participate, but the signaling cascades involved remain to be elucidate.

We have recently shown that Lipocalin 2 (Lcn2), a siderophore chelating protein, markedly increased in acquired cysts after 3/4 nephrectomy. Here, we show that Lcn2 was also overexpressed in cystic tubular epithelia of jck (juvenile cystic kidney) mice, an inherited model of PKD similar to the human autosomal dominant polycystic cystic disease (ADPKD). Notably, Lcn2 was not simply a marker of renal lesions, but a crucial mediator of cyst progression. In fact, inactivation of Lcn2 gene dramatically prevented cyst progression in both acquired and inherited experimental PKD. The beneficial effect was associated to a marked decrease of cell proliferation in cystic epithelia. In addition, we discovered that Lcn2 was a critical transcriptional target of EGFR and mediated its mitogenic effect. EGFR inhibition prevented Lcn2 up-regulation and protected the kidney from cyst progression in mice expressing a dominant negative EGFR isoform. In this context, HiF1-a was crucially required for EGFR-induced Lcn2 overexpression. These data were relevant to human ADPKD where urinary Lcn2 was increased particularly in patients who rapidly progressed to ESRF.

In conclusion, our results uncover a novel function of Lcn2 and a critical pathway leading to progressive cystogenesis and renal failure. Lcn2 acts, therefore, as a tubular growth regulator, the overexpression of which identifies patients with progressive PKD and, more importantly, regulates cyst growth.

Disclosure of Financial Relationships: nothing to disclose

**PUB346**

**Ectodysplasin A2 Receptor (EDA2R), a Gene Implicated in Male Pattern Baldness Is Underexpressed in *Cilia (-)* Oakridge Polycystic Kidney (orpk) Mice Cell Line** Soundarapandian Vijayakumar, Soumyaroop Bhattacharya, Aditi Mulgund. *Pediatrics, University of Rochester Medical Center, Rochester, NY.*

Oakridge polycystic kidney mice *(orpk)* with mutation in a protein involved in cilia formation called IFT88/polaris develop polycystic kidney disease. *Tg737orpk* mutants also exhibit defects in the patterning of the skull and teeth. Recent studies show that primary cilia may regulate sonic hedgehog (Shh) activity in the control of molar tooth number. *Cilia (-) orpk* mutant cells (pCDNA) and *cilia (+)* rescued cells that had stable transfection of polaris gene (BAP2) were obtained from Dr. Bradley Yoder (University of Alabama at Birmingham). Matrigel cysts from rescued cells had a well defined lumen whereas cysts from mutant cells had multiple lumens or no defined lumen. These results suggested that these two lines are phenotypically different and hence we investigated the two cell lines using genome-wide expression profiling to identify novel genes regulated by IFT88. We performed genome-wide expression profiling from high quality RNA extracted from pCDNA (orpk mutant; n=3) and BAP2 (rescued; n=3) cell lines, using Affymetrix GeneChip® mouse Gene 1.0 ST Array. Preliminary analysis using Significance Analysis of Microarrays (SAM) on normalized expression intensities identified 14 probe sets representing 5 annotated genes (Xsist, Aldh1a1, A1g2, Ttc39b and Ncl) as overexpressed in pCDNA samples while 1 gene (Eda2r) was underexpressed in the same when compared to BAP2. Preliminary immunostaining studies show that EDA2R may be localized in the basal bodies of rescued (BAP2) cell lines and its expression is severely decreased in mutant (pCDNA) cells. EDA/EDA2R is implicated in the regulation of shh signaling in the context of hair follicle, salivary gland and tooth development. This novel finding is very significant in the understanding of how primary cilia may regulate shh signaling. Also, recent gene linkage studies implicate the role of EDA2R in male-pattern baldness (androgenetic alopecia), and this raises the possibility that EDA2R could also be one of the "second hit" factors in PKD cystogenesis.

Disclosure of Financial Relationships: nothing to disclose

**PUB347**

**NGAL in ADPKD Families: A Genotype-Phenotype Correlation** Grazia Maria Virzì,[1] Valentina Corradi,[1] Fiorella Gastaldon,[1] Massimo De Cal,[2] Dinna N. Cruz,[1] Claudio Ronco.[1] *[1]Nephrology, S Bortolo Hospital; [2]Medical Surgical Sciences, University Padua.*

Autosomal dominant polycystic kidney disease (ADPKD) represents 8-10% of the causes of ESRD in the world. The rate of progression of renal insufficiency is variable, and ESRD occurs in only 50% of affected subjects.

There are few biomarkers for quantification or monitoring kidney function. Creatinine (Cr) is an unreliable parameter to describe kidney function.

One of the most promising biomarkers in clinical nephrology is NGAL, massively released from kidney tubular cells after harmful stimuli.

Recent studies suggest a possible role for NGAL in CKD.

Our aim was to investigate the role played by NGAL and its correlations with CysC, Cr, Urea, eGFR in ADPKD families.

We measured NGAL, CysC, Cr, Urea in 18 ADPKD patients on dialysis (ADPKD-RRT), 36 of their relatives not on dialysis (ADPKD-NoRRT), 38 wild-type relatives (WT) and 30 healthy controls (Ctr).

Plasma NGAL was measured using point-of care test. CysC, Cr and Urea were measured with standard methods in clinical laboratory.

| | ADPKD-RRT | WT | ADPKD-NORRT | P |
|---|---|---|---|---|
| NGAL pg/mL | 611(537,676) | 60(60,61) | 66(60,113) | <.001 |
| Cr mg/dL | 8.8(6.9,10.2) | 0.8(0.8,0.9) | 1.1(0.8,1.5) | <.001 |
| CysC mg/L | 6.08(5.6,6.5) | 0.71(0.6,0.8) | 0.84(0.7,1.5) | <.001 |
| Urea mg/dL | 127(109,141) | 33(30,40) | 41(31,70) | <.001 |

Plasma NGAL and CysC values in ADPKD-RRT were significantly higher than Ctr (median 61.7 pg/ml, p<.001).

ADPKD-RRT pts had significantly higher NGAL, CysC, Cr and Urea levels compared to ADPKD-NoRRT and WT.

ADPKD-NoRRT pts had higher NGAL, CysC, Urea and Cr levels compared to WT relatives.

NGAL levels were similar between WT group and Cont (p>.05).

A strong correlation between NGAL and RRF was observed (NGAL/eGFR: r=-0.81; NGAL/Cr:r=.89; NGAL/Urea:r=.7; NGAL/CysC:r=.95, all with p<.001).

In conclusion, we studied NGAL in the context of genotype-phenotype. In ADPKD, NGAL was higher in patients already on RRT compared to their affected relatives not on RRT. WT group had normal NGAL levels; so WT relatives could be a better Ctr group because they share same genes.

Disclosure of Financial Relationships: nothing to disclose

**PUB348**

**Development of a High Resolution Melt (HRM) Method for Mutation Screening in PKD2 Gene in ADPKD Families** Grazia Maria Virzì,[1] Alice Bruson,[2] Valentina Corradi,[1] Fiorella Gastaldon,[1] Massimo De Cal,[3] Dinna N. Cruz,[1] Maurizio Clementi,[2] Claudio Ronco.[1] *[1]Nephrology, S Bortolo Hospital; [2]Pediatrics-Clinical Genetics, University Padua; [3]Medical and Surgical Sciences, University Padua.*

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary diseases. Mutations of 2 genetic loci (PKD1 and PKD2) can lead to renal cysts. Mutations in PKD2 are expected to be responsible for 15% of cases of ADPKD. The PKD2 gene consists of 15 exons in the 4q21.2 region; it encodes a 5.4 kb transcript; polycystin-2 consists of 968 amino acids.

We apply high-resolution melt analysis (HRM) as a new method of mutation scanning in the PKD2 gene prior to sequencing to select only mutation positive samples.

The method is based on DNA amplification in the presence of a double stranded DNA (dsDNA) intercalating fluorescent dye and on the transition of the double-stranded DNA molecule to its two single strands. DNA denaturation or melting has been used for many years to study DNA structure and composition. Initially, fluorescence is high in a Melt analysis because the sample starts as dsDNA, but fluorescence increases as the temperature is raised and DNA dissociates into single strands. The observed melting behavior, typical of each sample, can be discriminated according to sequence and length. Amplification and HRM conditions for 16 fragments of the PKD2 gene were optimized. HRM analysis were performed with the Rotor Gene 6000 (Qiagen).

Regions where the patient and control samples produce a common profile were not further evaluated, while those regions where the patient profile deviates from the control were assessed by DNA sequencing.

In conclusion, we developed a screening method using HRM analysis prior to direct sequencing, where only positive samples (according to reference) are further sequenced. With this approach, all positive and negative patients were successfully distinguished, and the results obtained were in absolute concordance with the direct sequence analysis.

HRM for the PKD2 genotyping is a simple and sensitive technique, to detect known and unknown variants, that could significantly reduce the time and cost of screening for mutations.

Disclosure of Financial Relationships: nothing to disclose

**PUB349**

**Autophagic Dysfunction Is Associated with Autosomal Recessive Polycystic Kidney Disease** Shixuan Wang,[1] Xuefeng Su,[1] Maoqing Wu,[1] Ayumi Takakura,[1] Patricia D. Wilson,[2] Jing Zhou.[1] *[1]Medicine, Brigham and Women's Hospital, Boston, MA; [2]Pediatrics, Medical College of Wisconsin, Milwaukee, WI.*

Autophagy is a catabolic process for cells to respond to stressful conditions such as starvation and hypoxia. It has been linked to many types of human disorders. Thus far, no reports have been published about the association between autophagy and either autosomal dominant (AD) or recessive (AR) polycystic kidney diseases (PKD), the most common inherited kidney disorders. Here, we demonstrate dysregulation of autophagy in patients with ARPKD. Compared to that in normal individuals, LC3 expression level and LC3 II/I ratio were reduced in ARPKD patients, indicating defective autophagy. Furthermore, mTOR signaling was activated, as p70 S6 kinase was hyper-phosphorylated at site T389. In conditional knockout (CKO) mice of Pkd1, mTOR signaling was also activated; however, autophagy regulation exemplified by ratio of LC3 II/I and expression level of beclin 1 seemed normal. Taken together, these data suggest that mTOR-autophagy axis may play a distinct role in AD- and ARPKD and autophagy may be potentially a way for therapeutic intervention.

Disclosure of Financial Relationships: nothing to disclose

**PUB350**

**Type II Diabetes in Patients with Autosomal Dominant Polycystic Kidney Disease** Wei Wang, Berenice Y. Gitomer, Kim Mcfann, Xiang-Dong Yan, Robert W. Schrier. *Department of Medicine, University of Colorado Denver, Aurora, CO.*

**Purpose:** Autosomal dominant polycystic kidney disease (ADPKD) is the most common potentially lethal genetic disorder affecting 1 in 400 live births. Diabetes is also very common affecting 7.8% of the United States population. Type II diabetes mellitus accounts for more than 90% of patients. Moreover, the epidemic of obesity and diabetes is increasing within the US and worldwide. In this regard, there is information from our ADPKD center that body mass index has increased significantly in ADPKD subjects (1985-1992 vs. 1992-2001). However, the impact of type II diabetes in ADPKD patients has not been well studied.

**Methods:** In order to assess the effect of Type II diabetes on severity of ADPKD we undertook a survey of patients from our ADPKD clinical database. Forty-four ADPKD patients with type II diabetes and available clinical information were identified. Eighty-eight patients with ADPKD but without diabetes who were age and sex matched to those with diabetes were selected for comparison.

**Results:** Those patients with diabetes had significantly larger kidney volumes than those with ADPKD alone [Geometric mean (95% CI)],2456(1510-3992)$cm^3$ vs. 1358(1186-1556)$cm^3$, p=0.02. There were no statistical differences between comparison groups based on occurrence of hypertension, ESRD or death. However, among those whose age at hypertension was known, the patients with both ADPKD and diabetes had earlier median(95% CI) age at onset of hypertension compared to those with ADPKD alone, 31(23-40) vs.38(35-42) years, p=0.04. Patients with ADPKD and diabetes tended to have an earlier median age of death than those with ADPKD alone 63(49-no upper) vs. 65(61-71) years, p=0.09.

**Conclusion:** Patients with ADPKD and type II diabetes have larger renal volumes and tend to die at a younger age compared to those patients with ADPKD alone. Exercise and weight loss should therefore be a particular recommendation in patients with ADPKD.

Disclosure of Financial Relationships: nothing to disclose

**PUB352**

**Caspase 3 Stimulates Migration and Cord Formation of Ureteric Bud Cells: A Potential Role in Reduced Ureteric Bud Branching by Maternal Nutrient Restriction** Midori Awazu,[1] Michio Nagata,[2] Mariko Hida.[1] *[1]Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan; [2]Department of Pathology, Tsukuba University, Tsukuba, Ibaraki, Japan.*

We previously reported that maternal nutrient restriction (50%, NR) reduces the size of metanephroi and inhibits ureteric bud branching in the offspring. While developmentally regulated signaling molecules such as ERK, p38, PI3K/Akt, and ß catenine are downregulated and upregulated at embryonic day 15 and 18, respectively, cleaved caspase 3 is down regulated at both time points in metanephroi from the offspring of NR. Inhibition of caspase 3 is reported to suppress metanephros growth and ureteric bud branching, but the mechanism remains unclear. Recently, caspase 3 is implicated in functions other than apoptosis including cell proliferation and motility. We therefore examined the role of caspase 3 in cord formation, migration, proliferation, and apoptosis using a mice ureteric bud cell line. Ureteric bud cells were cultured in 3D collagen type 1 gels with FGF2 100 ng/ml in the presence or absence of specific caspase 3 inhibitors Ac-DNLD-CHO or Ac-DEVD-CHO at 2, 20, and 200 µM. Cord formation was observed after several days of culture. Both the length and the number of cord structure were significantly decreased by Ac-DNLD-CHO or Ac-DEVD-CHO in a dose dependent manner. Total cell number was not affected by caspase 3 inhibition. Apoptotic cells detected by Hoechst staining were rarely observed (0.8±0.6%) and the number was also not altered by caspase 3 inhibition. FGF2-stimulated cell migration assessed by a modified Boyden chamber method, was inhibited significantly by Ac-DNLD-CHO in a dose dependent manner (2 µM 36±3, 20 µM 28±2, 200 µM 2±1 vs vehicle 84±6). The expression of cleaved caspase 3, assessed by immunoblot was more intense in the fetal rat kidney and decreased by neonatal day 7. The expression was significantly less in the NR kidney, but the difference disappeared by neonatal day 7. In conclusion, caspase 3 may promote ureteric bud branching by stimulation of cell migration not apoptosis. Reduced ureteric branching in maternally nutrient deprived offspring may be mediated by suppressed caspase 3 activity.

Disclosure of Financial Relationships: nothing to disclose

**PUB353**

**Dysregulation of mTOR Signaling Pathway in Rat Metanephros by Maternal Nutrient Restriction** Mariko Hida,[1] Michio Nagata,[2] Midori Awazu.[1] *[1]Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan; [2]Department of Pathology, Tsukuba University, Tsukuba, Ibaraki, Japan.*

Maternal nutrient restriction produces offspring with fewer nephrons. While apoptosis has been suggested to be the cause, the mechanism is not fully elucidated. We previously reported that signaling pathways important for kidney development, ERK, p38, PI3K/Akt, and ß catenin were downregulated and upregulated by maternal nutrient restriction at embryonic day 15 (E15) and 18 (E18), respectively, in rat fetal kidney. Mammalian target of rapamycin (mTOR) is a kinase downstream of PI3K/Akt that integrates growth factor stimulation and nutrient availability with protein synthesis and cell growth. We examined the expression of activated mTOR in rat fetal kidney under nutrient-restricted conditions. The role of mTOR on proliferation was also investigated in a metanephric mesenchymal cell line MS7. The offspring of dams given food ad libitum (CON) and those subjected to 50% food restriction throughout pregnancy (NR) were examined. Metanephros size became significantly smaller in NR after E14. At E18, total kidney protein, DNA content, and protein/DNA were significantly decreased in NR (62%, 70%, and 87%, respectively). The expression of phosphorylated mTOR (Ser2448, P-mTOR), assessed by immunoblot, was decreased (0.4-fold) and increased (1.8-fold of CON), at E15 and E18, respectively, in NR. Immunohistochemically, P-mTOR expression was found in metanephric mesenchyme and glomerular precursors. In NR kidney, P-mTOR expression was decreased at E13 and E15, but was increased at E18 in the nephrogenic zone in association with increased expression of Ki67. Rapamycin, an inhibitor of mTOR, at 1 µM inhibited serum-stimulated proliferation of cultured metanephric mesenchymal cells by 30%. The expression of L-type amino acid transporter LAT1, a known activator of mTOR, was decreased at E15 in NR (0.4-fold of CON), but the difference was no longer observed at E18. In conclusion, the decreased mTOR activity at E15 may have a role in the inhibited metanephros growth under maternal nutrient restriction. Increased activity at E18 is thought to be a compensatory response which may be mediated by increased LAT1.

Disclosure of Financial Relationships: nothing to disclose

**PUB354**

**Reprogramming Human Kidney Epithelial Cells to Nephron Progenitors Requires More Than Epigenetic Destabilization with Growth Factors or Small Molecules** Caroline E. Hopkins, Jessica Ineson, Melissa H. Little. *Institute for Molecular Bioscience, University of Queensland, Australia.*

The adult kidney has a limited capacity to repair. Reprogramming adult kidney cells to a nephron progenitor-like phenotype may represent an alternative for cell therapy purposes. In this study, we have investigated whether adult kidney cells can be reprogrammed to a nephron progenitor-like state via growth factor signaling, small molecules and nuclear reprogramming. Our results show that the human proximal tubular cell line HK2s can be reprogrammed to undergo an epithelial to mesenchyme transition in response to TGFB1, however this does not lead to a kidney progenitor cell phenotype based on in vitro assays of renal potential. In contrast, we have found that treatment of HK2s with VPA, a HDAC inhibitor associated with reprogramming leads to increased expression of kidney progenitor markers assessed via qRTPCR. VPA treated HK2 cells upregulated key CM transcription factors, including Six2, Pax2, Osr1, Eya1 and Hoxa11, and underwent a mesenchymal morphological transition. However, when assessed for their ability to contribute to a CM field within a developing kidney ex vivo, these cells could not act as nephron progenitors. In order to deepen the CM attractor state, we have undertaken a screen for reprogramming factors via sequential pooling of 15 kidney developmentally-specific transcription factors. In this way, we have identified several pools of transcription factors that, together with VPA treatment, lower E-cadherin expression whilst inducing most elements of a genuine CM gene regulatory network. We are currently evaluating the relative renal potential of each positive pool to identify the most efficient transcription factor combination for reprogramming renal epithelial cells to a CM phenotype. This is the first report demonstrating targeted reprogramming within the kidney field. These results concur with the observations of the field that reprogramming involves overcoming multiple epigenetic barriers in order to both destabilize the gene regulatory network specific to the initiating cells and fully activate the network required for the desired attractor endpoint.

Disclosure of Financial Relationships: nothing to disclose

**PUB355**

**Reactive Oxygen Species (ROS) Produced by NADPH Oxidase Contribute Ureteric Bud Branching and Nephrogenesis** Sato Matsuura, Shuji Kondo, Kenichi Suga, Yukiko Kinoshita, Maki Urushihara, Toshiaki Tamaki, Shoji Kagami. *Department of Pediatrics and Pharmacology, University of Tokushima, Tokushima-city, Tokushima, Japan.*

Ureteric bud branching and nephrogenesis are performed through large-scale proliferation and apoptosis during renal development. ROS produced by NADPH oxidase considered to be involved in cell behaviors including proliferation and apoptosis. However the role of ROS remains to be determined during renal development. We first investigated

the expression of NADPH oxidase and ROS production in rat embryonic (E14, E15, E16, and E18) and postnatal (P1, P7, P20, and P42) kidneys. We further determined the role of ROS in ureteric bud branching and nephrogenesis using NADPH oxidase inhibitors. Immunohistochemistry revealed that the NADPH oxidase components (Nox1, 2, 3, 4, p22phox, p47phox, and p67phox) were expressed on epithelial cells in ureteric bud branches and immature glomerular cells and epitherial cells in nephrogenic zone. Western blotting also demonstrated all of these components were expressed in embryonic and postnatal kidneys. ROS production detected by DHE assay was strongly observed in ureteric bud branches and nephrogenic zone corresponding with NADPH oxidase localization. Organ culture of E14 kidneys for 2 days showed that the inhibition of NADPH oxidase with diphenyleneiodonium (DPI) ($1x10^{-5}$ M) and apocynin ($1x10^{-3}$ M) significantly decreased the number of ureteric bud branches (71.4%, $p<0.05$) and tips (66.7%, $p<0.05$) associated with reduced level of ROS production. This was also associated with decreased level of phosphlylated ERK1/2 and increased level of cleaved caspase-3 ($p<0.05$). Organ culture of E18 kidneys for 2 days showed that its inhibition reduced the size of nephrogenic zone ($P<0.01$) accompanied with reduced levels of ROS production, pax-2 expression, PCNA positive cells, and p-ERK1/2, and increased level of cleaved caspase-3. These results first demonstrate that ROS produced by NADPH oxidase might play an important role in ureteric bud branching and nephrogenesis through regulation of proliferation and apoptosis.

Disclosure of Financial Relationships: nothing to disclose

## PUB356

**NR3 Subtypes of the N-methyl-D-aspartate (NMDA) Receptor Expression in Developing Male and Female Rat Kidneys** Karen A. Munger,[1,2,3] Laura Mandler,[1,3] Rachel F. Reynen,[3,4] Jason L. Petersen.[1,2,3] *[1]Basic and Applied Research, Avera Research Institute, Sioux Falls, SD; [2]Internal Medicine, University of South Dakota, Sioux Falls, SD; [3]Research and Development, VAMC, Sioux Falls, SD; [4]Biology, Augustana College, Sioux Falls, SD.*

The NMDA receptor is a widely recognized glutamate receptor in the brain. NMDA receptors are positive ion channels for the net influx of calcium. Kidney function is largely unknown. The NR3 subtype of the NMDA receptor is only found in developing brain. Interestingly, the NR1/NR3 receptor complex requires only glycine rather than a combination of glycine and glutamate to activate the channel.

Male and female rat kidneys were examined at post-partum day 1, 7, 14 and 21 for NMDA content. Protein was isolated from kidneys and run on an 8% SDS Page gel and transferred to nitrocellulose membrane. Antibodies to the NMDA receptor subtype NR3A and B were applied to the membrane, followed by secondary antibodies. Positive bands were visualized with a chemiluminescent probe. Histological sections were examined in Bouins fixed tissues, sectioned at 7 microns and probed with primary anti-NR3 antibodies with CY5 labeled secondary probes. Sections were photographed on a fluorescent confocal microscope. PCR was conducted for NR3A and B message in kidney tissues.

RESULTS:

NMDA NR3 receptor subtypes are found in developing rat kidney. Interestingly, the NR3A is only found in the collecting ducts, whereas NR3B is widely expressed in all tubules EXCEPT the collecting duct. In addition, the NR3 subtype is expressed early in kidney development and protein expression peaks at 7-14 days after birth and then disappears. There are also gender-dependent differences in NR3B expression, with females having lower levels and peaking at day 7, whereas males have higher levels of NR3B expression and peak expression later, at day 14. RT-PCR results parallel the protein expression. These results echo our previous results of higher NR2A expression in males and also point to possible developmental differences in the NR3 expression. Future studies will focus on the regulation of the renal NR3 receptors and functional differences.

Disclosure of Financial Relationships: nothing to disclose

## PUB357

**Intrauterine Programming of Tubular Sodium Reabsorption in a Rat Model of Placental Insufficiency** Kai D. Nüsken,[1] Kerstin U. Amann.[2] *[1]Department of Pediatric Nephrology, University of Cologne, Cologne, Germany; [2]Department of Nephropathology, University of Erlangen-Nuremberg, Erlangen, Germany.*

**Introduction:** Bilateral ligation of the uterine arteries in rats (LIG) serves as a model of placental insufficiency. The offspring shows both intrauterine growth restriction (IUGR) and developmental programming, including persisting alteration of renal function.

**Methods:** Parameters of kidney function and morphology in offspring of dams which underwent either LIG or sham operation (SOP) were compared with those of untreated controls (C). Weight gain, blood and urinary parameters were recorded regularly up to the age of 30 weeks. Glomerular, tubular, interstitial and vascular characteristics and markers of inflammation (ED-1) and proliferation (PCNA) were determined at the age of 30 weeks. Renal gene expressions were quantified by RT-PCR.

**Results:** At puberty (age 7 weeks), both LIG and SOP offspring showed catch-up growth, decreased urinary sodium and increased urinary potassium and aldosterone ($p<0.001$). Concomitantly, plasma sodium was increased and potassium reduced. However, adult LIG offspring (age 30 weeks) showed an elevated urinary loss of sodium despite an increased renal gene expression of the mineralocorticoid receptor, the 11β-hydroxysteroide dehydrogenase type 2 and the β-subunit of the epithelial sodium channel. PCNA protein expression in the thick ascending loop of Henle (TAL) was concomitantly increased.

**Conclusions:** In puberty, SOP activates tubular sodium reabsorption similar to LIG, which may contribute to catch-up growth. In adult LIG offspring, increased PCNA in the TAL indicates need for tubular repair processes. As the TAL is crucial for sodium reabsorption, tubular damage in this part of the nephron may outweigh the activation of the ENaC, thus resulting in a loss of sodium.

Disclosure of Financial Relationships: nothing to disclose

## PUB358

**Maternal Undernutrition Dysregulates Apoptosis in Offspring Kidneys** Cynthia C. Nast,[1] Sanaz Tafti,[2] Mina Desai,[2] Michael Ross,[2] Thomas R. Magee.[2] *[1]Pathology, Cedars-Sinai Medical Center, Los Angeles, CA; [2]Ob-Gyn, Harbor-UCLA Medical Center, Torrance, CA.*

**Background:** Maternal undernutrition (MUN) induces offspring kidney nephron deficits by uncertain mechanisms. We evaluated MUN-induced kidney RNA, then protein expression before and after birth to assess the role of apoptosis. **Design:** Pregnant rat dams were 50% food restricted from embryonic day (E)10. Male offspring E20 and postpartum day (P) 1 kidneys were removed. E20 whole kidney RNA was hybridized using rat Agilent DNA microarrays. E20 and P1 kidneys were analyzed by Western blot and slides stained by TUNEL for morphometry and WT1 and Pax2 for apoptosis localization.

**Result:** E20 kidney microarray showed 198 known genes with ≥1.5 fold change. Analysis of ontological groups and signaling pathways revealed 4 significantly upregulated genes in apoptotic signaling pathways; Fas ligand (7.0 fold), TNFα (1.6 fold), TNFR1a(1.6 fold), and AKT2 (1.5 fold). Apoptosis protein expression was altered as shown below.

E20 and P1 Western Blot Data

| | E20 Kidney | E20 Kidney | P1 Kidney | P1 Kidney |
|---|---|---|---|---|
| | Fold Change | P value | Fold Change | P value |
| Fas Ligand membrane | 1.4 | 0.14 | 1.3 | 0.98 |
| Fas Ligand soluble | Not detected | -- | 1.0 | 0.93 |
| FAS | 2.2 | 0.002 | 1.9 | 0.05 |
| BAX | 0.9 | 0.52 | 4.5 | 0.01 |
| Bcl2 | 1.8 | 0.003 | 0.6 | 0.03 |
| Caspase 3 | 1.2 | 0.69 | 1.6 | 0.03 |
| p53 | 1.4 | 0.28 | | |

Kidney TUNEL showed significantly increased apoptosis in the P1 nephrogenic zone (NZ) (MUN 2.2 ±.3 vs C 1.6 ±.5, $p < 0.05$), primarily in mesenchyme and renal vesicles; E20 TUNEL trended upward ($p<0.07$)

**Conclusion:** Apoptosis is upregulated in P1 MUN offspring kidneys in NZ mesenchyme and renal vesicles, thus impairing postpartum nephron formation. Pro- and anti-apoptotic proteins are increased at E20; however ↑BAX and ↓Bcl-2 at P1 suggest parturition effects cell survival. MUN increased apoptosis may be a direct effect and/or due to dysregulated developmental signaling pathways such as Notch, which we previously have shown to be reduced by MUN at E20. Therefore P1 upregulated apoptosis likely is an important mechanism inducing nephropenia in MUN progeny.

Disclosure of Financial Relationships: nothing to disclose

## PUB359

**Von Hippel Lindau Syndrome: A Review of Patients Attending the Irish National Tertiary Referral Center over the Past Twenty Years** Frank J. O Brien, Peter J. Conlon. *Department of Nephrology, Beaumont Hospital, Dublin, Ireland.*

Introduction

Von Hippel-Lindau disease (VHL) is a syndrome characterized by variety of tumours. This disease is genetic and can present in childhood, adolescence or in adult life. This study aims to characterize the presentation, natural history and renal manifestations of patients attending our institution with this condition. We also examine changes in method of presentation over the past 20 years.

Methods

A retrospective chart review was carried out on all patients coded as having VHL by the Hospital Inpatient Enquiry Scheme (HIPE) at Beaumont Hospital. Age, sex, mode of presentation, presence or absence of end stage kidney disease (ESKD) and genotype were recorded. Presence or absence of the characteristic tumours of VHL was also noted, as were the initial presenting features of these tumours.

Results

18 patients were identified has having VHL. The most frequent mode of presentation was altered neurological signs (56%), with a proportion presenting with haematuria (33%). Patients diagnosed prior to 2000 were more likely to have presented with significant complications of VHL, while those diagnosed after this time were more likely to have been diagnosed via screening. Five patients (27%) developed end stage kidney disease as a result of nephrectomy for bilateral renal cell carcinoma. These patients all required haemodialysis. The average time spent on dialysis was 1.8 years (range 6 months-5 years). Three of these subsequently received a transplant, two cadaveric and one living donor. Two patients developed unilateral renal cell carcinoma. Nine patients (50%) had coincidental central nervous system haemangioblastomas. One person was noted to have died secondary to metastatic renal cell carcinoma.

Conclusion

Our institution is unique in Ireland, consisting of tertiary referral centres for nephrology, urology/transplant and neurosurgery. The prevalence of VHL noted above is similar to that described internationally. Our patient group has similar rates of ESKD, but higher rates of transplantation when compared to similar studies. This study highlights the significant renal burden of this illness. It also highlights the importance of screening.

Disclosure of Financial Relationships: nothing to disclose

**PUB360**

**Counting Glomerular Number in Developing Mice** Jianyong Zhong,[1] Haichun Yang,[2] Valentina Kon,[1] Agnes B. Fogo,[2] Iekuni Ichikawa,[1] Ji Ma.[1] *[1]Pediatrics, Vanderbilt University, Nashville, TN; [2]Pathology, Vanderbilt University, Nashville, TN.*

Attenuation in acquisition of normal nephron number *in utero* has been linked to a variety of diseases and may be compensated by postnatal interventions. Abnormal kidney size/nephron number *in utero* also characterizes some mice generated by transgenic technologies. These novel biologic insights and animal models require a reproducible and practical approach to determine the number of nephrons at various stages from a limited amount of tissue. Although the literature contains a few studies, they report a wide range of glomerular number. Further complicating this issue is the fact that traditional methods for glomerular number counting were developed primarily in adult rats and have not been validated in developing mice which are characterized by small and rapidly growing glomeruli. In the present study we tested the hypothesis that a stereological approach utilizing 2-dimentional sections to estimate the nephron number in the whole kidney encompassed in the Weibel-Gomez method can be used to obtain reliable and reproducible data in the developing kidney of the mouse.

Serial sections of mouse kidneys revealed that starting from 7 dpp, almost every glomerulus can be identified by WT-1 and synaptopodin staining. Despite continued increase in age, kidney weight and glomerular size, the number of glomeruli assessed by Weibel-Gomez method remained stable at 7, 10, 14, 18, 21, 25 and 28 dpp, averaging 19495±534 glomeruli per kidney (mean±SE, n=4-5 mice for each time point). There was no difference in glomerular number between the left and right kidneys. A small adjustment of the coefficient in the Weibel-Gomez equation was required for assessments in mice. These results were confirmed by using the fractionator/dissector method as well as by different examiners using the Weibel-Gomez method.

We conclude that, the Weibel-Gomez method is a reliable and reproducible method of counting the glomerular number in developing mice. The reference equation can be used as a practical alternative for the fractionator/dissector procedure and emerging imaging technologies that both require exhausting the small amount of tissues in mice.

Disclosure of Financial Relationships: nothing to disclose

**PUB361**

**Association of Vascular Endothelial Growth Factor Gene Polymorphisms and Vascular Complications in Patients with Type 2 Diabetes According to Heritage** Kenneth Earle,[1] Karima Zitouni,[2] Diane Harry,[2] Kamini Kalidas,[2] Lorna Tinworth,[2] Steve Jeffery.[2] *[1]St George's Hospital NHS Trust London, United Kingdom; [2]St George's University London, United Kingdom.*

**Introduction and aim**

Deregulation of vascular endothelial growth factor(VEGF) is associated with coronary syndromes and microvascular diabetes complications. It is unknown if genetic determinants of VEGF are related to the epidemiological observation of a greatly increased risk of renal and cardiovascular disease(CVD) in Indo-Asian(IA) and African-Caribbean(AC) patients with type 2 diabetes compared with those of Caucasian(CAN) heritage in the UK

**Methods**

We genotyped 369 consecutive clinic attendees in North London,UK of AC(n=90), IA(n=47) and CAN(n=232) heritage for the -460(C/T) and +405(G/C) VEGF polymorphisms by polymerase chain reaction-restriction fragment length polymorphism. Presence of CVD, retinopathy determined by direct fundoscopy, and nephropathy according to serial urinary albumin:creatinine ratios>3mg/mmol and/or pre-end-stage renal dysfunction, hemoglobin A1c(HbA1c) blood pressure and serum cholesterol was obtained from medical records

**Results**

The mean(SD) age and duration of diabetes of the AC, IA and CAN groups were, 58.3(12.2), 59.5(10.8), 57.4(15.3) years and 16.3(9.0), 17.9(7.5), 17.0(10.4) years respectively. There were no differences in HbA1c, blood pressure or total cholesterol.There was no difference in the distribution of the TC, TT, CC and the CG, GG, CC genotypes of the -460 and +405 VEGF polymorphisms between the groups

The proportion of patients with CVD(n=31) carrying the CC genotype was higher than those carrying the TC and TT genotypes (24vs 5vs 10%; p=0.01).There was an association of CVD with carriage of the CC genotype in the IA subgroup(p<0.01). In the whole cohort, the GG genotype was associated with retinopathy(p=0.01) and with marginal significance with nephropathy(p=0.05)

**Conclusion**

In these patients with type 2 diabetes, a genetically determined disturbance of VEGF regulation may be of relevance to the higher incidence of CVD in the IA heritage group, but appears not to relate to differences in renal complications in either the AC or IA groups compared with those of CAN heritage

Disclosure of Financial Relationships: nothing to disclose

**PUB362**

**TaqMan Genotyping Analysis of NADPH Oxidase Gene Locus in Acute Kidney Injury** Mary Celine R. Perianayagam,[1] Hocine Tighiouart,[2] Daniel T. O'Connor,[3] Bertrand L. Jaber.[1] *[1]Department of Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Biostatistics Research Center, Tufts Medical Center, Boston, MA; [3]Center for Human Genetics and Genomics, University of California, San Diego, CA.*

**Background**. Although oxidative stress plays an important role in acute kidney injury (AKI), the association of oxidative stress-related genetic variants and clinical outcomes are unknown.

**Methods**. We examined the association of 4 tag-single nucleotide polymorphisms (SNPs) (Ref-SNP No. rs8854, rs4673, rs37946244 and rs4782390) in the NADPH oxidase gene locus with dialysis requirement or the composite of dialysis requirement or in-hospital death in 262 hospitalized adults with AKI. Dominant and additive multivariable logistic regression analyses were performed.

**Results.** Observed and expected genotype frequencies were not different and allele frequencies met Hardy Weinberg equilibrium (P>0.05). Within each SNP, baseline characteristics were not different between genotype groups in respect to age, sex, race, and APACHE II score. In the dominant model, on univariate analysis, the rs8854 A-allele group experienced 50% and 59% lower odds for dialysis requirement or the composite outcome, respectively, as compared to the GG genotype group. This association with the composite outcome persisted after adjustment for sex, race, and APACHE II score. In the additive model, there was no independent association between each copy of the rs8854 minor A-allele and outcomes, suggesting lack of a gene dose effect. The remaining 3 tag-SNPs were not associated with the outcomes of interest.

| rs8854 A-allele (vs GG genotype) | OR | 95%CI | P |
|---|---|---|---|
| Dialysis requirement | | | |
| -Unadjusted | 0.50 | 0.22,1.11 | 0.09 |
| -Adjusted | 0.52 | 0.22,1.19 | 0.12 |
| Dialysis requirement or in-hospital death | | | |
| -Unadjusted | 0.41 | 0.19,0.89 | 0.02 |
| -Adjusted | 0.40 | 0.17,0.93 | 0.03 |

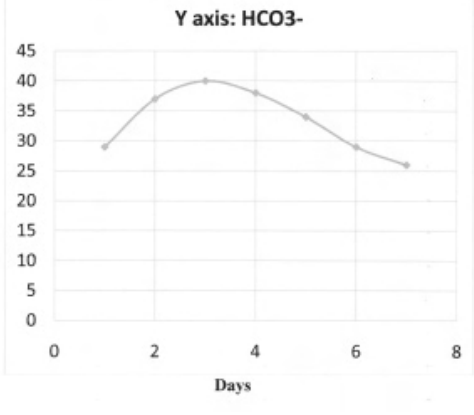
**Conclusion.** Of the 4 tag-SNPs in the NADPH oxidase gene locus examined in this pilot study, only one SNP located in the promoter region (rs8854) was associated with lower requirement for dialysis or in-hospital death. Larger studies are needed to confirm these relationships.

Disclosure of Financial Relationships: nothing to disclose

**PUB363**

**Regional Citrate Anticoagulation for Slow Continuosus Ultrafiltration (SCUF) Complicated by Severe Metabolic Alkalosis** Mourad Alsabbagh,[1] Edward A. Ross.[1] *[1]Division of Nephrology, University of Florida, Gainesville, FL; [2]Division of Nephrology, University of Florida, Gainesville, FL.*

Slow Continuous Ultrafiltration is a safe and efficient treatment for patients with fluid overload who are hemodynamically unstable, have low urine output, and don not need dialysis or hemofiltration (HF) for solute clearance.Anticoagulation needs to be sustained for these long treatments, and this can be clinically challenging as many patients have contraindications to systemic anticoagulation with heparin. Regional Citrate Anticoagulation would be an alternative option; however, we believe this can be problematic due to citrate kinetics. We present two cases in which patients received Anticoagulant Citrate Dextrose Solution A (ACD-A 225 ml/hr) pre-filter and had net UF of approximately 300 ml/hr: severe metabolic alkalosis developed with serum bicarbonate levels rising from 24-25 to 39-41 mmol/l [PH =7.70, PCO2=29.7, HCO3=37.3.




This is in contrast to our CVVH protocol using the same rate of ACD-A, in which there is approximately 2 L/hr of HF and no alkalosis; In this paper we discuss with detailed calculations how the metabolic alkalosis develops with using SCUF to emphasize the importance of accounting for the kinetics of citrate clearance into the HF, thereby avoiding acid base disturbances. Clinicians need to be aware of the risk of metabolic alkalosis when

using modalities with low rates of ultra- or hemofiltrate, and decrease or discontinue the citrate. In SCUF modality (compared with CVVH) the considerable net positive balance of citrate accumulate results in the development of severe metabolic alkalosis, despite net losses of bicarbonate into the ultrafiltrate.

Citrate Balance in SCUF in 3 days

| SCUF | Day 0 | Day 3 |
|---|---|---|
| Bicarb Balance (g/D) | -1.1 | -1.8 |
| Citrate Balance ( mmol/D) | +584.6 | +570.2 |

Net positive balance of citrate accumulate results in the

Disclosure of Financial Relationships: nothing to disclose

**PUB364**

**Microbiologic Treatment Efficacy in Continuous Renal Replacement Therapy (CRRT)** Daniel A. Caroff, Francis P. Wilson, Jeffrey S. Berns, Peter P. Reese. *Renal, Electrolyte, and Hypertension, University of Pennsylvania, Philadelphia, PA.*

Background: Despite theoretical and practical advantages, studies have not found that CRRT confers improved survival compared to intermittent hemodialysis (IHD) for acutely ill patients with AKI. We postulated that higher antibiotic clearances with CRRT cause low blood levels leading to poorer bacteriologic and clinical outcomes. This study aims to identify differences in treatment of bloodstream infection between patients on continuous venovenous hemodialysis (CVVHD) and IHD.

Methods: ICU patients receiving CVVHD (≥ 48 hrs) or IHD (≥ 2 treatments over 72 hrs) and positive blood cultures were identified over a 2-yr period. We studied the duration of positive cultures from initial IV antibiotic dosing, type of vascular access, antibiotic regimen, frequency of obtaining blood cultures, and microbiologic data.

Results: We identified 27 CVVHD and 21 IHD patients. The CVVHD cohort had a longer average duration of bloodstream infection than the IHD cohort (66.3 hrs vs. 56.9 hrs; p=0.08) that approached statistical significance. There was no significant difference in the rates of Gram-positive or Gram-negative infections between the cohorts. There was a higher rate of fungemia in patients with non-tunneled dialysis catheters compared to patients with tunneled catheters (p=0.04). The most commonly used antibiotics were Vancomycin, Cefepime, and Linezolid with similar usage in both groups. 61% of CVVHD patients died compared to 63% of IHD patients. On average, CVVHD patients had fewer blood cultures drawn after the first positive culture than IHD patients (0.61 vs 0.74 per day; p=0.07).

Conclusions: Our results suggest a longer average duration of bloodstream infection in patients on CVVHD compared to IHD. This may in part account for poor outcomes in septic CRRT patients. There was a higher incidence of fungemia in patients with NTDCs. Also, blood cultures were drawn at a lower rate in the CVVHD cohort, perhaps related to lower body temperature in CRRT than IHD. Future studies should explore whether more aggressive antimicrobial therapy during CRRT is necessary to improve treatment and survival of infected patients on CRRT.

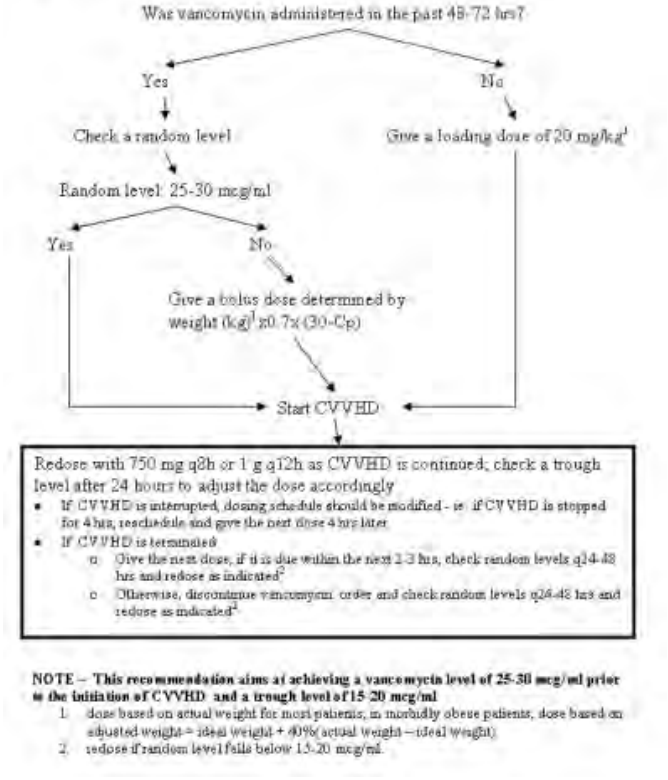
Disclosure of Financial Relationships: nothing to disclose

**PUB365**

**Vancomycin Dosing in Morbidly Obese Patients Undergoing CVVHD** Wei Chen, Julie L. Chen, Danielle Garcia, Payal Lakhani, Ladan Golestaneh. *Department of Medicine (Renal Division) and Pharmacy, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY.*

This study is to evaluate serum vancomycin removal in morbidly obese patients receiving continuous venovenous hemodialysis (CVVHD). Three morbidly obese patients with body mass indices (BMI) > 35 and oliguric renal failure were enrolled in a prospective, observational study. CVVHD was initiated 2 hours after the administration of a vancomycin loading dose. The loading dose of 20 mg/kg was given to achieve a target concentration of 25-30 mcg/ml. Blood flow, ultrafiltration and dialysate flow rates were individualized by the renal service. Vancomycin levels were measured at time 0, 2, 4, 8 hours. All three patients were obese, with BMI of 45, 70 & 36. The dialysate flow rate was 2 L/hr for all three patients, but blood flow and ultrafiltration rates varied within a limited range. The calculated half-life of vancomycin was 10.7, 14 & 11.7 hours, and the clearance was 72.4, 62.2, 73.6 ml/min. Vancomycin levels fell by 40.4%, 32.8%, 37.7% after 8 hours of CVVHD. When the data is extrapolated to 24 hours using calculated half-life, vancomycin levels fell below the therapeutic range (15 mcg/ml) after 12 hours. This study shows that vancomycin is effectively cleared by CVVHD in morbidly obese patients. Based on our results, we make the following recommendations: 1)for anuric or oliguric patients, to maintain therapeutic vancomcyin levels during CVVHD, an adequate loading dose is indicated to ensure a vancomycin level of 25 to 30 mcg/ml before the initiation of CVVHD; 2)in obese patients, we recommend a loading dose of 20 mg/kg based on adjusted body weight instead of actual weight; 3)vancomycin 1 gm q12hr or 750 mg q8hr is required to maintain a vancomycin trough of 15 to 20 mcg/ml during CVVHD; and the dosing regimen should be modified if there is any interruption in CVVHD.

**Figure 1. Recommended dosing regimen**




Disclosure of Financial Relationships: nothing to disclose

**PUB366**

**Acute Kidney Injury Requiring Dialysis: Shift CVVHD Dose Technical Details Mortality** Luis A. Concepcion. *Medicine Division of Nephrology, Scott & White Hospital Texas A&M Health Science Center, Temple, TX.*

The treatment of ARF requiring dialysis is controversial regarding the modality and dose.MATERIAL&METHODS:Analysis of a 6 month cohort of AKI patients treated with shift CVVHD (Nxstage 8 hours 40 Liter dialysate per sesion),demographics,lab data and survival obtained from the EMR,technical and monitoring details from the dialysis run sheet pre post bun kt/v urr per standard methods,data as mean and SD.RESULTS: 39 patients developed AKI requiring dialysis (43.6% sepsis,28.2% CV surgery, 28.2% other ) Mortality 39% (52.9% sepsis, 62.6%CV surgery ,18%other)8.1d on dialysis 19.2 days in the hospital ,196 treatments analyzed.Mean age 55.9y(19)weight 106kg(62)dialyzed for 7.1h (1.6)QB 300ml/min(45)dialysate K 2.75mEq/L(0.5)Vpress 197(46)heparin 1374units(1600)hypotension/hour 0.15(0.3) MAPpre 82.3 mmHg(15)MAPpost 83.3 mmhg (14)UF 3.4L(1.7)UF 483ml/h (248)URR 44.5% (14.6)kt/v per sesion 0.81(0.32) CVVHD dose 55.8ml/kg/h(21.2).Non survivors had a higher albumin(2.2vs1.9g/dl)lower phosphorous (4.7 vs 5.7 mg/dl)lower predialysis BUN(68vs78mg/dl)p<0.05.No difference in URR,kt/v,dose ccvhd.

CONCLUSION:Shift CVVHD is a method of RRT that can be used for AKI requiring dialysis,the survival is similar to other methods,the dose of dialysis as measured(URR kt/v ml/kg/min) did not differ between survivors and non survivors suggesting that other factors affect the survival outcome.

Disclosure of Financial Relationships: nothing to disclose

**PUB367**

**Genius® Dialysis System (Genius): A Safe and Efficient Method for Critically Ill Patients** Veronica T. Costa e Silva, Regina C. R. M. Abdulkader, Luis Yu, Gillene S. Ferreira, Renato Antunes Caires. *Nephrology Service, University of S Paulo School of Medicine, Sao Paulo, Brazil.*

Genius is well suited for hybrid therapies especially for critically ill patients. Adequacy and complications have not been studied. Thus, we retrospectively analyzed all dialysis with Genius system performed in adult ICUs during March 2010. The following complications were evaluated: hypotension, coagulation system (leading to dialysis interruption), cardiac arrhythmia, reduction of prescribed blood flow and no attainment of prescribed ultrafiltration (UF). Data are presented as mean ± SD or %. During the studied period, 195 dialysis were performed in 40 patients (4±3/patient). *Patients' characteristics*: age 58 ± 14 years, 55% male, 60% on vasopressors, 70% septic, 73% with acute kidney injury. *Dialysis' characteristics*: venous access by temporary catheter in 95% (51% femoral and 41% internal jugular veins), high-flux membrane in all (FS80 in 68%), blood flow = dialysate flow (250 mL/min in 68% of dialysis), no anticoagulation in 65% and duration of 6 hours in

58%. *Adequacy*: blood urea decreased from 118 ± 58 to 78 ± 41 mg/dL (P<0.01, 69 ± 27% reduction), and potassium decreased from 4.2 ± 0.8 to 4.0 ± 0.7 mEq/L (P<0.01). Prescribed UF was 1.6 ± 0.9 L which was not attained in 27% of dialysis. However, patients had their volume balance decreased from 1.6 ± 1.4 L in pre-dialysis day to 0.2 ± 1.6 L in dialysis day (P<0.01). *Complications:* 78% of the dialysis did not present any complication and 6% presented more than one complication. Main complications were hypotension in 20% (7% required introduction or increase in vasopressors), access problems in 32%, reduction in prescribed blood flow in 9% and clotting of the system in 17%. Clotting was associated (Fischer´s test) with access not working well (P<0.01) and with the need for decreasing blood flow (P<0.01) but not with the absence of anticoagulation (P=0.68). Cardiac arrhythmia occurred in 3 dialysis and bacteremia in 1. *In conclusion*, Genius may be an alternative for dialysing critically ill patients, allowing reduction of positive fluid balance. Complications are infrequent and mainly associated with problems of vascular access.

Disclosure of Financial Relationships: nothing to disclose

## PUB368

**Transition from Continuous to Intermittent RRT: Analysis of an International ICU Cohort** Dinna N. Cruz,[1] Roberto Fumagalli,[2] Manuel E. Herrera-Gutiérrez,[3] Paola Inguaggiato,[4] Detlef Kindgen-Milles,[5] Aníbal Defensor Marinho,[6] Rene Robert,[7] Claudio Ronco.[1] *[1]S Bortolo Hosp, Vicenza, Italy; [2]Milano Bicocca Univ, Monza, Italy; [3]Carlos Haya Hosp, Malaga, Spain; [4]S Croce e Carle Hosp, Cuneo, Italy; [5]Univ Hosp, Dusseldorf, Germany; [6]Santo Antonio General Hosp, Porto, Portugal; [7]Univ Hosp, Poiters, France.*

**Background**

In the management of Acute Kidney Injury (AKI) in the ICU, it is common practice to transition from CRRT to intermittent RRT (IRRT) as the patient clinically improves but continues to need renal support. However, there are no published data on this practice. Our aim was to describe the clinical circumstances present when patients are shifted to IRRT.

**Methods**

We identified 26 ICU patients in the DoReMi study who were started on CRRT then subsequently shifted to IRRT. We compared their characteristics on the first and last day of CRRT (i.e. the day before starting IRRT) using paired tests.

**Results**

The cohort was 69% male, median age 70 yr; SAPS II and SOFA scores on ICU admission were 49 and 12, respectively; pre-morbid serum creatinine (sCr) was 1.1 mg/dL. After a median of 6 (IQR 3-12) days on CRRT, they were transitioned to IRRT. On the last day of CRRT, they were less likely to be on vasopressors, and had significantly improved MAP, SOFA, urea, sCr and PaO2/FiO2 (Table 1). However, urine output was low. Two of 26 patients had to shift back to CRRT within 7 days, of whom 1 died in the ICU.

**Conclusion**

Patients are shifted from CRRT to IRRT when hemodynamic and metabolic status have improved. Patients with low urine output need continued renal support, albeit at lower intensity, and transition to IRRT may be an appropriate choice.

Table 1

| | First CRRT day | Last CRRT day | p |
|---|---|---|---|
| **SOFA** | 12.4 ± 3.2 | 10.4 ± 4.3 | 0.003 |
| **Vasopressor use (%)** | 92.3 | 42.3 | <0.001 |
| **MAP (mmHg)** | 97 (90 to 107) | 107 (97 to 119) | 0.004 |
| **$PaO_2/FiO_2$** | 228.2 ± 107.4 | 243.2 ± 87.7 | 0.41 |
| **sCr (mg/dl)** | 2.7 (1.5 to 3.9) | 1.6 (1.1 to 1.8) | <0.001 |
| **Urea (mg/dl)** | 97 (67 to 146) | 63 (51 to 85) | 0.007 |
| **Urine Output (ml/d)** | 1167 (120 to 1550) | 20 (0 to 700) | 0.003 |

Disclosure of Financial Relationships: Honoraria: Speaker Honoraria for Biosite/ Inverness Medical.

## PUB369

**How To Dialyze a Whale** Andre A. Kaplan,[1] Allison Tuttle.[2] *[1]Dept Med, UConn Hlth Ctr, Farmington, CT; [2]Mystic Aquarium, a Division of Sea Research, Inc., Mystic, CT.*

We report our efforts in providing renal replacement therapy for a 1050 kg. Beluga whale with AKI. Given the likely increased adipose stores we estimated total body water of 500 liters, thus calculating that dialytic requirements would be approximately 10 times that of a human. Hemodialysis did not appear a rational choice due to the anticipated difficulty in providing prolonged hemoaccess. PD had been previously tried unsuccessfully in a pilot whale (Ward DM, Proc Int Assoc Aquatic Animal Med, 1994 (abs). We therefore considered intestinal dialysis. Projected goal was to provide 30 liters/day of mannitol at 20% and to replace diarrhea output with 100 liters per day of hypotonic saline with bicarbonate. The whale had been trained to swallow a gastric tube and infusions were provided 5 times daily. It became quickly evident that the maximum tolerated gastric infusion was only 10 liters daily, with a concomitant 30 liters per day of parenteral fluid replacement with D5W, NS and bicarbonate. Lab values were obtained twice daily.

Results over a 15 day period:

Intestinal Dialysis in a Beluga Whale

| | day 1 | day 4 | day 7 | day 10 | day 13 | day 15 |
|---|---|---|---|---|---|---|
| **BUN** | 186 | 180 | 160 | 140 | 140 | 136 |
| **CR** | 13.3 | 14.5 | 14.7 | 16.6 | 18.3 | 19.4 |
| **K** | 6.9 | 6.1 | 4.8 | 4.4 | 4 | 4.1 |
| **HCO3** | 10.3 | 14 | 17.5 | 25.4 | 27.2 | 29.4 |
| **pH** | 6.99 | 7.04 | 7.08 | 7.22 | 7.32 | 7.38 |
| **Ca** | 8 | 8.9 | 9.2 | 9 | 9.1 | 9 |
| **Phos** | 10.4 | 11.8 | 11.1 | 10.4 | 10.3 | 9.5 |

As previously described with intestinal dialysis, removal of creatinine was inefficient. Continued increase in creatinine also supports our contention that improvements in nitrogen, potassium and acid base balance were the result of the treatment and not a return of endogenous renal function. Despite apparent success in dialytic goals, the whale expired on day 15. Preliminary postmortem evaluation confirmed clinical diagnosis of ATN. The cause of the ATN has not yet been identified. Although a definitive cause of death has not yet been determined, we believe intestinal dialysis represents a reasonable option in the treatment of AKI in the whale.

Note: Allison Tuttle is DVM, Diplomate, ACZM

Disclosure of Financial Relationships: nothing to disclose

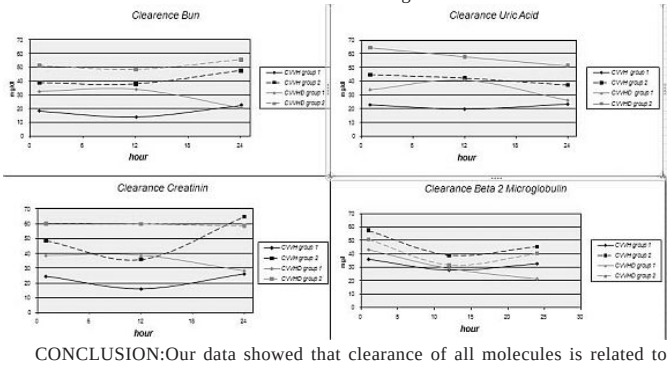
## PUB370

**Solutes Removal during Continuous Renal Replacement Therapy in Critically Ill Patients with a New High Polysulfone-Based Hemofilter (CUREFLO®): Convenction Versus Diffusion** Nicola Marchionna,[1,3] Matteo Floris,[1,3] Alessandra Brendolan,[1] Federico Nalesso,[1] Monica Zanella,[1] Claudio Ronco.[1,3] *[1]Nephrology, St Bortolo Hosp, Vicenza, Italy; [2]IRRIV, Italy.*

BACKGROUND:Solutes removal during continuous renal replacement therapy(CRRT) can be obtained by convention,diffusion and membrane adsorption.The best modality for CRRT is currently uncertain and it is poorly understood how transport of different solutes,changes over time.

METHODS:We compared,in critically ill patients,small molecular(creatinine,BUN,uric acid)and middle molecular weight(b2 microglobulin)solutes clearance and filter lifespam,during 7 continuous veno-venous dialysis(CVVHD)and 7 continuous veno-venous hemofiltration(CVVH)sessions.ACF-130W hollow-fiber dialyzer(1.3sqm,Asahi Kasei Kuraray Medical co.Ltd)was used.Patients were treated by CRRT using the Multifiltrate Fresenius machine.We compared different treatment conditions in CVVH(Group 1:Qb200 ml/min,Qr1500 ml/h in postdiluition,Qf -100 ml/h vs Group 2:Qb250 ml/min,Qr2500 ml/h in postdiluition,Qf -200 ml/h)and CVVHD(Group 1:Qb200 ml/min,Qd2500 ml/h,Qf -100 ml/h vs Group 2:Qb250 ml/min,Qd3500 ml/h,Qf -200 ml/h).Samples were collected before sessions and after 1,12 and 24h from the beginning.We also evaluated sieving coefficient of BUN,creatinine,uric acid,albumin,B2 microglobulin,myoglobin,alfa 2 macroglobulin

RESULTS:All sessions reached targeted 24h duration and at the end hemofilters displayed no clotting with a very low blood residual in the filter both in CVVH than in CVVHD treatments.Mean clearances are shown in Figure 1.




CONCLUSION:Our data showed that clearance of all molecules is related to filtration dose.Small molecular clearances are better in CVVHD during all long treatment. Moreover,middle molecular weight are better removed during CVVH.CUREFLO® filter is safe and its removal performances are suitable for the most advanced techniques in CRRT.

Disclosure of Financial Relationships: nothing to disclose

## PUB371

**Diffusive and Convective Solute Clearance during Continuous Renal Replacement Therapy with a New High Polysulfone-Based Hemofilter (CUREFLO®)** Nicola Marchionna,[1,3] Matteo Floris,[1,3] Alessandra Brendolan,[1] Federico Nalesso,[1,3] Monica Zanella,[1] Francesco Garzotto,[1,3] Dinna N. Cruz,[1,3] Pasquale Piccinni,[2] Claudio Ronco.[1,3] *[1]Nephrology, St Bortolo Hosp, Vicenza, Italy; [2]ICU, St Bortolo Hosp, Vicenza, Italy; [3]IRRIV, Vicenza, Italy.*

BACKGROUND: Solute exchanges during continuous renal replacement therapy (CRRT) can be obtained by convention, diffusion and membrane adsorption.The purpose of the study is the assessment of safety and efficacy of post-dilution continuous hemofiltration (CVVH) and hemodialysis (CVVHD) with CUREFLO®, a new high-flux polysulfone-based hemofilter

METHODS: Ten treatments (5 CVVH, 5 CVVHD) were performed in critically ill patients with acute kidney injury. ACF-130W hollow-fiber dialyzer (1.3 sqm, Asahi Kasei Kuraray Medical co.Ltd) was used. Patients were treated by CRRT using the Multifiltrate Fresenius machine. The treatment conditions were: CVVH(Qb 200 ml/min;Qr 1500 ml/h in postdiluition;Qf -100 ml/h) CVVHD(Qb 200 ml/min;Qd 2500 ml/h;Qf -100 ml/h. Samples were collected before the sessions and after 1, 12 and 24h from the beginning. We evaluated the clearance of BUN, creatinine, uric acid and sieving coefficient(SC)of BUN,creatinin,uric acid,albumin,B2microglobulin,myoglobin,alfa 2 macroglobulin.We also assessed the thrombogenicity through phothos of dialyzer for residual blood

RESULTS:Clearances for both treatments and SC of CVVH are showed.

| CVVH | | | |
|---|---|---|---|
| *Clearances* | *1h* | *12h* | *24h* |
| BUN | 18.3±10 | 14±12.8 | 22.5±4.9 |
| Creatinin | 24.4±11.9 | 16±17.1 | 26±6.8 |
| Uric Acid | 22.9±13.2 | 19.8±13.1 | 23.4±8.4 |
| *Sieving Coefficents* | *1h* | *12h* | *24h* |
| BUN | 1±0.08 | 1±0.07 | 1±0.04 |
| Creatinin | 1±0.07 | 1.1±0.07 | 1.1±0.04 |
| Uric Acid | 1.1±0.01 | 1.1±0.07 | 1.1±0.05 |
| Albumin | 0±0.01 | 0±0.01 | 0±0.01 |
| Beta 2 Microglobulin | 0.9±0.08 | 0.89±0.14 | 1±0.16 |
| Myoglobin | 0.58±0.26 | 0.38±0.16 | 0.31±0.15 |
| Alfa 2 Macroglobulin | 0.25±0.10 | 0.21±0.05 | 0.22±0.07 |
| | | | |
| CVVHD | | | |
| *Clearances* | *1h* | *12h* | *24h* |
| BUN | 18.3±10 | 14±12.8 | 22.5±4.8 |
| Creatinin | 24.4±11.9 | 16±17.1 | 26±6.8 |
| Uric Acid | 22.9±13.2 | 19.8±13.1 | 23.4±8.4 |

No adverse reactions were observed during the study.All sessions reached targeted 24h duration and at the end hemofilters displayed no clotting with a very low blood residual in the filter

CONCLUSION:The clearances for small molecules is greater in CVVHD than in CVVH as it is expected and the CUREFLO® shows a sieving profile suitable for removal of larger solutes through high filtration rate.The use of CUREFLO® is safe and its removal performances are suitable for the most advanced techniques in CRRT.

Disclosure of Financial Relationships: nothing to disclose

**PUB372**

**Outcomes in Intensive Care Unit Patients with Elevated Serum Lactate Requiring Renal Replacement** Robert M. Murithi, Warangkhana Wongba, William S. Burtnett, Mohamed Saad, Rosemary Ouseph. *Medicine, University of Louisville, Louisville, KY.*

Acute kidney injury (AKI) increases the mortality in patients in the intensive care unit. The mortality of AKI from sepsis is 75%. Methods: This study is a retrospective chart review of patients admitted to the intensive care units at local tertiary care centers from January 1, 2007-December 31, 2009. Inclusion criteria included age ≥ 18 years, diagnosis of sepsis, lactate level ≥ 2mmol/L, and acute kidney injury defined as an increase of 30% in serum creatinine or if baseline value was unknown a creatinine ≥ 1.5 mg/dL. Logistic regression was performed to see if there is a difference in patient survival in patients with lactate levels ≥ 2 mmol/L who require renal replacement therapy (RRT). Data is shown as mean ± s.d. Results: 51 cases were identified. The demographics of the population were 63% men, 76.5% Caucasian, 76% on pressors, average age 60 ± 17 years, baseline creatinine 1.42 ± .76, lactate 6 ± 4 mmol/L, and bicarbonate 20 ± 6 mEq/L. The overall mortality rate was 37%. Among patients requiring RRT (18% of study group) mortality was 67%. The mean time to initiate RRT was 2±1.7 days (3.3 ± 3.2 in survivors vs 1.5 ± .54 in nonsurvivors) . The mean time on RRT was 5.6 ± 4 days among survivors and 3.6 ± 2.8 days in patients who died. The overall withdrawal of care rate was 35%, among RRT it was 50%. Among patients who died 3 had an increase in lactate levels and 5 required increasing pressor support. There was no statistically significant difference in demographics or initial laboratory data between survivors and nonsurvivors. Conclusion: There was aggressive withdrawal of care by intensive care teams in patients determined to have low survival. Despite earlier initiation of renal replacement therapy nonsurvivors had increasing lactate levels and required increasing pressor support. The effect of interventions outlined by the Surviving Sepsis Campaign on outcomes in patients requiring RRT need further study.

Disclosure of Financial Relationships: nothing to disclose

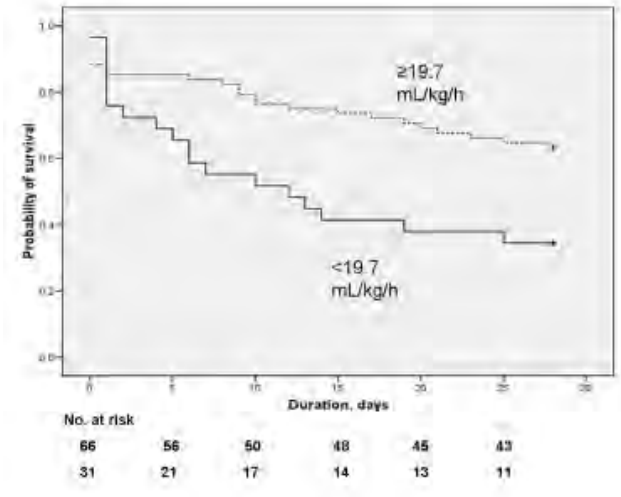
**PUB373**

**Delivered Dose of Continuous Venovenous Hemofiltration Predicts Outcome in Septic Patients with Acute Kidney Injury: A Retrospective Study** Shaikh Azam Nurmohamed,[1] Mark Koning,[2] Marc G. Vervloet,[1] Johan Groeneveld.[2] *[1]Nephrology, VU University Medical Center, Amsterdam, Netherlands; [2]Intensive Care, VU University Medical Center, Amsterdam, Netherlands.*

**Background:** The leading cause of acute kidney injury (AKI) in the intensive care unit is sepsis and its occurrence contributes to mortality and morbidity. Timing and dose of continuous venovenous hemofiltration (CVVH) remains controversial, particularly in sepsis. The objective of this study is to examine which CVVH characteristic best predicts mortality in sepsis-induced AKI.

**Methods:** We retrospectively studied all consecutive patients with sepsis-induced AKI requiring CVVH in a 1.5-year period. Patient, sepsis and CVVH characteristics, including timing, dose, mode, type of substitution fluid and of anticoagulation, and azotemic control were evaluated. Primary outcome was survival at day 28 after start of CVVH.

**Results:** Of the 97 patients, 43 (44%) died up to day 28 after start of CVVH. In univariate analyses, the delivered dose of CVVH was about 10% higher in survivors than non-survivors (median 23 vs 20 mL/kg/h, P=0.01), because non-survivors had a higher body weight, while the substitution fluid flow rate is fixed in our institution. In multivariate analyses, a lower delivered CVVH dose contributed to predict higher mortality, independently of non-renal organ failure, type of substitution fluid and azotemic control. In a Kaplan-Meier curve, a delivered dose <19.7 mL/kg/h was associated with shorter survival (P=0.006).




**Conclusion:** Our retrospective data suggest that in sepsis-induced AKI requiring CVVH, the delivered dose, rather than timing, mode of administration and azotemic control by CVVH, and organ failure are independent predictors of mortality. This argues for prospective studies on the effect of doses >19.7 mL/kg/h during CVVH in septic patients with AKI.

Disclosure of Financial Relationships: nothing to disclose

**PUB374**

**Value of Troponin (Tn) T as a Marker of Ischemic Heart Disease in Patients on Hemodialysis (HD). A Case Report** Ma del Carmen Prados Soler, Mª Dolores Del Pino Pino, Remedios Garofano, Clara Moriana, Manuel Ángel Rodríguez. *S. Nephrology, C. H. Torrecárdenas, Almería, Andalucía, Spain.*

**Introduction:**

Patients with chronic kidney disease (CKD) have a high rate of mortality associated with cardiovascular disease. The cardiac troponins (Tn) are biomarkers with great specificity for myocardial damage; their elevation is a criterion for the diagnosis of acute myocardial infarction (AMI)

Measurement of baseline TnT values in patients on hemodialysis is of great interest given the greater likelihood that these patients have elevated baseline levels. This non-specific increase may indicate less myocardial damage, an inflammatory response or a state of chronic volume overload

**Case Report:**

An 82-year-old man with stage 5 CKD on HD since January 08. He had a history of hypertension, type 2 diabetes mellitus and hypercholesterolemia. Ischemic cardiopathy: AMI, with a double coronary bypass and a pacemaker due to AV block

As an innovation, HD patients have their baseline Tn measured each 4 months. In this case, the serial baseline values of TnT were: 0.06, 0.1 and 0.09 ng/mL

He was admitted to the Cardiology Service with progressive dyspnea. Physical examination is normal. Cardiac markers: progressive elevation of TnT: 0.17 – 5.05 – 6.13 ng/mL. ECG: Pacemaker rhythm, with no capture or stimulation failure. Diagnosis: AMI of undetermined location

Forty-eight hours later the patient presented to the Emergency Service with sudden dyspnea. Auscultation: Bibasal crepitations up to the midfields. Cardiac markers: myoglobin 202, TnT 6.71 ng/mL. Echocardiogram similar : Left ventricle not dilated, walls not thickened, weight loss and septoapical dyskinesia. Diagnosis: Acute pulmonary edema. Given an emergency HD session – ultrafiltration, but died 60 minutes later; the ECG showed electrical beat due to pacemaker with electromechanical dissociation

**Conclusions:**

- In patients on HD, baseline measurements of TnT levels represent an improved strategy, as in a particular patient with symptoms of ACS, it is fundamental to compare the TnT value at that time with baseline figures

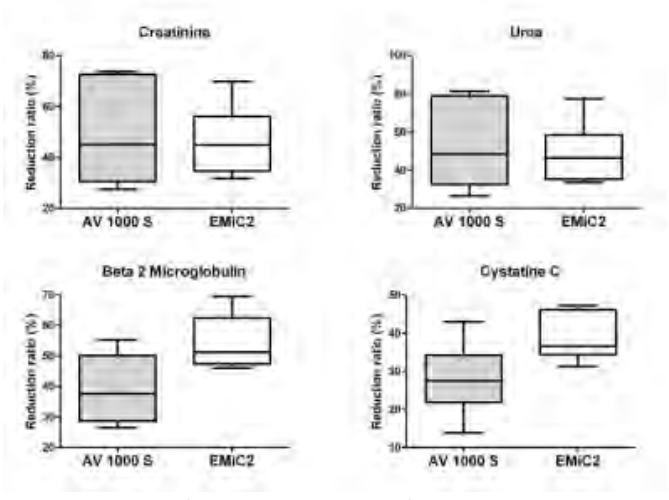
- Baseline measurement of TnT is particularly interesting in patients with a pacemaker, as ECG is not useful in these patients

Disclosure of Financial Relationships: nothing to disclose

**PUB375**

**Superior Elimination of Middle Molecules by the EMiC2 Dialyzer in Comparison to the AV 1000 S Dialyzer – A Prospective Comparative Cross-Over Study** Julius Schmidt, Carsten Hafer, Sajoscha A. Sorrentino, Hermann G. Haller, Jan T. Kielstein. *Department of Nephrology and Hypertension, Medical School Hannover, Hannover, Germany.*

**Background:** Removal of middle molecules is suggested to be advantageous in critically ill patients. Aim of the study was to compare middle molecule clearance of two dialyzers identical in surface area and material but different membrane porosity. **Methods:** Randomized cross-over trial. 10 ICU patients with AKI received two consecutive extended dialysis (ED) sessions starting either with EMiC2 dialyzer (Polysulfone, 1.8 $m^2$, Fresenius Medical Care (FMC) Germany) or AV1000S dialyzer (1.8 $m^2$, Polysulfone, FMC) using the GENIUS system®. All patients were treated using the 90 L GENIUS batch dialysis system (FMC). Blood / dialysate flow: 150 ml/min, treatment time 10 h. **Results:** Treatment with both dialyzers was well tolerated by all patients (4F/6M, mean age 43 years). There was a marked difference in dialyzer clearance of the middle molecules beta2-microglobulin (EMiC2: 51.4 ± 1.8 ml/min, AV 1000S: 40.5 ± 1.9 ml/min, p=0.0003) and cystatin c (EMiC2: 46.1 ± 1.4 ml/min, AV 1000S: 33.3 ± 1.7 ml/min, p=< 0.0001) **)** was also reflected by the more pronounced reduction of serum levels of these compounds ([b2M] EMiC2: 54.3 ± 3.6 %, AV 1000S: 39.1 ± 4.5 %, p=0.025, [cysc] EMiC2: 38.9 ± 2.6 %, AV 1000S: 28.0 ± 3.9 %, p=0.043).




In line with this we found a higher total amount of middle molecules in the collected ultrafiltrate and dialysate in the EMiC2 group ([b2M] EMiC2: 122.1 ± 17.3 mg, AV 1000S: 71.2 ± 11.9 mg, p=0.033, [cysc] EMiC2: 26.1 ± 4.3 mg, AV 1000S: 12.7 ± 3.1 mg, p=0.027)**.** There was no difference in the total amount of eliminated albumin per ED. **Discussion:** The EMiC2 dialyzer is more effective in eliminating middle molecules as compared to the AV1000S dialyzer. The clinical relevance of this finding should be evaluated in the future.

Disclosure of Financial Relationships: nothing to disclose

**PUB376**

**Different Modes of Blood Purification for Acute Kidney Injury Following Multiple Bee Stings** Ling Zhang, Li Zhou, Ping Fu. *Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

**Correspondence :** Prof. Ping FU

**Objective** This study is to investigate the effect of different modes of blood purification for the treatment of acute kidney injury (AKI) following multiple wasp stings.

**Methods** 87 patients with AKI due to wasp stings during 2001-2009 were retrospectively analyzed. 82 cases with AKI were recieved blood purification: (1)CVVH group:early intervention of continuous venovenous hemofiltration (CVVH) for at least 48h was performed, then replaced by intermittent hemodialysis (IHD) when conditions of patients were stable. (2)CVVH+PE group: plasmapheresis was performed on Day 1 and Day 2 in addition to CVVH. (3)IHD group: IHD was performed three times per week. (4) PD group: continuous ambulatory peritoneal dailysis (CAPD) was performed.

**Results** (1)CVVH group: 33 cases (91.7%) discharged improved, but 3 case died. (2)CVVH+PE group: 9 cases (90%) discharged improved, but 1 case died. (3)IHD Group: 22 cases (73.3%) improved but 3 cases died, 2 cases progressed to end stage renal disease(ESRD) and 3 cases withdrawed. (4)PD group: no patients improved, 2 cases died, 3 cases progressed to ESRD and 1 case withdrawed. Survival rate and recovery of the renal function in groups of CVVH, CVVH+PE and IHD groups were better than PD group. There was no significant differences of survival rate and recovery of the renal function among CVVH, CVVH+PE and IHD groups. But in the early stage, the decrease in total bilirubin, creatine kinase and white blood cells are more significant in groups of CVVH and CVVH+PE than IHD group. Adverse events (low blood pressure) in CVVH and CVVH+PE group were less than IHD group (P<0.05). Patients in CVVH+PE group progressed into polyuria (16.1±8.3 vs.23.2±11.2 days) and normal renal function (26.4±11.8 vs.34.2±16.1 days) earlier than CVVH group (P<0.05).

**Conclusions** Results from this study indicate that early CVVH treatment might be helpful in patients with impaired organ function and might reduce hospitalization days. But whether it can increase the survival rate of patients needs to be confirmed by further studies. CVVH combined PE might represent as a novel approach. CAPD may not be the first choice because of the low clearance rate.

Disclosure of Financial Relationships: nothing to disclose

**PUB377**

**Epoetin Alfa SQ Dosing May Be Reduced to Once Per Week in Hemodialysis Patients** Alexandre S. Ackad,[1] Christopher D. Parisi,[2] Julius M. Gardin.[1] *[1]Medicine, Hackensack University Medical Center, Hackensack, NJ; [2]Department of Patient Care, Hackensack University Medical Center, Hackensack, NJ.*

Purpose: To assess the feasibility of reducing the frequency of SQ EPO dosing, while maintaining a hemoglobin target of 11 to 12 gm/dl.

Methods: Retrospectively studied were (n= 299) prevalent HD patients from 1/1/09 through 12/31/09. Patients were gradually switched from the conventional EPO administration of 3 doses per week SQ to a once per week dose that was equivalent to the sum of their previous weekly EPO doses. Concurrent iron replacement protocol was unchanged. Excluded were incident HD patients less than 2 months on HD, those requiring more than 90,000 iu EPO/w, and patients with a mean Hb =>of 13 gm/dl. Hb values were the average of all 2009 end-of-the-month values. The EPO dose and Ferrous Gluconate (FERR) dose per week were computed from the cumulative monthly EPO dose and the total number of outpatient treatments.

Patients were divided into three groups: Group A (n= 124) received 1 or less injection/w, Group B (n= 137) between 1 and 2.5 doses/w, and Group C (n= 38) 3 or more doses per week. One way ANOVA followed by a Tuckey post-test was used for statistical analysis.

Results:

Table 1

| | Group A | Group B | Group C | P value |
|---|---|---|---|---|
| Mean Hb gm/dl | 11.69(11.61-11.79) | 11.30(11.10-11.33) | 11.18(10.80-11.21) | <0.001 |
| Mean EPO iu/wk | 2907(2580-3234) | 4782(4225-5338) | 8250(6176-10325) | <0.001 |
| Mean FERR iu/wk | 17.18(15.28-19.07) | 18.91(16.91-20.91) | 18.29(15.50-21.09) | 0.4167 |

Conclusion: Reducing SQ EPO administration from 3 times per week to 1 time per week in our chronic prevalent HD population was done without compromising the overall Hb target, and was accompanied by a substantial reduction in average weekly EPO dosage.

Disclosure of Financial Relationships: nothing to disclose

**PUB378**

**Iron Sucrose Maintenance Therapy in Hemodialysis (HD) Patients and Erythropoiesis-Stimulating Agent (ESA) Sparing** George R. Aronoff,[1] Michael Brier,[1,2] Claudy Mullon,[3] Phaneth Keo,[3] Ann Mooney,[3] Norma J. Ofsthun,[3] Jose A. Diaz-Buxo.[3] *[1]Department of Medicine, Univ. of Louisville Sch. of Med, Louisville, KY; [2]Dept. of Med., VA Medical Center, Louisville, KY; [3]Fresenius Medical Care NA, Waltham, MA.*

**Background:** The treatment of anemia in HD patients includes the administration of an ESA and iron. In addition to IV iron replacement, HD patients also need maintenance IV iron to sustain iron sufficiency and Hgb levels within recommended limits. To test the hypothesis that HD units giving maintenance IV iron and having a higher percentage of patients with transferrin saturation (TSAT) between 20-50% would use less ESA to maintain desired Hgb levels, we examined iron usage, ESA doses and Hgb levels from a large hemodialysis data base.

**Methods:** We compared facility level data from 153 Fresenius dialysis clinics with 40 patients using 80% iron sucrose from Jan-Dec 2008. A subset of facilities with a greater-than-median % patients with Hgb 10-12 g/dL and less-than-median Epo dose was identified (N=49). The average quarterly % of HD patients with 3-month average TSAT in range 20-50% was used to rank facilities. Based on this method, 16 of 49 clinics (group 1) had a higher % TSAT in range and 9 of 153 clinics (group 2) with lower % TSAT. Algorithms, iron indices, Hgb levels, Epo doses were compared for all 25 facilities.

**Results:**

Average quarterly % of patients and 3-month average TSAT, Ferritin, Hgb and Epo

| Group | % Pts w/TSAT 20-50% | TSAT (%) | Ferritin (ng/ml) | Hgb (g/dl) | % Pts w/Hgb 10-12 g/dl | Epo units/ Administration |
|---|---|---|---|---|---|---|
| 1 | 90 | 30.4 | 674 | 11.6 | 62.5 | 6,412 |
| 2 | 69 | 26.6 | 540 | 11.5 | 59.9 | 7,665 |

Iron replacement doses were used in both groups, however, Group 1 used iron maintenance of 100mg every other week (N=5), 100 mg q month (N=8), and either 25 or 50 mg q week (N=3). Group 2 used smaller maintenance doses of 25 or 50 mg q week (N=8). The maintenance algorithms targeted TSAT 20-50% and serum ferritin 100-800 ng/ml.

**Conclusion:** A higher % of patients with a 3 month average TSAT of 20-50%, higher mean TSAT and ferritin but comparable hemoglobin levels were achieved with 16% less Epo using iron sucrose maintenance doses of 100 mg every other week or 100 mg once a month.

Disclosure of Financial Relationships: Consultancy: AMAG
Amgen
Affymax
Fresenius Medical Care
NovartisResearch Funding: Merck
NxStage.

**PUB379**

**Transfusion Practices of In-Hospital Hemodialysis Patients in the Past 5 Years** George N. Coritsidis, Saad A. Bhatti, Kayode C. Lawrence, Salwa Rhazouani. *Division of Nephrology, Elmhurst Hospital Center-Mt Sinai School of Medicine, Elmhurst, NY.*

Introduction

With the upcoming bundling process the issue of possible increases in transfusions of ESRD patients has arisen. In-hospital transfusion practices have much improved in the last 5-10 years after the TRICC trial (N Engl J Med. 1999). Given the importance of avoiding transfusions in ESRD we were interested to see if there have been changes in transfusion practice for these patients.

Methods

Medical records were reviewed for all dialysis patients admitted to Elmhurst Medical Center for the periods of 2004/2005 and 2009/2010. For those patients that received transfusions the following were recorded: reasons for transfusion (H=hemorrhage, C=cardiac, A=anemia of chronic disease), transfusion trigger hemoglobin levels (Hb) and number of units given.

Results

Between 2004 and 2005, 158 patients on dialysis were admitted of which 22 were transfused: 46% H; 46% A and 9% C. 54 units were given in total. Between 2009 and 2010, 239 patients on dialysis were admitted of which 19 were transfused: 58% H; 42% A; 0% C. 46 units were given in total.We present our findings.

Comparison of transfusion practices: 2004/05 vs 2009/10

| | 2004-05(n=158) | 2009-10(n=239) | p value |
|---|---|---|---|
| Age (yrs) | 57.8±3.38 | 51.3±3.33 | NS |
| Hb trigger for transfusion (g/dL) | 6.61±0.132 | 6.74±0.057 | NS |
| Units Transfused (per patient) | 2.45±0.19 | 2.48±0.26 | NS |
| TRc | 6.96 | 5.61 | NS |

NS=non significant; TRc=ESRD patients transfused per 100 ESRD patient admissions per year

Conclusions

Over the last five years there has been a trend towards decreased transfusion of ESRD patients admitted to our hospital. Furthermore, fewer units were given for reasons other than hemorrhage, though this did not reach significance. On average, there was no difference in the trigger HB levels over the years. However, in both time periods they were below that of the TRICC trial of 7 g/dl. Limiting transfusions in ESRD patients, where reasonable, has benefical effects for future transplantation.

Disclosure of Financial Relationships: Consultancy: Consultant for Amgen Inc and Cerner.

**PUB380**

**Once Monthly C.E.R.A. in Hemodialysis Patients Provides Stable Hb Levels in All Patient Groups – Results from the SESAM Study** Frank Dellanna,[1] Thomas Weinreich,[2] Frank Leistikow.[3] *[1]Dialysezentrum Karlstrasse, Düsseldorf, Germany; [2]Dialysezentrum, Villingen-Schwenningen, Germany; [3]Nierenzentrum Mannheim, Mannheim, Germany.*

**INTRODUCTION AND AIMS:** CERA (Mircera®) was approved as a new long acting ESA for patients (pts.) suffering from renal anemia. The SESAM trial was one of the first observational studies to test stability of anemia control of CERA in routine clinical practice in hemodialysis pts.

**METHODS:** This non-interventional study was conducted in 92 German centers. Data of 924 hemodialysis pts. were collected over 8 months/9 visits. Analysis of the data was done in a descriptive way.

**RESULTS:** 910 pts. formed the Full Analysis Set (FAS), and for 710 pts. (Per Protocol Set, PPS), data on application of CERA and Hb measurements at least twice between visits 7 and 9 were available. In 856 pts. information about previous ESA trt. was recorded. Most pts were pretreated with Epoetin beta and Darbepetin alfa(42.7% / 22.7%). Although not for all pts. of the PPS unique identification for a subgroup was possible or information was missing, the pt. numbers with data available were sufficent for subgroup analysis:

- Route of admin.: 124 (s.c.) / 394 (i.v.)
- Age: 201 (<=65) / 342 (>65)
- Gender: 296 (m) / 247 (f)
- Previous ESA trt.: 373 (Epoetin) / 133 (Darbepoetin)
- Diabetic pts: 205 (yes) / 338 (no)

Results from subgroup analysis (PPS) for the evaluation period are given in Table 1:

Table 1: Individual deviation from mean during evaluation period in different subgroups (% of pts)

| | | **<= 1 g/dl** | **1-1,5 g/dl** | **1,5-2 g/dl** | **>2 g/dl** |
|---|---|---|---|---|---|
| Route of admin. | s.c./i.v. | 95.2/87.8 | 3.2/7.6 | 0.8/3.8 | 0.9/0.8 |
| Age | <=65/>65 | 88.1/90.4 | 7.0/6.4 | 4.5/2.3 | 0.5/0.9 |
| Gender | m/f | 90.9/87.9 | 6.1/7.3 | 3.0/3.2 | 0.0/1.6 |
| Previous ESA trt | Epoetin/Darbepoetin | 89.5/89.5 | 7.0/4.5 | 2.9/4.5 | 0.5/1.5 |
| Diabetic pts | yes/no | 90.7/88.8 | 6.3/6.8 | 2.4/3.6 | 0.5/0.9 |

**CONCLUSIONS:** Once monthly CERA trt. maintained stable Hb values with low intraindividual variability in this non-interventional study. Although some minor differences could be observed, results were consistent throughout all subgroups, thus showing that once-monthly CERA is suitable for all pts. on hemodialysis.

Disclosure of Financial Relationships: Honoraria: Roche, Shire, Sandoz, Amgen, Novartis, Hexal, Medice, Astellas.

**PUB381**

**CKD Epidemiology and Renal Anemia Treatment with NeoRecormon in Everyday Clinical Practice in Haemodialysed Patients in Latvia, Poland and Serbia. The Results of Non-Interventional, Observational Study** Maciej Drozdz,[1] Boleslaw Rutkowski,[2] Jolanta Malyszko.[3] *[1]Nephrology Department, Collegium Medicum Jagiellonian University, Cracow, Poland; [2]Nephrology, Transplantology and Internal Diseases Department, Medical University of Gdansk, Gdansk, Poland; [3]Department of Nephrology and Transplantology with Dialysis Unit, Medical University of Bialystok, Bialystok, Poland.*

ESA are routine treatment for renal anemia.However the approach toward renal anemia may differ in different countries.The aim of the study was to compare the retrospective data on CKD epidemiology and renal anemia management and prospective data on 12 months of standard ESA therapy between the countries. It was a non-interventional,observational study.Data of 383 patients were analysed.The most common reasons of CKD were:pyelonephritis in Latvia,hypertensive nephrosclerosis and glomeluronephritis in Serbia,diabetic nephropathy and pyelonehritis in Poland.Mean serum creatinine and eGFR at CKD diagnosis were similar in all countries.Patients from Serbia had significantly higher creatinine concentration and lower creatinine clearance at predialysis care,hemodialysis start and at start of ESA therapy.The rate of patients with early ESA therapy (>3 months before start of hemodialysis)was 11,6% in Serbia,6,2% in Latvia,11,2% in Poland.In Serbia 74,4% of patients started ESA therapy after hemodialysis start.During the retrospective period the mean weekly doses of ESA were comparable in Poland and Serbia (5470±2670 vs 4485±2234 units/week)and higher in Latvia(9323±3627 units/week).Patients from Poland had higher than patients from Serbia and Latvia hemoglobin values at start of CKD(10,6±2,3 vs 10±2,1 and 9,6±2,2 p=0,02),at predialysis care(11,2±1,8 vs 10,2±2,4 and 10±1,6 p=0,02)at start of hemodialysis 9,4±1,6 vs 8,7±1,6 and 9,2±2;p=0,02).During prospective phase the mean weekly dose of epoetin beta remained statistically higher in Latvia.The rate of patients with blood transfusions was highest in Poland(44%)(Serbia 7%,Latvia6% p<0,001).Results:There are country specific differences in the renal anemia management.Patients in Latvia received higher doses of ESA.Patients from Poland more often receive blood transfusions.

Disclosure of Financial Relationships: nothing to disclose

**PUB382**

**No Malnutrition, No Chachexia Is Feasible in Chronic Dialysed Children: Apply Daily On-Line Hemodiafiltration** Michel Fischbach. *Children's Dialysis Unit, University, Strasbourg, France.*

Despite major advances in the understanding and management of uremic growth failure, 35 % to 50 % of children with chronic kidney disease (CKD) still grow up to become adults of small stature. The final adult height achieved is correlated with the height deficit recorded at the time of kidney transplantation. A degree of catch up growth does occur after kidney transplantation in childhood, but it is often limited. Growth retardation in children with CKD causes significant difficulties in their daily lives, often limiting psychosocial integration. Additionally, growth retardation is associated with a greater number of hospital admissions and an increased risk of mortality. Growth failure is the common end point of a variety of pathologies which result in growth hormone resistance including. In children on chronic dialysis, linear growth may be improved by ensuring optimal clinical care is given. This includes maximising nutritional support in order to prevent malnutrition. Further management options include the administration of rhGH treatment and the use of more frequent and intensive dialysis sessions i.e. daily on line hemodiafiltration (D-OL-HDF), which combines increased dialysis convective flow with ultrapure dialysate, in order to limit cachexia. Since 2002 (N=21children) we deliver D-OL-HDF in children, 6 times a week 3hours, convective flow of 18 to 27 L/m². Despite normal/free diet, no need for phosphate chelators, normal blood pressure with 3/21children needing one drug, normal echcardiac function, and catch up growth. This "perfect" growth package is in part related to reduced inflammation (CRP, beta 2 microglobulin), normal diet/appetite, and overall a low time average deviation of bicarbonate (optimal control of acidosis). No malnutrition, no cachexia is faesible in chronic dialysed children. The only limitation should be costs (human, economical)of in centre intensive daily HDF.

Disclosure of Financial Relationships: nothing to disclose

**PUB383**

**Treatment of Anaemia in Patients on Haemodialysis (HD) in Catalonia: Relationship between Dosis of Erythropoiesis Stimulating Agents (ESA), Haemoglobin (Hb) and Mortality** Joan Fort,[1] Aleix Cases,[2] Alberto M. Martinez-Castelao,[3] Roser Deulofeu Vilarnau,[4] Emma Arcos Fuster,[4] Jordi Comas Farnes.[4] *[1]Nephrology, Hospital Vall Hebron, Barcelona, Spain; [2]Nephrology, Hospital Clinic, Barcelona, Spain; [3]Nephrology, Hospital Bellvitge, Barcelona, Spain; [4]Catalan Health Service, Catalan Renal Registry Committee, Barcelona, Spain.*

Objective

Relationship with mortality and Hb levels and ESA treatment in patients on HD

Methods

An observational, prospective study using data from the Catalan Renal Registry. Patients who began HD in Catalonia between 2004 and 2007 (n=3.904). To analyse both types of ESA together (Epoetin and Darbepoetin Alpha) we used conversion factor Darbepoetin Alpha, in mcg, to Epoetin (in IU) 1:200. The Resistance Index (RI); quotient between the dosis of ESA adjusted for weight and Hb level. Andersen-Gill model, with robust variance

estimation, adjusted according to the ESA dosis, HB level, RI and other known risk factors, to study the relationship between mortality risk, Hb level and the dosis of ESA.

Results

The mean age of the patients was 65.5 years of whom (64% male). The mean Hb during follow-up decreased as of 2005, from 12.2 g/dl to 11,7 g/dl in 2007 ($p < 0.05$). A negative relationship between ESA weekly doses and Hb levels was found. Patients with doses over 16.000 IU had a lower average Hb level of 2.22 units ($p < 0.05$).

The relative mortality risk in patients with Hb level < =11 g/dl was significantly higher than the risk in patients with over 11 g/dl ($p < 0.005$). There was no decreased mortality risk in the groups with higher Hb levels ($p > 0.05$). Regarding ESA, there were no significant differences between the non-treated patients and the rest.

The relative mortality risk in patients with RI over 10 was greater than in patients with RI under 10. This risk increased progressively as the patients became more resistant. There were no differences in mortality risk between the groups with lower RI ( < =5 and > 5-10).

Conclusions

Patients with the highest Hb levels, had better survival rates (cut-off point Hb > 11 g/dl) and required lower ESA doses. The patients with greatest mortality risk were those who showed the most resistance to treatment.

Disclosure of Financial Relationships: nothing to disclose

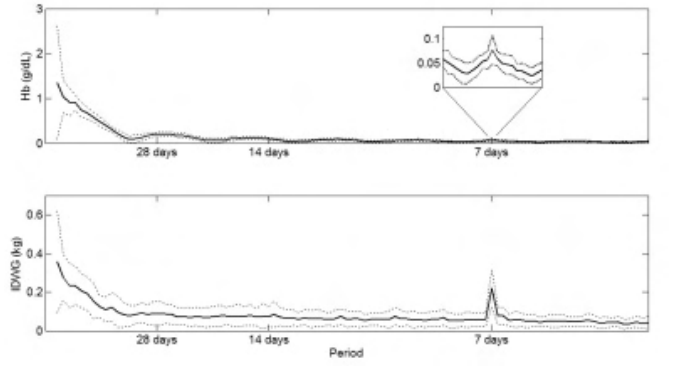
## PUB384

**Association between Interdialytic Weight Gain and Hemoglobin Variability**
Adam Gaweda,[1] Brian Harris Nathanson,[4] Michael J. Germain,[3] George R. Aronoff,[1] Alfred A. Jacobs,[1] Michael Brier.[2] *[1]Department of Medicine, University of Louisville, Louisville, KY; [2]Department of Veterans Affairs; [3]Baystate Medical Center, Springfield, MA; [4]OptiStatim, LLC, Longmeadow, MA.*

Hemoglobin (Hb) variability is common in ESRD patients. It is caused by multiple factors, including interdialytic weight gain (IDWG). In this retrospective cohort study, we quantify the association between IDWG and Hb variability.

We studied 49 ESRD patients treated at Baystate Medical Center, Springfield, MA between March 2007 and July 2008. The Hb was measured every treatment using Crit-Line III (Hemametrics Inc.). IDWG was defined as a difference between the current pre-dialysis and the preceding post-dialysis weight. To analyze the variability of Hb and IDWG we used Fourier series. It represents longitudinal data as a series of periodic components of different amplitude and frequency (frequency spectrum). We compared the frequency spectra of Hb and IDWG using mixed effect regression.

The mean IDWG within the study population was 2.70 (±0.15) kg. Regression analysis revealed positive correlation between IDWG and Hb variability ($R^2 = 0.49$). The IDWG has a significant periodic component with periodicity of 7 days and amplitude of 0.22 (±0.01) kg. The amplitude of the corresponding Hb component is 0.07 (±0.004) g/dL per kg.




Mean frequency spectra (continuous line) and 95% CI (dotted line) for Hb and IDWG.

Interdialytic weight gain associated with increased Hb variability varies prominently on a weekly basis consistent with weekend weight gains. From a population perspective, intraweek weight gains have limited impact on Hgb variability, however this impact may be significant in a few patients. This supports mid- or late week Hb determinations for anemia management.

Disclosure of Financial Relationships: nothing to disclose

## PUB385

**Protein Energy Wasting (PEW) in Hemodialysis: A Mediterranean Perspective**
Carolina Gracia-Iguacel, Jorge Enrique Rojas-Rivera, Beatriz Fernández, Jesus Egido, Alberto Ortiz. *Nephrology, Fundacion Jimenez Diaz, Madrid, Spain.*

**Background:**

Malnutrition is common in hemodialysis (HD) patients and is associated with increased morbidity and mortality.Until recently, there was not a common nomenclature that allow a detailed study of the problem.The purpose of the present cross-sectional study was to asses for the first time in a Southern European country the prevalence of the recently defined PEW and to study determinants of PEW in this population since most published information in Europe has been obtained in Northern Europe, which has different dietary and social habits.

**Patients and methods:**

Eighty-eight stable HD patients from a single unit were studied.Clinical, biochemical and anthropometric measurements which are criteria of PEW were studied.Statistical analyses were performed using statistical software spss 5.version.

**Results:**

PEW was present in 36% of the patients. PEW patients presented a significantly higher EPO resistance index (ERI) (15.8 ±11.3 vs 9.38 ± 7.7 UIEPO/kg/week/hb, p=0.006) and lower levels of transferrin (161 ±42 vs 162 ± 33 mg/dl, p=0.046); and triglycerides (122 [81–160] vs 166 [112 – 227] mg/dl ,p=0.009).In the univariate analysis: age (p=0.006),CO2 (p=0.014),hsCRP (p=0.035) and 25 hidroxyvitamin D (p=0.006) levels were associated with the presence of PEW.In a multinomial logistic regression analysis higher age and the presence of metabolic acidosis were independently associated to higher odds of having PEW,we found non significant trend for hsCRP (table 1).

| | Odds ratio ( 95%CI) | P |
|---|---|---|
| CO3H-≤17mEq/L | 0.15(0.03-0.63) | 0.01 |
| Age≥72 years | 6.80(1.66-27.78) | 0.008 |
| hsCRP≥0.9 ng/mL | 0.31(0.09-1.05) | 0.06 |
| 25 hydroxi≤15ng/mL | 1.18(0.22-6.30) | 0.11 |
| Hb≤9.5g/dL | 0.45(0.081-2.49) | 0.36 |

**Conclusions:**

This observational study indicates that the prevalence of PEW in HD patients from a Southern European country is high.The presence of PEW is a determinant of EPO resistance.We identify a correctable factor that increased the risk of PEW: metabolic acidosis.Further studies should address the influence of correction of metabolic acidosis on the prevalence of PEW.

Disclosure of Financial Relationships: nothing to disclose

## PUB386

**Endotoxaemia in Haemodialysis: A Novel Factor in Erythropoetin Resistance**
Laura E. A. Harrison,[1] James O. Burton,[1] Cheuk-Chun Szeto,[2] Philip K. T. Li,[2] Chris W. McIntyre.[1,3] *[1]Department of Renal Medicine, Royal Derby Hospital, United Kingdom; [2]Medicine & Therapeutics, Chinese University of Hong Kong, Hong Kong; [3]School of Graduate Entry Medicine and Health, University of Nottingham, United Kingdom.*

Endotoxin (ET) derived from gut translocation is a driver of systemic inflammation, and more directly, a wide variety of other pathophysiological responses. Severe endotoxaemia is an underappreciated, but common finding, in haemodialysis (HD) patients. Resistance to erythropoietin (EPO), previously identified as a predictor of mortality risk, and associated with inflammation and malnutrition, is also wide spread. This study aims to explore the potential link between previously unrecognised endotoxaemia and EPO Resistance Index (ERI) in HD patients.

50 established HD patients were studied at a routine HD session. Data collection included weight, BMI, ultrafiltration volume, weekly EPO dose and blood sampling pre and post HD. ERI was calculated as ratio of total weekly EPO dose to body weight (U/kg) to haemoglobin level (g/dL).

Patient age was 62±14 yrs and dialysis vintage 38 months [IQR 18-70]. Mean Hb was 11.3±1.3g/dL with a median EPO dose of 10,000 [IQR 7,500-20,000] u/wk and ERI of 13.7 [IQR 6.9-23.3] (U/Kg)/(g/dL). Mean pre-HD serum ET levels were significantly elevated at 0.69±0.30 EU/ml.

Natural logarithm of ERI correlated to predialysis ET levels (r=0.324, p=0.03) with only relatively weak association with hsCRP (r=0.280, p=0.07). Log ERI also correlated with UF volume, a driver of circulatory stress (r=0.295, p=0.046) previously identified to be associated with increased intradialytic endotoxin translocation. There were no significant correlations with BMI, ktV, albumin, PTH or ferritin levels in this group characterised by good control of these important clinical variables.

This is the first evidence that endotoxaemia driven by HD-induced circulatory stress may play a role in ERI and its associated mortality. It raises the possibility that elevated EPO doses may in part merely be identifying patients subjected to significant circulatory stress and suffering the myriad of negative biological consequences arising from sustained systemic exposure to ET.

Disclosure of Financial Relationships: nothing to disclose

## PUB387

**Protein Nutrition in Patients Switching Low-Flux to High-Flux Hemodialysis**
Tai Yeon Koo,[1] Jung-Sik Park,[1] Gheun-Ho Kim.[2] *[1]Department of Internal Medicine/Divison of Nephrology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [2]Department of Internal Medicine/Divison of Nephrology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [3]Department of Internal Medicine/Divison of Nephrology, Hanyang University College of Medicine, Seoul, Korea.*

**Background**:Malnutrition is related to morbidity and mortality in dialysis patients. High-flux dialyzer has been used to improve nutritons in hemodialysis patients. However,several clinical trials have shown conflicting results regarding impact of flux intervention on nutritons.**Methods**:We retrospectively evaluated effects of changing membrane flux on nutriton in 29 hemodialysis patients who switched low-flux to high-flux membranes.After flux intervention, patients were followed for 24 months and parameters were compared with basal values.Dry weight,serum albumin,total protein,cholesterol and normalized protein catabolic rate(nPCR) were obtained quarterly and predialysis β2-microglobulin(β2MG),cystatinC,hs-CRP,Kt/Vurea, urea reduction ratio(URR) and β2MG reduction ratio yearly.**Results**:During follow-up,Kt/Vurea,URR, hs-CRP,serum cholesterol

and dry weight wasn't significantly affected by flux intervention.However,predialysis β2MG and cystatinC were significantly decreased and β2MG reduction ratio was significantly increased.Serum albumin was significantly declined early,but recovered. Serum total protein decreased early but significantly increased, and nPCR significantly increased after 1 year.

Comparison of nutritional parameters between low-flux and high-flux hemodialysis

| | -3month | 6month | 12month | 18month | 24month |
|---|---|---|---|---|---|
| Albumin(g/dL) | 4.8±0.3 | 4.3±0.4* | 4.5±0.4* | 4.5±0.4* | 4.7±0.3 |
| Protein(g/dL) | 7.2±0.4 | 7.0±0.6 | 7.1±0.5 | 7.3±0.5 | 7.6±0.5* |
| nPCR(g/kg/day) | 1.1±0.2 | 1.1±0.3 | 1.2±0.4* | 1.2±0.4* | 1.3±0.3* |

Values are described as mean±SD.*p<0.05 by paired t-test.

**Conclusion:**Flux intervention may produce biphasic response in nutrition, initial decline and final improvement, in hemodialysis patients.The nutrition may have been aggravated in early by enhanced middle molecular clearances but improved in late by increasing protein intake.

Disclosure of Financial Relationships: nothing to disclose

**PUB388**

**Factors Predicting Erythropoietin Resistance among Maintenance Hemodialysis Patients** Salman Rasheed Mallick,[1] Anca C. Rafiroiu,[1] Saima Iqbal,[1] Mahboob Rahman,[2] Charity Kankam,[1] Malik Ladha,[1] Gita Verma,[1] Osama W. Amro.[1] *[1]Internal Medicine, Saint Vincent Charity Medical Centre, Cleveland, OH; [2]Division of Nephrology, University Hospitals of Cleveland, Case Western Reserve University, Cleveland, OH.*

Individual response to erythropoietin-stimulating agents among end stage renal disease patients is affected by multiple factors, many of them still poorly quantified. The aim of this study was to identify and quantify potential factors that can modify the response to erythropoietin (EPO) in hemodialysis patients. The study used a retrospective, longitudinal, multicenter design and included in its final sample 1456 patients from 15 hemodialysis units in Northeast Ohio that were followed up to two years. The outcome measure used to evaluate the dose–response effect of EPO therapy was the erythropoietin resistance index (ERI), calculated as the weekly weight-adjusted dose of EPO divided by the hemoglobin level. Mean ERI for the entire group was 15.11±14.26 U/kg/week/g per 100 ml. ERI was negatively impacted by being a female, having a low body mass index, low serum iron, low serum ferritin and low albumin levels. Antecedents of diabetes, congestive heart failure or neoplasm, or the normalized protein catabolic rate had no effect on ERI. Angiotensin converting enzyme inhibitor or angiotensin receptor blocker use, alkaline phosphatse, intact parathyroid hormone and, urea reduction ratio were also significantly associated with ERI but Statin use and K t/v were not. This study has several strengths, including its unique nature based on a large population-based sample, the diversity of the HD population analyzed and the variety of biological markers assessed. Clarifying the predictors of ERI among ESRD patients can be of tremendous importance given the magnitude of this problem in the US population.

Disclosure of Financial Relationships: nothing to disclose

**PUB389**

**Clinical and Biochemical Correlates of Extracellular Mass (ECM)/ Body Cell Mass (BCM) Ratio in Hemodialysis (HD) Patients (Pts)** Neal Mittman, Rakesh R. Sheliya, Meghna M. Desai, Brinda Desiraju, Jyotiprakas Chattopadhyay, Morrell M. Avram. *Avram Division of Nephrology, Long Island College Hospital, Brooklyn, NY.*

Malnutrition is a strong predictor of mortality in hemodialysis (HD) pts. Bioimpedance analysis (BIA) has been validated as a useful tool to measure body composition in dialysis pts. Extracellular mass (ECM)/ body cell mass (BCM) ratio is a highly sensitive index of malnutrition, i.e. increased ECM/BCM ratio (EB ratio). We have previously reported that a single hemodialysis treatment does not change extracellular water/intracellular water ratio. The objective of the present study was to explore the relationship between EB ratio, clinical and biochemical parameters in our HD pts. We enrolled 49 pts, recorded demographic, clinical and biochemical data, and followed them over 9 years. BIA was used to determine ECM and BCM pre-dialysis. The mean age was 51 years. Fifty-five percent were women. The majority (81%) were African-American, and 40% were diabetic. The mean EB ratio was 1.37 ± 0.3 (range: 0.70-2.42). Pts with EB ratio in the highest tertile had significantly poorer survival than pts with EB ratio in the lowest tertile (2.4 vs 4.2 yrs; p=0.031). The results of univariate regression analysis is shown below.

Univariate regression analysis: predictors of ECM/BCM ratio

| Variables | Beta Coefficient | p values |
|---|---|---|
| Age (years) | 0.43 | 0.002 |
| Gender (Female) | -0.53 | <0.0001 |
| Months on dialysis | 0.29 | 0.046 |
| Albumin (g/dL) | -0.295 | 0.068 |
| Creatinine (mg/dL) | -0.55 | <0.0001 |

Higher EB ratio was associated with female gender, with increased age and greater months on dialysis at enrollment, and with lower somatic protein stores (serum creatinine). In conclusion, EB ratio is a strong independent risk factor for mortality, most likely due to its association with nutritional status, and therefore may be a useful prognostic tool in HD pts. The use of serial measurements to impact survival, ideally in association with aggressive management of protein energy malnutriton, needs to be evaluated in prospective trials.

Disclosure of Financial Relationships: nothing to disclose

**PUB390**

**Repatriation of ESA Prescribing and Administration in Haemodialysis (HD) Patients Reduces Cost without Affecting the Efficacy of Erythropoiesis Response** Reena A. Popat,[1] Christopher J. Kirwan,[2] Martin J. Raftery,[2] Magdi Yaqoob.[2] *[1]Pharmacy & Medicines Management, Barts and The London NHS Trust; [2]Nephrology and Transplantation, Barts and The London NHS Trust.*

After a London wide initiative to create savings on the procurement of ESA therapy, we undertook a process of repatriation to bring back the prescribing and administration of ESA therapy from community to the hospital for HD patients under our care. We investigated whether repatriation of ESA therapy maintained or improved anaemia management whilst creating savings for the health economy.

**Method:** Patients analysed were those maintained on the same ESA preparation and on HD for 6 months pre and post repatriation. Hb, ESA dose, PTH, CRP and ferritin levels were recorded for the study period and type of vascular access. Mean Hb and ESA dose per patient pre and post repatriation and percentage of Hb measurements greater than 10.5g. dL pre and post repatriation was compared.

**Results:** 210 patients (127 male) with a mean age of 57.3 years were recruited. 74 patients were Asian, 73 Caucasian 61 Afro Caribbean and 2 other. 133 patients had permanent vascular access.

There was no statistical difference in Hb pre and post repatriation in all patients (10.8g. dl and 11g.dl respectively) and when analysed according to vascular access: Pre repatriation permanent: 10.8g.dl, temporary 10.9g.dl; post repatriation both permanent and temporary access patients mean Hb was 11g.dl. The percentage of patients with an Hb of greater than 10.5g.dl prior to repatriation was 61% vs. 63.6% post repatriation (NS).

There were no difference in the ESA dose between all groups pre and post repatriation: 10831iu vs. 104262iu respectively (NS) and when analysed according to access: Pre repatriation permanent: 10223iu, temporary: 11881iu and post repatriation permanent: 10256iu, temporary: 10860iu (p 0.059).

CRP, PTH and ferritin were similar in all groups' pre and post repatriation.

Approximately $3 million has been saved as a result of repatriation.

**Conclusion:** Repatriation of ESA prescribing has made significant cost savings whilst maintaining Hb control at similar ESA requirements suggestive of no compromise in the efficacy.

Disclosure of Financial Relationships: nothing to disclose

**PUB391**

**The Changes of Insulin (Ins) Resistin (Rs) and Adiponectin (Adp) Concentrations and the Changes of HOMA-IR (H-IR) in 4-Hour Hunger Test (HT) in Malnourished (MN) and Obese (OB) HD Pts in Comparison with the Control Group (CG)** Katarzyna Romejko-Ciepielewska, Katarzyna Szamotulska, Zbigniew Bartoszewicz, Stanislaw Niemczyk. *Nephrology, Military Institute of Medicine, Warsaw, Poland.*

1. Evaluation if there are diff. in Ins, Rs, Adp conc. changes and in H-IR in 4-hour HT between HD MN, normal-weight(NW) and OB pts and HD pts and the CG.

87 pts. Group 1-37 HD: 12 pts BMI < 21,0, 12 - 21,0 – 25,9, 13 ≥ 30,0. CG-50 pts: 13 pts BMI < 21,0, 15- 21,0–25,9, 22 ≥ 30,0.We ex. the Ins, Rs, Adp and glucose conc. in fasting state(FS) at 8:00 a.m. (the last meal at 8:00 p.m. the prev. day) at 12.

Ins in FS was significantly(SS) higher in MN HD in comp. with MN CG: 9,19 vs 5,24, p=0,008. In HD Ins in FS falls with the rise of BMI, vs to CG.

In FS the fall of H-IR value with the rise of BMI in HD: 1,68, 1,22, 0,95. In the CG IR rose with the rise of BMI: 1,21, 1,70, 3,86. The SS diff. bt HD OB and CG : p<0,001.

Rs in FS was SS higher in HD pts vs CG: 15,06 vs 4,72 p<0,001 in MN pts; 17,87 vs 5,73 p<0,001 in NW pts; 17,53 vs 5,22 p<0,001 in OB pts.

Adp in FS bt HD and healthy pts. Adp conc. in FS fall with the rise of BMI: 247,61, 149,50, 123,21 in HD pts and 155,19, 101,62, 98,27 in CG pts.

In 4-hour HT Ins conc. falls SS in CG: 5,24 vs 2,93 p<0,001; 6,27 vs 4,54 p<0,001; 14,93 vs 9,34 p<0,001, The SS fall of Ins in 4-h HT was observed in MN HD pts 9,19 vs 7,01, p=0,005. In 4-h HT H-IR values fall SS in the CG: 1,21vs 0,64 p<0,001 in MN, 1,70 vs 1,13 p<0,001 in NW, 3,86 vs 2,40 p<0,001 in OB pts. In HD H-IR values fall SS in MN 1,68 vs 1,36 p=0,001 and in NW 1,22 vs 0,95 p=0,042.

In 4-HT test Rs conc. rises SS in the CG NW 5,73 vs 6,17 p=0,092, in OB pts 5,22 vs 6,11, p=0,020 and in HD NW : 17,87 vs 19,21 p=0,027. The fall of Rs in 4-HT in HD MN: 15,06 vs 14,16 and in HD OB pts:17,53 vs 17,13.

In 4-HT test the SS diff. in Adp conc. in the CG. In HD with correct BMI we observe the SS rise of Adp conc. (p=0,032) and in OB HD pts the SS fall of Adp conc. (p=0,033).

1. High Ins and high IR in FS and in 4-h HT in HD MN may be the hormonal cause of anorexia.

2. Rs and Ins chang and H-IR chang in FS and in 4-h HT do not confirm the hypothesis that Rs develops IR

Disclosure of Financial Relationships: nothing to disclose

**PUB392**

**Renal Anemia Treatment with NeoRecormon in Everyday Clinical Practice in Haemodialysed Patients in Latvia, Poland and Serbia. The Results of Non-Interventional, Observational Study** Boleslaw Rutkowski,[1] Jolanta Malyszko,[2] Maciej Drozdz.[3] *[1]Nephrology, Transplantology and Internal Diseases Department, Medical University of Gdansk, Gdansk, Poland; [2]Department of Nephrology and Transplantology with Dialysis Unit, Medical University of Bialystok, Bialystok, Poland; [3]Nephrology Department, Collegium Medicum Jagiellonian University, Cracow, Poland.*

ESA are routine treatment for CKD anemia.However the approach toward renal anemia treatment may differ among countries.The aim of the study was to compare retrospective data and prospective data on 12 months of ESA therapy in ESRD patients in Latvia, Serbia and Poland.The study was conducted in 50 centers, 383 patients' data were analyzed(221 males,162 females;aged 19-90(57,4±14,9)years;259 from Poland,81 from Latvia,43 from Serbia).The percentage of patients with early ESA introduction(>3 months before hemodialysis start)was 11,6% in Serbia,6,2% in Latvia and 11,2% in Poland. In Serbia most of patients(74%)started ESA therapy after hemodialysis start.In Poland ESA therapy was introduced 32±172 days before dialysis start.This was earlier than in Latvia(23±251) and Serbia(4±206)(p<0,001).Patients from Poland had higher than patients from Latvia and Serbia Hb concentrations at CKD diagnosis and at predialysis care. During retrospective phase most patients in Serbia received NeoRecormon subcutaneously (95%),while in Poland subcutaneous route of administration was used in 51% of patients and in Serbia 69%(p<0,001).The dose of epoetin beta was higher in Latvia (9300±3600 units/week) than in Serbia (5600±3300)and Poland(4500±2200) during retrospective phase of the study and remained statistically higher during prospective phase.Patients from Latvia had signicantly higher than patients from Serbia and Poland Hb concentrations during all study visits (11,72g/gl (±1,3) vs 11,16g/dl(±1,3) and 11,0g/dl(±1,3)).The rate of patients with blood transfusions was higher in Poland (44%) (in Serbia(7%) and Latvia (6%)(p<0,001). Results:In Poland ESA therapy started earlier and patients had higher Hb levels on CKD diagnosis and predialysis care.The dose of epoetin beta and Hb levels were highest in Latvia.

Disclosure of Financial Relationships: nothing to disclose

**PUB393**

**Safety of a Single Administration of 1 gram of IV Ferumoxytol in Hemodialysis Patients with Iron Deficiency Anemia (IDA)** Dawn M. Sabau,[1] Erin S. Nowak,[1] Joseph E. Bender III,[2] William Strauss,[2] Naghmana Bajwa.[2] *[1]Liberty Dialysis, Kokomo, IN; [2]AMAG Pharmaceuticals, Inc., Lexington, MA.*

Iron deficiency is the most common cause of anemia and is nearly universal in the hemodialysis population. Intravenous (IV) iron is commonly used for the management of iron deficiency anemia (IDA) in these patients. *Feraheme*® (ferumoxytol) Injection is a novel IV iron therapy that has been approved in the US for the treatment of IDA in adults with CKD. Ferumoxytol consists of an iron oxide core with a unique carbohydrate coating (polyglucose sorbitol carboxymethylether), that is isotonic, has a neutral pH and has lower free iron content than other IV iron products. Ferumoxytol is currently approved for an initial 510 mg IV injection followed by a second 510 mg IV injection 3 to 8 days later; a second course of therapy can be given to patients with persistent or recurrent IDA.

A small regional dialysis clinic has had recent experience administering 1.02 g of ferumoxytol (2 vials of 510 mg each), as a single injection in 12 hemodialysis patients. These patients were predominantly male (8/12) and ranged in age from 45-75 years. These administrations were well tolerated. There were no adverse reactions reported in these patients, including IV iron class reactions (e.g., hypotension or anaphylactoid reactions), except for one of the 12 patients who experienced a mild rash on the torso and legs 4 days post ferumoxytol administration; this event resolved within 10 days without any treatment. This patient is a 66-year-old male with a history of allergies to 6 classes of drugs and no previously reported reactions to IV irons. No adverse events were reported in the remaining 11 patients. Although the sample size is small, ferumoxytol demonstrated good tolerability when administered as a single injection of 1.02 g to these patients. These results suggest that further exploration of a 1 gram dosing paradigm is warranted.

Disclosure of Financial Relationships: nothing to disclose

**PUB394**

**Once-Monthly Anemia Management Maintains Stable Hemoglobin Levels in Hemodialysis Patients – Results from the HbDay Study** Piotr Seniuta, Thierry Baranger, Valerie Drouillat, Frank Bergé. *Hemodialysis, Polyclinique Bordeaux Nord Aquitaine, Bordeaux, France.*

**Objective:** Methoxy polyethylene glycol-epoetin beta is a continuous erythropoietin receptor activator (C.E.R.A.) and provides the maintenance of stable hemoglobin (Hb) levels in hemodialysis patients with once-monthly administration. Chronic kidney disease patients with renal anemia undergoing treatment with an erythropoiesis stimulating agent (ESA) can be given supplementary iron to maintain the Hb target. The HbDay study evaluates the maintenance of stable Hb levels with an intravenous supplementary iron (iron hydroxy/dextran), on the same day as the subcutaneous administration of C.E.R.A. every 4 weeks.

**Material and Methods :** This "real life" observational study is based on a 9 month period in a single centre. The data on Hb level, iron status and ESA treatment was collected retrospectively for a 3 month period prior to the once-monthly anemia management and during the next 6 months. 125 hemodialysis patients were evaluated (48 % female), the mean duration on dialysis is 5 years and the mean age is 73 years. Age distribution is: < 65 years, 25%, 65-75 years, 21%, 75-85 years, 38%, ≥ 85 years, 16%.

**Results :** The Hb level and iron status are stable during the study. The mean Hb level is 11.1 ±1.33 g/dl in baseline (one week before the start of C.E.R.A.) and 10.9 ±1.21 g/dL, during the evaluation at W24 p=0.242. 56% of the patients are between 10-12 g/dL in baseline; and 66% during the evaluation. During the evaluation at W24, the mean serum ferritine is 363 µg/L, the mean transferrine saturation is 26%, the median dose of iron hydroxyl/dextran is 200mg/month and the median dose of C.E.R.A. is 150µg/month (45% patients with the same dose as the baseline, 33% decrease and 22% increase).

**Discussion :** The Hb levels can be maintained in hemodialysis patients with both administrations of iron supplementation and C.E.R.A. on the same day every 4 weeks. **Conclusions :** This "real life" study in a intensive dialysis center shows that the once-monthly anemia management can be effective. This is an opportunity to simplify the organization of dialysis centers and as a result make them more cost effective.

Disclosure of Financial Relationships: Consultancy: RocheResearch Funding: Affymax, bBraun, Roche, NovoNordisc; Honoraria: Roche.

**PUB395**

**Cumulative Safety Outcomes with Ferumoxytol in a Medium-Sized Dialysis Organization (MDO)** Amit Sharma,[1] Denise Vanvalkenburgh,[2] Kellie Y. Becker,[2] Betsy J. Lahue.[3] *[1]Boise Kidney and Hypertension Institute, Boise, ID; [2]Liberty Dialysis, Mercer Island, WA; [3]AMAG Pharmaceuticals, Lexington, MA.*

**Purpose:** Conduct a retrospective review of the safety and anemia outcomes associated with implementation of a ferumoxytol IV iron management protocol.

**Methods:** Anemia outcomes and lab values were collected across all clinics of a medium-sized dialysis organization (MDO) from April 2009 through April 2010. Adverse events (AEs) reported for patients receiving ferumoxytol treatments were summarized from first dose through June 13, 2010. The overall rate of adverse events was calculated and types of events were examined. Hemoglobin, TSAT and ferritin values were evaluated at the unit level for the 13 month period of observation.

**Results:** Starting in October 2009, nearly 54% (46/85) of the MDO clinics began administering doses of ferumoxytol, however ferric gluconate and iron sucrose made up >75% of the total IV iron grams used in 2009. Feraheme use increased from 22% of grams in Q4 2009 to 48% in Q1 2010, and in April, 84% (73/87) of MDO sites were administering ferumoxytol, comprising 63% of total iron grams used. To date (6/13/2010), 2515 patients have received 7457 doses of ferumoxytol. Forty-six (46) events were reported during this timeframe in 7457 doses (0.62%); these included nausea (16), hypotension (8), vomiting (7), dyspnea (7), rash (6), wheezing (1) and fatigue (1). Comparing 2009 to 2010 monthly anemia outcomes for the MDO clinics, there were no apparent differences in the proportion of patients with hemoglobins >12g/dL , TSATs >50% and ferritin >500 ng/dL.

**Conclusions:** This MDO has gained a large experience with ferumoxytol following successful implementation of a ferumoxytol IV iron management protocol. There were very few adverse events and the types of adverse events observed were as expected for IV iron products. Ferumoxytol appears to be well tolerated for the treatment of iron deficiency anemia in the dialysis population.

Disclosure of Financial Relationships: Consultancy: Amgen, AmagResearch Funding: AMAG, AFFYMAX, Luitpold, AMGEN; Honoraria: AMGEN, AMAG; Scientific Advisor: AMAG, AMGEN.

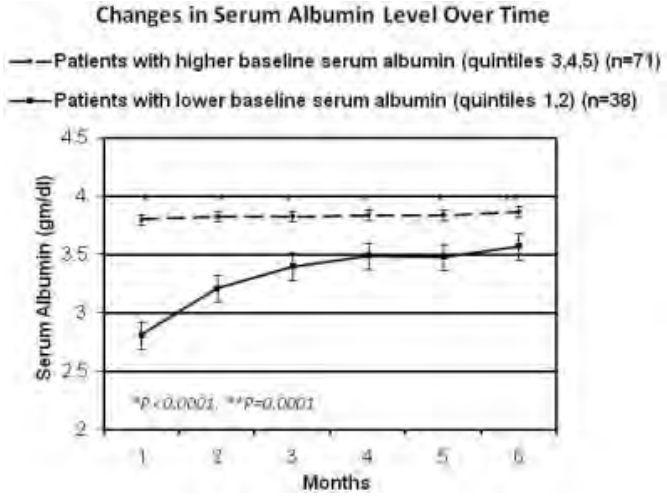
**PUB396**

**Does Serum Albumin Level Improve in Hemodialysis Patients after Starting Dialysis?** Arif Showkat,[1] Fridtjof Thomas,[2] Elizabeth A. Tolley.[1,2] *[1]Medicine, University of Tennessee, Memphis, TN; [2]Preventive Medicine, University of Tennessee, Memphis, TN.*

Malnutrition is a common problem in chronic hemodialysis (CHD) patients (pts). The purpose of this study is to evaluate the changes in nutritional status as measured by serum albumin (SA) in mostly African-American (AA) CHD pts, during the first 6 months after starting dialysis.

Retrospective chart review study. 109 incident CHD pts, 98% were AA; 60% were male; 44% had diabetes mellitus (DM), and 10% had human immunodeficiency virus (HIV) infection. Mean age was 50.44±13.49 years.The following variables were used for nutritional parameters: SA, normalized protein catabolic rate, serum transferrin, serum creatinine, and body mass index. Values of the variables were collected on each pt for the first 6 months after starting dialysis. The repeated measures of SA were modeled utilizing the mixed-effects modeling approach.

Baseline SA level was predicted by the presence of DM and patient's age. During the 6-month follow-up period, most of the improvement in SA level was found among the pts with baseline SA level in the lower two quintiles.




Patient's age was the only significant predictor of the change in SA levels during the 6-month follow-up.

The Coefficent Estimates for the Fixed Effects in the Identified Model

| | Coefficients | S.E. | P |
|---|---|---|---|
| Variables affecting baseline SA | | | |
| Intercept | 3.55 | 0.06 | <0.0001 |
| Age | 0.101 | 0.04 | 0.012 |
| DM | -0.20 | 0.07 | 0.0064 |
| Variables affecting the changes in SA during 6 months | | | |
| Month | 0.05 | 0.009 | <0.0001 |
| Age | -0.01 | 0.007 | 0.0071 |

In incident CHD pts, SA level over time improved only in a small group of pts and was affected by patient's age and baseline SA level.

Disclosure of Financial Relationships: nothing to disclose

**PUB397**

**Treatment of Confirmed B12 Deficiency in Hemodialysis Patients Improves Epogen Requirements** Norbert Shtaynberg, Majed Samarneh, Michael M. Goldman, Morton J. Kleiner, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background. Vitamin B12 deficiency may have deleterious effects on overall functionality and may increase erythropoietin stimulating agent (ESA) resistance in end stage renal disease (ESRD) patients on maintenance hemodialysis, yet little is known about its' prevalence in this population.

Methods. Vitamin B12 as well as MMA levels were drawn from ESRD patients prior to hemodialysis. All patients with MMA levels greater than 700nmol/L had peripheral smears evaluated for hyper-segmented neutrophils. Those who were positive were considered to be B12 deficient and underwent treatment with intramuscular vitamin B12 injections for a total of 4 months. Post treatment MMA levels and blood smears were obtained. Erythropoietin dosages were monitored pre and post treatment.

Results. There was a 58% (60/103) prevalence of vitamin B12 deficiency as defined by a positive MMA level and a positive blood smear. Out of 52 patients with positive smears, 36 (69.2%) were negative on repeat analysis after B12 treatment.

Mean epogen (EPO) dosages significantly decreased by 16,572±41,902 units from baseline to the post-B12 treatment period (p=.0082, Wilcoxon Signed Rank test). From June through August, the mean EPO usage was 82,066.7±47,906.3 and from January through March, the mean EPO usage was 65,495.0±39,690.7. Post-B12 treatment hemoglobin levels were 11.5 ± 1.1 g/dL. There were 26 patients (52%) receiving iron supplementation during the B12 treatment period and 24 (48%) not receiving supplementation.

Conclusion

Vitamin B12 supplementation was associated with a decrease in the mean dose of ESA administration while maintaining a stable hemoglobin level. Although there is a high variability in the dosing, this was also seen in the patients who were not B12 deficient. Maintaining serum vitamin B12 levels has been shown to improve functionality, and may allow a decrease in the use of ESA's and their toxicities and significant costs.

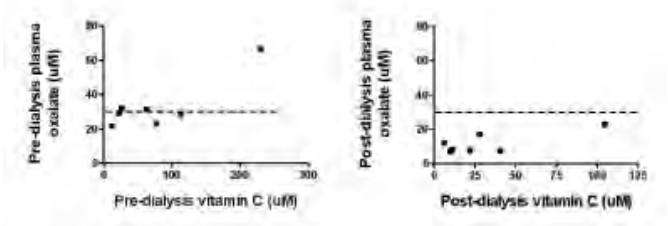
Disclosure of Financial Relationships: nothing to disclose

**PUB398**

**Plasma Oxalate and Vit C Levels before and after Hemodialysis** William D. Sirover,[1] Thilagavathi Venkatachalam,[1] Robert L. Benz,[4] Lawrence S. Weisberg,[1] Nathan W. Levin,[2] Peter Kotanko,[2] Garry J. Handelman.[3] *[1]Cooper Medical Institute, Camden, NJ; [2]Renal Research Institute, New York, NY; [3]University of Massachusetts, Lowell, MA; [4]Lankenau Hospital, Wynnewood, PA.*

Plasma oxalic acid accumulates between treatments in HD patients, and is then cleared by hemodialysis. Although oxalate levels before treatment can exceed supersaturation (βCaOx>1), which usually occurs when oxalate is >30 μM, efficient hemodialysis is able to bring oxalate levels to <30 μM. At that concentration, oxalate can dissolve and permanent oxalate tissue deposits are unlikely. Supplemental vit C can increase pre-dialysate oxalate in HD patients, and is a cause for concern in the design of studies that use vit C for anemia management.

We screened 42 HD patients for pre-dialysis vit C level, and then identified a subset with either low (<30 μM) or high (>60 μM) plasma levels. Plasma vit C and oxalate were measured just before dialysis, right after dialysis, at 24 hours, and at then just prior to the next treatment. The graph shows oxalate and vit C measurements for 7 patients; the figure on the left is pre-dialysis, and the figure on the right is post-dialysis.



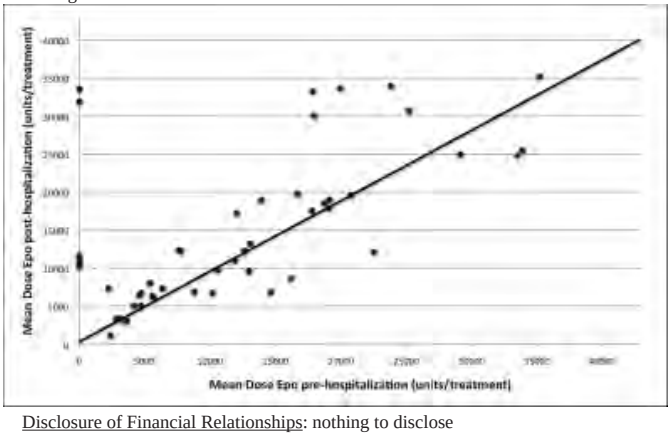

Predialysis, 3 of 7 patients were at βCaOx>1 (dotted line), but after dialysis all patients were below βCaOx. One patient had a Kt/V=1, predialysis oxalate 72 μM, and predialysis vitC 240 μM, but this patient also was below the oxalate saturation threshold following dialysis. Our findings indicate thathigh-flux dialysis effectively clears oxalate; however if patients are administered supplemental vit C, it would be prudent to ensure that Kt/V goals are met, and to make occasional determinations of pre and post dialysis plama oxalate levels. Patients in the therapeutic range of vitC (60-80 μM) to optimize EPO dose may not experience oxalosis, as plasma is cleared to below the oxalate supersaturation threshold, after adequate hemodialysis treatment (Kt/V>1.2).

Disclosure of Financial Relationships: nothing to disclose

**PUB399**

**Impact of Hospitalization on Hemoglobin (Hb) Levels and Erythropoietin Stimulating Agent (ESA) Use in Patients with ESRD on Hemodialysis: A Single Center Experience** Wojciech Sokolowski,[1,3] Vishalakshmi Batchu,[2] Jodumutt Ganesh Bhat,[1] Premila Bhat.[1,2] *[1]Atlantic Dialysis Management Services, LLC, Ridgewood, NY; [2]Wyckoff Heights Medical Center, Brooklyn, NY; [3]New York Medical College, Valhalla, NY.*

Variability in ESA responsiveness and dosage is of clinical and financial importance for the dialysis community. Hospitalization may lead to reduced Hb levels and increased Epoetin alfa (Epo) use. We reviewed the charts of patients at a large urban dialysis unit with a formal anemia management protocol targeting Hb 10-13 g/dL who missed dialysis due to hospitalization from 1/1/2007 through 12/31/2007. Patients were included in the analysis if hospitalized for ≥48 hours and returned to the dialysis unit for subsequent care. Patients who initiated dialysis <1 month prior to hospital admission were excluded and those with multiple admissions were included in the analysis for only the first of their series of hospitalizations. During the first three months of the study period, 60 patients were hospitalized 79 times. 49 patient admissions were included in this interim analysis (remainder not analyzed due to missing data (n=13), repeated hospitalization (n=9), death (n=6) or transfer (n=2) during follow-up period). Mean age was 63.0 years, 70% were male, and mean length of stay (LOS) was 9.4 days. Compared with baseline, mean Hb was unchanged in the 3 months after hospital stay (12.09 vs. 11.94 g/dL) but administered Epo dose was significantly increased (12,529 vs. 15,417 units/treatment, p=0.02). 30/49 patients had a net increase in mean dose of Epo and 13/49 patients had increases of ≥5000 units/treatment. This effect showed no significant interaction with age, sex, LOS, or serum albumin. In conclusion, hospitalization is associated with increased ESA use to maintain Hb at target levels.




Disclosure of Financial Relationships: nothing to disclose

**PUB400**

**Prognosis of Hemodialysis Patients with Chronic Heart Failure Relates to Patient's Muscle Bulk** Oonishi Takahiro. *Nephrology, Yamada Red Cross Hospital, Ise, Mie, Japan.*

Purpose: Dialyzed patient's muscle bulk is related creatinine index (CrID). Moreover,CrID is not only related muscle mass of the patient but also their vital prognosis.

In general, the skeletal muscle blood flow and disturbances of peripheral circulation relate, and are said that it is important when the condition is understood in the state of chronic heart failure. We hypothyzed that the poor prognosis of herat failure was low CrID , but high CrID was related not poor prognosis. Method: The heart failure incidence of 1.5 years, serm albumin, CrID, BNP, Hb, and the left ventricle ejection fraction(LVEF) examined . For nine people (7 men, 2 women, and 12.8 average dialysis period years) who had been diagnosed as a chronic cardiac failure in January, 2009. Result: Three persons died during this studyperiod ,one person was acute respiratory failure ,one person was arrhythmia due to acute myocardial infarction,one person was septicemia due to ovarian abscess. The albumin and CrID of the case who had died were intentionally low, and BNP was intentionally high. As for Kt/V and PCR, a significant difference was not seen. Conclusions: It was thought that the muscle bulk took part patient with chronic heart failure's prognosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB401**

**The Combination Therapy of Intradialytic Parenteral Nutrition and Oral L-Carnitine Administration Could Prevent the Progression of Malnutrition-Inflammation – Atherosclerosis Syndrome** Masataka Tsunoda. *H.N.Medic Sapporo-Higashi, Hokkaido, Japan.*

**Backgrounds:** Chronic dialysis patients with protein energy malnutrition (PEM) have occasionally treated by intradialytic parenteral nutrition (IDPN), but controversial arguments still exist as to whether or not IDPN improves PEM. The lipid metabolism disorder caused by L-carnitine(LC) deficiency has supposed to lead the muscle loss in dialysis patients and to be related to the ineffectiveness of IDPN. In this study, we treated the patients with PEM by the combination of IDPN and LC. **Methods:** Six patients, whose nutritional conditions had not improved by IDPN, were enrolled to the study. 200ml of 6.1 % amino acids solution, 200ml of 50% dextrose solution and 100ml of 20% soy lipid solution were infused to the blood line throughout each entire dialysis session. 300mg of LC was orally administered for 20 minutes before each dialysis session. Total energy intakes including with IDPN ranged 30- 35Kcal/Kg/day, and it was recognized appropriate for dialysis patients. Body compositions by bioimpedance method, serum levels of C-reactive protein (CRP), and amino acid profile were evaluated every 3 months for 12 months. **Results:** During the IDPN treatment alone, the fat mass had gradually increased while the muscle mass had decreased. With LC add-on, however, the muscle mass dramatically increased. The levels of serum CRP significantly decreased 6 months after the administration of LC (0.058±0.033 to 0.038±0.020 mg/dl; $p<0.05$). Amino acid profile showed interesting changes; both levels of taurin(109±85.5 to 127±92.7 nmol/ml; $p<0.05$) and methionine(25.8±1.97 to 30.4±3.40nmol/ml; $p<0.01$) increased, and total homocysteine (tHcy) decreased significantly 6 months after the administration(65.2±46.5 to 50.7±34.8 nmol/ml; $p<0.05$). **Conclusions:** The combination therapy of IDPN and LC ameliorated the nutritional status of the patients through the improvement of lipid metabolism. Furthermore, it reduced the serum levels of CRP and tHcy, which is closely related to the acceleration of atherosclerosis in dialysis patients. The combination therapy could be a recommendable strategy for the prevention of Malnutrition-Inflammation-Atherosclerosis syndrome.

Disclosure of Financial Relationships: nothing to disclose

**PUB402**

**Intraobserver Validation of an Oscilometric Technician for Assessment of Ankle-Brachial Index** Zaida Noemy Cabrera Jimenez, Rosilene M. Elias, Isac Castro, Joao Egidio Romao, Jr. *Nephrology, University of Sao Paulo School of Medicine, Sao Paulo, SP, Brazil.*

**Background:** The easiest widely-used method for the diagnosis of peripheral arterial disease is the measurement of ankle-brachial index (ABI). ABI has been shown to be a good predictor of mortality among patients on hemodialysis. However, the variability intra-observer in measuring ABI among patients on hemodialysis has not been formally tested

**Methods**: We prospectively included 124 thrice-weekly incident hemodialysis patients (85 men; age 51 ± 20 years old). Two oscillometric devices were used to measure blood pressure in upper and lower extremities simultaneously. Measurements were performed on both sides pre and post dialysis (baseline), and were repeated in two consecutive dialysis sessions (time 1 and 2)

**Results**: The Friedman test showed no difference in across multiple measurements in right systolic blood pressure (SBP) and right diastolic blood pressure (DBP) ($p=0.205$ and $p=0.212$, respectively). The same was observed for left side SBP and DBP ($p=0.982$ and $p=0.160$, respectively). Wilcoxon test showed no difference between pre and post ABI (Table 1).

Pre vs post ABI

| | Right ABI pre dialysis | Right ABI post dialysis | p | Left ABI pre dialysis | Left ABI post dialysis | p |
|---|---|---|---|---|---|---|
| Baseline | 1.03 (1.18 , 1.33) | 1.09 (1.18 , 1.31) | 0.161 | 0.98 (1.20 , 1.31) | 1.08 (1.20 , 1.30) | 0.890 |
| Time 1 | 1.05 (1.18 , 1.29) | 1.03 (1.16 , 1.28) | 0.582 | 1.06 (1.20 , 1.30) | 1.08 (1.19 , 1.28) | 0.933 |
| Time 2 | 1.07 (1.18 , 1.29) | 1.08 (1.20 , 1.30) | 0.114 | 1.08 (1.19 , 1 .32) | 1.09 (1.22 , 1.34) | 0.623 |

DBP (time 2) and SBP (baseline) presented no self-correlation, and perfectly fit between confidence interval, generating the most reliable ABI (time 2 post and time 1 pre dialysis)

**Conclusion**: This prospective study showed a good intra-observer agreement in measuring blood pressure by an oscilometric device to calculated ABI, in incident patients in hemodialysis. The most reliable measurement could be determined to calculate the ABI. Further studies will be necessary to test the applicability of this method in identify cardiovascular risk in this population

Disclosure of Financial Relationships: nothing to disclose

**PUB403**

**Effective Cardiac Therapy, Anemia Full Correction with Erythropoeitin, However Intensified Intravenously Iron Application Results in Significant Regression of Left Ventricular Hypertrophy (LVH) in Patients with Very Long Time Survival on Hemodialysis (HD)** Hannelore B. Hampl. *Internal Medicine, Nephrology, Charite University Berlin.*

**Aim** of the study was to find out the effect of adequate cardiac therapy in combination with full anemia correction on regression of LVH; because a suffered heart needs additionally energy (oxygen) to heal! Secondary question: What could be the reason for increased events in presence of normal hemoglobin (Hb) described by others?

384 patients (84 diabetics), (age 59.5±7.8 years/HD since 14.1±8.5 years; 260 female) were regularly echocardiographically controlled since II/97 until 12/08, the LVH as Mass index ($g/m^2$) was evaluated. During anemia correction on the way to normal Hb iron parameters (transferrin- saturation (TFS), ferritin, iron levels) and thrombocyte counts (ThCs) were checked 3- monthly. High target doses of β- blockers, ACE- inhibitors, ATI-receptor blockers, aldosteron blockers were given.

**Results:** before/after therapy: Blood pressure (mmHg) 162±13/87±16 to 132±12/81±5, $p<0.001$; Hb (g/dl) 11.6±2.2/14.3±1.5, $p<0.001$; ThCs ($mm^3$) 302 800±79 000/180 000±60 000, $p<0.01$; TFS (%) 16.5±5.3/45.5±5.2, $p<0.01$; iron (µmol/L) 9.6±3.4/21.3±5.4, $p<0.01$**;** ferritin (µg/L) 1 175±854/1 477± 1 101, n.s. LVH-Regression:total(48%);g/m2**:** 166.9±30.3/112.9±11.7, $p<0.001$**;** partial(26%), 212.1±40.7/158.6±37.7**,** $p<0.01$; not successful (26%), 176.5±40.4/184.39.2,n.s. Increase of 1g/dl Hb decreases LVH of 5.0 g/m2 in non-diabetics; however in only 2.5g/m2 in diabetics! Multivariate analysis**:** cardiac therapy ($p<0.03$),esp. β- blockers($p<0.01$), Hb correction ($p<0.01$) are independent, significant prognostic factors for LVH regression! Our cardiac/non cardiac mortality over time: 10/16%; no increase in events. **Conclusion:** Effective cardiac therapy decreases significant cardiac mortality. Iron therapy has to be intensified i.v. to prevent functional iron deficiency responsible for activated thrombocytosis inducing fatal strokes/cardiac events. Full anemia correction is not a risk factor for increased mortality and events. In contrast to non- diabetics, diabetics show in particular a higher dependency of their cardiac function on normal (!) oxygen availability.

Disclosure of Financial Relationships: nothing to disclose

**PUB404**

**Reactive Thrombocytosis Due to Iron Deficiency Anemia: Has an Aggravating Risk Factor for Cerebro/Cardio Vascular Events (CCVEs) during Full Renal Anemia Correction as Yet Not Been Taken into Consideration?** Hannelore B. Hampl. *Nephrology, University, Berlin, Germany.*

Randomized controlled trials(RCTs) in uremics have found an increased risk for CCVEs when hemoglobin targets >13g/dl during therapy with erythropoeitin/ESAs. The mechanism of these harmful events with erythropoeitin/ESA therapy to full anemia correction has not been elucidated. Aim of the study was to find out a relationship between iron deficiency and thrombocyte counts(**ThCs)** on the way to anemia correction. Erythropoiesis needs iron. **Oral iron absorption is disturbed in uremics .**

We reviewed(1998-2007) the iron status(transferrin g/L,-saturation, TSAT %, iron µmol/L, ferritin µg/L, Hb g/dl, mean corpuscular hemoglobin concentration, MCHC g/dl, hypochromic RBCs %, fibrinogen mg/dl) and correlated these factors to **ThCs /mm3 during anemia correction** in 384 HD-pats.; age 59.5±7.8 yrs; 176 f, time on HD 13.5±8.5 yrs. Intensified **iron application only intravenously** on the way to full anemia correction of Hb 14g/dl with epoietin β of 15 000 IU(correction phase); **only 5000 IU/pat./week(maintained phase) without increased CCVEs compared to RCTs** has had the following results(before/after correction): Remarkable, there is a significant negative correlation between iron parameters and ThCs: the higher the MCHC(29.9/**36.1,**$p<0.01$); the lower the hypochromic cells(16.9/**0.7,**$p<0.001$; the higher the TSAT(27.2/**46.6**, $p<0.001$); the higher the iron level( 52.6/**75.2**,$p<0.001$); the higher the ferritin values (500.1/**1537**,$p<0.001$); the lower fibrinogen levels(741.3/**189.4**,$p<0.001$), **the lower thrombocyte counts** 310 000/**187 522.** Absolut transferrin was diminished (167.6/148.5,$p<0.05$) due to chronic inflammation in uremics and therefore **faster** saturated. **Conclusion:** Iron status has to be intensified (TSAT>40%) and applicated only iv due to poor oral iron absorption during full anemia correction in uremics to prevent functional iron deficiency which is well known for inducing reactive thrombocytosis via thrombopoeitin receptor being responsible for severe CCVEs like in RCTs. **ESA therapy to raise Hb requires a large amount of iron iv, raising Hb to normal requires an even greater amount routinely causing functional iron deficiency.**

Disclosure of Financial Relationships: nothing to disclose

**PUB405**

**Analysis of the Causes of Death and Mortality Risk Factors in Patients Treated with Hemodialysis** Wenlv Lv, Xiaoqiang Ding, Jie Teng, Jianzhou Zou, Yimei Wang, Yihong Zhong. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

**Purpose:** To analyse the causes of death in maintenance hemodialysis patients and explore the risk factors affecting survival of hemodialysis patients. **Methods:** The data were retrospectively analysed during the periods 1 Jan. 1998 through 31 Dec. 2008. Cox regression analysis was used to find mortality risk factors**. Results:** 237 of 648 maintenance hemodialysis patients died in the study period. Their average age at the beginning of hemodialysis was 63(19-87) yeas old and men accounted for 60.8%. The median survival time was 9.6 (0.03-156.80)months. The main causes of death were cardiovascular disease (20.3%), cerebrovascular disease(16.5%) and infection (24.9%). 33.3% of the deaths occurred within 3 months of hemodialysis. Results of Cox regression analysis: (1) Age (HR=1.036, 95%CI 1.013-1.061, $P<0.01$), Charlson comorbidity index(CCI)≥5(HR=6.408, 95%CI 1.893-11.694, $P<0.01$) and baseline blood albumin(HR=0.943, 95%CI 0.895-0.993, $P<0.05$), history of congestive heart failure(HR=2.463, 95%CI 1.265-4.797, $P<0.01$) and cerebrovascular diseases (HR=2.532, 95%CI 1.084-5.912, $P<0.05$) were the mortality risk factors. (2) The history of cerebrovascular diseases(HR=7.741, 95%CI 2.817-12.273, $P<0.01$)and DN (HR=3.659, 95%CI 1.299-8.311, $P<0.05$) were the risk factors of death due to cardiovascular and cerebrovascular diseases. (3) Age(HR=1.075, 95%CI 1.004-1.151, $P<0.05$), blood albumin(HR=0.908, 95%CI 0.799-0.988, $P<0.05$) at the beginning of hemodialysis were the risk factors of death from infection. (4) Blood albumin(HR=0.883, 95%CI 0.819-0.953, $P<0.01$) and eGFR(HR=0.853, 95%CI 0.729-0.922, $P<0.01$) at the beginning of hemodialysis, the history of congestive heart failure before hemodialysis(HR=3.011, 95%CI 1.231-5.365, $P<0.05$) were the risk factors of death within 3 months of hemodialysis. **Conclusion:** Cardiovascular and cerebrovascular diseases, infection are the main causes of death in maintenance hemodialysis patients. Older patients, CCI≥5, lower blood albumin,DN, underlying cardiovascular and cerebrovascular disease, and late initiation may be the risk factors of Mortality.

Disclosure of Financial Relationships: nothing to disclose

**PUB406**

**Aortic Arch Calcification Score Predicts Mortality in Peritoneal Dialysis Patients** Francesca K. Martino, Ching Yan Goh, Ilenia Filippi, Pierluigi Di Loreto, Rossella Torregrossa, Maria Pia Rodighiero, Carlo Crepaldi, Claudio Ronco. *S Bortolo Hospital.*

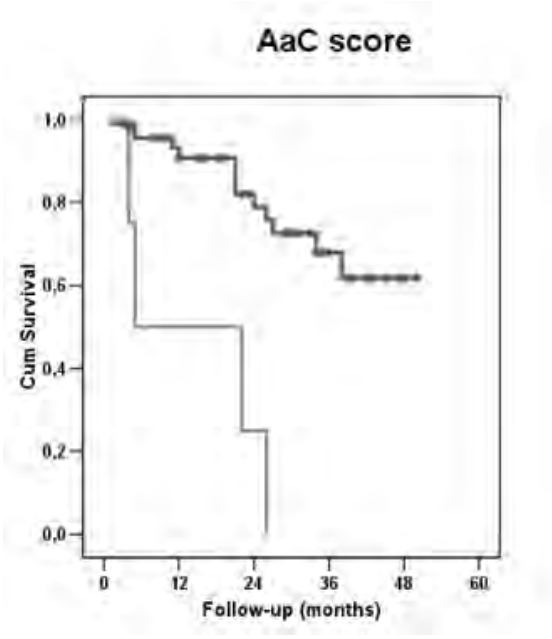
**Background:** Vascular Calcification (VC) is associated with cardiovascular disease (CVD) and mortality. Several radiological imaging are used to evaluate VC extension but some are expensive and not always easily accessible. However, quantification of aortic arch calcification (AaC) by chest Xray (CxR) is cheap and easily available. We aimed to access the prognostic role of AaC score in mortality of peritoneal dialysis patients (PD pts).

**Method:** We performed a retrospective study on 57 pts who started their PD in 2006 or 2007. We measured the AaC score (0:no calcification, 1:small spot of calcification, 2: one or more thick calcifications, 3: circular calcification) from pts CxR on their 1st clinic visit.

Baseline characteristics of pts were evaluated: age, primary renal disease (PRD), duration of RRT, history of CVD and biochemical parameters like Albumin, Hb, Ca, P and PTH.

Kaplan-Meier method was used to calculate the survival rate, and differences were assessed with log rank statistic. Multivariable Cox regression models addressed the time to death. All statistical tests were performed with SPSS version 16.

**Results:** The median follow-up period was 29 months (IQR17-36.5). All cause mortality was 29.8%. In univariable analyses, the age of pts at initiation of PD (p=0.0076), AaC score ≥2 (p=0.0001), PRD (p=0.0015), history of CVD (p=0.032) were significantly associated with mortality. In multivariable analyses, we found that high AaC score ≥2 (HR:4.058; p=0.035) and diabetic nephropathy (DN) (HR:7.332;p=0.007) were the only 2 independent predictors of all cause mortality.




**Conclusion:** In this analysis, we conclude that high AaC score ≥2 and DN are independent predictors of mortality in PD pts.

Disclosure of Financial Relationships: nothing to disclose

**PUB407**

**The Association of Prorenin and Biomarkers for Cardiovascular Disease in Non-Diabetic Normotensive Hemodialysis Patients** Yoshiyuki Morishita, Shiho Hanawa, Junko Chinda, Eiji Kusano. *Nephrology, Jichi Medical University, Shimotsuke, Tochigi, Japan.*

**Background.** Circulating prorenin contributes to the pathogenesis of tissue damages leading to cardiovascular disease (CVD) in diabetic mellitus (DM) and hypertension (HT) patients by activating tissue renin-angiotensin-aldosterone (RAS) system and intracellular signaling by binding (pro)renin receptor (PRR); however, little is known about its roles in hemodialysis (HD) patients.

**Aim**. We evaluated the association of circulating prorenin and CVD predictive biomarkers in non-diabetic normotensive HD (nonDM-NT-HD) patients.

**Methods.** We measured prorenin and PRR to investigate the association of prorenin and CVD predictive biomarkers, such as brain natriuretic peptide (BNP), high-sensitivity C-reactive protein (hs-CRP), 8-hydroxydeoxyguanosine (8-OHdG) and diacron-reactive oxygen metabolite (d-ROM) in fourteen nonDM-NT-HD patients and ten healthy volunteers (control).

**Results.** Prorenin did not increase in nonDM-NT-HD patients compared to control (nonDM-NT-HD: 175.7±131.6 pg/m vs. control: 164.7±109.9 pg/m, (N.S.)). *PRR mRNA* expression increased in nonDM-NT-HD patients (nonDM-NT-HD: 1.5 vs. control: 0.9 ($p<0.0001$)). Although prorenin was not correlated with hs-CRP, it was significantly correlated with BNP, 8-OHdG and d-ROM (BNP; r=0.766; $p<0.001$, 8-OHdG; r=0.712; p=0.003, d-ROM r=0.735; p=0.002) in nonDM-NT-HD patients. Prorenin was not correlated with plasma renin activity (PRA), angiotensin I (ATI), angiotensin II (ATII) and aldosterone (Ald), whereas PRA was significantly correlated with ATI and ATII in nonDM-NT-HD patients. PRA, ATI, ATII and Ald were not correlated with CVD predictive biomarkers in nonDM-NT-HD patients.

**Conclusions.** Circulating prorenin was associated with CVD predictive biomarkers independent of circulating RAS in nonDM-NT-HD patients. It may contribute to the development of CVD in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB408**

**Echocardiographic Changes after Conversion from Conventional Hemodialysis to In-Center Thrice Weekly Nocturnal Hemodialysis** Albert M. Osei, Lissa Sugeng, Jay D. Shah, Diane M. Fodor, Lynn Weinert, Bharathi V. Reddy. *Medicine, Section of Nephrology, University of Chicago, Chicago, IL.*

**Background:** Approximately 70-80% of stage V CKD patients have some features of left ventricular hypertrophy (LVH) before initiation of dialysis. Conventional 4-hour thrice weekly hemodialysis (CHD) fails to cause regression of LVH. There is evidence that a longer and or more frequent hemodialysis sessions as done in home hemodialysis (HHD) lead to regression of LVH. HHD may not be feasible for all dialysis patients. In-center nocturnal hemodialysis (INHD) is a new modality that is done three nights a week, 8 hours per session. The effect of INHD on left ventricular mass (LVM) and left ventricular ejection fraction (LVEF) is unknown.

**Design, setting, participants and measurement:** This retrospective analysis assessed the impact of conversion from CHD to INHD on LVH and LVEF. In 13 patients who switched from CHD to INHD, adequacy of dialysis, blood pressure (BP), calcium, phosphorous, protein catabolic ratio and hemoglobin (Hb) were determined before and 6 months after conversion. Changes in LVM and LVEF were reviewed in 6 patients who had 2D echocardiograms before and 1 year after conversion to INHD.

**Results:** Nine of 13 patients were converted to INHD because of volume overload. There was no significant difference in pre-dialysis systolic BP before and after conversion to INHD. However, there was a significant reduction in average post dialysis systolic BP from 134 (+/-22) mmHg to 113 (+/- 15) mmHg (P= 0.01). There was no significant difference in Hb before and after conversion. In six patients who had echocardiographic data, baseline mean LVM was 150(+/- 44) gm/m$^2$ and LVEF was 40(+/-14) %. After switching to INHD, 4 of 6 patients had regression of LVM to a mean of 106(+/-31) mg/m$^2$and 5 patients had improvement of LVEF to a mean of 53(+/-3) %.

**Conclusion:** Regression of LVM may be achieved with INHD and this could be related to improved fluid management with longer dialysis sessions. However, this is a small retrospective study. Prospective randomized trials comparing the effects of CHD and INHD on LVM and LVEF are needed.

Disclosure of Financial Relationships: nothing to disclose

## PUB409

**Concurrent Use of Clopidogrel and Proton Pump Inhibitors Does Not Influence Prognosis of Coronary Heart Disease in Hemodialysis Patients**

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**Background**: Dual antiplatelet therapy (APT) with aspirin and clopidogrel is recommended for patients with acute coronary syndromes (ACS) and/or percutaneous coronary intervention (PCI). Current consensus recommendations state that these patients should receive a proton pump inhibitor (PPI) to reduce gastrointestinal (GI) bleeding. Recent analyses have suggested that PPIs may weaken the antiplatelet effects of clopidogrel by involving CYP (cytochrome P450)2C19. Although ACS is common in hemodialysis patients and PCIs are widely performed, little attention has been paid to this point in hemodialysis patients.

**Design**: Retrospective cohort study using medical records of Juntendo University Hospital to identify who received coronary angiography (CAG) from January 2008 to December 2009.

**Patients**: Thirty-six hemodialysis patients who were hospitalized for CAG were examined.

**Measurements**: Baseline and follow-up drug use was assessed. Primary outcomes were re-hospitalizations for severe cardiovascular disease.

**Results**: During the observation period, 69% of concurrent users and 40% of non-concurrent users had cardiovascular events (p=0.169). There was no significant difference in the time to coronary events between concurrent users and non-concurrent users (p=0.27, log-rank test). The hazard ratio (HR) associated with concurrent use of clopidogrel and PPIs for serious cardiovascular disease was 1.13 (95% confidence interval (CI): 0.46 to 2.57). The significant factors for severe cardiovascular disease in hemodialysis patients were female gender (HR: 5.66, CI: 1.47 to 23.71), serum LDL-cholesterol (HR: 1.08, CI: 1.03 to 1.14) and serum phosphate (HR: 0.50, CI: 0.22 to 0.99).

**Limitations**: The selection bias by cardiologists and severity of coronary heart disease in each patient could not be assessed.

**Conclusion**: In hemodialysis patients with serious coronary heart disease treated with clopidogrel and PPIs, the corresponding point estimate for serious cardiovascular disease is not increased.

Disclosure of Financial Relationships: nothing to disclose

## PUB410

**Short-Term Outcomes of Cardiac Surgery in Patients on Chronic Dialysis**

Takeshi Yamamoto,[1] Jun Matsuda,[2] Hiroyuki Kadoya,[2] Masanobu Takeji,[2] Megumu Fukunaga,[1] Atsushi Yamauchi.[2] *[1]Nephrology, Toyonaka Municiple Hospital, Toyonaka, Japan; [2]Nephrology, Osaka Rosai Hospital, Sakai, Japan.*

*Background*. Long-term dialysis remains a major risk factor for cardiac surgeries, especially for those who have diabetic nephropathy. One of our strategies for these patients is an intensive perioperative management by the collaboration between the surgeons and the nephrologists. This study aimed to elucidate whether our strategy is appropriate.

*Methods*. Between 2005 and 2008, 36 chronic dialysis patients(36/460 total cardiac surgeries, 7.8%) underwent cardiac surgery, including coronary artery bypass grafting(CABG), valve surgery, combined CABG and valve procedures, and other cardiac surgeries, in our institution. The mean age was 64.8 years old (38 to 77 years). Twenty patients had diabetic nephropathy(group D) and 16 patients were without diabetic nephropathy(group ND). All patients were dialysed the day before surgery. In the postoperative period, we managed all the patients in ICU. In most instances, we started continuous hemodialysis(CHD) the day after the operation and controlled water and electrolyte balance appropriately until the circulatory conditions reached stable. Preoperative profiles and perioperative and short-term results were examined, with comparing group D with group ND.

*Results*. A part of results are shown in the Table.

*Conclusions*. With intensive perioperative management and advanced technology, cardiac surgery can be done in chronic dialysis patients safely with acceptable early mortality and morbidity.

| | Group D(n=20) | Group ND(n=16) | P value |
|---|---|---|---|
| Age(y.o.) | 64.4±9.2 | 65.3±8.5 | 0.76 |
| Hb (g/dl) | 9.9±1.4 | 11.3±1.4 | 0.006 |
| HbA1c(%) | 6.9±1.3 | 5.2±0.9 | <0.001 |
| Duration of dialysis(months) | 51.9±50.6 | 151.7±124.3 | 0.007 |
| Operation : CABG(+) | 18(90%) | 6(37.5%) | <0.01 |
| Intubation time(hrs) | 74.5±110.8 | 27.1±28.8 | 0.08 |
| ICU stay(days) | 10.0±7.0 | 7.0±3.8 | 0.11 |
| CHD time(hrs) | 54.8±76.8 | 41.9±28.3 | 0.51 |
| Hospital stay(days) | 45.9±72.5 | 25.3±9.7 | 0.22 |
| 1 year survival rate(%) | 80.4 | 93.8 | 0.54 |
| Reoperation for bleeding | 3(15%) | 1(6.3%) | 0.77 |
| Mediastinitis | 3(15%) | 0 | 0.31 |

Disclosure of Financial Relationships: nothing to disclose

## PUB411

**Provision of Dialysis Services in Libya**

Wiam A. Alashek,[1] Chris W. McIntyre,[1] Maarten W. Taal.[2] *[1]School of Graduate Entry Medicine, University of Nottingham, Derby, United Kingdom; [2]Department of Renal Medicine, Royal Derby Hospital, Derby, United Kingdom.*

**Purpose:** Dialysis is entirely funded by the public health care sector in Libya but there is no registry to gather national data. This study aimed to investigate dialysis provision and practice in Libya. **Methods:** A cross-sectional study of all Libyan dialysis facilities was undertaken from April to July 2009. Data were collected using a structured questionnaire. **Results:** 2430 patients were treated in 40 centres (adult population prevalence 624 per million). Thirty-two (80%) dialysis units were located in the north of the country where 88.5% of inhabitants live. Only 3 centres offered peritoneal dialysis. There were a total of 173 functioning haemodialysis (HD) stations (1 machine to 3.4 patients). Separate room/s were allocated for virus positive patients in 92.5% of units while dedicated machine/s were reserved in 7.5%. Hand wash facilities were available in each HD room in 22 centres (55%). No dialyzer reuse was permitted. The total number of doctors was 114 (doctor to patient ratio 1:22). Two remote centres were operating without doctors. The number of trained dialysis nurses was 639 (nurse to pateint ratio 1:3.8). 52.5% of patients were offered scheduled consultations in outpatient clinics. Patients received 4-12 h of HD per week. Kt/v was routinely calculated for some patients in 10% of centres, whereas urea reduction ratio was the main method of adequacy monitoring in 25%. 70% of centres used single pre-dialysis urea measurement monthly to monitor adequacy. Haemoglobin was monitored monthly in all units but only 50% monitored iron status. Serum calcium and phosphorus were measured in 85% of centres and serum calcium only in 5%. No assessment of blood minerals was performed in 10%. Monitoring of serum albumin was done in 57.5% of centres. 55% of centres experienced intermittent deficiency in laboratory supplies resulting in failure to perform required blood tests. **Conclusion:** Dialysis services are appropriately distributed. In general, the provision of dialysis is adequate but several areas for improvement have been identified. It is hoped that this study will facilitate the development of a Libyan Renal Registry.

Disclosure of Financial Relationships: nothing to disclose

## PUB412

**Could Hemodialysis Patients Maintaining a Low Phosphorus Diet?**

Eleni Chelioti,[1] Antonios Zagorianos,[1] Athanasios Georgiou,[1] Antonis Ntalianis,[2] Prokopis Papazafeiris,[1] Gabriel Papadakis.[1] *[1]Dept. of Nephrology and Renal Unit, General Hospital of Piraeus, "Tzaneio", Athens, Greece; [2]Dept. of Sociology, University of Crete, Crete, Greece.*

Purpose: In the majority of hemodialysis (HD) patients, phosphorous (P) intake largely exceeds the amount of P removed during HD. The use of P binders is universal in this population. The reduction of P intake is considered an additional measure for the prevention of P retention. Aim of this study was to evaluate the knowledge of low phosphorous diet, the compliance to intake daily a low phosphorous diet and the optimal reception of phosphate binders.

Method: A descriptive study was performed in our Renal Unit. Participants were 29 HD patients (21 males/8 females, mean age 61±14years, mean period on dialysis 98±77months, mean Kt/V 1.4±0.1), who were undergoing dialysis three times per week. All patients had been prescribed phosphate binders. According to an adjusted questionnaire from Kidney Early Evaluation Program-Quality of life, patients completed a questionnaire concerning compliance with phosphorous binders, knowledge of low phosphorous diet and if consumed daily a low phosphorous diet. Statistical analysis based on using descriptive statistics such as mean ±SD and percentages.

Results: The percentage of patients who had knowledge of a low phosphorous diet was 82.7%(24/29) but the majority of them continued to be on a high phosphorous diet. The frequency of HD patients who consumed daily a high phosphorous diet was 38%(11/29) and thrice a week was 55. 2%(16/29). Only, 6.8%(2/29) of HD patients were on a low phosphorous diet. Moreover,79.3% (23/29)of them received the prescribed phosphate binders and 82.6 %(19/23) were receiving phosphate binders regularly. The percentage of HD patients who received calcium carbonate, sevelamer or combinations of these was 43.5%, 34, 8% and 21.7% respectively.

Conclusion: The majority of HD patients had a very good knowledge of a low phosphorus diet but their compliance to a low phosphorous diet was poor. Moreover, the reported receiving of phosphate binders were good. It is necessary to optimize the compliance of a low phosphorus diet and the use of phosphate binders by hemodialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB413**

**The Influence of Continuous Quality Improvement on Outcomes in Maintenance Hemodialysis Patients** Xian-Guang Chen, Hua Wu. *Nephrology Department, Beijing Hospital, Beijing, China.*

**Objective** To observe the influence of continuous quality improvement(CQI) on outcomes in maintenance hemodialysis patients. **Methods** According to the KDOQI guideline the CQI team improved the objective laboratory result from 2004 to 2009 using the four-step process called PDCA cycle-plan: plan, do, check and act and then analyzed the relationship between the improvement and mortality rate. **Results** There were 75 hemodialysis patients in 2004 and 117 patients in 2009. They had no significant difference in age and gender. The URR in 2009(69.6±8.74%) was higher than that in 2004(65.23±6.46%, p<0.001). In 2009 there were better levels of hemoglobulin(111.1±13.61g/L vs. 98±17.39g/L, p<0.001), serum phosphorus(1.56±0.49mmol/L vs. 1.77±0.61mmol/L, p=0.013) and albumin(39.1±2.81g/L vs. 37.86±4.31g/L, p=0.034)than those in 2004. Serum calcium (2.34±0.2mmol/L vs. 2.41±0.3mmol/L, p=0.081), iPTH (393.6±443.91pg/ml vs. 427.75±380.99pg/ml, p=0.615) and C-reactive protein(0.6±0.88mg/dl vs. 0.96±1.47mg/dl, p=0.081)had no difference in the two groups. According to KDOQI guideline, the rates of the result within the recommended range were showed in table1. Parts of the result were compared with DOPPS 2007. In 2009 there were longer hemodialysis time (57.67±51.06 months vs. 32.11±32.37 months, p<0.001) than that in 2004. Mortality rate in 2009 was slightly better than that in 2004(8.5% vs. 14.7%, p=0.185). **Conclusions** According to KDOQI guideline, continuous quality improvememt can improve anemia, serum phosphorus, nutrition levels and hemodialysis patients' outcomes.

Table1:the rates within the recommended range

| | DOPPS 2007(%) | 2004 n=75(%) | 2009 n=117(%) |
|---|---|---|---|
| HB(110-120g/L) | 26 | 18.7 | 35.9a |
| HB(>110g/L) | 64.7 | 29.3 | 58.9 a |
| iPTH(150-300pg/ml) | 29.6 | 22 | 31.6 a |
| Serum calcium(2.1-2.37mmol/L) | 50.8 | 40 | 48.7 |
| Serum phosphrate(1.13-1.78mmol/L) | 49.8 | 48 | 52.1 |
| URR(>65%) | | 53.3 | 76.9 a |
| CRP(<0.8mg/dl) | | 70.7 | 82.8 |
| ALB(>40g/L) | 33.6 | 41.3 | 51.3 |

a p<0.05 compare with result of 2004

Disclosure of Financial Relationships: nothing to disclose

**PUB414**

**Immunogenicity and Efficacy of a Pandemic Influenza A (H1N1) 2009 AS03-Adjuvanted Vaccine in Patients with End-Stage Renal Disease** Ralf A. Dikow,[1] Isabella Eckerle,[1] Vedat Schwenger,[1] Martin G. Zeier,[1] Paul Schnitzler,[2] Claudia Sommerer.[1] *[1]Nephrology, University Hospital, Heidelberg, Germany; [2]Virology, University Hospital, Heidelberg, Germany.*

**BACKGROUND**

Patients with end-stage renal disease have a reduced response to vaccinations due to uraemia-related immune dysfunction. To increase immunogenicity of vaccines, antigens can be formulated with adjuvants. The new tocopherol-containing adjuvant system $AS03_A$ has not been tested in patients with end-stage renal disease so far.

**METHODS**

Patients on dialysis were immunized with an inactivated split-virion A/California/7/2009 H1N1v pandemic vaccine with the adjuvant system $AS03_A$, either only with a single dose at day 0 or with a second dose at day 21. Antibodies against pandemic influenza A (H1N1) 2009 were tested with an ELISA in every patient three months after vaccination and compared to non-immunized dialysis patients.

**RESULTS**

In total, 294 dialysis patients were enrolled. 169 patients were vaccinated with either one dose (64 patients) or two doses (105 patients) of pandemic H1N1 vaccine, 125 patients remained unvaccinated. Influenza A H1N1 vaccination resulted in 40/63 (63.5%) patients with one vaccination and in 93/105 (88.6%) patients with two vaccinations in a positive Pandemic New Influenza IgG ELISA > 11 AU. Multivariate regression analysis revealed (i.) former response to hepatitis B vaccination and (ii.) number of vaccination doses as independent factors for the response to influenza A H1N1 vaccination. No episode of influenza A (H1N1) illness occurred in any of the groups within the study period of six months after vaccination. Use of the adjuvant system $AS03_A$ caused only mild to moderate local symptoms in 143/169 (84.6%), but no serious adverse events.

**CONCLUSION**

We report the first study of pandemic H1N1 vaccine adjuvanted with $AS03_A$ in patients with end-stage renal disease. Influenza A (H1N1) AS03-adjuvanted vaccine is immunogenic, effective and save in these patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB415**

**Are Small Dialysis Organizations (SDOs) Prepared for Income Changes under the CMS Proposed Payment System?** Fredric O. Finkelstein, Alan S. Kliger. *Hospital of St. Raphael, Yale, New Haven.*

Background. The CMS Proposed Payment System for dialysis contained few concrete instructions for dialysis facilities. It has been suggested that small dialysis organizations (SDOs, Independents and Regionals with < 50 units) have fewer resources to prepare for the new payment model, placing them at risk when the final rule is published. Our objective in this study was to determine how SDOs were preparing for potential income losses.

Method. Using an SDO database developed from CMS files and other government data sources, we drew a quota sample of 75 SDO facilities designed to represent the population of SDO facilities in the 48 contiguous states by region, number of stations, rural/suburban/urban, and high/low minority location. Facility managers were queried in three separate interviews over a six week period about their awareness, preparation and potential changes. Interviews were part of a larger study designed to estimate PPS payments from patient data. Facilities were compensated for participation in the study.

Results. Of the more than 300 facilities contacted, 23% had joined Large Dialysis Organizations (LDOs), 42% were out of business or unreachable. Of the eligible facilities, nearly half declined to participate. Only 59% said they had read the CMS report, and only 24% said they understood the report very well; 47% looked up their estimated payments under the new bundle. Among those responding to detailed interviews, if their payments were 5% lower than expected, 45% said they would stay in business "only with deep cuts in costs", 30% said they would go out of business, and 25% said they would continue to operate without radical changes**.** If payments were cut 5% below current levels, 56% said they were "Very likely/ Likely" to lose experienced nurses, due to either staffing cuts or attrition.

Conclusions. The number of SDO facilities has declined sharply since 2007. A majority were unaware or unprepared for the changing payment model and anticipated a variety of cuts in their income, staff and operational capabilities. These results suggest the 2011 transition will not proceed smoothly.

Disclosure of Financial Relationships: nothing to disclose

**PUB416**

**Evolution of Quality Parameters and Mortality in Haemodialysis Patients in Catalonia: Catalan Registry of Renal Patients** Joan Fort,[1] Aleix Cases,[2] Alberto M. Martinez-Castelao,[3] Roser Deulofeu Vilarnau,[4] Emma Arcos Fuster,[4] Jordi Comas Farnes.[4] *[1]Nephrology, Hospital Vall Hebron, Barcelona, Spain; [2]Nephrology, Hospital Clinic, Barcelona; [3]Nephrology, HUB, Barcelona, Spain; [4]Catalan Renal Registry Committee.*

Objectives

To study the evolution of some quality indicators for Haemodialysis (HD) and survival in patients initiating HD during the period 2002-2007.

To compare survival of patients returning to HD following a renal transplant (RT) with those initiating HD for the first time.

Methods

Using data from the Renal Catalan Registry,we studied patients initiating HD who did not die during the first 90 days of treatment (n=4,494). From the initiation of HD until the end of the follow-up period, univariate (by year of initiation) and bivariate (by year of initiation and year of follow-up) analyses were performed.We calculated a model of parametric survival adjusted by repeated-measure, time-dependent variables.

Results

From 2002 the mean (sd) Kt/V increased progressively until 2007 (1.30 (0.27) to 1.45 (0.27), together with the percentage of patients with Kt/V>1.3 (39.2% to 70.0%)and those undergoing 12 hours of HD weekly (51.1% to 60.0%.There was a progressive increase in tunneled catheter use (8.1% to 13.9%)while arteriovenous fistula (AVF) remained stable (76.8% to 76.4%). The mean (sd) Haemoglobin (Hb) increased from 2002 to 2005 (11.5 g/dl (1.69) to 12.4 g/dl (1.36)) and then decreased (11.9 g/dl (1.35) in 2007). We observed a progressive increase in the number of patients with Hb levels between 11 and 13 g/dl (46.5% to 56.4%). In 2005 and after adjusting for risk factors, there was a drop in the relative mortality risk compared to 2002 (RR=0.63). Patients with greater risk of death where those who had a Kt/V<1.3, were on dialysis <12 hours weekly, and who initiated HD with catheter and Hb<11 g/dl. The relative mortality risk was not significantly different for those patients returning to HD following RT.

Conclusions

Kt/V, weekly hours of HD and percentage of patients with Hb between 11 and 13g/dl improved during the studied period. The percentatge of AVF remain stable. From 2005 a reduction in mortality was observed. Patients reinitiating dialysis following renal transplant had similar evolution of HD quality parameters and survival to those initiating HD for the first time.

Disclosure of Financial Relationships: nothing to disclose

**PUB417**

**Survival after Starting RRT in Patients with Adult Polycystic Kidney Disease (ADPKD)** Richard Haynes,[1] Farhad Kheradmand,[2] Christopher G. Winearls.[1] *[1]Oxford Kidney Unit, Oxford Radcliffe Hospitals NHS Trust, Oxford, Oxfordshire, United Kingdom; [2]Department of Urology, Oxford Radcliffe Hospitals NHS Trust, Oxford, Oxfordshire, United Kingdom.*

Aim: to investigate whether survival after beginning of RRT had improved for patients with ADPKD from 1971 to present day.

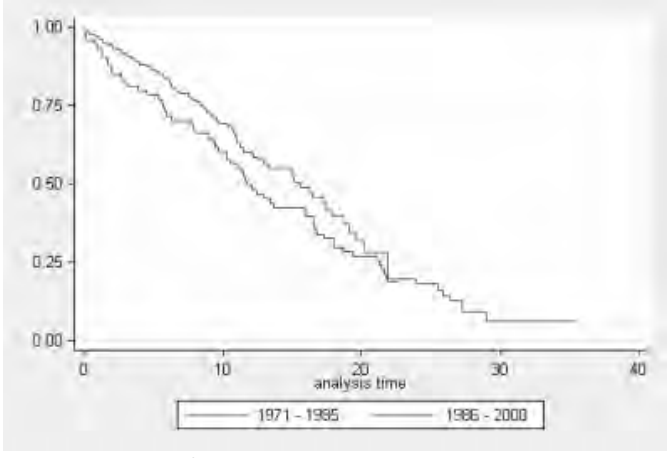
Methods: basic characteristics of all adult patients with ADPKD starting RRT in our unit from 1971-2000 were collected. Relative risk of death were estimated using Cox regression.

Baseline characteristics of study population, by period.

| | All patients | Period | | p value |
|---|---|---|---|---|
| | | 1971 - 1985 | 1986 - 2000 | |
| Number | 265 | 74 | 191 | |
| Age at start of RRT ± SD | 53.5 ± 10.0 | 51.1 ± 8.4 | 54.4 ± 10.4 | 0.017 |
| Number male (%) | 120 (45) | 38 (51) | 82 (43) | 0.22 |
| Number transplanted (%) | 192 (72) | 52 (70) | 140 (73) | 0.62 |
| Comorbidities (%): | | | | |
| Cardiac disease | 15 (6) | 7 (10) | 8 (4) | 0.10 |
| Cerebrovascular disease | 4 (2) | 0 (0) | 4 (2) | 0.21 |
| Peripheral vascular disease | 0 (0) | 0 (0) | 0 (0) | n/a |
| COPD | 2 (1) | 0 (0) | 2 (1) | 0.38 |

COPD = chronic obstructive pulmonary disease

Results: 265 patients with ADPKD started RRT of whom 152 died (median follow-up 11 years). Age (HR 1.08; 95% CI 1.06-1.10) and RRT mode (transplant v dialysis as time-varying covariate; HR 0.22; 95% CI 0.16-0.31) were associated with survival in unadjusted analyses. In adjusted model period was associated with survival (HR 0.67; 95% CI 0.47-0.97). Median survival increased from 11.7 to 15.5 years.




Over same period, life expectancy in general population had increased only slightly, suggesting improvements observed here were specific for ESRD population.

Conclusions: survival for patients with ADPKD on RRT has improved more than that expected from secular trends from 1971 to 2000. RRT now provides almost 2/3 of lifespan expected (from general population) compared to about 1/2 in 1971.

Disclosure of Financial Relationships: nothing to disclose

**PUB418**

**Patient and Technique Survival among Independent and Assisted Peritoneal Dialysis Patients** Jay S. Hochman, Pietro Ravani, Rob Quinn. *Internal Medicine, University of Calgary, Calgary, AB, Canada.*

Peritoneal dialysis (PD) is an effective form of home based renal replacement therapy. As the dialysis population ages worldwide, there are an increasing number of patients with barriers to self care PD. Many of these barriers can be overcome by home care assisted PD. For this reason many centers have developed and implemented home care assisted PD programs. Despite the growing interest and utilization of assisted PD programs, little is known regarding technique and patient survival.

In this study, we will use data from a prospectively maintained dataset that captures baseline characteristics and outcomes of all incident dialysis patients between January 1, 2004 and the present. The adjusted PD technique survival of patients treated with assisted PD will be compared to those treated with self-care PD. Death and transplantation will be handled as competing risks for the purposes of our analysis. In addition, we will describe the reasons for technique failure and their relative frequency according to time on therapy. This work will help us better understand the outcomes of patients treated with home care assisted PD and will provide targets for future therapeutic interventions to improve outcomes among PD patients.

At the time of abstract submission ethics approval is pending. However, results will be available for presentation at renal week.

Disclosure of Financial Relationships: nothing to disclose

**PUB419**

**Trends in Prevalence and Morbidity in HIV-Infected Dialysis Patients** Paul L. Kimmel, Paul W. Eggers. *NIDDK, NIH, Bethesda, MD.*

HIV infection is the fourth most common correlate of kidney disease in young male African American US ESRD patients. Previously we showed incidence remained stable and prevalence had increased through 2000, due to improved survival among HIV-infected (HIV+) dialysis patients, as the number of patients living with AIDS and in the US ESRD program increased, presumably because of availability of highly active antiretroviral therapies (HAART) in the US. Morbidity in this population has not been evaluated. Using previously developed methodology we evaluated trends in prevalence and morbidity of HIV+ patients in the ESRD dialysis program.

A retrospective cohort study was performed using the USRDS, analyzing patients receiving dialysis from 1995 to 2007. Presence of HIV/AIDS was ascertained through the CMS 2728 cause of ESRD code and by examination of billing codes, a method previously used by us and validated by Hebert et al. Hospitalization rates were calculated per 1,000 person years as in the USRDS Annual Data Report.

The prevalence of HIV+ patients increased from 2397 in 1995 to 8761 in 2007, while the percentage of HIV+ patients in the ESRD program has remained stable between 1.4 and 1.6% of the population since 2000. HIV/AIDS accounted for between 6.6 and 6.9% of black men aged 25 to 44 in the program from 2000 to 2007. Hospitalization rate in the ESRD program was 218/1000 in 2007, while that of HIV+ patients was 22.4% higher at 267/1000. There was an approximately 2.5 fold increase in the number of hospitalizations for infection in HIV+ patients compared to non-HIV/AIDS patients, a disparity not noted in other diagnostic groups.

HIV+ patients remain an important group in the ESRD program. The numerical prevalence of HIV infection has increased but the proportion has remained relatively stable. The morbidity of HIV+ patients is increased compared with uninfected patients, with the greatest disparity noted in rates of infectious causes of hospitalization.

Disparities in hospitalization for infections suggest better approaches to prevention, surveillance, prophylaxis and treatment interventions may reduce morbidity and improve survival of HIV+ ESRD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB420**

**The Prevalence and Predictors of Anaemia in European Children with Established Renal Failure** Leah Krischock, Karlijn J. Van Stralen, Kitty J. Jager, Franz S. Schaefer, Enrico E. Verrina, Jaap Willem Groothoff, Eleanor Jane Tizard. *On Behalf of the ESPN/ ERA-EDTA Registry Study Group, Amsterdam Medical Centre, Amsterdam, Netherlands.*

Purpose of study: Determine the prevalence of anemia in European children with Established Renal Failure (ERF), identify risk factors for anemia in this patient population, and determine the adequacy of treatment of anemia.

Methods: Data were collected from 2028 patients on dialysis, and 2313 transplant patients aged 17 years or under from 2000 to 2009, from 20 European countries contributing to the ESPN/ERA-EDTA Registry. Percentages were weighted according to the number of hemoglobin (Hb) measurements available. There was an average of 3.8 measurements per patient. Linear mixed model analyses were used to determine effect of parameters on Hb levels.

Results: Comparing the results with European adult guidelines and British paediatric guidelines, 46% of dialysis and 26% of transplant patients were anemic. 90% of dialysis patients were treated with erythropoietin stimulation agents (ESA), while only 10% of transplant patients were on an ESA.

In dialysis patients, younger age was strongly associated with lower Hb levels. Haemodialysis (rather than peritoneal dialysis), and a primary diagnosis (PRD) of cystic kidney disease were also associated with lower Hb levels, while congenital anomalies of the kidneys and urinary tract were associated with higher Hb levels.

In transplant patients, predictors for a lower Hb level were again younger age as well as female gender. Patients with a PRD of HUS had higher Hb levels compared with all other PRDs.

Conclusions: We have identified anemia is a common and significant problem in European children with ERF. Children on haemodialysis are more prone to anemia than those on peritoneal dialysis and those who have been transplanted. Younger children, particularly those under six years of age, are most at risk. Evidence based European guidelines are needed to help paediatric nephrologists optimize management of anemia in children requiring renal replacement therapy. The management of anemia in those particularly at risk should be specifically targeted.

Disclosure of Financial Relationships: nothing to disclose

**PUB421**

**Decrease in the Number of Undocumented Patients Starting Hemodialysis in a New York City Hospital over the Last Three Years** Mary C. Mallappallil, Reisha T. Browne, Win Kyaw, Moro O. Salifu. *Internal Medicine, Division of Nephrology, SUNY HSCB Downstate at Brooklyn, New York, NY.*

Purpose : We hypothesized that there is a change in the number of undocumented patients who present themselves to the inner city hospitals in New York City for hemodialysis over the past three years.

Methods: We looked at the number of incident patients starting hemodialysis in our center over the past three years from 2007 to 2009, analyzed physician and social worker notes, billing specialist input and social security numbers for all patients to determine their immigration status.

We are the largest Municipal hospital based dialysis center in New York City with a referral base of 22 community hemodialysis centers. Our hemodialysis population is unique with patients from the Caribbean ethnic minority, who are emergently started on hemodialysis in the hospital. With no prior medical care dialysis is started with a catheter after which, on discharge they are transferred to out patient hemodialysis units. They become eligible in New York State for emergency Medicaid which covers the cost of care for hemodialysis.

In 2007, we started 138 new patients on hemodialysis of which 58 (42%) were undocumented patients. In 2008, we started a total of 115 patients on hemodialysis of which 39 (34%) were undocumented and in 2009, we started 105 patients on hemodialysis of which 27 (26%) were undocumented patients with no medical insurance. Using chi square analysis the decrease in number of undocumented immigrant patient for the three years is significant with *p* value at 0.005, which is an unexpected decrease.

The average creatinine of patients starting hemodialysis in 2007 was 9.5mg/dL and in 2009 is 9.9mg/dL.

We correlated the decrease in the incident number of new undocumented immigrant dialysis patients to the trend of decrease in the illegal immigrant population reported by the center of immigration studies and data from Homeland Security from 2007 to 2009 due to economic reasons and increased immigration enforcement.

We still have a significant number of undocumented immigrant patients on hemodialysis compared to the most other institutions and the United States Renal Data System.

Disclosure of Financial Relationships: nothing to disclose

## PUB422

**Outcomes of Patients Starting Hemodialysis with Serum Creatinine Greater Than 30mg/dL** Mary C. Mallappallil, Reisha T. Browne, Win Kyaw, Moro O. Salifu. *Division of Nephrology, Department of Internal Medicine, State University of New York at Brooklyn, Downstate Medical Center and Kings County Hospital Center, Brooklyn, NY.*

We report 8 cases of patients with serum creatinine (SCre) > 30mg/dL (GFR < 2ml/min) who presented for initial hemodialysis (HD) in our hospital over the past 3 years. We analyzed the mortality of these patients at one year based compared to those with SCre <30md/dL.

Observational study of 149 patients who started hemodialysis over the past three years. We analyzed patient mortality at 1 year with serum creatinine >30mg/dL (n=8) and compared to all those with SCre<30mg/dL (n=141).

Many of our patients started HD in the hospital, without prior medical care. All belong to ethnic minority groups & are usually started on HD with a catheter.

After 1year on HD, 1 of the 8 patients with SCre > 30mg/dL died (12.5%) compared to 20 of 141 patients (15%) in the group with SCre < 30mg/dL. In the group with Scre > 30mg/dL, the mean SCre and Body Mass Index(BMI) was 34.2mg/dL and 30 respectively. With SCre < 30mg/mL, the subset that survived 1 year had a mean SCre and BMI of 10.3mg/dL and 27.9 and the subset that died had mean SCre and BMI of 7.3mg/dL and 25 respectively.

In those with SCre> 30mg/dL, 7 were placed on chronic HD, 2 had prior medical care and medical insurance at presentation. All spoke English & presented symptomatically to the hospital.

Clinical Characteristics of Patients with Serum Creatinine greater than 30mg/mL starting Hemodialysis

| patient number | Age and sex | serum creatinine md/dL at start of HD | GFR at start of HD ml/min | Body Mass Index | Alive/ one year | type 2 diabetes | Hypertension |
|---|---|---|---|---|---|---|---|
| 1 | 35M | 37.7 | 1.6 | 33 | yes | no | yes |
| 2 | 50F | 31.4 | 1.4 | 35 | yes | yes | yes |
| 3 | 47M | 30.5 | 1.9 | 30 | yes | no | yes |
| 4 | 56M | 30.5 | 1.9 | 22 | yes | no | yes |
| 5 | 52M | 36.7 | 1.5 | 34 | yes | no | yes |
| 6 | 28M | 33.5 | 1.9 | 31 | yes | no | yes |
| 7 | 51F | 30.3 | 1.4 | 29 | no | no | no |
| 8 | 22M | 42.5 | 1.5 | 26 | yes | no | no |

We found that very high serum creatinine > 30mg/dL with high BMI, had better one year mortality (12.5%) than the average patient starting hemodialysis our center (15%) and this may be due to the larger muscle mass. We need larger groups to determine the significance of our findings.

Disclosure of Financial Relationships: nothing to disclose

## PUB423

**A Survey of Influenza Vaccination Status among Dialysis Staff in the South Chicago Area** Albert M. Osei, Bharathi V. Reddy, Pradeep V. Kadambi, Michelle A. Josephson, Woojin James Chon. *Department of Medicine/ Nephrology, University of Chicago, Chicago, IL.*

**Background:** The advisory committee on immunization practices includes dialysis patients in the high risk category of those needing influenza vaccinations. Some studies have concluded that dialysis patients do not achieve the same level of protection after vaccination as non-dialysis patients. The use of antiviral medications in patients on hemodialysis is plagued with uncertainty about the optimal dosing. Thus, influenza infection prevention among dialysis patients must include reducing their viral exposure during outbreaks. Hemodialysis patients spend at least 12 hours each week in close proximity to nurses and technicians. The rate of influenza vaccination among the dialysis staff is unknown and so are the factors affecting the rate of vaccination.

**Design, Setting, and Participants:** We distributed a single page survey on influenza vaccination status, reasons for declining vaccination, facility policies and patient inquiries. The target group was dialysis nurses and technicians in the south side of the city of Chicago and the surrounding suburbs.

**Results:** In all, 108 individual responses representing 58% return rate were received from 10 different units that allowed their staff to participate. 62% of the respondents were dialysis technicians and 32% were nurses with 6% unknown. For the current influenza season, 55% received the seasonal influenza vaccine and 35% reported receiving the H1N1 vaccine. 22% reported having never received any type of influenza vaccine. The stated reasons for not receiving vaccination included: "never had the influenza vaccination" at 14%, "not in a high risk category" at 9%, and "previous skin reaction" and "vaccination not made available" at 6% each. 84% reported availability of vaccines from their facilities. 67% reported having been asked their opinion on influenza vaccination on at least one occasion by a patient. Of those asked, 81%recommended it, 7% advised against it and 12% offered no opinion.

**Conclusion:** Influenza vaccination among hemodialysis staff is suboptimal. Education of dialysis staff and a policy mandating vaccination may need to be considered.

Disclosure of Financial Relationships: nothing to disclose

## PUB424

**A Comprehensive Multicentric Assesment of Haemodialysis Facilities** Eduardo Parra,[1] Dolores Arenas,[2] Maria Fernanada Martínez,[3] Fernando Alvarez-Ude.[4] *[1]Nephrology, Hospital Reina Sofía de Tudela, Tuela, Navarra, Spain; [2]Nephrology, Hospital Perpetuo Socorro, Alicante, Elche, Elda, Spain; [3]Nephrology, Hospital Casa de la Salud, Valencia, Spain; [4]Nephrology, Hospital General de Segovia, Segovia, Spain.*

INTRODUCCTION:

Nowadays, we do not have a comprehensive system to assess haemodialysis (HD) facilities, considering clinical performance measures (CPMs), health related quality of live (HRQoL), patient´s satisfaction and costs, simultaneously. The aim of this study is to analyze the results of the HD therapy in 6 different facilities.

MATERIAL AND METHOD:

This is an observational and prospective study. Two centres were public and four private (with agreement with our National Health Service). We collected several HD results over 2008.

RESULTS:

Centres showed no significant differences in demographic, kind of renal disease and Charlson Co-morbidity Index. The table shows the results of each centre.

| CENTRES | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| Type of centre | | PUBLIC | PUBLIC | PRIVATE | PRIVATE | PRIVATE | PRIVATE |
| Mean of patient´s months on HD | | 37.3 | 30.6 | 47.0 | 167.4 | 39.9 | 27.9 |
| CLINICAL PERFORMANCE MEASUREMENTS | | | | | | | |
| | Kt/v (% > 1,2) | 90.0 | 88.2 | 81.4* | 94.0 | 96.3 | 87.8 |
| | Hb (% 11-13 gr/dl) | 51.2 | 62.2 | 51.1 | 66.2 | 61.1 | 56.1 |
| | Ca (% 8,4-9,5 mg/dl) | 68.3 | 75.7 | 60.0 | 69.2 | 68.5 | 58.5 |
| | P (% 2,7-5 mg/dl) | 68.3 | 70.3 | 46.7* | 69.2 | 72.2 | 51.2 |
| | VASCULAR ACCESS (% native arteriovenous fistula) | 48.8* | 73.0 | 71.7 | 79.7 | 77.8 | 75.6 |
| | ANNUAL MORTALITY | 16.1 | 22.9* | 19.1 | 11.4 | 10.0 | 10.8 |
| SATISFACTION AND QUALITY OF LIFE | | | | | | | |
| | SATISFACTION KBD | | | | | | |
| | ESCALE 0-100 | 91.6 | 97.6 | 87.5 | 85.1 | 89.0 | 87.3 |
| | HEALTH RELATED QUALITY OF LIFE | | | | | | |
| | SF-36 MSS | 53.1 | 46.8 | 45.8 | 46.5 | 49.1 | 51.1 |
| | SF-36 PSS | 31.7 | 33.0 | 32.5 | 37.0 | 35.4 | 35.9 |
| COST | | | | | | | |
| | ANNUAL COST PER PATIENT (€) | 42.574 | 39.289 | 32.872 | 29.786 | 35.461 | 35.294 |

*p < 0.05, MSS: mental summary score, PSS: physical summary score

CONCLUSION:

There were differences in some CPMs between centres but not in those related to HRQoL and patient´s satisfaction. There was a great variability in cost between different centres. In our study, lower cost and private management did not imply poorer outcomes.

Disclosure of Financial Relationships: nothing to disclose

## PUB425

**A Novel Comprehensive System To Assess Haemodialysis Facilities** Eduardo Parra,[1] Dolores Arenas,[2] Maria Fernanada Martínez,[3] Fernando Alvarez-Ude.[4] *[1]Nephrology, Hospital Reina Sofía, Tudela, Navarra, Spain; [2]Nephrology, Hospital Perpetuo Socorro, Alicante, Elche, Elda, Spain; [3]Nephrology, Hospital Casa de la Salud, Valencia, Spain; [4]Nephrology, Hospital General de Segovia, Segovia, Spain.*

INTRODUCCTION:

Nowadays, we do not have a comprehensive system to assess haemodialysis (HD) facilities. The aim of this study is to find a suitable, clear and acceptable way of assessing HD facilities.

MATERIAL AND METHOD:

This is an observational and prospective study. Several HD variables were collected over 2008 in 6 different facilities. The evaluation system gives, by consensus of investigators, a relative weight to each selected indicator. The maximum number of points was assigned to the facility with better results for each selected indicator and cero to the one with the poorest result. The other facilities get points proportionally to that clearly-defined range. HD variables considered and the points assigned were: A) clinical performance measurements (CPMs), which included Kt/v (10 points), haemoglobin (10), calcium (10), phosphorous (10), type of vascular access (10) and annual mortality (20), B) Health Related Quality of

Live (HRQoL), estimated using the SF-36® test (10), C) patients satisfaction, using KBD® test (10), D) annual cost per patient for each centre (10).

For instance, if a centre (the best one) has an 80% of patients with haemoglobin on target (11-13 g/dl), and another (the worst one) has 60%, they will get 10 and 0 points respectively; a center with a 70% will get 5 points.

RESULTS:

Centres showed no differences in age, sex, time on HD, kind of renal disease and Charlson Co-morbidity Index. The total score of each centre was 40.7, 53.5, 24.4, 76.8, 76.3 and 51.8.

CONCLUSION:

We believe that this way of assessing HD facilities may fulfil the requirements of simplicity, clarity, proportionality, discrimination, acceptability and comprehensiveness as well as correlation with CPMs and being focused into improvement of centres. The generalization of such a system of assessing HD centres may contribute to improve the outcomes and efficiency of the health system.

Disclosure of Financial Relationships: nothing to disclose

**PUB426**

**Outcome of Acute Admission in Haemodialysis or Transplant Patients over a 12 Month Period. Requirements for High-Dependency Care and the Effect of Ethnicity** Darren S. Parsons, Adam Mclean, Neill D. Duncan, David Taube, Tom Cairns. *Imperial College Kidney and Transplant Institute, West London Renal and Transplant Centre, Imperial College Healthcare NHS Trust, London, United Kingdom.*

**Background**

The West London Renal and Transplant Centre (WLRTC) serves a population of 2 million. It provides inpatient services for 2800 patients on renal replacement therapy: 1350 dialysis (HD/PD) and 1450 transplant patients (TX).

**Methods**

We have analysed the outcome of acute admission in patients on renal replacement therapy during a 12 month period to examine outcome, length of stay (LOS), requirements for additional organ support beyond dialysis (eg inotropic support, non-invasive ventilation) in our renal high-dependency ward (RHDU) and the effect of patient ethnicity on admission rates.

**Results**

From 31.5.2009 to 31.5.2010 there were 3873 elective and 2633 acute admissions to WLRTC. Acute admissions comprised: HD/PD 1270, TX 1038 and Nephrology 325. LOS was HD/PD 12 ± 20 (median 5, 1- 272) and TX 7 ± 16 (median 3, 1-273) days. Death during acute admission occurred in 98/1270 HD/PD (7.5%) and 15/1038 TX (1.4%). During the same time period outpatient deaths on renal replacement were 59 HD/PD and 14 TX. Overall death on the programme during 12 months: HD/PD 60/1350 (4.4%) and TX 29/1450 (2.0%). Of the acute admissions, 369/2308 (16.0%) episodes required 2 organ support (duration 1-45 days) and 101/2308 (4.4%) required 3 organ support (duration 1-29 days) beyond basic renal replacement therapy in RHDU.

| Ethnicty | HD/PD Programme | HD/PD Admissions | TX Programme | TX Admissions |
|---|---|---|---|---|
| Number | 1350 | 1270 | 1450 | 1038 |
| Caucasian | 34.3% | 37.4% | 51.5% | 46.3% |
| Afro-Caribbean | 17.5% | 19.4% | 10.8% | 16.0% |
| Indo-Asian | 23.8% | 19.5% | 20.8% | 20.5% |
| Other/Unknown | 24.3% | 23.7% | 23.7% | 17.2% |

Comparison of Ethnicity on Programme (HD/TX) compared to Acute Admissions

**Conclusions**

Our inpatient mortality rates are low in both HD/PD and TX acute admissions. A significant proportion of acute admissions required additional organ support beyond dialysis. Analysis by ethnicity showed little significant impact on the requirement for acute admission in HD/PD or TX patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB427**

**Quality Accreditation of a Public Hemodialysis (HD) Unit in Spain. Our Experience** Mª Dolores Del Pino Pino, Ma del Carmen Prados Soler, Remedios Garofano, Clara Moriana, Manuel Ángel Rodríguez. *Nephrology, Complejo Hospitalario Torrecárdenas, Almería, Spain.*

In May 2006 the current HD unit was opened. It consists of 5 rooms, 41 monitors, 26 posts, three shifts a day (Mondays to Saturdays), 100 fixed patients, with 100% occupation of dialysis posts, 15.870 sessions performed during the last year and a total of 50 staff. Quality accreditation of the HD Unit aims to encourage the continued improvement of the Service, through three important points: "What is not recorded does not exist", "What is not measured cannot be improved", "Consider solely those records and documents that can benefit the Unit".

Accreditation Process: Evaluation by the Andalusian Health Care Quality Agency (AHCQA) performed of 86 standards, corresponding to four blocks: I. The citizen, center of the Health System, II. Organization of patient-centered activity, III. Professionals, IV. General support and safety processes, and V. Results. The development of the accreditation process was as follows: Phase 1: Preparation phase – March 2008; Phase 2: Self-evaluation and internal improvement – from March 2008 to June 2009; Phase 3: External evaluation – July 2009. Decision of the Certification Committee: Accreditation of Advanced Quality, thus becoming the only public HD Unit accredited in Andalusia. By complying with more than 90% of the standards, the AHCQA considered our strong points to be: user satisfaction, participation and rights, accessibility and continuity of care, activities related to the promotion of health care programs, structure and installation of general, support and safety processes; Phase 4: Follow-up self-evaluation during 5 years.

Conclusions: Being the sole public HD Unit accredited by the AHCQA has endowed our hospital with recognition by the Health Authorities; for the patients, we have surpassed their level of satisfaction, confidence and trust in us, and for our Service it has led us to seek out areas for improvement. We consider ourselves to be a solid working team, desiring continuous improvement and hoping to obtain even better accreditation levels. We encourage HD Units to follow our experience as it is the best way to promote continuous improvement.

Disclosure of Financial Relationships: nothing to disclose

**PUB428**

**Demographics of Undocumented Residents with End-Stage Renal Disease in the Houston Community** Rajeev Raghavan, David Sheikh-Hamad. *Department of Medicine, Division of Nephrology, Baylor College of Medicine, Houston, TX.*

Introduction: Currently there is no uniform national policy regarding health care for undocumented residents (UR). Since both care delivery and compensation vary between states, confusion and ethical dilemmas are abound. The term UR include persons who have either crossed the U.S. border illegally or are immigrants who entered legally, but are now out-of-status. It is not known exactly how many UR require renal replacement therapy (RRT). Combining the Urban Institute estimate of 11 million UR with data from the United States Renal Data System (USRDS) database, at least 6000 UR in the U.S. require RRT. In this work, we sought to evaluate the demographics of this patient population in the Houston public hospitals and clinics.

Methods: One hundred and eighty-five UR currently receive dialysis in the Houston community. Sixty of these patients receive maintenance hemodialysis. Demographic information was obtained via patient interview and chart review.

Results: When compared to patients in the USRDS, this patient population is younger and demonstrates lower prevalence of diabetes (42.8 *vs* 60 years; and 37% *vs* 44%, respectively; $p<0.05$). 95% of the patients are Hispanic and only 23% graduated high school. Most patients have spent over 30% of their life in the United States before commencing dialysis (average 16.5 years); and 53% received pre-dialysis care. Only 3% of the patients had knowledge of their kidney disease prior to immigrating to the US. Importantly, none of the patients want to return to their home countries because of established roots here and perceived poor outcomes for patients on dialysis in their home countries.

Conclusions: The number of UR needing RRT in the Houston public hospital system is large and imposes a significant financial burden on the community. Adequate solutions require uniform policy nation-wide. The use of non-standard dialysis is one approach that may benefit these patients while containing costs. Immigration reform is critical if these patients were to benefit from the new health care reform.

Disclosure of Financial Relationships: nothing to disclose

**PUB429**

**Successful Treatment of Colon Cancer with Oxaliplatin/5-Flurouracil/ Luecovorin in a HIV Positive Patient with End Stage Renal Disease on Hemodialysis** Divya Salhan, Abhijit Kontamwar, Kenar D. Jhaveri, Michael D. Gitman. *The Raggio Institute and the Division of Kidney Diseases and Hypertension, NSLIJ Health System, Manhasset, NY.*

Little is known about the dosing and efficacy of cancer treatment in hemodialysis (HD) patients. Here we report the successful use of oxaliplatin(OX) for the treatment of colon cancer in a HIV positive patient with end stage renal disease (ESRD) on hemodialysis (HD).

A 57 year old HIV positive Caucasian male with ESRD on HD was evaluated for the treatment of colon cancer. He had developed ESRD secondary to the use of tenovofir and NSAIDS and had been on HD for one and a half years. A CT scan revealed a large polypoid mass in the caecum and he was subsequently diagnosed with stage 3 A colon carcinoma. The carcinoembrionic antigen level was 2.1ng/ml. He was on antiretroviral therapy. The patient received modified FLOX chemotherapy which included: OX 40mg/m2, L-luecovorin(LV) 360 mg/m2, 5-Flurouracil(5FU) 400mg/m.The patient received 4 cycles of chemotherapy. Each cycle consisted of 6 weeks of chemotherapy with a 2 week break between the cycles. The OX and LV were administered over 2 hours and 5 FU was administered as an IV push 1 hour into the LV infusion. HD was performed within 1 hour of OX administration. The HD was done via Optiflux F160 dialyzer for three and a half hours with a blood flow rate of 400ml/min. The patients laboratory toxicity profile was followed closely throughout the course of chemotherapy. According to the National Cancer Institution toxicity criteria the patient tolerated the chemotherapy well with Grade 0 toxicity. He received full treatment without interruption. The most recent CEA level was 1.2ng/ml.The HIV viral load (vl) pre and post treatment was 50 and 93 RNA copies/ml respectively.

With the rising number of dialysis patients requiring chemotherapy, clinicians will need a safe and effective therapy to treat this population. We report that the reduced dose modified FLOX regimen was safe and effective in our HIV positive ESRD patient and should be considered as an option for other ESRD patients with colon cancer.

Disclosure of Financial Relationships: nothing to disclose

**PUB430**

**Anemia Management in Hemodialysis Patients: A Report from Binocrit® Observatory Study (BOS)** Malik Touam,[1] Pablo A. Urena.[2] *[1]AURA, Paris, France; [2]Clinique du Landy, Levallois-Perret, France.*

This was prospective national observatory. 83 nephrologists described their practices when including hemodialysis pts (HD) during the period from april 2009 to april 2010. ESA treatment by Binocrit ® is initiated in HD adults, without iron deficiency and whose hemoglobin (Hb) is stable during the last three months before inclusion.

332 HD (mean age 65 y, male 55%, 9 y on dialysis, diabetes 24%) are included. At baseline the mean Hb, ferritin (Fe) and TSAT were respectively 10.8 ± 3 g/dl, 220 ± 56 µg/l and 21 ± 7%. The optimal Hb was [10-12 g/dL] or [11-13 g/dl] for 90.3% and 9.7% of nephrologists. This target relates to respectively 93% and 7% of HD. If Hb> 13 g/dl, 32.3% of nephrologists suspend ESA, while 67.7% reduce the dosage. In 87% of HD, iv route for iron therapy was used . Fe target is [200-500 mg / l] in 52.8% , and [500-800 mg / l] in 47.2% of cases. TSAT target is [15-30%] in 89.7% and> 30% in 10.3% of cases. When Fe is above the target, 81% of nephrologists suspend iron treatment, and 19% reduced dosage or frequency of administration. The interval between iron injection and blood sampling for iron status: 7 d in 16.1% , between 7-14 d in 12.9% and no standardised protocol in 51.6% of cases. At initiation of ESA therapy Hb is monitored weekly in 12.9%, every two weeks in 54.8%, every month in 32.4% of cases. In the maintenance phase, it is monitored every two weeks (35.5%) and monthly (64.5%). In multivariate analysis (taking into account age, sex, vintage on dialysis, diabetes, inflammation, Fe and TSAT target), there is no significant difference between the HD where the target Hb was 10-12 g/dl and those where the target Hb was 11-13 g/dl.

Conclusion: for 90% of nephrologists Hb target was 10 -12 g/dl for 93% of HD. A minority of nephrologists are choosing a high Hb target in 7% of pts, whose main characteristics are not different from the majority of HD. The iron therapy is administered iv in the majority of HD. However, its biological monitoring does not seem to obey a strict protocol in 51.6% of nephrologists. 30% of nephrologists believe that the optimal Fe is [500-800 mg / L], whereas only 10.3% are an TSAT> 30%.

Disclosure of Financial Relationships: nothing to disclose

**PUB431**

**Effect of Regional Distribution of Dialysis Satelites on Travel Time and Access to Care** Lilyanna Trpeski,[1] Stanley S. Fenton,[2] Charmaine E. Lok.[2] *[1]Ontario Renal Network, Cancer Care Ontario, Toronto, ON, Canada; [2]Nephrology, Toronto General Hospital, Toronto, ON, Canada.*

Background: Providing dialysis care is influenced by direct and indirect patient factors. Access to dialysis care is an indirect factor that may affect long term patient outcomes. Rural satellite centers were designed to facilitate dialysis by reducing travel times for patients who live long distances from urban dialysis centers in Ontario, Canada.

The aim of this study was to explore the impact of having satellite centers on patients' commuting distances and travel times from their homes to their dialysis centers, almost 2 decades after satellite centers were first introduced in Ontario.

Methods: Prevalent dialysis patients at the end of 2009, abstracted from the Ontario Renal Reporting System, were analyzed. The patients' residential and the dialysis facilities' postal codes were used in the validated "Great Circle Distance" formula which calculates the shortest distance between 2 points using SAS software. The travel time was calculated based on the average driving speed of 70 km/h.

Results: At the end of 2009, 9,626 patients were receiving dialysis in 27 centers and 42 satellites in Ontario. The mean distance from home to the dialysis facility for all in-centre patients was 13.1km. In 10 of 14 Health Regions, 95% of patients live up to 50 km from the dialysis center. Travel time greater than 1 hour was observed in 0.8% to 15% of the patients by Health Region.

| Factors in Peritoneal Dialysis Patients | Hazard Ratio PD | Upper CI | Lower CI | Factors in Hemodialysis Patients |
|---|---|---|---|---|
| Age 45-64 yrs | 2.093 | 2.46 | | Age 45-64 yrs |
| >65 years | 3.499 | 4.14 | 2.96 | >65 yrs |
| Ethnicity: Black | 0.634 | 0.82 | 0.49 | Ethnicity: Black |
| Indigenous | 1.701 | 3.82 | 0.76 | Indigenous |
| Other | 0.648 | 0.76 | 0.55 | Other |
| 120-160 incident patients | 1.041 | 1.39 | 0.78 | 330-534 incident patients |
| 160-247 incident patients | 1.042 | 1.37 | 0.79 | 534-643 incident patient |
| >247incident patients | 1.217 | 1.62 | 0.92 | >643 incident patients |
| Sex: female | 1.031 | 1.18 | 0.9 | Sex: female |
| diabetes | 1.818 | 2.09 | 1.58 | diabetes |
| coronary artery disease | 1.549 | 1.77 | 1.36 | coronary artery disease |
| peripheral vascular disease | 1.595 | 1.88 | 1.35 | peripheral vascular disease |
| cerebrovascular disease | 1.159 | 1.39 | 0.97 | cerebrovascular disease |
| lung disease | 1.362 | 1.76 | 1.05 | lung disease |
| smoking | 1.094 | 1.39 | 0.86 | smoking |
| BMI <18.5 | 1.534 | 1.98 | 1.19 | BMI <18.5 |
| BMI 24.9-29.9 | 0.784 | 0.91 | 0.68 | BMI 24.9-29.9 |
| BMI >=30 | 0.86 | 1.05 | 0.7 | BMI >=30 |

Conclusion: The regional distribution of dialysis centers is effective in ensuring timely access to care in the majority of the patients (90%) in Ontario. However, there are 3 regions where patients are more likely to travel >1 hour, one way, to receive dialysis. Additional strategies, such as telemedicine, is necessary to improve access and monitoring of dialysis care for patients with long commute times.

Disclosure of Financial Relationships: nothing to disclose

**PUB432**

**Comparison of Two Models of Telemedicine in Remote Dialysis Units in Northern Quebec** Murray L. Vasilevsky,[1] Claude Sicotte,[2] Khalil Moqadem,[2] Johanne Desrochers,[1] Madeleine St-Gelais.[1] *[1]Nephrology, McGill University Health Centre, Montreal, QC, Canada; [2]Department of Health Administration, University of Montreal, Montreal, QC, Canada.*

Telemedicine was introduced to improve the surveillance of patient care in two remote satellite units serving First Nation Cree patients in the James Bay region of Northern Quebec. An electronic database (Nephrocare) accessible from the University center was operative in both sites and linked to the University center. The telemedicine model differed between sites. One model (Unit A) consisted of weekly or bi-weekly rounding on each patient by the nephrologist, using a mobile telemedicine station in the satellite unit. The second model (Unit B) consisted of a weekly videoconference between the nephrologist and satellite nurses to review charts and resolve problems. All Cree patients older than 18 and dialyzed for a minimum period of 9 months prior to and after introduction of telemedicine were included. The impact of telemedicine on outcome measures was compared between Unit A and Unit B. Data was extracted retrospectively from monthly lab tests and chart review and subjected to ANOVA with repeated measures. 28 patients were included in the analysis. There were no differences in pre-dialysis systolic and diastolic BP, serum Hb, serum albumin, phosphate, PTH, glucose, glycosylated Hb and KT/V between units. The number of prescription medication changes and transfers to the university center was the same. Telemedicine provides better security for patients and staff in remote settings. Different models of telemedicine to support dialysis in remote settings may be equally effective and should be adapted according to the local needs of the particular unit.

Disclosure of Financial Relationships: Honoraria: Astra-Zeneca, Janssen, Amgen, Roche, Shire.

**PUB433**

**Impact of Vascular Access Blood Flow Monitoring on Access Survival in Hemodialysis Patients** Amir Abdi Pour,[2] Seyed-Ali Sadjadi,[1] Navin Jaipaul,[1] James I. McMillan.[1] *[1]Nephrology Section, VA Loma Linda Health Care System, Loma Linda, CA; [2]Nephrology Division, Loma Linda University Department of Medicine, Loma Linda, CA.*

Vascular access thrombosis results in suboptimal outcome in patients on hemodialysis. Numerous studies have shown divergent results of the impact of monitoring on access survival. We conducted a retrospective review to determine failure rate of the first usable vascular access during the first year of use in 102 United States Veterans with (Group 1) and without (Group 2) blood flow monitoring, using the saline dilution technique over a 10 year period. Table 1 shows the demographics and impact of surveillance.

Table 1: Baseline Characteristics and Study Endpoints

| Variable | Group 1( N=48) | Group 2( N=54) | P value |
|---|---|---|---|
| Age (±SD), years | 69±9 | 63±11 | 0.40 |
| Male gender, n | 48 | 52 | 0.17 |
| Diabetes, n | 30 | 37 | 0.52 |
| Hypertension, n | 40 | 49 | 0.26 |
| Peripheral Vascular Disease, n | 7 | 10 | 0.59 |
| Coronary ArteryDisease, n | 24 | 21 | 0.25 |
| Congestive Heart failure, n | 21 | 27 | 0.65 |
| Access type, n | | | 0.27 |
| AV fistula | 26 | 35 | |
| AV graft | 22 | 19 | |
| Access failure, n | | | |
| Overall | 12 | 4 | 0.01 |
| AV fistula | 1 | 1 | |
| AV graft | 11 | 3 | |
| Infection, n | 6 | 2 | 0.09 |
| Revision, n | 14 | 5 | 0.01 |
| Thrombosis, n | 19 | 3 | <0.001 |
| Fistulogram, n | 18 | 34 | 0.01 |

**Results**: There was no significant difference in demographics, comorbidities, or type of access between the two groups. Overall access loss and revision were higher and statistically significant in group 1 vs. group 2 (Table 1). Access thrombosis was also significantly higher in group 1 vs. group 2 (Table 1). Graft failure was significantly higher in the first year compared to native fistula failure in both groups (n= 14 vs. 2; p value <0.001). Although not statistically significant, access infection tended to be higher in group 1 vs. group 2 (Table 1). **Conclusion**: Access surveillance decreases the rate of access thrombosis and failure but requires more procedures and interventions. It appears that access surveillance has a greater impact on preventing graft loss vs. native fistula loss during the first year of follow-up.

Disclosure of Financial Relationships: nothing to disclose

## PUB434

**Deadlock in Hemodialysis Angioaccess (DHDAA)** Thanh Cao Huu, Cridlig Joelle, Michele Kessler, Luc Frimat. *Nephrology, CHU Nancy, ??Vandoeuvre, France.*

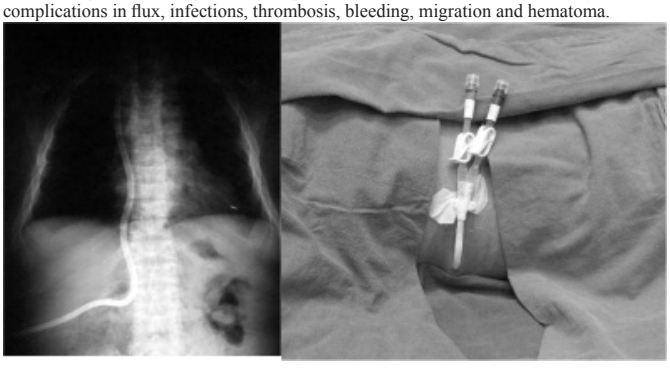
The authors reported observations of deadlock in hemodialysis angioaccess (DHDAA) of 15 patients (9 F & 6 M) with mean age at 59±18 years , treated by hemodialysis for 127±86 months. Co-morbidity as diabetic mellitus and obesity were noted in 8 patients, arteriopathy and coronaropathy in 12, calciphylaxis and chronical inflammatory diseases in 4. Risk factors incriminated of DHDAA were: high incidence of central veins catheterism (CVC) (6±4 catheters per patient including 4±3 non-tunnelled-catheters and 3±3 permanent tunnelled catheters (PTC) (Canaud) per patient.. Only 1 patient did receive 2 subclavian catheters before DHDAA situations. They received up to 11±6 surgical angioaccess failures including fistula, PTFE, or shunt. Only 3 patients received radio-cephalic in forearms as the first angioaccess while 12 did receive proximal fistula or PTFE. Femoral accesses including catheters, PTFE and saphen vein bypasses were attempted in 9 patients. Stenoses of cave system-central veins occurred in most of the patients (stenoses of internal jugular veins:13, brachio-cephalic veins:13, subclavian veins:10, femoral & iliac veins: 3). Thank to Angioplasties,11 permanent tunnelled catheters were inserted and 6 new angioaccess operated. Without the need of angioplasty, 3 s/clavian PT catheters, 2 femoral vascular accesses and 1 brachial PTFE bypass were performed. These last angio-access comprised aggressive but still fragile solutions since: 1 patient was to be transplanted in emergency, 7 still kept PT catheters, 7 could receive proximal Fistula or PTFE AA (including 2 ligations, 2 stenoses treated by angioplasties and 2 stenoses treated by PTFE bypass). Peritoneal dialysis were contraindicated or failed in all and transplantation was possible in another patient. Hospitalisations were frequent, unpredictable and stressful. 4 Patients died 1 to 6 months after the last AA. In conclusion, the situation of DHDAA in our 15 patients was the combination of numerous angioaccess failures and CV catheters & stenoses. Management of DHDAA are time consuming. Multi-disciplinary efforts and patience are needed.

Disclosure of Financial Relationships: nothing to disclose

## PUB435

**Percutaneous Trans-Lumbar (PTL) Dialysis Catheter Placement, Unto Superior Vena Cava (SVC), Guided by Computed Tomography, a Case Report** Javier Castillo Tapia,[1] Alejandro Chavez,[1] Salvador Mendoza,[1] Patricia Peña,[1] Armando Avila,[2] Juan Rochin Teran,[2] Leticia Marquez,[1] Luis Alberto Evangelista Carrillo,[1] Abel Puentes Camacho,[1] Jorge Andrade-Sierra,[1] Leonardo Pazarin,[1] Mario Sandoval Sandoval,[1] Enrique Rojas-Campos,[3] Alfonso M. Cueto-Manzano,[3] Benjamin Gomez-Navarro.[1] *[1]Nephrology and Organ Transplant, IMSS, Guadalajara, Jalisco, Mexico; [2]Radiology and Imagenology, IMSS, Guadalajara, Jalisco, Mexico; [3]Medical Research Unit in Renal Diseases, IMSS, Guadalajara, Jalisco, Mexico.*

**Introduction**: In HD when common vascular accesses (VA) are unavailable it is necessary to consider other sites.The first Latin-American, PTL placement unto SVC, CT guided. **Case**. Female 28 yrs. ESRD (vesicoureteral reflux). 2 renal transplants (1992,1999), from LRD (father) and CD, lost for AR at 36 and 16 mo. HD since Feb-2002, 18 VA (6 mahurkar (MAH), 6 tunelized catheters, 3 native and 3 graft fistulas [GF]). Hepatitis B and C positive; cross-match positive (99%), PRA HLA C-I (100%) and C-II (100%). Last VA lost (GF-right femoral ), perforation and bleeding (August 28 2009) at HD. We placed a MAH useful for 1 week in a subclavian branch (Sept. 2, 2009). We planned a PTL catheter placement unto SVC, guided by CT, as a last option. **TECHNIQUE**: Initial CT to measure distance skin-IVC. Punction at the lumbar back between the iliac ridge-lumbar spine; IVC was reached after 3-attempts using the Seldinger technique; corroborated by pulsation absence and CT. Using a hydrophilic wire (guide), catheter was introduced from IVC to SVC, CT-corroborated . Skin tunnelization to the right flank. The catheter max. flux reached was 450 ml/hr, recirculation 5%, Kt/V 1.6. June 15, 2010: SCr 12.2mg/dL, Uric acid 9.5mg/dL, P 3.8 mg/dL, Ca 8.5mg/dL, Hb10.5g/dL, Alb 3.6g/dL. It has 120 catheter/days, with no complications in flux, infections, thrombosis, bleeding, migration and hematoma.



Disclosure of Financial Relationships: nothing to disclose

## PUB436

**Case Study: HeRO Vascular Access Device Long-Term Outcomes in Renal Transplant Patient with Multiple Co-Morbidities** Howard E. Katzman. *Department of Vascular Surgery, University of Miami, Miami, FL.*

**Purpose:** To report long-term outcomes 47 months after implant of the novel HeRO vascular access device in a catheter-dependent Hispanic male with a history of two renal transplants and multiple co-morbidities.

**Methods:** The HeRO device was implanted July 25, 2006, in a 31 year-old Hispanic male as part of the HeRO Food and Drug Administration clinical trial. This catheter-dependent patient required dialysis since the age of 8 due to congenital deformity. His exhaustive vascular access history included multiple fistulas, grafts and catheters at the time of HeRO implant. Co-morbidities included hypertension, hepatitis C, central venous stenosis in both brachiocephalic locations, thyroid papillary carcinoma and total thyroidectomy with no history of smoking or diabetes. This patient continues to be followed beyond the clinical trial requirements for evaluation of long-term outcomes.

**Results:** A HeRO device was successfully placed over-the-wire utilizing the existing dialysis catheter tract in the right internal jugular vein. A femoral bridging catheter was placed and used for acute dialysis until the HeRO device could be cannulated, approximately one month post implant. To-date, this patient has been followed for 47 months post HeRO implant. Two percutaneous HeRO interventions have been required to maintain patency, the first at 42 months post implant and the second at 44 months post implant. At approximately 46 months post implant, the original HeRO graft was replaced with a new HeRO graft due to pseudoaneurysms in the graft segment, which resolved the thrombosis issues. No infections have been reported to-date.

**Conclusion:** This case report demonstrates that patients ineligible for an upper extremity access due to central venous stenosis may experience the long-term benefits of a subcutaneous arteriovenous access by receiving the HeRO device.

Disclosure of Financial Relationships: nothing to disclose

## PUB437

**Early Thrombectomy of Arteriovenous Dialysis Grafts: Is It Worthwhile?** Vijay Mudunuri, Jeremy C. O'Neal, Michael Allon. *Nephrology, University of Alabama at Birmingham, Birmingham, AL.*

Introduction:

In spite of the National Kidney Foundation's initiative to increase use of autogenous fistulas for vascular access, many patients continue to undergo hemodialysis with arteriovenous (AV) grafts. Clotted AV grafts are treated with endovascular or surgical thrombectomy. The outcomes of thrombectomies performed within 2 months of graft creation were assessed.

Methods:

We retrospectively analyzed the outcomes of all AV grafts placed at our medical center over a 5 year period that required thrombectomy within 60 days of creation. Technical success was defined as the immediate restoration of graft patency. Primary patency was calculated from declot to first intervention and cumulative patency from declot to permanent graft failure. We also compared the outcomes for grafts undergoing thrombectomy at ≤30 days vs 31-60 days.

Results:

Of 709 AV grafts placed, 98 grafts (14%) clotted within 60 days of creation and underwent percutaneous or surgical thrombectomy, including 63 (9%) within 30 days of graft creation, and 35 (5%) at 31-60 days after creation. Grafts clotting within 30 days of creation typically underwent surgical thrombectomy and those clotting after 30 days usually underwent percutaneous thrombectomy. The immediate technical success was 82%. The median primary graft patency was 14 days and median cumulative graft patency was 38

days, and was similar at ≤ 30 vs 31-60 days. Thrombosis within 30 days was likely due to poor arterial inflow and thrombosis at 31-60 days was commonly due to stenosis at the venous anastomosis.

Outcomes of early declot of AV grafts

| | <=30 days | 31-60 days | p value |
|---|---|---|---|
| No. of patients | 63 | 35 | |
| Anatomic abnormalities | Laceration of graft wall-1, Redundant graft-2, Kinked graft-1, Adherent clot-1 | Stenosis at venous anastomois-23 | |
| Declot- Surgery/ Interventional Radiology | 60/ 3 | 10/ 25 | <0.001 |
| Primary patency | | | |
| Median, days | 11 | 23 | 0.46 |
| 90 days | 19% | 31% | |
| 180 days | 17% | 22% | |
| Cumulative patency | | | |
| Median, days | 27 | 163 | 0.29 |
| 90 days | 34% | 51% | |
| 180 days | 32% | 45% | |

Conclusion:

Thrombectomy within 2 months of graft creation is associated with poor primary and cumulative patency, and may not be worthwhile.

Disclosure of Financial Relationships: nothing to disclose

**PUB438**

**Superior Vena Cava Syndrome Secondary to Permcath Catheter for Hemodialysis (HD)** Ma del Carmen Prados Soler, Mª Dolores Del Pino Pino, Remedios Garofano, Clara Moriana, Manuel Ángel Rodríguez. *S. Nephrology, C. H. Torrecárdenas, Almería, Andalucía, Spain.*

**Introduction:** Thrombosis or stenosis of the superior vena cava (SVC) and/or brachiocephalic trunk (BCT) in patients with central catheters for HD is frequently, secundary to alterations of hemostasis and barotrauma, whether or not accompanied by endovascular infection. The symptoms are shoulder girdle edema, dyspnea and jugular vein ingurgitation. Angioplasty, endoluminal treatment of the stenosis requires implantation of a stent to prevent restenosis

**Case Report:** 72-year-old woman with chronic kidney disease, on HD since 05, via a Permcath catheter.On 07 she required a change of the permanent catheter due to failure of the previous one. In November 09 she presented catheter dysfunction due to lack of flow, for which she was given urokinase. Physical examination of note showed: facial and cervical edema, rest of the examination are normal and vital constants too. Laboratory studies are normal. A superior cavography showed: stenosis of the SVC in the upper third, no filling of the right BCT due to thrombosis, with abundant collateral circulation via the intercostal braches to the azygos vein, which in turn drained into the SVC, and partial thrombosis of the left BCT. Given that stent implantation requires withdrawal of the permanent catheter and that in this case there was great difficulty obtaining another vascular access, it was initially decided to systemic anticoagulation for 6 months. Later, depending on the findings of a radiological control and the clinical situation the possibility of endoluminal treatment will be assessed

**Conclusions:**

- Controversy exists in the superior vena cava syndrome concerning the treatment of choice. Conservative treatment with oral anticoagulation may be a good alternative in some cases, sometimes achieving clinical and radiological resolution of the syndrome, with endoluminal treatment being reserved as a second choice

- Given the increased frequency of the use of central venous catheters for HD for long periods of time and that venous stenosis or occlusion is usually asymptomatic, early diagnosis is important to permit its resolution

Disclosure of Financial Relationships: nothing to disclose

**PUB439**

**Proactive Monitoring of Hemodiaysis Vascular Accesses Reduces Declot Procedures at an Out-Patient Vascular Center** Jack E. Rubin.[1] [1]*Los Angeles Vascular Center, Inglewood, CA;* [2]*J. Joseph Hewett, Los Angeles Vascular Center, Inglewood, CA.*

Proactive monitoring by nephrologists in their dialysis centers as well as routine vascular access flow rate determination at Los Angeles Vascular Center (LAVC) was responsible for reducing the incidence of declot procedures in vascular accesses of patients sent there. In 2007, 36% of all vascular salvage procedures performed there were declots, this percentage fell to 31% in 2008 and settled at 24% in 2009. This was a reduction of 31% in the number of declots done from 2007 to 2009. In conjunction with this fall in declot procedures, there was also an increase in the percentage of angioplasties done at LAVC. In 2007, 30% of vascular salvage procedures done there were angioplasties, it rose to 37% in 2008 and it reached 46% in 2009. This was a increase of 51% in the number of angioplasties done in 2009 compared to 2007.

PERCENTAGE OF DECLOTS AT LAVC

| | | |
|---|---|---|
| 36 | 31 | 24 |
| 2007 | 2008 | 2009 |

This reduction in the number of declot procedures was due to proactive montioring of patients' vascular accesses by their attending nephrologists and the nursing staff at the patients' dialysis centers as well clinic visits at LAVC to check blood flow rates of their accesses. Patients were referred to LAVC for consistent reductions of KT/V measurements, evidence of access blood flow rates < 600 ml/min, increased venous pressure found while patients were on dialysis, and post-dialysis bleeding, all of which are compatible with a stenosis forming their vascular access. The restult of this proactive monitoring was both lower costs to insurers both due to the fact that angioplasties are not as costly to perform and there were fewer in- hospital admissions for patients due to their not being able to be declotted in time, in a hospital setting, to return to their dialysis centers for their scheduled dialysis sessions.

Disclosure of Financial Relationships: Ownership: I am a shareholder in Los Angeles Vascular Center.

**PUB440**

**Reasons Why and Ways How To Convert an Access Center to a Vascular Center** Jack E. Rubin,[1] Samuel Awuah,[2] J. Joseph Hewett.[1] [1]*Los Angeles Vascular Center, Inglewood, CA;* [2]*RMS Lifeline, Vernon Hills, IL.*

Los Angeles Vascular Access Center (LAVAC) opened in June of 2007 to perform vascular salvage procedures on hemodialysis patients. Within 3 months of its opening it performed it first lower extremity angiogram (LEA). This was done as the number of people with peripheral arterial disease (PAD), between 10-20 million, was much larger than the number of patients with ESRD, 425,000. LEA's are in table 1.

Number of Lower Extremity Angiograms Done at LAVC

| | | |
|---|---|---|
| 10 | 73 | 163 |
| 2007 | 2008 | 2009 |

LAVAC did 13 renal angiograms in 2008 and in 2009 it did 37 and changed its name to Los Angeles Vascular Center (LAVC) to better reflect its performing procedures other than vascular salvage. LAVC started doing venous ablation (VA) procedures and in 2009 and performed 14. In 2009 pain management done by implantation of spinal column stimulators as well as epidural injections of steroids was started.

LAVC was able to diversify due to its having a practice model different from most other vascular access centers. It always had an IR, rather than an IN performing its procedures because an IR is better equipped to perform procedures on the larger sized arteries of the legs and kidneys. Additionally, IN's are not trained in vein ablation techniques. LAVC was fortunate to have an IR that had training in pain management and has successfully placed spinal column stimulators (SCS) for patients with chronic pain in the past.

In summary, by employing an IR, a vascular access center can become a vascular center which can perform an increased number of cases apart from merely doing vascular salvage. This allows a wider range of patients which can be treated there to correct both PAD and RAS as well as doing VA procedures. In addition to the larger number of patients aso available for treatment, the number of referring medical personnel can be increased. Instead of having referrals only from nephrologists, the center's referrals can then come from podiatrists, wound care centers, internists as well as gynecologists for VA's. An additional service line, pain management, can also be done, as it is in LAVC, by an IR trained in pain management.

Disclosure of Financial Relationships: Ownership: I am a shareholder in Los Angeles Vascular Center.

**PUB441**

**Clinical Outcomes of Arteriovenous Grafts (AVG) Placed Prior to Hemodialysis (HD) Initiation** Roman A. Shingarev, Ivan D. Maya, Michael Allon. *Medicine, University of Alabama at Birmingham, Birmingham, AL.*

Pre-HD patients usually receive an arteriovenous fistula (AVF) as their first vascular access, with AVG placement reserved for HD patients who have had a failed AVF. Little is known about the outcomes of AVG placed in pre-HD patients. We compared the outcomes of AVG placed pre-HD with those placed post-HD at a large dialysis center.

We retrospectively queried a prospective, computerized vascular access database, to identify 417 patients with a new AVG placed during a 5-year period, including 69 placed pre-HD and 348 post-HD. We compared baseline demographic and clinical characteristics, cumulative AVG survival (from surgical placement to permanent failure), and primary AVG patency (from surgical placement to first intervention) between the two AVG groups.

The two patient groups were similar in baseline demographics (age, sex, race) and co-morbidities (diabetes, hypertension, coronary artery disease, peripheral vascular disease, and cerebrovascular disease). Of the 65 patients with pre-HD AVG who eventually started HD, 39 (or 60%) were able to use the AVG at a median of 60 days after AVG surgery. Cumulative AVG survival was similar in patients with pre-HD and post-HD AVG (median, 583 vs 657 days; HR 1.21 (95% CI, 0.86-1.75), p = 0.25; 1-yr patency 57 vs 60%; 2-yr patency 39 vs 48%; 3-yr patency 31 vs 40%). Primary graft patency was also similar in patients with pre-HD and post-HD AVG (median, 155 vs 99 days; HR 0.84 (95% CI, 0.65-1.11), p = 0.23; 1-yr patency 26 vs 20%; 2-yr patency, 11 vs 11%).

AVG placed pre-HD have similar clinical outcomes to those placed pre-HD, with comparable cumulative survival and primary patency. Pre-HD AVG placement is a viable option in selected CKD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB442**

**Can In-Center Intermittent Peritoneal Dialysis (IPD) Provide the Necessary Adequacy for New Dialysis Patients?** Alp Akonur,[1] Steven Guest,[1] Arshia Ghaffari,[2] James A. Sloand,[1] J. Ken Leypoldt.[1] *[1]Renal Division, Baxter Healthcare Corporation, McGaw Park, IL; [2]Department of Medicine, University of Southern California, Los Angeles, CA.*

Although national and international expert nephrology groups have published recommendations to initiate dialysis when the glomerular filtration rate (GFR) falls within 6-10.5 ml/min/1.73m², no clinical trials have compared outcomes in patients starting dialysis with full versus incremental dialysis dose (Pollock et al, 2007). We used the 3-pore model to evaluate the combined effectiveness of increasing doses of three times per week in-center intermittent PD and residual GFR in achieving the recommended urea clearances.

Two dialysis modalities were examined: 6 dwells / 12L total volume (low-dose IPD) and 50% tidal / 24L total volume (high-dose IPD). 8-hour dialysis duration and 1.5% dextrose solution were assumed with 2L fill volume except in tidal mode. PD Adequest 2.0 (Baxter Healthcare, Deerfield, IL, USA) and typical patient kinetic parameters were used to model urea clearances. The minimum residual GFR required to achieve a total weekly urea Kt/V of 1.7 was calculated.

In the absence of any dialysis, the minimum residual GFR necessary to achieve a weekly urea Kt/V of 1.7 was calculated to be approximately 10 ml/min/1.73m². We found the 12L, low-dose IPD modality to be adequate for patients with GFR greater than 6.8 ml/min/1.73m². The 24L, high-dose tidal IPD modality was adequate for patients with GFR greater than 5.5 ml/min/1.73m². These results suggest that in-center intermittent PD may be a viable option for patients with residual GFR greater than approximately 5 ml/min/1.73m² and could serve as a bridge when new patient training is delayed or home support is temporarily lost. Compared with full-dose PD (i.e. 24-hour, 7 days per week), intermittent PD may help eligible patients achieve the recommended clearance targets while minimizing excessive glucose exposure.

| | No Dialysis | Low-Dose IPD | High-Dose IPD |
|---|---|---|---|
| Calculated IPD Kt/V | 0 | 0.5 | 0.7 |
| Residual Kidney Kt/V | 1.7 | 1.2 | 1.0 |
| Residual GFR, ml/min/1.73m² | 9.7 | 6.8 | 5.5 |

Disclosure of Financial Relationships: Employer: Baxter Healthcare Corporation; Ownership: Baxter Healthcare Corporation.

**PUB443**

**Hiccups as Sole Symptom of Peritonitis in a Peritoneal Dialysis Patient: Case History and Discussion** Sholey Argani. *Renal Section, Veteran's Affairs Medical Center, Washington, DC.*

A 60 year old diabetic patient whose history included mild retinopathy, hepatitis C virus infection, and hypertension, commenced peritoneal dialysis (PD) for his chronic kidney disease stage 5. Initial PD prescription provided 4 exchanges of 2.5 % dextrose on cycler at night, with a last fill of icodextran. Approximately five months after initiating peritoneal dialysis, the patient presented with four days of persistent hiccups. Physical examination at this time revealed an afebrile well nourished man with blood pressure 105/62 mm Hg, heart rate 72 beats/min. His abdomen was soft with no erythema at the PD catheter site.

On blood chemistry, glucose measured 76 mg/dl, BUN 40 mg/dl, creatinine 10.6 mg/dl, sodium 132 mmol/L, Potassium 2.8 mmol/L, Chloride 94 mmol/L, and bicarbonate 26 mmol/L.

Peritoneal dialysate appeared faint yellow, with 561 non red blood cells/cub mm. On differential, there were 70% segmented neutrophils, 14% lymphocytes, 15 % monocytes, and 1% eosinophils. The culture of peritoneal fluid was unrevealing, but intravenous vancomycin was administered. Within 24 hours the hiccups had completely resolved. This patient received intraperitoneal vancomicin for two weeks, as well as prophylactic oral fluconazole.

The myriad causes of hiccups (singultus) include irritation of the vagus or phrenic nerves, central nervous system pathology, and metabolic derangements such as alcohol intoxication, hyponatremia and uremia. [1,2] One case report [3]noted hiccups due to acidic lactate solution in a PD patient; these resolved with the change to a ph-neutral dialysate. However, in our review of the literature this is the only case of hiccups as the only presenting symptom of culture negative PD peritonitis.

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Disclosure of Financial Relationships: nothing to disclose

**PUB444**

**Peritoneal Dialysis Outcomes during First Year Treatment of Incident Diabetic Patients** Patricia Quadros Branco,[1] Anabela S. Rodrigues,[2] Maria Augusta Cabrita Silva Gaspar,[1] Ricardo Vizinho,[1] Maria João Carvalho,[2] Manuel A. Amoedo,[4] Rui Castro,[5] Ana Paula Bernardo,[6] Jose Diogo Barata,[1] António Cabrita.[2] *[1]Nephrology Department, Santa Cruz Hospital, Portugal; [2]Santo Antonio Hospital; [3]Sao Joao Hospital; [4]Espirito Santo Hospital; [5]Tras os Montes e Alto Douro Hospital; [6]Amato Lusitano Hospital.*

BACKGROUND: Whereas diabetes mellitus (DM) is not a consistent risk factor for peritoneal dialysis technical survival, other co-morbid diseases such as cardiovascular disease (CVD) are common in DM patients (pts) and may predict technical survival. AIM: Compare early technical survival of incident PD DM pts with non-DM pts. METHODS: Prospective multicentre, cohort study of every incident PD pts in a national public health care system, in a reference area of 10 million people. We collected baseline data, hospital admissions, peritonitis, transplants, CV events and deaths, PD prescription, dialysis efficacy, anaemia. Pts were followed for the first 12 months. Technique failure was defined as switching dialysis modality; we censored for death. RESULTS: 238 pts, 98 APD and 140 CAPD, were included. 24.8% DM. Age 53±15 years, 56.7% male; 12 pts died; 13 switched treatment dialysis modality (6 infection, 7 underdialysis). DM pts were older (57±12 vs 52±16 years, p =0.013) and had higher prevalence of CVD (55% vs 21% p<0.001). There were no differences in dialysis efficacy targets, peritoneal transport, anaemia, serum albumin, peritonitis rate. In multivariate analysis DM was positively (HR=10.1; p= 0.02) and daily fluid removal negatively (HR =0.9; p= 0.01) associated with mortality. After adjustment for age, non-optional PD, DM, daily fluid removal, icodextrin utilization, baseline CVD was associated with short-term technique failure (HR = 5.2; p=0.02 ). CONCLUSION: In this model DM was not predictor of early dialysis modality switch. Fluid removal optimization may influence the prognosis of these pts.

Disclosure of Financial Relationships: nothing to disclose

**PUB445**

**Haemodialysis (HD) Patients Are Still Capable of Choosing Peritoneal Dialysis (PD)** Jean-Louis D. Christophe, Jean-Philippe Lengelé, Florence Hubert, Marylène Tarin. *Service de Néphrologie, GHdC - Hôpital St-Joseph, Gilly, Belgium.*

Once end-stage renal disease (ESRD) patients are in HD, they rarely choose to be transferred to PD. Those transfers mainly happen because of HD technique failure. We wanted to know how often and how our PD patients come from HD.

We retrospectively studied the 50 patients who started PD in our center between February 1996 and May 2010. Only two patients who started PD after long-term failure of renal transplantation following HD were excluded.

Of the 48 remaining patients, 15 (31 %) had been transferred to PD in spite of HD being their first mode of treatment. Of these, 5 were referred very late in the course of their renal disease or progressed rapidly and had to be taken urgently in HD before being informed on alternative techniques of renal replacement therapy (RRT). They later chose PD and were transferred after a mean time in HD of 122 days (range 44-322). Six other patients had received the information on the different modalities of RRT and had chosen PD before reaching ESRD, but had a rapidly progressive disease or an intercurrent event that urged us to start with HD. PD was initiated a mean of 69 days (range 11-247) later, one patient feeling so well in HD that he had an arteriovenous fistula before eventually asking to be transferred to PD.

Finally, four patients had deliberately chosen HD as their first mode of treatment but changed their mind later on (mean time in HD 434 days, range 216-861) : two did so after a local re-information meeting including current and former (transplanted) PD patients; one was psychologically intolerant to HD; another one was bored from travelling to the HD center. It is of note than none of the 15 patients was transferred to PD because HD was becoming practically impossible e.g. due to vascular access failure.

**Conclusions**: patients who are in HD may very well be transferred to PD on a voluntary basis, even after being treated for years in HD. Information on PD should be regularly given to HD patients, by doctors and nurses but patient-to-patient information should also be stimulated. The medical and paramedical staff must pay attention to HD patients' needs that might change in time and be met by PD.

Disclosure of Financial Relationships: nothing to disclose

**PUB446**

**Peritonitis Occurring Less Than 1 Month after Training Explained by Maladaptive Connector Set for Peritoneal Dialysis (PD)** George N. Coritsidis,[1] Dharmeshkumar V. Sutariya,[1] Barbara Beach.[2] *[1]Division of Nephrology, Mt. Sinai School of Medicine at Elmhurst Hospital Center, Elmhurst, NY; [2]Peritoneal Dialysis Unit, Elmhurst Hospital Center, Elmhurst, NY.*

Introduction

Early peritonitis in PD can be due to surgical complications. Any peritonitis is associated with diminished technique survival. We recently experienced an unusually high infection rate soon after commencement of PD in 4 patients. After reviewing our PD training process and having ruled out surgical issues, we examined the possibility of a malfunction within the system due to complaints of leakage.

Methods

After a review of charts, history and physical examinations of affected patients, the common finding was the evidence of leakage in 3 patients despite normal exit sites in all. The company of the plastic connector sets was contacted.

Results

All patients had catheter flushes by day 7. Infection was first identified by symptoms and abnormal cell count. On average, peritonitis occurred within 1 month of training and 2 months of catheter placement. Two patients had relapses. 5 of 6 episodes grew Staphylococcus Epidermidis.

Patient Characteristics

| Patient | Training Date | Infection date | Cell Count (per cu.mm.) | 2nd Episode of Peritonitis | Organism |
|---|---|---|---|---|---|
| 1 | Day 15 | Day 34 | 12240 | Day 79 | Staph. epidermidis (both episodes) |
| 2 | Day 20 | Day 17 | 950 | Day 72 | Staph. epidermidis (both episodes) |
| 3 | Day 12 | Day 21 | 330 | none | culture negative |
| 4 | Day 34 | Day 110 | 1600 | none | Staph. epidermidis |
| Average | Day 20.45 | Day 45.4 | 3780 | | |

Date of surgery is considered day 0

All patients were initially treated with vancomycin, ceftazidime and antifungal prophylaxis, with prompt resolution of the peritonitis. On contact with the manufacturer, it was revealed that similar episodes were reported but were resolved after structural changes. A repeat company investigation indicated a new error in the current connector size, and titanium connectors were dispatched. There were no new infections after the change to titanium connectors.

Conclusions:

In diagnosing early peritonitis soon after PD catheter placement, the possibility of a malfunction anywhere along the system should be entertained and examined.

Disclosure of Financial Relationships: Consultancy: Consultant for Amgen Inc and Cerner.

**PUB447**

**Peritonitis Prevention in Peritoneal Dialysis Patients with Domestic Animals**

Sunil George, Cheryl Laveglia, Nand K. Wadhwa. *Department of Nephrology, Stony Brook University Hospital, Stony Brook, NY.*

Evidence based literature suggests that a companion animal/pet can help a person better cope with stressful life events, prevent loneliness, decrease depression, improve activities of daily living, and increase social interactions. Many peritoneal dialysis (PD) patients keep pets as social support. However the close interaction with these pets can increase the risk for peritonitis particularly with Pasteurella multocida. In our dialysis unit we encountered a patient with recurrent peritonitis with this organism. This prompted us to evaluate the prevalence of pets in peritoneal dialysis patients and to incorporate a training session about pets when the patient initiates PD. Questionnaires related to the interactions of the patients with the domestic animals during treatments were distributed to all PD patients. The patient population included 17 males and 9 females with a mean age of 47 years. The patients were followed over 520 patient-months. The survey of our PD population showed that 23 % (6/26) have pets at home. Two patients including the patient with recurrent peritonitis reported that pets had frequent contact with the dialysis bags and tubing or direct contact with the patient during set up. Three patients allowed the pets to enter the room occasionally during treatment. One patient never allowed his pets to interact during connections, exit site care or while he was on automated PD. We instituted a training session for patients who kept domestic animals as pets. The training included interventions including patient education, counseling on hygiene and placement of barriers limiting the pet's access to the dialysis equipment. Appropriate interventions taken by the patient with recurrent peritonitis have kept him free of peritonitis for two years. Subsequently we have no episode of peritonitis related to pets in our unit. Thus we recommend a periodic questionnaire and an educational session for all PD patients who have or intend to have companion animals as social support.

Disclosure of Financial Relationships: nothing to disclose

**PUB448**

**The Effect of Twenty Four Hour Peritoneal Rest in Peritoneal Dialysis Patient**

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**Background:** Peritoneal dialysis (PD) is superior to hemodialysis (HD) in protection of residual renal function. The present study was conducted to evaluate whether peritoneal resting could improve continuous ambulatory peritoneal dialysis (CAPD) patient's peritoneal function. We assumed that patients who have some residual renal function may be able to choose whether normal dose daily PD or high dose PD and one day peritoneal rest. We investigated that whether twenty four hour peritoneal rest might lead to improve peritoneal methothelial cell and peritoneal membrane function. **Method:** As a single-center prospective observational study, we analyzed 5 PD patients. They kept residual renal function, and their peritoneal transport characteristics categories (checked using a peritoneal equilibration test: PET) were High average or High, from initiating dialysis therapy for more than six months. We adjusted the PD prescription to keep peritoneal rest and the minimal dose of total (peritoneal and kidney) Kt/Vurea of at least 1.7 per week. We reperformed a PET and examined a mean surface area of peritoneal mesothelial cell. **Results:** The mean PD period was 541±240 days. The mean enforcement period of peritoneal rest was 143±77 days. The mean surface area of peritoneal mesothelial cell was significantly improved (before peritoneal rest: 343.8±9.2μm$^2$ vsersus after peritoneal rest :300±11.2μm$^2$, P=0.019 ). Peritoneal membrane function did not change significantly in this study (the dialysate/plasma ratio of creatinine (D/P); P=0.19, the dialysate 240 min/ initial dialysate ratio of glucose (D/Do); P=0.18). **Conclusion:** In this study, the peritoneal rest improved a mean surface area of peritoneal mesothelial cell. This result clearly indicated that peritoneal rest improved morphological changes. Twenty four hour peritoneal rests may improve peritoneal dysfunctions in patients with residual renal function.

Disclosure of Financial Relationships: nothing to disclose

**PUB449**

**A Comparison of Short Term Outcomes after Cardiothoracic Surgery (CTS) in End Stage Renal Disease (ESRD) Patients by Dialysis Modality**

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The number of ESRD patients undergoing CTS has increased over recent years, but concerns exist regarding the outcomes of patients treated with peritoneal dialysis (PD) during the immediate post surgical period. We sought to examine short term outcomes in both PD and hemodialysis (HD) patients who underwent CTS in this retrospective cohort study. We reviewed records for all ESRD patients who received CTS at our center between January 1, 1994 and December 31, 2008, including those who underwent coronary artery bypass grafting (CABG), valve replacement, and combined CABG/valve replacement. All PD patients who received CTS during the study period were included in our analysis. Two controls matched for age, sex, diabetes status and Charleston Comorbidity score (CCS) were obtained for each PD patient from the pool of HD patients who received CTS. The student's t-test, Wilcoxon rank sum, Fisher's exact test and Yates chi square corrected for continuity were used wherever appropriate.

| | PD (n=36) | HD (n=72) | p value |
|---|---|---|---|
| Mean age (years) | 58.8±9.4 | 59.3±9.1 | 0.78 |
| Males (%) | 24 (67) | 44 (61) | 0.73 |
| Surgery type (%) | | | 0.34 |
| CABG | 28 (78) | 48 (67) | |
| valve replacement | 5 (14) | 19 (26) | |
| CABG + valve replacement | 3 (8) | 5 (7) | |
| Diabetes Mellitus (%) | 26 (72) | 54 (75) | 0.92 |
| Mean CCS | 6.6±2.0 | 6.8±2.2 | 0.66 |
| Elective Surgery (%) | 13 (37) | 32 (45) | 0.57 |
| Median CSU LOS in days (IQR) | 2 (1.75-5) | 4(2-6.25) | 0.02 |
| Median hospital LOS in days (IQR) | 9.5 (7-13) | 11 (7-17) | 0.19 |
| Median intubation time in hours (IQR) | 24 (24-24) | 24 (24-48) | 0.07 |
| Post operative infection (%) | 2 (6) | 16 (22) | 0.03 |
| Death during hospitalization (%) | 3 (8) | 8 (11) | 0.33 |

CSU = cardiac surgical unit; LOS = length of stay; IQR = interquartile range; CRRT = continuous renal replacement therapy

Only 2 (6%) of our PD patients required conversion to HD during their hospital stay. The incidence of post-operative infection and median CSU LOS were lower for PD patients. Our findings suggest that PD patients undergoing CTS generally do not require conversion to HD and do not fare worse than similar HD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB450**

**Fluid Overload – A Frequently Encountered, Easily Ignored and Difficultly Controlled Problem in Peritoneal Dialysis Patients Associated with Cardiac Hypertrophy and High Pro-BNP and Adiponectin Levels**

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**INTRODUCTION AND AIMS:** Fluid overload in dialysis patients may induce heart failure but fluid depletion may decrease residual renal function. This study is to evaluate fluid status in hemodialysis (HD) and peritoneal dialysis (PD) patients by chest X-ray, cardiac echo and body composition spectroscopy and the effect of fluid control in PD patients by dietary management and monitored by BCM.

**METHODS:** 96 regular HD patients and 98 PD patients were recruited. Body compositions were checked by BCM. Chest X-ray, Cardiac echo, blood biochemistry were checked concomitantly. For PD patients, they were further randomized into 2 groups (control and intervention). Body compositions were measured every month in intervention group and at enrolling and end point of study in control group. Chest X-ray, Cardiac echo, nutritional markers, pro-brain natriuretic peptide (BNP) and cytokines were checked at the point of enrollment and the end point of study. The statistic methods included unpaired t test, Chi-square test and person correlation.

**RESULTS:** There was no significance in cardio-thoracic ratio and percentage of cardiomegaly between PD and HD patients. However, PD patients had higher percentage of LVH . Besides, PD patients had significantly higher blood pressure than HD patients. The degree of overhydration was higher in PD patients . The degree of overhydration was associated with LV mass in PD patients. Despite of intensive diet education and monitored by BCM, fluid overload, evidenced by high Pro-BNP and adiponectin levels, is still a common problem in PD patients.

**CONCLUSIONS:** Fluid overload in PD patients is presented as higher BP, more usage of anti-HT agents, and higher overhydration measured by BCM. In comparison to chest-x-ray, BCM is a more sensitive and easy tool for control of fluid overload in PD patients. However, by dietary management it is still not satisfactory to achieve the goal despite of monitored by BCM. To decrease the complications in PD patients, we should pay more attention to the fluid status.

Disclosure of Financial Relationships: nothing to disclose

## PUB451

**Ten-Year Experience with Fungal Peritonitis in Peritoneal Dialysis Patients: Antifungal Susceptibility Patterns in a North-American Center** Jasmin Levallois,[1] Annie-Claire Nadeau-Fredette,[1] Denis Ouimet,[1] Michel Vallee.[1] *[1]Service de Nephrologie, Hopital Maisonneuve-Rosemont, Montreal, QC, Canada; [2]Service de Microbiologie, Hopital Maisonneuve-Rosemont, Montreal, QC, Canada.*

Objective : To describe the clinical and microbiologic features associated with fungal peritonitis in peritoneal dialysis (PD) patients at Hopital Maisonneuve-Rosemont (HMR), from August 1996 to July 2006.

Methods : Cases were retrieved from the microbiology laboratory culture registry. Antifungal susceptibility was determined by the CLSI M27A2 method.

Results : Among 288 PD patients (total follow-up of 7258 patient-months), 9 patients were found with fungal peritonitis. Candida spp. were identified in all of them, with a majority of non-albicans Candida species. Resistance to fluconazole, itraconazole or voriconazole was as frequent as potential resistance to amphotericin B. No isolate was resistant to caspofungin and one was resistant to micafungin. Prior bacterial peritonitis was frequent (66%). All patients had their PD catheter removed and all of them survived.

**Table 1: Antifugal susceptibility of 9 cases of fungal peritonitis (MICs)**

| Sex/Age | Species | Ampho B | Fluco | Itraco | Vorico | Caspofung | Micafung |
|---|---|---|---|---|---|---|---|
| M/75 | *C. albicans* | 0.5 | 1 | 0.12 | 0.12 | 0.12 | 0.25 |
| M/51 | *C. glabrata* | **2 R** | **16 I** | **32 R** | **32 R** | 0.5 | 0.06 |
| M/67 | *C. krusei* | 1 | **R*** | 0.5 | 0.5 | 1 | 2 |
| M/78 | *C. parapsilosis* | 0.5 | 1 | 0.12 | 0.016 | 1 | **16 R** |
| M/69 | *C. parapsilosis* | 0.125 | 2 | 0.06 | 0.008 | 0.25 | 2 |
| F/62 | *C. parapsilosis* | **2 R** | 0.25 | 0.06 | 0.016 | 0.5 | 0.06 |
| F/56 | *C. tropicalis* | 0.25 | 0.5 | 0.12 | 0.03 | 0.03 | 0.25 |
| M/35 | *C. tropicalis* | 0.25 | 0.5 | 0.25 | 0.06 | 0.12 | 0.5 |
| M/61 | *C. tropicalis* | **2 R** | 4 | **1 R** | 0.5 | 1 | 0.25 |

**R** = resistant **I** = intermediate

**R*** = *C.krusei* are assumed to be intrinsically resistant to fluconazole.

Conclusions : In our institution, fungal peritonitis in PD patients is rare. All cases were caused by Candida species. Variable susceptibility patterns were observed, which may influence the initial empirical antifungal therapy and underscore the importance of individual speciation and susceptibility testing of invasive Candida isolates

Disclosure of Financial Relationships: nothing to disclose

## PUB452

**Serum Parathyroid Hormone Level (PTH) Varies with Vitamin D Dosing Schedule in Peritoneal Dialysis (PD) Patients** Susie Q. Lew. *Department of Medicine, George Washington University, Washington, DC.*

Oral vitamin D therapy is used in the management of secondary hyperparathyroidism in ESRD PD patients. Time dependent changes in PTH level following oral vitamin D ingestion is not known, but is important information used to adjust vitamin D dosage.

The aim is to determine the temporal change in PTH level following oral vitamin D administration in ESRD PD patients.

Blood samples for PTH were obtained either 1 day (A), 2 days (B) or 3 days (C) after oral vitamin D ingestion for 2 consecutive months, respectively. Results are given as mean ± SD. Significant if $p \leq 0.05$ #.

Results

| (n=22) | A | B | C | A vs B | B vs C | A vs C |
|---|---|---|---|---|---|---|
| PTH pg/ml | 382±264 | 447±312 | 451±319 | p<0.04 # | p<0.93 | p<0.18 |
| calcium mg/dl | 8.8±0.5 | 8.9±0.6 | 8.7±0.7 | p<0.10 | p<0.07 | p<0.70 |
| phosphorus mg/dl | 5.5±1.4 | 5.3±1.6 | 5.3±1.3 | p<0.40 | p<0.98 | p<0.39 |

PD patients have a lower PTH level when obtained the day after oral vitamin D ingestion. PTH level trends upward with longer intervals between ingestion and blood draw.

Larger prospective studies are required to identify the optimal time to sample blood for PTH level in PD patients.

Disclosure of Financial Relationships: Consultancy: Amgen, GenzymeResearch Funding: Mitsubishi Tanabe Pharma Corporation

Cardiokine

Affymax; Honoraria: Amgen, Genzyme, OrthoBio, Fresenius.

## PUB453

**Estimated GFR as a Measure of Dialytic Clearance in Patients on Peritoneal Dialysis** Umair M. Malik,[1] Khurram Mumtaz,[1] Manish Jain,[1] Saurabh A. Pande,[1] Kanwal Raghav,[2] Imara Dissanayake,[1] Eric J. Bloom,[1] Rasib Raja.[1] *[1]Nephrology, Albert Einstein Medical Center, Philadelphia, PA; [2]Internal Medicine, Albert Einstein Medical Center, Philadelphia, PA.*

Background: In PD patients weekly Measured Creatinine Clearance (MCC) is an estimate of dialysis adequacy and correlates with long term patient well being and mortality. Currently, besides doing a 24 hour dialysate and urine collection to calculate MCC, there is no other practical way to estimate small molecule clearance provided by dialysis from readily available blood tests.

Objective: The goal of this study was to determine whether Modification of Diet in Renal Disease (MDRD) and Cockroft-Gault estimated GFR correlates with peritoneal dialysis delivered creatinine clearance.

Methods: Measured Creatinine Clearnce (MCC) for 34 patients was calaculted by collecting 24-hour peritoneal dialysate and residual urine and checking serum creatinine the following day. In our study, values for Measured Creatinine Clearance (MCC) are presented in ml/min/1.73 m2 and were obtained by dividing the weekly creatinine clearance by 10,080 (the number of minutes in a week) and normalizing for body surface area. SPSS software was used to compare mean Measured creatinine clearance (MCC) and estimated MDRD and Cockfoft-Gault GFR.

Results: For the group as a whole, the Modiication of Diet in Renal disease (MDRD) estimated GFR predicted the Measured creatinine Clearance (MCC) from the serum creatinine reasonably well. The mean Measured Creatinine Clearance (MCC) for the study group as a whole was 6.8 +/- 3.3 ml/min/1.73 m2, and the value predicted by the Modification of Diet in Renal Disease (MDRD) equation was 5.6 +/- 2.5 ml/min/1.73 m2. The Cockcroft-Gault equation predicted a mean value of 8.5+/-3.3 ml/min/1.73 m2.

Conclusions: Modification of Diet in Renal disease (MDRD) and Cockcroft -Gault estimated GFRs approxiamate closely with Measured Creatinine Clearance (MCC) and can therefore be used as surrogate measures of small molecule clearance in patients on peritoneal dialysis. The conclusions from this study need to be prospectively confirmed in a larger cohort of peritoneal dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

## PUB454

**Clinical Impact of Complementary Therapy of Peritoneal Dialysis and Hemodialysis in Japan; a Retrospective Multicenter Study** Yukio Maruyama, Keitaro Yokoyama, Masaaki Nakayama, Chieko Higuchi, Tsutomu Sanaka, Yoshihide Tanaka, Ken Sakai, Sonoo Mizuiri, Yasushi Otsuka, Satoru Kuriyama, Teruhiko Maeba, Hideaki Iwasawa, Toshiyuki Nakao, Tatsuo Hosoya. *EARTH (Evaluation on the Adequacy of Renal Replacement Therapy) Study Group, Tokyo, Japan.*

Background and Aims: Although complementary therapy of peritoneal dialysis (PD) and hemodialysis (HD) is widely performed to correct underdialysis and/or overhydration in Japan, its clinical efficacy is still unknown.

Methods: In this retrospective multicenter study, we recruited 106 patients (57±11 years and 77 males) changed the therapy from PD alone to complementary therapy of PD and HD.

Results: The median duration of PD alone and subsequent complementary therapy were 3.0 and 1.3 years, respectively. During the first three months after changing the therapy, body weight, serum creatinine and the dialysate to plasma creatinine ratio decreased significantly. On the other hand, hemoglobin level elevated.

Conclusions: Complementary therapy of PD and HD is an effective way to control fluid status and correct inadequate solute removal. Additionally peritoneal transport rate was thought to be improved.

Time course changes

| | Before the initiation of complementary therapy | Three months later | P value |
|---|---|---|---|
| BW (kg) | 65.1±14.8 | 63.7±13.8 | <0.01 |
| Systolic BP (mmHg) | 145±21 | 144±19 | 0.77 |
| Diastolic BP (mmHg) | 81±14 | 81±10 | 0.83 |
| Urine volume (mL/day) | 150 (0-2000) | 75 (0-1900) | <0.01 |
| Amount of the PD solution (mL/day) | 8300±1600 | 8200±1400 | 0.56 |
| Ultrafiltration rate (mL/day) | 1000 (-500-2350) | 975 (-1000-2150) | 0.51 |
| Hb (g/dL) | 8.7±1.6 | 10.3±1.3 | <0.01 |
| BUN (mg/dL) | 59.8±14.8 | 57.4±13.5 | 0.09 |
| Cr (mg/dL) | 13.0±3.4 | 12.4±3.0 | <0.01 |
| β2-m (pg/mL) | 34.6±6.9 | 34.0±6.9 | 0.27 |
| D/P cr | 0.68±0.12 | 0.60±0.12 | <0.01 |
| Total Kt/V (/week) | 1.8±0.4 | N.A. | |
| Total Weekly Ccr (L/week) | 49.9±13.1 | N.A. | |

Disclosure of Financial Relationships: nothing to disclose

**PUB455**

**Infected Titanium Adapter: A Common Cause of Relapsing Peritonitis** Gautam M. Phadke, Amandeep Gill, Diptesh Gupta, Kunal Chaudhary, Madhukar Misra. *Internal Medicine, Nephrology, University of Missouri, Columbia, MO.*

Relapsing peritonitis,is defined as an episode of peritonitis with the same genus/species that caused the preceding episode of peritonitis within 4 weeks after completing the course of antibiotics. We report a case-series of five patients with relapsing peritonitis due to infected titanium adapter.

Retrospective analysis of patients with relapsing peritonitis. Patients with pre-sternal Missouri Swan-neck Catheter were analyzed.

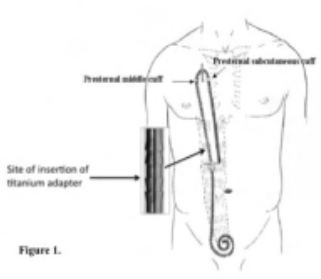


Figure 1.

All five patients had the first episode of peritonitis after 4 months of initiation of PD. In all five patients, first peritonitis presented with classical signs and symptoms of peritonitis viz. abdominal pain, cloudy effluent. Subsequent presentations were variable: asymptomatic with cloudy effluent, minimal abdominal discomfort. The commonest organism isolated was Coag. negative Staphylococcus (Epidermidis in 3/5 and Hominis is 1/5). 1 out of 5 patients had culture negative peritonitis. During relapses, none of the patients had clinical evidence of exit-site infections or subcutaneous tunnel infection. The average number of relapses prior to diagnosis of titanium adapter (TA) infection was 3. 1 patient had to be changed to HD due to catheter loss due to infection in the immediate proximity of the adapter. 4 patients successfully underwent change of TA and no subsequent episodes of peritonitis were reported for 6 months after the change of the adapter.

Infected TA is not an unusual cause of relapsing peritonitis. Non-diagnosis or delayed diagnosis of infected TA, is due to lack of awareness amongst providers who routinely take care of patients with pre-sternal catheter; and due to lack of diagnostic tools to detect this type of infection. Changing the TA, in most circumstances resolves the problem, thus preventing catheter replacement or catheter loss.Only 1 out of 5 patients was lost to HD.

Disclosure of Financial Relationships: nothing to disclose

**PUB456**

**Effect of New PD Fluid Connecting System on the Prevention of CAPD Peritonitis** Young Rim Song,[1] Youngsu Kim,[1] Sung Gyun Kim,[1] Soo Jin Kim,[1] Sejoong Kim,[2] Hyung Jik Kim.[1] *[1]Department of Internal Medicine, Hallym Univ. Sacred Heart Hospital, Anyang, Gyeonggi-do, Korea; [2]Department of Internal Medicine, Gachon University of Medicine and Science, Incheon, Korea.*

Purpose: Continuous ambulatory peritoneal dialysis (CAPD) peritonitis increases mortality and morbidity of patients with peritoneal dialysis and has harmful effects on the adequacy of peritoneal dialysis. Main cause of peritonitis is contact contamination from catheter manipulation in exchanging fluid. This study was performed if the new developed transfer set (staysafe$^r$ Catheter Extension Luer-Lock, Fresenius Medical Care, Germany) could decrease the incidence of CAPD peritonitis.

Method: We compared control group with traditional transfer set and treatment group with new transfer set. Control group was enrolled patients on CAPD from January to June 2007 and treatment group was patients from May to October 2008. Follow-up period was 12 months. Primary end point was the incidence of CAPD peritonitis. Secondary end points were the incidence of exit site infection and peritonitis free survival.

Result: There were no differences of basic characteristics between 36 patients in control group and 32 in treatment group. The incidence of peritonitis in treatment group was significantly less than that in control group (0.30 vs. 0.70 episodes/year, respectively, p=0.021). The peritonitis free survival of treatment was also significantly better than that of control group (p=0.017). There was no difference in exit site infection rate between two groups (0.11 vs. 0.22, respectively, p=0.283). The causative microorganisms of both groups had no differences and the differences of the amount and duration of use of antibiotics were insignificant.

Conclusion: This study suggests that the use of the new transfer set could decrease the incidence of CAPD peritonitis and increase peritonitis free survival time.

Disclosure of Financial Relationships: nothing to disclose

**PUB457**

**Barriers to International Peritoneal Dialysis Evidence-Based Medicine and Quality Practices: Implications for Guideline Development** Manish M. Sood,[1] Mauro Verrelli,[1] Gemini Tanna,[2] Paul Komenda,[1] Claudio Rigatto,[1] Daniel Schwartz.[3] *[1]Nephrology, University of Manitoba, Winnipeg, MB, Canada; [2]Nephrology, University of Toronto, Toronto, ON, Canada; [3]Nephrology, University of British Columbia, Abbotsford, BC, Canada.*

**Introduction:** Little is known regarding barriers to guideline implementation in the Nephrology community. We set out to identify barriers, specifically in evidence-based practice and measurement of quality indicators, in an international cohort of peritoneal dialysis (PD) Practioners.

**Methods:** Subscribers to an online nephrology education site (Nephrology Now) were invited to participate in an online survey. Nephrology Now is a non-profit, monthly mailing list that highlights clinically relevant articles in Nephrology. 475 physicians supplying PD care participated in an online survey assessing their use of evidence-based medicine (EBM) and continuous quality indicators (CQI) in their PD practice. Ordinal logistic regression was utilized to determine relationships between baseline characteristics and EBM and CQI practices.

**Results:** The majority of physicians were Nephrologists (89.7%). 50.4% worked in academic centres and 77.3% utilized a personal digital assistant (PDA) device. The geographic regions of our respondents were 13.5% Canadian, 34.5% USA, 19.4% European, 4.4% Australian, 5.3% South American, 10.7% African and 12.2% Asian. Adherence to PD clinical practice guidelines were generally strong however lower adherence was associated with countries with lower total health care expenditure as percentage gross domestic product, not using PDA, length of physicians practice and smaller (<20 patients per centre) PD practice.

**Conclusions:** International variation in guideline adherence may be influenced by a country's health care expenditure, physicians PDA use and experience and size of PD practice. These barriers may impact future guideline development and implementation

Disclosure of Financial Relationships: nothing to disclose

**PUB458**

**A Rare Cause of Peritoneal Dialysis Related Peritonitis: Leclercia Adecarboxylata** Christin M. Spatz, Apurva Lapsiwala, William Brian Reeves. *Nephrology, Hershey Medical Center/PSU, Hershey, PA.*

A 38 year old male with end-stage renal disease on peritoneal dialysis secondary to diabetic nephropathy and prior failed renal transplantation presented with abdominal pain of two days duration. He was afebrile and his abdominal exam was remarkable for diffuse tenderness. Peritoneal fluid was cloudy with 3,499 nucleated cells 94% neutrophils. Fluid culture grew *Leclercia Adecarboxylata* and *Acinetobacter Baumanii*. *L. adecarboxylata* was pansusceptible to Cephalosporins and Aminoglycosides. *A. baumanii* was resistant to Cefazolin but susceptible to Cefepime and Aminoglycosides. He was treated with intraperitoneal Cefepime and 72 hours later his cell count had decreased to 1,943 nucleated cells, however, the patient had not clinically improved. Intraperitoneal Gentamicin was then added to Cefepime. After 48 hours, his abdominal pain resolved. Repeat fluid testing showed only 62 nucleated cells and peritoneal culture showed no growth. He was discharged home on hospital day 6 with intraperitoneal Gentamicin and Cefepime to complete 21 days of antibiotics.

*Leclercia adecarboxylata* is a gram negative bacillus usually found in the setting of polymicrobial infections. The rarity of infection alone may suggest *Leclercia* requires other pathogens to cause clinically significant disease. Literature review shows most cases of *L. adecarboxylata* are pan-susceptible. Although our patient's cultures were initially susceptible to beta lactams, it is likely resistance occurred leading to treatment failure. Our case had a good outcome with early broadened antibiotic coverage for presumed beta-lactamase resistance. This organism, although infrequently isolated, may represent therapeutic challenges in the future if not identified early and treated aggressively.

Disclosure of Financial Relationships: nothing to disclose

**PUB459**

**Nephrologists' Perceptions Regarding the Eligibility Criteria for Peritoneal Dialysis (PD): A Conjoint Analysis Study** Hisako Yoshida,[1] Kazuhiko Tsuruya,[1] Shunsuke Yamada,[2] Toshiaki Nakano,[2] Masatomo Taniguchi.[2] *[1]Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.*

**Purpose**: The purpose of this study was to investigate nephrologists' perceptions regarding the eligibility criteria for PD.

**Methods:** We developed three types of questionnaires that can reveal the perception of the nephrologists as to PD using conjoint analysis (CA); 1) the eligibility criteria for PD, 2) the nephrologists' cognition for ''high self-care ability'' and 3) factors that determine ''clinical status of PD patients'' as good. These questionnaires were answered by 10 nephrologists (male: 9, age 35±13 years). The model of ''the eligibility criteria for PD'' was composed of 16 profiles of hypothetical patient with end-stage renal disease. They were designed to have seven characteristics (i.e. sex, age, diabetic status, weight, living situation, patients' compliance with treatment, cardiac function), as was referred to previous report (Thamer et al, Am J Kidney Dis, 2000). The following question was asked in each hypothetical patient; ''Do you recommend PD for this patient?''. Similarly,

we developed the other two questionnaires using the Self-Management Capacities Scale (Su et al. J Adv Nurs, 2009).

**Results:** CA revealed that ''compliance with treatment'' was the most important factor that affects eligibility criteria for PD, but failed to reveal the dominant factors regarding ''self-care ability'' that influence nephrologists' perception in the dialysis modality selection. However, as to the questionnaire of ''clinical status of PD patients'', the factors concerning ''fluid control'' (e.g. blood pressure level, sodium intake and BNP level) affected the nephrologists' perceptions in the assessment of PD patients.

**Conclusion:** This study revealed that the nephrologists' perception regarding the eligibility criteria for PD was most influenced by ''compliance with treatment''. In the future study, we want to clarify the association between the nephrologists' perceptions and the clinical practice in the actual patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB460**

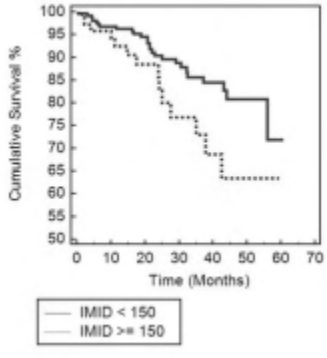
**Index of Mortality Bound to the Inflammatory Status in Dialysis (IMID): A New Predictive Index of Mortality in Dialysed Patient** Dumoulin Alexandre. *Nephrology, Centre de Dialyse, Beziers, France.*

**Introduction**: In spite of the progress in the hold in charge of the dialysis patients, the mortality of the dialysis patients remains always extremely elevated.The main objective of this survey is to determine an index of mortality clean to the dialysis based on characteristic elements of the chronic inflammation and notably on the ß2 Microglobulin (ß2M), raised in routine in dialysis allowing to define the IMID.

**Material and methods**: The survey rests on the assessment of nutritional and inflammatory parameters at a population of incidental dialysis patients on the period of the 01.01.2005 to the 01.01.2010. From these parameters: Albumin, Pre-albumin, C reactive protein, Orosomucoid and ß2M, the calculation of the IMID has been achieved to the hold in charge of the patient and way quarterly $IMID_{Max}$. The dialysis treatment (haemodialysis HD or haemodiafiltration HDF) prescribed has been considered like a therapeutic in intension to treat.

**Patient:** The survey is about 605 patients distributed in 229 women and 376 men whose respective middle age (year) is of 72.1±13.1 and 69.8±15.3. On the set of the incidental patients, 133 deceased patients permitted to determine the Lti (the life span).

**Results**: More the IMID is raised, the Lti more is short ($p<0.001$). The actuarial curve shows a meaningful difference between the patients having to the entry an IMID superior to 150 ($p<0.03$).




Among the deceased patients, the IMID is representative of neoplasy or an inflammatory death. Difference doesn't exist between both dialysis modality (HD vs HDF) concerning the $IMID_{Max}$ and the Lti for the deceased patients.

**Conclusion :** The IMID is an index of choice in the prognosis of mortality carriers of neoplasy or having an inflammatory syndrome (like infection). The HDFdoesn't bring an improvement in the prognosis of mortality.

Disclosure of Financial Relationships: nothing to disclose

**PUB461**

**Rat Bladder as a Model for Oxidative Damage from Intravenous Iron (IVFe) Preparations** George R. Bailie,[1] Hsin Li,[2] Amy B. Pai,[1] Robert M. Levin.[2] *[1]Albany Nephrology Pharmacy Group, Albany College of Pharmacy & Health Sciences, Albany, NY; [2]Department of Pharmaceutical Sciences, Albany College of Pharmacy & Health Sciences.*

Background: Many serum biomarkers have been used to examine oxidative stress associated with IVFe. The rat bladder is sensitive to oxidative damage and might be a sensitive model. We examined bladder contractility responses after IVFe.

Methods: Five Groups of 6 adult SD rats received 1 or 5 mg/kg IVFe or saline intravenously each week for 4 doses. IVFe included iron sucrose (IS), ferric gluconate (FG), high MW iron dextran (ID), ferric carboxymaltose (FCM) and ferumoxytol (FMX). Animals were sacrificed 7 days after the last dose, serum obtained via cardiac puncture and 2 full thickness bladder strips were placed in baths containing Tyrode's solution for contractility studies. Strips were successively stimulated with 2, 8 or 32 Hz field stimulations (80 V, 1 msec duration; acts via synaptic transmission), carbachol (10 mM; acts via direct muscarinic cholinergic stimulation) and KCl (120 mM; acts via direct muscle depolaration - does not need synaptic transmission or receptor stimulation). All responses recorded as mg tension/100 mg strip weight.

Results: At 1 mg/kg, no changes in bladder weight were noted in any treatment group. There was an increased bladder weight at 4 weeks only in the FG 5mg/kg group vs. control ($p<0.05$). Contractile response to carbachol and KCl were significantly reduced in FG group and showed dose-response activity (Table shows 5 mg/kg responses only).

Contractile response (mg tension/100 mg strip weight) after 5 mg/kg IVFe doses

| Stimulus | Control | IS | FCM | FMX | ID | FG |
|---|---|---|---|---|---|---|
| 2 Hz | 15.3 | 12.1 | 12.3 | 13.4 | 12.7 | 14.3 |
| 8Hz | 39.2 | 35.7 | 33.3 | 39.3 | 40.0 | 39.1 |
| 32 Hz | 57.4 | 51.2 | 45.1 | 54.2 | 57.9 | 52.4 |
| Carbachol | 41.7 | 41.8 | 31.4 | 40.4 | 37.4 | 42.9^ |
| KCl | 23.3 | 22.2 | 22.1 | 27.6 | 27.0 | 29.7* |

^$p<0.05$ vs control. *$p<0.05$ vs FG 1 mg/kg

Conclusions: FG increased bladder mass and has a dose-dependent negative effect on the contractile responses on bladder smooth muscle vs. other IVFe products following carbachol and KCL. Future studies will investigate this mechanism further and determine if this can be a test for the oxidative properties of IVFe products.

Disclosure of Financial Relationships: Consultancy: Fresenius Medical Care, American Regent, Vifor Pharma, Genzyme; Honoraria: Fresenius Medical Care, American Regent, Vifor Pharma.

**PUB462**

**Assessment of CRP and ESR Measurement as Inflammatory Markers in ESRD Patients with Hyperferritinemia** Roger F. Carbajal Mendoza, Hemant Magoo, Donald I. Baumstein, Alf M. Tannenberg. *Nephrology, Metropolitan Hospital Center/New York Medical College, New York, NY.*

Introduction: There is no perfect marker for occult inflammation in patients on chronic hemodialysis. The serum measurements of inflammatory markers may help to determine whether the presence of hyperferritinemia in dialysis patients is due to iron overload or inflammation. Currently, no consensus exists regarding which marker should be used to designate inflammatory states versus non-inflammatory states in patients with end stage renal disease (ESRD). Considering C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) as markers of inflammation and erythropoeitin (EPO) hyporesponsiveness as a consequence of inflammation, our objective was to determine if high levels of CRP and ESR, as well as hyperferritinemia correlate with high level of erythropoeitin resistance index (ERI) as an indicator of poor response to EPO.

Method: This observational study included 50 chronic hemodialysis patients. We measured hemoglobin, hematocrit, serum albumin, transferrin saturation, ferritin, CRP, ESR in monthly laboratory evaluation. The ERI was calculated as weekly weight adjusted dose of EPO divided by the hemoglobin level. The average age was 52.6 years and 62% of patients were male. The ERI average was 12.9. The average ferritin, CRP and ESR levels were 803 ng/ml (range 21-1650 ng/ml), 12.3 mg/L (range 0.07- 88.6 mg/L), 22.2 mm/hr (range 1-60 mm/hr) respectively.

Results: We did not find a linear correlation between either CRP and ERI ($R^2$: 0.0005) or ESR and ERI. In addition there was no clinically significant correlation between ferritin and ERI ($R^2$: 0.1127), Transferrin saturation and ERI ($R^2$: 0.133). There was no correlation between ESR and Ferritin ($R^2$: 0.0493), nor CRP and Ferritin ($R^2$: 0.0033).

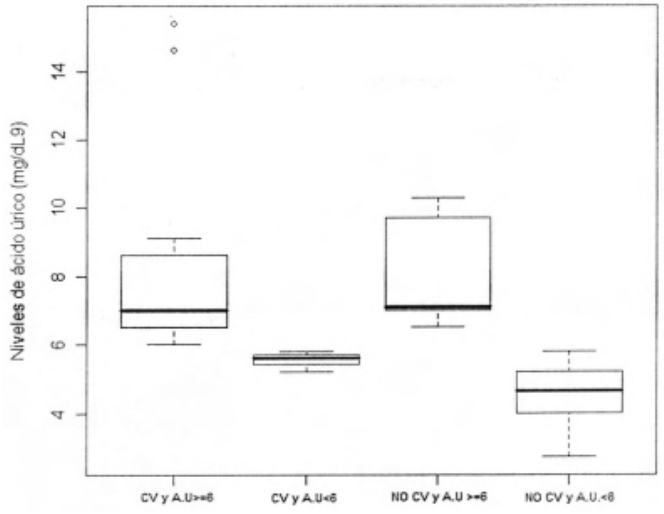
Conclusion: The data presented shows no correlation between CRP or ESR with ferritin as indicators of inflammation in our chronic hemodialysis patients. This suggests that higher levels of ESR and CRP may not be helpful to differentiate hyperferritinemia due to inflammation versus iron overload.

Disclosure of Financial Relationships: nothing to disclose

**PUB463**

**Uric Acid, Vascular Calcification and Atherosclerosis in Chronic Renal Disease** Luis Gerardo D'Marco, Dayana J. Olmos. *Nephrology, Ruiz y Paez University Hospital, Bolivar, Venezuela.*

Clinic and epidemiological studies have shown that cardiovascular (CV) disease is related to an increase in the mortality rate of patients with chronic renal disease (CRD). Uric acid (UA) is strongly associated with renal disease and CV risk, and its levels are especially increased in people with hypertension or patients undergoing dialysis. Interest in the association between UA levels and CV risk has been renewed in recent years. The mechanism by which UA can inflict damage is still unknown. **OBJECTIVE**: The purpose of this research was to relate the presence of vascular calcification (VC) and atherosclerosis studied by carotid echography, UA levels and other laboratory parameters in 50 patients with CRD undergoing dialysis. **METHODS:** Fisher Test was used to determine whether or not the differences were significant, which was decided based on p–value obtained in the test (<0.05). **RESULTS**: The age of patients averaged 45.34 years (SD: ± 17.2) and most of patients were female (56%). The predominant dialysis method was hemodialysis (52%) for an average period of 34.96 months (SD: ± 35.48). The average laboratory test values were: hemoglobin (8.6 g/%), urea (141.2 mg/dl) (p=0,012), creatinine (7.4 mg/dl), UA (6.9 mg/dl) (p=0.040), total cholesterol (188.1 mg/dl), triglycerides (206.5 mg/dl), albumin (3.4 mg/dl), calcium (8.6 mg/dl), phosphorus (6.5 mg/dl), and Ca x P product (55.4). VC was observed in 56% of patients; 46% had echographic criteria for atherosclerosis (intima-media thickness ≥ 0.9 mm) with an overall average of 0.89 mm (SD: ±0.28) (p=0.0006), being higher in patients with hypertension and diabetes Mellitus; this group also posed an increased predisposition to atherosclerosis and VC (p=0.01).




**CONCLUSIONS**: According to these results, we conclude that UA levels over 6 mg/dl pose an increased risk of VC and adverse CV events.

Disclosure of Financial Relationships: nothing to disclose

**PUB464**

**Influence of CKD, RRT, Inflammation and Proteolytic Enzymes on the Development of Atherosclerosis in Carotids** Wieslaw Klatko,[1] Stanislaw Niemczyk,[2] Katarzyna Szamotulska,[4] Tomasz Wisniewski,[1] Leszek Paczek.[3] *[1]Department of Nephrology, Regional Specialistic Hospital, Ciechanow, Poland; [2]Department of Nephrology, Military Medical Institut of Warsaw, Warsaw, Poland; [3]Department of Immunology, Trnsplantology and Internal Medicine, Medical University of Warsaw, Warsaw, Poland; [4]Department of Epidemiology, National Research Institute of Mather and Child, Warsaw, Poland.*

Atherosclerosis vascular disease is the most frequent complication in pts with chronic kidney desease undergoing hemodialysis and peritoneal dialysis. Intima media complex (IMC) measuring has been the non-invasive imaging test for evaluation of cardiovascular risk. Little is known how proteolytic enzymes activites influence on development of atherosclerosis.

The study was carried out on 60 pts in the following groups:

Group1 (n-30) undergoing HD

Group2 (n-10) undergoing PD

Group3 (n-10) pts with CKD without dialysis

Group4 (n-10) control group

Methods:

IMC of common carotid artery was measured bilaterally at the level 1cm from the bulb, two in each side on the far wall. The mean IMC was recorded as the average of the left and right IMC.

Blood samples were taken in the morning before dialysis, for pts undergoing hemodialysis before dialysis.

Results:

Medians

| Parameters\Groups | HD | PD | CKD | Controls |
|---|---|---|---|---|
| Collagenase mcU/ml | 36.89* | 51.89** | 35.83* | 30.59 |
| Cathepsin B mcU/ml | 45.83** | 45.67** | 37.63* | 21.43 |
| Elastase mcU/ml | 45.21** | 42.01* | 40.26* | 32.38 |
| CRP mg/l | 6.72* | 6.43 | 2.00 | 1.44 |
| IMC | 0.75*** | 0.65*** | 0.55 | 0.50 |

*)p< 0.05, ** )p<0.01, ***p)<0.001 for comparison to control group (t-test after logarithmic transformation)

Conclusion:

Relation between development of atherosclerosis in carotids and age was observed in all groups treated with dialysis. IMC among patient undergoing hemodialysis and peritoneal dialysis is higher and more related to age than among pts treated without dialysis and control group. Duration of RRT, urea, creatinine, albumin, cholesterol, TGL, troponin I serum concentration were not correlated with IMC.It has been observed that pts undergoing HD, PD and with CKD without dialysis have higher elastase, collagenase, cathepsin B activity and higher CRP concentration.

Disclosure of Financial Relationships: nothing to disclose

**PUB465**

**Dry Weight, Pre-Dialysis Systolic Blood Pressure (SBP) and Intima-Media Thickness (IMT) Predict Pre-Dialysis Brain Natriuretic Peptide (BNP) Levels** Paolo Lentini,[1-2] Luca Zanoli,[3] Valentina Pellanda,[1] Vincenzo Catena,[1] Alexandra Chronopoulos,[1] Massimo De Cal,[2] Claudio Ronco,[4] Marco Baiocchi,[1] Roberto Dell'Aquila.[1] *[1]Nephrology-Intensive Care Unit, St. Bassiano Hospital, Bassano Del Grappa (Vi), Italy; [2]University of Padua, Padua, Italy; [3]Internal Medicine, University of Catania, Catania, Italy; [4]Nephrology, St Bortolo Hospital, Vicenza, Italy.*

Background: High BNP levels are correlated with cardiovascular (CV) damage and poor outcomes in hemodialysis (HD) patients. Aim: To determine the independent predictors of vascular damage in a population of chronic HD patients. Materials and Methods: All 74 patients enrolled in our HD program were included. Median pre-dialysis BNP (183 pg/mL) was used as a cut-off to create two subgroups. Hematological and biochemical variables were obtained by a pre-dialysis blood draw. All patients underwent a doppler of the supra-aortic blood vessels 30-45 days before BNP analysis. All dopplers were executed by the same operator. Student t test and chi-square test were used for comparison. Univariate predictors of BNP were studied in multivariate logistic regression analysis. The model was tested in ROC curve analysis. Results:Univariate analysis: Patients with BNP>183pg/ml had significantly lower Hgb, weight and dry weight,higher SBP,pulse pressure,IMT,carotid stenosis and CV events. Logistic regression analysis: Dry-weight(1kg increase,OR 0,93,95%CI 0.88-0.98,p<0.05), pre-dialysis SBP (1mmHg increase,OR 1.03,95%CI 1.01-1.06, p<0.05),and IMT (0.1mm increase, OR 1.21, 95%CI 1.04-1.40, p<0.05) were selected as independent predictors of pre-dialysis BNP>183 pg/ml.

Table 1.Logistic regression analysis for BNP pre dialysis > 183 (pg/ml)

| | OR | 95.0% CI | p |
|---|---|---|---|
| Dry weight (Kg) | 0,925 | 0,876 0,977 | 0,005 |
| SBP pre dialysis (mmHg) | 1,032 | 1,007 1,057 | 0,011 |
| IMT (0.1 mm) | 1,208 | 1,041 1,402 | 0,013 |

Hosmer-lemeshow Goodness-of-Fit test was not significant (p=0.264), Nagelkerke R-square was 0.33. The Area under ROC curve for the selected model was 0.78 (95%CI 0.67-0.88, p<0.0001). Conclusions: Dry weight, pre-dialysis SBP and IMT predict higher levels of pre-dialysis BNP.

Disclosure of Financial Relationships: nothing to disclose

**PUB466**

**The Effluent Free Radical Level Is a Potential Predictor of Technique Failure and Mortality in Peritoneal Dialysis Patients** Hiroshi Morinaga,[1] Hitoshi Sugiyama,[2] Keiichi Takiue,[1] Masashi Kitagawa,[1] Tatsuyuki Inoue,[1] Shinji Kitamura,[1] Yohei Maeshima,[1] Hirofumi Makino.[1] *[1]Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [2]Center for CKD and Peritoneal Dialysis, Kayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

INTRODUCTION AND AIMS: Residual renal function (RRF) is associated with oxidative stress and survival in peritoneal dialysis (PD). This study investigated the association between oxidative stress and renal anemia, RRF and prognosis in PD.

METHODS: The levels of free radical in the overnight dwell of peritoneal effluent from 45 patients were determined by electron spin resonance (ESR) spectrometry using α-phenyl-N-tert butylnitrone (PBN) as a spin trapping agent. The levels of hemoglobin, plasma level of β2-microglobulin (β2MG), the effluent level of 8-hydroxy-2´-deoxyguanosine (8-OHdG), the daily urine volume and adequacy of dialysis at the baseline were also measured. The effect of these factors on the composite endpoint (withdrawal from PD or mortality) in patients followed for twenty-four months was analyzed.

RESULTS: A significantly positive correlation was observed between the level of effluent free radicals (EFR) and plasma β2MG (R = 0.3708, P = 0.0122) or effluent 8-OHdG (R = 0.3676, P = 0.0130), and a negative correlation between the level of EFR and hemoglobin (R = -0.3393, P = 0.0226), daily urine volume (R = -0.3335, P = 0.0252) or re

Disclosure of Financial Relationships: nothing to disclose

**PUB467**

**Anti-Inflammatory Effects of Oral Paricalcitol in Hemodialysis Patients** Juan F. Navarro,[1,2] Carmen Mora,[2] María L. Méndez,[1] Mercedes Muros,[3] Javier García.[1] *[1]Nephrology Service, University Hospital Nuestra Señora de Candelaria (HUNSC); [2]Research Unit, HUNSC; [3]Clinical Biochemistry, HUNSC, Santa Cruz de Tenerife, Spain.*

Hemodialysis (HD) patients are affected by an inflammatory syndrome, which has been related to diverse complications. Moreover, inflammation is a strong factor in the development and progression of atherosclerotic disease. During the last years, a growing interest has developed related to the pleiotropic effects of vitamin D in CKD. Paricalcitol, a selective vitamin D receptor (VDR) activator, has demonstrated immunomodulatory effects in experimental studies. However, data clinical data about this effect are very scarce. The aim of the present study is to analyze in HD patients the anti-inflammatory effects of oral paricalcitol.

Eighty patients were initially considered for inclusion: 55 were excluded and 25 subjects (mean age, 62 years; mean HD time, 12 months; 56% diabetics) were included in the study. All of them were previously treated with intravenous calcitriol, and after a 4-weeks wash-out

period, oral paricalcitol (1 µg/day) was administered for 12 weeks. Baseline serum calcium (Ca), phosphorus (P), Ca-P product, and iPTH were 9.1 mg/dl, 4.8 mg/dl, 44.2 $mg^2/dl^2$ and 317 pg/ml, respectively. At the end of the study, these parameters did not change, except for iPTH, which decreased to 302 pg/ml ($p<0.05$). Serum concentrations of high-sensitive C-reactive protein (CRP), interleukins (IL)-1,6 and 10, and tumor necrosis factor-alpha (TNFa) were measured. After paricalcitol administration, serum IL levels did not change. However, CRP and TNFa experienced a significant decrease: 5.9±2.3 vs 7.2±3.4 mg/l ($p<0.001$), and 7.1±2 vs 7.6±2.6 pg/ml ($p<0.05$), respectively. The percent decrease of these parameters were 4% and 14%, respectively, respect to their basal values. The ratio between pro- (IL-1, IL-6 and TNFa) and anti-inflammatory (IL-10) cytokines experienced a beneficial change: IL-6/IL-10 ($p=0.05$) and TNFa/IL-10 ($p=0.01$). In conclusion, oral paricalcitol administration to HD patients is associated with modulation of inflammatory process, specifically with a reduction of CRP and TNFa, as well as an improvement of IL-6/IL-10 y TNFa/IL-10 ratios.

Disclosure of Financial Relationships: nothing to disclose

**PUB468**

**Comparative Study between the Inflammatory and Metabolic Profile of Peritoneal Dialysis (PD) Versus Hemodialysis (HD)** Karina F. Pinheiro, Victor Sato, Bruno C. Silva, Márcia Fernanda Arantes Oliveira, Hugo Abensur. *Nephrology, Hospital das Clinicas - University of São Paulo School of Medicine, Sao Paulo, Brazil.*

Fibrinogen which is involved in the coagulation system and leptin which is directly correlated to the nutritional state and to body total fat are related to higher inflammatory state and consequently higher cardiovascular(CV)risks.To evaluate metabolic characteristics and inflammatory markers through fibrinogen, leptin and the C-reactive protein(CRP)in patient's maintained PD and HD.We studied 63 prevalent patients in dialysis at only one university center(48 patients on HD and 15 on PD).Fibrinogen, leptin, lipids, albumin and CRP were compared between the two groups.Statistic analyze between 2 groups was carried using chi-square test for qualitative variables and t-test or Mann-Whitney for quantitative variables with normal distribution or not, respectively.Clinical-laboratorial characteristics of the patients on PD compared to patients on HD were as follow: age 48±18 vs. 47±18($p>0.05$); hypertension 100% vs 69%($p=0.013$);obesity evaluated through body mass index(BMI) 25.67±6.9Kg/$m^2$ vs 22.39±3.9Kg/$m^2$($p = 0.012$); diabetes 27% vs. 4%($p=0.01$); fibrinogen 511±141mg/dL vs. 380±115mg/dL($p=0.001$); CRP 14.18±13.82 vs 4.18±5.01($p < 0,001$); albumin 3.87±0.46g/dL vs 4.16 ± 0.32g/dL($p = 0.011$) and hemoglobin 10.3±1.15g/dL vs 11.5±1.67g/dL($p < 0.001$). Leptin median was 35,6ng/mL on PD and 8,2ng/mL on HD patients($p=0.006$).The lipid profile among patients maintained PD and HD were: cholesterol 190±46mg/dL vs 154±36mg/dL($p = 0.003$); LDL 104.4±41.3mg/dL vs. 83.9±28.7mg/dL($p=0.035$); HDL 54.8±22.2mg/dL vs. 42±15.1mg/dL($p=0.014$) and triglycerides 156.8±115.2mg/dL vs. 140.8±61.8mg/dL($p>0.05$).There was no significant statistic difference regarding the number of CV events, nor as to the Kt/V standard.Statins use among patients maintained PD and HD was 53% and 19%($p=0.008$) .Patients maintained PD showed higher levels of fibrinogen, leptin and CRP when compared to patients on HD.Therefore, as these subjects were more hypertensive and presented worse lipid profile and higher BMI, it can be attributed to a higher inflammatory status and worse volume control.

Disclosure of Financial Relationships: nothing to disclose

**PUB469**

**Arteriovenous Fistula Failure: Role of Cardiovascular Structure and Function** Zoe C. L. Pittman,[1] Shvan Korsheed,[1] Stephen G. John,[1] Chris W. McIntyre.[1,2] *[1]Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]University of Nottingham, United Kingdom.*

Primary and secondary arteriovenous fistula (AVF) failure is a major barrier to increasing AVF utilisation. Previous observational studies of access failure have largely focussed on demographic factors, partially describing patients likely to suffer thrombotic loss of AVFs. Therefore, we have undertaken a further detailed study of access failure as a function of *pre-operative* vascular anatomy and cardiovascular performance (local and systemic).

We studied 43 patients, two weeks prior to AVF formation and followed up for at least a year. In addition to pre-operative vascular mapping, demographic and biochemical data, patients underwent a comprehensive cardiovascular assessment including transthoracic echocardiography, pulse wave analysis and pulse wave velocity (PWV). Factors associated with failure were identified by multivariate analysis.

Primary failure occurred in 13/43 patients, secondary failure occurred in 11/30 during the subsequent follow up period of 17.8 (± 8.8) months. Arterial diameter and arterial blood flow predicted primary failure ($r^2 = 0.254$, OR 1.096 (1.018–1.179) $p=0.004$ and $r^2=0.247$, OR 1.033 (1.004–1.062) $p=0.005$ respectively). The only other predictor was carotid radial (CR) PWV ($p= 0.052$). For secondary failure CR-PWV and carotid femoral PWV were tending to significance ($p=0.059$ and $p=0.065$ respectively). Despite a significant degree of cardiac contractile dysfunction (mean LVEF 44.9 ± 12.5%), no measures of systemic or central CV performance were found to be associated with risk of access failure.

Primary and secondary AVF loss is dependant on peripheral vessel size and viscoelastic properties, with reduced upper limb arterial compliance being associated with a reduced propensity for access failure. These data do not support the contention that poor cardiac contractile status is likely to result in failure to establish native vascular access.

Disclosure of Financial Relationships: nothing to disclose

**PUB470**

**A Bioimpedance Study Assessing the Relationship between Inflammation and Body Composition in a Haemodialysis Population** Siddhesh Mukund Prabhavalkar, Julie E. Browne, Ying Kuan. *Renal Unit, Altnagelvin Area Hospital, Londonderry, United Kingdom.*

Introduction

Patients on maintenance haemodialysis (MHD) have excess morbidity and mortality in comparison to general population. The development of malnutrition, inflammation, and atherosclerosis (MIA) syndrome has been postulated to be a major reason for this. MHD is also associated with changes in body composition, with decrease in lean body mass, which may be a consequence of MIA. Multifrequency bioimpedance represents a novel and an accurate way of assessing body composition in this population. This is a cross-sectional study assessing the relationship between inflammation, as measured using C reactive protein and body composition.

Method

Data was collected from 54 patients on maintenance haemodialysis which included Gender, Age, duration on dialysis, CRP, albumin, total cholesterol, and HDL cholesterol. Bioimpedance measurements (% skeletal muscle mass (%SMM), % body fat mass (%BFM)) were obtained prior to dialysis from the subjects. Statistical analysis was performed using Medcalc Stat package.

Results

Data was obtained from 54 patients (34 males and 20 females). Mean age was 62.1 years +\- standard deviation (SD) 14.98. CRP positively correlated with %BFM ($p=0.005$, $r^2=0.14$) whilst negatively correlated with %SMM ($p=0.003$, $r^2=0.16$). In multiple regression model, CRP was shown to be independently associated with %BFM ($p=0.0069$) as well as %SMM ($p=0.0083$). However duration on dialysis, albumin, age of subjects and total cholesterol were not shown to be associated with body composition indexes.

Conclusion

The results of this cross-sectional study suggest that body composition of patients on MHD may be affected by inflammation. These changes can be postulated to be part of the phenomenon of MIA, and suggests that interventions that reduce inflammation and improve lean muscle mass may serve to improve prognosis of patients on dialysis. This study also supports the use of bioimpedance as a measure of nutrition, providing information on MHD patients independent of other clinical biomarkers.

Disclosure of Financial Relationships: nothing to disclose

**PUB471**

**Effect of Atorvastatin on Cardiac Troponin-T and High Sensitivity C-Reactive Protein in Maintenance Hemodialysis Patients** John White,[1] Harold M. Szerlip.[2] *[1]Medicine, Medical College of Georgia, Augusta, GA; [2]Medicine, University of Arizona, Tucson, AZ; [3]Augusta, GA.*

In asymptomatic dialysis patients, elevated cardiac troponin T (cTNT) and hs-CRP levels are associated with increased cardiovascular events and may represent modifiable risk factors. We postulated that low level elevations in cTnT were related to chronic inflammation. The purpose of this study was to investigate the association of cTnT and hs-CRP over time in maintenance hemodialysis (HD) patients and to ascertain whether treatment with an HMG-CoA reductase inhibitor would reduce both cTnT and hs-CRP.

Methods: We conducted a prospective cohort study enrolling 42 asymptomatic HD patients at 2 outpatient dialysis facilities. Levels of cTnT and hs-CRP were analyzed at baseline, then weekly for 4 weeks to determine variability. Patients with elevated cTnT >= 0.1 ng/ml were then treated with atorvastatin x 6 months. The relation between clinical factors, cTnT, and hs-CRP were assessed as well as the effect of atorvastatin on final cTnT and hs-CRP.

Results: At baseline (n = 42), cTnT was detectable in 89% and 28.6% (n = 12) had cTnT >= 0.1. Measured levels of hsCRP (n = 31) were low risk in 29% (< 3.1 mg/L), high risk in 42% (3.1-10), and markedly elevated (>10) in 29%. Individual variability for cTnT was low with 80% of values within 0.02 from the mean. Contrastingly, hs-CRP individual coefficient of variation averaged 42%. There was no relation between cTnT and hs-CRP. Age (r = 0.33) and time on dialysis (r = 0.42) were moderately related to cTnT. Atorvastatin treatment had no effect on cTnT or hs-CRP levels in 8 patients.

Conclusions: Elevations in cTnT in patients on chronic hemodialysis do not vary significantly over time. In contrast there appears to be marked variation in hs-CRP indicating varying degrees of inflammation. There is no relationship between inflammation as assessed by hs-CRP and elevations in cTnT. Atorvastatin had no effect on either of these biomarkers.

Disclosure of Financial Relationships: nothing to disclose

**PUB472**

**Immediate Inhibitory Effect of Acidic Electrolyzed Water on Respiratory Tract Infection (New Influenza H1N1pdm Virus) and Urinary Tract Infection In Vitro** Tokunori Yamamoto,[1] Masahiko Katayose,[2] Kyoichiro Yoshida,[2] Nobuo Achiwa,[2] Naoto Sassa,[1] Norihisa Matsukawa,[1] Masashi Katoh,[1] Yasushi Yoshino,[1] Kazuo Mizutani,[1] Ryohei Hattori,[1] Momokazu Gotoh.[1] *[1]Urology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Advanced Technology Development Section, Hoshizaki Electric Co., Ltd., Toyoake, Japan.*

Because of their low immunity, chronic kidney disease (CKD) patients have significantly higher risks of death and complications caused by infection than non-CKD patients. Urology department providing dialysis treatment, kidney transplantation, and urinary tract infection treatment is one of the departments where many compromised hosts can stay or visit as either inpatients or outpatients. Although vaccination is recommended to prevent pathogens with higher infection risks, CKD patients are less capable of obtaining and maintaining antibodies and likely to lose immunity earlier than healthy individuals.

In this study, acidic electrolyzed water generated by electrolysis technology (hereinafter called "electrolyzed water (EW)") was examined in vitro for respiratory tract infection (California/4/2009 (A-H1N1) pdm, obtained from US CDC) and urinary tract infection. EW was added with virus suspension and inoculated into embryonated eggs. The hemagglutinating properties observed after 2 days of incubation were used to calculate 50% egg-infective dose.

The experiment showed that EW (pH 2.7, available chlorine concentration 20 ppm) effectively inhibited more than 99.99% of H1N1 within 10 seconds. Similar efficacy was also observed for urinary tract infection. Low pH of EW has high and instant sterilizaiton effect against clinical isolated drug resistance bacterium (Staphylococcus aureus (MRSA), Enterococcus faecalis, Pseudomonas aeruginosa, Acinetobacter baumannii). It was suggested that EW is effective in preventing infection in urology clinics and hospitals apt to have many compromised hosts.

Disclosure of Financial Relationships: nothing to disclose

**PUB473**

**Elevated Interleukin-18 in End-Stage Renal Disease Compared to Healthy Controls and Chronic Kidney Disease** Kenneth Yong. *Nephrology, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia.*

***Introduction***

Interleukin(IL)-18 is an inflammatory cytokine with pro-atherogenic properties that has been implicated in the pathogenesis of atherosclerosis and is an independent predictor of cardiovascular death in the general population. End-stage renal disease (ESRD) is a state of chronic inflammation characterised by accelerated atherosclerosis. Although serum IL-18 levels have been demonstrated to be elevated in dialysis-dependent ESRD patients, serum IL-18 level in chronic kidney disease (CKD) patients remains unknown.

***Methods***

This was a cross-sectional study of healthy controls, stage 3-4 chronic kidney disease (CKD) patients, and stage 5 pre-dialysis CKD patients. Fasting serum levels of IL18 were measured using a commercially available ELISA method (MBL Co, Ltd) with level of detection of 7.5pg/mL.

***Results***

Mean age was similar in all 3 groups. Serum levels of IL18 were significantly elevated in stage 5 CKD patients (n=15, mean±SD 413.5 ± 176.1 pg/mL) compared with stage 3-4 CKD patients (n=20, 260.8 ± 77.8 pg/mL; p=0.006) and healthy controls (n=20, 258.7 ± 132.6 pg/mL; p=0.005). IL18 levels were similar between the CKD and healthy control group (p=0.949).

***Conclusion***

Serum IL18 levels were significantly elevated only in advanced CKD patients and may in part explained the greater risk of atherosclerosis in the group of patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB474**

**Spondylodiscitis in Hemodialysis Patients – Still a Risk in an Era of Fistulas and Catheter Replacement** Masoud Afshar,[1,2] Robert F. Reilly.[1,2] *[1]Medicine, VA North Texas Health Care System, Dallas, TX; [2]Medicine, UT Southwestern Medical Center at Dallas, Dallas, TX.*

Spondylodiscitis (infection of the vertebral disc and adjacent vertebral bodies) is a devastating complication of access-related bacteremia in hemodialysis patients. We report a series of 13 patients seen in our unit with spondylodiscitis over the past 10 years. The most common presentation was back pain with localized tenderness over the spinal segment involved (11/13). The majority of patients were diabetic (10/13). Only 3 had fever and 5 an elevated WBC count on presentation. As previously reported the most common access was a permcath (9/13). Five of 9 permcath patients had a bacteremic episode in the preceeding 3 months. All were treated with permcath removal and placement of a new catheter after several days of antibiotic therapy. Catheter salvage was not attempted. Surprisingly an AV fistula served as vascular access for 3. Blood cultures were positive in 8 of 13 and all were Staphylococcal species (epidermidis-2, MSSA-3, MRSA-4). The lumbosacral spine was the most commonly involved spinal segment (9/13). An associated epidural or psoas abscess was present in 5. C-reactive peptide (CRP) levels were elevated, >10 mg/dL in 7 of 12 patients in which they were measured. Erythrocyte sedimentation rate (ESR) was measured in 10 and was >60 mm/hr except in one patient (42 mm/hr). Eight patients had an albumin concentration <3.2 g/dL and serum hemoglobin concentration <11 gm/dL. Most patients recovered fully, however, two were left with significant neurologic deficits. Three died within six months of initial presentation (two with continuing infection and one of coronary artery disease). The typical affected hemodialysis patient in our series was a diabetic presenting with back pain. Despite the fact that most laboratory tests showed evidence of an inflammatory state (elevated ESR, elevated CRP, decreased albumin, and decreased hemoglobin) fever and an elevated WBC count were often absent. Importantly, although most cases occurred in patients with permcaths, a significant number (3/13) had an AV fistula for their vascular access. In our hands replacement of infected catheters was not protective.

Disclosure of Financial Relationships: nothing to disclose

**PUB475**

**Design of a Prospective Observational Study To Evaluate the Prospective Payment System (PPS) Impact on Small Dialysis Organizations (SDOs)** Irene Agodoa, Lei Lei, Denise Globe. *Amgen inc, Thousand Oaks, CA.*

**Background:** Beginning January 1, 2011, the Centers for Medicare and Medicaid Services (CMS) has proposed to implement a bundled prospective payment system that would establish a combined payment for composite rate and separately billable dialysis services. A case mix adjustment model has been developed to attempt to align reimbursement with costs. However, SDOs may not have a sufficiently large risk pool over which to spread and mitigate financial risk and may not have adequate resources to treat patients requiring high resource utilization. To understand the impact of the new policy on this vulnerable dialysis segment, a registry has been designed to describe trends in treatment patterns of SDOs prior to and during the implementation of the PPS. **Design:** A multi-center, multi-year observational study of End Stage Renal Disease (ESRD) patients receiving dialysis at SDOs (defined as part of a chain that has fewer than 50 dialysis units within the entire association). Enrollment begins in 2010 with ~4.5 to 6 months of subject data collection during this baseline year, and up to 48 months of subject data collected after the initiation of the PPS. Approximately 50 small dialysis units from throughout the United States (US) will be enrolled. Site selection will be geographically diversified by inclusion of interested facilities to represent the 9 US Census Bureau Divisions. Primary study endpoints are the proportion of patients within each facility meeting each of the CMS ESRD PPS quality metrics over time. Secondary endpoints include facility operating characteristics (ie ownership and closures) and patient characteristics, treatment patterns, and clinical outcomes reported in aggregate per facility over time. To maintain study integrity, participating facilities will not be individually identified. Descriptive analyses will be conducted to estimate treatment patterns and patient characteristics at the facility level before and during CMS reimbursement policy changes. This study will provide a comprehensive data repository that will enable real-time assessment of treatment trends in SDOs prior to and during the period of PPS implementation.

Disclosure of Financial Relationships: Employer: Amgen; Ownership: Stock - Amgen.

**PUB476**

**Improved Blood Flow and Adequacy with Streamline Bloodlines** Joan E. Arslanian, Carl M. Lockman, Yvette C. Parker, Chaim Charytan. *NY Hospital of Queens, Flushing, NY.*

Dialysis adequacy (Kt/V) is a determinant of mortality. Kt/V is a function of blood flow (BF), dialysate flow, dialyzer type and treatment time. Increasing blood flow can be economically and logistically simple, but must be combined with attention to arterial pressure (AP).

We evaluated the Medisystems Streamline (SL) bloodline with improved hemodynamics (lower turbulence,no blood-air contact) by raising blood flows based on arterial pressures. We hypothesized that we would 1) improve Kt/V for patients with different vascular accesses and starting BF with little impact on AP; and 2) further improve Kt/V for patients with already high Kt/Vs.

In a cross-over evaluation of 202 patients, we compared Kt/V 1) on conventional bloodlines; and 2) on SL. We recorded spKt/V and treatment values for average delivered BF, average AP, actual treatment times, average dialysate flows, dialyzer types and accesses. We excluded patients with changes in treatment times, dialysate flows, dialyzers or accesses between periods. We calculated significance using paired t-test and McNemar's test.

With SL, we showed significantly increased BF with a minimal 4% increase in AP. Average Kt/V and the percent of patients with Kt/V >=2.0 also improved significantly, regardless of starting BF and access type.

| | Conv. Bloodlines | SL Bloodlines | Change | p-Value | N |
|---|---|---|---|---|---|
| **Avg Arterial Pressure(mmHg)** | **-161** | **-167** | **4%** | **0.01** | **202** |
| **Avg Blood Flow (mL/min)** | **378** | **449** | **19%** | **<0.001** | **202** |
| % patients BF> 425mL/min | 1% | 74% | 73% | <0.001 | 202 |
| **Avg Blood L Processed*** | 83 | 99 | 20% | | |
| **Avg Kt/V** | 1.94 | 2.11 | 9% | <0.001 | 130 |
| >=1.6 | **95%** | **95%** | **0%** | **NS** | **130** |
| >=2.0 | **39%** | **73%** | **34%** | **<0.001** | **130** |
| **% Achieving Kt/V>=2.0 By Patient's Conventional BF (mL/min):** | | | | | |
| BF<350 | 41% | 76% | 35% | 0.04 | 17 |
| BF 351-375 | 37% | 80% | 43% | <0.001 | 30 |
| BF 376-424 | 39% | 79% | 40% | <0.001 | 57 |
| BF >425 | 42% | 77% | 35% | 0.08 | 26 |
| **% Achieving Kt/V>=2.0 By Access Type:** | | | | | |
| Fistula | 37% | 79% | 43% | <0.001 | 101 |
| Graft | 48% | 78% | 30% | 0.023 | 23 |

*Overall Average BF X Average Treatment Time

Disclosure of Financial Relationships: nothing to disclose

**PUB477**

**Bioimpedance Analysis and the Duration of the Hemodialysis Session** Carlo Basile, Pasquale Libutti, Anna Lucia Di Turo, Francesco Casucci, Nicola Losurdo, Annalisa Teutonico, Carlo Lomonte. *Division of Nephrology, Miulli General Hospital, Acquaviva delle Fonti, Italy.*

Bioelectrical impedance (BIA) is a measurable property of electrical ionic conduction of soft tissue. Impedance is composed of resistance (R) and reactance (Xc). Physiologically, measured R correlates strongly with total body water. In man, more than 90% of impedance is composed of R. Aim of this study was the investigate R and Xc during hemodialysis (HD) sessions of different duration.

This is a two-step crossover study. All HD sessions utilized high-flux FX80 dialyzers and the GENIUS® single-pass batch dialysis system (FMC, Germany), which consists of a closed dialysate tank of 90 l. **Step A:** 22 stable white prevalent anuric uremic patients underwent two standard (~ 4h) bicarbonate HD sessions in a random sequence. The sessions were pair-matched as far as the dialysate and blood volume processed (90 l), volume of ultrafiltration and dialysate Na+ (140 mmol/l) and K+ concentrations (2 mmol/l) are concerned. One HD session had the dialysate Ca++ concentration 1.5 mmol/l; the other 1.25 mmol/l. **Step B**: 11 out of the 22 patients underwent one standard (~ 4h) and one long-hours (~ 8h) slow-flow bicarbonate HD session in a random sequence. The sessions were pair-matched as described in step A with the only exception that dialysate Ca++ concentration was 1.5 mmol/l in both treatments. R and Xc were determined at the start and the end of each HD session, injecting 800 µA at 50 kHz alternating sinusoidal current with a standard tetrapolar technique.

Solute mass balances were not significantly different in both steps, except for Ca++ mass balance in step A. R and Xc values did not show any statistically significant difference in step A. Post-dialysis R, Δ R (the difference between post- and pre-dialysis) and percent increase of R values were significantly higher in the 8h sessions in step B ($P < 0.0001$, 0.02 and P 0.02, respectively).

In conclusion, 8h slow-flow bicarbonate HD sessions were associated with post-dialysis R values significantly higher than the corresponding ones of the 4h sessions. If higher R values may represent a proxy of a correct dry body weight, it remains a matter of future research.

Disclosure of Financial Relationships: nothing to disclose

**PUB478**

**Removal of Uremic Retention Solutes in Standard Bicarbonate Hemodialysis and Long-Hours Slow-Flow Bicarbonate Hemodialysis** Carlo Basile,[1] Pasquale Libutti,[1] Anna Lucia Di Turo,[1] Francesco Casino,[2] Luigi Vernaglione,[3] Sergio Tundo,[1] Pasquale Maselli,[1] Annalisa Teutonico,[1] Carlo Lomonte.[1] *[1]Division of Nephrology, Miulli General Hospital, Acquaviva delle Fonti, Italy; [2]Division of Nephrology, Matera, Italy; [3]Division of Nephrology, Manduria, Italy.*

Many studies already stressed the importance of hemodialysis (HD) time in the removal of uremic toxins. In those studies, however, also the amount of dialysate and/or processed blood was altered. Aim of this crossover study was to investigate the isolated effect of time on removal of uremic toxins.

Eleven anuric HD patients underwent 2 bicarbonate HD sessions (~ 4h and ~ 8h) in random sequence. The GENIUS® single-pass batch dialysis system and the high-flux FX80 dialyzers (FMC, Germany) were utilized. GENIUS® consists of a closed dialysate tank of 90 l, thus offering the opportunity of effecting mass balances of any solute in a very precise way. The volumes of blood and dialysate processed, of ultrafiltration and dialysate composition were prescribed to be the same. Small molecules (urea, creatinine, phosphorus and uric acid), the middle molecule $ß_2$M and protein-bound compounds (homocysteine, hippuric acid, indole-3-acetic acid and indoxyl sulfate) were investigated. Total solute removals (solute concentration in the spent dialysate x 90 l, i.e. the volume of dialysate) (TSR), clearances (TSR of a solute / area under the plasma water concentration time curve of the solute) (K), total cleared volumes (K*time) (TCV), and dialyzer extraction ratios (K/blood flow) (ER) were determined.

TSR, TCV and ER were statistically significantly larger during prolonged HD for all small and middle molecules (at least, $P = 0.01$). The percent increases of TSR were 26.6 % for phosphorus and 39.2% for $ß_2$M ($P = 0.005$). No statistically significant difference was observed for protein-bound solutes in any of the above parameters.

In conclusion, small and middle molecules are removed more adequately from the deeper compartments when performing a prolonged HD. Hence, factor time is very important for these retention solutes. The kinetic behavior of protein-bound solutes is completely different because of the strength of their protein binding.

Disclosure of Financial Relationships: nothing to disclose

**PUB479**

**Hemodynamic Stability in Long-Hours Slow-Flow Bicarbonate Hemodialysis** Carlo Basile, Pasquale Libutti, Anna Lucia Di Turo, Luigi Vernaglione, Francesco Casucci, Nicola Losurdo, Annalisa Teutonico, Carlo Lomonte. *Nephrology, Miulli Hospital, Acquaviva delle Fonti, Italy.*

The interplay of Na+, K+ and Ca++ mass balance (MB) with ultrafiltration volume ($V_{UF}$) is crucial in hemodynamic stability during hemodialysis (HD).

This two-step crossover study utilized high-flux FX80 dialyzers and the GENIUS batch dialysis system (FMC, Germany). **Step A:** 22 stable HD patients underwent two standard (4h) bicarbonate HD sessions in random sequence. The sessions were pair-matched for the dialysate and blood volume processed (90 l), $V_{UF}$ and dialysate Na+ and K+ concentrations ((140 and 2 mmol/l). One HD session had dialysate Ca++ concentration 1.5, the other 1.25 mmol/l. **Step B**: 11 patients underwent one 4h and one 8h slow-flow bicarbonate HD session in random sequence. The sessions were pair-matched as described in step A except for dialysate Ca++ concentration (1.5 mmol/l in both treatments). Na+, K+ and Ca++ concentrations were measured in fresh and spent dialysate to determine solute MB. Systolic (SBP), diastolic and mean arterial pressure (MAP) trends were analyzed. Plasma volume (PV) changes were computed from plasma total protein concentrations.

All dialysis sessions were uneventful. **Step A:** mean Na+ MB and K+ MB were not significantly different, whereas Ca++ MB was less positive with dialysate Ca++ concentration of 1.25 mmol/l. Intradialysis decreases of mean SBP, MAP, PV and $V_{UF}$ were not significantly different between the two treatments. **Step B:** all solute MBs were not significantly different between the two treatments. SBP decreased significantly during the 4h runs, whereas was stable during the 8h ones ($P<0.0001$ and P=NS, respectively). Significantly lower intradialysis decreases of mean SBP ($P<0.02$) and MAP ($P<0.04$) occurred in the 8h sessions, despite not different mean $V_{UF}$ (2.9 l). The slope of PV in the first 4 h was less steep during the 8h sessions ($P<0.0001$). PV decrease was significantly higher at the end of the 4h HD runs ($P<0.04$).

In conclusion, a better hemodynamic stability was achieved in the 8h sessions despite not different $V_{UF}$ and solute MBs. A better PV preservation can explain the better hemodynamic stability peculiar to long-hours HD treatments.

Disclosure of Financial Relationships: nothing to disclose

**PUB480**

**Elevated Plasma Brain Natriuretic Peptide (BNP) Levels in Hemo-Dialysis (HD) Patients and Its Lack of Correlation with Volume Status** Robert L. Benz,[1,2,3] Rob J. Mathews,[1] Mark R. Pressman,[2,5] Michael M. Chernick,[4] Albert A. Keshgegian.[2,3,6] *[1]Nephrology, Lankenau Hospital, Wynnewood, PA; [2]Lankenau Institute for Medical Research, Wynnewood, PA; [3]Mainline Health, Wynnewood, PA; [4]Biostatistics, Lankenau Institute for Medical Research, Wynnewood, PA; [5]Pulmonary & Sleep Medicine, Lankenau Hospital, Wynnewood, PA; [6]Pathology, Lankenau Hospital, Wynnewood, PA.*

**Background**: In HD patients, BNP can be elevated in the absence of overt circulatory overload. We evaluated the presence of elevated BNP levels and it's correlation with pre/post HD volume status in ESRD patients.

**Methods**: 22 patients on maintenance high-flux HD without evidence of overt circulatory overload were studied. Volume status was assessed by history, clinical exam, and weight measurements. Pre/post-HD (1st of week) and pre- next HD (mid-week) measurements of BNP were collected and compared to patients' interdialytic weight gains (IDWG), weights pre/post/pre-HD, volume ultra-filtered (U.F.), and HD treatment variables.

**Results**: 19/22 HD patients had elevated BNP levels. Mean (1st of week) pre-HD BNP was 846.2pgm/ml; mean post-HD BNP was 895.4 pgm/ml; and mean pre mid-week BNP level was 755.2 pg/ml (nl<100pgm/ml). Mean group post-HD weights matched mean group EDW goal. Mean U.F. was 2.65 L and 2.55 respectively. Mean KT/V was 1.66. There was no significant change in pre/post/pre-BNP. There was no correlation with BNP and U.F. [p=0.615, 0.682, and 0.646 (pre/post/pre) respectively]. Post-HD BNP levels did not correlate with KT/V ($p = 0.927$).

**Conclusion**: BNP is elevated in HD patients. Despite patients achieving EDW, BNP did not change significantly pre/post-HD and did not correlate with total U.F. or KT/V. These results suggest volume may not be the causative factor for BNP elevation and that BNP is not cleared effectively with high-flux HD. Measuring BNP in HD patients may be misleading in assessing for circulatory overload states.

Disclosure of Financial Relationships: nothing to disclose

**PUB481**

**Circulatory Refilling Capacity of Patients in Hemodialysis** Olga R. Carmona. *Nephrology Center, University of Uruguay, Montevideo, Uruguay.*

**Background**. There is know that fluid overload require more ultrafiltration with the risk of lost of plasma volume preservation increasing the possibility of intradialytic hypotension(IH).The most common etiology of IH is the rapid fluid removal in a short time . Plasma Refilling Rate (PRR) mitigate the blood volume reduction by ultrafiltration.

**Objective**: Analize the correlation between PRR with IH and Hemodynamics Parameters at the end of Hemodialysis (HD).

**Methods**.29 patients on conventional HD were studied. Systolic Blood Pressure(SBP), Diastolic Blood Pressure (DBP), Heart Rate(HR), Ultrafiltration Rate (UFR) were measured at 10 minutes intervals throughout HD.Blood samples were obtained to measure Hematocrit used to calculate Plasma Volume (PV) pre ,second hour and at the end of HD. PRR was calculate with the difference between PV and UFR at the second hour and at the end of HD.Student t test was used to compare PRR at the second hour and the end of HD. Pearson correlation was used to establish the relation between the IH and hemodynamicsParameters,with PRR at the end of HD.

**Results**

A significant drop of PRR at the end of HD respect at the second hour were calculated:PRR,second hour :796,94ml±297,08ml;PRR at the end of HD:468,82ml±335,32ml (t= -3,74 ,p<0,001).

Correlation between Hemodynamics Parameters and PRR at the end of HD

| Hemodynamics Parameters | Mean ±StD | Correlation with PRRend | Significance:p<0,05 |
|---|---|---|---|
| IH | 0,38±0,49 | -0,788 | 0,0001 |
| PAS (mmHg) | 145,86±28,26 | 0,442 | 0,016 |
| PAD(mmHg) | 87,37±18,88 | 0,286 | 0,133 |
| HR (beats/min | 78±13,35 | 0,143 | 0,458 |
| UFR(ml/min | 891,03±26,921 | 0,455 | 0,013 |
| FR (ml) | 2867,86±858,10 | 0,400 | 0,032 |

38 % of the patients suffer hypotensive episodes at the end of HD.There was a significant negative Pearson Correlation between IH episodes with the PRR at the end of HD Besides there was a significative correlation between PAS , UFR and FR with the PRR .

**Conclusion**: A low Blood Pressure at the end of HD can be a sign of a too rapid Ultrafiltration in relation to the circulatory refilling capacity.

Disclosure of Financial Relationships: nothing to disclose

**PUB482**

**Heparin Influence on the Lipid Profile in Dialysis** Maria Mar Castilla, Francisca Lopez, Juan Payan Lopez, Cayetana Moyano Peregrin. *Nephrology, Hospital Costa del Sol, Marbella, Malaga, Spain.*

Purpose of the study

To evaluate the variations in the lipid profile during 3 dialysis sessions performed with different regimens of anticoagulation (unfractionated heparin (UFH), low molecular weight heparin (LMWH) and without heparin).

Methods

Prospective, randomized, controlled trial in 12 chronic hemodialysis (HD) patients. Measurement of triglycerides (TG), cholesterol (Col), HDL and LDL pre-HD and post-HD, in 3 dialysis sessions with the same breakfast (25g fat) and different anticoagulation. Values expressed as mean ± standard deviation. Statistical analysis Wilcoxon test (comparing dialysis: UFH vs no Heparin, LMWH vs no Heparin and UFH vs LMWH).

Results

Results

| | Percentage of increase during a dialysis session (mg/dL) | | | |
|---|---|---|---|---|
| | TG | Total Cholesterol | HDL | LDL |
| Unfractionated heparin | 28%(±29) | 8%(±9) | -0.2%(±10)* | 1% (±22) |
| Bemiparina | 38%(±31) | 4 %(±10) | 8%(±15) | -4%(±35) |
| Without Heparin | 64%(±43) | 7%(±10) | 14%(±16)* | -9%(±12) |

*p= 0.036

UFH drops HDL, the opposite occurs with the LMWH and dialysis without heparin. These differences are significant only when comparing the variations of HDL on dialysis with UFH with those of no heparin dialysis.

The larger increase of TG in dialysis without heparin versus dialysis with UFH and LMWH, can be justified because of the vascular endothelial lipoprotein lipase (LPL) release induced by heparin.

Conclusions

1. Decreases HDL cholesterol in dialysis conducted with unfractionated heparin and increases in dialysis without heparin.

2. The type of heparin used (unfractionated heparin vs bemiparin) has not shown significant differences on the lipid profile during a single session of hemodialysis under the conditions of this study.

Disclosure of Financial Relationships: nothing to disclose

**PUB483**

**End-Stage Renal Disease (ESRD) Prospective Payment System (PPS): Identifying and Assessing Impact on Hospital-Based Dialysis Centers (HBDCs)** George N. Coritsidis,[1] Anjali Acharya,[1] Ross Miller,[2] Kelly J. Ko,[2] Akhtar Ashfaq,[3] Irene Agodoa.[3] *[1]NYC Health and Hospitals Corporation, New York, NY; [2]Cerner LifeSciences, Beverly Hills, CA; [3]Amgen Inc, Thousand Oaks, CA.*

**Introduction:** HBDCs may be at increased financial risk under the proposed ESRD PPS. This has implications for quality and access to care for dialysis patients. This qualitative study was done to understand how HBDCs perceive the PPS. **Methods:** From 10/2009 to 2/2010, an online questionnaire targeted all HBDCs in the US (N=811) that provide outpatient and inpatient dialysis treatment to adults. Of these HBDCs, 698 were successfully contacted, 305 agreed to participate, and 127 completed the 37-question survey. Facilities could give multiple responses. To monitor consequences longitudinally, follow-up data will be collected over the next 3 yrs. **Results:** Hospital types included: community teaching (46), community non-teaching (36), academic (30), public (18), and other (7). Of these, 31% were affiliated with small hospitals (<200 beds) and 44% reported that other dialysis centers in the community were somewhat unavailable (9%) or scarcely available (35%). Mean reported distance to the nearest facility was 29 miles overall; 56 miles for those serving rural populations, and 24 and 42 miles for those reporting access as somewhat unavailable and scarcely available, respectively. The PPS was perceived as favoring large dialysis chains and potentially having a negative impact on quality and access to care. Dialysis centers affiliated with smaller hospitals were more concerned with financial risk *vs.* those affiliated with larger hospitals (85% *vs* 75%). There was concern that this may lead to closure, while those affiliated with larger hospitals were more concerned with being over-burdened due to closure of smaller nearby centers. Facilities treating <100 patients/yr reported they were at least somewhat unprofitable (57%) compared to facilities serving >100 patients/yr (31%). **Conclusions:** HBDCs believe they will be adversely affected by the PPS, which could potentially lead to decreased access to care for patients, especially smaller facilities serving rural populations.

Disclosure of Financial Relationships: Consultancy: Consultant for Amgen Inc and Cerner.

**PUB484**

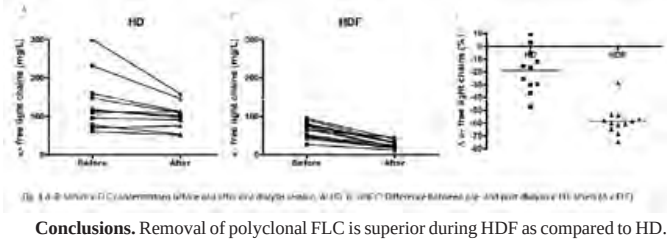
**Polyclonal Free Light Chains Are More Effectively Removed by Online Hemodiafiltration Than by Low-Flux Hemodialysis** Claire H. Den Hoedt,[1,2] Neelke C. Van Der Weerd,[3] R. W. Wulkan,[1] Albert H. Mazairac,[2] Muriel Grooteman,[3] E. Lars Penne,[2,3] Michiel Bots,[4] Peter J. Blankestijn,[2] Pieter M. Ter Wee,[3] Marinus A. Van Den Dorpel.[1] *[1]Maasstad Hospital, Rotterdam; [2]UMCU, Utrecht; [3]VUmc, Amsterdam; [4]Julius Center, Utrecht, Netherlands.*

**Background.** Serum κ- (22.5 kDa) and λ-FLC (45 kDa) concentrations increase with worsening renal function and have been associated with reduced neutrophil function. FLC may contribute to infection risk and inflammatory state in dialysis patients.

**Purpose.** To investigate whether polyclonal FLC are more effectively removed by online hemodiafiltration (HDF) than by low-flux hemodialysis (HD).

**Methods.** Blood samples were obtained in 12 HDF and 12 HD patients, at the beginning and end of 1 dialysis session. κ- and λ-FLC concentrations were determined by nephelometry with high-specificity immunoassays. Differences in FLC were compared by Mann-Whitney tests.

**Results.** κ-FLC changed from 116 mg/L (median, total range: 61-300) to 102 mg/L(51-158) in HD patients and from 66 mg/L (27-95) to 23 mg/L (12-46) in HDF. λ-FLC changed from 107 mg/L (58-201) to 103 mg/L (65-190) in HD and from 69 mg/L (37-336) to 50 mg/L (26-313) in HDF. Pre-dialysis levels were significantly different between the two groups (κ: p=0.002 and λ: p=0.03). The median difference in serum κ-FLC before and after dialysis was -17% (-47 to +9) in HD patients and -60% (-75 to -29) in HDF (p<0.001, for difference in Δ change). For λ-FLC this was -3% and -28% respectively (p=0.002). Weight change and residual kidney function did not differ between HD and HDF.




**Conclusions.** Removal of polyclonal FLC is superior during HDF as compared to HD. The lower pre-dialysis FLC levels in HDF suggest a sustained decrease in FLC. Further studies are needed to establish the association between FLC and clinical endpoints.

Disclosure of Financial Relationships: nothing to disclose

**PUB485**

**High-Flux Membranes in "Standard" Hemodialysis Technique Versus On-Line Hemodiafiltration** Carlo Donadio, Angeliki Kanaki, Danika Tognotti, Valentina Batini, Elena Donadio, Raffaele Caprioli, Alberto Lippi, Ettore Balestreri. *Internal Medicine - Nephrology, University of Pisa, Pisa, Italy.*

Bicarbonate hemodialysis is often performed using low-flux membranes, which do not clear the blood from "middle" molecules. On-line hemodiafiltration (HDF) with high-flux (HF) membranes is needed to remove β2-microglobulin (β2M) and other middle molecules. On-line HDF is performed by means of sophisticated dialysis monitors.

Aim of this study is to compare dialytic efficiency in the removal of small and middle molecules of HF membranes in "standard" HD technique in comparison with the use in on-line HDF technique.

Patients and methods. Eight patients, 31-78 years, dialytic vintage 11-228 months, in stable conditions and nutritional status, treated with HDF technique with HF membranes (HDF100 Fresenius 3 pts, Polyflux 210H 5 pts).

Dialytic efficiency, tolerability, and effects on inflammation, and cardiovascular parameters were assessed using three HF dialyzers (Triacetate: Nipro N190FH; Helixone: Fresenius FX1000; Polyamide: Gambro Polyflux 210H) in "standard" HD in comparison with on-line HDF technique.

Patients were treated in randomized rotation with each HF dialyzer in HD and HDF technique for 2 weeks, after an equilibration period of 1 month with usual HDF treatment. Blood- and dialysate-flow were maintained constant. Reinfusion fluid, in post-dilution during HDF, was 19.5±2.8 L.

The removal of small molecules was very high (urea reduction ratio 76.2-78.8 %) and similar with all membranes either in HD or in HDF. Phosphate removal ranged 39.3-58.6%. The removal of β2M was very high, 62-75%, in HDF and 60-68% in HD. β2M removal was significantly higher in HDF vs HD only with Poly210H. A significant removal of myoglobin, homocysteine and BNP was found with all membranes in HD and HDF. TNF concentrations decreased after all HDF dialysis and after HD with Poly210H e N190FH. A slight increase in IL10 was found with N190FH. IL6 was unchanged. The analysis of ultrafiltrate fluid demonstrated occasionally a modest concentration of albumin.

Conclusion. HD performed with HF dialyzers could be a cost-effective and efficient alternative treatment, simpler and cheaper than HDF.

Disclosure of Financial Relationships: nothing to disclose

**PUB486**

**Altered Sleep Architecture in Patients with End-Stage Renal Disease** Rosilene M. Elias,[1] Douglas Bradley,[2] Christopher T. Chan.[1] *[1]Nephrology, Toronto General Hospital, University Health Network, Toronto, ON, Canada; [2]Respirology, Toronto General Hospital, University Health Network, Toronto, ON, Canada.*

**Background and Hypothesis:** Although sleep disturbances in End Stage Renal Disease (ESRD) are widely recognized, the effect of uremia on sleep architecture is poorly investigated. We hypothesize that ESRD patients exhibit impaired sleep architecture independent of sleep-related breathing disorders.

**Methods**: We studied 57 ESRD patients (42 men) and 57 controls (46 men) who had undergone polysomnography. Control subjects were matched to the ESRD patients by age (52.9 ± 1.7 vs. 51.7 ± 2.2 yrs, p=0.67), body index mass (31.3 ± 0.8 vs. 29.4 ± 1.1 kg/m$^2$, p=0.18), periodic leg movement index (25.1 ± 4.4 vs. 27.7 ± 7.1 leg movements/hr sleep, p=0.75), and frequency of apneas and hypopneas/hr sleep (apnea-hypopnea index: 41.5 ± 3.9 vs. 41.0 ± 4.7, p=0.94). Sleep architecture was assessed by quantifying total sleep time, sleep efficiency (% of time asleep/sleep period time after lights out), amount and proportion of time spent in slow-wave (N3 sleep) and rapid-eye movement (REM) sleep, and the frequency of arousals from sleep (ArI).

**Results**: ESRD patients had shorter total sleep time, diminished sleep efficiency and reduced REM sleep (Table 1). Univariable analysis showed that total sleep time and REM sleep were independent related to ESRD (p=0.002 and p=0.008, respectively).

Sleep variables

| | Control (n=57) | ESRD (n=57) | p |
|---|---|---|---|
| Total Sleep Time (min) | 323.2 ± 10.22 | 276.9 ± 12.39 | 0.0018 |
| Sleep Efficiency (%) | 76.69 ± 2.07 | 69.61 ± 2.9 | 0.0497 |
| REM sleep (min) | 48.26 ± 3.29 | 34.46 ± 3.88 | 0.0078 |
| REM sleep (%) | 14.74 ± 0.98 | 11.09 ± 1.16 | 0.0175 |
| N3 sleep (min) | 37.9 ± 6.1 | 33.7 ± 4.0 | 0.5663 |
| N3 sleep (%) | 12.2 ± 1.7 | 11.5 ± 1.4 | 0.7716 |
| Arousals Index | 38.8 ± 24.6 | 49.0 ± 42.6 | 0.1210 |

**Conclusion:** ESRD patients exhibited impaired sleep architecture, independently of ssleep apnea, characterized by reduced sleep eiciency, total and REM sleep time. Prospective evaluation of the effect of intensifying uremia clearance on sleep morphology therefore warrants further examination.

Disclosure of Financial Relationships: nothing to disclose

**PUB487**

**Evaluation of Alternative Epoetin alfa Dosing Regimens To Reduce Hemoglobin Variability Using Pharmacokinetic Pharmacodynamic (PKPD) Model-Based Simulation** Bill Frame,[1] Diane R. Mould,[1] Thomas Comstock,[2] Sameer Doshi,[2] Juan Jose Perez Ruxio.[2] *[1]Projections Research, Poenixville, PA; [2]Amgen Inc, Thousand Oaks, CA.*

**Purpose:** To assess the impact of alternative Epoetin alfa (EA) dosing algorithms on within subject hemoglobin (Hb) variability in patients on hemodialysis (HD) using PKPD model-based simulation. **Methods:** A validated PKPD model of Hb response to EA in patients on HD with anemia was used to simulate Hb profiles in a cohort of 3000 virtual subjects, from which patients with stable dose and Hb levels between 10-12 g/dL were selected and randomly assigned to each of the 3 dosing arms (N≥750 subjects per arm). For each arm, doses were simulated to be administered thrice weekly and adjusted monthly; reduced by 25% if the Hb increased >2 g/dL/month; held if Hb ≥13 g/dL until ≤12 g/dL, then resumed at 25% lower dose. Dosing per label was compared with Dosing Algorithms A and B, which simulated graded dose changes of 10% and 25% at defined Hb values. The target Hb ranges for arms A and B were set to 10-12 g/dL and 10.5-11.5 g/dL, respectively. Subject doses were simulated to be titrated per the assigned algorithm for 6 months followed by a 6 month evaluation period. After Hb simulation, Hb variability was determined as the within subject Hb standard deviation (SD) during the evaluation period and % of values within the Hb range of 10-12 g/dL. **Results:** 2613 simulated subjects were randomized to the 3 dosing algorithms. Summary statistics show similar within subject Hb variability for the 3 dosing algorithms.

| Statistic, Mean (SD) | Label Dosing | Dosing Algorithm A | Dosing Algorithm B |
|---|---|---|---|
| N | 853 | 837 | 923 |
| Hb mean (g/dL) | 11 (0.8) | 11 (0.88) | 11 (0.79) |
| Within subject Hb SD (g/dL) | 1 (0.4) | 1 (0.4) | 1 (0.38) |
| Proportion Hb within 10-12 g/dL (%) | 59.2 (23.8) | 58.3 (24.3) | 62.6 (23.5) |

**Conclusions:** Results suggest that graded dosing approaches for EA will not result in reduced Hb variability within subjects and that Hb variability observed in prevalent patients on dialysis is not due to the dosing algorithm, but may be due to patient factors including intercurrent clinical events, which were not considered in this simulation exercise.

Disclosure of Financial Relationships: Consultancy: Owner of an S corp that consults for Projections Research.

**PUB488**

**Evaluation of Inpatient Urea Reduction Ratios in Chronic Hemodialysis Patients** Meghana R. Gaiki, Maria V. Devita, Michael F. Michelis. *Division of Nephrology, Department of Medicine, Lenox Hill Hospital, New York, NY.*

End stage renal disease (ESRD) patients on hemodialysis (HD) are frequently admitted to the hospital for dialysis and nondialysis related medical issues. There are no guidelines to measure adequacy of the HD prescription in a hospital setting despite there being core measure requirements for other medical diagnoses.

We sought to measure hemodialysis adequacy using urea reduction ratios (URRs) in chronic hemodialysis patients upon admission to the hospital and compare each patient's data to their outpatient (OP) hemodialysis prescription (HDRx) and URR. Information regarding URRs, HD access, blood flow rate (Qb), dialyzer clearance, duration of the HD session, ultrafiltration volume and blood pressures was recorded for both settings. The dialysate flow rate was 500ml/min for all HD sessions.

Data was collected for 19 patients. In the hospital, fifteen patients (80%) had a lower HDRx and 12 (63%) patients had URRs < 65%. All 12 had a lower HDRx, in terms of lower dialyzer clearance (n=7), shorter duration of HD session (n=10), lower Qb (n=9) or a combination of these variables (n=11). Discernible reasons for lower HDRx included: postoperative pain (n=2), hemodynamic instability (n=4), access problems (n=2), patient request (n=3) or a combination of these issues (n=3). Four patients had lower HDRx due to unclear reasons. Of interest, 6 (50%) of the 12 patients had URRs < 65% in the OP setting in the month just prior to admission. The remaining 7 (37%) of the 19 patients maintained adequate URRs in both settings.

In conclusion, a majority of the patients had URRs <65% and lower HDRx in the hospital due to concurrent medical problems including hemodynamic instability, postoperative pain and access problems. In some instances there was no apparent justification for the lower HDRx. It is important to monitor changing patient variables before each HD session. It may be worthwhile to measure dialysis adequacy in the hospital several times to optimize clearances.

Disclosure of Financial Relationships: nothing to disclose

**PUB489**

**Connective Tissue Growth Factor Is Effectively Removed by Online Hemodiafiltration, While It Is Not Removed by Low-Flux Hemodialysis** Karin G. Gerritsen,[1] Claire H. Den Hoedt,[1,3] Alferso C. Abrahams,[1] Tri Q. Nguyen,[2] Marinus A. Van Den Dorpel,[3] Muriel Grooteman,[4] Roel Goldschmeding,[2] Peter J. Blankestijn.[1] *[1]Nephrology, University Medical Center Utrecht, Netherlands; [2]Pathology, University Medical Center Utrecht, Netherlands; [3]Internal Medicine, Maasstad Hospital, Rotterdam, Netherlands; [4]Nephrology, VU Medical Center, Amsterdam, Netherlands.*

Connective tissue growth factor (CTGF) is an important profibrotic factor in chronic kidney disease (CKD). Previous studies also indicated pro-inflammatory properties and a potential role in atherosclerosis. The aminoterminal fragment (N-CTGF, 18 kD) is the predominant form in plasma. In CKD, CTGF accumulates in plasma. It is postulated that (N-) CTGF is a middle molecular weight uremic toxin, which could be removed by convective therapies. We investigated whether CTGF is removed by online hemodiafiltration (HDF) as compared to low-flux hemodialysis (HD).

We measured plasma CTGF at the beginning and end of 1 dialysis session in 12 HDF and 12 HD patients. CTGF levels are depicted as medians with ranges. Mann Whitney test was used for comparison of differences between pre- and post-dialysis CTGF levels.

Median pre-dialysis plasma CTGF was 2.6 nmol/L (1.0-7.0) in HD as compared to 2.7 nmol/L (1.4-4.7) in HDF (P=0.95). Median post-dialysis plasma CTGF was 2.8 nmol/L (1.1-7.3) in HD as compared to 0.91 nmol/L (0.31-2.6) in HDF. The median difference in plasma CTGF before and after dialysis was +13% (-30 to +37) in HD and -63% (-86 to -45) in HDF (P<0.001) (Fig. 1). Weight change, ultrafiltration volumes and residual kidney function did not differ between HD and HDF.

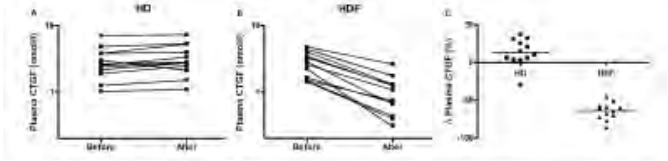



Fig. 1. A-B. Plasma CTGF levels before and after one dialysis session. A: HD. B: HDF. C: Difference between pre- and post-dialysis CTGF levels (Δ CTGF).

Our data show that CTGF is effectively removed by HDF while it is not removed by conventional low flux HD. Regarding the profibrotic and potential pro-inflammatory properties of CTGF, removal by HDF may have beneficial prognostic implications in dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB490**

**Impact of Phosphate Binder Choice on Missed In-Center Dialysis Treatments: The Perspective of a Small, Mid-Size, and Large Dialysis Organization in the US** Daniel Grima,[1] Jay B. Wish,[2] Lisa Bernard,[1] Elizabeth S. Dunn,[3] David C. Mendelssohn.[4] *[1]Cornerstone Research Group Inc., Burlington, ON, Canada; [2]University Hospitals Case Medical Center, Cleveland, OH; [3]Genzyme Corporation, Cambridge, MA; [4]Humber River Regional Hospital, Weston, ON, Canada.*

**Purpose:** Missed in-center dialysis treatments, including those due to hospitalization, have been associated with compromised patient outcomes and increased healthcare resource utilization. The objective of this study was to quantify the impact of sevelamer vs. calcium based binders (CBBs) on missed in-center dialysis treatments among hyperphosphatemic dialysis patients from the perspective of a small, mid-size, and large dialysis organization in the US. **Methods:** A model was developed in Excel to estimate missed dialysis sessions among three hypothetical cohorts of hyperphosphatemic patients treated with either sevelamer or CBBs. The cohorts were characterized by their size to represent a small, mid-size or large dialysis organization (75, 30,000 and 120,000 patients, respectively). In any given month, a patient in the model could receive dialysis treatments within the center, experience a hospitalization or die. Treatment-specific monthly survival rates, hospitalization rates, length of stay and binder dosages were derived from the published literature. A dialysis schedule of 3 treatments per week was assumed. Analyses were conducted for a time horizon of 1 year. **Results:** The use of CBBs was associated with an increased number of missed in-center dialysis treatments compared to the use of sevelamer over 1 year. The magnitude of sevelamer's impact on maintaining in-center dialysis treatments increased with the size of the dialysis population.

Missed In-Center Dialysis Treatments due to Hospitalization

| | Calcium | Sevelamer | Difference |
|---|---|---|---|
| Small (N=75) | 446 | 395 | 51 |
| Mid (N=30,000) | 178,714 | 158,142 | 20,572 |
| Large (N=120,000) | 714,857 | 632,571 | 82,286 |

**Conclusions:** Treatment of hyperphosphatemic dialysis patients with sevelamer relative to CBBs is associated with a reduction in missed in-center dialysis treatments across small, mid-size and large dialysis organizations.

Disclosure of Financial Relationships: Consultancy: Daniel Grima is an employee of and shareholder in Cornerstone Research Group, Inc. and as such, has received funds as an consultant to Genzyme Corporation.

**PUB491**

**Inclusion of Dialysis Costs in Cost-Effectiveness Analyses of Therapies for Patients on Dialysis: A Case Study of Sevelamer for the Treatment of Hyperphosphatemia** Daniel Grima,[1] David C. Mendelssohn,[2] Elizabeth S. Dunn,[3] Lisa Bernard.[1] *[1]Cornerstone Research Group Inc., Burlington, ON, Canada; [2]Humber River Regional Hospital, Weston, ON, Canada; [3]Genzyme Corporation, Cambridge, MA.*

**Purpose:** There is uncertainty as to whether the cost of dialysis is related to a therapy that extends the life of a patient on dialysis, but does not impact the need for or the extent of dialysis, and consequently, uncertainty around whether dialysis costs should be included in cost-effectiveness analyses (CEAs) of these therapies. This study examined the cost-effectiveness of sevelamer versus calcium-based binders (CBBs) as treatment for hyperphosphatemia in dialysis patients and, within this context, the suitability of including dialysis costs in such CEAs. **Methods:** A Markov model estimated life years, incremental cost per life year (LY) gained and incremental quality-adjusted life year (QALY) gained. Treatment-specific survival was derived from the Dialysis Clinical Outcomes Revisited (DCOR) study and extrapolated using Weibull regression. The base case analysis used resource use and survival data for patients ≥ 65 years combined with Canadian unit costs and utility weights from the literature. Analyses were conducted for a 10-year time horizon using the Alberta Health Care System perspective, with costs and outcomes discounted at 5% per year. **Results:** In the case where dialysis costs were excluded, sevelamer resulted in a gain of 1.02 LYs and 0.62 QALYs per patient compared with CBBs, producing ratios of $20,847/LY and $34,175/QALY gained. Inclusion of dialysis costs resulted in ratios above $90,000/LY and $150,000/QALY gained. **Conclusions:** No therapy that extends the life of dialysis patients, without decreasing the need for or the extent of dialysis, can be cost-effective if dialysis costs are included. As a result, patients requiring dialysis could be denied access to life-extending therapies, simply because dialysis is costly. We conclude that dialysis costs should not be included in CEAs of such therapies and recommend that health economic guidelines, practice and public policy in this area be consistent, ethical and non-discriminatory.

Disclosure of Financial Relationships: Consultancy: Daniel Grima is an employee of and shareholder in Cornerstone Research Group, Inc. and as such, has received funds as an consultant to Genzyme Corporation.

**PUB492**

**Endotoxaemia in Haemodialysis Patients Is Not Associated with Excessive Hydration** Laura E. A. Harrison,[1] Cian Chan,[1,2] James O. Burton,[1] Simon J. Davies,[2] Cheuk-Chun Szeto,[3] Philip K. T. Li,[3] Chris W. McIntyre.[1,4] *[1]Department of Renal Medicine, Royal Derby Hospital, United Kingdom; [2]Department of Nephrology, University Hospital of North Staffordshire, United Kingdom; [3]Medicine & Therapeutics, Chinese University of Hong Kong, Hong Kong; [4]School of Graduate Entry Medicine and Health, University of Nottingham, United Kingdom.*

Endotoxin (ET) has a wide range of adverse effects on cardiovascular function; driving inflammation, atherosclerosis and cardiovascular mortality. ET translocation from gut to circulation has been associated with fluid overload in heart failure and may be a factor in endotoxaemia in PD patients. ET levels are grossly elevated in haemodialysis (HD) patients, but assessment with indirect measures of fluid status (left atrial volume, BNP) has not demonstrated a relationship with endotoxaemia. We aimed to assess endotoxaemia with reference to absolute measures of body composition.

14 established HD patients were studied using a gold standard Deuterium dilution (De) method and Bioimpedance Analysis (BIA) to assess total body water (TBW) pre and post HD. Deuterium concentration was measured in breath by flowing-afterglow mass spectrometry, ensuring full equilibration. BIA was measured using a multifrequency, multisegmental device. Blood samples were taken pre and post HD.

Patient age was 65±15 yrs and dialysis vintage 19 months [IQR 9-60]. Mean ET pre-HD was 0.72±0.39 EU/ml, which inversely correlated to $TBW_{De}$ pre and post HD (r= -0.657 in both groups, $p<0.05$) as well as to BMI (r=-0.565, p=0.035). However, there was no relationship between TBW indexed to weight (TBW/body weight) and ET levels. $TBW_{De}$ and $TBW_{BIA}$ correlated pre and post HD (r=0.956 and 0.943, $p<0.001$).

HD results in severe endotoxaemia, but factors influencing this are not fully understood. In contrast to heart failure, excessive hydration does not appear to be a significant driver of endotoxaemia in HD. Furthermore, relative obesity appears to be associated with lower levels of serum ET. This may relate to lean/fat mass hydration or relative protection against bowel hypoperfusion as a result of omental tissue load and could be related to the observed positive effects of obesity on HD survival.

Disclosure of Financial Relationships: nothing to disclose

**PUB493**

**Relative Blood Volume Monitoring during Hemodialysis: A Qalitative Indicator of Fluid Overload** Jasmine Ion Titapiccolo,[1] Manuela Ferrario,[1] Francesco Garzotto,[2] Maria Gabriella Signorini,[1] Dinna N. Cruz,[2] Flavio Basso,[2] Alessandra Brendolan,[2] Federico Nalesso,[2] Ulrich Moissl,[3] Ciro Tetta,[3] Sergio Cerutti,[1] Claudio Ronco.[2] *[1]Department of Bioengineering, Politecnico di Milano, Milano, Italy; [2]San Bortolo Hospital, Vicenza, Italy; [3]Fresenius Medical Care Deutschland GmbH, Germany.*

**Background.** Inadequately removed fluid volume may lead to chronic fluid overload which can lead to hypertension, left ventricular hypertrophy and heart failure. This exploratory study aims at identifying new parameters obtained from continuous Blood Volume Monitoring allowing an indirect evaluation of hydration status.

**Methods.** 33 chronic HD patients from the dialysis unit of the San Bortolo Hospital in Vicenza were enrolled. Before each HD treatment, fluid overload (FO) was assessed with a whole body bioimpedance spectroscopy device (BCM, Fresenius Medical Care). During HD, blood volume reduction (BVR%) was monitored by a real-time online ultrasonic blood volume monitor (BVM, Fresenius Medical Care). Over the first 30 minutes and the last 30 minutes the BVR% values were linearly interpolated and their slopes were assessed. The parameters considered in this work are: initial BVR% slope (slope$BV_0$), final BVR% slope (slope$BV_{end}$), and BVR% reduction, which corresponds to the difference between the initial value (100%) and the final value.

**Results.** Patients with higher FO (>2.5l) have a significantly lower slope$BV_0$ and lower BVR% reduction in respect with the patients with FO<2.5l, whereas the final slope was not significantly different between the two groups.

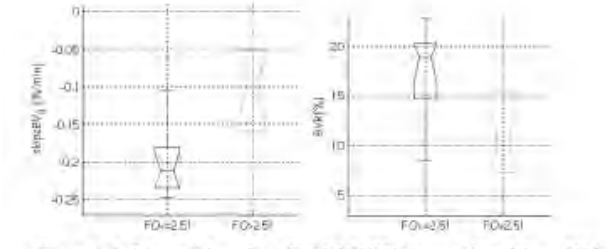



Figure 1. Left panel, boxplot of initial BVR% slope values (slope $BV_0$). Right panel, boxplot of BVR% reduction in the two populations: not overhydrated (FO≤2.5l) and overhydrated patients (FO>2.5l).

**Conclusion.** The slope of the RBV% values, estimated in the first 30 minutes of treatment, was found to be a useful parameter to identify previously unrecognized states of overhydration. The proposed parameter can be used as a qualitative indicator of fluid overload.

Disclosure of Financial Relationships: nothing to disclose

**PUB494**

**Systematic Review of Effects of Erythropoiesis-Stimulating Agents (ESAs) on Fatigue among End-Stage Renal Disease (ESRD) Patients on Dialysis**
Kirsten L. Johansen,[1] Dennis A. Revicki,[2] Fredric O. Finkelstein,[3] Christopher Evans,[4] Shaowei Wan,[5] Irene Agodoa.[5] *[1]UCSF, San Francisco, CA; [2]United BioSource Corp, Bethesda, CA; [3]Hospital of St Raphael, Yale University, New Haven, CT; [4]MapiValues, Inc., Boston, MA; [5]Amgen Inc, Thousand Oaks, CA.*

**Purpose**: Improvement in health related quality of life remains an important goal of treating patients with ESRD. Recent data highlighted the impact of ESA therapy on improving physical function and exercise tolerance in this population. The purpose of the present study was to review the literature to better understand the effect of ESA therapy on dialysis patients' perception of fatigue. **Method:** A systematic literature review was conducted on the effects of ESAs on fatigue using MEDLINE and EMBASE, limited to articles published in English from 1988 to 2010. In addition, reference lists of identified papers were searched. To meet inclusion criteria, the studies had to report data on a measure of fatigue before and after an intervention with any ESA among adult patients undergoing dialysis. **Results:** 76 studies were identified; 13 examined the effects of ESAs on fatigue outcomes and met inclusion criteria. Of those, 3 randomized controlled trials (RCT) used Kidney Disease Questionnaire (KDQ) to measure fatigue. The incremental increase in KDQ fatigue score between baseline and follow-up (24 weeks or 48 weeks) was higher when baseline hemoglobin (Hb) level was lower. Mean KDQ fatigue score changes were 0.16 (median change), 0.46, and 1 in the ESA treatment group with achieved Hb level of 13.5 g/dL, 12.2 g/dL, and 10.2 g/dL (low)/11.7 g/dL (high) compared with baseline Hb level of 11.1 g/dL, 9-11 g/dL, and 7 g/dL respectively (clinically meaningful difference in KDQ score is 0.5). Four studies measured SF-36 vitality scores and found a 0.9 to 24.8% improvement in vitality at various follow-up times. **Conclusion:** Treating anemia is associated with an improvement in fatigue among dialysis patients. Additional research is needed to examine the benefit of treating anemia to current Hb targets on other anemia-associated symptoms among dialysis patients.

Disclosure of Financial Relationships: Research Funding: Amgen, Abbott Laboratories; Scientific Advisor: Amgen Nephrology Advisory Board.

**PUB495**

**Interdialytic Weight Gain: Predictors and Associations**
Kotagal Shashi Kant,[1,2] Heather Duncan,[1,2] Karthikeyan Meganathan,[3] Zona E. Saylor,[1,2] Mahmoud T. El-Khatib,[1,2] Abhijeet Goyal.[1,2] *[1]Nephrology and Hypertension, Univ Cincinnati Coll Med, Cincinnati, OH; [2]Dialysis Clinic, Inc, Cincinnati, OH; [3]Public Health Sciences, Univ Cincinnati, Cincinnati, OH.*

High cardiovascular morbidity and mortality remains a vexing problem for HD patients. High IDWG (interdialytic weight gain) is associated with increased mortality but also with better nutrition. Adherence to diet and HD time are factors controlled by patients. We assessed the contribution of dialysis time, microinflammation, demographic, laboratory and HD treatment specific factors on IDWG in a cohort of HD patients.

**Methods:** 97 HD pts treated for at least one month were included in this analysis of treatments Jan-Mar 2010. All treatments at one outpatient dialysis center, with an interdialytic interval of 2 or 3 days, were reviewed for IDWG, treatment duration, BVP (blood volume processed) & EPO dose given, as well as pre- and post-sitting blood pressures and weights. IDWG per day was estimated by dividing the IDWG by the interval; these values were then normalized (nIDWG) to the patient's EDW (estimated dry weight). The cohort was divided into two groups based on the median value, hi-nIDWG & lo-nIDWG. All treatment parameters, as well as common labs, EPO dose/kg and basic demographics were described for these two groups.

**Results:** nIDWG was positively correlated with number of BP meds and EPO dose/kg. Hi-nIDWG was correlated negatively with Hgb, corrected calcium, BVP & EDW. A multivariable logistic regression model showed that BVP and corrected Ca remained significantly associated with nIDWG. Comparison of the highest and lowest quartiles of nIDWG showed the same differences we found in analysis of the entire cohort.

**Conclusions:** Our results suggest that patient specific variables are associated with hi-nIDWG. Surprisingly patients with lower EDW had higher nIDWG, as well as lower BVP values despite equivalent HD times. Hemodynamic instability is possibly responsible for the lower BVP. We will examine echocardiographic parameters for further explication of this finding. Our results support the survival disadvantage of lower body mass in HD patients. Further study is needed of this problem.

Disclosure of Financial Relationships: nothing to disclose

**PUB496**

**Adjustments of Dialysate Potassium Concentration in Response to Daily Changes in Serum Potassium in Chronic Outpatient Hemodialysis**
Csaba P. Kovesdy,[1,2] Jun Ling Lu,[3] Sandra M. Malakauskas.[1] *[1]Salem VA Medical Center, Salem, VA; [2]University of Virginia, Charlottesville, VA; [3]Salem Research Institute, Salem, VA.*

Abnormal serum potassium (SeK) is associated with increased mortality in dialysis patients. Dialysate potassium concentration (DiK) is adjusted based on monthly SeK measurements in chronic outpatient hemodialysis (HD). Short term fluctuations in SeK may require more frequent adjustments, but the ideal frequency of SeK measurement is unknown.

We measured SeK before each HD session for 4 weeks in 36 outpatients undergoing 3x/week HD, with adjustment of DiK as needed. We examined correlations of SeK and of the number of implemented adjustments in DiK with various clinical characteristics using Pearson correlation coefficients.

Patients were all males, mean (SD) age was 68.6 (9.9) years, 53% were black and mean (SD) baseline SeK was 4.9 (0.6) mmol/L. At baseline 47% and 53% of patients used a DiK of 2.0 and 3.0 mmol/L, respectively. Higher SeK was associated with dialysis on the first day of the week and with younger age, white race, lower KT/V and DiK, and higher protein catabolic rate. Changes in DiK were implemented 68 times (16% of all the treatments, median (range) of 2 (0-5) changes/month/patient). The number of changes/patient in DiK did not correlate with clinical characteristics (Table).

Adjustments in DiK are warranted in a significant proportion of outpatient dialysis sessions based on short term fluctuations in SeK. The current paradigm of once-a-month SeK monitoring is inadequate and could result in the application of inappropriate DiK concentrations, which could potentially contribute to a higher incidence of arrhythmias. Routine implementation of more frequent SeK monitoring should be considered in chronic outpatient HD.

Correlation of the number of DiK adjustments/patient/month with clinical characteristics

| | correlation coefficient | p value |
|---|---|---|
| Age | 0.26 | 0.13 |
| Race | -0.13 | 0.5 |
| BMI | -0.23 | 0.17 |
| Treatment time | -0.12 | 0.5 |
| Type of access | 0.03 | 0.8 |
| KT/V | -0.03 | 0.8 |
| nPCR | 0.01 | 0.9 |
| Serum K | -0.28 | 0.11 |
| Serum albumin | 0.18 | 0.3 |
| Dialysate K | -0.21 | 0.2 |

Disclosure of Financial Relationships: Consultancy: GenzymeResearch Funding: Abbott, Genzyme, Shire; Honoraria: Genzyme, Novartis, Shire.

**PUB497**

**Is Bioimpedance Spectroscopy (BIS) Useful in Determination of Dry Weight (DW) in the Elderly Dialysis Patients?**
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The aim of this pilot study was to test the applicability of bioimpedance spectroscopy (BIS) and to describe the hydration status and body composition (BC) of a cohort of elderly dialysis patients above 75 years in our dialysis unit.

A total of 34 pts > 75 years were registered, 10 were excluded (1 due to language problems, 4 were too ill, 1 transplanted and 4 due to practical problems), 24 pts included in the study. To quantify fluid status and assess BC (lean tissue mass and adipose tissue mass), whole body BIS assessments was made using the Fresenius Body Composition Monitor (BCM). Electrodes were placed on hand and foot, in a supine position, and the measurements were done before dialysis session.

BCM results.

| | |
|---|---|
| Age m | 81.4 years |
| Blood pressure (before dialysis) m | 158/76 mm/Hg |
| BMI Body mass index (after dialysis) m | 24.7 kg/m² |
| LTI (lean tissue index) m | 10.8 (10 pt < 10th percentile) |
| FTI (fat tissue index) m | 13.1 (8 pt > 90 th percentile) |

m= mean

Overhydration (OH) was registered in 17 pts, i.e. moderate in 9 ( OH > 1.0 L and < 2.5 L), serious in 8 (OH > 2.5 L). Only four pts in each groups was planned for ultrafiltration. Residual renal function: diuresis (D)> 1 L/ 24 h in 9 pts, 7 pts D: 0.5 - 1 L/ 24 h and 8 pts anuric. Se-albumin 37.5 g/l (36 - 45). According to BMI, 1 pt had underweight (< 18.5), 15 pts had normal weight (18.5 – 24.9), 6 pts overweight (25.0 – 29.9) and 2 pts were obese (BMI > *30).* The BCM revealed a low mean LTI (very low LTI in 10 pts) whereas the mean FTI was high (very high FTI in 8 pts) indicating protein energy wasting (PEW) with muscular depletion combined with fat gain. The BCM enables more precise estimates of the pts's nutritional status than se-albumin and BMI, indicating PEW in a substantial proportion of elderly dialysis patients.The results indicate that the BCM may be helpful to detect overhydration (OH > 2.5L) and to determine dry weight in elderly dialysis patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB498**

**Does Hemodiafiltration Alter Protein Binding of Drugs Compared to Conventional Hemodialysis?**
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BACKGROUND: Online high efficiency hemodiafiltration (ol-he-HDF), which combines convection and diffusion, showed improved clearance of middle weight molecules and protein-bound solutes compared to conventional hemodialysis (HD), and better control of the inflammatory state. This may interfere with drug protein binding. METHODS: We compared protein binding of phenytoin (an acidic drug bound to albumin) and quinine (a basic drug bound to alpha-1 acid glycoprotein) in predialysis serum of 25 patients on chronic renal replacement therapy for at least 6 months (12 ol-he-HDF patients with reinfusion

volume >23 liters per dialysis session and 13 conventional hemodialysis patients; all with *Kt/V* ≥1.2) and 11 healthy control volunteers. Protein binding was measured using in vitro equilibrum dialysis with radiolabeled $^{3}$H-Quinine and $^{14}$C-Phenytoin added to each subject's serum.

Percentage of free phenytoin and quinine

| | Albumin (g/L) | AAG (g/L) | % free phenytoin | % free quinine |
|---|---|---|---|---|
| PreHDF | 37±3* | 1.1±0.4* | 17.2±1.3* | 12.2±2.8 |
| PreHD | 35±3* | 1.3±0.3* | 19.1±4.5* | 12.8±4.8 |
| Controls | 41±2 | 0.7±0.2 | 11.9±0.5 | 13.7±2.0 |

Data are presented as group mean ± SD; *p<0.05 versus normal subjects (two-sample t-test); AAG, alpha-1 acid glycoprotein; HDF, online high efficiency hemodiafiltration; HD, conventional hemodialysis.

RESULTS: Levels of free phenytoin were increased in end-stage renal disease patients compared with controls. No differences were observed between HD and ol-he-HDF patients for protein binding with phenytoin. Levels of free quinine were similar in ol-he-HDF patients, HD patients and controls. CONCLUSIONS: Online high efficiency hemodialfiltration does not seem to alter significantly predialysis protein binding of drugs compared to conventional hemodialysis.

Disclosure of Financial Relationships: nothing to disclose

**PUB499**

**Assessment of Hemoglobin (Hb) Responsiveness to Epoetin alfa (EPO) in Patients on Hemodialysis (HD) Using a Population Pharmacokinetic Pharmacodynamic (PKPD) Model** Diane R. Mould,[1] Thomas Comstock,[2] Sameer Doshi,[2] Juan Jose Perez Ruxio.[2] *[1]Projections Research, Phoenixville, PA; [2]Amgen Inc, Thousand Oaks, CA.*

**Purpose**: Develop a population PKPD model describing the effect of EPO on Hb response in HD patients for use in simulation of alternative EPO dosing. **Methods**: A PKPD model was developed using patient demographics, clinically relevant covariates, and EPO and Hb concentrations from 4 clinical trials (15 subjects with PK and 118 subjects with Hb). EPO PK were described using an open 2 compartment model with linear elimination; predicted individual time courses served as the input function to the PD model. A maturation-structured cytokinetic model consisting of 5 compartments linked in a catenary fashion was used to describe the time course of Hb, following a zero-order process and stimulated by EPO according to an Emax function. Visual predictive checks using data from 3 studies were performed to qualify the model. **Results**: The PK and PD of EPO were well described by the model. PK parameter estimates were similar to values previously reported. Population mean (%CV) parameters were clearance (L/h)0.64(27); central volume (L)9.4(49); intercompartmental clearance (L/h)0.12(52); and peripheral volume (L)3.7(67). The PD model described two subpopulations: i. with Hb based on their EPO dosing and ii. with Hb not apparently related to their EPO dosing. The second subpopulation accounted for 18.6% of the subjects and included those who experienced serious infections, bleeding or other intercurrent events that could cause a substantial change in Hb. Parameter estimates from the PD model were physiologically reasonable and consistent with published reports. Population mean (%CV) parameters were maximum effect (g/dL/d)0.17(5.7); $SC_{50}$(Units/L)11(32); and red blood cell lifespan (d) 73(2.8). **Conclusions:** Hb response to EPO in stable patients on HD is successfully described using a population PKPD model. The model provides a platform for simulation of Hb using different dosing strategies. Further work is needed to model the variability observed in patients with intercurrent events, such as infection and inflammation that can influence Hb response to EPO.

Disclosure of Financial Relationships: Consultancy: Dr Mould is a paid consultant of Amgen.

**PUB500**

**Distributions of and Correlations among Retained Organic Solutes in Hemodialysis Patients: The Retained Organic Solutes and Clinical Outcomes in Hemodialyis (ROSCO) Study** Laura C. Plantinga,[1] Timothy W. Meyer,[2] Thomas H. Hostetter,[3] Josef Coresh,[4] Michal L. Melamed,[3] Nancy E. Fink,[4] Natalie Plummer,[2] Zhe Quan,[3] Pooja C. Oberai,[4] Neil R. Powe.[1] *[1]University of California, San Francisco, CA; [2]Stanford University, Palo Alto, CA; [3]Albert Einstein College of Medicine, Bronx, NY; [4]Johns Hopkins University, Baltimore, MD.*

The accumulation of organic waste products is thought to contribute to poor outcomes in hemodialysis (HD) patients, but little is known about the distributions and inter-correlations of these putative uremic toxins. In a cohort of 521 incident HD enrolled 1995-1998 from 71 US clinics, we measured and compared levels of unique organic solutes [p-cresol sulfate (PCS), indoxyl sulfate (indican), methylamine (MMA), and dimethylamine (DMA)]. Using thawed plasma samples from ~6 mo after start of dialysis, we measured the solute levels by high-performance liquid chromatography with fluorescence detection. Quality control analyses with duplicate samples showed good agreement for all five solutes (r=0.89-0.99, P<0.001 for all). Median (interquartile range) values were: PCS, 3.1 (1.9-4.3) mg/dl; indican, 1.6 (1.0-2.4) mg/dl; MMA, 49.6 (43.2-56.2) μg/L; and DMA, 18.3 (16.1-20.5) μM; all levels were far greater than those in healthy individuals. Most inter-correlations were statistically significant (P<0.05) but weakly positive, with PCS and indican being the most highly correlated (r=0.33), followed by DMA and indican (r=0.19) and MMA and indican (r=0.18). PCS levels were higher in patients who were male, non-obese, without history of gastrointestinal disease, and with baseline creatinine ≥7 mg/dl and BUN ≥50 mg/dl. Indican levels were higher in those with fewer comorbidities; higher creatinine and BUN; and lower white blood cell counts. Higher MMA and DMA were associated with higher creatinine; higher DMA was additionally associated with no residual urine at baseline, obesity, and non-white race. In summary, levels of retained organic solutes vary markedly by HD patient and the solutes are only weakly correlated with each other. Individual solute levels are associated with different patient factors and may contribute to variation in patients' symptoms.

Disclosure of Financial Relationships: nothing to disclose

**PUB501**

**Hemodialysis Prescription Optimization Decreases Intradialytic Hypotension: A Quality Assurance Study** Davina J. Tai, Joslyn D. Conley, Jennifer M. MacRae. *Medicine, University of Calgary, Calgary, AB, Canada.*

Intradialytic hypotension (IDH) is a common complication of hemodialysis (HD). We have previously shown that preventative strategies for IDH are underutilized (Tai ASN 2008). In this quality assurance/quality improvement (QA/QI) study, we determined the effect of deliberate HD prescription optimization on the incidence of IDH and the prevalence of IDH-prone patients in the Foothills Medical Centre HD unit in Calgary, AB. Two 4 week study periods were compared: the control period (Aug 19-Sept 15, 2007) and the intervention period (Sept 6-Oct 3, 2009). The intervention period occurred after 2 months of weekly didactic education for rounding HD physicians and focused HD prescription optimization during bedside HD rounds. HD prescriptions were modified as deemed clinically appropriate by rounding HD physicians. IDH was defined as a drop in systolic blood pressure (SBP) ≥ 20 mmHg (if the pre-HD SBP was ≤ 100mm Hg, then IDH was defined as a drop in SBP ≥ 10 mmHg) with associated symptoms or intervention. Patients were classified as IDH-prone if IDH occurred in 30% of their HD sessions over 4 weeks. The control and intervention groups consisted of 91 and 82 patients, respectively. Patients in the intervention group were younger (62 years vs 70 years, p=0.01) and had less diabetes (43% vs 59%, p=0.03). The incidence of IDH was 11% in the intervention group vs 17% in the control group (p=0.0002). 13% of patients in the intervention group were IDH-prone vs 23% in the control group (p=0.10). Patients in the intervention group were prescribed multiple preventative strategies more frequently than in the control group (p=0.02). HD prescriptions in the intervention group utilized lower dialysate temperatures (89% vs 55%, p<0.001) and combination sodium and ultrafiltration profiling (20% vs 8%, p=0.02) more frequently than in the control group. At present, there is no standard of care in managing IDH. Our results illustrate that a QA/QI program to optimize HD prescriptions significantly decreases the frequency of IDH in chronic HD patients. More studies are needed to identify superior prophylactic and therapeutic options for IDH.

Disclosure of Financial Relationships: nothing to disclose

**PUB502**

**Exercise Increases Intradialytic Urea Clearance in Chronic Hemodialysis Patients** Davina J. Tai,[1,2] Kristen Parker,[2] Joslyn D. Conley,[1,2] Jennifer M. MacRae.[1,2] *[1]University of Calgary, Calgary, AB, Canada; [2]Southern Alberta Renal Program, AB, Canada.*

Hemodialysis (HD) adequacy is based on urea kinetics and urea clearance. Low blood flow to muscles during HD is a rate-limiting factor to urea clearance. Theoretically, intradialytic exercise increases muscle perfusion, thereby increasing urea clearance and HD adequacy. We performed a retrospective study to determine the effect of active intradialytic exercise on intradialytic urea clearance in patients from 2 community HD units in Calgary, AB. Patients were eligible if they were on HD at least 3 times per week for at least 3 months and were participating in a voluntary intradialytic exercise program consisting of active stationary cycling supervised by an exercise therapist. The primary outcome was change in average intradialytic urea clearance (ΔKurea) with exercise as measured by ionic dialysance during HD. Thirty-one patients with a mean age of 70 years were included in the study. Intradialytic exercise began at a mean time of 61 minutes into HD, with a mean total exercise time of 34 minutes. The average Kureas pre-exercise, during exercise, and post-exercise were 220mL/min, 245mL/min, and 220mL/min, respectively. With intradialytic exercise, there was a significant increase in Kurea (ΔKurea = +25mL/min [95% CI 15 to 36; p<0.0001]) which returned to baseline once exercise was stopped (ΔKurea= -24mL/min [95% CI -34 to -16; p<0.0001]. Our study illustrates that intradialytic exercise is associated with increased intradialytic urea clearance. More studies are needed to determine if intradialytic exercise translates into improved clinical outcomes for HD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB503**

**Effects of Protein-Leaking Dialyzer on Plasma Pentosidine Concentration, a Marker of Carbonyl Stress, in Hemodialysis Patients** Hidenori Yamazaki, Fumihiro Tomoda, Tsutomu Koike, Takeshi Hayashi, Hiroyuki Kinuno, Satoshi Kagitani, Taizo Nakagawa, Hiroshi Inoue. *The Second Department of Internal medicine, University of Toyama, Toyama, Japan.*

Objective: Over 90% of plasma pentosidine (PEN), a marker of carbonyl stress is bound to albumin. Because only the free fraction of PEN is available for diffusion through dialysis membrane, the removal of PEN is low in conventional hemodialysis (HD). However, the effects of protein-leaking dialyzer on albumin-bound solutes such as PEN remained to be elucidated in hemodialysis patients.

Design and Methods: In the present study, the removability of PEN was compared between two types of triacetate dialyzer with different membrane pore sizes, FB-UH and FB-G (having pore radius of 76 and 60Å, respectively). Eight HD patients were treated with FB-UH and FB-G by crossover manner in the same condition for three months each.

Plasma PEN and the leakage of albumin into dialysate effluent were measured at the end of each treatment. Removal rate of solutes was calculated from their pre-HD and post-HD blood levels.

Results: Although the removal rate of small molecular solutes such creatinine and urea did not differ between the two dialyzers, $\beta_2$-microglobulin was removed more efficiently by FB-UH compared with FB-G. At pre-HD levels, plasma PEN was lower in FB-UH compared with FB-G. Plasma PEN decreased after HD in FB-UH, but did not change in FB-G (0.35±0.10 to 0.26±0.07 vs 0.70±0.09 to 0.70±0.14 μg/mL; HD × dialyzer interaction, $p<0.05$). Consequently, the removal rates of PEN were greater in FB-UH than in FB-G (24.7±2.5 % vs 4.9±7.0 %, $p<0.05$). The leakage of albumin into dialysate effluent was also greater in FB-UH compared with FB-G (2.41±0.41 g vs 0.51±0.02 g, $p<0.05$). Additionally, the removal rate of PEN was correlated positively with the leakage of albumin into dialysate effluent ($r=0.56$, $p<0.05$) in the combined measurements of both dialyzers.

Conclusion: The protein-leaking dialyzer, FB-UH reduced carbonyl stress efficiently in HD patients. The reduction of carbonyl stress in FB-UH was attributed to the removal of albumin-binding PEN via leakage into dialysate effluent.

Disclosure of Financial Relationships: nothing to disclose

**PUB504**

**Effect of Different Dialyzers on Clearance of beta-2 Microglobulin and Small Weight Molecule Toxins in Hemodialysis Patients** Jihong Yang, Hua Wu, Tianqing Tang, Xian-Guang Chen. *Department of Nephrology, Beijing Hospital, Beijing, China.*

**Objective:** To compare effect of different dialyzers on clearance of beta-2 microglobulin(β2-MG) and small weight molecule toxins in maintain hemodialysis patients. **Methods:** Sixty-seven stable Chinese hemodialysis (HD) patients were recruited into this one time HD or hemodialysisfiltration (HDF) study in January, 2010. The exclusion criteria were: on HD less than 3 months; central venous catheter; heart failure and acute infection. The patients were divided into B1-1.6H (PMMA membrane) HD, F7APS (Fresenius Polysulfone) HD, FX60(Helixone) HD and APS900 (ASAHI Polysulfone) HDF four groups by different dialyzers. They had 3 times HD per week, each HD lasted 4 hours. Blood chemistry, hemoglobin and β2-MG were measured before and after HD or HDF session. **Results:** Of the 67 patients, average age was 60.8±3.4 years , ratio of male to female was 36 to 31, duration on HD was 65.2±46.2 months, body weight was 64.4±10.7kg, hemoglobin was 115.0±11.6g/L, serum albumin was 39.9±2.34g/L. The age, gender, proportion of patients with diabetic, body weight, hemoglobin and serum albumin was not significantly different among four groups. Reduction rate of serum urea in B1-1.6H,F7APS, FX60 and APS900 group was 66.9%, 68.8%, 69.2% and 69.0% respectively; Reduction rate of creatinine was 63.3%, 66.2%, 63.2% and 61.5%; And reduction rate of serum phosphorus was 48.6%, 54.6%, 53.1% and 53.2% . There was no significantly difference in the reduction rate of urea, creatinine and phosphorous among four groups. The value of KT/V of FX 60 group was highest, APS 900 group ranked second, F7APS group third and B1-1.6H group was lowest, difference between highest group and lowest group was significant; Reduction rate of β2-MG in APS900, FX60, B1-1.6H group was 77.7%, 52.3% and 29.8% respectively, whereas concentration ofβ2-MG in F7APS group increased 15.4% after dialysis. Difference between each two groups was significant. **Conclusions:** High flux HD had the best effect on the clearance of small weight molecule toxins; Except HDF could effectively reduced the concentration of β2-MG, high flux and special middle flux (PMMA membrane) HD also had the same role.

Disclosure of Financial Relationships: nothing to disclose

**PUB505**

**Severe Lactic Acidosis with Renal Failure in Multiple Mieloma (MM): A New Case Report** Antonio Di Felice, Emiliana Ferramosca, Lucia B. De Sanctis, Antonio Santoro. *Division of Nephrology Dialysis and Hypertension, St. Orsola-Malpighi University Hospital, Bologna, Italy.*

Type B lactic acidosis has been rarely described in association with MM. Only 2 case reports has documented lactic acidosis in MM.

CASE REPORT: The patient, male, 51 year old, was admitted in our hospital because of increasing bone pain and rapidly worsening of renal failure. MM with severe bone lesions was diagnosed in 2003. He underwent 2 autologous and 1 allogenic bone marrow transplantation. After transplant failure he was treated with cycles of chemotherapy (thalidomide, dexamethasone, bortezomib) and radiation therapy, interrupted for intolerance signs. His MM was deemed to unresponsive to therapy. On admission patient was non toxic, Blood Pressure 130/80 mmHg, heart rate 80 bpm, diuresis 2.500 ml. Laboratory tests showed creatinine 4.8 mg/dl, BUN 230 mg/dl, Glicemia 96 mg/dl, Hb 10.9 gr/dl, WBC 9.500/mmc, PLT 254.000/mmc, PTT-ratio 1.0, INR 1.11, Na 132 mEq/lt , K 6.0 mEq/lt, Ca 8.6 mg/dl, CPK 135 U/L. Arterial blood gas determination showed a severe metabolic acidosis with $HCO3^-$ 4.0 mMol/lt; arterial lactate level was 132 mg/dl. After i.v. bicarbonate administration, continuous renal replacement treatment with CVVHDF was started in order to obtain a rapid removal of lactate and a better control of bicarbonate levels; contemporarily a new course of dexamethasone and thiamine treatment were started. However, in spite of the treatment and the presence of normal diuresis, lactic acid levels increased up to >230 mg/dl, with persisting acidosis and progressive worsening of general conditions. No other causes of lactic acidosis than MM were found (neither prolonged hypoxia, nor particular pharmacological treatment or thiamine deficiency). The course was complicated by a severe sepsis and the patients deceased after 15 days without recovering of renal function.

In *conclusion*, our case is very probably the third case of type B lactic acidosis associated with MM. This disease, often with severe prognosis, rarely described in patients with rapidly progressive and refractory MM, may be underestimated. Therefore it should be interesting to dose lactate in all patients with severe MM.

Disclosure of Financial Relationships: nothing to disclose

**PUB506**

**Mechanisms of Aldosterone Action in the Intercalated Cells of the Collecting Ducts** Kahori Hori,[1] Takanori Nagai,[1] Yuichiro Izumi,[2] Yushi Nakayama,[3] Yukiko Hasuike,[1] Masayoshi Nanami,[1] Yoshinaga Otaki,[1] Katsumasa Kawahara,[4] Takeshi Nakanishi,[1] Hiroshi Nonoguchi.[1] *[1]Division of Kidney and Dialysis, Dept of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [2]LKEM, NHLBI, NIH, Bethesda, MD; [3]Nephrology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan; [4]Physilology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan.*

Aldosterone palys a major role for urine acidification and acid excretion in the collecting ducts. Aldosterone receptor, namely mineralocorticoid receptor (MR), has been believed to be localized in the principal cells and non-type A intercalated cells but not in the type A intercalated cells of the collecting ducts. We have established a new cell line of the intercalated (IN-IC) cells from SV40 transgenic rats. We examined the mehcanisms of aldosterone action via MR in the IN-CI cells. RT-PCR revealed that IN-IC cells have MR, 11ß hydroxysteroid dehydrogenase type 2 (11ß HSD2), vasopressin V1a receptor, H-ATPase, HK-ATPase, Rhesus blood group C glycoprotein (Rhcg), AE1, pendrin but not vasopressin V2 receptor, aquaporin 2 and ENaC, showing the characteristics of type A intercalated cells. The effects of aldosterone on MR, 11ß HSD2, H-ATPase, HK-ATPase, Rhcg, AE1, and pendrin were examined using real time PCR and Western blot. Aldosterone dose-dependently increased mRNA and protein expressions of MR, 11ß HSD2, HK-ATPase, Rhcg, AE1 and pendrin. In contrast, aldosterone decreased H-ATPase protein expression. Gene silencing of MR by 5 nM siRNA with 80 nM oligofectamine abolished the effects of aldosterone on mRNA and protein expressions of MR, 11ßHSD, H-ATPase, HK-ATPase, Rhcg, AE1 and pendrin. Vasopressin also increased H-ATPase, HK-ATPase and Rhcg but not AE1and pendrin expressoins. In contrast, aldosterone decreased mRNA expresson of V1a vasopressin receptor. These data show that 1) type A intercalated cells have MR, 11ß HSD2 and vasopressin V1a receptor, 2) effects of aldosterone on acid-base regulation is completely through MR and there are no non-MR pathway in the type A intercalated cells, 3) vasopressin via V1aR modulates aldosterone action on acid-base regulation.

Disclosure of Financial Relationships: nothing to disclose

**PUB507**

**Regulation of the SAT1 (SLC26A1) Transporter in Kidney by Acid-Base Status and Dietary Sulfate Intake** Nilufar Mohebbi,[1,2] Ming Zeng,[1] Wolfgang Krick,[3] Gerhard Burckhardt,[3] Birgitta C. Burckhardt,[3] Carsten A. Wagner.[1] *[1]Institute of Physiology, University of Zurich, Zurich, Switzerland; [2]Division of Nephrology, University Hospital Zurich, Zurich, Switzerland; [3]Institute for Vegetative Physiology and Pathophysiology, University of Göttingen, Göttingen, Germany.*

The SAT1 anion exchanger is expressed basolaterally in the renal proximal tubule and the liver and in vitro is able to mediate transport of anions such as sulfate, chloride, bicarbonate, or oxalate. In the kidney the transporter has been implicated into the basolateral release of reabsorbed sulfate into blood. We have previously observed in microarray and proteome studies that 2 and 7 days of $NH_4Cl$-induced acidosis increased SAT1 mRNA and protein in mouse kidney. Here we studied the in vivo regulation of SAT1 in rat kidney and liver. Rats were given NaCl, $Na_2SO_4$, NaCl + $NH_4Cl$, or $Na_2SO_4$ + $NH_4Cl$ for 7 days to dissect the regulation by acidosis or sulfate repletion/depletion. $NH_4Cl$ induced similar metabolic acidosis in both groups with increased urinary acid excretion. $NH_4Cl$ together with NaCl as well as dietary sulfate alone stimulated urinary sulfate excretion. Addition of sulfate to the diet reduced mRNA expression of SLC13A1 (NaSi) but had no effect on renal SAT1 mRNA and protein. $NH_4Cl$-loading with NaCl reduced NaSi mRNA but did not alter SAT1 mRNA. Combined $Na_2SO_4$ and $NH_4Cl$ loading decreased NaSi mRNA but again did not affect SAT1 mRNA. In kidney, $NH_4Cl$ enhanced SAT1 protein expression 3-4 fold. In contrast, in liver, $Na_2SO_4$ or $NH_4Cl$ alone increased SAT1 mRNA whereas the combination had surprisingly no effect. Thus, regulation of SAT1 by sulfate and acidosis may be organ-/cell-specific. The exact role of SAT1 in the transport of sulfate or other substrates requires further elucidation.

Disclosure of Financial Relationships: nothing to disclose

**PUB508**

**Defining the Buffering Process by a Triprotic Acid without Relying on Stewart-Electroneutrality Considerations** Minhtri K. Nguyen, Liyo Kao, Ira Kurtz. *Medicine, UCLA, Los Angeles, CA.*

Upon the addition of protons to an aqueous solution, a component of the $H^+$ load will be bound i.e. buffered. In an aqueous solution containing a triprotic acid, $H^+$ can be bound to 3 different states of the acid as well as to $OH^-$ ions that are derived from the auto-ionization of $H_2O$. In quantifying the buffering process of a triprotic acid, one must define the partitioning of $H^+$ among the three states of the acid and also the $OH^-$ ions in solution in order to predict the equilibrium pH value. However, all previous quantitative approaches that model triprotic acid titration behaviour and used to predict the equilibrium pH rely on the mathematical convenience of electroneutrality/charge balance considerations.

This fact has caused confusion in the literature, and has led to the assumption that charge balance/electroneutrality is a causal factor in modulating proton buffering (Stewart formulation). However, as we have previously shown, although charge balance can be used mathematically as a convenient tool in deriving various formulae, electroneutrality per se is not a fundamental physicochemical parameter that is mechanistically involved in the underlying buffering and proton transfer reactions. The lack of distinction between a mathematical tool, and a fundamental physicochemical parameter is in part a reason for the current debate regarding the Stewart formulation of acid-base analysis. We therefore posed the following question: Is it possible to generate an equation that defines and predicts the buffering of a triprotic acid that is based only on $H^+$ partitioning without incorporating electroneutrality in the derivation? Towards this goal we derived our new equation utilizing: 1) partitioning of $H^+$ buffering; 2) conservation of mass; and 3) acid-base equilibria. In validating this model, we compared the predicted equilibrium pH with the measured pH of an aqueous solution consisting of $Na_2HPO_4$ to which HCl was added. The measured pH values agreed with the predictions of our equation within experimental error. Our results provide further important evidence that one can mathematically model the chemistry of acid-base phenomenology without relying on electroneutrality considerations.

Disclosure of Financial Relationships: nothing to disclose

**PUB509**

**Renal Dysfunction in American Cutaneous Leishmaniasis (ACL)** Rodrigo Alves de Oliveira,[1] Lucia Andrade,[3] Antonio C. Seguro,[3] Talita R. Sanches,[3] Leonardo Oliveira Teotonio,[1] Claudio Gleidiston Lima Silva,[1] Lucyo Flavio Bezerra Diniz,[1] Elizabeth De Francesco Daher.[2] *[1]Internal Medicine, Federal University of Ceará - School of Medicine - Campus Cariri, Barbalha, Ceara, Brazil; [2]Internal Medicine, Federal University of Ceará - School of Medicine - Campus Fortaleza, Fortaleza, Ceara, Brazil; [3]Nephrology Department, University of São Paulo School of Medicine, Sao Paulo, Brazil.*

Renal dysfunction in ACL has been attributed to antimonial treatment but might be attributable to ACL itself. To test this hypothesis, we evaluated 37 ACL patients before treatment. We tested glomerular and tubular renal function, comparing the results with those obtained for 8 control subjects. We also tested urine and plasma osmolality (Uosm and Posm) before and after administration of desmopressin (after a 12-h fast), as well as plasma bicarbonate (Pbic), urinary pH (UpH) and plasma pH (PpH) before and after oral administration of $CaCl_2$ (acidification test). In addition, we quantified AQP2, NHE3, NKCC2, H-ATPase and pendrin. None of the patients had glomerular dysfunction (ClCr, 109±31 ml/min). Urinary concentrating defect was identified based on a post-test U/Posm ratio <2.8 in 27 patients (77%) or Uosm <700 mOsm/kg in 22 (63%). There was no statistical difference between pre- and post-test osmolality. Urinary AQP2 expression was significantly lower in patients than in controls (99.5±0.5 vs. 38.5±12%, p=0.006), whereas that of NKCC2 was significantly higher (102±2.5 vs. 147±12%, p=0.02). Urinary acidification defect (post-test UpH >5.45) was seen in 17 patients (46%; p=0.006 vs. controls). Pre-test Pbic was <21 mEq/L in 12 patients (32.5%), and pre-test PpH was <7.35 in 14 (38%). NHE3 expression was significantly higher in patients than in controls (100±0.6 vs. 176±15%, p=0.015), as was that of H-ATPase (98±0.2 vs. 190±8%, p=0.04) and pendrin (110±7.0 vs. 152±10%, p=0.05). The urinary concentrating defect is likely caused by downregulation of AQP2 expression, and the increased NKCC2 expression probably represents a compensatory mechanism. ACL is associated with dysregulation of major acid-base transporters and may thus impair urinary acidification.

Disclosure of Financial Relationships: nothing to disclose

**PUB510**

**Application of a New Delta Current Method for Determining the Substrate Stoichiometry of Electrogenic Sodium Bicarbonate Cotransporters NBCe1-A and NBCe2-C** Xuesi Max Shao,[1] Liyo Kao,[2] Ira Kurtz.[2] *[1]Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

The stoichiometry of electrogenic NBC transporters is an important determinant of their function. If an electrogenic transporter has a fixed stoichiometric ratio q, q can be determined by the reversal potential $E_{rev}$ with known intra- and extra-cellular concentrations of the transported species if a specific blocker is available. If there are other transporters/channels in the membrane, a widely used approach is to calculate delta $E_{rev}$ by altering concentrations of the transported species. This approach is based on the assumption that the effects of other channels and transporters on $E_{rev}$ are additive. However, the Goldman-Hodgkin-Katz (GHK) equation suggests otherwise: $E_{rev}$ is a *logarithmic* function of the sum of different conductances rather than being additive. In the present study, we therefore developed a new delta current (ΔI) method to estimate q based on a general model of transporter function (including symporters and antiporters) that does not require a blocker. ΔI instead of delta $E_{rev}$ is determined by altering the external concentration of a transported ion thereby eliminating other currents. The ratio of ΔIs at membrane voltages $V_2$ and 0 is: $\Delta I_2/\Delta I_o = (\exp(FV_2/2RT))^{q-1}$. Therefore, $q = (2RT/FV_2)\ln(\Delta I_2/\Delta I_o)+1$. We tested this ΔI methodology in patch-clamp experiments of HEK-293 cells expressing NBC1e1-A or NBCe2-C. Our data show that the ΔI equation accurately calculates a stoichiometry ratio of $HCO_3^-:Na^+$ of 2 demonstrating the utility of this new methodology for measuring the stoichiometry of electrogenic transporters.

Disclosure of Financial Relationships: nothing to disclose

**PUB511**

**Effects of Respiratory and Metabolic Acidosis on Transcription, Expression, and Distribution of NHE3 in OKP Cells** Pedro Henrique I. Silva, Nancy A. Reboucas. *Physiology and Biophysics, University of São Paulo, Sao Paulo, Brazil.*

The $Na^+H^+$ exchanger isoform 3, NHE3 (Slc9-a3), is essential for $H^+$ secretion and $HCO_3^-$ reabsorption in renal proximal tubules. Our goal in the present study was to evaluate the influence of respiratory (RA) and metabolic acidosis (MA) on expression and sub-cellular localization of NHE3 in opossum kidney proximal tubule cells (OKP).

Confluent OKP cells were submitted to acidosis for 24 h, either by addition of HCl to the medium or by increasing $pCO_2$ (80 or 160 mmHg) in the incubator chamber. Control cells were grown at pH 7.4, $[HCO_3^-]$ 24 mM, and $pCO_2$ 40 mmHg. MA: mild, pH 7.21±0.02, $[HCO_3^-]$ 15.4±0.9; severe, pH 6.95±0.07, $[HCO_3^-]$ 8,5±1.8. RA: mild, pH 7.11±0.03, $[HCO_3^-]$ 24.5±0.3; severe, 6.86±0.01, $[HCO_3^-]$ 29.7±1.0. NHE3-mRNA levels, measured by real time RT-PCR, was similarly increased in mild (46±10.8%) and severe (35.7±8.4%) MA, although it remained unchanged in both types of RA. NHE3-mRNA stability, measured after inhibition of transcription with actinomycin D, did not change in either types of acidosis. Increased activity of the promoter gene, evaluated by the expression of the reporter gene Firefly luciferase, was observed in severe MA when the region spanning -2,095 to +55 was evaluated, but remained unchanged on the segment -152/+55.

A 58.4±6.8% increase in the relative NHE3 protein abundance, evaluated by Western blot of total proteins, was detected in severe MA and remained unchanged in mild or severe RA. Also, an increase of 107±15.4% in the amount of cell-surface NHE3-protein, evaluated by biotin binding, was observed in severe MA, and by 51.0±25.5% in severe RA, but the latter was not significant.

We conclude that metabolic acidosis increases the expression of NHE3 gene by mechanisms that alter the transcription efficiency, but not the mRNA stability. The transcriptional activity seems to be affected not by the low pH, but by the low levels of $HCO_3^-$, since respiratory acidosis with equal low pH had no effect on the promoter activity. Metabolic acidosis, in addition to increasing NHE3 expression at mRNA and protein levels, also increases NHE3 recruitment to the apical membrane, an effect that was not significant in respiratory acidosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB512**

**Sodium Bicarbonate and Progression to Renal Failure in a Majority African American CKD Patient Population** Lakshmi Turlapati, Raeesa Mirza, Rania Abdel-Rahman, Rachel Ward, Travis Kauffmann, Meredith Whitacre, Susan Tober. *Division of Nephrology & Hypertension, Brody School of Medicine at East Carolina University, Greenville, NC.*

**Background**

A study by de Brito-Ashust et al demonstrated that sodium bicarbonate may delay the progression of renal failure to end stage renal disease (ESRD) among chronic kidney disease (CKD) patients. However, this study was conducted on a largely Caucasian patient population located in the U.K. The purpose of this study was to examine the effect of sodium bicarbonate (NaHCO3) on the rate of progression to ESRD among a predominately African American population in an academic medical center in the southeastern U.S.

**Methods**

We conducted a retrospective cohort study to examine the impact of NaHCO3 on the rate of change in GFR. Inclusion criteria consisted of patients older than 18 years with a GFR ≤35. The primary endpoint was the rate of change in GFR. Data was collected until the patient's most recent clinic visit or start of dialysis. The treatment group included patients receiving NaHCO3, and the controls were patients who were not treated but had serum bicarbonate levels similar to those of the treatment group. Independent sample t-tests were used to compare the mean rate of change in GFR between groups.

**Results**

Two hundred charts were reviewed. Of these, 60.5% were African American and 30.8% were Caucasian. The average patient was 62.5 (±14.78) years and most patients were female (54.5%). Average serum bicarbonate level at initiation of treatment with NaHCO3 was 19 mmol/l. Thirty-five patients were in the treatment group, and there were 28 controls. Compared to the control group, the mean rate of change in GFR was slower among patients treated with NaHCO3 (-4.07 ± 6.83 vs. -3.94 ± 7.65), but the difference was not statistically significant (95% CI-2.71-4.77, p>.05).

**Conclusion:**

This retrospective study indicates that NaHCO3 is not associated with decreased progression of ESRD among predominately African American CKD patients. Population differences may account for the findings in this trial compared to previous reports. This finding needs to be confirmed in a prospective, randomized trial.

Disclosure of Financial Relationships: nothing to disclose

**PUB513**

**SIADH Due to Neurocysticercosis with a Novel Treatment Strategy** Jenny Blau,[1] Julianna Barsony,[2] Joseph G. Verbalis.[2] *[1]Internal Medicine, Georgetown University, Washington, DC; [2]Division of Endocrinology and Metabolism, Georgetown University, Washington, DC.*

We report a case of SIADH as a result of neurocysticercosis for the first time, and highlight the application of the new V2 receptor antagonist tolvaptan for long-term control of symptomatic SIADH. The larval stage of the pork tapeworm preferentially invades the central nervous system causing neurocysticercosis (NCC), with potential for associated

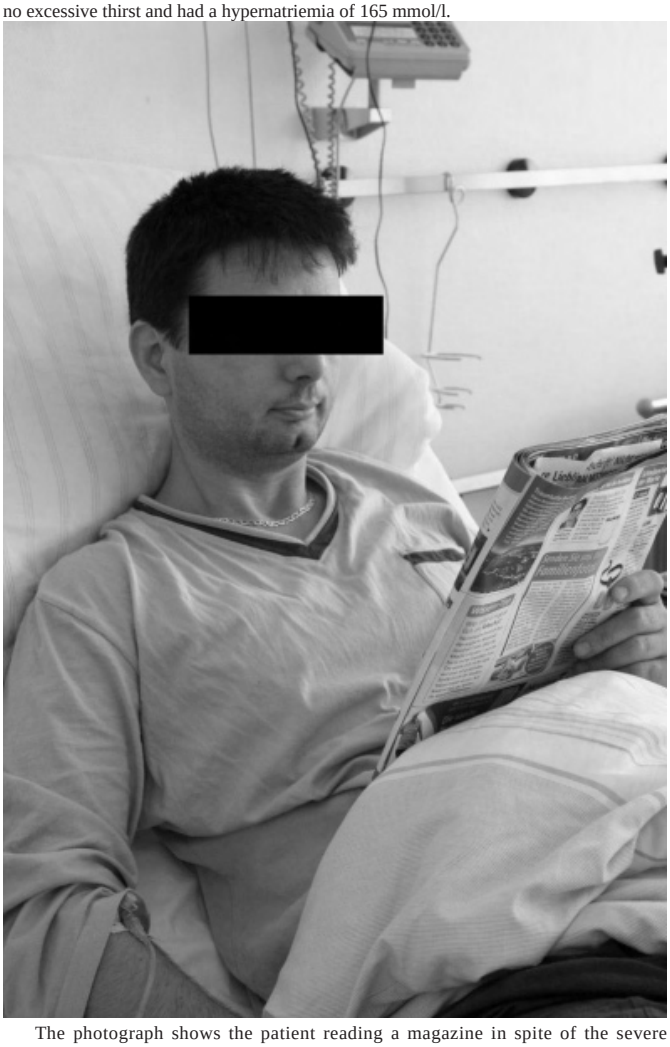
endocrine disruptions due to inflammatory and mass effects. NCC associated syndrome of inappropriate antidiuretic hormone secretion (SIADH), presenting as hyponatremia, has not been reported. A 33-year old Mexican male presented with headache, nausea, vomiting, altered mental status and gait instability that progressively worsened. MRI imaging revealed a 3.5x3.2 cm cyst occupying the third ventricle with hydrocephalus and thickening of the superior and mid portions of the infundibulum with a left shift. CSF ELISA was positive for anti-cysticercus antibody. SIADH was diagnosed based on euvolemic hyponatremia ([Na+]=123 mmol/L), hypoosmolality (273 mOsm/kg H2O), inappropriately elevated urine osmolality (685 mOsm/kg H2O), and urine sodium (UNa=168 mmol/L), with normal thyroid and adrenal function. The patient underwent surgery and received antihelmintic therapy with abendazole and dexamethasone. Pathology demonstrated absence of direct invasion of the hypothalamus or the pituitary, and noted astrocyte inflammatory reaction. After initial trials of fluid restriction and hypertonic NaCl, persistent hyponatremia responded to escalating doses of tolvaptan (from 15 mg to 60 mg final). Follow-up for 3 months demonstrated normal serum [Na+] (141-143 mmol/L) on tolvaptan 60 mg daily, with no side-effects. This first case presentation of NCC associated SIADH addresses an emerging problem in the U.S., due to rising immigration rates from endemic countries in Latin America, Asia and Africa. The lessons learned from the application of tolvaptan for control of symptomatic SIADH extend beyond NCC. In addition to fluid and electrolyte control, the long-term adverse consequences such as osteoporosis and fractures should also be preventable.

Disclosure of Financial Relationships: nothing to disclose

**PUB514**

**A Case of Upward Reset Osmostat** Martin D. Brzoska, Ernst H. Scheuermann, Christoph Betz. *Medicine, Division of Nephrology, Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany.*

We present a case of upward reset osmostat in a 34 year old male with type 1 diabetes mellitus. At the time of clinical workup of the disorder the patient was asymptomatic with no excessive thirst and had a hypernatriemia of 165 mmol/l.



The photograph shows the patient reading a magazine in spite of the severe hypernatremia. However, he had experienced slight disturbances of speech and gait when a serum sodium of 180 mmol/l had been detected two months before.

Our workup included a fluid deprivation test with measurement of serum and urine electroytes, urine osmolarity, plasma ADH concentration and adrenal hormones. Pituitary and hypothalamic lesions as well as solid tumors were ruled out with radiographic imaging.

Producing a urine osmolarity of 900 mosmol/l the patient showed intact renal and hypothalamic reaction to fluid deprivation. We detected no osmotic diuresis or associated chronic kidney disease whatsoever. Volume expansion in turn led to the excretion of a dilute urine and showed no effect on serum sodium concentrations and osmolarity.

We therefore made the diagnosis of upward reset osmostat, which is described as a rare selective disorder of the osmoreceptors with ADH secretion being primarily governed by changes in volume.

Disclosure of Financial Relationships: nothing to disclose

**PUB515**

**Severe Hyponatremia Caused by Portal Vein Thrombosis without Cirrhosis of Liver – A Hope with Tolvaptan!** Penchala S. Mittadodla,[1] Robert S. Gayner.[1] *[1]St Luke's Hospital, Bethlehem, PA; [2]Bethlehem, PA; [3]Bethlehem, PA.*

Hyponatremia is commonly seen in patients with cirrhosis of the liver with up to 50% of patients having a sodium level less than 135 meq/l. Hyponatremia is a predictor of death with cirrhosis of liver highlighting the need for adequate treatment or correction. We report a rare case of a post-splenectomy patient who developed treatment unresponsive hyponatremia caused by portal vein thrombosis without cirrhosis of the liver ultimately treated with tolvaptan successfully.

A 55-year-old Caucasian male was admitted with a 2-week history of progressively worsening lethargy and abdominal distension secondary to ascites. The patient had a serum Na of 116 meq/l on admission. An extensive workup was performed including a serum osm of 264 mmol/kg, TSH of 2.2 uIu/ml, cortisol level of 14.7 mcg/dl, urinary osmolarity of 337 mmol/kg, urine sodium of 14 mmol/l, normal ejection fraction by echo, normal LFTs (except elevated alkaline phosphatase) and creatinine of 0.9-1.3 mg/dl.

The patient had a splenectomy for hypersplenism, secondary to chronic myelomonocytic leukemia (currently in remission), four weeks prior to this presentation. His baseline serum sodium pre-operatively and up until 4 weeks post operatively ranged between 134 to140 meq/l. Past medical history was notable for ascites, hypertension and anemia.

A CT of the abdomen and abdominal ultrasound revealed portal vein thrombosis with portal hypertension and ascites. His liver appeared normal. A Liver biopsy was performed and showed moderate portal inflammation with mild piecemeal necrosis and bridging fibrosis suggestive of portal vein thrombosis but no evidence of cirrhosis. Transsinusoidal pressure gradient was measured at 7 mmHg.

His hyponatremia was very resistant to fluid restriction, lasix, and aldactone. Tolvaptan, a vasopressin 2 antagonist, was administered in the hospital and his sodium improved to 134 meq/l after 4 days and thereafter ranged between 129 to 133 meq/l with tolvaptan treatment. Additionally, he was treated with warfarin for portal vein thrombosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB516**

**A Weakness of Cola** Penchala S. Mittadodla,[1] Robert S. Gayner,[1] Gloria Fioravanti.[1] *[1]St Luke's Hospital, Bethlehem, PA; [2]Bethlehem, PA; [3]Bethlehem, PA.*

Although the effects of caffeine intake have long been recognised, there is very little published literature about chronic excessive intake of cola causing severe electrolyte disturbances. We report a case of cola induced hypokalemia leading to severe muscle weakness. The importance of a detailed history and systematic approach in the investigation of hypokalemia is discussed.

A 43-year old Caucasian male was admitted with 2-week history of escalating weakness and inability to ambulate. He denied any other symptoms. His past medical history was non-contributory and he was not taking any medications. He consumed alcohol occasionally. Physical examination revealed normal blood pressure and was significant only for symmetrically reduced 3/5 strength in all extremities. Lab data revealed hypokalemia with serum potassium of 2.3mmol/l; all other labs were normal except elevated serum CK at 2814 U/l with a normal TSH, random cortisol, aldosterone and sedimentation rate. Urinary electrolytes were not done prior to supplementation of pottasium. His dietary history obtained the next day revealed that patient consumed 5-6 liters of cola/day. With the cessation of cola consumption and administration of potassium supplements, the hypokalemia and muscle weakness resolved.

Caffeine, an ingredient of cola, is known to have a weak diuretic action and can cause hypokalemia if consumed in excess of 500-600mg/day. Cola has 10-15mg of caffeine per 100ml, therefore our patient had been consuming at least 800-1000mg/day for the last 4-6 months. Inadequate dietary intake of potassium along with excessive renal loss due to diuresis would have led to his presentation. Many potential causes of hypokalemia can be eliminated after a detailed history, dietary review and laboratory evaluation. Measurement of blood pressure, serum electrolytes, urine electrolytes and evaluation of acid-base status should be the initial step in the diagnosis of hypokalemia. Subsequent evaluations such as measurement of serum aldosterone, renin and cortisol levels may be needed in certain cases.

Disclosure of Financial Relationships: nothing to disclose

**PUB517**

**Severe Acidosis Due to Propylene Glycol Overdose** Uma Krishna Pakkivenkata, Edward A. Ross. *Division of Nephrology, Univ. of Florida, Gainesville, FL.*

In cases of apparent overdose it is often challenging to ascertain the exact poison when laboratory results are not immediately available. Clinicians need to be aware that agents which are increasingly used as alternatives to ethylene glycol, may themselves

have toxicity. The case below illustrates severe acidosis in which treatment for suspected methanol or ethylene glycol overdose was also efficacious for the unexpected final diagnosis of propylene glycol (PG) poisoning.

A 58 year old male with alcohol dependence was admitted with altered mental status. He complained of blurred vision, dizziness and subjective fever. Physical exam was remarkable only for the impression of alcohol intoxication. Notable laboratory findings were: serum Na 135, K 3.3, Cl 83, $CO_2$ 5 meq/L; BUN 22, Cr 2.4 mg/dl; serum osmolality measured 360 and calculated 331 mOsm/kg, lactate 8.5 mmol/L, EtOH 185 mg/dl, salicylate undetectable; ABG pH 6.89, $HCO_3$ 3.2, $pCO_2$ 16; urinalysis no crystals. With increased anion and osmolal gaps methanol or ethylene glycol toxicity was suspected. He was started on fomepizole and was emergently hemodialyzed for 5 hours, normalizing the anion gap. Days later methanol and ethylene glycol levels returned undetectable, however the PG level was very high at 200 mg/dl making it the sole identifiable cause of the acidosis.

PG (mol wt 76) is used as an emulsifier for IV benzodiazepines, and toxicity due to excess dosing has been reported. It is also now used in antifreeze as a substitute for ethylene glycol. PG is metabolized to pyruvic acid, acetic acid and lactic acid. The parent compound is renally cleared and thought primarily responsible for toxicity, rather than metabolites: CNS depression, arrhythmias, ocular irritation, and GI disturbances. It is rapidly cleared by hemodialysis.

Our patient's altered mental status precluded a history of this overdose. It is imperative that clinicians be aware of growing industrial use of PG, and add this possibility to the differential diagnosis for patients in whom ethylene glycol or methanol toxicity are being considered. Fortunately hemodialysis will efficiently remove PG, as well as many other small suspected toxins, while final identification is awaited from the laboratory.

Disclosure of Financial Relationships: nothing to disclose

**PUB518**

**A Novel Case of Adult-Onset Bartter's Syndrome** Wiroon Sangsiraprapha, Daniel Addison, Evan A. Longfield, Biruh Workeneh. *Internal Medicine, Baylor College of Medicine, Houston, TX.*

Bartter's Syndrome is an autosomal recessive disorder marked by normal blood pressure with profound hypokalemia, metabolic alkalosis, hypomagnesemia, and hypercalciuria. This is caused by a range of mutations in genes encoding for ion transport proteins in the thick ascending limb of the loop of Henle. Although commonly a childhood disease, adult-onset Bartter's syndrome has been noted into the third decade of life. We present this case to add to the sparse literature available in this rare condition.

A 34 year-old Latin American female with a history of multiple DVTs, endometriosis, asthma, and chronic UTIs which was treated with chronic gentamicin presented with persistent hypokalemia that was resistant to replacement therapy. Physical examination revealed normal blood pressure, while laboratory investigation revealed profound hypokalemia with kaliuresis, hypocalcemia with hypercalciuria, hypomagnesemia, and metabolic alkalosis. Urine diuretic screen was negative and renal ultrasonography was normal. Based on the clinical and laboratory findings she was diagnosed with classical Bartter's syndrome.

Bartter's syndrome may present in both the antenatal period as well as in the more common neonatal form. Disease presentations well into the third decade of life have been reported but no reports describe chronic disease presenting after the third decade of life. Additionally, cases of transient Bartter's syndrome associated with aminoglycoside use have been described however aminoglycoside-associated disease lasted no longer than 6 weeks following the cessation of therapy. Our patient went on to have persistent Bartter's after more than a year of aminoglycoside discontinuation. This leads us to believe that our patient has disease consistent with classical Bartter's syndrome. We postulate that long-term use of gentamicin may have played a role in activating a disease-causing mutation leading to her unusually late disease-onset.

**Conclusion**: The classic form of Bartter's syndrome may present beyond the third decade of life, and adult-onset Bartter's syndrome may be associated with gene activating drugs.

Disclosure of Financial Relationships: nothing to disclose

**PUB519**

**FGF23: An Indicator of Protein Intake in Children with Normal and Mildly Impaired Renal Function?** Justine Bacchetta,[1,2] Pierre J. Cochat,[1] Laurence Dubourg,[1] Isidro B. Salusky,[2] Katherine Wesseling-Perry.[2] *[1]Hospices Civils de Lyon & Universite de Lyon, Bron, France; [2]David Geffen School of Medicine, UCLA, Los Angeles.*

Fibroblast Growth Factor 23 (FGF23) is a phosphaturic factor and a suppressor of 1α hydroxylase activity in the kidney. Although its role in phosphate and vitamin D metabolism have been demonstrated in CKD patients, little is known in subjects with normal renal function or early CKD.

We performed a sub-group analysis of the INU23 study (influence of GFR and age on FGF23 levels in pediatric CKD) in the subgroup of children with renal risk but normal renal function or early CKD, after excluding patients. with solid organ transplantation, nephrotic syndrome and genetic disorders associated with specific bone abnormalities.

A total of 120 children (65 boys, age 10.7±3.9 yrs, BMI 17.4±3.6) were included. Mean±SD (range) for biological parameters were: GFR (inulin clearance) 109±18 (70-150) ml/min/1.73m$^2$, PTH 31±12 (7-86) pg/ml, 25OHD 25±10 (7-69) ng/l, C-terminal FGF23 (Immutopics$^R$) 38±41 (2-376) RU/ml, intact FGF23 (Kainos$^R$) 35±17 (2-78) pg/ml and uric acid (UA) 253±69 (95-452) µmol/l.Univariate Spearman analysis found significant associations between UA and GFR (r=-0.21; p=0.02), age (r=0.33; p<0.001), body weight (r=0.41; p<0.001), height (r=0.37; p<0.001), BMI (r=0.34; p< 0.001), PTH (r=0.34; p<0.001), C-terminal FGF23 (r=0.19; p=0.04), intact FGF23 (r=0.26; p=0.005), phosphate reabsorption rate (RRP, r=-0.36; p< 0.001), TmP/GFR (r=-0.28; p=0.02) and serum proteins (r=0.19; p=0.04). Multiple linear regression analyses using backward stepwise procedures demonstrated that uric acid could predict TmP/GFR, as well as PTH and C-terminal FGF23 serum levels. A similar trend was observed for intact FGF23. Since phosphate, protein, and purine intakes are closely linked, therefore the association between uric acid, markers of urine phosphate excretion and serum FGF23 suggests that circulating levels of FGF23 may be an early nutritional indicator of high protein and phosphate intakes in a pediatric population with normal or mildly impaired GFR.

Disclosure of Financial Relationships: nothing to disclose

**PUB520**

**Shank2 Redistributes with NaPiIIa during Regulated Endocytosis** Evgenia Dobrinskikh, Hector Giral-Arnal, Yupanqui A. Caldas, Moshe Levi, R. Brian Doctor. *Medicine, University of Colorado, Denver, Denver, CO.*

Serum phosphate levels are acutely impacted by the abundance of sodium phosphate co-transporter IIa (NaPiIIa) in the apical membrane of renal proximal tubule cells.PDZ domain-containing proteins bind NaPiIIa and likely contribute to the delivery, retention, recovery and trafficking of NaPiIIa. Shank2 is a distinctive PDZ domain protein that binds NaPiIIa. To begin defining the role of Shank2 in regulating NaPiIIa activity, distribution and abundance, the present in vivo study followed the fates of NaPiIIa and Shank2 during regulated NaPiIIa endocytosis. Rats were maintained on a low phosphate diet and then plasma phosphate levels were acutely elevated by high phosphate feeding to induce the recovery, endocytosis and degradation of NaPiIIa. Western blotting of renal cortical tissue from mice given high phosphate feed showed NaPiIIa and Shank2 underwent degradation. Quantitative immunofluorescence analyses, including microvillar vs intracellular intensity ratios and intensity correlation quotients, showed that Shank2 redistributed with NaPiIIa during the time course of NaPiIIa endocytosis. Further, NaPiIIa and Shank2 trafficked through distinct endosomal compartments (clathrin, early endosomes, lysosomes) with the same temporal pattern. These in vivo findings indicate that Shank2 is positioned to coordinate the regulated endocytic retrieval and down regulation of NaPiIIa in rat renal proximal tubule cells.

Disclosure of Financial Relationships: nothing to disclose

**PUB521**

**Down-Regulation of Paracellin-1 in Diabetic Rats as a Possible Cause of Hypomagnesemia in Diabetes** Kaori Takayanagi, Taisuke Shimizu, Takatsugu Iwashita, Yosuke Tayama, Juko Asakura, Koichi Kanozawa, Hajime Hasegawa. *Dept of Nephrol & Hypertens, Saitama Med Center, Saitama Med Univ, Kawagoe, Saitama, Japan.*

Hypomagnesemia is a well known clinical feature of diabetes mellitus, and is recently revealed to be involved in the development of insulin resistance. Although the hypomagnesemia in diabetes is known to be provoked by the decrease in the renal Mg reabsorption, its molecular mechanisms have not been elucidated. In this work, we studied the changes in the expression of Mg transporting molecules in diabetic rats.

Kidneys were sampled from male SD rats at 4 weeks after streptozotocin (STZ) injection (60 mg/kg, ip). Changes in the expression and distribution of Mg transporting molecules were studied by immunohistochemistry and real time PCR.

In results, fractional excretion of Mg (FEMg) was significantly decreased in STZ-rats (0.49±0.12% in STZ-rats vs 0.64±0.11% in control), although serum Mg concentration was not decreased. In STZ-rats, gene expression of paracellin-1 (PCLN), which is a principal Mg pathway in tight junction of ascending loop of Henle (TAL), was significantly decreased (68.6±14.9% in STZ-rats vs 100.7±6.7% in control rats), although gene expression of calcium sensing receptor (CaSR) was similarly down-regulated (46.2±3.2 in STZ-rats vs 106.7±17.7 in control rats). Gene expressions of TRPM6, TRPM7, NKCC2, ROMK and NCC were all unchanged in STZ-rats. Immunohistochemistry of PCLN showed diminished immunoreactivity in the superficial and juxtamedullary lesions of cortex in the STZ-rats. Similarly, immunoreactivity of CaSR was also diminished in the cortex.

Present study suggested that the hypomagesemia in diabetes might be provoked by the decreased reabsorption of Mg in TAL which might be principally resulted from down-regulation of PCLN. The decreased Mg reabsorption is known to be noticed in microalbuminuric stage of diabetic nephropathy. PCLN-related inhibition of Mg reabsorption may indicate the interstitial damage of early diabetic nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**PUB522**

**Renal Hypophosphatemia and SIADH Associated with Small Cell Carcinoma: Report of a Case and Review of the Literature of 9 Cases** Ekamol Tantisattamo, Roland C. K. Ng. *Department of Medicine, University of Hawaii, John A. Burns School of Medicine, Honolulu, HI.*

*Background*: Acquired isolated renal phosphate wasting associated with tumor known as oncogenic osteomalacia is a rare paraneoplastic syndrome caused by overproduction of fibroblast growth factor 23. Oncogenic osteomalacia is usually associated with benign mesenchymal tumors. Syndrome of inappropriate antidiuretic hormone secretion (SIADH), on the other hand, is a common paraneoplastic syndrome caused by small cell carcinoma (SCC). Concomitant oncogenic osteomalacia and SIADH associated with SCC is very rare with only 4 other cases reported in the literature. We report a case of SCC-related renal wasting hypophosphatemia and concurrent SIADH and review the literature reporting 9 other cases of SCC associated with oncogenic osteomalacia.

*Case report*: A 60-year-old Caucasian male presented with nausea, vomiting, and generalized malaise due to persistent hyponatremia for 2 months despite fluid restriction. Laboratory workup comfirmed SIADH. Serum phosphorus was less than 1 mg/dl and remained low in spite of phosphate replacement. Fractional excretion of phosphorus was 41% (normal < 5%) and renal tubular maximum resorptive capacity of phosphorus factored for GFR was 0.6 mg/dl (normal 2.5-4.2 mg/dl). Serum calcium was 9 mg/dl and total 25-Hydroxy vitamin D was 34 ng/ml. Chest x-ray was unremarkable. Chest and abdominal CT scan showed a left apical lung mass, left hilar adenopathy, and liver metastasis. Bronchoscopy with biopsy confirmed SCC. His serum phosphorus normalized after receiving the first cycle of chemotherapy.

*Conclusions*: Almost half of reported cases of renal phosphate wasting associated with SCC concomitantly presented with SIADH. These cases had initial serum phosphorus level lower and survival periods shorter than those without SIADH. This rare dual paraneoplastic syndrome and low serum phosphorus may be a poor prognostic sign. In addition, both renal phosphate wasting and SIADH usually occur in a short period of time before identification of SCC. Therefore, renal wasting hypophosphatemia with concomitant SIADH/hyponatremia should prompt a search for SCC rather than a benign mesenchymal tumor.

Disclosure of Financial Relationships: nothing to disclose

**PUB523**

**Opossum Kidney (OK) Cells Demonstrate Molecular and Electrophysiological Properties Consistent with "Leaky" Proximal Tubular Epithelia** R. Todd Alexander, Jelena Borovac, Andrew C. Rasmussen. *Department of Pediatrics & Physiology, University of Alberta, Edmonton, AB, Canada.*

The proximal tubule (PT) reabsorbs the majority of glomerular filtrate. A significant amount of water and electrolytes are (re)absorbed from this nephron segment via the paracellular pathway. The molecular mechanisms mediating this are incompletely understood. This is in part explained by the lack of a well characterized cell culture model suitable to the study of paracellular transport across a leaky epithelia such as the PT. We therefore set out to develop and characterize a cell culture model with which to study PT paracelluar transport. To this end, we employed Opossum kidney (OK) cells. We first measured their transepithelial resistance, with a EVOM Epithelial Voltohmmeter, as they became polarized. We found, by 4 days after plating, a resistance of $9.78 \pm 1.52\ \Omega/cm^2$, that did not increase further. Next, we employed Ussing chambers to perform dilution potential measurements. Consistent with a fixed paracellular pore that is not altered by voltage, we observed a linear relationship between voltage and current applied across the monolayer. These studies demonstrated a pNa/pCl ratio of 0.962 ± 0.006, consistent with preferred paracellular anion flux. This was maintained across a range of dilutions between 0.25 – 4 (apical to basolateral). The absolute flux of $Na^+$ was found to be 4.9*10-8 ± 0.1*10-9 cm/s and $Cl^-$ to be 5.1*10-8 ± 0.1*10-9 cm/s. This is in keeping with the high conductance of this cell line (0.05 ± 0.01 S/cm²). We then employed a radiotracer to measure calcium diffusion across monolayers of OK cells and found a flux of 214 ± 43 nmol/hr/cm². In order to understand the molecular basis of these findings we set out to determine the expression of claudins (tight junction proteins implicated in paracellular ion fluxes) in this model system. We were able to identify and clone by homology claudin-4, -6, -11, -12, -15 and -20 from this cell line. In summary, we have characterized the molecular and electrophysiological phenotype of a low resistance, renal epithelial cell culture model with which to study PT paracellular transport.

Disclosure of Financial Relationships: nothing to disclose

**PUB524**

**Evidences That Ser 418 Can Modulate hSGLT1 Distribution in MDCK Cells** Olivia Beloto-Silva, Maria Oliveira-Souza. *Physiology and Biophysics, University of Sao Paulo, Sao Paulo, SP, Brazil.*

The regulation of $Na^+$/glucose co-transporter (SGLT1) is essential for the provision of glucose to the body and, thus it is important for maintenance of blood glucose at near constant levels. The activity and cellular distribution of SGLT1 is modulated by the phosphorylation state of the protein involving protein kinases PKA or PKC. One of the known phosphorylation sites is at Serine 418 (S418). In this study we investigated the effect of cellular distribution of human SGLT1 (hSGLT1) and a mutation changing S418 to a histidine (hSGLT1-*S418H*). Endogenous SGLT1 and SGLT2 expression, in *wild type* MDCK cells (*wt*MDCK), was accessed by analysis of mRNA. The genes encoding for hSGLT1 and hSGLT1-*S418H* were ligated into the fluorescent vector pEGFP-N1 and transfected into *wt*MDCK cells. Immunoblotting was used to confirm if hSGLT1 and hSGLT1-*S418H* were being expressed after the transfection. Immunofluorescence was used to provide information about the cellular distribution of hSGLT1 and hSGLT1-*S418H*. Our results show no expression of mRNA for SGLT1 or SGLT2 was detected in *wt*MDCK cells (nontransfected), what makes this cell a model system for studying SGLT1 or SGLT2 co-transporters. The distribution of hSGLT1, in transfected cells, was predominantly in the plasma membrane. In contrast, the distribution of hSGLT1-*S418H* was predominantly in the cytosol, but will be further confirmed with experiments of biotinylation. Thus, this work demonstrates that presence of Serine 418 is required for proper cellular distribution of hSGLT1. Moreover, the phosphorylation of S418 appears to be necessary for the insertion of the protein into the plasma membrane of MDCK cells, which can probably modify the glucose transport by SGLT1. Financial Support: FAPESP

Disclosure of Financial Relationships: nothing to disclose

**PUB525**

**Clinical Features of Hyponatremia: Changes Related to Increasing Use of Thiazide Diuretics** Inhye Cha, Won-Yong Cho, Sang-Kyung Jo, Hyoung-Kyu Kim. *Department of Internal Medicine, Division of Nephrology, Korea University Anam Hospital, Seoul, Republic of Korea.*

Background : Hyponatremia is the most frequent electrolyte disorder in clinical practice. Use of thiazide diuretics markedly increased, and thiazide-induced hyponatremia seems to be frequently encountered. Purpose : Evaluate the incidence, etiologies of hyponatremia for 6years(2004-2009). Assess the prevalence and characteristics of thiazide-induced hyponatremia. Methods : Retrospective cohort study. Total 322 patients who admitted with hyponatremia were included. Results : 174(54%) patients presented hyponatremia. 208(64.6%) were female, mean age was 69.9 years-old, average plasma sodium was 118.9mEq/L on admission. Most common current medical histories were hypertension(56.5%) and diabetes mellitus(29.8%). Most common causes were thiazide diuretics(37.6%) and SIADH(18.9%). Most common clinical manifestations were general weakness(44.4%) and nausea(22%). Thiazide-induced hyponatremia show a tendency to increase from 2004 to 2009(P<0.001), combination type drug use were also increasing. In thiazide-induced hyponatremia compare to those from other causes, portion of female was higher (73.6% vs 64.6%, P<0.001), mean age was older (74.7 vs 69.9 years-old, P<0.001) and incidence of cerebrovascular accident was higher (19.8% vs 11.5%, P<0.001). Conclusion : The number of hyponatremia increased for recent 6years. The percentage of thiazide-induced hyponatremia is still increasing, and thiazide-containing combination drug is becoming a main cause of thiazide-induced hyponatremia. Thiazide-induced hyponatremia seems to occur mainly in old age, female and who has history of cerebrovascular event. Therefore more cautions might be necessary in those patients with thiazide use.

Disclosure of Financial Relationships: nothing to disclose

**PUB526**

**RLY5016: A Novel Therapeutic Polymer To Control Serum Potassium: Physical, Chemical & In Vitro Properties** Jamie Cope, Kalpesh N. Biyani, Han-Ting Chang, Eric F. Connor, Angela Lee, Mingjun Liu, Deidre L. Madsen, Paul Mansky, Jonathan A. Mills, Florence Roger, Jerry Buysse. *Relypsa, Santa Clara, CA.*

Potassium (K) is the main intracellular electrolyte required for fundamental processes such as membrane activation, ion and solute transport, and the regulation of cell volume. Serum K levels ≥5.5mEq/L (hyperkalemia) can lead to muscle weakness, ventricular tachycardia and death. Precise control of K homeostasis, therefore, is critical. Acute or chronic hyperkalemia can arise from tissue trauma, renal insufficiency, insulin deficiency or resistance, conditions that interfere with the normal workings of the renin-angiotensin-aldosterone-system (RAAS) and/or the use of RAAS blocking drugs. For half a century, a cation exchange resin, sodium polystyrene sulfonate (Na-PSS; e.g. Kayexalate, Kionex), has been used to treat hyperkalemia. Recently its efficacy, as well as the safety of the doses of sorbitol with which it is most often co-administered, has been questioned.

RLY5016 is a new, high capacity, non-absorbed, cation exchange polymer intended for the control of serum K. The RLY5016 polymer comprises low swelling beads with an average diameter of 100uM. The counter-ion present on RLY5016 is calcium (rather than sodium), which is preferable for chronic heart failure or hypertension patients with chronic renal insufficiency in whom control of sodium intake is important.

RLY5016 releases most or all of its calcium under low gastric pH conditions and therefore becomes available for binding other cations when the pH increases upon exit from the stomach. Because of its novel chemical composition, RLY5016 has a binding capacity approaching double that of Na-PSS when tested in vitro at pH6-6.5 (Table).

RLY5016 binds up to 1.5mEq K per gram of polymer in human colonic and fecal extracts, where abundant interfering ions are available to compete for binding sites. This polymer is therefore capable of binding K in the complex milieu of the colon and increasing K excretion through this route.

| Polymer | Final pH | K Bound (mEq/g) |
|---|---|---|
| RLY5016 | 6.21 ± 0.02 | 8.53 ± 0.13 |
| Kayexalate | 6.35 ± 0.02 | 5.09 ± 0.05 |

Disclosure of Financial Relationships: Employer: Relypsa, Inc.; Ownership: Relypsa, Inc.

**PUB527**

**Acute Management of Patients with Hyperkalemia: A Systematic Review** Meghan J. Elliott,[1] Paul E. Ronksley,[2] Catherine M. Clase,[3,4] Sofia B. Ahmed,[1] Brenda Hemmelgarn.[1,2] *[1]Department of Medicine, University of Calgary, Calgary, AB, Canada; [2]Department of Community Health Sciences, University of Calgary, Calgary, AB, Canada; [3]Department of Medicine, McMaster University, Hamilton, ON, Canada; [4]Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, ON, Canada.*

Hyperkalemia occurs commonly and, if left untreated, can lead to fatal cardiac arrhythmias. We undertook a systematic review of the randomized trial evidence supporting available therapies in the acute management of hyperkalemia.

A previous systematic review included studies to 2003 (Mahoney BA et al, 2005). We conducted an all-language search of MEDLINE and EMBASE from 2003 to 2009 that combined the search theme, hyperkalemia, with the sensitive RCT filter. Eligible articles included RCTs, quasi-RCTs and crossover studies of pharmacological and non-pharmacological interventions for hyperkalemia in adults.

Our search retrieved 2158 potentially relevant citations; 9 were reviewed in full text (Kappa=0.94). Five (n=164) of these in addition to 11 studies (n=212) from the previous review were included. There was insufficient data from studies for meta-analysis to be performed. Intravenous (IV) insulin and dextrose reduced serum $K^+$, on average, by 0.5 mmol/L within 15 minutes and persisted 1 to 2 hours. A similar decrease occurred within 30 minutes of treatment with both nebulized and IV beta$_2$-agonist formulations. Nebulized albuterol and IV insulin together were more effective than either alone (decrease in $K^+$ 1.2 + 0.87 mmol/L at 60 minutes). Results for IV bicarbonate were equivocal. There was no appreciable benefit to resin or fludrocortisone over placebo. No major adverse events were reported with any of the interventions. Most studies were small and of variable quality.

The evidence guiding emergency interventions for hyperkalemia is limited and based on small studies with poor reporting and methodological limitations. Based on this systematic review, appropriate first line therapies include nebulized or inhaled beta-agonists and IV insulin with dextrose. Combination therapy appears more effective than either intervention alone. Further well-designed studies in this area are needed.

Disclosure of Financial Relationships: nothing to disclose

## PUB528

**Analysis of Characteristrics of Hyponatremic Patients Hospitalized Via Emergency Department** Eun Hee Jang,[1] So Yeon Choi.[2] *[1]Nephrology, School of Medicine, Jeju National University Hospital, Jeju, Jeju-do, Korea; [2]Internal Medicine, Seoul Adventist Hospital, Seoul, Korea.*

Hyponatremia is the most common electrolyte disorder. We evaluated the causes and clinical manifestations of symptomatic hyponatremia. We retrospectively reviewed clinical records of the hyponatremic patients who had been admitted from January 2005 to June 2009. We enrolled 68 patients(22 male, age 67.8±15.9 years, mean±SD) without liver cirrhosis, heart failure, chronic kidney disease or septic shock. We analyzed data to evaluate the differences of clinical manifestations according the age, sex, symptoms, taking medications(especially diuretics such as thiazide), urine sodium concentrations and the degree of hyponatremia. Sodium concentration([$Na^+$]) was 116.9±7.1 mEq/L and serum osmolalilty was 247.8±19.4 mOsm/kg. Urine sodium concentration and osmolality were 68.0±55.7 mEq/L and 382.8±150.4 mOsm/kg respectively. There was no difference in serum [$Na^+$] according to age, sex. Thirty four patients (50%) had nausea and vomiting and 21(30.9%) patients showed neurologic symptoms. Patients with neurologic symtoms seemed to have lower serum osmolarity than patients without neurologic symptoms(242.9± 19.2 vs 250.21±19.5 mOsm/kg, $p = 0.08$). The main causes of symptomatic hyponatremia were diuretics(35, 51.4%), poor oral intake(17, 25.0%) and SIADH(6, 8.8%). Initial choice of fluid therapy were discontinuation of offending drugs with free water restriction(8, 11.7%), 0.9% normal saline infusion(33, 48.5%) and hypertonic saline(20, 29.4%). Thiazide-induced hyponatremia(35, 51.4%) was common in elederly female patients(24) and showed more hypo-osmolar than hyponatremia due to other causes(240.0±17.8 vs 256.5 mOsm/kg, $p < 0.05$). However, in 19 cases of thiazide-induced hyponatremia, serum [$Na^+$] was recovered quickly by isotonic fluid infusion, discontinuing thiazide without hypertonic saline with no neurologic complications. Thiazide diuretics may be the main cause of symptomatic hyponatremia. Especially in elderly patients, thiazide should be carefully administered with frequent electrolyte monitoring.

Disclosure of Financial Relationships: nothing to disclose

## PUB529

**Hyponatremia with Aliskiren: Pseudohyporeninemic Hypoaldosteronism** Rahul S. Koushik.[1,2,3] *[1]UTHSC, TX; [2]International Kidneycare Foundation, TX; [3]SAKDC, San Antonio, TX.*

Introduction: Direct rennin inhibition (DRI, Aliskiren, Tekturna®, Rasilez®) has supplemented ACEI, ARB and spironolactone in renin-angiotensin-aldosterone system (RAAS) antagonism. Long term side effects of this drug are unknown. Three cases of hyponatremia on aliskiren, are presented.

Case 1: A 72 year-old obese Latino woman with CKD IV, DM2, HTN, CHF and remote uninephrectomy presented with dizziness, confusion, weakness after a recent increase in loop diuretic. Fifteen months ago, alsikerin 300 QD was added to a regimen of irbesartan 300 QD, lisinopril 40 BID, metoprolol 100 BID, nifedipine 60 BID and furosemide 80 BID. Symptoms and sodium slowly normalized on holding ACEI, ARB and aliskiren.

Case 2: A 60 year-old Latino woman with CKD III, DM2, HTN and hypothyroidism presented with dizziness, weakness, atypical chest discomfort. Two years ago aliskiren 150 QD was started to supplement lisinopril 40 QD and HCTZ 12.5 QD. She responded to IV fluids and withholding aliskiren/ACEI.

Case 3: A 74 year-old African-American woman with CKD IV, DM2 and HTN presented with a subcutaneous abscess and uncontrolled diabetes. Two years ago, aliskiren 300mg was added to her regimen of olmesartan 40 BID, amlodipine 10 QD, furosemide 40 BID and clonidine 0.2 BID.

BP and labs

| Case | BP | Sr Na | Sr K | Ur Na | BNP | Sr urate | eGFR |
|---|---|---|---|---|---|---|---|
| 1 | 110/75 | 117 | 5.0 | 25 | 633 | 9.3 | 22 |
| 2 | 82/50 | 134 | 4.4 | 112 | 5 | NA | 23 |
| 3 | 134/70 | 131 | 4.6 | 64 | 44 | 10.5 | 29 |

Ur Na= urine sodium, BNP=brain natriuretic peptide, NA=not available

Discussion: Patients presented with diverse symptoms, pre-renal labs (high BUN/creatinine ratios and sr. uric acid) and hyponatremia long after aliskiren was started (mean 21.3 months). They responded to holding RAAS blockers. Aliskiren therapy may result in a physiological state of *pseudohyporeninemic hypoaldosteronism* that interferes with the protective, renin-mediated, 'aldosterone escape' of chronic diuretic use.

Conclusions: We have reached a new zenith in RAAS antagonism. Aliskiren with other RAAS blocking agents and diuretics may increase susceptibility to severe hyponatremia. Urine electrolytes may not be useful in assessing volume depletion in aliskiren treated patients

Disclosure of Financial Relationships: nothing to disclose

## PUB530

**Hospital Admissions for Hyperkalemia with Trimethoprim-Sulfamethoxazole – A Cohort Study of 267,212 Older Women with Urinary Tract Infections** Ngan Lam,[1] Matthew A. Weir,[1,2] Amit X. Garg.[1,2,3] *[1]Department of Medicine, Division of Nephrology, University of Western Ontario, London, ON, Canada; [2]Department of Epidemiology and Biostatistics, University of Western Ontario, London, ON, Canada; [3]Institute for Clinical Evaluative Sciences, Toronto, ON, Canada.*

Background: Trimethoprim-sulfamethoxazole (TMP-SMX) is a commonly used antibiotic which may cause hyperkalemia by blocking the amiloride-sensitive sodium channels in the cortical collecting duct. This association has been described in case reports and small studies.

Objective: To obtain population-based estimates of the risk of hospital admissions for hyperkalemia following treatment with TMP-SMX in elderly women for urinary tract infection (UTI).

Design: Retrospective, population-based, cohort study.

Methods: We utilized linked health care administrative databases in Ontario, Canada from January 1, 1997 to March 31, 2009 to identify elderly women with simple UTIs who were treated with TMP-SMX. We compared their results to those treated with other antibiotics. The primary outcome was a hospital admission for hyperkalemia within 10 days of antibiotic initiation.

Results: The cohort consisted of 267,212 women. The primary outcome rate per 100,000 patients was 53.7 in the TMP-SMX group. This represented a 3.0-fold increased risk compared to amoxicillin, the referent drug. No such association was observed with the other antibiotics. Only 2.5% of women admitted for hyperkalemia had an outpatient potassium level measured in the days following their TMP-SMX initiation.

Conclusion: TMP-SMX therapy increases the risk of hospitalization for hyperkalemia. Physicians should be aware of this adverse event and consider a standing order to measure serum potassium three days after a TMP-SMX prescription in older patients at risk of hyperkalemia.

Association between antibiotic use and hospital admissions for hyperkalemia

| | Event rate (per 100,000 persons) | Odds Ratios [95% CI] | |
|---|---|---|---|
| | | Unadjusted | Adjusted |
| TMP-SMX | 53.7 | 2.30 [0.82, 6.51] | 3.05 [1.07, 8.67] |
| Ciprofloxacin | 15.5 | 0.66 [0.19, 2.35] | 0.66 [0.19, 2.36] |
| Norfloxacin | 9.0 | 0.38 [0.11, 1.31] | 0.46 [0.13, 1.58] |
| Nitrofurantoin | 9.8 | 0.42 [0.12, 1.43] | 0.45 [0.13, 1.55] |
| Amoxicillin (reference) | 23.3 | 1.00 | 1.00 |

Disclosure of Financial Relationships: nothing to disclose

## PUB531

**Spontaneous Severe Hypokalemia (HK) of Uncertain Etiology Presenting with Quadriplegia in a Rural South Indian Population** Sreejith Parameswaran,[1] Rathinam Swaminathan,[2] Jai Radhakrishnan.[3] *[1]Nephrology, JIPMER, Pondicherry, India; [2]Medicine, JIPMER, Pondicherry, India; [3]Medicine, Columbia U, NY, NY.*

Spontaneous, severe HK manifesting as quadriplegia in the absence of overt renal function abnormalities has been increasingly observed at our center( during just the months of May & June 2010, a total of 10 such pts were admitted to our center). In this preliminary report, we describe the clinical and biochemical features of 4 such pts (2F, age 34-44 yrs). 3 pts presented with flaccid quadriparesis and 1 pt with neck weakness. 2 pts reported nocturia. All pts were normotensive, none were diabetic. 2 pts experienced previous episodes and had discontinued K supplements.

Relevant biochemistries are shown:

Table 1:

| | Serum K (mEq/L) | Serum HCO3 (mEq/L) | Serum Creatinine (mg/dl) | Urine K (mEq/L) | Urine Osm (mosm/l) |
|---|---|---|---|---|---|
| Patient 1 | 2.1 | 27 | 1.0 | 8.6 | 204 |
| Patient 2 | 1.8 | 22 | 1.1 | 8.02 | 175 |
| Patient 3 | 1.9 | 36 | 0.8 | 12.7 | 457 |
| Patient 4 | 2.3 | 50 | 1.1 | 5.91 | 108 |

All the patients had normal s.creatinine, 2 of 4 patients had severe metabolic alkalosis, and developed low $iCa^{+/}$carpopedal spasm following K+ correction. All had low urine $K^+$ suggesting extrarenal loss or transcellular shift of $K^+$. None of the patients had h/o vomiting or loose stools, they denied h/o intake of drugs known to produce HK. Our center is located in southern peninsular India with a 'tropical dry climate' with high ambient temperatures.

March to June are hot and dry with temperatures up to 39$^0$C. All our patients lived in a rural setting and were of low socioeconomic status. . Rice was their staple diet, consumed three times a day; their financial status did not allow consumption of high $K^+$ foods(meat, vegetables and fruits). We hypothesize that extremely low dietary $K^+$ coupled with

excessive loss of $K^+$ in the sweat resulted in severe HK. Periodic paralysis is a possibility but is a rare entity.

In summary, we report, for the first time from our country, a high incidence of hypokalemic paralysis postulated to be from a very low consumption of $K^+$ coupled with excessive loss in sweat. Further studies on the epidemiology and etiology are underway.

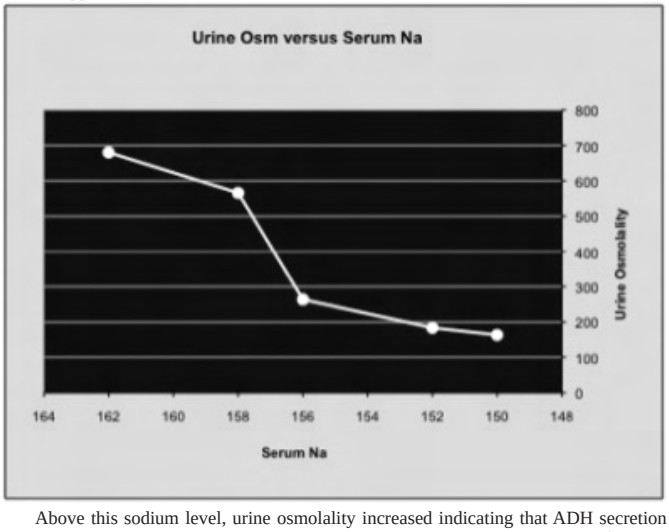
Disclosure of Financial Relationships: nothing to disclose

**PUB532**

**Neurosarcoidosis Presenting as Hypernatremia: Interplay of Reset Osmostat and Relative Hypodipsia** Kanwal Raghav,[1] Saurabh A. Pande,[2] Nina Mingioni,[1] Eric J. Bloom.[2] *[1]Internal Medicine, AEMC, Philadelphia, PA; [2]Nephrology, AEMC, Philadelphia, PA.*

Preface: Pathophysiology underlying hypernatremia entails either excess salt intake or free water loss. Antidiuretic hormone (ADH) release and thirst center stimulation due to rising plasma osmolality, act as homeostatic mechanisms. Together, they minimize free water loss and increase fluid intake, maintaining serum sodium within a narrow range. Rarely, the problem lies within this regulatory system impairing either the thirst mechanism or osmotic regulation of ADH release.

Case: 32-year-old woman with sarcoidosis presented with generalized weakness. Labs showed serum sodium of 172mmol/L and urine osmolality of 1302mOsm/k. Trending serum sodium and urine osmolality revealed that osmolalities dropped dramatically when sodium approached 157mmol/L.




Above this sodium level, urine osmolality increased indicating that ADH secretion and its action on kidney was intact. MRI showed enhancement anterior and inferior to third ventricle suggestive of neurosarcoidosis. Desmopressin with increased water intake, stabilized serum sodium nearing 154mmol/L.

Hypothesis: Since patient showed signs of thirst, but resultant water intake was insufficient to manage the serum osmolality, a relative hypodipsia was proposed. Also since urine osmolality fell dramatically below a sodium concentration of 157mmol/L, a reset osmostat with the body recognizing higher than normal sodium as the norm was coexistent. Neurosarcoidosis affecting the thirst center [medial preoptic nucleus] and osmoreceptors [subfornical organ and organum vasculosum of lamina terminalis] was the culprit lesion.

Discussion: Hypodipsia with essential hypernatremia due to reset osmostat is a rare entity. There is an anomalous thirst mechanism along with defective osmoreceptors which results in a chronic, fluctuating hypernatremia. treatment is uncertain.

Disclosure of Financial Relationships: nothing to disclose

**PUB533**

**Human Platelet Lysates as a Serum Substitute in Renal Epithelial Cell Culture** Caroline Rauch, Elisabeth Feifel, Gerhard Gstraunthaler. *Division of Physiology, Innsbruck Medical University, Innsbruck, Austria.*

Cultured renal epithelia form monolayers of differentiated, polarized cells. Grown on permeable supports, an apical and a basolateral compartment are separated by the cultured epithelium, that enable to study epithelial transport in vitro. Some requirements must be met by the in vitro system: retention of the polar cell architecture and junctional assembly, presence of vectorial transport, sidedness of cellular uptake routes, and retention of segment-specific metabolic and transport properties. Thus, culture conditions, culture media and supplements have a significant impact on in vitro epithelial function. In this respect, the quality of fetal bovine serum (FBS) seemed to be of major importance. In early studies a high lot-to-lot variablity of FBS was found that substantially influenced the differentiation of Xenopus laevis A6 epithelia and the generation of a transepithelial potential difference (PD) and resistance (TEER), and batch-testing of FBS was required.

We recently reported on the use of human platelet lysates (PL) as a replacement for FBS. PL preparation was optimized for growth factor content, quantified by ELISA. The growth promoting capacity of PL was tested on renal cell lines, for which growth characteristics, phenotypes, and differentiation end points are well established. PL support growth, proliferation and differentiation, as assessed by dome formation, of proximal tubule-like LLC-PK1, HK-2 and distal tubule-like MDCK cells. Proliferation was monitored by determination of cell density and by resazurin or WST-8 assays. Proliferation rates were identical in culture media with 10% FBS or 5% PL. To biochemically determine the mitogenic potential of PL, the stimulation of ERK1/2 MAP kinase was determined. Addition of PL to quiescent LLC-PK1 cultures resulted, like FBS, in specific phosphorylation, and thus activation, of ERK1/2. In addition, TEER was monitored in filter-grown LLC-PK1 and MDCK epithelia. Both epithelia generated a TEER of 150-250 Ω•cm2 in PL-supplemented media, comparable with FBS.

PL are a valuable, animal-derived component-free substitute for FBS in renal epithelial cell culture, with a proven high batch-to batch uniformity.

Disclosure of Financial Relationships: nothing to disclose

**PUB534**

**Progesterone: The Missing Factor in Potassium Homeostasis in Mice and Men** Amel Salhi,[1] Anie Azroyan,[1] Aurelie Edwards,[2] Gilles Crambert.[1] *[1]Centre de Recherche des Cordeliers, INSERM/UPMC/CNRS, Paris, France; [2]Department of Chemical and Biological Engineering, Tuft University, Medford, MA.*

Modern dietary habits are characterized by high-Na+ and low-K+ intakes, each of which has been correlated with a higher risk for hypertension. In this study, we examined whether long-term variations in the intake of Na+ and K+ induce lasting changes in the plasma concentration of circulating steroids by developing a mathematical model of steroidogenesis in mice. An interesting finding of the mathematical model is that mice increase their plasma progesterone levels specifically in response to K+ depletion. This prediction was confirmed by experimental measurements in both male mice and men. We then focused on the putative role of progesterone as an in vivo modulator of renal function under potassium restriction conditions. Our results indicate that progesterone regulates renal K+ handling both in males and females, independently of its role in reproduction. We established that the increase in progesterone production by male mice is time-dependent and is correlated with decreased urinary K+ content. The progesterone-dependent ability to retain K+ efficiently is due to a RU486-sensitive stimulation of the "colonic" H,K-ATPase expressed in kidney. Our results suggest the existence of a hereto unknown regulatory process involving progesterone, its nuclear receptor, the colonic H,K-ATPase and renal K+ retention.

Disclosure of Financial Relationships: nothing to disclose

**PUB535**

**Involvement of H,K-ATPase Type2 in Renal Potassium Retention during Pregnancy** Amel Salhi, Gilles Crambert. *Centre de Recherche des Cordeliers, UPMC / CNRS, Paris, France.*

Progesterone, a steroid hormone involved in the female menstrual cycle, pregnancy (supports gestation) and embryogenesis, is also a neuromediator acting in the central nervous system, suggesting that this hormone may have other functions larger than those usually described. Moreover, different types of progesterone receptors are found in non reproductive tissues, such as kidney.

Previous studies from our group have recently shown that adrenal progesterone is a hormone produced in a chronic potassium deficient diet, and that it acts on kidney to allow optimal potassium reabsorption by stimulating the H,K-ATPase type 2 (HKA).

Our objective was to determine the role of progesterone in different circumstances known to induce renal potassium retention, for instance in pregnancy.

We have confirmed that a positive potassium balance is induced during pregnancy, regardless of food intake, and this retention is correlated to a stimulation of H,K-ATPase type2 expression (3 fold increase at day 16)

To examine the hypothesis that HKA is important during pregnancy, we have mated wild type mice and established a time course of serum progesterone level, urinary potassium excretion and renal expression of the HKA in female during pregnancy. We have also examined the phenotype of female deficient for HKA during pregnancy.

Preliminary results suggest that HKA is stimulated in kidney of pregnant females and that the lack of HKA results in hardship during parturition. These difficulties are not linked to the genotype of fetuses and potassium replenishment through absorption of enriched water will allow optimal gestation/delivery.

Disclosure of Financial Relationships: nothing to disclose

**PUB536**

**Gordon's Syndrome: A Case Report of Normotensive Variant** Erdal Sarac. *Department of Internal Medicine, St. Elizabeth Health Center - NEOUCOM, Youngstown, OH.*

Gordon's syndrome, considered a monogenic form of low-renin hypertension, is a rare, autosomal-dominant disease characterized by hypertension, hyperkalemia, hyperchloremic metabolic acidosis and normal glomerular filtration rate.

Here we report an unusual presentation of Gordon's syndrome with normal blood pressure in a 42 year-old Caucasian female with persistent hyperkalemia. Past medical history included endocarditis, classic migraine, non-aneurysmal sub-arachnoid hemorrhage, bronchial asthma, and osteoporosis. The patient complained of muscle weakness and had unremarkable physical examination and normal blood pressure. Laboratory evaluation revealed: normal serum sodium, chloride. bicarbonate level and glomerular filtration rate. Baseline plasma renin activity, aldosterone and cortisol levels were within normal range. An ACTH stimulation essay was performed:

ACTH Stimulation Essay

| Laboratory Results | Baseline | Post-ACTH |
|---|---|---|
| Urine K (mmol/L) | 4.9 | 8.7 |
| Serum ACTH (ng/dl) | 19 | 9 |
| Serum aldosterone (ng/dl) | 2.1 | 26 |
| Urine Na (mmol/L) | 66 | 93 |
| Serum K (mmol/L) | 5.7 | 5.7 |

Additional testing revealed normal 24 h urine aldosterone, HbA1C, 1,25 (OH)2 Vitamin D, intact PTH level, CK total, CT abdomen and pelvis, renal ultrasound, and renal scan. A diagnosis of a primary selective defect in potassium secretion, such as the one associated with Gordon's syndrome, was made. Treatment with hydrochlorothiazide was started with subsequent return of serum potassium level to normal.

To date, about 100 cases of Gordon syndrome have been described in the literature. Both familial and sporadic forms have been seen. Normotensive forms of Gordon's syndrome were also described and is mostly seen in adults. This case demonstrates the need to be aware of a normotensive variant of Gordon syndrome especially in adult patients with unexplained hyperkalemia.

Disclosure of Financial Relationships: nothing to disclose

**PUB537**

**Treatment of Hyponatremia Is Associated with Enhanced Cognitive Function** Rick P. Vaghasiya, Maria V. Devita, Georgia Panagopoulos, Michael F. Michelis. *Division of Nephrology, Department of Medicine, Lenox Hill Hospital, New York, NY.*

Hyponatremia occurs in 15% to 30% of hospitalized patients, making it the most common electrolyte abnormality in this population. Mild hyponatremia has generally been considered asymptomatic, however, a recent study showed that patients with mild to moderate chronic hyponatremia had an increased risk of falls, as well as gait and attention impairments. The present study was designed to assess cognition levels using the Mini-Mental Status Exam (MMSE) in patients with varying degrees of hyponatremia pre and post serum sodium (SNa) improvement.

Twenty-four hospitalized patients with SNa values $\leq$ 134 meq/L from a single center were included. The MMSE was administered to these patients and scores recorded out of a maximum of 30. The MMSE was repeated when the investigators felt the SNa improved appreciably. Initial SNa levels were approached therapeutically with 0.9% NS, fluid restriction, vasopressin receptor antagonists, withholding medications, or 3% NS as clinically indicated. The MMSE were administered at least 72 hours apart and the individual questions were altered when the test was repeated. Pre and post SNa improvement MMSE scores were compared.

The initial SNa levels of the 24 patients ranged from 117 to 134 meq/L with a mean of 124.3 meq/L (SD±4.4) and post-improvement SNa ranged from 127 to 143 meq/L with a mean of 133.7 meq/L (SD± 4.1, p=0.016). The mean SNa improvement was 9.4 meq/L. Overall, 21 of the 24 patients (88%) had an increase in MMSE score after improvement in SNa. Of those 21 patients, 7 patients had a pre SNa level $\geq$ 127 meq/L. Nine patients had a 4-10% increase in MMSE score, 8 patients had an 11-20% increase, 3 patients had a 21-35% increase and one patient had a 100% increase in the MMSE score after SNa improvement.

Treatment of hyponatremia at all levels was associated with improved cognitive function. These findings suggest that careful monitoring of serum sodium levels is important in all patients, even in those with a mild degree of hyponatremia. Attempting to correct hyponatremia to improve cognitive function should be encouraged.

Disclosure of Financial Relationships: nothing to disclose

**PUB538**

**Diabetic Ketoacidosis in Haemodialysis-Dependent Patients-"A Rare Occurence"** Bhavani Adusumilli. *Internal Medicine, William Beaumont Hospital, Royal Oak, MI.*

The occurrence of diabetic ketoacidosis (DKA) in hemodialysis-dependent (HD) patients has been rarely reported in the literature and involves a different treatment algorithm compared to those with preserved renal function. Here, we present the clinical course and management of hypertension and DKA in a patient with end stage renal disease on chronic hemodialysis.

A 32-year-old African-American male with insulin dependent diabetes mellitus and chronic renal failure on hemodialysis presented with nausea and bilious vomiting. He reported not taking insulin for two days because of decreased appetite. On presentation his vital signs included a blood pressure of 180/125, respiratory rate of 22 breaths/min, and heart rate of 91 beats/min, temperature of 36.1 $^{o}$ C. Clinical examination revealed strong odor of ketones, dry mucous membranes, poor capillary refill and clear breath sounds. Serum biochemistry measurements showed serum glucose of 281mg/dl, sodium of 137 mEq/L, potassium of 3.9 mEq/L, chloride of 99 mEq/L, anion-gap of 21 with bicarbonate of 17 mEq/L, BUN of 31 and serum creatinine of 14.59 mmol/L. Other relevant results included beta-hydroxybutyrate level of 1.91 mmol/L, lipase of 37 units/L and serum osmolality of 300mosm/L. Chest X -ray and blood and urine cultures were unremarkable. He was started on regular insulin infusion with frequent monitoring of blood glucose and serum electrolytes. Hemodialysis was initiated with rapid correction of metabolic abnormalities.

This case illustrates the main issues facing clinicians managing DKA in HD patients. Unlike severe hyperglycemia in preserved renal function, which routinely causes clinically significant extracellular volume (EC) deficits, DKA in HD patients causes EC expansion. Furthermore, the same degree of hyperglycemia causes less rise in serum tonicity in HD patients compared to patients with preserved renal function primarily because of the absence of osmotic diuresis in dialysis patients. Although sole therapy with insulin infusion is adequate to treat severe hyperglycemia in majority of HD patients, associated high anion gap metabolic acidosis requires hemodialysis.

Disclosure of Financial Relationships: nothing to disclose

**PUB539**

**Exploring Molecular Mechanisms behind the Aquaporin-2 Translocation in Renal Principal Cells Using Small Molecule Inhibitors** Jana Bogum,[1, 2] Vedrana Tabor,[1] Jens Furkert,[1] Jens von Kries,[1] Walter Rosenthal,[3, 4] Enno Klussmann.[1] *[1]Leibniz Institute for Molecular Pharmacology (FMP), Berlin, Germany; [2]Department of Biology, Chemistry and Pharmacy, Free University Berlin, Berlin, Germany; [3]Max Delbrueck Center for Molecular Medicine (MDC), Berlin, Germany; [4]Department of Molecular Pharmacology and Cell Biology, Charite - University Medicine, Berlin, Germany.*

Arginine-vasopressin (AVP) regulates aquaporin-2(AQP2)-mediated water reabsorption from primary urine by binding to the vasopressin V2 receptor on the surface of renal collecting duct principal cells. Stimulation of the receptor is followed by an increase in cAMP and activation of protein kinase A (PKA) that phosphorylates AQP2 at C-terminal Serine 256. This, in turn, leads to a redistribution of AQP2 from intracellular vesicles into the plasma membrane and facilitates water reabsorption from primary urine. Only few proteins involved in the control of AQP2 have been identified. Thus, molecular mechanisms underlying the transport of AQP2 are largely unknown. In a cell-based screening approach we identified novel small molecules inhibiting the cAMP-dependent AQP2 redistribution. Through detailed characterization of effects of the small molecules and identification of their targets we aim to discover proteins controlling the AQP2 translocation to the plasma membrane of renal principal cells. Such novel inhibitors of the AQP2 redistribution might be useful to develop an efficient therapy for diseases associated with excessive water retention, such as chronic heart failure (CHF), liver cirrhosis or SIADH (syndrome of inappropriate antidiuretic hormone hypersecretion).

Disclosure of Financial Relationships: nothing to disclose

Disclosure of Financial Relationships: nothing to disclose

**PUB540**

**SIADH in a Patient with Fetanyl Patient-Controlled Analgesia** Roger F. Carbajal Mendoza, Hemant Magoo, Donald I. Baumstein, Alf M. Tannenberg. *Nephrology, Metropolitan Hospital Center/New York Medical College, New York, NY.*

Introduction: Hyponatremia is the most common electrolyte disorder in hospitalized patients. We report a case of hyponatremia secondary to syndrome of inappropriate secretion of antidiuretic hormone (SIADH) associated with Fentanyl Patient-Controlled Analgesia (PCA) in the setting of treatment of abdominal pain. To the best of our knowledge there is only one prior case report of fentanyl and SIADH which involved fentanyl transdermal patch.

Case: A 55 year old African American female patient was treated with fentanyl PCA pump for abdominal pain secondary to pancreatitis. The patient developed euvolemic hypoosmolar hyponatremia while receiving pain control with fentanyl PCA. Serum osmolality was 272 mOsm/kg while urine osmolality was 415 mOsm/kg suggesting inappropriate antidiuresis. Urine sodium of 139 mmol/L argues against volume contraction. Serum creatinine, thyroid function test and cortisol levels were normal consistent with adequate renal, thyroid and adrenal function.There was no recent use of diuretics.

Conclusion: We found an association between fentanyl PCA and hyponatremia with resolution of hyponatremia once the drug was discontinued.

Disclosure of Financial Relationships: nothing to disclose

**PUB541**

**Pioglitazone-Induced Sodium Retention in Rat Is Independent of alpha Subunit of Epithelial Sodium Channel (ENaC$\alpha$) Expression in the Distal Nephron** Jinping Li, Bala Sundara Ponnam, Hongbao Ma, Shyan-Yih Chou. *Renal and Hypertension Division, Brookdale University Hospital, NY.*

***Purpose:*** To investigate the role of ENaC$\alpha$ in sodium retention induced by pioglitazone (PGZ).

***Methods:*** Male SD rats were maintained on a 1% NaCl diet; the experimental group was fed on the same diet plus PGZ. To suppress the production of aldosterone, a separate group of rats were maintained on a 3% NaCl diet with or without PGZ. To further suppress aldosterone production, an angiotensin converting enzyme inhibitor was combined with an angiotensin receptor antagonist. In this group, the rats were fed on a 3% NaCl diet and received enalapril plus olmesartan with or without PGZ. All PGZ-treated rats received PGZ at the dose of 40 mg/kg bw/day for 5 days. Western blotting was then performed and the data were expressed as mean ± SE in arbitrary densitometry units normalized to ß-actin signals.

***Results:*** In the group of rats maintained on a 1% NaCl diet, the protein expression for ENaC$\alpha$ in the medulla was similar between the control and PGZ-treated rats (band density, 0.536 ± 0.016 in PGZ-treated rats, *vs* 0.604 ± 0.040 in control). In the cortex, the band density for ENaC$\alpha$ was 0.676 ± 0.110 in PGZ-treated rats, similar to 0.746 ± 0.156 in pair-fed control rats. In PGZ-treated and control rats, there was a linear correlation between ENaC$\alpha$ and serum and glucocorticoid-induced kinase-1 protein expression (P = 0.01). In the group of rats maintained on a 3% NaCl diet, the expression for ENaC$\alpha$ in the medulla was 0.996 ± 0.182 in PGZ-treated rats, similar to 0.573 ± 0.049 in pair-fed control rats. In

the cortex, the band density for ENaCα was 1.256 ± 0.193 in PGZ-treated rats, similar to 1.320 ± 0.104 in pair-fed control rats. In PGZ-treated rats maintained on a 3% NaCl diet while receiving enalapril and olmesartan, the band density for ENaCα in the medulla was 0.604 ± 0.070, similar to 0.602 ± 0.032 in pair-fed rats receiving enalapril and olmesartan. In the cortex, the band density for ENaCα was 0.189 ± 0.016 in PGZ-treated rats, similar to 0.304 ± 0.050 in control rats.

***Conclusion:*** Sodium retention induced by PGZ is independent of expression of ENaCα in the apical membrane of the distal nephron.

Disclosure of Financial Relationships: nothing to disclose

**PUB542**

**Drug Interaction between Celecoxib and Methotrexate (MTX) in Organic Anion Transporter 3-Transfected Renal Cells and in Rats** Akimitsu Maeda,[1] Shuichi Tsuruoka,[2] Kentarou Ushijima,[4] Yoshikatsu Kanai,[3] Hitoshi Endou,[5] Akio Fujimura.[4] *[1]Pharmacy, Aichi Cardiovascular and Respiratory Center, Ichinomiya, Aichi, Japan; [2]Nephrology, Univ. of Tsukuba, Tsukuba, Ibaraki, Japan; [3]Pharmacology, Osaka Univ., Osaka, Japan; [4]Pharmacology, Jichi Medical Univ., Shimotsukua, Tochigi, Japan; [5]Pharmacology, Kyorin Univ., Mitaka, Tokyo, Japan.*

MTX has a clinically important pharmacokinetic interaction with nonsteroidal anti-inflammatory drugs (NSAIDs) mainly through its competition for tubular secretion via the renal organic anion transporter 3 (OAT3). Celecoxib, cyclooxygenase 2 inhibiter, has not been reported to interact with MTX, but the mechanisms are unclear why the interaction is not occurred. We have previously reported the usefulness of human OAT3-transfected renal tubular cells for screening of the drugs which interfere with the pharmacokinetics of MTX. The purpose of this study was to evaluate the effect of celecoxib on MTX tubular secretion using a renal cell line stably expressing human OAT3 (S2-hOAT3), and to evaluate the pharmacokinetic interaction of the two drugs in rats. [$^3$H]MTX uptake into S2-hOAT3 cells was significantly inhibited by celecoxib in a concentration-dependent manner and the Ki value was 35.3 μM. However, MTX serum concentrations and urinary excretion of MTX over 24 h in rats were not affected by celecoxib (50, 200 mg/kg). Celecoxib serum concentrations in the rats were increased by the increase in celecoxib dosage and the maximum drug concentration (Cmax) was 20.6 μM (celecoxib 200 mg/kg), which did not reach the Ki value obtained in the in vitro study. These results indicated that celecoxib inhibited the secretion of MTX via hOAT3, which suggested that celecoxib was a substrate of hOAT3. However, co-administration of the two drugs at clinical dosage did not affect the pharmacokinetics of MTX, because the serum concentrations did not reach the Ki value. Although the accumulation study using S2-hOAT3 cells was useful to predict the interaction between the new drug and MTX in vivo, a comparison of the Ki value with the Cmax in clinical dosage was necessary to evaluate the degree of this interaction.

Disclosure of Financial Relationships: nothing to disclose

**PUB543**

**Changes in Serum Aldosterone Relate to Reductions of Extracellular Volume in Hemodialysis Patients** Jochen G. Raimann,[1,2] Li Liu,[1,2,5] Fansan Zhu,[1] Stephan Thijssen,[1,2] Mary Carter,[1] Andrew S. Bomback,[4] Vimal K. Derebail,[3] Philip J. Klemmer,[3] Nathan W. Levin,[1] Peter Kotanko.[1,2] *[1]RRI, NYC; [2]BIMC, NYC; [3]UNC, Chapel Hill; [4]Columbia University, NYC; [5]Peking University First Hospital, Beijing.*

Background

Water and salt homeostasis in healthy subjects is tightly regulated by the renin - angiotensin -aldosterone system (RAAS). In hemodialysis (HD) patients (pts) a right-shifted relation between extracellular volume (ECV) and serum aldosterone was demonstrated (Bomback, 2009). This study aimed to investigate temporal changes of aldosterone following ECV reduction in HD pts.

Methods

Chronic HD pts (BMI < 40 kg/m2) underwent gradual reduction of post-HD weight. ECV, total body water (TBW) and aldosterone were measured pre-HD at baseline (BL) and after post-HD weight reduction. TBW and ECV were measured by segmental bioimpedance spectroscopy (Zhu, 2006). Serum aldosterone was determined by ELISA (Alpco Diagnostics). Changes in the aforementioned parameters were assessed by paired T-Test. Data are shown as mean±SD.

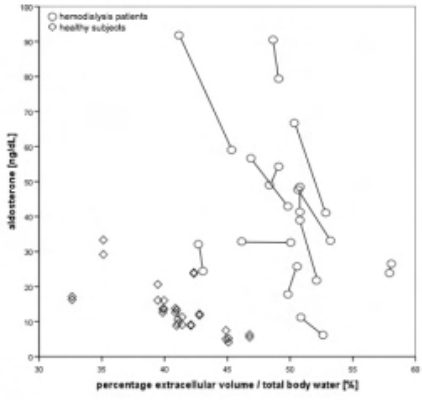
Results

Thirteen HD pts (age 55±14 yrs, 9 females, 8 blacks, BMI 27±6 kg/m2) were enrolled over a period of 53±39 days. ECV changed by -1.2±2.6 L (P=0.11), ECV/TBW (BL: 49.3±3.7%; end: 49.1±4.0%, P=0.8) and aldosterone (BL: 40.7±23.5 ng/dL versus 42.1±19.9 ng/dL, P=0.7) did not change significantly. Changes in aldosterone correlated significantly with changes of ECV/TBW ($R^2$=0.57, P<0.01; Figure 1).

Conclusion

Serum aldosterone levels are higher in HD pts compared to healthy controls (Figure 1) and its relationship to ECV/TBW is shifted to the right. The inverse relationship between changes of ECV/TBW and accompanying changes in aldosterone seen in HD pts suggests an intact RAAS sensitive to ECV changes.

Figure1: Serum aldosterone versus ECV/TBW in healthy subjects (data from Bomback, 2009) and the 13 HD patients in the present study.




Disclosure of Financial Relationships: nothing to disclose

**PUB544**

**Prophylactic Tolvaptan for Chemotheraputic Hydration in a Central Nervous System Lymphoma Complicated by Syndrome of Inappropriate Antidiuretic Hormone** Maura A. Watson, Frank P. Hurst, Stephen W. Olson. *Medicine/Nephrology Service, Walter Reed Army Medical Center, Washington, DC.*

**Case:** A 59 year old female with primary central nervous system (CNS) lymphoma required high volume sodium bicarbonate infusion during rituxan and methotrexate infusion due to acute kidney injury (AKI) in the setting of prior treatment cycles. During the infusion, the patient developed symptomatic hyponatremia with a sodium nadir of 120. Urine and serum sodium, creatinine, and osmolarity were consistent with the diagnosis of syndrome of inappropriate antidiuretic hormone (SIADH). Serum sodium normalized and symptoms resolved after discontinuation of volume replacement and initiation of free water restriction. The V2-receptor antagonist tolvaptan was added to her subsequent chemotherapy cycle with prophylactic high volume sodium bicarbonate infusion and successfully prevented repeat symptomatic hyponatremia. There also was no repeat AKI. Urine osmolarity declined from 694 to 344 mOsm/kg over four days, suggesting effective urinary free water loss induced by tolvaptan.

**Discussion:** To our knowledge, this represents the first reported case of the successful use of tolvaptan to permit chemotherapeutic hydration in the setting of SIADH. SIADH is a common disorder seen in CNS malignancies and can complicate chemotherapeutic regimens requiring intravenous fluids. Tolvaptan is a selective oral vasopressin 2-receptor antagonist that increases free water excretion to reverse the effects of SIADH. Previous studies have focused on the potential clinical benefit of tolvaptan in cardiac heart failure, cirrhosis and acute brain injury. This case suggests a potential treatment niche for vasopressin receptor antagonists in patients with SIADH in the setting of disease processes that require aggressive hydration such as contrast prophylaxis, hypercalcemia, rhabdomyolysis and chemotherapeutic hydration.

Disclosure of Financial Relationships: nothing to disclose

**PUB545**

**In Utero Exposure to Maternal Diabetes Lead to Glucose Intolerance and HTA without Major Effect on Lipid Metabolism** Bertrand Blondeau,[1] Celine Fassot,[1] Jean-Paul Duong van Huyen,[2] Martine D. Lelievre-Pegorier.[1] *[1]INSERM U872, Centre de Recherche des Cordeliers, Paris, France; [2]Departement de Pathologie, Hopital Europeen Georges Pompidou, Paris, France.*

We have previously demonstrated in the rat that in utero exposure to maternal diabetes impairs renal development leading to a reduction in nephron number (Nehiri et al, Diabetes 2008). Recent evidence shows that adult metabolic disease may originate from adverse fetal environment that will alter organ development and function in postnatal life. Here we analyzed the in utero exposure to maternal diabetes on the development of a metabolic syndrome in the offspring.

Pregnant rats were made diabetic (plasma glucose around 20 mM) with a single injection of streptozotocin injection on day 0 of gestation. Offspring from diabetic mothers (DMO) and from control mothers (CMO) were followed from birth to 12 months of age. In these animals, metabolic parameters such as glucose tolerance, insulin sensitivity as well as plasma lipid levels and pancreatic insulin and morphology were studied.

As compared to controls, DMO offspring had a normal birth weight and an impaired postnatal growth that persisted throughout life. Metabolic tests revealed that DMO offspring show an impaired glucose tolerance as soon as 6 months associated with a decreased insulin secretion but normal insulin sensitivity. In older animals (12 months of age), glucose intolerance and both impaired insulin secretion and sensitivity were still observed. In DMO offspring we measured high blood pressures, decreased fasting plasma triglycerides levels and normal plasma NEFA, HDL-cholesterol and total cholesterol.

Altogether, these results show that this model of in utero exposure to maternal diabetes leading to normal birth weight induced glucose intolerance and increased blood pressure,

without major effects on lipid metabolism. It is suggested that a fetal hyperglycaemic environment program hypertension and glucose intolerance but does not alter lipid metabolism.

Disclosure of Financial Relationships: nothing to disclose

**PUB546**

**Renalase Belongs to a New Class of Catecholamines Metabolizing Enzymes** Gary V. Desir, Peili Wang, Guoyong Li, Lieqi Tang, Heino Velazquez. *Medicine, Yale School of Med, VACHS, West Haven, CT.*

Renalase is an FAD containing protein that is expressed in kidney, heart, skeletal muscle, and small intestine, and is secreted into blood. In vitro data suggest it specifically degrades catecholamines and functions as a soluble amine oxidase. However, recombinant renalase metabolized catecholamines at a rate low enough to cast doubt on its physiological relevance as an amine oxidase. To gain further insight into its kinetic properties, we performed additional computer-based domains analyses, and noted the presence of a Rossmann fold, and significant similarities with some members of the NADB_Rossmann superfamily, suggesting the presence of a NAD(P) binding site. In vitro assays revealed that hRenalase has intrinsic NADH oxidase activity in the absence of catecholamines (Km=9.39±0.87 μM, Vmax=17.19±1.22 nmol/min/mg protein). NADPH is not metabolized to a significant degree. A saturating concentration of NADH (250 μM) stimulated renalase-mediated epinephrine metabolism by 17.9±1.1 fold ($p<0.000001$). The estimated Km for epinephrine is 17.09±4.28 μM, with a Vmax of 22.18±0.11 nmol/min/mg protein. Epinephrine metabolism is autocalytic since the rate increases by 2 fold over the first 30 minutes. While catalase was ineffective, superoxide dismutase inhibited epinephrine metabolism completely, indicating a superoxide anion dependent mechanism. We conclude that recombinant hRenalase1 belongs to a new class of FAD-containing, NADH-dependent catecholamines metabolizing enzymes, and its kinetics parameters for epinephrine compare favorably to those of MAO-A and MAO-B. We speculate that renalase is a physiologically relevant modulator of epinephrine levels.

Disclosure of Financial Relationships: nothing to disclose

**PUB547**

**Comparative Study of Additional Antihypertensive Effect of Magnesium Supplement with or without Angiotensin-II Receptor Blocker in Hypomagnesemic Rats** Yang Wook Kim,[1] Tae Hee Kim,[2] Hyun Seung Lee,[2] Kyu-Bok Jin.[1] *[1]Nephrology, Internal Medicine, Haeundae Paik Hospital, Inje University, Pusan, Korea; [2]Nephrology, Internal Medicine, Busan Paik Hospital, Inje University, Pusan, Korea.*

**Introduction;** Mg is an essential element critically involved in vascular function and influences blood pressure(Bp) regulation by modulating vascular tone and reactivity. Several studies have shown that serum Mg is inversely associated with blood pressure and plasma renin activity. The purpose of this study was to evaluate the additional effect of dietary Mg supplement with angiotensin receptor blocker on Bp in hypomagnesemic rats.

**Methods;** Fifty five S-D male rats were used. The rats were divided into Mg-deficient and control groups. Mg-free and Mg-contained diet was adminitrated. After 14 weeks, ten Mg-deficient and five control rats were treated with Mg. Another group of ten Mg-deficient rats were received angiotensin receptor blocker(ARB; losartan , 30 mg/Kg, intraperitoneal), and ten Mg-deficient rats received ARB plus Mg in the same way.

Systolic Bp was measured by tail-cuff method using a plethysmography. During this experimental period, serum Mg, calcium, potassium, angiotensin, aldosterone, 1,25-dihydroxyvitamin D and vasopressin were measured.

TGF-b1, AQP-2 of renal tissue extract and immunohistochemistry for TGF-beta1 were preformed.

**Results;** At 14 weeks, systolic Bp was significantly higher in Mg-deficient rats than in control rats ($P$=0.034).

In the hypomagnesemic rats, treatment with Mg resulted in a decrease of systolic Bp, which was further decreased by losartan and losartan/Mg combination. Treatment with losartan/Mg combination resulted in a decrease of systolic Bp most. However, the difference did not reach statistical significance. The serum calcium, angiotensin II, aldosterone and 1,25-dihydroxyvitamin D were higher while the vasopressin level lower in Mg-deficient rats. AQP-2 expression was increased in hypomagnesemic group, decreased in losartan group. There were no differences of pathological findings and expression of TGF-b in tissue of kidney and heart among groups.

**Conclusion;** The oral magnesium supplement can reduce the blood pressure in hypomagnesemic hypertension.

Also, Magnesium supplementation may enhance the effect of antihypertensive medication of angiotensin-II receptor blocker.

Disclosure of Financial Relationships: nothing to disclose

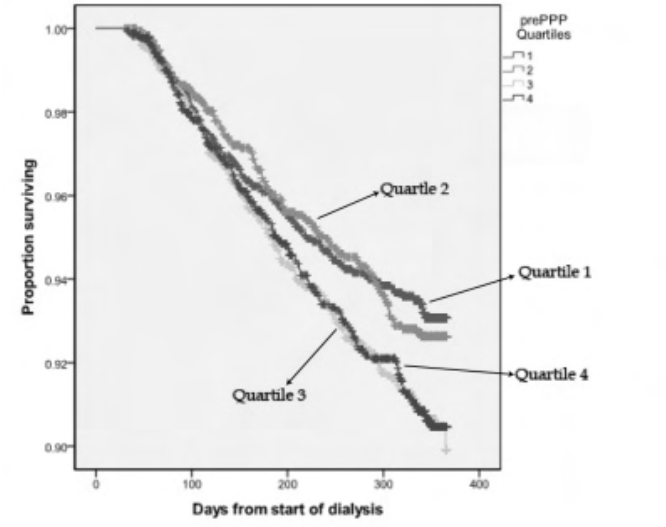
**PUB548**

**Impact of Pulse Pressure on Patient Outcomes in Incident Hemodialysis Patients** Len A. Usvyat,[1] E. Lars Penne,[1,2] Olga Sergeyeva,[1] Stephan Thijssen,[1,2] Peter Kotanko,[1] Nathan W. Levin.[1] *[1]Renal Research Institute, NY, NY; [2]Beth Israel Medical Center, NY, NY.*

High pulse pressure is associated with increased mortality in general population. The aim of this study was to examine the specific effects of this relationship in hemodialysis (HD) patients while also adjusting for systolic blood pressure (SBP) and change in SBP over time.

All RRI in-center HD patients who initiated dialysis between Jan 2000 and Jan 2009 were included. Pre-HD SBP (preSBP) and diastolic blood pressure (preDBP) were recorded each treatment in the first year of HD. Mean preSBP (preSBP) and the pulse pressure as percent of preSBP (prePPP = 100%*[preSBP - preDBP]/ preSBP) of the first 30 days were calculated. The linear slope of preSBP (preSBPSLOPE) was computed per patient using all preSBP values in year one to demonstrate change in preSBP: increased, decreased, and stable. Patients were stratified based on quartiles of prePPP: quartile 1 (14-44%), 2 (44-48%), 3 (48-52%), 4 (52-74%).

7077 pts were included (56% male, 39% black, 53% diabetic, age [SD]: 62.2 [15.8] yr). Using Kaplan-Meier analysis, quartiles 1 and 2 of prePPP had the longest survival time while quartiles 3 and 4 shortest survival ($p<0.05$ using log-rank test).




Using Cox regression (adjusted for gender, race, diabetes, preSBP, preSBPSLOPE), quartile 4 had the highest risk of death (HR=1.36, 95% CI: 1.05-1.77), followed by quartile 3 (HR=1.26, 95% CI: 0.98-1.62). No differences were observed between quartile 1 and quartile 2.

The differences in prePPP groups disappeared after adjusting for age as a continuous variable. This finding was further confirmed by a subgroup analysis stratified by age groups, suggesting that prePPP does not alter patient outcomes in dialysis population.

In contrast to the general population, pulse pressure is not a significant predictor of mortality in HD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB549**

**Blood Pressure Characterization by Radiotelemetry in Rat Offspring after Maternal Low Protein Diet** V. Matti Vehaskari,[1,2] Tyrus Stewart,[1] Jeannine Ory Ascani,[2] Johnny Porter.[3] *[1]Pediatrics, LSU Health Sciences Center, New Orleans, LA; [2]The Research Institute for Children, New Orleans, LA; [3]Physiology, LSU Health Sciences Center, New Orleans, LA.*

Maternal protein restriction in rats has been widely used to induce intrauterine growth restriction (IUGR) and adult hypertension, usually measured by the tail cuff method, in the offspring. The present study was undertaken to characterize the BP profile in unstressed IUGR offspring by radiotelemetry.

Pregnant dams were subjected to 6% protein (LP) or control 20% protein (C) diet throughout second half of pregnancy. Radiotelemetry transmitter was implanted in subgroup of LP (N=7) and C (N=6) offspring at 7-10 months of age. Baseline telemetry recording was started 5 days later, followed by 1 week of recording on high Na (3%) diet. Renal cortical and medullary tissue for epinephrine and norepinephrine assay by HPLC was collected at sacrifice.

LP pups had 15% lower birth weight. By tail cuff method, elevated daytime systolic BP in LP pups was present by 8 weeks of age and persisted until 10 months of age (LP 133 vs. C 119 mmHg, P=0.014) but the difference disappeared under anesthesia (LP 119 vs. C 119 mmHg). Continuous telemetry monitoring in awake animals revealed no significant differences between LP and C pups during day or night in systolic (day 119 vs. 121, P=0.48; night 120 vs. 124 mmHg, P=0.33) or diastolic (day 90 vs. 89, P=0.77; night 94 vs. 92 mmHg, P=0.57) pressures. Appropriate BP dipping during inactive (day) period and decrease in heart rate was observed in both groups. High Na diet induced similar small BP increase (4-6 mmHg) in both groups. Norepinephrine content (ng/mg wet weight) was increased in LP kidneys but it reached statistical significance only in the cortex (P=0.033). There were no differences in renal epinephrine content.

In conclusion, continuous telemetry monitoring revealed no sustained hypertension in this model of IUGR. Higher tail cuff BP may be due to enhanced stress reactivity of LP rats during handling. Therefore, the reduced nephron number and later nephrosclerosis described in this model of prenatal programming may be dissociated from systemic hypertension.

Disclosure of Financial Relationships: nothing to disclose

**PUB550**

**Nicorandil Has the Micociculatory Renoprotective Effect on Renal Ischemia-Reperfusion Model in Mice** Tokunori Yamamoto, Hideki Mizuno, Naoto Sassa, Norihisa Matsukawa, Masashi Katoh, Yasushi Yoshino, Kazuo Mizutani, Ryohei Hattori, Momokazu Gotoh. *Urology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.*

[Aim]
Nicorandil (Nic), an angina treatment which has ATP sensitive K channel opening and nitrate like activity, is known to have cardioprotective effect for ischemic cardiac disease. It is considered that angina and acute renal failure have a common point as ischemic disease. In this study, we investigated the protective effect of Nic on renal ischemia reperfusion model in mice.
[Method]
C57BL/6 mice (8 weeks old, male) were anesthetized by pentobarbital. After the uninephrectomized of right kidney, left renal artery and vein were clamped for 30 min. One day after reperfusion, serum creatinine (sCr) and urea nitrogen (sUN) were measured. Nic was administrated from one day before operation by drinking water (6, 30 mg/kg/day). peritubular capillary blood flow(PTC) and diameter of afferent arteriole (Af)were measured by intravital microscopy(Yamamoto T et al AJP Renal 2001).
[Result]
Nic slightly but significantly reduced sCr and sUN in a dose dependent manner (sCr, Control; 2.6±0.3, Nic 6; 2.3±0.1, Nic 30; 2.1±0.2, sUN, Control; 218.8±1.2, Nic 6; 191.4±7.8, Nic 30; 191.3±9.6, mean±SE, n=11-13). PTC significantly increased treatment group than control group( 0.41 plus minus0.09 VS 0.22 plus minus0.11micro/sec,$p<0.05$ ). Diameter of Af dilated treatment group than control group(15.2plus minus1.8VS 10.8plus minus2.1micrometer ,$p<0.05$ )
[Conclusion]
Nicorandil improved not only ischemic cardiac damage but also ischemic microcirculatory renal damage.
Disclosure of Financial Relationships: nothing to disclose

**PUB551**

**Telmisartan Improves Metabolic Profile in Obese Patients with Arterial Hypertension** Marcin Adamczak,[1] Malgorzata Kubik,[1] Jerzy Chudek,[1, 2] Andrzej Wiecek.[1] *[1]Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland; [2]Department of Pathophysiology, Medical University of Silesia, Katowice, Poland.*

Purpose of study: There are several lines of evidence suggesting that telmisartan may improve cardiometabolic profile. The aim of the study was to estimate changes of insulin resistance, plasma levels of adiponectin and of proinflamatory cytokines after long-term antihypertensive treatment with telmisartan in obese hypertensive patients.
Patients and methods: 34 previously untreated obese adults (BMI between 30 and 40 $kg/m^2$) with arterial hypertension were enrolled into the study. Euglycemic-hyperinsulinemic clamp technique was applied for measurement of glucose cells uptake (M value) and calculation of M to insulin plasma concentration ratio (M/I). M and M/I values, body fat content (by DEXA method), as well as plasma concentration of adiponectin, its high molecular weight fraction (HMW), leptin and markers of inflammation (hsCRP, TNFα, IL-6, IL-8) were estimated before and after 6-month telmisartan therapy.
Results: 25 patients completed the study. Telmisartan therapy was followed by 14.2% decrease of systolic and by 19.6% decrease of diastolic blood pressure. Body weight and fat mass did not change significantly. Both M and M/I values increased significantly (24.4% and 38.6%, respectively). Plasma concentrations of hsCRP and IL-8 decreased significantly, while plasma IL-6 and TNFα concentrations only tended to decline. Plasma levels of total adiponectin and its HMW fraction increased after treatment with telmisartan by 10.8% and by 23.5%, respectively.
Conclusions: 1. Telmisartan monotherapy improves insulin sensitivity in obese patients with hypertension. 2. The additional potential benefit of telmisartan therapy is the increase of plasma adiponectin concentration and suppression of microinflammation that may contribute to slowing of atherosclerosis development and reduction of cardiovascular risk in obese hypertensive patients.
Disclosure of Financial Relationships: nothing to disclose

**PUB552**

**Gender Disparities in the Awareness and Control of Hypertension** Abdulkareem Alsuwaida, Mohammed A. Al-Ghonaim. *Medicine, King Saud University, Riyadh, Saudi Arabia.*

***Background***
Hypertension is an important risk factor for the commonest cause of death among men namely, cardiovascular diseases. The purpose of this study was to provide data concerning gender difference in the awareness, treatment, and control of hypertension in Adult men.
***Methods***
We conducted a cross sectional study in Riyadh, the capital of Saudi Arabia. Subjects were asked if they had been told by a physician that they had hypertension or were on a blood pressure medication. Blood pressure was measured using standardized Joint National Committee (JNC) protocol.
***Results***
The study sample consisted of 814 adults who were at least 18 years old. Of the estimated 27.6 % people with hypertension, 38.6 percent were unaware of their hypertension, 29.8% were aware of their condition but were not being treated, and among those whom been treated 40.8 percent remained uncontrolled. Independent predictors of a lack of awareness of hypertension were an age of at least 45 years, male sex, and BMI greater than 30. The extent of awareness and control of hypertension did not differ significantly by monthly income, educational level, physical activities or smoking status.
***Conclusions***
Awareness and control of hypertension is low in men, making them public health priorities. Achieving more stringent blood pressure control will require increased attention by physicians and public education to improve the awareness and control of hypertension.
Disclosure of Financial Relationships: nothing to disclose

**PUB553**

**High Incidence of Albuminuria Associated with Cardiovascular Risk Factors in Japanese Hypertensive Patients: Cross-Sectional Study with a Nationwide Internet Survey (AVA-E Study)** Koichi Asahi,[1] Yoshihiro Tani,[1] Kenichi Tanaka,[1] Kenji Baba,[3] Yasushi Itakura,[3] Kunitoshi Iseki,[2] Tsuyoshi Watanabe.[1] *[1]Department of Nephrology and Hypertension, Fukushima Medical University, Fukushima, Japan; [2]Dialysis Unit, University Hospital of the Ryukyus, Nishihara, Okinawa, Japan; [3]Dainippon Sumitomo Pharma Co., Ltd., Tokyo, Japan.*

Objective: The present study aims to elucidate the real status and risk factors associated with abnormality of urinary albumin excretion (UAE) in Japanese hypertensive patients. Method: Demographic and clinical data on 8,963 hypertensive outpatients who underwent qualitative tests for urinary albumin/creatinine ratio (quali-ACR) from September 2009 to March 2010 were collected via the Internet from 639 nationwide registered physicians (AVA-E study conducted by Dainippon Sumitomo Pharma Co., Ltd.). Results: Mean office blood pressure and eGFR were 138.3/78.7 mmHg and 69.7 mL/min/1.73 m2, respectively. Qualitative urinary protein test (quali-UP: a dip-stick method) were simultaneously tested for 94.9% of the patients and revealed the incidence of (-), (±), (+), (++), and (+++) are 71.9%, 14.5%, 8.4%, 3.7%, and 1.4%, respectively. Quali-ACR showed the abnormally high range in 42.9% of overall patients and 35.4% of the 7,355 patients with (-) or (±) on quali-UP. The incidence of CKD (eGFR<60ml/min/1.73m2) was 20.8%. Reported comorbid risk factors were smoking (17.8%), diabetes (34.9%), high LDL-C, (41.0%), low HDL-C (10.9%), high TG (30.1%), dyslipidemia (57.2%) and histories of myocardial infarction (3.5%) and stroke(5.4%). Multivariate logistic regression analyses identified age, BP, BMI, diabetes, dyslipidemia, number of comorbidities and eGFR as risk factors for abnormalities in quali-ACR. Interpretation: Abnormal UAE are highly frequent and associated with cardiovascular risk factors in Japanese hypertensive patients on usual outpatient care.
Disclosure of Financial Relationships: nothing to disclose

**PUB554**

**Combination of Irbesartan and Enalapril Versus Amlodipine on Hypertensive Retinopathy in Patients with Chronic Kidney Disease** Eleni Chelioti,[1] Antonios Zagorianos,[1] Adrianna Mitropoulou,[2] Athanasios Georgiou,[1] Prokopis Papazafeiris,[1] Gabriel Papadakis.[1] *[1]Dept. of Nephrology and Renal Unit, General Hospital of Piraeus, "Tzaneio", Athens, Greece; [2]Dept. of Ophthalmology, General Hospital of Piraeus, "Tzaneio", Athens, Greece.*

Purpose:Aim of this study was to evaluate the effect of combination of irbesartan and enalapril or amlodipine on retinal microvasculature changes in hypertensive patients with chronic kidney disease (CKD).
Method: Twenty-three patients (12males/11females, age range 45-85 years) with untreated hypertension (Systolic Blood Pressure, SBP>160 mmHg and Diastolic Blood Pressure, DBP >90 mmHg) and CKD stage 3(mean Clearance creatinine 39±7ml/min/1.73m2) were randomized to received a combination of irbesartan 150 mg/day and enalapril 10mg/day (n=13) or amlodipine 10mg/day (n=10) in a double-blind study for 12 months. Measurements of blood pressure, retinal microvasculature( arteriolar sclerosis and narrowing) using a fundoscopic exam and renal function were evaluated at baseline and at the end of the study.
Results: Both the combination of irbesartan and enalapril and single treatment with amlodipine reduced blood pressure (BP) in all patients (BP≤130/80mmHg $p<0.001$). Accompanying the reduction in BP, there were an improvement in arteriolar sclerosis and narrowing ($p<0.03$ and $p<0.02$ respectively). Although, both in group BP reduction was associated with a significant ameliorate of renal function, mean clearance creatinine 52±10ml/min/1.73m2 ($p<0.05$). Moreover, we found out an inverse relation between arteriolar narrowing and left ventricular hypertrophy.
Conclusion: Either double antihypertensive treatment or single treatment can induce regression of retinal microvascular abnormalities associated with reduction of BP. However, fundoscopy is a simple and non invasive exam that may be useful in the assessment of target organ damage. These results should be interpreted with caution because the sample is small and may limit the power of the study.
Disclosure of Financial Relationships: nothing to disclose

## PUB555

**A Dose-Response Relationsship between NOS-Inhibition with L-NMMA and Blood Pressure in Healthy Man** Frank H. Christensen, Thomas Larsen, Jesper N. Bech, Erling B. Pedersen. *Departments of Medical Research and Medicine, Holstebro, Denmark.*

**Purpose:** Nitric oxide is involved in the regulation of blood pressure, but the importance of this regulation in healthy man is still unclear. The present study investigated the effects of increasing doses of the Nitric Oxide synthase (NOS) inhibitor L-NMMA on blood pressure in healthy man.

**Methods:** Twelve healthy young men (18-30 years) were included in a cross-over, placebo-controlled, single-blinded study. On 4 separate study days, the subjects were randomised to receive placebo or one of 3 doses of L-NMMA: 3.0 mg/kg bolus IV injection followed by 2.0 mg/kg/hour IV infusion for one hour, 4.5mg/kg bolus injection followed by 3.0 mg/kg/hour IV infusion for one hour or 6.0 mg bolus IV injection followed by 4.0 mg/kg/hour IV infusion for one hour. Blood pressure (BP) and heart rate (HR) were repeatedly followed every 5 to 10 minutes during infusion period. Four days before each study day, the subjects were given 3 gr. NaCl pr day as a supplement to their usual diet.

The differences in BP and HR were assessed in steady state period using area under curve (AUC) in relation to baseline. Differences are presented in medians with interquartile ranges in brackets. Statistics were performed with Friedman's test

**Results:**

Baseline diastolic (DBP) and systolic blood pressure (SBP) and HR were not different between groups (119.5/62.1 ± 7.5/ 5.9 mmHg, 52.9 ± 7.5 beats/min, Mean ± SD). IV infusion of L-NMMA increased both DBP and SBP and decreased HR. From 20-60 min a stable BP and HR was observed with less than 10% difference in the variables. The calculated AUC increased in a dose-dependent way both for DBP and SBP (placebo: -28 (90) / 18 (217) , 3.0mg: 151 (103) / 71 (100), 4.5mg: 219 (113) / 68 (218), 6.0mg: 283 (51) / 217 (252) mmHg*min, $p < 0.001$, $p = 0.025$) The calculated AUC for heart rate decreased in a dose-dependent way (placebo: -21 ± (159), 3.0mg: -95 ± 192, 4.5mg: -163 (160) mmHg*min, 6.0mg: -178 (136) mmHg*min, $p = 0.021$).

**Conclusions:**

NOS inhibition with continuous L-NMMA infusion causes a dose dependent increase in DBP and SBP and a dose dependent decrease in heart rate.

Disclosure of Financial Relationships: nothing to disclose

## PUB556

**Discordant Changes in Vascular Compliance and Proteinuria with Drug Therapy in CKD Patients** Debbie L. Cohen, Kevin Sterling, Raymond R. Townsend. *Medicine, Renal Division, University of Pennsylvania, Philadelphia, PA.*

Chronic kidney disease (CKD), proteinuria and increased arterial stiffness are associated with increased cardiovascular disease (CVD) risk. We performed a prospective randomized trial of subjects with stage 1-3 CKD with proteinuria > 500 mg/dL. All subjects were already on renin angiotensin suppressing therapy with either an ACE ***or*** ARB prior to enrollment. Subjects were then randomized to 3 treatment groups in addition to their current treatment: group 1 - ACE+ARB, 2 - eplerenone, 3 - isosorbide mononitrate. Subjects were followed for a period of 4 months and had pulse wave velocity (PWV) and central pulse pressure measured (SphygmoCor PV device, AtCorMedical, Sydney, Australia] at baseline and 4 months to determine if changes in urine protein excretion resulted in changes in vascular compliance depending on drug therapy assignment in patients with CKD and proteinuria. Results (baseline minus 4 month value) are shown in the table below.

| | Group 1 (n=5) | Group 2 (n=13) | Group 3 (n=11) |
|---|---|---|---|
| Δ Urine Protein g/day | 0.32±0.2 | 1.04±0.4* | -0.2±0.3 |
| Δ SBP mm Hg | 13.4±14.9 | 9.7±6.4 | 1.0±5.4 |
| Δ PWV m/sec | 0.87±0.9 | -1.13±1.15 | 0.9±1.17* |

*p = 0.02

7 subjects were randomized to group 1 with dual RAAS blockade (with 2 drop outs), 13 subjects were randomized to RAAS + eplerenone, 11 subjects were randomized to RAAS + Nitrate. Urine protein excretion was decreased in group 1 and 2 but was increased with the addition of the nitrate in group 3. BP declined in all 3 groups over the treatment period however PWV decreased in group 1 and 3 but increased in group 2.

Conclusion:

1. Significant decrease in proteinuria in RAAS + eplerenone despite an increase in PWV.

2. Slight non-significant increase in proteinuria but significant decrease in PWV in RAAS and nitrate.

3. Changes in protein excretion on drug therapy added to RAAS foundation are not predicted by changes in PWV.

Disclosure of Financial Relationships: nothing to disclose

## PUB557

**Primary Hypertensive Nephrosclerosis Has Become a Less Common Cause of ESRD** Amit J. Joshi, George Dunea, Peter D. Hart. *Division of Nephrology, Stroger Hospital of Cook County, Chicago, IL.*

**Background:** The role of hypertension in hastening the progression of renal disease is undisputed, but the prevalence of hypertensive nephrosclerosis as a primary cause of end-stage renal disease (ESRD) continues to be overestimated.

**Methods:** We reviewed by clinical criteria the diagnoses of patients starting dialysis in the past 2 years (June 2006-May 2010) at a large inner city hospital. A presumed diagnosis of primary hypertensive nephrosclerosis was made on the basis of excluding other causes of ESRD and finding a long history (>10 years) of hypertension, gross cardiomegaly, relatively small kidneys on ultrasound, and proteinuria < 2g/d.

**Results:** The estimated overall prevalence of primary hypertensive nephrosclerosis was 7%. In African American patients 38% had diabetic nephropathy, 22% had glomerular disease, 14% had hypertensive nephrosclerosis, and 27% had other causes. In Hispanics, 57% had diabetic nephropathy, 27% had glomerular disease, 14% had other causes, and only 2% had primary hypertensive nephrosclerosis.

Table 1

| Etiology of ESRD | African American(n=129) | Hispanic (n=118) | Others (n=35) |
|---|---|---|---|
| Diabetes | 49 (38%) | 68 (57%) | 14 (40%) |
| Glomerular disease | 28 (22%) | 32 (27%) | 13 (37%) |
| Hypertensive nephrosclerosis | 18 (14%) | 2 (2%) | none |
| HIV | 11 (8%) | none | none |
| Lupus | 6 (5%) | 2 (2%) | 1 |
| Others | 17 (13%) | 14 (12%) | 4 |

**Conclusion:** We found that in the past 2 years the overall prevalence of primary hypertensive nephrosclerosis was 7%. In African Americans in our hospital, it has declined over the past 2 decades from 22% to 14%. We suggest that nephrologists reporting to networks and registries should alter their diagnostic criteria.

Disclosure of Financial Relationships: nothing to disclose

## PUB558

**Effect of Dietary Potassium Supplement on Blood Pressure, Augmentation Index and the Renin-Aldosterone System in Healthy Humans** Solveig K. Matthesen, Thomas G. Lauridsen, Erling B. Pedersen, Henrik Vase. *Departments of Medical Research and Medicine, Holstebro Hospital, Holstebro, Denmark.*

**Purpose:**

Animal experiments and studies in humans have shown that potassium treatment can reduce blood pressure due to a reduced contraction of vascular smooth muscle cells. We wanted to test the hypothesis that dietary potassium supplement would decrease peripheral and central blood pressure(CBP), pulse wave velocity (PWV) and augmentation index (AI) in healthy humans.

**Methods:**

The effect of potassium 100 mmol /day was measured in a randomized, placebo-controlled, cross-over study of 22 healthy humans. The treatment periods were 4 weeks separated by a 2 week wash-out period. The participants received a diet standardized according to weight and physical activity during the last four days of each treatment period. The diet was composed of 55 % carbohydrates, 15 % proteins and 30 % fat. Daily fluid intake was 250 ml/1000KJ, and sodium content in the diet was 150-200 mmol depending on the energy demand. We measured 24-hour-BP using Kiwex 2430, CBP, PWV and AI using Sphygmocor, GFR using the constant infusion technique with 51-Cr-EDTA, plasma concentrations of renin (PRC) and aldosterone (PAC) using RIAs, and fractional excretion of potassium ($FE_K$) and sodium ($FE_{Na}$).

**Results:**

Urinary potassium excretion increased during the intervention (placebo: 75± 22 mmol/24 hour versus potassium supplement: 168 ±37 mmol/24 hour, $p< 0.05$, means±SD). $FE_K$ increased (13±4 to 29±7, $p< 0.05$). $FE_{Na}$ , 24-hours BP, PWV, CBP, AI and GFR were not significantly changed. During potassium supplementation, an increase was measured in PRC (7±4 to 10±8 mIU/ l, $P<0.05$) and PAC (378±217 to563±362 pmol/l, $P<0.05$).

**Conclusions:**

Urinary potassium excretion was significantly increased during the intervention indicating that the participants complied with the diet. Neither 24-hours-BP, CBP nor AI changed significantly during intervention. The lack of decrease in blood pressure might be attributed to an antagonizing effect induced by the increase in the activity of the renin-aldosterone system.

Disclosure of Financial Relationships: nothing to disclose

## PUB559

**Plasma Nitrite in Patients with Renal Impairment: A Pilot Study** Daniel Schroeder, Jens Passauer. *Nephrology, University Hospital Carl Gustav Carus, Dresden, Germany.*

**Background:** Plasma nitrite (NO2) is now regarded as an important storage compound of nitric oxide (NO) which significantly contributes to vasodilatation and organ protection during ischemia. At present little is known about the status of this physiological principle in patients with renal impairment.

**Methods:** In a pilot study we determined baseline plasma nitrite levels in patients with renal insufficiency (RI, n=19), in patients on dialysis (D, n=30) and in healthy control subjects (C, n=11) by chemiluminescence (NOA280, Sievers instruments, CO). In addition we measured changes in NO2 levels during 8 minutes of complete forearm ischemia in HD (n=5) and C (n=5). Finally we determined changes in NO2 during a four-hour dialysis session in D (n=20).

**Results:** NO2 was 182±92 nmol/L in D, 162±95 in RI and 67±28 in C showing an inverse correlation to GFR (MDRD, $r=0.35$;$p<0.005$). During hemodialysis NO2 decreased to 82±25% (1h), 84±43 (2h) and 63±21 (4h) of the pre-dialysis baseline level. During forearm ischemia we observed a significantly more pronounced decrease in NO2 to 80±10% (2min), 73±12 (6min) and 59±11 (8min) of baseline in D compared to C (91±12 ; 84±15 and 82±13% ; $p<0,05$ by ANOVA).

**Conclusion:** 1) Renal impairment is associated with an increase in plasma nitrite. 2) The high NO2 plasma concentrations observed in D can not be explained by the dialysis procedure itself. 3) There are significant differences in nitrite metabolism during ischemia between dialysis patients and control subjects. Further studies have to clarify the significance of these observations and to unravel the underlying mechanisms.

Disclosure of Financial Relationships: nothing to disclose

**PUB560**

**Role of CD4$^+$CD28$^-$ T Cells in Pathogenesis of Cardiovascular Events in Patients on Haemodialysis** Nihil Chitalia,[1] Behnam Zal,[2] Christina Baboonian,[2] Debasish Banerjee.[1] *[1]Renal Medicine, St. George's Hospital, London, United Kingdom; [2]CV Sciences, St. George's University of London, London, United Kingdom.*

**Background**

ESRD patients have a high cardiovascular (CV) mortality; not explained by traditional risk factors. Inflammation and macrophage activation have been proposed as a key pathogenic mechanism in the development and progression of atherosclerosis in these patients. A human heat shock protein 60 (hHSP60; a stress protein upregulated in inflammation) specific sub-population of CD4$^+$ cells, CD4$^+$CD28$^-$ , present in coronary artery disease (CAD) engage in vascular damage via Killer Immunoglobulin-like Receptor 2DS2 (KIR2DS2). Also, CD4$^+$CD28$^-$ cells produce IFN-γ which is a potent activator of macrophages contributing to plaque rupture.

This study investigated the expansion and activation of CD4$^+$CD28$^-$ cells in ESRD patients.

**Methods and Results**

15 patients (mean age 61.9±9), men 60%, DM 33%, hypertensives 94%, current smoking 0% and 10 healthy controls were studied. CD4$^+$CD28$^-$ cells were isolated from peripheral blood by FACS and cloned using irradiated allogeneic feeder cells.

Three patients (20%) had increased number of CD4$^+$CD28$^-$ cells that constituted 6-9% of the total CD4$^+$ count. None of the controls had CD4$^+$CD28$^-$ cells. Analysis of clones (n=112) by RT-PCR showed 45%, 49% and 51% of cells in each patient expressed the KIR2DS2, largely in the absence of the corresponding inhibitory KIRs (95%). KIR2DS2 positive clones exibited autoreactivity against target cells pulsed with hHSP60. As DAP-12 is essential for effector function, we tested the hHSP60 reactive cells for DAP-12 mRNA. 92% of 2DS2 positive clones expressed DAP-12 and were reactive to hHSP60. These clones produced IFN- γ upon antigen exposure.

Proportion of killer hHSP60-specific T cells in HD patients were notably higher compared to pre-dialysis patients (41% vs 15%; studied separately).

**Conclusion**

The higher frequency of killer CD4$^+$CD28$^-$ cells in these patients is of significance which may suggest their gradual expansion with progressive renal impairment. We therefore propose a mechanism outside the traditional risk factors that may account for CV events in these patients.

Disclosure of Financial Relationships: nothing to disclose

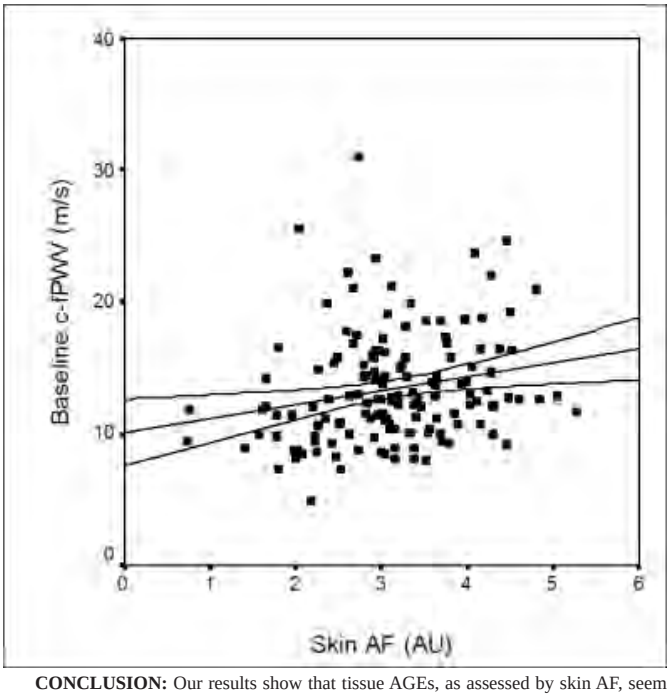
**PUB561**

**Tissue Advanced Glycation End-Products Are Not Associated with the Progression of Aortic Stiffness in Hemodialysis Patients** Véronique Couture,[1,2] Mihai Silviu Utescu,[1,2] Simon Desmeules,[1,2] Karine Marquis,[1,2] Mohsen Agharazii.[1,2] *[1]Medicine, Université Laval, Québec, QC, Canada; [2]Medicine, CHUQ-HDQ, Québec, QC, Canada.*

**INTRODUCTION**: Among haemodialysis (HD) patients, an increased aortic stiffness is associated with increased cardiovascular disease (CVD). It has been suggested that skin autofluorescence (AF), a measure of tissue advanced glycation end-products (AGEs), predicts mortality in HD patients and is associated with arterial stiffness in patients with end-stage renal disease (ESRD). The purpose of this study is to examine whether there is an association between skin AF and aortic stiffness in HD patients and whether skin AF can predict the progression of aortic stiffness.

**METHODS:** This is a cross-sectional observational study with longitudinal follow-up in 205 (118 men) chronic HD patients. At baseline, skin AF was assessed by the AGE-Reader® and expressed as Arbitrary Units (AU) while aortic stiffness was evaluated before the second HD session of the week by the measurement of carotid-femoral pulse wave velocity (c-fPWV). A follow-up was also performed one year after the baseline examination in order to study the impact of tissue AGEs on the progression of aortic stiffness and merely 131 patients (70 men) completed the study.

**RESULTS:** Baseline values for skin AF and c-fPWV were 3.14±0.86 AU and 13.2±4.1 m/s respectively while c-fPWV at follow up was 13.9±4.2 m/s. Skin AF correlates positively with baseline c-fPWV (r=0.216, *p=0.009*). Conversely, there is no correlation between skin AF and the progression of aortic stiffness.




**CONCLUSION:** Our results show that tissue AGEs, as assessed by skin AF, seem to be associated with aortic stiffness at baseline in HD patients. However, skin AF is not associated with the rate of progression of aortic stiffness in this population. These results suggest that tissue AGEs may not be involved in the progression of aortic stiffness.

Disclosure of Financial Relationships: nothing to disclose

**PUB562**

**The Utility of Ultrasonography To Show Arterial Media Calcifications (AMC) in Young People with End Stage Renal Disease Not Yet on Dialysis** Annibale Marinelli, Franco Della Grotta. *Nephrology and Dialysis, Riuniti Hospital Anzio, Anzio, Roma, Italy.*

In young people, arterial media calcifications are linked to diabetes and to vintage dialysis and they increase the risk of cardiovascular mortality. It is unknown if AMC are present in end stage renal disease before starting dialysis. We have evaluated the development of AMC and arterial intimal calcifications (AIC) with ultrasonography; infact, even if it isn' t used for this purpose, it permits a clear distinction between the two kinds of lesions besides showing AMC in an initial stage (linear deposition of calcium salts as spots or as segments). We have studied 44 subjects with the inclusive age between 25 and 55 years; 25 (15M/10F; mean age 41±9 years) were affected by chronic renal failure (MDRD 14±5 ml/min) and 19 were healthy volunteers (8M/11F; mean age 41±8 years). The exam was performed with trasversal and longitudinal scansions of the common and superficial femoral arteries (until the lower part of the thigh). The nephropaties were constituited by nefroangiosclerosis (4), diabetes type I (4), diabetes type II (1), chronic pyelonephritis (3), unknow etiology (4). In comparison to healthy subjects, patients were more hypertensive (22/25, p<0,001) and they had higher values of phosphate (4,8 mg/dl ± 0,9 vs. 3,6 ±0,4, p<0,001) and PTHi (318±258 pg/ml vs. 70±40, p<0,001). Vascular calcifications (AIC plus AMC) were evident in 44% of patients and in the 10% of the controls (p<0,05); isolated AIC were higher in chronic kidney disease patients although not in a significant way (6 vs 2). On the contrary, we observed AMC only in the end stage renal disease ( 7 cases,p<0,05). Taking into account all patients affected by renal damage, AIC and AMC were associated rispectively with age (49±4 y., vs 39±8, p<0,01) with and with diabetes (5/7, p<0,001). Although we have evaluated only a few patients, this study shows that, despite bone turn-over alterations, the development of AMC in young people with end stage renal disease is connected primarily with diabetes.

Disclosure of Financial Relationships: nothing to disclose

**PUB563**

**Plasma Asymmetric Dimethyl L-arginine Levels in End Stage Renal Disease Patients with Pulmonary Hypertension** Jeffrey M. Turner,[1] Ariel Meyer,[1] Zhe Quan,[1] Daniel Spevack,[1] Michal L. Melamed,[1] Timothy W. Meyer,[2] Thomas H. Hostetter,[1] Amanda C. Raff.[1] *[1]Albert Einstein College of Medicine, Bronx, NY; [2]Stanford University School of Medicine, Palo Alto, CA.*

**Introduction:** End stage renal disease (ESRD) patients are at increased risk for developing pulmonary hypertension and those with pulmonary hypertension are at increased risk for mortality. Asymmetric dimethyl L-arginine (ADMA), an inhibitor of endogenous nitric oxide synthase, is associated with primary pulmonary hypertension and has previously been found to be elevated in some patients with renal failure. ADMA may contribute to endothelial dysfunction in ESRD patients with pulmonary hypertension. We

hypothesized that ESRD patients with pulmonary hypertension would have higher levels of plasma ADMA than ESRD patients without pulmonary hypertension as well as controls without renal disease.

**Methods:** We studied 20 ESRD patients that had available echocardiogram data from a single outpatient hemodialysis unit. Eleven patients with PASP > 35mmHg were selected from a previously identified pulmonary hypertension cohort and 9 patients without pulmonary hypertension were selected as a comparison group. In addition, plasma from 10 control subjects without renal disease was collected. Plasma ADMA levels were measured in each group.

**Results:** Patients with ESRD had significantly higher levels of ADMA than normal controls 0.722 +/- 0.143 μM/L vs. 0.484 +/- 0.072 μM/L (p<0.001). ADMA plasma levels in ESRD patients with and without pulmonary hypertension were 0.766 +/- 0.161 μM/L vs. 0.684 +/- 0.108 μM/L (p=0.21) respectively. These 2 groups were similar in regards to sex, age, PTH, calcium, phosphorous, and hemoglobin. The mean Kt/V was > 1.2 in both groups.

**Conclusions:** ADMA levels are increased in patients with ESRD as compared to normal controls without renal failure. However, we were unable to detect a difference in plasma ADMA levels in ESRD patients with and without pulmonary hypertension.

Disclosure of Financial Relationships: nothing to disclose

## PUB564

**Plasmatic Pentosidine Levels and Vitamin D Dosing Are Associated with Accelerated Progression of Aortic Stiffness in Hemodialysis Patients** Mihai Silviu Utescu,[1,2] Véronique Couture,[1,2] Karine Marquis,[1,2] Simon Desmeules,[1,2] Mohsen Agharazii.[1,2] *[1]Medicine, Université Laval, Québec, QC, Canada; [2]Medicine, CHUQ-Hôtel Dieu de Québec, Québec, QC, Canada.*

INTRODUCTION: Aortic stiffness as measured by carotido-femoral pulse wave velocity (c-f PWV) is associated with increased cardiovascular (CV) mortality in hemodialysis (HD) patients. However, the progression rate and the determinants of aortic stiffening in HD are not completely elucidated. The accumulation and generation of advanced glycation end products (AGEs), endothelial dysfunction and vascular calcification resulting from a disturbed calcium phosphate metabolism could play a role.

METHOD: The c-fPWV) was measured in 109 chronic (more than 3 months) HD patients (49 females). Fifteen months (14±5) after the baseline measurements we re-evaluated the c-fPWV. Demographic and anthropometric data (age, sex) , history of CV disease and diabetes, dialysis vintage, residual renal function, laboratory parameters (calcium, phosphate, PTH, albumin, CRP), plasmatic levels of pentosidine (AGEs) and endothelin-1 (endothelial dysfunction) and finally, medication dosage (alfacalcidol, sevelamer hydrochloride and calcium carbonate) were evaluated at baseline.

RESULTS: During the follow-up period, c-fPWV increased from 13.13±3.79 to 14.25±3.88 m/s (p<0.001). After adjustments for the changes in mean blood pressure and heart rate, the c-fPWV progression rate per year was of 0.54±1.19m/s (p<0.01). Determinants of adjusted c-fPWV progression rate per year expressed as percent value from baseline c-fPWV were assessed with a multivariate analysis using baseline clinical, dialysis, laboratory, medication dosage data, mean blood pressure and heart rate as independent variables. In this model, only Alphacalcidol dosing (R2 =0.095; p<0.005) and plasmatic pentosidine levels (R2=0.049; p<0.05) were retained as independent determinants of c-fPWV progression rate. Endotheline-1 plasmatic levels positively correlated with pentosodine levels (r=0.404; p<0.001) but were not retained as determinant.

CONCLUSION: Plasmatic levels of pentosidine and vitamin D dosing seem to be independent predictors of an accelerated progression of AS in HD patients.

Disclosure of Financial Relationships: nothing to disclose

## PUB565

**Resistive Index Measured by Renal Doppler Ultrasound Reflects Arteriolosclerosis in Renal Biopsy Specimen** Yohei Doi,[1] Fumiki Yoshihara,[1] Hatsue Ishibashi-Ueda,[2] Satoko Nakamura,[1] Yoshio Iwashima,[1] Hideaki Takata,[1] Takashi Fujii,[1] Takeshi Horio,[1] Yuhei Kawano.[1] *[1]Hypertension and Nephrology, National Cerebral and Cardiovascular Center, Suita, Oosaka, Japan; [2]Pathology, National Cerebral and Cardiovascular Center, Suita, Oosaka, Japan.*

**Background, Purpose**

Resistive index (RI), calculated from the renal doppler waveform, has been shown to be relevant to progression of renal dysfunction and survival rate. However, clinical significance or related factors of RI have not been fully clarified. The purpose of the present study was to clarify the correlation between RI , clinical parameters and renal histology.

**Materials and Methods**

Of 46 consecutive patients underwent renal biopsy, 35 patients who underwent renal doppler ultrasound were enrolled. Clinical diagnosis of the 35 patients were Mesangial proliferative glomerulonephritis(n=13), Membranous nephropathy (n=6), Diabetic nephropathy (n=6) and others (n=10). Biopsy specimens were evaluated as follows, 1. interstitial fibrosis was quantitated using a software for image analysis, 2. arteriolosclerosis was evaluated as the ratio of luminal diameter to the outer diameter, 3. glomerulosclerosis and tubular atrophy were assessed by means of a semi-quantitative method.

**Results**

The characteristics of participants were age 58.6±14.9 years, BMI 24.9±3.9, blood pressure 138.7±19.2 / 75.7±10.5 mmHg, blood urea nitrogen(BUN)19.9±6.8 mg/dL, serum creatinine (S-Cr) 1.29±0.59 mg/dL, hemoglobin (Hb) 12.8±2.4 g/dL, urinary protein excretion 3.3±3.0g/day, urinary N-acetyl-β-glucosaminidase 15.6±19.8 U/L, urinary β2-microgloburin 1309±4901 μg/dL, RI 0.68±0.09. In univariate regression analysis, RI was significantly and positively correlated with age (r=0.467), BUN (r=0.510), S-Cr (r=0.544), interstial fibrosis (r=0.348), tubular atrophy (r=0.340) and negatively with arteriolosclerosis (r=-0.408) and Hb (r=-0.335). Stepwise multiple regression analysis showed age, S-Cr, arteriolosclerosis were independent related factors for RI (β-coefficient=0.309, 0.444, -0.277, respectively).

**Conclusion**

RI was increased in associated with age, renal dysfunction and arteriolosclerosis. Arteriolosclerosis may be one of the determinants of RI.

Disclosure of Financial Relationships: nothing to disclose

## PUB566

**Rosiglitazone (RGZ) Improves the Natriuretic/Diuretic Response to Atrial Natriuretic Peptide (ANP) but Not cGMP-Mediated Renal Vasodilatation in Rats with Congestive Heart Failure (CHF)** Ilia Goltsman,[1] Elena Ovcharenko,[1] Zaid Abassi,[1] Aaron Hoffman,[1] Xinkang Wang,[2] Giora Z. Feuerstein,[2] Joseph Winaver.[1] *[1]Physiology and Biophysics, Faculty of Medicine, Technion-IIT, Haifa, Israel; [2]Translational Medicine, Pfizer, Collegeville, PA.*

**Background/Aims:** The natriuretic/diuretic response to ANP and endothelial-dependent (ED) and -independent (EI) vasorelaxation, both cGMP-mediated, are blunted in rats with aorto-caval fistula (ACF), an experimental model of CHF. Recently, we reported that chronic treatment with RGZ, a PPARγ-agonist, improved the natriuretic response to ANP in rats with CHF (Goltsman et al., ASN Renal Week 2009, abstract TH-PO063). In the present study we explored the effects of RGZ treatment on ED and EI renal vasodilatation in rats with ACF.

**Methods:** The natriuretic/diuretic responses and urinary cGMP (UcGMP) excretion in response to rat ANP (15 and 50 μg/kg/hr) were assessed by clearance methodology in CHF rats following 2-week RGZ (30 mg/kg/day, p.o) or vehicle (Ve) treatment, and in sham-operated controls (N=6-10). The capacity of isolated glomeruli and collecting ducts to generate cGMP in response to ANP ($10^{-11}$-$10^{-6}$M) was tested. The ED and EI renal vasodilatory responses to ACh (1-100 μg/kg/min) and SNAP (10, 30 μg/kg/min), respectively, were studied by ultrasonic flowmetry.

**Results:** CHF rats treated with RGZ exhibited significantly enhanced natriuretic and diuretic responses compared with Ve, as represented by peak $U_{Na}V$; control: 11.2±1.8 μEq/min, CHF+Ve: 3.66±1.2* μEq/min, CHF+RGZ: 11.2±2.3† Eq/min (P<0.05 vs. *control and †CHF+Ve). However, these improvements were not matched by increased UcGMP excretion. Also, RGZ treatment did not alter the capacity to generate cGMP in response to ANP in isolated renal tissues. Finally, the blunted renal vasodilatory responses to ACh and SNAP were not improved in CHF rats treated with RGZ.

**Conclusions:** In rats with CHF, chronic RGZ treatment enhances the natriuretic response to ANP, but not the blunted cGMP-mediated vasodilatation. The data may also suggest that RGZ selectively enhances signaling mediated by particulate rather than soluble guanylate cyclase, probably at a step beyond cGMP generation.

Disclosure of Financial Relationships: nothing to disclose

## PUB567

**Single Centre Study on Outcomes Following Renal Artery Stenting** David Makanjuola,[1] Matthew Lumley,[1] Hugh Gallagher.[1] *[1]Nephrology, St. Helier Hospital, Carshalton, United Kingdom; [2]Nephrology, St. Helier Hospital, Carshalton, United Kingdom; [3]Nephrology, St. Helier Hospital, Carshalton, United Kingdom.*

Background

Renal artery stenosis causes hypertension, renal dysfunction and is associated with cardiovascular events. We examined the results of all patients who had renal artery stenting for atherosclerotic disease since 2003 to present at our unit.

Methods

Data were collected on BP, antihypertensives taken, creatinine pre-procedure, at 6 months and at end of follow up; whether the patient was taking an ACE-I/ARB pre-procedure and if not, whether they had been introduced post procedure.

Results

48 patients had renal artery stenting. Mean age was 72.0+/-1.4years, mean follow up was 19.7+/-2.3months.

Outcomes following renal artery stenting

| | Change in Creatinine (μmol/L) | Change in Systolic BP(mmHg) | Change in Diastolic BP(mmHg) | Change in number of antihypertensive medications |
|---|---|---|---|---|
| 6 months | 12.4, CI -0.8 to 25.6, P value< 0.06 | -13.9, CI -5.6 to-22, P value< 0.002 | -7.3, CI -3.3 to -11.3, P value< 0.0007 | -0.2, CI -2.0 to 1.7, P value< 0.4 |
| End of follow up | 9.2, CI -2.0 to 20.4, P value< 0.1 | -7.9, CI -3.9 to -11.9, | P value< 0.0003 | -0.05, CI -0.5 to 0.4, P value< 1 |

17 were on ACE-I or ARBs pre-procedure, 26 were not and 5 patients had missing information. Of the 26 patients post procedure 9 were started on an ACE-I/ARB. Therefore, of the patients who were not on an ACE-I/ARB pre-procedure 39% were able to start following the procedure.

Discussion

Our findings show that in our patient group, renal artery stenting for atherosclerotic renovascular disease led to a significant reduction in systolic and diastolic BP at 6 months and end of follow up. Revascularisation however had no impact on the number of antihypertensive agents prescribed. The ASTRAL trial only included patients in whom

there was uncertainty whether or not they would benefit from revascularisation. Therefore, those patients most likely to derive benefit may have been excluded. Our experience suggests that revascularisation may enable a significant proportion of patients to be able to commence ACE-I/ARBs.

Disclosure of Financial Relationships: nothing to disclose

**PUB568**

**Retinal Venules Dilate after an Episode of Haemodialysis in End-Stage Kidney Failure** Foong Kien Newk-Fon Hey Tow,[1] Qi-Lun Ooi,[1] Tien Y. Wong,[2, 3] Ryo Kawasaki,[2] Peter F. Mount,[4] Deb J. Colville,[1] Judith A. Savige.[1] *[1]Medicine, The University of Melbourne (Northern Health), Melbourne, VIC, Australia; [2]Center for Eye Research Australia, The University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, VIC, Australia; [3]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore; [4]Department of Nephrology, Austin Health, Melbourne, VIC, Australia.*

**Background:** Retinal vessel appearance reflects changes in the systemic microvascular circulation. This study examined the effects of haemodialysis on retinal vascular caliber.

**Patients and Methods:** Twelve males and 12 females with a median age of 62.5 years (range 30-87) underwent routine haemodialysis using Gambro high-flux dialysis membranes. Twenty (83%) patients had hypertension and 10 (42%) had diabetes. Retinal images were recorded using a non-mydriatic camera (Canon CR5-45NM) immediately before and 30 minutes after dialysis. Retinal vascular calibers were measured by a trained grader using a computer-assisted vessel measurement system (University of Wisconsin, Maddison, WI, USA) and Knudtson's formula. Measurements were summarised as Central Retinal Artery Equivalent (CRAE) and Central Retinal Vein Equivalent (CRVE).

**Results:** Patients had a mean of 2.0 L fluid removed ($p<0.0001$), and their mean arterial pressures decreased from 91.5 mm Hg to 84.7 mm Hg ($p=0.058$). Retinal arterioles dilated in males (mean change 6.9 um, CI 1.30 - 12.5, $p= 0.020$) but not in females after dialysis. Retinal venules dilated in all patients (mean change 12.7 um, CI 7.3 - 18.3, $p<0.001$). The change in CRVE correlated positively with the volume of fluid removed per Kg body weight (coefficient 5.869, $p=0.042$), and negatively with the change in mean arterial pressure (coefficient - 0.364, $p= 0.046$). Vessel calibre returned to baseline by 2 hours.

**Conclusions:** Retinal vascular dilatation after dialysis probably occurs secondary to the release of systemic vasoactive factors that affect all microvascular beds and contribute to post-dialysis hypotension. Retinal vascular imaging allows the examination of dynamic physiological changes in the microvascular circulation.

Disclosure of Financial Relationships: nothing to disclose

**PUB569**

**Uric Acid Is Causally Associated with Blood Pressure in a Controlled Setting: Results from a Mendelian Randomization Analysis of GLUT9** Afshin Parsa, Eric M. Brown, Matthew R. Weir, Braxton D. Mitchell, Patrick F. Mcardle. *Medicine, University of Maryland School of Medicine, Baltimore, MD.*

BACKGROUND: Elevated serum levels of uric acid (UA) are consistently associated with hypertension, but the directionality of the association remains debated. We used, in a controlled setting, a Mendelian randomization approach to demonstrate the direction of the association between UA and blood pressure (BP). Using a recently described functional single-nucleotide polymorphism, rs16890979 (Val253Ile), in the GLUT9 gene that is associated with lower UA levels, we examined the unconfounded association between genotype and BP.

METHODS: Genotyping was performed on 516 "healthy" participants in the HAPI Heart Study to identify variants of a genetic locus reliably associated with serum uric acid level (GLUT9). Participants were on prepared fixed standardized high and low sodium diets (280 and 40 meq per day) for 6 days each and BP measures were based on ambulatory mean 24 hour BP measures. All of our participants were free of diuretic or other anti-hypertensive medication use. Using Mendelian randomization analysis, relationships between genotype and both UA and BP were assessed.

RESULTS: Each copy of the minor Ile allele conferred approximately a 0.44 mg/dl reduction in uric acid ($p = 3.2x10-11$). On the high sodium diet each copy of the Ile allele was associated with a mean decreases in systolic BP of 2.2 mmHg ($p = 0.0058$). The effect of the genotype was attenuated, but still significant on the low sodium diet with a mean decreases in systolic BP of 1.48 mmHg ($p = 0.038$).

CONCLUSION: Decreases in serum uric acid concentration, as directly mediated by genetic variants of GLUT9, are causal of decreases in BP and may be modified by dietary sodium intake.

Disclosure of Financial Relationships: nothing to disclose

**PUB570**

**ACE/ACE2 Ratio in Serum and Kidney Cortex Is Increased in Sexually Mature Male Mice** Marta Riera, Julio Pascual, Judit Rigol, Maria Jose Soler. *Nephrology, Parc de Salut Mar. Fundació IMIM, Barcelona, Spain.*

It has been shown that there are gender differences in the expression of some of the components of the Renin-Angiotensin System (RAS). Gender differences in renal and cardiovascular diseases seem to involve the overexpression of various components of the RAS, including angiotensin converting enzyme (ACE), the angiotensin II type 1 receptor, and angiotensinogen. Previous studies demonstrated that serum ACE activity is increased in 8-week old male mice as compared to female controls. We previously showed that serum ACE2 activity is altered in healthy male mice and human. Gender differences in ACE/ACE2 ratio have not been widely studied.

The aim of this study is to investigate whether there are differences between healthy male and female mice in ACE/ACE2 activity ratio in serum at different ages (1,3 and 6 month-old-mice). We also studied ACE/ACE2 ratio in kidney cortex from male and female mice at 1 and 3-months. For this purpose, serum and renal tissue was obtained from male and female mice at 1, 3 and 6 months of age. ACE and ACE2 activities were measured using respective fluorescent assays.

Serum ACE/ACE2 ratio was increased in males as compared to females at sexual maturity, 3 and 6 months of age (3 month-old:1.00±0.13 vs 1.31±0.09; 6 month-old: 1.00±0.06 vs 1.65±0.11 arbitrary units; $p=0.06$ and $p<0.05$, respectively). Additionally, ACE/ACE2 ratio in kidney cortex was increased in 3-month old male mice as compared to female mice (1.00±0.20 vs 2.39±0.67, $p<0.05$). We did not observe any difference in ACE/ACE2 ratio in serum and kidney cortex from 1-month old male mice as compared to females, when animals are sexually immature.

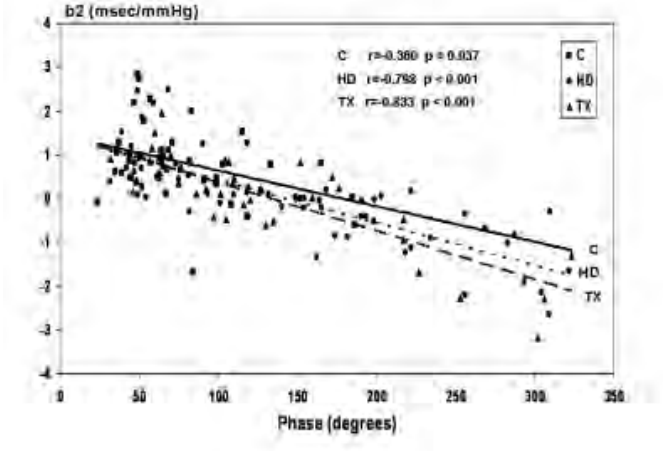
In conclusion, ACE/ACE2 ratio is increased in plasma and kidney cortex from sexually mature male mice as compared to female. ACE/ACE2 imbalance in male mice may increase Angiotensin II formation and decrease its degradation that will lead angiotensin II accumulation in serum and kidney in male mice. Angiotensin II accumulation in kidney and serum might be associated to the accelerated kidney disease progression and increased cardiovascular risk in males.

Disclosure of Financial Relationships: nothing to disclose

**PUB571**

**Delayed Effect of Blood Pressure Fluctuations on Heart Rate in Patients with End-Stage Renal Disease** Dan Sapoznikov, Dvora Rubinger. *Nephrology and Hypertension Services, Hadassah University Medical Center, Jerusalem, Israel.*

The magnitude and the time delay of the baroreflex may be affected by decreased autonomic activity in the uremic state. The present study was undertaken to assess the delayed effect of systolic blood pressure (SBP) fluctuations on heart rate in patients with end stage renal disease. To evaluate the magnitude and the time delay of heart rate response, continuous interbeat intervals (IBI) and SBP were monitored in non-diabetic patients on chronic hemodialysis (HD, n=68), in patients after renal transplantation (TX, n=39) and in age-matched controls (C, n=34). A 4-term multivariate model for prediction of IBI differences from present and past SBP differences was calculated by the least square method. The frequency domain variables LF$\alpha$ , phase shift and lag time were also calculated. The $b_1$ model coefficient, representing the dependence of IBI difference with the first previous SBP difference, was markedly lower in HD patients than in controls, but increased after TX. $b_1$ strongly correlated with age and with the baroreceptor indices LF$\alpha$ coefficient and baroreflex slope. The correlations of $b_2$ coefficient, the 2nd model term, with the phase shift between SBP fluctuations and IBI are shown in Figure1:




Significant correlations were also found between $b_2$ and the lag time in both HD ($r=0.724$, $p<0.001$) and TX ($r=0.827$, $p<0.001$) patients. The lag time in controls (0.5-1.75 sec) was markedly lower than in HD and TX patients (up to 3.2 sec). These findings show: 1. In patients with end stage renal disease, there is a significant time delay in the heart rate response to the blood pressure variations. 2. Renal TX improves baroreceptor indices, but has no significant effect on the time delay of heart rate response to blood pressure oscillations. The prolonged delay may contribute to the circulatory instability in patients with chronic kidney disease.

Disclosure of Financial Relationships: nothing to disclose

**PUB572**

**The Effect of Cyclooxygenase-2 Inhibition on Systemic and Renal Hemodynamic Function and the Renin Angiotensin System in Healthy Humans** Magdalena A. Sarna,[1] Brenda Hemmelgarn,[1,2] Daniel A. Muruve,[1,2] Jennifer M. MacRae,[1,2] Shubhra Singh,[1] Yan Shi,[1] Darlene Y. Sola,[1] Sofia B. Ahmed.[1,2] *[1]Department of Medicine, University of Calgary, Calgary, AB, Canada; [2]Alberta Kidney Disease Network, AB, Canada.*

Even in healthy humans, cyclooxygenase-2 (COX-2) inhibition results in loss of kidney function, though the mechanism by which this occurs is unclear. COX-2-derived vasodilatory prostaglandins play a prominent role in arterial vasoregulation through interactions with the renin angiotensin system (RAS). Accordingly, we examined the role of COX-2 in control of baseline systemic and renal hemodynamic function and in the response to angiotensin II (AngII) in healthy humans. We hypothesized that COX-2 inhibition would result in a decrease in GFR (glomerular filtration rate), RPF (renal plasma flow) and RAS components and would increase the pressor response to AngII challenge.

Nine healthy subjects (4 females and 5 males) were studied in high salt balance, a state of maximal RAS suppression. Circulating components of the RAS (plasma renin activity (PRA), AngII, and aldosterone), blood pressure (BP) and renal hemodynamics [GFR, RPF, filtration fraction (FF)] were measured at baseline and in response to graded AngII infusion pre- and post-14 days of COX-2 inhibition with 200 mg celecoxib daily. Compared to baseline, there was a decrease in all circulating components of the RAS (PRA (p=0.15), AngII (p=0.36) and aldosterone (p=0.03)) following COX-2 inhibition. Blood pressure was unaffected by COX-2 inhibition (p=0.37 MAP, p=0.30 systolic, p=0.90 diastolic BP). There was a decrease in GFR (p=0.03) and RPF (p=0.02) post COX-2 inhibition with a resultant increase in FF (p=0.02). Compared to baseline, GFR was maintained while RPF decreased and FF increased in response to AngII after COX-2 inhibition. There was no difference in the circulating RAS components or pressor response to AngII pre- vs post-COX-2 inhibition.

The results suggest that COX-2 modulates RAS activity and renal hemodynamics at baseline and in response to AngII in healthy humans. COX-2 inhibition is associated with glomerular hyperfiltration and this may represent the mechanism by which COX-2 inhibition predisposes to loss of GFR.

Disclosure of Financial Relationships: nothing to disclose

**PUB573**

**Relation between Metabolic Abnormalities Associated with Arterial Dysfunction in Pediatric Dialysis and Transplant Patients** Hanan K. Tawadrous, Haroon Kamran, Anil K. Mongia, Louis Salciccioli, Morris J. Schoeneman, Jason Lazar. *Pediatric Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.*

Background

We previously demonstrated that higher augmentation index (AI), greater carotid intima-media thickness and endothelial dysfunction are present in patients with end stage renal disease on dialysis (D) compared to transplant recipients (Tx) and healthy controls. The etiology of increased arterial stiffness is uncertain but may be related to metabolic abnormalities present in D patients that improve after transplantation.

Objective

To assess metabolic abnormalities associated with increased arterial stiffness in pediatric D patients.

Methods

We studied 44 patients:15 on D,14 Tx (6 with living related donor (LRD), and 8 with deceased donor transplant (DDRT),and 15 healthy age, gender and ethnicity matched controls (C). CIMT and brachial artery flow-mediated increase in arterial diameter (FMD) was measured by high-resolution ultrasound. Heart rate corrected to 75 bpm (AI75) was measured by applanation tonometry. Hemoglobin (Hgb) and 25 hydroxy Vit D were obtained in all groups. PTH, calcium, phosphate and calcium x phosphate product (CaxP) levels were obtained in D. Mean values between groups were compared using ANOVA and pairwise comparisons.

Results

In all pts, FMD and PWV were each significantly correlated with Hgb, renal group (C,Tx,D) and with VitD on univariate analyses. AI75 was correlated with Hgb and renal group, but not Vit D. Hgb and Vit D were significantly correlated (r=.51,p=.001). On multivariate analyses, FMD was independently associated with renal group whereas PWV (p=.10) and AI75 (.005) were associated with Hgb. In the D group, AI75 was also correlated with P level(r=-.74, p=.004) and Ca x P (r=-.71, p=.007), but not with PTH or Ca. In D,cumulative Epogen dose was unrelated to any of the vascular parameters upon partial correlation with Hgb.

Conclusion

Metabolic and vascular parameters differ among D, Tx, and C. Anemia appears to play an important role in the development of arterial dysfunction in pediatric renal patients, whereas P and CaxP products may be contributory in those on D. High Epogen dosage does not appear to be detrimental.

Disclosure of Financial Relationships: nothing to disclose

**PUB574**

**Prolonged Dual Antiplatelet Therapy Did Not Decrease the Incidence of Restenosis after PTRA-Stenting — A Six-Year Study** Xin Zhang,[1] Mei Wang,[2] Li Zuo,[1] Wei Qin,[1] Haiyan Wang.[1] *[1]Nephrology, Peking University First Hospital; Institute of Nephrology, Peking University, China; [2]Nephrology, Peking University People's Hospital, China.*

**Background**: Dual antiplatelet therapy for at least 1 month is recommended for post-PCI patients with bare metal stents, but it is not yet known if this period is optimal for post-PTRAS patients.

**Objective:** To test if a prolonged dual antiplatelet therapy can lower instent-restenosis in renal arteries.

**Method:** Data from 2003 to 2009 was retrieved from the ARAS register database.

Patients were included if they: had unilateral or bilateral renal artery stenosis≥50% by angiography and were implanted BM-Stents; took aspirin plus clopidogrel for at least 1 month post procedure; were regularly followed up for at least 12 months.

Paients were divided into 1-month group (dual antiplatelet therapy for 1 month) and prolonged group (dual antiplatelet therapy for longer than 1 month). Restenosis was defined as a combination of Peak Systolic Velocity>180 cm/s and Renal Aortic Ration(RAR)>2.5:1 on color duplex sonography.

**Results:** A total of 116 patients with 141 stents were analyzed. Mean age was 67.7±8.7 years, and 89 % were male. The mean time for follow up was 31.1±18.8(4~81)months. One patients died (0.9%) and 32 had non-fatal cardiovascular events (27.6%). Eighteen patients were dignosed restenosis(15.5%).

The 1-month group had 18 patients and the prolonged group had 98. Baseline data including age, gender, and atherosclerotic comorbities and the intervention parameters including percentage of stenosis, the reference vessel diameter, and the stent size were all comparable. The period of clopidogrel pretreatment was longer in prolonged group(3.3±4.2 *vs* 1.9 ±1.5days, P<0.05). At the end of follow up, the restenosis rate did not differ between two groups(17/98 *vs* 1/18, P>0.05). Incidence of bleeding events was comparable.

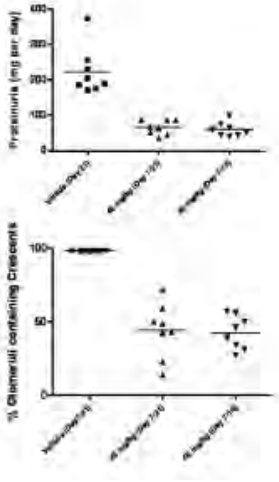
**Conclusion:** In our retrospective study, aspirin with prolonged clopidogrel did not lower the incidence of restenosis after PTRA-stents. The optimal period for dual antiplatelet therapy needs to be studied in prospective clinical trials.

Disclosure of Financial Relationships: nothing to disclose

**PUB575**

**Spleen Tyrosine Kinase Inhibitor R788 Is Effective in Reducing the Severity of Late Stage Glomerulonephritis and That This Effect Persists after Treatment Is Withdrawn** John P. McDaid,[1] Jennifer Smith,[1] Gurjeet Bhangal,[1] Esteban S. Masuda,[2] H. Terence Cook,[1] Charles D. Pusey,[1] Frederick W. K. Tam.[1] *[1]Imperial College Kidney and Transplant Institute, Imperial College, London, United Kingdom; [2]Rigel Pharmaceuticals Inc, South San Francisco, CA.*

Spleen tyrosine kinase (Syk) is crucial tyrosine kinase in the regulation of signal transduction upon activation of Fc receptors by immune complexes leading to proinflammatory cytokine production. Immune complex deposition in the kidney is an important cause of renal failure and the induction of diseases such as crescentic glomerulonephritis. Using the Wystar Kyoto rat model of glomerulonephritis and the Syk inhibitor R788 (fostamatinib disodium), an oral prodrug of the selective Syk inhibitor R406, we have previously shown that Syk inhibition is effective in the reduction of severity of glomerulonephritis. In this study we demonstrate that Syk inhibition is effective in reducing the severity of late stage disease at 21 days and also that the reduction in proteinuria by 70% (p<0.0012) and glomerulular crescents by 50% (p<0.001) compared to vehicle control group was maintained even when the treatment was withdrawn at day 14. Further investigations into the effect of Syk inhibition on fibrosis, a key feature of this late stage disease, by examining fibrotic markers alpha smooth muscle actin, fibronection, type IV collagen and cytokines TGFβ and CTGF are ongoing.

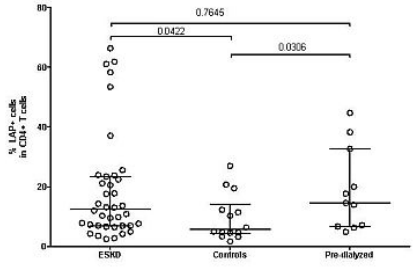



Disclosure of Financial Relationships: nothing to disclose

**PUB576**

**Modulation of CD4$^+$/LAP$^+$ in Uremic Peripheral Blood** Pascal Meier,[1] Regine Audran,[2] Rachel Meier.[1] *[1]Nephrology, CHCVs, Sion, Switzerland; [2]Immunology, CHUV, Switzerland.*

CD4$^+$/CD25$^+$ regulatory T cells (Tregs) are essential for maintaining self-tolerance and immune homeostasis. Several molecules including transforming growth factor beta (TGF-β) have been linked to the function and differentiation of Tregs. We have demonstrated that some uremic toxins (i.e. oxidized LDL) may induce Tregs cell cycle arrest and apoptosis affecting their suppression capacity and finally promote a constant micro-inflammatory state in patients with end-stage kidney disease (ESKD). In this study, we analyzed markers of regulatory populations in human peripheral blood from ESKD patients. Frequency of natural Tregs CD4$^+$/FOXP3$^+$/CD25$^{hi}$/CD69$^-$ or CD45RO$^+$ or of CD4$^+$/CD127$^{lo}$/CD25$^{hi}$ was lower than in pre-dialyzed patients and controls.




On the contrary, we observed an increased frequency of a population of CD4$^+$ T cells expressing membrane TGF-β and of its latency-associated peptide (LAP) in ESKD and in pre-dialyzed patients (Mann-Whitney p values). The role of this population of CD4$^+$/CD127$^+$/CD25$^{lo/int}$ sequestering TGF-β on their surface, suggesting a regulatory function, is not yet elucidated. Thus, we have characterized in ESKD patients, the modulation of a population of LAP$^+$ and m TGF-β$^+$ CD4 T cells that is different from the classic dysfunctional CD4$^+$/FOXP3$^+$/CD25$^{hi}$ natural Tregs. Our findings provide the opportunity to identify CD4$^+$/LAP as a therapeutic target for the treatment of the micro-inflammatory state encountered in ESKD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB577**

**Sirolimus Modulates Development and Progression of HIV-Associated Nephropathy (HIVAN) by Altering Plasminogen Activation Inhibitor (PAI)-1 Gene Expression** Shabina Rehman, Shitij Arora, Dileep Kumar, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.*

Renin-Angiotensin System (RAS) has been demonstrated to contribute to the progression of renal failure in several human and animal modes of chronic kidney diseases. Modalities which prevent the production or block the effect of Angiotensin (Ang II) have been demonstrated to slow down the progression of HIVAN. Recently, we reported that sirolimus attenuated the development of HIVAN (Am J Pathol. August, 2010). Since Ang II mediates it profibrotic effect through PA-1, we asked whether sirolimus modulated the progression of HIVAN by altering renal tissue expression of PAI-1.

Four weeks old Tg26 animals (n=6) were administered either vehicle or sirolimus (5 mg/kg/day, intra-peritoneal) for 14 days (group A) or 28 days (group B). To determine the role of Ang II in PAI-1 mediated mediated renal injury in HIVAN, Tg26 mice (n=3) were treated with either saline or telmisartan (300 µg/day, by miniosmotic pump) for two weeks. At the end of experimental periods, kidneys were isolated and prepared for histology. In addition, RNA was extracted evaluated for expression of PA-1 gene by RT-PCR.

In group A, the vehicle treated mice showed 1+ to 2+ glomerulosclerosis and 2+ to 3+ tubular dilatation, two rapamycin-treated mice showed no glomerular sclerosis and only mild dilatation of tubules and one rapamycin treated mouse showed 0 to 1+ glomerulosclerosis and 1+ to 2+ tubular dilatation. In group B, vehicle treated animals showed two-fold advanced glomerular and tubular lesions vs. rapamycin-treated animals. Rapamycin-treated animals showed 5-fold decrease in renal tissue PA-1 gene expression when compared with vehicle treated Tg26 mice. Tg26 mice showed enhanced tubular cell expression of PAI; however, telmisartan not only diminished development renal lesions in Tg26 mice but also inhibited tubular cell expression of PAI-1. These findings indicate Ang II contributes to the tubular cell expression of PAI-1 in HIVAN and rapamycin may be slowing down renal lesions in HIVAN by attenuating PAI-1 transcription.

Disclosure of Financial Relationships: nothing to disclose

**PUB578**

**Thrombin Stimulates Synthesis of M-CSF, GM-CSF, MCP-1, and MIF in Human Proximal Tubular Epithelial Cells (PTEC) in Culture** Yuko Shimaya, Michiko Shimada, Hideaki Yamabe, Takeshi Fujita, Reiichi Murakami, Norio Nakamura, Ken Okumura. *Nephrology, Hirosaki University, Hirosaki, Aomori, Japan.*

Although Colony-stimulating factors (CSFs) were originally defined as haematopoietic cell growth factors, it has been suggested that CSFs are involved in various inflammatory conditions. However, it's role and expressions in the kidney has not well elucidated. We have been investigated the effect of thrombin in the kidney cells since thrombin exists in urine of the patients with glomerulonephritis, and urinary procoagulant activity has been suggested in the tubulointerstitial injury.

In this study, we used thrombin with or without argatroban which is a synthetic thrombin inhibitor to stimulate human PTEC and examined the expressions of Macrophage Colony Stimulationg Factor (M-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), Monocyte Chemoattractant Protein-1 (MCP-1) and Migration Inhibitory factor (MIF). Protein levels in the cell supernatants were measured by ELISA, and mRNA levels were quantified by realtime RT-PCR.

M-CSF, GM-CSF and MCP-1 protein levels were largely increased ($P$<0.01) and MIF were slightly increased ($P$<0.05) with thrombin (0.5-5.0u/ml, 24-72hours) in a dose and time dependent manner, and decreased with argatroban ($P$<0.01). Besides, 6h incubation with thrombin (1.0 or 5.0u/ml) increased mRNA levels of M-CSF, GM-CSF and MCP-1 dose-dependently($P$<0.05) and mRNA levels of MIF did not change.

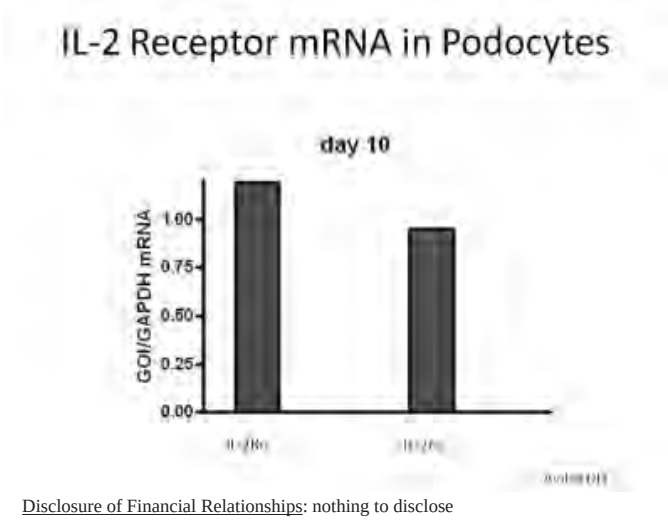
Collectively, thrombin stimulated the production of M-CSF, GM-CSF, and MCP-1 by cultured PTEC and the possibly related to the tubulointerstitial injury in glomerulonephritis via blood coagulation process.

Disclosure of Financial Relationships: nothing to disclose

**PUB579**

**Expression of the IL-2 Receptor in Murine Podocytes** Diego H. Aviles,[1] Jeannine Ory Ascani,[1] David J. Tate,[2] William E. Smoyer,[3] Arnold H. Zea.[2] *[1]Pediatrics, LSU Health Sciences Center/ Children's Research Institute, New Orleans, LA; [2]Stanley Scott Cancer Center, LSU Health Sciences Center, New Orleans, LA; [3]Pediatrics, Nationwide Children's Hospital, Columbus, OH.*

Nephrotic syndrome (NS) is a renal disease that results in massive proteinuria and edema. Patients with NS secondary to FSGS have poor responses to treatment and a high risk for developing renal failure. The renal podocyte plays an important role in maintaining the structural integrity of the glomerular filtration barrier. While mutations affecting the podocyte account for only a minority of the cases of NS, there is evidence that cytokines play a role in many forms of NS. We have shown that patients with NS due to FSGS have increased IL-2 production, although the underlying mechanism for IL-2 induced proteinuria is unknown. **Hypothesis**: IL-2 induced proteinuria is due to a direct effect on the podocyte. **Methods:** Differentiated murine podocytes were used to measure the IL-2 receptor expression in the presence and absence of IL-2 by qRT-PCR, flow cytometry and Western blotting. Apoptosis was measured by flow cytometry, while cytokine production was measured in supernatants with a Bioplex cytokine kit. **Results:** We found that murine podocytes expressed the IL-2 receptor, and that they produced IL-2 when stimulated with exogenous IL-2. IL-2 also induced podocyte apoptosis. **Conclusions:** We conclude that murine podocytes express a functional IL-2 receptor, and that they produce IL-2 in response to IL-2 exposure. Our results thus establish the ability of podocytes to both produce and respond to IL-2, a prime target to our current treatment for NS, although whether such stimulation in NS represents an autocrine or paracrine response to IL-2 remains uncertain.




Disclosure of Financial Relationships: nothing to disclose

**PUB580**

**Vitamin D Antibacterial Activity in Cells from Peritoneal Dialysate Effluent**
Justine Bacchetta, Rene Chun, Katherine Wesseling-Perry, Martin Hewison, Isidro B. Salusky. *Orthopedic Hospital Research Center & Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles.*

CKD patients are vitamin D deficient (precursor 25OHD and active $1,25(OH)_2D$) and both forms of the vitamin stimulate innate antibacterial activity in human monocytes. Infections are the main cause of morbidity in peritoneal dialysis (PD) pts. Thus, we hypothesized that peritoneal monocytes from PD pts may be targets for vitamin D therapy with potential unrecognized effects in the prevention of infections. Cells from 23 dialysate effluents (12 pts, age 16±3 yrs, serum 25OHD 29±9 ng/ml) were studied. Cells were isolated by centrifugation of 24 hour-dialysate effluent and evaluated for: 1) cell phenotype by flow cytometry; 2) baseline *in vivo* expression of genes associated with vitamin D function: vitamin D receptor (VDR], 24-hydroxylase (CYP24A1), 1α-hydroxylase (CYP27B1) and antibacterial cathelicidin (LL37); 3) gene expression following treatment of peritoneal monocytes *in vitro* with 25OHD (100 nM) or $1,25(OH)_2D$ (5 nM). There was a wide range of peritoneal cell number among pts (median 826,380; r 24,500-34,848,000 cells/l). Analysis of total cell populations from PD effluent before *in vitro* culture showed a significant correlation between the expression of CYP27B1 and LL37 ($r= 0.728$; $p<0.001$), and between the expression of CYP24A1 and both CYP27B1 and LL37 ($r= 0.698$ and 0.573, respectively, $p<0.01$). Serum 25 OHD levels did not correlate with the expression of vitamin D-dependent genes in PD cells. Crucially, expression of antibacterial LL37 by peritoneal monocytes was further enhanced after *in vitro* treatment with either 25OHD (10-fold) or $1,25(OH)_2D$ (100-fold).This is the first demonstration that, similar to peripheral blood monocytes, peritoneal monocytes have a functional system for vitamin D metabolism and activity. Induction of antibacterial LL37 by these cells appears to be dependent of expression of CYP27B1, and is enhanced by the addition of either inactive or active vitamin D. These data highlight a potentially important new function for vitamin D supplementation in the prevention of infectious related complications in PD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB581**

**Differential Effects of Age and Cytomegalovirus-Seropositivity on Circulating T Lymphocyte Subsets of End-Stage Renal Disease Patients**
Michiel G. H. Betjes, Elly L. E. A. De Wit, Nicolle H. R. Litjens. *Nephrology, Erasmus Medical Center, Netherlands.*

**Background:** Patients with end-stage renal disease (ESRD) have an impaired T cell immunity which may be related to the profound changes in total number and distribution of their circulating T cell subsets. Cytomegalovirus (CMV)-seropositivity is increasingly recognized as a major determinant of accelerated T cell ageing and as such may contribute to impaired T cell immunity.

**Patients and Methods**: In a cross-sectional study the absolute numbers of circulating T lymphocytes and CD4pos and CD8pos T cells were determined of 139 ESRD patients (60% CMV-seropositive) and compared to healthy individuals matched for age and CMV serostatus. T cell subsets were further characterized by their differential expression of CD45RO and CCR7. Expression of the markers CD28 and CD57 on memory T cells was analyzed to establish the degree of T cell differentiation in more detail.

**Results:** CMV-seropositivity is associated with a significant expansion of both CD4 and CD8 memory populations in healthy individuals already at a young age. The total number of CD8 memory T cells is not affected by age in CMV-seropositive individuals and remains expanded by an average 60% compared to CMV-seronegative individuals. In addition, the degree of T cell differentiation is increased in CMV-seropositive healthy individuals. A modest age-related contraction of the naïve CD4pos T cell population was associated with CMV-seropositivity, in contrast to the CMV-independent pronounced decrease in the number of the CD8pos naïve T cells. ESRD patients showed a profound naïve T cell lymphopenia at all ages. Within their CD4pos T cell compartment, CMV-seropositivity aggravated the contraction of naïve T cells and increased the number of differentiated memory T cells. The remarkable CMV-seropositivity related expansion of memory CD8pos T cells was not observed, but the degree of terminal differentiation was increased.

**Conclusion:** CMV seropositivity is associated with pronounced age-related changes like expansion and increased differentiation of circulating CD4pos and CD8pos T cell populations, and aggravates the T cell dysregulation observed in ESRD patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB582**

**Synergistic Antifungal Effect of Caspofungin and Human Polymorphonuclear Leukocytes (PMNs) from Chronic Haemodialysed Patients and Renal Transplant Recipients Against *Candida albicans***
Franca Giacchino,[1] Giuliana Banche,[2] Valeria Allizond,[2] Daniela Scalas,[2] Janira Roana,[2] Giuseppe Garneri,[1] Rosaria Patti,[1] Rosanna Coppo,[3] Vivian Tullio,[2] Chiara Merlino,[2] Narcisa Mandras,[2] Anna Maria Cuffini.[2] *[1]Nephrology and Dialysis Unit, Civil Hospital, Ivrea, Turin, Italy; [2]Department of Public Health and Microbiology, University of Turin, Turin, Italy; [3]Nephrology Dialysis Transplant Unit, Regina Margherita Hospital, Turin, Italy.*

**Background.**Invasive fungal infections(IFIs) are difficult to eradicate especially in immunocompromised host: uremic patients are highly susceptible to fungal infections due to impaired phagocyte-dependent host defences. Hence, antifungal drugs that positively influence phagocyte activity may be crucial for IFI resolution.

**Objectives**.The aim of this study was to evaluate the effect of caspofungin, a new echinocandin, on the functions of PMNs from healthy subjects, haemodialysed patients and renal transplant recipients towards *Candida albicans*.

**Study design and Methods**.PMNs were separated from venous blood samples of 40 healthy donors, 68 haemodialysed patients and 65 renal transplant recipients. The effects of caspofungin on either phagocytosis or intracellular killing by PMNs towards *C.albicans* were investigated by incubating yeasts and PMNs with caspofungin. Drug-free controls were included.

**Results.**A diminished PMN efficiency was found in haemodialysed patient and renal transplant recipient PMNs, with reduced both phagocytosis and fungicidal activity towards intracellular yeasts, in comparison with healthy subject PMNs. As the majority of systemically acting antifungal drugs, caspofungin did not significantly improve phagocytic activity. Conversely, the fungicidal activity of uremic patient PMNs was significantly potentiated by caspofungin after 60'and 90' of incubation, in comparison with drug-free controls($p<0.01$).

**Conclusions.**Our findings provide evidence that caspofungin is able to restore the depressed intracellular killing by uremic patient PMNs, through a synergistic effect with PMNs towards *C.albicans* and may constitute effective therapeutic option for IFI treatment in patients with altered phagocyte-dependent innate immunity.

Disclosure of Financial Relationships: nothing to disclose

**PUB583**

**Proliferation and Activation during Tubulointerstitial Injury in the Native Kidney**
Victoria J. Ingham, Neil S. Sheerin. *Institute of Cellular Medicine, Newcastle University, Newcastle-upon-Tyne, United Kingdom.*

Tubulointerstitial (TI) inflammation and fibrosis is seen with progressive renal failure and may be a common pathway to renal failure. The role and function of TI lymphocytes in the pathogenesis of injury is unknown.

Methods

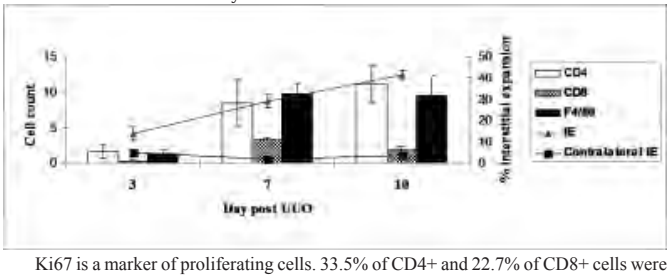
We used the unilateral ureteric obstruction (UUO) model of TI fibrosis to assess the composition and phenotype of infiltrating leukocytes.

Four animals were sacrificed at 3, 7 and 10 days after UUO. Tissue sections were analysed for:

- TI injury with Periodic acid Schiff and immunohistochemical (IHC) αSMA staining
- Cellular infiltration with quantification of CD4+, CD8+ and F4/80+ cells by IHC
- T cell proliferation by immunofluorescent dual staining of CD4+ or CD8+ cells with Ki67
- T cell activation by dual staining of CD4+ or CD8+ cells with CD69

Results

There was a positive correlation between cellular infiltration and injury. Few T cells were present at day 3 post UUO. By day 7, CD4+ and CD8+ T cells increased 6 and 11 fold compared to day 3. CD4+ T cell infiltration predominated over CD8+ cells with a CD4+: CD8+ ratio of 2.5:1 and 6:1 at days 7 and 10. There was negligible T cell infiltration in contralateral and normal kidneys.




Ki67 is a marker of proliferating cells. 33.5% of CD4+ and 22.7% of CD8+ cells were found to be Ki67+ at day 10 post UUO.

CD69 suggests lymphocyte activation. CD4+ activation peaked at day 7. More CD8+ lymphocytes were activated at later time points post UUO suggesting a requirement for initial CD4+ help ($p<0.05$).

Conclusions

Lymphocytes are not simply trafficking through injured kidney but show evidence of proliferation and activation. Since Chapman et al. (Virology 2005) showed that T cell proliferation only occurs in response to antigen recognition, this work suggests a loss of tolerance to self antigen in this non-immunologically mediated model of TI fibrosis. As yet the specific antigen(s) are unknown.

Disclosure of Financial Relationships: nothing to disclose

**PUB584**

**Immune Response to Hepatitis B Vaccine in ESRD Population**
Lakshmi P. Nadimpalli,[1] Prajwol R. Pant,[1] Earl C. Smith,[1] Janos Molnar,[1] Ashok K. Singh,[2] Krishnamurthy P. Gudehithlu,[2] Andres Serrano.[1] *[1]Department of Nephrology, Mt. Sinai Hospital/Chicago Medical School, Chicago, IL; [2]Department of Nephrology, John H. Stroger Hospital of Cook County, Chicago, IL.*

End-Stage Renal Disease (ESRD) patients have a low seroconversion rate compared to the general population, following Hepatitis B vaccination (HBV). It has been reported an association between high TNFα levels, a marker of chronic inflammation, and immunocompromized state in chronic hemodialysis (HD) patients. We conducted a prospective study to identify clinical parameters that can predict seroconversion, and to

evaluate the possible association between inflammation (measured by inflammatory markers -TNFα, CRP & ferritin) and the immune response following HBV. Thirty five chronic HD patients who were negative for hepatitis B surface antigen and antibody, received 40 µg of HBV in the deltoid muscle at 0, 1 & 6 months. Immune response to HBV was evaluated 1 month after completion of the vaccination series by quantitative antibody (Ab) titers. Clinical parameters and inflammatory markers were compared between patients who responded and those who did not. Patients who responded to HBV (22 or 63%) were younger than those who did not (53.95 +/- 15.47 vs. 68.85 +/- 9.67, p=0.018). There were no differences in other parameters (sex, ethnicity, type of access, number of years on HD, urea reduction ratio, serum iron, hemoglobin, albumin, PTH, calcium, phosphorus and bicarbonate) and in the use of erythropoietin stimulating agents or vitamin D analogues between the two groups. We specifically evaluated the association of inflammatory markers (TNFα, CRP, ferritin) with seroconversion after HBV, but there were no differences between the two groups. Patients were also stratified according to their Hepatitis B Ab titers, but there were no differences in any of the parameters described.

***Conclusion:*** The immune response to HBV in chronic HD is low compared to the general population. Younger age seems to be associated with an adequate response to HBV. We did not identify any association between inflammation, measured by inflammatory markers (TNFα, CRP & ferritin), and the lack of seroconversion after HBV.

Disclosure of Financial Relationships: nothing to disclose

**PUB585**

**Micro- and Macrovascular Dysfunction in Systemic Lupus Erythematosus** Susann Patschan, Martha Potulski, Daniel Patschan, Gerhard A. Mueller. *Department of Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany.*

Patients with systemic lupus erythematosus (SLE) suffer from vasculitis of small arteries/arterioles. The disease is associated with a 17-fold higher risk for artherosclerosis. Aim of the study was (I) to analyze the regenerative activity of the endothelial progenitor cell (EPC) system and (II) to measure pulse wave velocity in SLE.

A total of 39 SLE patients and 21 healthy controls were included into the study. EPC regeneration was evaluated by a colony-forming unit assay, total peripheral circulating EPCs were measured by cytometric analysis. Pulse-wave velocity (PWV) was quantified by tonometric analysis.

SLE patients, as compared to controls, displayed significant mobilization of total CD133+ and of CD133+/Flk-1+ cells (81 ±19 vs. 25 ±20%, p<0.001 and 0.92 ±0.84 vs. 0.46 ±0.47, p=0.02). In contrast, the regenerative activity of EPCs was markedly affected (25 ±29 vs. 45 ±31, p=0.01). Pulse wave velocity, a parameter of macrovascular damage, did not differ between patients with SLE and healthy controls (mean blood pressure levels in SLE patients were normotensive).

In summary our data show (I) a dramatic increase in circulating CD133+ cells and a significant increase in peripheral CD133+/Flk-1+ cells in SLE, reflecting intact EPC mobilization. (II) However, the regenerative activity of the EPC system was affected, which points towards the inability of adequate vascular repair. Since PWV analysis did not show differences between (normotensive) SLE and healthy controls, signs of microvascular dysfunction (e.g. dimished EPC proliferation) may not allow to conclude on structure/function of the larger arterial blood vessels and vice versa. Thus, both functional vascular elements must be analyzed separately.

Disclosure of Financial Relationships: nothing to disclose

**PUB586**

**Risk Assessment and Renal Morbidity after Acute Stroke** Rupesh Raina. *Department of Nephrology and Hypertension, Cleveland Clinic Foundation, Cleveland, OH.*

Although acute stroke is an emergency disease and shares the same atherosclerotic risk factors with ischemic heart disease, the association of renal failure and stroke is poorly investigated.

We assessed the prevalence and predisposing factors for renal insufficiency in patients who sustained acute stroke and investigated outcomes (hospital discharge disposition (HDD) and length of stay (LOS)).

628 patients from a single center with acute ischemic and hemorrhagic stroke during 2007 and 2008 were evaluated. AKI, defined as serum creatinine.>0.3mg/dl or percentage increase of 50% from baseline on admission and discharge was assessed. Risk factors considered were age, gender, race, BUN value and history of hypertension, diabetes mellitus, hyperlipedemia ( HPL), coronary artery disease (CAD), and stroke. The mean age for all members was 63 years old, and the ages ranged from 18 to 96. The median length of stay was 6 days.

Of the 628 patients with acute ischemic and hemorrhagic 90 (14.3%) had renal insufficiency at admission. the prevalence of renal insufficiency at admission ranged from 4.1% (430) to 17.2% (431).

Multivariable models for renal insufficiency at admission showed males (p<0.001), those with hypertension (p=0.002), diabetes (p=0.003), stroke (p=0.027) and CKD (p<0.001) history had significantly higher risk of renal insufficiency at admission. No significant differences were observed in history HPL or CAD.

Of the those with hypertension (p=0.021)and CKD(p<0.001) also had significantly higher risk of renal insufficiency at discharge. In addition, non-whites (p<0.001) and older patients (p<0.01)also showed increased risk of renal insufficiency at discharge.

Conclusion: Renal failure is prevalent in acute stroke patients at time of admission and is associated with poor outcome at discharge. Consistent implementation of renal protective management and more broad use of drugs with potential renal protective properties may result in better outcome.

Disclosure of Financial Relationships: nothing to disclose

**PUB587**

**HIVAN Phenotype Is Associated with Downregulation of MicroRNA-21 and MicroRNA-192** Divya Salhan, Dileep Kumar, Ashwani Malhotra, Pravin C. Singhal. *Medicine, LIJ Medical Center, New Hyde Park, NY.*

HIV-associated nephropathy (HIVAN) phenotype is characterized by proliferarion of podocytes and tubular cells. However, the exact pathophysiology of proliferative phenotype is not clear. Since, we recently reported that epithelial mesechymal transition (EMT) contributed to pathogenesis of HIVAN phenotype in Tg26 mice (AJP 2010), a mouse model of HIVAN, we asked whether microRNAs were associated with this process. Micro RNAs have been reported to be differentially expressed in various pathological and physiological states. The precise role of microRNAs, endogenous RNA oligonucleotides which specifically target mRNA and regulate gene expression in HIVAN is not known. Several studies have also identified micro RNAs as key regulators of EMT and may enforce the epithelial phenotype.

FVB/N (control) and Tg26 mice aged 16 wks (n=3) were sacrificed and kidneys were harvested for renal histology, immunohistochemical studies, and RNA extraction. Renal cortical sections were immunolabeled for EMT markers (α-SMA, vimentin, FSP1, ZEB2, and E-Cadherin).Total RNA from renal tissue (n=3) was isolated by Triozol reagent (Invitrogen).cDNA was synthesized using SuperScript Enzyme Mix (Invitrogen).Real-Time qPCR was performed (according to the manufacturer's instructions) to detect the Mir-192 and Mir- 21 by using SYBR Green Universal Kit (Invitrogen) using forward primers for Mir-21, Mir-192 and universal qPCR primers (Invitrogen).

Renal histology in TG26 mice showed classical HIVN phenotype in the form of collapsing variant of focal segmental glomerulosclerosis and microcystic dilatation of tubules. Moreover, in all Tg26 mice, variable numbers of glomerular and tubular cells showed expression of α-SMA, vimentin, FSP1, and loss of E-Cadherin; whereas, control mice did not show any abnormality. Renal tissue expression of Mir-21 and Mir-192 were decreased in Tg26 (Mir21, 5-fold and Mir 192, 4.5-fold; n=3) vs. control mice(n=3). These results demonstrate that downregulation of Mir-21 and Mir-192 was associated with the induction of EMT, a suggested feature of HIVAN phenotype.

Disclosure of Financial Relationships: nothing to disclose

**PUB588**

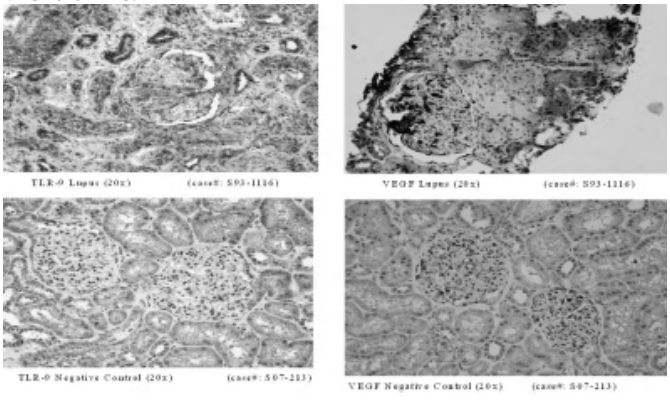
**Role of VEGF and TLR 9 in Lupus Nephritis** Mohammad A. Samih,[1] Sofia Rubinstein,[1] Leah Balsam,[1] Marianne Frieri,[1] Ahmad Aljada,[3] Hui Liu.[2] *[1]Department of Internal Medicine, Nassau University Medical Center, East Meadow, NY; [2]Department of Pathology, Nassau University Medical Center, East Meadow, NY; [3]Department of Biomedical Sciences, School of Health Professions and Nursing Sciences, Long Island University, CW Post C.W. Post Campus, Brookville, NY.*

Introduction: Vascular endothelial growth factor(VEGF) is a key regulator of angiogenesis, binds to receptors on all endothelial cells, including glomerular endothelial cells. TLR9, one of the Toll-like receptor family, implicated in pathogenesis of autoimmunity especially in SLE and the induction of T-helper 1 immune response.

Objective: We compared glomerular and tubulointerstitial expression of both proteins in biopsies from lupus nephritis with normal controls.

Methods: 10 lupus nephritis(LN) kidney biopsies were collected, with 10 normal controls. Immunohistochemistry was performed using the Vectastain Elite ABC kit. Slides were deparaffinized, dehydrated, permeabilized with 0.1% Saponin in PBS, treated with 0.3% hydrogen peroxide and blocked with serum containing PBS and 0.1% Saponin. Slides were incubated with antibodies against VEGF and TLR9 monoclonal antibody, incubated with biotin labeled anti-mouse for 0.5 hour followed by Vectastain ABC solution, incubated with ImmPACT DAB Substrate, stained with hematoxylin and eosin, mounted and microscopically score at 10x and 20x.Clinical renal scoring was also assessed.

Results: VEGF intensity score was greater in the glomeruli vs. the tubules. TLR9 intensity score was greater for renal tubules vs. VEGF. Both stains were negative in the healthy controls. There was no correlation with class severity and intensity of staining of VEGF and TLR9.



Conclusion: This is the first study that examined the combined expression of VEGF and TLR9 in LN in human samples. Both proteins could be a potential future therapeutic target.

Disclosure of Financial Relationships: nothing to disclose

**PUB589**

**CCR5 Deficiency Aggravates Murine Lupus Nephritis** Jan-Eric Turner,[1] Hans-Joachim Paust,[1] Sabrina Bennstein,[1] Philipp Bramke,[1] Oliver M. Steinmetz,[1] Erik M. Disteldorf,[1] Joachim Velden,[2] Rolf A. Stahl,[1] Ulf Panzer.[1] *[1]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]Insitut für Pathologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

The recruitment of T cells and monocytes is a characteristic feature of human and experimental lupus nephritis and closely correlated to loss of renal function. The chemokine receptor CCR5 is expressed on monocytes and T cell subsets and is supposed to play an important role in recruiting these leukocytes into inflamed organs.

In this study, we investigated the functional role of CCR5 by using the MRL/MpJ-Faslpr (MRL/lpr) mouse model of systemic lupus erythematosus that resembles the human disease. CCR5-/- mice were backcrossed into the MRL/lpr background for at least seven generations. Analysis of 6-month-old CCR5-/- MRL/lpr mice showed aggravated nephritis with more glomerular tissue damage, increased albumin to creatinine ratio ($20.1 \pm 4.3$ vs. $0.9 \pm 0.43$; $P < 0.01$) and augmented T-cell recruitment ($9.9 \pm 1.4$ vs. $4.7 \pm 0.7$ CD3+ cells / high power field; $P < 0.01$) compared to their wild-type littermates. The relative distribution of IFNγ- and IL-17-producing CD4+ T cells, FoxP3+ regulatory T cells, and IFNγ-producing CD8+ T cells in the kidney and the secondary lymphoid organs of MRL/lpr mice were unaffected by CCR5 deficiency. The mechanism underlying the aggravation of the nephritis in CCR5-/- MRL/lpr mice remains to be elucidated.

Disclosure of Financial Relationships: nothing to disclose

**PUB590**

**Neutrophils Are an Important Source of IL-17 in Human Inflammatory Diseases** Joachim Velden,[1] Hans-Joachim Paust,[2] Jan-Eric Turner,[2] Oliver M. Steinmetz,[2] Saskia Schröder,[2] Ursula Kneissler,[1] Elion Hoxha,[2] Erik M. Disteldorf,[2] Hans-Willi Mittrücker,[3] Rolf A. Stahl,[2] Udo Helmchen,[1] Ulf Panzer.[2] *[1]Nierenregister, Institut für Pathologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [3]Institut für Immunologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

BACKGROUND: The pathogenic role of interleukin-17A (IL-17) in autoimmune diseases has been demonstrated in numerous transgenic mouse models. It is further supported by a promising anti-IL-17 approach for the treatment of rheumatoid arthritis in humans. IL-17-driven inflammation has primarily been attributed to the actions of Th17 cells. However, the cellular sources of IL-17 in human tissues remain to be fully elucidated.

PURPOSE: The primary goal of this study was to identify and characterize intrarenal IL-17 containing ($IL\text{-}17^+$) cells in human ANCA-associated glomerulonephritis (ANCA-GN).

METHODS: Human kidney biopsies of ANCA-GN and tissue samples of other inflammatory diseases were analyzed by immunohistochemical and immunofluorescent microscopy co-localization studies. Neutrophils were isolated from healthy human blood and were either analyzed by immunofluorescent microscopy, or stimulated *in vitro* and analyzed by ELISA.

RESULTS: Neutrophils were by far the largest $IL\text{-}17^+$ cell population in acute necrotizing ANCA-GN, in chronic active Crohn's ileocolitis, in psoriasis dermatitis, and in non-autoimmune inflammatory diseases such as infectious tonsillitis. The second most frequent $IL\text{-}17^+$ species were mast cells, whose contribution was increased in chronic as compared to acute ANCA-GN. The amounts of $IL\text{-}17^+$ T cells were negligible throughout in all specimens. All neutrophils from healthy human blood constitutively contained IL-17, which they were able to release upon stimulation *in vitro*.

CONCLUSIONS: Neutrophils are the predominant IL-17-laden effector cells in human ANCA-associated glomerulonephritis, Crohn's disease and psoriasis. The concept of Th17 cells being the main source of IL-17, at least in these human autoimmune diseases, is thus challenged.

Disclosure of Financial Relationships: nothing to disclose

**PUB591**

**Increased Levels of Anaphylatoxin (C5a) and Bradykinin in End-Stage Renal Disease Patients on Maintenance Hemodialysis** Vinod K. Bansal,[1] Evangelos Litinas,[2] Korosh Sharain,[2] Debra Hoppensteadt,[2] Jawed Fareed.[2] *[1]Department of Nephrology, Loyola University Medical Center, Maywood, IL; [2]Department of Pathology, Loyola University Medical Center, Maywood, IL.*

Besides the upregulation of inflammatory mediators end stage renal disease (ESRD) patients maintained on hemodialysis are subjected to periodic exposure to heparin and contact activation due to procedural settings. Recently the presence of a heparin contaminant, namely hypersulfated chondroitin sulfate was linked with the adverse reactions and deaths observed in these patients (Kishmoto, et al. N J Med 2008). To validate this report we measured both the C5a anaphlatoxin and bradykinin levels in ESRD patients prior and after maintenance hemodialysis. The control group comprised of 40 normal healthy individuals were included to establish the normal level of these mediators. Sandwich Elisa methods utilizing monoclonal antibodies which are specific for either human C5a or bradykinin were used in these studies. Both the C5a and bradykinin were elevated in pre-dialysis samples from ESRD patients (C5a: 3.2±0.6 ng/ml vs 14.2± 4.6 ng/ml, bradykinin: 6.4±1.8ng/ml vs 9.3±2.4ng/ml). Moreover, dialysis itself produced an increase in both the C5a and bradykinin levels. The postdialysis samples were further increased up to 60%, suggesting that dialysis and heparinization itself result in the up regulation of these mediators. Supplementation of heparin to the plasma also resulted in the generation of both C5a and bradykinin. The plasma samples included in these studies represents patients who were not treated with the contaminant heparin. These results suggest that both C5a and bradykinin are up-regulated in ESRD patients and this level can be further augmented by dialysis and heparinization. Therefore, additional factors may have contributed to the complex adverse reaction profiles and deaths in patients administrated with contaminated heparin.

Disclosure of Financial Relationships: nothing to disclose

**PUB592**

**Penicillin G Reduces Podocyte Injury Induced by Puromycin Aminonucleoside: Role of Transmembrane Transporters** Mary Artero,[1] Cristina Zennaro,[2] Lorella Pascolo,[3] Marco Stebel,[2] Claudio Tiribelli,[2] Maria Pia Rastaldi,[4] Michele Carraro.[2] *[1]Azienda Ospedaliero-Universitaria, Trieste; [2]University of Trieste; [3]Sincrotrone SpA, Trieste; [4]Fondazione Policlinico, Milan, Italy.*

We evaluated the effect of penicillin G, which is a substrate for several families of small organic molecule transporters, on podocyte damage induced by puromycin aminonucleoside (PAN).

Sprague Dawley rats were injected with PAN or normal saline. Two subgroups of PAN rats were treated with penicillin G before PAN injection. After 10 days the rats were killed, and the kidneys were excised to determine filtration characteristics and for histologic and morphometric analysis. Glomerular adhesion and cell viability in podocyte culture were studied using both established lines of differentiated human podocytes and primary culture from isolated rat glomeruli. Transport in live cells was studied using fluorescent penicillin. Expression of podocyte membrane transporter messenger RNA was measured .

PAN treatment resulted in proteinuria and hypoalbuminemia, mitigated in rats pretreated with penicillin G. Kidney:body weight ratio and glomerular hypertrophy were reduced in pretreated animals. Albumin permeability correlated with proteinuria and was comparable to the control group in PAN rats pretreated with penicillin G. Healthy glomeruli pretreated with penicillin G demonstrated permeability characteristics which depended on the doses of both penicillin and PAN. PAN inhibited glomerular adhesion and podocyte outgrowth on collagen IV substrate in a dose-dependent fashion in cell cultures in which penicillin G was removed. Cultures in which penicillin G was not removed showed normal adhesion, according to the dose of the antibiotic. Penicillin G also increased podocyte viability 48 h after PAN exposure. Intracellular fluorescent penicillin was clearly visualized in podocytes after incubation. Podocyte gene expression was detected for OATP and P-glycoprotein transporters and the monoamine transporter PMAT.

Penicillin G reduces podocyte injury induced by PAN in the intact animal and in culture, possibly by blocking membrane transporters, particularly of the OATP family, or an intracellular target.

Disclosure of Financial Relationships: nothing to disclose

**PUB593**

**Presence of C1q Deposits Is Associated with Better Prognosis in Children with Primary Focal Segmental Glomerulosclerosis (FSGS)** Nataliya Chorny, Morris J. Schoeneman, Anil K. Mongia, Sreevidya Kusuma, Shella Mongia. *Pediatric Nephrology, SUNY Downstate, Brooklyn, NY.*

Primary FSGS is the most common cause of steroid resistant nephrotic syndrome in children. Progression to ESRD (End Stage Renal Disease) is common (>60%), but some patients have prolonged remission with normal renal function. Prognostic indicators of FSGS have not been clearly defined. We performed a retrospective review of 44 children with primary FSGS diagnosed between 1996 -2009. Ages at onset ranged from 2.5 years to 19 years, and mean follow-up period was 6.3 years (1.5 -16) 33 were males and 11 were females. 27 patients were black, 9 Hispanic, 5 Caucasian and 4 other. 14 patients progressed to ESRD over 2 to 8 years. 30 patients (68%) entered prolonged remission, 14 in complete without proteinuria and 17 in partial remission with persistent proteinuria but without nephrotic syndrome. We examined clinical, laboratory and histologic variables at onset, in an attempt to determine prognostic indicators. Our data revealed that 3 variables at onset had a significant impact on prognosis. They were: presence of C1q deposits (p=0.002), GFR at presentation (p=0.002), and percentage of global glomerulosclerosis (p=0.045). Furthermore the patients with C1q deposits needed less immunosuppressive medications to achieve and stay in remission. Serum albumin, Urine PC ratio, Hemoglobin, and Cholesterol were not significantly associated with development of ESRD.

Characteristics of children with remission vs ESRD

| Features at onset | Remission (n=30) | | ESRD (n=14) | | |
|---|---|---|---|---|---|
| | Mean | S.D | Mean | S.D | p-value |
| GFR at presentation | 123 | 38 | 81 | 38 | 0.002 |
| C1q Deposits | N=8 | | 0 | | 0.002 |
| % of Global Glomerulosclerosis | 7.8 | 8 | 26 | 27 | 0.04 |
| Urinary P/C ratio | 4.9 | 6.3 | 8 | 10 | 0.23 |
| Normal blood pressure | n=23 | | n=9 | | 0.21 |

We conclude that presence of C1q deposits is associated with better prognosis and requires lesser immunosupression. This group of patients could represent a different variant of FSGS.These features may assist in guiding the type and intensity of initial immunosuppressive and/or vasoactive therapy.

Disclosure of Financial Relationships: nothing to disclose

**PUB594**

**The Renoprotective Effect of Podocyte-Targeted HO-1 Expression Is Unsustainable Following Glomerular Immune Injury** Pu Duann,[1] Ling-Mei Chiang,[2] Elias A. Lianos.[1] *[1]Medicine, Robert Wood Johnson Med School, New Brunswick, NJ; [2]Pediatrics, Chang-Gung Memorial Hospital, Keelung, Taiwan.*

Induction of heme oxygenase (HO)-1 is a key defense mechanism against oxidative stress. Compared with tubules, glomeruli are refractory to HO-1 upregulation in response to injury. This can be a disadvantage as it may be associated with insufficient production of cytoprotective heme-degradation metabolites (biliverdin, CO). We, therefore, explored whether targeted HO-1 expression in glomeruli can reduce proteinuria in immune injury induced by an anti-glomerular basement membrane (GBM) antibody (Ab). We employed a 4.125-kb fragment of a mouse nephrin promoter downstream to which a FLAG-tagged human (h)HO-1 cDNA sequence was inserted and generated transgenic mice from the FVB/N parental strain, in which immunolocalization (using anti-FLAG antiboby) of the transprotein (FLAG-hHO-1) in glomerular epithelial cells (GEC) was validated. Urine protein (Up) factored by urine creatinine (Uc) excretion in transgenic (Tg) mice with anti-GBM Ab injury assessed on days 3 and 6 of injury was significantly lower compared with wild-type (Wt) controls. Up/Uc on day 3: Tg 2.62 ±1.41, Wt 5.48 ±2.56, (p=0.02). Day 6: Tg 3.43 ± 1.94, Wt 8.34 ± 4.76. (p= 0.019). This antiproteinuric effect dissipated at later time points. Up/Uc on day 9: Tg 41.51 ± 68.71, Wt 41.60 ± 58.2. Day 12: Tg 67.58 ± 38.71, Wt 71.60 ± 48.9 (p= 0.98). To explore the mechanism of this non-sustainable salutary effect, we explored changes in levels of the FLAG-hHO1 transprotein in the course of immune injury. Western blot analysis of FLAG-hHO1 in protein lysates coupled with densitometry revealed a progressive decrease in transprotein levels. Compared to levels prior anti-GBM treatment, FLAG-hHO1 was reduced to 62% on day 2, 23% on day 3 and became undetectable on days 6, 9 and 12. We conclude that the unsustainable salutary effect of GEC-targeted HO-1 expression is due to degradation of the transprotein, which occurs via an as yet unknown mechanism.

Disclosure of Financial Relationships: nothing to disclose

**PUB595**

**Glucocorticoid Action in Podocytes Is Modulated by ABCB1 and Can Induce ABCB1 Activity** Tad Eichler,[1] Danica Petrovic-Djergovic,[1] Seetharamaih Chittiprol,[1] Richard F. Ransom.[1,2] *[1]Center for Clinical and Translational Research, The Research Institute at Nationwide Childrens Hospital, Columbus, OH; [2]Pediatrics, The Ohio State University School of Medicine, Columbus, OH.*

The primary treatment for nephrotic syndrome is oral glucocorticoids (GC), and we have previously reported that GCs can both protect from and ameliorate podocyte injury. GC are substrates for the ABC family of multiple drug resistance efflux pumps, primarily ABCB1. We previously found that ABCB1 is expressed in podocytes both *in vitro* and *in vivo*. In order to demonstrate a functional role for ABCB1 in GC action against nephrosis *in vivo*, we induced experimental nephrotic syndrome in rats puromycin aminonucleoside (PAN) injection. Separate groups of rats were injected daily with the ABCB1 inhibitor verapamil (VER), with the synthetic GC, methylprednisolone (MP), or with VER and then MP (VER+MP). Proteinuria was measured in 24 hr urine by the Bradford assay. The amount of protein in 24 h urine was similar in PAN, VER, MP groups at both 10 (372 ± 9 mg/24 h) and 12 days (289 ± 83 mg/24 h) after PAN injection, while proteinuria in VER+MP was dramatically reduced (97 and 44 mg/24 h at 10 and 12 d). The injurious effect of PAN on cultured podocytes was slightly reduced by low doses of either VER or the synthetic GC, dexamethasone (DEX), while a synergistic protective effect was evident in cells treated with VER+DEX. We further hypothesized that GC can increase the expression or activity of podocyte ABCB1, which may be a mechanism of induced steroid resistance in nephrotic syndrome. We found that the both the quantity and activity of ABCB1 protein increased (131% and 157% of controls) in cultured human podocytes increased after DEX treatment. In addition, the mRNA expression of the GC-regulated genes, FKBP5 and TSC22D3, was increased in a dose-dependent manner by DEX, and this induction was significantly greater in podocytes pre-treated with VER. These results suggest a significant role for ABCB1 in both the therapeutic action of GC in nephrotic syndrome and in the development of steroid resistance via enhancement of podocyte ABCB1 activity.

Disclosure of Financial Relationships: nothing to disclose

**PUB596**

**Focal Segmental Glomerulosclerosis Induced by Lithium Treatment** Vince Faridani,[1,2] Csaba P. Kovesdy.[2,3] *[1]Cariolion Clinic, Roanoke, VA; [2]Salem VA Medical Center, Salem, VA; [3]University of Virginia, Charlottesville, VA.*

Lithium carbonate (Li) is a medication commonly used to treat bipolar affective disorder. The drug is known to produce a number of renal side effects, including nephrogenic diabetes insipidus, chronic interstitial nephropathy, renal tubular acidosis and minimal change disease. We report a rare case of focal segmental glomerulosclerosis (FSGS) in the context of chronic Li use.

We describe a 52 year old male with a history of hypertension and bipolar disorder, who presented with a gradual elevation in his serum creatinine and nephrotic range proteinuria. His medications included clonazepam, diltiazem, lamotrigine, paroxetine, simvastatin and lithium carbonate for the last 15 years. Physical examination was unremarkable. Initial laboratory workup showed serum creatinine 2.0 mg/dl (eGFR 35), blood cholesterol 264, triglycerides 460, HDL 42, LDL 144. Urine analysis showed a specific gravity of 1.004, large blood on dipstick, 30 mg/dl protein, pH of 5.5 and sediment showing 1-3 red blood cells, spot urine protein-creatinine ratio indicated 3 grams of protein/24hrs. Renal ultrasound showed normal sized kidneys and multiple small simple cysts. A kidney biopsy was performed which revealed FSGS and moderate patchy interstitial fibrosis.

FSGS associated with chronic Li exposure has only been described in 3 previous pediatric case reports. In our patient the histological finding of FSGS accompanied by limited foot processes effacement, glomerulomegaly and proteinuria without hypoalbuminemia suggests secondary FSGS, possibly related to Li exposure. It is unclear how lithium could induce FSGS. It has been theorized that lithium could interact with anionic sites of the glomerular capillaries known to limit the passage of molecules and thus cause proteinuria. Cessation of lithium can facilitate the resolution of nephrosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB597**

**Puromycin Aminonucleoside Increases Podocyte Intercellular Permeability Via Oxidative Stress** Tae-Sun Ha. *Pediatrics, Chungbuk National University, Cheongju, Chungbuk, Korea.*

Puromycin aminonucleoside (PAN)-induced nephrosis is a well-described model of human idiopathic nephrotic syndrome because PAN injection into rats results in increased glomerular permeability with the characteristic ultrastructural changes in glomerular epithelial cells (GEpC; podocytes) similar to human nephrosis. To investigate the role of zonula occludens (ZO)-1 and oxidative stress on PAN-induced podocyte phenotypical changes and hyperpermeability *in vitro*, we cultured rat GEpCs in media containing various concentrations of PAN. Morphological assessment revealed that *in vitro* PAN induced not only the ultrastructural changes of GEpC, such as shortening and fusion of microvilli, but also separation of the intercellular gaps and ZO-1 and polymerization of F-actin resulting in increased intercellular permeability. Oxidative stress level after PAN treatment was markedly higher than that of basal level. PAN induced the inner cytoplasmic translocation of ZO-1 protein and also reduced ZO-1 protein amount and mRNA expression in a dose-dependent manner. These phenotypical changes of podocyte caused by PAN were prevented by the antioxidative effect of vitamin C. Our results demonstrated that the glomerular hyperpermeability caused by intercellular ZO-1 disturbances via oxidative stress would be the mechanism of proteinuria in experimental PAN-induced nephrosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB598**

**Circulating Galactose-Deficient IgA1 in IgA Nephropathy: Findings from a Large Case-Control Cohort from North China** Ping Hou,[1,3] Krzysztof Kiryluk,[1] Yifu Li,[1] Zina Moldoveanu,[2] Bruce A. Julian,[2] Jan Novak,[2] Ali G. Gharavi,[1] Hong Zhang.[3] *[1]Medicine, Div. of Nephrology, Columbia University, NY; [2]Medicine and Microbiology, University of Alabama at Birmingham, AL; [3]Nephrology, Peking University First Hospital, Beijing, China.*

Gal-deficient IgA1 (Gd-IgA1) is a key pathogenic factor in development of IgA nephropathy (IgAN). The aims of this study were to evaluate levels of circulatory Gd-IgA1 as a predictor of IgAN and examine their clinical correlates.

We measured plasma Gd-IgA1 using HAA lectin-based ELISA in a large case-control cohort from Beijing (496 IgAN cases and 499 healthy controls). We calculated the ROC curve and estimated the sensitivity and specificity of Gd-IgA1 in the diagnosis of IgAN. In addition, we correlated high Gd-IgA1 levels with clinical characteristics of our patients.

Among cases, 52% were males; 89% had microscopic and 28% gross hematuria, 75% had mild (0.3-3 g/24h) and 20% severe (>3 g/day) proteinuria, 49% had hypertension on presentation. Average age was 32.5 years (+/-11.2) and eGFR was 83.5 +/- 33.9 ml/min/1.73 m2 at the time of biopsy. Plasma Gd-IgA1 level was significantly elevated in IgAN patients compared to controls (P=5.0x10-78); this difference was robust to adjustments for age and gender. The area under the ROC curve was 0.85 (95%CI: 0.83-0.88, p =1.9x10-82), with sensitivity and specificity of 74% and 81%, respectively. Among cases, high Gd-IgA1 levels were associated with a greater degree of renal dysfunction, as reflected by a significant inverse correlation of Gd-IgA1 with eGFR at biopsy (r=-0.2, P=9.9 x 10-6). On average, CKD stage IV-V patients had higher plasma Gd-IgA1 compared to patients with CKD stage I-III (P = 5.4 x 10-9). In addition, Gd-IgA1 level positively correlated with systolic BP (r=0.2, p=1.2x10-3) and diastolic BP (r=0.1, p=0.025). In contrast, associations of serum total IgA and IgA1 with either eGFR or BP were not significant. Gd-IgA1 level did not correlate with proteinuria or hematuria.

In summary, this is the largest study to date demonstrating that plasma levels of Gd-IgA1 are important predictors of IgAN and strongly correlate with the degree of renal dysfunction.

Disclosure of Financial Relationships: nothing to disclose

**PUB599**

**Is Low Birth Weight a Signal for Podocyte Damage and a Risk Factor for Secondary Focal Segmental Glomerulosclerosis?** Yohei Ikezumi,[1] Toshiaki Suzuki,[1] Tamaki Karasawa,[1] Hiroya Hasegawa,[1] Hiroshi Kawachi,[2] Hiroko Nishimura,[3] Makoto Uchiyama.[1] *[1]Department of Pediatrics, Niigata University Medical and Dental Hospital; [2]Department of Cell Biology; [3]Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata-City, Japan.*

**Background:** Recent studies show an increased incidence of focal segmental glomerulosclerosis (FSGS) and that steroid-sensitive FSGS is often associated with enlarged glomeruli and a reduced number of nephrons. We examined in children whether secondary FSGS also shows these traits and whether low birth weight (LBW) may be a signal/risk factor for early FSGS.

**Materials and Methods:** We examined the birth weight of patients (n = 206) who received renal biopsy in our institute between 1993-2009. Fifteen patients were diagnosed with secondary FSGS, and 5 of these (33.3%, significantly higher than the overall LBW rate in Japan, 9.7%) had a record of LBW. We used PAS and/or PAM-stained paraffin-embedded tissues to quantify the glomerular cells, with separation of cells in the endocapillary, mesangial, and extracapillary (podocyte) areas. Cells were counted in at least six full-shaped glomeruli (visible vascular and urinary poles) without sclerotic lesion. Twelve biopsy specimens from age-matched non-FSGS patients (minimal pathology) with normal birth weight (NBW) were used as control.

**Results:** Glomeruli were significantly larger in LBW-FSGS vs. control (91% larger; $P < 0.001$) or vs. NBW-FSGS patients (75%; $P < 0.001$). The number of mesangial area cells was significantly lower (25%) in LBW-FSGS vs. NBW-FSGS ($P < 0.05$). The number of podocytes was 70% lower in LBW-FSGS vs. controls ($P < 0.001$) and 62% lower vs. NBW-FSGS ($P < 0.001$). NBW-FSGS patients vs. controls also showed 23% fewer podocytes ($P < 0.05$).

**Conclusion:** These results show that 1) LBW may correlate with development of early FSGS; and 2) in LBW-FSGS kidneys, there were fewer podocytes whereas the glomeruli were enlarged, suggesting that LBW (and prematurity) may predispose children to podocyte loss and to secondary FSGS. LBW may be deemed a risk factor and a means of its early discovery. (Supported by JSPS KAKENHI 22591177 and 21390307)

Disclosure of Financial Relationships: nothing to disclose

**PUB600**

**Glomerular Volume and Mesangial C4d Deposition on IgA Nephropathy: Markers of Higher Risk of Progression to Renal Failure** Monica X. Inofuentes, Bernardo Moguel, Virgilia Soto, Maria Carmen Avila-Casado. *Nephropathology, Instituto Nacional de Cardiologia, Mexico City, Mexico.*

IgA nephropathy (IgAN) is the most frequent glomerulopathy worldwide has a wide spectrum of clinical presentation, and a similarly range of hystopathological findings.

Several previous studies had identified clinical and/or histopathological prognostic factors in IgAN, but the significance of these factors still controversial.

**AIMS.**

The aim of this study was to identify histopathological indicators of progression to ESRD, by means mesangial C4d deposition and glomerular volume (GV).

**METHODS.**

This retrospective cohort study included patients with IgAN who underwent renal biopsy at our center from January 2004 to December 2007. C4d was marked by immunohistochemistry in paraffin embedded tissue, then cut off into 3- to 4μm sections; and stained with PAS. Evaluation of glomerular volume were done with digital video camera coll-Snap with image analyzer Vx51

**RESULTS.**

Thirteen consecutive renal biopsies from clinical diagnosis of haematuria/proteinuria (mean age 38 ±10: male 69%/ female 31%) were included; 70% had mesangial C4d positive. Mean GV was $8.86\mu m^3 x 10^6 \pm 4\ \mu m^3$. .C4d positive, were associated with an increased in serum uric acid *(p=0.02)*, systolic blood pressure (*p=0.001)* and proteinuria (*p=0.04)* the group with an increased GV (47%) were associated with systolic hypertension (*p=0.05)*, hyperuricemia (*p=0.003) and* increased in serum creatinine (*p=0.03)*.

**CONCLUSION.**

C4d positive and increased GV are associated with progression of renal damage in IgAN. Such factors could be relevant in naturally occurring in IgAN.

Disclosure of Financial Relationships: nothing to disclose

**PUB601**

**Expression of Phosphorylated (p) PKCε in Experimental Crescentic Glomerulonephritis (CGN)** Vassiliki N. Karavana,[1] Elias A. Lianos.[1,2] *[1]Medicine and Thorax Foundation, University of Athens, Athens, Greece; [2]Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ.*

PKCε, a DAG-dependent, $Ca^{2+}$- independent kinase attenuates extent of fibrosis following tissue injury, suppresses apoptosis and promotes cell quiescence. In CGN, glomerular epithelial cells (GEC) contribute to fibro-cellular crescent formation while they also transdifferentiate to a mesenchymal phenotype. To explore whether activated PKCε is expressed in these lesions, we employed a rat model of CGN. Administration of rabbit antibody against rat glomerular basement membrane (anti-GBM) in rats pre-immunized with rabbit Ig resulted in extensive CGN by day 10-12 and heavy proteinuria. Controls included animals that received: a) no anti-GBM Ab, and b) Puromycin Aminonucleoside (PAN) (15mg/100g) to cause non-immune GEC injury associated with comparable degree of proteinuria. Phosphorylation at $Ser^{729}$ of PKCε is critical for kinase activation and cellular compartmentalization. Using an antibody against PKCε phosphorylated (p) at $Ser^{729}$, we assessed immunolocalization of the kinase on days 10-12 of CGN. In glomeruli of control animals, pPKCε was undetectable. In glomeruli of animals with CGN, pPKCε was expressed exclusively in GEC. Marked pPKCε expression was also present in cells comprising fibrocellular crescents, which had acquired a mesenchymal phenotype (negative for GEC markers, positive for α-smooth muscle actin). In renal tubules, identified using lectin staining, there was weak to absent pPKCε expression in proximal tubular cells. In contrast, there was strong expression in distal convoluted tubules and in medulary and cortical collecting tubules. These were surrounded by inflammatory infiltrates and fibrosis. pPKCε localization in glomeruli following PAN was non specific. We conclude that pPKCε expression in GEC and crescentic lesions is specific for immune-mediated injury and may provide a defense mechanism against progression.

Disclosure of Financial Relationships: nothing to disclose

**PUB602**

**Novel Expression of Claudin-5 in Glomerular Podocytes** Ryo Koda,[1,2] Linning Zhao,[1] Eishin Yaoita,[1] Yutaka Yoshida,[1] Sachiko Tsukita,[3] Atsushi Tamura,[3] Masaaki Nameta,[4] Ichiei Narita,[2] Tadashi Yamamoto.[1] *[1]Department of Structural Pathology, Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; [2]Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; [3]Laboratory of Biological Science, Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University, Osaka, Japan; [4]Cooperative Laboratory of Electron Microscopy, Niigata University, Niigata, Japan.*

Tight junctions are the main intercellular junctions of podocytes of the renal glomerulus under nephrotic conditions. Their requisite components, claudins, still remain to be clarified. We have measured the mRNA levels of claudin subtypes by quantitative RT-PCR using rat isolated glomeruli. Claudin-5 was found to be expressed most abundantly in glomeruli. Mass spectrometric analysis of membrane fractions from isolated glomeruli also confirmed only a predominant expression of claudin-5 without any detection of other claudin subtypes. In situ hybridization and immunolocalization studies revealed that claudin-5 was localized mainly in glomeruli where podocytes were the only cells expressing claudin-5. Claudin-5 protein was observed on the entire surface of podocytes including apical and basal domains of the plasma membrane in the normal condition, and was inclined to be concentrated on tight junctions in puromycin aminonucleoside nephrosis. Total protein levels of claudin-5 protein in isolated glomeruli were not significantly upregulated in the nephrosis. These findings suggest that claudin-5 is a main claudin expressed in podocytes and that the formation of tight junctions in the nephrosis may be due to local recruitment of claudin-5 rather than due to total upregulation of the claudin protein levels.

Disclosure of Financial Relationships: nothing to disclose

**PUB603**

**Molecular Mechanism for Angiotensin II Induced Proteinuria** Eva Koenigshausen, Magdalena Woznowski, Ivo Quack, Sebastian A. Potthoff, Lars C. Rump, Lorenz Sellin. *Department of Nephrology, Heinrich Heine University, University Hospital, Duesseldorf, Germany.*

**Introduction**

Microalbuminuria serves as an early marker for glomerular injury in hypertensive and diabetic patients. Inhibitors of the renin-angiotensin-aldosterone system but not calcium channel blockers reduce albuminuria in these patients. Albuminuria results from a defect in the glomerular filter that is composed of endothelium, basal membrane and podocytes with slit diaphragms. A major component of the glomerular slit diaphragm is nephrin, that is endocytosed upon bindung to the adaptor protein β-arrestin2.

**Methods**

Cells expressing the AT1-receptor or its mutant D125AR126L, nephrin and β-arrestin2 were stimulated with Angiotensin II (Ang II). After cell lysis, co-immunoprecipitation with subsequent westernblot analysis was performed. For the inhibitor studies, cells were pretreated with the AT1-inhibitors 60 min before stimulation with Ang II. The effect of Ang II on the β-arrestin2 binding motif was studied by using two nephrin mutants. For the endocytosis assay, cells were stimulated with Ang II and incubated with biotin before cell lysis.

**Results**

Ang II stimulation increases the protein interaction between nephrin and β-arrestin2. This Ang II effect is dependent on the AT1-receptor and can be inhibited by AT1-receptor blockers. The G-protein signalling is essential for the Ang II effect, as the AT1-receptor mutant D125AR126L abolishes all G-protein signalling and inhibits the Ang II mediated increase of the nephrin β-arrestin2 interaction. Phosphorylation of T1120 and T1125 of the nephrin C-terminus is essential for the binding of β-arrestin2 even after stimulation with Ang II. Stimulation with Ang II increases endocytosis of nephrin.

**Conclusion**

Ang II weakens the integrity of the slit diaphragm through increase of nephrin endocytosis and is perceived to promote proteinuria. This previously unknown molecular effect of Ang II could help to understand the molecular mechanisms of Ang II induced proteinuria beyond hemodynamic effects.

Disclosure of Financial Relationships: nothing to disclose

**PUB604**

**Angiotensinogen Copies Have Inverse Relationship with Podocyte Nephrin Expression** Dileep Kumar, Hersh Goel, Divya Salhan, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.*

Nephrin is reported to be an important constituent of slit diaphragm. It is widely believed that slit diaphragm acts as a glomerular filtration barrier for the filtration of mid and large molecular proteins. Loss of nephrin has been reported to contribute to the development of glomerular proteinuria in several models of chronic kidney diseases. We have previously demonstrated that chronic Ang II infusion in rats attenuated nephrin expression by podocytes (Am J Nephrol 28:500-507, 2008). Moreover, Ang II in *in vitro* studies promoted podocyte apoptosis (Am J Physiol 283:F173-80, 2002). In the present study we evaluated the effect of angiotensinogen (*Agt)* copies on podocyte expression of nephrin and associated renal injury.

Four months old FVBN mice with variable copies of *Agt* (*Agt*-2, *Agt*-3, *Agt*-4) were evaluated for their blood pressure levels, degree of proteinuria, blood urea nitrogen, serum Ang II levels. Kidneys were harvested and prepared for renal histology and immunohistochemical studies for nephrin expression, apoptosis and oxidative stress.

*Agt*-4 mice showed higher (P<0.01) levels of blood pressure when compared with *Agt*-2 mice. *Agt*-4 mice also showed an increase mean urinary protein-creatinine ratio when compared to *Agt*-2 mice. *Agt*-4 mice showed segmental glomerulosclerosis (15 ± 2.5% glomeruli) and tubulointerstitial fibrosis; whereas, *Agt*-2 mice did not show any overt renal lesions. *Agt*-4 mice showed enhanced renal tissue expression of oxidative stress and increased number of apoptotic cells when compared with *Agt*-2 mice. Interestingly, *Agt*-4 mice showed attenuated glomerular expression of nephrin when compared with *Agt*-2 mice. Mice with 3 *Agt* copies showed only mild attenuation of glomerular expression of nephrin. These studies suggest that higher *Agt* copies are associated with proteinuria and development of sclerosis by attenuating nephrin expression.

Disclosure of Financial Relationships: nothing to disclose

**PUB605**

**Glomerulotubular Interaction in IgA Nephropathy Induces Apoptosis of Tubular Epithelial Cells: Role of Crosstalk between Angiotensin II and Aldosterone** Joseph C. K. Leung, Loretta Y. Y. Chan, Sydney C. W. Tang, Kar Neng Lai. *Department of Medicine, The University of Hong Kong, Pokfulam, Hong Kong.*

Glomerulotubular crosstalk network is pivotal in inducing tubulointerstitial damage in IgA nephropathy (IgAN). We had previously shown that polymeric IgA1 (pIgA1) from IgAN patients activated the renin-angiotensin system (RAS) in mesangial cells and up-regulated the inflammatory cascade in proximal tubular epithelial cells (PTEC). We further examined the role of aldosterone (Aldo) in tubular injury.

PTEC expresses only the mineralocorticoid receptor (MR), but not 11beta-hydroxysteroid dehydrogenase type II or aldosterone synthesis, suggesting the lack of aldosterone synthesis by PTEC. Apoptosis, measured by cleaved PARP expression and caspase 3 activity, was induced in PTEC activated by conditioned medium prepared from mesangial cells (HMC) cultured with pIgA1 from IgAN patients (n=35) but not from normal subjects (n=32). Exogenous angiotensin II (AngII) and Aldo, but not purified pIgA1 protein, induced PTEC apoptosis in dose- and time-dependent manner. Significant increased expression of NADPH oxidase and intracellular reactive oxidative species (ROS) formation were also detected in PTEC cultured with AngII, Aldo or conditioned media prepared from IgAN patients. Pre-incubation of PTEC with AngII receptor subtype-II (AT2R) antagonist (PD123319) or MR antagonist (eplerenone), but not AT1R antagonist (losartan), partially suppressed the pIgA1-conditioned media induced ROS generation (25%) and apoptotic events (< 35%). Combined blockade with PD123319 and eplerenone achieved more than 95% inhibition of ROS generation and apoptosis. Interestingly, AngII and pIgA1 conditioned media, but not Aldo, up-regulated the expression of AT2R and MR in PTEC. Pre-incubated PTEC with PD123319, but not losartan or eplerone, abrogated these up-regulated AT2R and MR expression.

In conclusion, our in vitro data suggest that AngII and aldosterone, released by pIgA1 activated HMC, served as the mediators for glomerulotubular crosstalk in inducing apoptosis of PTEC through generation of ROS species. In addition, crosstalk between AngII and aldosterone could play a role in pIgA1 induced PTEC apoptosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB606**

**Ethanol Alters Expression of Cytochrome P450 (CYP) Isoforms in Podocytes** Ellen T. McCarthy,[1] Jianping Zhou,[2] Shuhua Wang,[1] Ryan Eckert,[1] Tarak Srivastava,[3] Ram Sharma,[2] Virginia J. Savin,[2] Mukut Sharma.[2] *[1]Kidney Institute, University of Kansas Medical Center, Kansas City, KS; [2]Kansas City VA Medical Center, Kansas City, MO; [3]Childrens Mercy Hospital, Kansas City, MO.*

Ethanol intake is associated with salutary effects on cardiovascular function as well as hypertension in larger doses. Likewise, 20-hydroxyeicosatetraenoic acid (20-HETE) has both anti- and pro-hypertensive effects. The effect of ethanol on podocyte expression of the CYP isoforms that make 20-HETE has not been described. We incubated immortalized podocytes with ethanol (1, 2, 5, 10 or 20 µl/ml) for 1, 8 or 24 hrs. We then examined expression of CYP4a12a, CYP4a12b and CYP4f13 using quantitative RT-PCR. Results were expresses as fold change over appropriate time controls. In preliminary studies we examined the effect of ethanol on podocyte cytoskeleton using fluorescence microscopy following actin staining. Ethanol (2, 5, 10 and 20 µl/ml) significantly increased expression of CYP4a12a in podocytes at 8 hr ($P$<0.05 vs control). The highest concentration of ethanol (20 µl/ml) caused a significant decrease in expression of CYP4a12a at 24 hr ($P$<0.02 vs control). Expression returned to baseline values by 24 hr at other concentrations. A similar pattern was seen in expression of CYP4a12b in podocytes, namely increased expression at 8 hr (5 and 10 µl/ml, $P$<0.01 vs control) and suppression by 20 µl/ml at 24 hr ($P$<0.001 vs control). Ethanol (20 µl/ml) increased expression of CYP4f13 in podocytes with a 2-fold increase being seen at 24 hr, though this did not reach statistical significance. Fluorescence microscopy showed marked derangement of the actin cytoskeleton after ethanol treatment (10 and 20 µl/ml) for 24 hr. In summary, we have shown that ethanol in meaningful concentrations induces podocyte expression of CYP450 isoforms capable of 20-HETE synthesis, namely CYP4a and 4f. We hypothesize that ethanol alters eicosanoid metabolism in glomerular cells, and that this effect varies with levels of ethanol exposure. The long-term effects of chronic ethanol exposure on podocytes is unknown. The effect of ethanol on eicosanoid metabolism in renal cells may in part explain the cardiovascular impact seen in humans.

Disclosure of Financial Relationships: nothing to disclose

**PUB607**

**The Cytoskeleton and Mechancial Properteis of Renal Glomerular Capillaries and Glomeruli** R. Tyler Miller,[1] Joel M. Henderson,[2] Leslie A. Bruggeman,[1] Jung Hee Suh,[4] Jeffrey H. Miner,[4] John R. Sedor,[1] Martin R. Pollak.[3] *[1]Medicine, CWRU/Metrohealth, Cleveland, OH; [2]Pathology, BU, Boston, MA; [3]Nephrology, Harvard/BIDMC, Boston, MA; [4]Medicine, Washinton Univ., St. Louis, MO.*

The mechanical properties of tissues and cells are important determinants of their differentiated state, function, and responses to injury, but are not well characterized or understood. Renal glomeruli in particular, primarily the capillary walls, are exposed to significant hemodynamic forces and must have mechanical properties that permit them to withstand and accommodate these forces. Understanding their mechanics is an important step towards understanding renal diseases characterized by abnormal expression or assembly of structural proteins and abnormal hemodynamics. We use atomic force microscopy (AFM) to measure the elastic properties of rat glomerular capillaries, and a newly developed technique, capillary micromechanics, to measure the elastic properties of whole glomeruli. The baseline Young's moduli of the capillary walls and intact glomeruli were approximately 2,500 Pa. Treatment with the actin-depolymerizing agents Cytochalasin D and Latrunculin B reduced the elastic moduli of capillaries and whole glomeruli by approximately 50%. Inhibition of non-smooth muscle myosin activcity reduced the elastic moduli by approximately 35%. Cytochalasin D and Latrunculin B reduced the G/F actin ratios of glomeruli, but did not disrupt their architecture. AFM studies of glomeruli from HIVAN and Col4a3-/- mice reveal that these glomeruli are significanly softer than controls at a time when pathology by light microscopy is minimal. Glomeruli have tightly controlled mechanical properties, those properties are dependent on the state of the actin cytoskeleton and non-muscle myosins, glomerular capillaries contribute significantly to the mechanical properties of whole glomerluli, and abnormal mechanical properteis of glomeruli may contibute to glomerular disease.

Disclosure of Financial Relationships: Scientific Advisor: Genzyme.

**PUB608**

**Novel Polarity Complex in Podocytes: Crumbs Homolog 2 and Its Interaction Partners** Masatoshi Nukui, Karl Tryggvason, Jaakko Patrakka. *Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden.*

Podocytes, glomerular visceral epithelial cells, are located on the outer part of the glomerular basement membrane found facing to the urinary space. They are highly polarized cells having extremely unique trait to maintain both a dynamic cell shape. Recent studies verify that conserved polarity protein complexes are fundamental regulators for podocyte morphology. These protein complexes include the Crumbs complex and the partitioning defective (PAR) complex.

Drosophila Crb is involved in the control of cell-cell adhesion and epithelial cell polarity. Crumbs homolog 2 (Crb2) is one of the three mammalian homologues of Drosophila Crb, and is a large transmembrane protein, whose function has been unknown. We confirmed that Crb2 is expressed mainly in the kidney and the brain by RT-PCR, and co-localizes with nephrin in podocyte foot processes by immunohistochemistry.

To investigate the function of Crb2, we started to look for interaction candidates of Crb2 by Y2H experiment using the intracellular part of Crb2 as a bait, and we found that Mmp5, Par6 and Lnx2 may interact with Crb2. Human kidney sections were stained with antibodies directed against these proteins, and the results confirmed that three candidates were present in podocyte foot processes. To confirm the interaction of Crb2, co-immunoprecipitation experiment was conducted. For further investigation, pull down experiment, using GST-Crb2 protein, accompanied with mass spectrometry analysis will be performed. Our result provides a new insight into protein complexes in podocyte, and shed new light on podocyte biology.

Disclosure of Financial Relationships: nothing to disclose

**PUB609**

**Immunomodulation of Experimental Nephrotic Syndrome Induced by PAN in Rats** Danica Petrovic-Djergovic,[1] Seetharamaih Chittiprol,[1] Richard F. Ransom.[1,2] *[1]Center for Clinical and Translational Research, The Research Institute at Nationwide Childrens Hospital, Columbus, OH; [2]Pediatrics, The Ohio State University School of Medicine, Columbus, OH.*

A variety of experimental evidence supports the association of minimal change nephrotic syndrome (MCNS) with atopy and a Th2 cytokine bias. The puromycin aminonucleoside (PAN) model of experimental MCNS in rats is thought to exert direct toxic effects on glomerular podocytes, though the responsiveness of the PAN model to glucocorticoid therapy suggests there may be an unappreciated immune contribution. We therefore measured cytokines in rats in order to examine a potential immunological contribution to the proteinuria induced by PAN. Blood was withdrawn from rats 10 days after PAN injection, and total protein was measured in 24 h urine collections. Cytokine levels were measure using a custom 14-plex Luminex xMAP assay (Millipore MAP Cytokine Panel) both in plasma and in supernatants of 2 d cultures of isolated peripheral blood mononuclear cells (PBMC) treated with LPS, PMA+ionomycin, or without stimulation. Consistent with an association between proteinuria and Th2 cytokines, the plasma concentration of IL-4 and IL-13 were greater at 10 days post-injection in PAN-treated rats than in controls (123 ± 46 vs. 51 ± 10 pg IL-4/ml and 244 ± 42 vs. 125 ± 34 pg IL-13/ml). However, the plasma concentration of interferon-γ, a Th1 cytokine, was also greater in PAN-treated rats than controls (292 ± 52 vs. 17 ± 12 pg IFNγ/ml). The mean plasma concentration of CCL5 (RANTES) was lower in PAN-treated animals than controls (14,500 ± 1830 vs. 25,600 ± 7260 pg CCL5/ml), while plasma concentrations of other cytokines were not significantly different. Surprisingly, less IL-4, IL-13, and IFNγ were released by un- and stimulated PBMC isolated from PAN-treated rats than from controls. In summary, these results suggest that the nephrosis induced in rats by PAN injection may have a previously-unappreciated immune component, though the decreased capacity of PBMC from PAN rats to release cytokines suggests an alternate source, possibly the kidney, for effector cytokines.

Disclosure of Financial Relationships: nothing to disclose

**PUB610**

**Are Circulating Fibrocytes an Indicator of Disease Severity in Proliferative Glomerulonephritis?** Christine M. Ribic, Limin Liu, Catherine M. Clase, Peter Margetts. *Division of Nephrology, McMaster University, Hamilton, ON, Canada.*

**Background:** Proliferative glomerulonephritis (GN) can be complicated by progression to end stage renal disease associated with interstitial fibrosis. Recent studies have postulated that circulating bone marrow derived progenitor cells called fibrocytes are recruited to injured tissue and contribute to fibrinogenesis. We aimed to quantify circulating fibrocytes in patients with newly diagnosed proliferative GN, and to examine fibrocytes as a biomarker of disease activity and prognosis. **Methods:** We recruited 11 patients in a single centre with proliferative GN undergoing renal biopsy and 6 volunteer healthy controls. Fibrocytes were defined as cells positive for CD45 and collagen-1 by flow cytometry and were quantified in enrolled patients and controls. The samples were run in replicates and the results with the most consistent control IgG staining were used. **Results:** Of the enrolled patients, 3 were women, mean age 58 (standard deviation [SD] 19) years; 8 had anti-neutrophil cytoplasmic antibody positive vasculitis, 1 had lupus, 1 had IgA nephropathy and 1 had immune-complex mediated GN. Renal biopsy confirmed proliferative GN in all cases. Median serum creatinine was 478 (SD 334) mmol/L. Fibrocytes were 4.9% of peripheral white cells in cases and 1.9% in controls (P = 0.36); absolute fibrocyte concentrations were 0.72 $x10^9$ cells/L in cases and 0.14 $x10^9$ cells/L in controls (P = 0.15). There was a significant association between serum creatinine and fibrocyte concentration (P = 0.03). There was also a significant correlation between follow-up serum creatinine and biopsy interstitial fibrosis score (P= 0.02). **Conclusion:** Our preliminary data show a 3-fold increase in circulating fibrocytes in patients with active proliferative GN compared with controls, and an association between fibrocytes and creatinine, that did reach statistical significance. Further work is needed to determine whether these suggestive findings indicate fibrocyte tissue injury and to develop the quanitification of fibrocytes as a biomarker for disease activity in active proliferative GN.

Disclosure of Financial Relationships: Research Funding: Astellas Pharmaceuticals.

**PUB611**

**Functional Consequences of NO Depletion on Glomerular Soluble Guanylyl Cyclase (sGC) and Protein Permeability** Mukut Sharma,[1] Ram Sharma,[1] Ellen T. McCarthy,[2] Virginia J. Savin,[1] Elias A. Lianos.[3] *[1]Nephrology Research, MBRF, KC VA Medical Center, Kansas City, MO; [2]Kidney Institute, KU Medical Center, Kansas City, KS; [3]Nephrology, UMDNJ-RWJMS, New Brunswick, NJ.*

In Chronic Kidney disease (CKD) circulating levels of asymmetric dimethylarginine (ADMA), a potent Nitric Oxide Synthase (NOS) inhibitor, increase. We have demonstrated that ADMA injures glomerular filtration barrier (GFB) by NO depletion thereby increasing superoxide ($O_2^{\cdot -}$) and decreasing cGMP. Further, GFB is protected by NO and cGMP, the ligand and product of sGC activation, respectively [KI 2005; AJP 2009]. NO-mediated activation of sGC requires reduced sGC heme iron ($Fe^{+2}$). However, NO depletion may affect GFB through additional mechanism(s). Presently we studied changes in albumin permeability ($P_{alb}$) in isolated rat glomeruli to determine whether ADMA injures GFB by altering the redox state of sGC heme iron. Glomeruli were incubated for 15 min at 37°C with ADMA at concentrations found in CKD patients, 2) ODQ, a potent oxidizer of NO-sensitive sGC heme iron, 3) a combination of ADMA (5μM) and ODQ (5μM). Results (Table 1), show that ADMA+ODQ had an additive effect on $P_{alb}$. HMR-1766 (1-10μM), an NO- and heme-independent sGC activator that stimulates sGC only when sGC heme-iron is oxidized, dose-dependently reversed the effect of ADMA+ODQ on $P_{alb}$. ADMA-induced increase in $P_{alb}$ was reversible by DETA-NONOate (NO donor), Tempol ($O_2^{\cdot -}$scavenger) and by 8-Br-cGMP (data not shown). Table 1.

| Group (n=15 each) | $P_{alb}$±SEM | Significance*P*value |
|---|---|---|
| Control | 0.005±0.09 | |
| ADMA 5μM | 0.65±0.05 | 0.001*vs.*Control |
| ODQ 5μM | 0.5±0.1 | 0.001*vs.*Control |
| ADMA 5μM+ODQ 5μM | 0.81±0.075 | 0.001*vs.*Control |
| ADMA 5μM+ODQ 5μM+HMR 1μM | 0.70±0.098 | |
| ADMA 5μM+ODQ 5μM+HMR 5μM | 0.51±0.056 | 0.001*vs.*ADMA+ODQ |
| ADMA 5μM+ODQ 5μM+HMR 10μM | 0.14±0.087 | 0.001*vs.*ADMA+ODQ |

We conclude that ADMA increases $P_{alb}$ by causing NO depletion. sGC heme iron oxidation, owing to the consequent $O_2^{\cdot -}$ excess, only partially accounts for this increase.

Disclosure of Financial Relationships: nothing to disclose

**PUB612**

**Glomerular Protective 8,9-Epoxyeicosatrienoic Acid (8,9-EET) Is Not Metabolized by Soluble Epoxide Hydrolase (sEH) in Mouse Podocytes** Mukut Sharma,[1,3] Jianping Zhou,[1] Ram Sharma,[1,3] Virginia J. Savin,[1,3] Tarak Srivastava,[2] Ellen T. McCarthy.[3] *[1]Nephrology Research, KC VA Medical Center, Kansas City, MO; [2]Nephrology, Children's Mercy Hospital UMKC, Kansas City, MO; [3]Kidney Institute, KU Medical Center, Kansas City, KS.*

Preservation of podocyte structure and function in the glomerular filtration barrier is critical for the treatment and prevention of proteinuria. Four EET regioisomers namely, 5,6-, 8,9-, 11,12- and 14-15-EET, mediate several biological processes in an autocrine/paracrine manner. We have recently discovered a unique protective effect of 8,9-EET in the glomerular filtration barrier. Since EETs are readily metabolized to biologically active dihydroxyeicosatrienoic acids (DiHETrE) by soluble epoxide hydrolase (sEH), it is not clear whether 8,9-EET or its vicinal diol 8,9-DiHETrE is responsible for the observed glomerular protection. We compared the effect of 8,9-EET (100 nM) and 8,9-DiHETrE (50-200nM) on glomerular albumin permeability *in vitro* ($P_{alb}$). We used the circulating permeability factor (FSPF) in FSGS patient plasma (20μL) to cause glomerular injury indicated by increased $P_{alb}$. Summary of Results (Table 1) shows that only 8,9-EET protected against FSPF.

| Table 1: Group | $P_{alb}$ ±SEM (n=15 each) | *P* value |
|---|---|---|
| Control | 0.005±0.07 | |
| 8,9-EET 100nM | -0.24±0.12 | |
| 8,9-HETrE 100nM | 0.12±0.079 | |
| FSGS | 0.75±0.08 | 0.001*vs.* Control |
| 8,9-EET 100 nM+FSGS | 0.06±0.11 | 0.001*vs.* FSGS |
| 8,9-HETrE 200nM+FSGS | 0.5±0.07 | 0.1 *vs.* FSGS |

We next examined the expression of sEH protein in differentiated mouse podocytes and isolated rat glomeruli using immunoblotting. When compared with the renal cortex, both podocytes and glomeruli showed negligible expression of sEH. Metabolism of 8,9-EET in glomeruli and podocytes appears to be different from that in tubules and the interstitium. We conclude that the glomerular protective effect of 8,9-EET is not due to 8,9-DiHETrE and that sEH is unlikely to contribute significantly to 8,9-EET metabolism in podocytes.

Disclosure of Financial Relationships: nothing to disclose

**PUB613**

**Coordinated Loss of Neuroglobin, Synaptopodin, and Nephrin in VHL-Deficient Podocytes** Brooke M. Steenhard, Kathryn S. Isom, Larysa Stroganova, Adrian T. Zelenchuk, Patricia St. John, Dale R. Abrahamson. *Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS.*

Neuroglobin (Ngb) is an ancient heme protein thought to bind or transport oxygen and/or reactive oxygen species. Discovered in brain, the function of Ngb is unclear, but a Ngb overexpressing transgenic mouse is protected from ischemia-induced injury to brain and heart. Ngb is expressed at either extremely low levels or absent in normal kidney. In isolated glomeruli from mice with a conditional deletion of von Hippel Lindau (VHL) in podocytes (Pod-Cre fVHL), Ngb is massively upregulated at both mRNA and protein levels. Glomeruli from Pod-Cre fVHL and wildtype littermates were isolated and assayed for other globin changes using quantitative real time RT-PCR. Compared to wildtype, cytoglobin (Cygb) mRNA levels are unchanged, but there is a small but significant decrease in alpha (Hba-a1) and beta (Hbb-b1) chains of hemoglobin in Pod-Cre fVHL glomeruli. Globin profiling was repeated in glomeruli from Alport mice lacking *Col4a3*, a component of the mature glomerular basement membrane. In contrast to Pod-Cre fVHL, there are no changes in Cygb, or Hbb-b1 in 4 week old Alport glomeruli, and Ngb is not upregulated (and only barely detectable). Ngb protein in Pod-Cre fVHL glomeruli is podocyte-restricted and overlaps with anti-synaptopodin and anti-nephrin labeling. Unlike Alport, where all glomeruli undergo rapid and diffuse fibrosis, kidneys from proteinuric Pod-Cre fVHL mice stained with Periodic acid-Schiff reveal a range of glomerular histology. Some glomeruli appear almost normal, with intense podocyte immunolabeling for Ngb, synaptopodin, and nephrin. Other glomeruli had degrees of fibrosis, with PAS-positive matrix in part or all of the glomerulus. Weak labeling for Ngb, synaptopodin and nephrin labeling are seen in less fibrotic regions, but all three markers are lost in areas of severe sclerosis. Our results suggest a possible reno-protective role for Ngb within Pod-Cre fVHL kidney.

Disclosure of Financial Relationships: nothing to disclose

**PUB614**

**Endoplasmic Reticulum (ER) Stress Precedes Podocyte Apoptosis at the Onset of Proteinuria in the db/db Mouse Model of Diabetic Nephropathy (DN)** Jianling Tao, Jie Ma, Hong Zhang, Jin Hong Li, Yubing Wen, Hang Li, Xuemei Li, Xue-Wang Li. *Division of Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences.*

Objective: Podocyte apoptosis was indicated as an early phenomenon of DN. ER stress induced podocyte apoptosis contributes to proteinuria in some animal models of podocyte injury. We hypothesized in db/db mouse model of DN, ER stress is involved in podocyte apoptosis coincidently with the onset of proteinuria.

Methods: Six, nine, twelve-week-old male C57BLKS/J db/db mice (n=7) and age-matched db/m control mice (n=5) were studied. Twenty-four hour urine protein output (24UP), blood free fatty acid(FFA), HbA1c, and fast blood glucose(FBG) were measured. The ratio of the mesangial area to glomerular area (M/G) was calculated. The glomerular apoptosis was assayed by TUNEL. The intensity of Glucose-regulated protein 78(GRP78) was co-localized with synaptopodin by immunofluorescence stain. Indicators of ER-associated apoptosis GRP78, C/EBP homologous protein (CHOP), Bcl-2 and Bax of renal cortical tissues were assayed by WB.

Results: The body weight, 24UP, blood FFA, HbA1c, and FBG of db/db mice were significantly higher than each age matched control($p<0.05$). The kidney weight was significantly higher in 12w db/db mice compared with 12w db/m mice. 24UP was significantly higher in 9w and 12w db/db mice compared with that of 6w db/db mice ($p<0.05$). M/G in 9w db/db mice was significantly higher than that of 9w db/m mice ($p<0.05$). There were no significant changes of M/G among three control groups. There were no significant changes in glomerular apoptosis detected either among three db/m or db/db mice groups or between db/m and db/db mice($p>0.05$). But confocal microscopy showed steadly increased expression of GRP78 in podocytes only in db/db mice with age increase. There were no significant changes of GRP78, CHOP, Bax and Bcl-2 protein in renal cortical tissues either between db/db with db/m groups or among db/db or db/m mice groups.

Conclusions: ER stress precedes podocyte apoptosis at the onset of proteinuria in db/db mouse model of DN.

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Disclosure of Financial Relationships: nothing to disclose

**PUB615**

**Involvement of Endoplasmic Reticulum (ER) Stress in Podocyte Apoptosis Induced by Saturated Fatty Acid Palmitate** Jianling Tao,[1] Xiong Zhong Ruan,[2] Hang Li,[1] Yubing Wen,[1] Xuemei Li,[1] Xue-Wang Li.[1] *[1]Division of Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China; [2]Centre for Nephrology, University College London Medical School, London, United Kingdom.*

Objective: Podocyte apoptosis was recently indicated as an early phenomenon of diabetic nephropathy.Pancreatic β-cells exposed to saturated free fatty acid paimitate undergo irreversible ER stress and consequent apoptosis, involving in the pathogenesis of insulin resistance. We hypothesized palmitate could induce podocyte apoptosis via ER stress.

Methods: Podocyte apoptosis was determined by DAPI stained apoptotic cell counting and TUNEL stain.The expressions of indicators of ER molecule chaperone and ER-associated apoptosis Glucose-regulated protein 78(GRP78),C/EBP homologous protein (CHOP),Bcl-2 and Bax were assayed by Western Blot and Real-time PCR.GRP78 and synaptopodin were co-localized by immunofluorescence stain.

Results: Palmitate significantly increased cultured murine podocytes TUNEL-positive cells and apoptotic cell percentage time-dependently when loading 0.5 mM (10h, 13h, and 15h compared with 0h, $p<0.05$) and dose-dependently when loading 0.25mM, 0.5 mM, 0.75 mM,and 1 mM of palmitate for 15h (compared with control, $p<0.05$). Palmitate time-dependently and dose-dependently increased the protein expression of GRP78,CHOP and BAX and decreased that of Bcl-2. Palmitate ranging from 0.25 mM to 1 mM loading for 12h significantly increased mRNA of GRP78,CHOP and BAX and decreased that of Bcl-2 compared with control($p<0.05$) with the maximum concentration in 0.75 mM. 0.5 mM of palmitate loading for 3h, 8h and 12h significantly increased mRNA of GRP78, CHOP and BAX and decreased that of Bcl-2 compared with 0h ($p<0.05$)with the maximum effect at 3h.Confocal microscopy demonstrated GRP78 expression was significantly increased when exposing to 0.5 mM of palmitate for 8h compared with control.

Conclusions: Palmitate could induce podocyte apoptosis via ER stress, suggesting podocyte apoptosis and consequent proteinuria caused by lipotoxic free fatty acid could be ameliorated by relief of ER stress.

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Disclosure of Financial Relationships: nothing to disclose

**PUB616**

**Silencing of Podocyte VEGF-A Induces Irreversible Glomerular Damage: Animal and Cellular Model** Delma Veron, Claudia A. Bertuccio, Pardeep Kumar Aggarwal, Alda Tufro. *Pediatrics, Yale University, New Haven, CT.*

Vascular endothelial factor-a (VEGF-A) is essential to the differentiation of endothelial cells and vascular morphogenesis. In the glomerulus, podocytes and endothelial cells express VEGF-A. Genetic deletion of VEGF-A in the endothelium leads to systemic endothelial degeneration, vascular thrombosis and swelling of glomerular endothelium, whereas VEGF-A knockout in podocytes caused thrombotic microangiopathy in mice. To study the mechanism involved in local injury induced by low glomerular VEGF-A we developed an inducible podocyte silencing VEGF-A mouse and cellular model.

We generated Tet-O-siVEGF transgenic mice by oocyte pronuclear injection of a Tet-On vector containing an shRNA targeting the first VEGF exon; their breeding with podocin-rtTA mice resulted double transgenic mice that silence VEGF-A in podocytes in a doxycycline-regulated manner.

Upon doxycycline induction for a week podocin-rtTA:tet-O-siVEGF mice decreased VEGF-A mRNA and VEGF-A protein levels to ~20% of single transgenic or non-induced controls, as determined by qPCR and ELISA in isolated glomeruli. Mice developed proteinuria, mesangial expansion, mesangiolysis, microaneurisms, and decreased glomerular volume. Glomerular ultrastructure evidenced endothelial cell swelling, GBM lamination, podocyte effacement and fusion. Both functional and structural damage was irreversible after removal of doxycycline.

In addition, we crossbred podocin-rtTA:tet-O-siVEGF mice with Immortomouse®, isolated and cloned conditionally immortalized podocytes, which expressed WT1, podocin, nephrin and CD2AP and silenced VEGF-A upon induction with doxycycline.

We conclude that both animal and cellular models are useful tools to study the pathophysiology and molecular mechanisms involved in the irreversible glomerular damage induced by low VEGF.

Disclosure of Financial Relationships: nothing to disclose

**PUB617**

**Sirolimus Inhibits Development of HIV-Associated Nephropathy (HIVAN) by Inhibiting Epithelial Mesanchymal Transposition** Iti Yadav, Anju Yadav, Madhuri Adabala, Dileep Kumar, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.*

HIV-associated nephropathy (HIVAN) is characterized by proliferative phenotype in the form of collapsing glomerulopathy and microcystic dilatation of tubules. Recently, epithelial mesenchymal transdifferetiation (EMT) of renal cells has been demonstrated to contribute to the pathogenesis of proliferative HIVAN phenotype (Am J Physiol. 298:F734-44, 2010). We hypothesize that sirolimus will not only inhibit renal cells EMT but will also attenuate manifestation of proliferative HIVAN phenotype.

Age (three weeks old) and sex matched HIVAN (Tg26) or control (FVB/N) mice in groups of six were administered ether normal saline (normal saline receiving Tg26 mice, TgNS; normal saline receiving control mice, CNS) or sirolimus (5.0 mg/Kg, intraperitoneal, every other day; sirolimus receiving Tg26 mice, TgS; sirolimus receiving control mice, CS) for either two weeks (protocol A) or eight weeks (Protocol B). At the end of the scheduled periods, blood and urine samples were collected; kidneys were harvested and processed for renal histology, immunohistochemical and mRNA studies.

TgNS showed enhanced ($P<0.01$) proliferation of both glomerular and tubular cells when compared to CNS in both protocols, A and B; on the other hand, TgS showed attenuated renal cell proliferation when compared with TgNS. TgNS also showed increased number of α-SMA-, vimentin-, and FSP1 + ive cells (glomerular as well as tubular) when compared with CNS (protocols A and B); however, TgS showed reduced number of α-SMA, vimentin, and FSP1 + ive renal cells when compared to TgNS. Renal tissues of TgNS showed enhanced expression of PCNA, α-SMA, and FSP1 by immunoblotting studies when compared with CNS. However, sirolimus attenuated renal tissue expression of PCNA, α-SMA, and FSP1 in Tg26 mice (both in protocol A and B). Interestingly, sirolimus preserved renal epithelial cell expression of E-cadherin in TgS. Since sirolimus also attenuated renal cell ZEB expression (a repressor of E-cadherin transcription), it appears that sirolimus may be attenuating renal cell EMT by preserving epithelial cell E-cadherin expression.

Disclosure of Financial Relationships: nothing to disclose

**PUB618**

**Role of Heparanase in the Pathogenesis of Minimal Change Nephrotic Syndrome Induced by Respiratory Syncytial Virus** Jinxiang Yu, Yuhong Tao, Zheng Wang. *Department of Pediatrics, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China.*

**Background:** Respiratory syncytial virus (RSV) is the most important triggering factor of minimal change nephrotic syndrome(MCNS).β-D-endoglycosidase heparanase is proposed to be important in the pathogenesis of proteinuria by selectively degrading the negatively charged side chains of heparan sulfate proteoglycans(HSPG) within the glomerular basement membrane(GBM).

**Objective:** to explore the role of heparanase in the pathogenesis of MCNS induced by RSV

**Methods:** SD rats were inoculated with $6\times10^6$PFU(plaque-forming unit) RSV, and killed on days 4,8,14,28,56 and 84 postinoculation ($RSV_4$, $RSV_8$, $RSV_{14}$,$RSV_{28}$, $RSV_{56}$ and $RSV_{84}$). The proteinuria and serum parameters were measured; renal histology was observed by light microscopy and transmission electron microscopy;RSV mRNA in the kidney and lung was confirmed by *in situ* hybridization. Anionic sites in GBM were quantitatively evaluated through polyethyleneimine(PEI) staining under transmission electron microscopy. Renal heparanase protein and mRNA expression level were determined by immunohistochemical staining and real-time quantitative reverse transcriptase polymerase chain reaction(real-time quantitative RT-PCR).

**Results:** After inoculation of RSV , the urinary protein increased, foot processes of glomerular epithelial cells were fusional and accompanied with hypoalbuminemia, particularly in rats inoculated with $6x10^6$PFU RSV on days 14-28 postinoculation when the changes of renal tissue under lightmicroscope and electron microscope (the fusion of foot processes were extensive) bear similarity to human MCNS essentially. Compared with those of normal control ,$RSV_{56}$ and $RSV_{84}$ groups, anionic sites in GBM significantly from $RSV_4$,

$RSV_8$, $RSV_{14}$ groups decreased ,and heparanase expression in $RSV_4$, $RSV_8$, $RSV_{14}$ group were elevated. There were a positive correlation between the expression level of heparanase and proteinuria, and a negative correlation between anionic sites and proteinuria.

**Conclusion:** Heparanase expression contributes to the pathogenesis of proteinuria in RSV nephropathy in rats.

Disclosure of Financial Relationships: nothing to disclose

**PUB619**

**Tacrolimus Reduces Nephrotic-Range Proteinuria of IgA Nephropathy Via Podocyte Actin Cytoskeleton Maintenance** Qingxian Zhang,[1,2,3] Li Zhu,[1,2,3] Sufang Shi,[1,2,3] Lijun Liu,[1,2,3] Jicheng Lv,[1,2,3] Hong Zhang,[1,2,3] Hai Yan Wang.[1,2,3] *[1]Renal Division, Peking University First Hospital; [2]Peking University Institute of Nephrology; [3]Key Laboratory of Renal Disease, Ministry of Health of China.*

**Background:** Recently, it was reported the Calcineurin (CaN) induced synaptopodin dephosphorylation, which could damage podocyte actin cytoskeleton and induce proteinuria, could be blocked by CaN inhibitor, cyclosporine A, in cultured podocyte and mice model. Our previous clinical study showed tacrolimus (FK506), another inhibitor of CaN, improved the proteinuria remission of steroids or steroids with cyclophosphamide / mycophenolate resistant nephrotic-range proteinuria in IgA nephropathy (IgAN) patients . We speculated FK506 reduced proteinuria in patients with IgAN by maintaining podocyte actin cytoskeleton through inhibiting CaN.

**Methods:** 12 IgAN patients with persistant nephrotic-range proteinuria after three to six months treatment with steroids or steroids plus cyclophosphamide/mycophenolate were administrated with FK506 at least for six months and their renal specimens were evaluated. Among them, 3 patients received repeated renal biopsy. 4 cases of peritumoral normal kidney tissue were used as normal control. Expression of CaN and synaptopodin were evaluated by immunohistochemical staining. In vitro, human podocyte were treated with puromycin aminonucleoside (PAN) with or without FK506. Integrity of podocyte actin cytoskeleton, expression of CaN and synaptopodin were detected after 48 hours treatment by Immunofluorescent staining.

**Results:** Compared to control, patients with IgAN showed increased expression of CaN and decreased expression of synaptopodin, which mainly displayed in podocyte. In those patients received repeated renal biopsy after complete proteinuria remission with FK506 therapy, the expression of CaN reduced and synaptopodin increased. In vitro, PAN induced podocytes showed F-actin lost, synaptopodin expressed weakly and disorderedly after 48 hours, and it could be partial recovered by application of FK506.

**Conclusion:** These results indicated that FK506 can maintain the integrity of podocyte actin cytoskeleton to reduce nephritic-range proteinuria in patients with IgAN.

Disclosure of Financial Relationships: nothing to disclose

**PUB620**

**Expression and Significance of Vascular Endothelial Growth Factor (VEGF) AND Its Receptor in Hepatitis B Virus-Associated Nephritis** Yongze Zhuang,[1] Xiaorong Zhong,[2] Yinghao Yu.[2] *[1]Nephrology, Fuzhou General Hospital, Nanjing Command PLA, Fuzhou, Fujian, China; [2]Pathology, Fuzhou General Hospital, Nanjing Command PLA, Fuzhou, Fujian, China.*

VEGF and its receptor may participate in the development and progression of chronic kidney diseases,but the role of VEGF and its receptor in HBV-associated nephropathy(HBV-GN)is unclear. The clinical and pathological data were retrospectively analyzed in 68 patients with HBV-GN diagnosed as by renal biopsy. The protein expression of VEGF,VEGF receptor 2(Flk-1) and thrombomodulin(TM)in renal biopsy specimens were determined by immunohistochemistry and tested by semi-quantitative method. VEGF and Flk-1 were weak or no expression in glomeruli of normal kidney tissues, while the expression of VEGF and Flk-1 were significantly increased in HBV-GN and significantly different among different pathological types. The expression of VEGF,Flk-1 and TM in sclerosing glomerulonephritis(SGN)group was significantly lower than those in other pathologic types. The expression of VEGF and Flk-1 were positively correlated in the degree of glomerular changes in the mild lesion group(57.35%), but was negatively correlated in the moderate and severe disease group; and that in sclerosis group (9cases,glomerular sclerosis rate>50%) were evidently decreased. 46cases(67.65%)of those patients had the renal vascular lesion, of which mild change in 21cases, moderate change in 17cases,severe lesion in 8cases. The expression of VEGF and Flk-1 in moderate and severe vascular lesions were significantly higher than those without vascular disease. The expression of VEGF and Flk-1 expression in 10 cases(eGFR <60ml/min)were significantly lower than other patients. The expression of VEGF and Flk-1 were positively correlated with the level of urine protein in the mild and moderate disease group. The percent of chronic renal insuffiiency in the group of VEGF<4points was obviously higher than 4-6 and >6points. In early lesions of HBV-GN, podocyte was injuried, promoting VEGF upregulation,which led to endothelial cell activation and Flk-1 upregulation and promote the formation of proteinuria; in late lesions,VEGF and its receptor were decreased, promoting glomerulosclerosis and progression of HBV-GN.

Disclosure of Financial Relationships: nothing to disclose

**PUB621**

**Clinical and Pathological Study on IgA Nephropathy with the Deposition of Hepatitis B Virus Antigens in Renal Tissue** Jian Chen,[1] Hongbin Zhong,[1] Yinghao Yu.[2] *[1]Nephrology, Dong Fang Hospital, Fuzhou, Fuzhou, Fujian, China; [2]Nephrology, Fuzhou General Hospital, Nanjing Command PLA, Fuzhou, Fujian, China.*

Objectives: To investigate the clinical and pathological characteristic, To evaluate whether there existed different subtypes of IgA nephropathy with the deposition of HBV antigens in renal tissue, and the pathological characteristics of it. Methods Retrospective study method was applied to analyze 120 IgA nephropathy accompanied by the deposition of HBV antigens and IgA nephropathy without deposition of HBV antigens. The control study was performed on clinical classification, histomorphology, immunological type, ultrastructure. The relevant factors were scored by semiquantitative analysis. Results Group A(37.5%) with massive proteinuria and nephrotic syndrome was significantly different from group B(19.17%)(P<0.05)and group A with haematuriu (7.5%) was significantly different from group B(19.17%)(P<0.05). Pathological Lee's classification of renal tissue:grade IV in group A(27.14%) was significantly higher than in group B(8.33%)(P<0.05),while grade II in group A(18.83%) was significantly lower than in group B(47.5%)(P<0.05). Immunohistochemical result: group A(60.0%) with IgA+IgG+IgM was significantly higher than group B(28.33%)(P<0.05);while group B(32.0%) with IgA+IgG was significantly higher than group A(10.83%)(P<0.05).The result of ultrastructure :51 cases of group A(42.5%) with Lesion of basement membrane was significantly more than only 3cases of group B(2.5%)(P<0.05);while 69 cases of group A(57.50%) with abnormality of foot cells was significantly more than 41 cases of group B(34.17%)(P<0.05);group A with much deposition under mesenterium, endothelium, basement membrane and epithelium was significantly more than group B(2.5%)(P<0.05). Conclusions Clinical presentation of IgA nephropathy with the deposition of HBV antigens in renal tissue is various. Massive proteinuria or nephrotic syndrome is the most common symptom, which pathological changes is more severity and type of IgA+IgG+IgM is more common. There are 42.5% cases companied with lesions of basement membrane.

Disclosure of Financial Relationships: nothing to disclose

**PUB622**

**Anti-C1q Antibodies: Association with Lupus Nephritis in Afrocaribbean Population in a French West Indies Island** Maryvonne R. Dueymes,[1] Christophe Deligny,[2] Raluca Ples,[3] Jean-Marc Roger Dueymes-Laporte.[4] *[1]Immunology Department, CHU, Fort de France, Martinique; [2]Internal Medicine Department, CHU, Fort de France, Martinique; [3]Anatomo-Pathology Laboratory, CHU, Fort de France, Martinique; [4]Nephrology Department, Centre Hospitalier, Lamentin, Martinique.*

Introduction

Renal involvement is a major complication of systemic lupus erythematosus (SLE) and is a strong determinant of morbidity and mortality. The prognosis of lupus nephritis indicate a need for identifying early biomarkers that predict nephritis development. Autoantibodies against C1q have been described in SLE as well as in other connective diseases. They have been considered as a marker of disease activity and presence of nephritis.

Objectives

Our aim was to compare titers of anti-C1q and anti-DNA in afrocaribbean SLE patients with (n=30) or without (n=30) subsequent biopsy-proven lupus nephritis.

Methods

Sera from 60 SLE patients based on the American college of Rheumatology (ACR) criteria, 20 rheumatoid arthritis without nephritis and 20 controls without auto-immune disease and nephritis were collected. Anti-C1q were detected by ELISA Kit (Bioadvance) and anti-DNA antibodies were measured by IF and ELISA (Phadia).

Results

Anti-C1q were detected in 24/30 patients (80%) with nephritis (in majority class IV and class V) and in 8/30 patients (28%) without renal disease. No anti-C1q were found in the two other groups, rheumatoid arthritis and controls. Anti-C1q titers were higher in SLE patients with nephritis than without (p<0,02). Anti-DNA were positive in SLE patients with nephritis (90%) and without nephritis (78%) (p=NS). Anti-C1q did not correlate with anti-DNA in all groups.

Conclusion

The study in Afrocaribbean SLE patients confirms previous finding in other populations the association of anti-C1q antibodies with nephritis and disease activity. Their presence or absence represent an non invasive biological marker in the follow up of SLE patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB623**

**Sunitinib Induced Nephrotic-Range Proteinuria** Stacy Z. Ker,[1] Jason I. Biederman,[1,2] Khaled M. Ismail,[1,2] Michael T. Keefe.[1,2] *[1]Botsford Hospital, Farmington Hills, MI; [2]Hypertension Nephrology Associates, PC, Livonia, MI.*

Presented are two cases, an 85 year old female and a 66 year old male with renal cell carcinoma (RCC) treated with nephrectomy followed by sunitinib for recurrence. Both patients had no proteinuria prior to sunitinib therapy. Nephrotic-range proteinuria of 11.6 g/g and 9.8 g/g was detected 21 months and 24 months, respectively, after starting sunitinib on a 4-week-on, 2-week-off cycle. Both patients were also noted to have uncontrolled blood pressures during the 4-week-on cycle of their treatment. Reduced estimated glomerular

filtration rate (eGFR) was also noted in both patients. Serum creatinine increased from a pre-sunitinib level of 2.1 mg/dL to 3.2 mg/dL 24 months post-therapy in the female and 1.9 mg/dL pre-sunitinib therapy to 4.16 mg/dL 25 months post-treatment in the male. Serologic work-up of nephrotic-range proteinuria was negative, with the exception of a weakly positive ANA in the female. Neither patient was diabetic and random glucose levels were normal. Sunitinib was not discontinued in either of the patients due to excellent treatment responses. Because of the poor long-term outcomes of patients with stage 4 RCC and presence of a solitary kidney, renal biopsy was not performed in either patient.

Proteinuria and hypertension are well known side effects of bevacizumab, a vascular endothelial growth factor (VEGF) inhibitor. Sunitinib, which inhibits multiple tyrosine kinase receptors, including VEGF receptors, was not shown to induce proteinuria in clinical trials. This phenomenon has only recently been described in animal models as a preeclampsia-like syndrome induced by sunitinib and a similar drug, sorafenib. Previous case reports of sunitinib induced renal failure with renal biopsy data have shown thrombotic microangiopathy but have presented with sub-nephrotic range proteinuria. Due to the poor long term survival of these patients and the paucity of biopsy data, we believe this clinical syndrome is under reported. Clinicians should monitor patients treated with the aforementioned drugs for hypertension, proteinuria and renal dysfunction.

Disclosure of Financial Relationships: nothing to disclose

**PUB624**

**The Gene Expression of TWEAK/Fn14 and IP-10/CXCR3 in Glomerulus and Tubulo-Interstitium of Patients with Lupus Nephritis** Jianxin Lu, Bonnie Kwan, Kai Ming Chow, Philip K. T. Li, Cheuk-Chun Szeto. *Department of Medicine & Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, HongKong, China.*

*Background* The role of TNF-like weak inducer of apoptosis (TWEAK)/Fn14 and interferon-inducible protein (IP-10) / CXCR3 axis in the pathogenesis of lupus nephritis remain elusive. *Methods* We quantified the mRNA expression of TWEAK, Fn14, IP-10 and CXCR3 in glomerulus and tubulointerstitium of 42 patients with lupus nephritis (LN group) and 10 healthy controls. *Results* As compared to controls, LN patients had higher glomerular expression of TWEAK and Fn14, but glomerular CXCR3 expression was lower in the LN group. Similarly, LN group had higher tubulointerstitial expression of TWEAK and Fn14, but lower tubulointerstitial expression of CXCR3, than controls. Glomerular TWEAK expression of class V nephritis was significantly higher than proliferative nephritis. Glomerular expression of CXCR3 significantly correlated with proteinuria (r=-0.532; p=0.019), whereas tubulointerstitial CXCR3 significantly correlated with serum creatinine (r=-0.447; p=0.029). *Conclusion* In patients with lupus nephritis, there is an increase in intra-renal expression of TWEAK and Fn14, and a decrease in CXCR3 expression. Intra-renal expression of CXCR3 correlates with proteinuria and renal function. Our findings suggest that TWEAK/Fn14 and IP-10/CXCR3 axis may contribute to the pathogenesis of lupus nephritis.

Disclosure of Financial Relationships: nothing to disclose

**PUB625**

**The Gene Expression of NGAL and TLR9 in Glomerulus and Tubulo-Interstitium of Patients with Lupus Nephritis** Jianxin Lu, Bonnie Kwan, Kai Ming Chow, Philip K. T. Li, Cheuk-Chun Szeto. *Department of Medicine & Therapeutics, Prince of Wales Hospital, HongKong, China.*

*Background* The role of Neutrophil gelatinase associated lipocalin (NGAL) and Toll-like receptor 9 (TLR9) in the pathogenesis of lupus nephritis remain elusive. Methods Laser microdissection of the snap-frozen kidney biopsy specimens was performed using the PALM Microlaser System. We quantified the mRNA expression of NGAL and TLR9 in glomerulus and tubulointerstitium of 42 patients with lupus nephritis (LN group) and 10 healthy controls. *Results* As compared to controls, LN patients had higher glomerular expression of TLR9. Similarly, LN group had higher tubulointerstitial expression of NGAL and TLR9. Both of Glomerular(r=0.554; p=0.001) and tubulointerstitial (r=0.379; p=0.043) TLR9 significantly correlated with proteinuria. As for NGAL, only tubulointerstitial NGAL significantly correlated with proteinuria(r=0.492; p=0.003), GFR(r=-0.386; p=0.022) and Cl(r=0.54; p=0.004). *Conclusion* In patients with lupus nephritis, there is an increase in intra-renal expression of NGAL and TLR9. Intra-renal expression of NGAL and TLR9 correlates with proteinuria and renal function. Our findings suggest that NGAL and TLR9 may contribute to the pathogenesis of lupus nephritis.

Disclosure of Financial Relationships: nothing to disclose

**PUB626**

**Effect of Rituximab on Resistant Idiopathic Membranous Glomerulonephritis (IMGN)** Filippo Mangione, Ciro Esposito, Francesca Castoldi, Luigi Villa, Clara Migotto, Nicoletta Serpieri, Noemi Maggi, Vittoria Esposito, Fabrizio Grosjean, Antonio Dal Canton. *Nephrology, Policlinico S.Matteo, Univ. of Pavia, Italy.*

IMGN may lead to progressive renal dysfunction. All the therapeutic strategies proposed for IMGN are burdened with side effects. Rituximab (RTX) has proven to be effective in patients with IMGN, however not in patients with refractory disease. We evaluated if RTX could be useful as a cyclosporine-sparing agent and in patients refractory to other treatments. RTX (1 g) was administered i.v. on day 1 and day 15. In cases of ongoing CYA therapy, CYA was tapered by 20% after the last infusion, and hence monthly if no increase in proteinuria was noted. Patients were followed up to 6 months. Complete remission was defined as proteinuria < 0.3 mg/24h; partial remission was defined as proteinuria < 1 g/24h. 6 patients (5 ♂, 1♀ ) were enrolled. 4 patients had CYA-dependent IMGN and 2 patients had refractory IMGN. Mean age was 52.8 years (range 40-77 y); mean time from first diagnosis of IMGN was 61.8 months (range 4-143 months). Mean baseline proteinuria was 3.06 ± 1.74 g/24h; 5/6 patients had proteinuria > 2 g/24h, and 2 of them had nephrotic proteinuria. Significant B cell (counted as CD19+ and CD20+ circulating cells) depletion occurred right after the first infusion and was maintained up to 6 months. At the end of the study, mean proteinuria levels were not significantly different from baseline (2.8 ± 2.7 g/24h; p=0.76). Only 1/6 patient (which was in the refractory group) reached complete remission. One patient in the CYA-dependent group discontinued CYA therapy with proteinuria steadily < 1 g/24h; another one afforded a 40% reduction in CYA dose, but with proteinuria > 1 g/24h. Two subjects remained nephrotic. There was a slightly increase of renal function at the end of the study period (77.67 ± 29.52 vs 88.17 ± 22.27 ml/min per 1.73 $m^2$, p = 0.24). Only two minor side effects were noted (pruritus without cutaneous rash, 1 hypotensive episode) during infusions. RTX can be safely used in patients with refractory IMGN or, as a cyclosporine-sparing agent, in cyclosporine-dependent IMGN however its efficacy remains to be proved.

Disclosure of Financial Relationships: nothing to disclose

**PUB627**

**Red Cell Exchange in the Management of Malaria Falciparum** Raeesa Mirza, Rania Abdel-Rahman, Tejas P. Desai. *Nephrology and Hypertension, East Carolina University, Greenville, NC.*

Learning objectives:

Although common worldwide, malaria is an extremely unusual occurrence in the United States. Therefore it can present a diagnostic and therapeutic challenge. By describing this case, we hope to highlight the role of red blood cell exchange transfusion in the treatment of severe Malaria Falciparum.

Case Information:

A 27 year old female with a history of type 2 diabetes was admitted to an outside hospital, complaining of fevers, chills and myalgia for one week. Initally she was thought to have influenza and treated conservatively. However, her clinical condition continued to deteriorate with the development of jaundice, hepatic dysfunction and acute renal failure. She was empirically started on multiple intravenous antibiotics, and transferred to our facility.

It was noted that she had recently returned from India two weeks prior to this admission. She denied any illness while she was there besides traveler's diarrhea. In light of this information, a blood smear was performed which revealed *Plasmodium falciparum*. The patient was found to have a 23% parasitemic burden. At this point an infectious disease consult was obtained, and the patient was started on quinidine and clindamycin intravenously.

Nephrology was consulted for a red blood cell exchange transfusion. A 1.5 liter exchange was perfomed on the day of admission. Blood smears were monitored before and after treatments, with the goal of reducing parasitemic burden to less than 10 %. Smears were then followed every 12 hours, and the patient required one additional exchange transfusion to achieve our goal.

She tolerated and responded well to the treatment, and after several days in the ICU with ventilatory and dialytic support, she recovered completely.

Summary:

As malaria is encountered so rarely in this country, few guidelines exist regarding management. After reviewing the literature we found cases of severe malaria falciparum responding to exchange transfusion. This case illustrates the indication for cytapheresis in the setting of malaria falciparum causing severe malaria.

Disclosure of Financial Relationships: nothing to disclose

**PUB628**

**A 120-Month Retrospective Analysis of De Novo HBV Infection in a Mayo Clinic Hemodialysis System – A Comparative Effectiveness Review and a Call for Change in Current US CDC Guidelines for HBV Immuno-Surveillance and Immuno-Prophylaxis** Macaulay A. Onuigbo,[1] Macaulay A. Onuigbo.[2] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Midelfort Clinic, Eau Claire, WI.*

Background

Infection with HBV is a serious public health issue. Complications of HBV infection include cirrhosis, liver failure and heptaocellular carcinoma. Hemodialysis (HD) exposes ESRD patients to significantly higher risk for HBV infection. Current US CDC guidelines and recommendations, practiced in US HD units, and worldwide, were established in 2001. A recent false positive HBsAg test following HBV immunization (Engerix B) led us to believe that changes to current guidelines are long overdue.

Objective

The aim was to determine the incidence/prevalence rates of de novo HBV infection among HD patients in five Mayo Clinic Hemodialysis units over a 120-month period and to assess the relative risks of a positive HBsAg test being a de novo HBV infection versus a false positive serology test following HBV immunization. A new patentable IT Software Program to help optimize immuno-prophylaxis and immuno-surveillance for HBV in HD patients and other high risk populations was to be developed. New changes to current US CDC guidelines would subsequently be enunciated.

Methods

A retrospectively acquired database of relevant HBV serology test results in the last ten years from patients attending five Mayo Clinic HD units was analyzed.

Results

Preliminary data show that we had a zero incidence of de novo HBV infection in our HD population during the last ten years. The only positive HBsAG test results were false positive following Engerix B vaccination.

Conclusion

Our preliminary data demonstrate that de novo HBV infection among HD patients in the US is very rare; it was absent in our unit over a 120-month period. False positive HBsAG tests may be more prevalent than de novo HBV infection. Current US CDC guidelines on HBV immuno-prophylaxis and immuno-surveillance, established in 2001, are due for a revision. Our new IT Software Program under development could maximize the efficacy and effectiveness of HBV immunoprophylaxis among high HD patients and high-risk populations, in general.

Disclosure of Financial Relationships: nothing to disclose

**PUB629**

**The Experimental Treatment and Its Mechanism of Total Flavonoids of Ajuga (TFA) on Mesangial Proliferative Glomerulonephritis (MsPGN)** Weihua Peng. *Nephrology, DongFang Hospital, Fuzhou, Fujian P.R.C., Fuzhou, Fujian, China.*

To observe the effect of TFA on Rats with MsPGN and its mechanism, we established the improved animal model of chronic serum sickness MsPGN. The positive urinary protein rats were randomly divided into the model control group(normal rats), the tripterygium wilfordii polycoride(TPG) group(0.018g•kg$^{-1}$•day$^{-1}$),the TFA high-dosage group(2.16g•kg$^{-1}$•day$^{-1}$),the TFA mid-dosage group(1.08g•kg$^{-1}$•day$^{-1}$) and the TFA low-dosage group(0.54g•kg$^{-1}$•day$^{-1}$). After 6weeks,24h urinary protein,blood biochemistry were detected. Rats Mesangium,GMC and ECM accumulation were observated under the light microscope.The content of MDA and SOD activity was detected by chemical colorimetric assay,the concentration of IL-1,TNF-αby radio immunoassay,the expression of TGF-$\beta_1$ by ELISA,and the expression of NF-κBp$^{65}$ in rats nephridial tissue with immunohistochemistry.

The excretion of urine protein in TFA groups and TPG group were more significantly decreased than the model group(P<0.01 or P<0.05).GMC in model group increased significantly, mesangial region became obviously widen, groundsubstance increased,the capillaries were pressed into narrowing or disappearing,those pathomorphological changed in TFA groups dramatically lessen(P<0.01 or P<0.05).There were no statistics difference in urine protein excretion and pathomorphological change among the first two TFA groups and TWG group(P>0.05).SOD activity in TFA groups were obviously higher than model control group,the expression of MDA,IL-1,TNF-α,TGF-$\beta_1$ and NF-κB p$^{65}$ were obviously decreased(P<0.05 or P<0.01),and Nuclear translocation of NF-κB was significantly inhibited.The effect of increasing SOD activity and decreasing the content of MDA in TFA mid-dosage group were better than TPG group(P<0.05).The relative analysis shows the expression of NF-κB p$^{65}$ was significantly positive correlation with the expression of Serum IL-1,TNF-α,TGF-$\beta_1$(r=0.566, P<0.05; r=0.669, P<0.05; r=0.598, P<0.05),and was significantly negative correlation with SOD activity(r=-0.825, P<0.01).The research indicated that TFA had a certain therapeutical effect on MsPGN which might be related to resist oxidative stress and decrease the expression of NF-κB, IL-1, TNF-α, TGF-$\beta_1$.

Disclosure of Financial Relationships: nothing to disclose

**PUB630**

**Occult Intrarenal Bacteria Cause Fibromyalgia: New Ideas of Pathogenesis** Russell E. Randall. *NA.*

Bacterial casts are pathognomonic for intrarenal infection. 278 patients (99% female) with a clinical diagnosis of fibromyalgia were studied. 65% were free of urinary symptoms but often had livido reticularis and palmar erythema, headaches, chronic fatigue, arthralgias and skin rashes. Low titer autoantibody (ana, anca, antithroid) positivity occured in 27%.

clean catch urines were cultured and the stained sediment examined using high power oil immersion and polarizing microscopy.

bacterial and/or celluar casts were present in 99%. Routine urine cultures identified organisms in only 80%. All urine specimens were further subjected to intentional dehydration and delayed microscopy at 30 minutes, one, three and 24 hours. 100% showed the formation of microcrystals, both free and within bladder or renal epithelial cells. These appeared to be derived from nuclear proteins from infected apoptotic cells. The precise composition of these crystals is being studied, but they resemble uric acid in a micro-form or amino acids from degradating nuclear proteins.

It is hypothesized that intrarenal reabsorption of bacterial-induced intracellular products of apoptosis initiates the symptoms known as fibromyalgia. The precice mechanisms are not yet known.

To be discussed: criteria that defines intrarenal infection, and the response to and the adequacy of antibiotic therapy.

Disclosure of Financial Relationships: nothing to disclose

**PUB631**

**Decreased Numbers of Plasmacytoid Dendritic Cells but Normal Counts of Monocyte Subpopulations in ANCA-Associated Vasculitis during Remission** Marten Segelmark,[1,2] Thomas Hellmark,[1] Mohamed A. Abdgawad.[1,2] *[1]Department of Nephrology, Lund University, Lund, Sweden; [2]Department of Nephrology, Skane University Hosptial, Lund, Sweden.*

Introduction: In most chronic inflammatory diseases there are reports of increased numbers of pro-inflammatory monocytes (CD14low/CD16+). AASV patients in remission are at an high risk of relapse, and AASV remission have been shown to be associated with increased levels of circulating cytokines and increased proportion of PR3+/CD177+ neutrophils.

Methods: We analyzed monocyte subpopulations and plasmacytoid dendritic cells (PDC)complete as well as comolete white blood cell count in 29 stable AASV patiens, 24 healthy controls (HC) and 14 renal transplant recipients (Tp).

Results: Compared to HC AASV had significantly more PMNs, and less PDCs and lymphocytes. Tp patients also had high PMNs and low lymphocyte counts, suggesting this to be secondary to treatment. However AASV had significantly less PDCs compared to the Tp patients. Neither Tp nor AASV patients had any deviations in the three major monocyte subpoplations.

PDCs and monocyte subpopulations in AASV

| Cells 10*6/l | AASV (n=32) | HC (n=23) | Tp (n=14) |
|---|---|---|---|
| Total white cells | 7697 (±3169 | 6291±1841 | 7471±1264 |
| PMN | 5755±2926 | 3848±1809*** | 5107±1014 |
| Lymphocytes | 1179±884 | 1839±471*** | 1542±563 |
| Total Monocytes | 548±290 | 491±144 | 636±276 |
| CD14+/CD16low | 469±258 | 419±138 | 547±266 |
| CD14+/CD16+ | 48±38 | 34±16 | 52±55 |
| CD14low/CD16+ | 32±29 | 38±17 | 37±25 |
| PBC | 3.8±4 | 10.3±5*** | 6.8±4* |

*=p<0.05 vs AASV, ***=p<0.001 vsAASV

Disucssion: Low circulating PBCs may indicate an increased recruitment of such cells to target organs. PBCs are the main source of type 1 interferons, suggesting a role for these cytokines in AASV. The change in monocyte subpoplation reported from many other inflammatory states were not seen in AASV remission.

Disclosure of Financial Relationships: Research Funding: Funding from GAMBRO AB, about $30.000 per year from 2008-2011.

**PUB632**

**Detection of PreS1/S2Ag and Other HBV Antigens in the Patients with HBV Associated Glomerular Nephritis** Chenggang Shi, Xiaolin Zeng, Hui Peng, Qiongli Yin, Chao Ma. *The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China.*

**Background** PreS1 is an HBV envelope protein. Immunogenicity of PreS1 is strong, and at the same time the PreS1 antigen is an index of virus replication. Our aim is to detect the expression of PreS1/S2-Ag and other antigens of hepatitis B in renal tissues of hepatitis B virus associated nephritis.

**Methods** PreS1/S2Ag,HBeAg,HBsAg,HBcAg in renal paraffin sections were detected by immunohistochemistry in 59 Patients, including 49 HBV carring nephrosis, 5 minor changed disease, 5 renal calculus or renal carcinoma. The status of antigen expression and clinical feathers were given statistical analysis.

**Results** (1) The four antigens were expressed in the renal tissue. The positive rates of the Pre-s1/s2Ag, HBeAg, HBsAg, HBcAg in the 49 patients with HBV carring nephrosis were 32.7%(16 cases), 38.8%(19 cases), 14.3%(7 cases), 46.9%(23 cases), respectively. All four antigens positive rate was 70.2% (36 cases). (2)The expression of PreS1/S2-Ag in the renal was correlation with the expression of HBcAg in renal(r=0.459, P<0.01). It was located in the cytoplasm of renal tubular and glomerular epithelial cells, endothelial and mesangial cells. (3)In the 36 cases of HBVGN there were 14 cases (38.9%) of membranous nephropathy (MN), 4 cases (11.1%) of membranoproliferative glomerulonephritis (MPGN), 8 cases (22.2%) of IgA nephrosis (IgAN), 1 case (2.8%) of minimal change glomerulopathy (MCD), 3 cases (8.3%) of mesangial proliferative glomerulonephritis (MsPGN), 6 cases (16.7%) of proliferativesclerosing glomerulonephritis (PSGN). (4)The positive expression rates of HBcAg through megatemperature and high pressure with gastric enzyme or simple megatemperature and high pressure to restore antigens are 48.9%, 14.6%. There was significant difference (P<0.01); (5)The occurrence of HBVGN was correlated with the status of serum HBeAg (r=0.367, P<0.05).

**Conclusion** PreS1/S2-Ag expresses in renal tissues with HBVGN .The method of antigen restored through megatemperature and high pressure with gastric enzyme can raise the positive expression rates of HBcAg in renal. To detect the four antigens can elevate the rate of diagnose of HBVGN.

Disclosure of Financial Relationships: nothing to disclose

**PUB633**

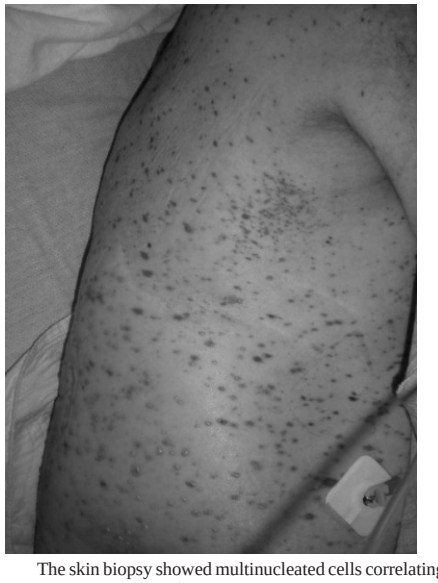
**Disseminated Herpes in a Patient Receiving Mycophenolate Mofetil and Prednisone for Membranous Nephropathy** Alejandro Solano Bayardo,[1] Sorour Rahgoshay,[1] Razi Syed,[1] Wajid M. Choudhry.[2] *[1]Internal Medicine, Unity Health System, Rochester, NY; [2]Nephrology, Unity Health System/University of Rochester, Rochester, NY.*

**Introduction**

Disseminated herpes has been associated in immunocompromised patients in general. Mycophenolate mofetil, has been rarely associated with disseminated herpes and its occurrence is mostly seen in patients with organ transplant.

**Case Discussion**

A 81 year old male with history of idiopathic membranous glomerulonephritis stage I-II, with 5.6 g/day proteinuria diagnosed in 2002; had been receiving treatment with mycophenolate mofetil at 2 g daily resulting in partial remission therefore dosage was reduced to 1 g a day in a year time. Later in 2008 the dosage was further reduced to 500 mg daily. He developed severe nephrotic syndrome 5 months later requiring increasing mycophenalate mofetil at 2 g a day and the addition of prednisone. Following this, the patient was admitted to the hospital with dyspnea and generalized pruritic and painful rash for 3 weeks and epigastric pain with vomiting for 2 days prior to admission. Physical exam revealed a diffuse erythematous papular rash with some vesicles involving his face, chest and back.



The skin biopsy showed multinucleated cells correlating with herpes infection and viral cultures were positive for varicella zoster virus. During hospitalization upper endoscopy showed multiple shallow ulcers and bronchoscopy showed mucosal ulcers with BAL positive for Aspergillosis. The patient received treatment with IV acyclovir and caspofungin with resolution of skin rash along with clinically recovery.

**Conclusion**

The presentation of our patient with disseminated herpes and pulmonary aspergillosis is most likely associated with immunosuppression caused by mycophenolate mofetil and prednisone in the setting of membranous nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**PUB634**

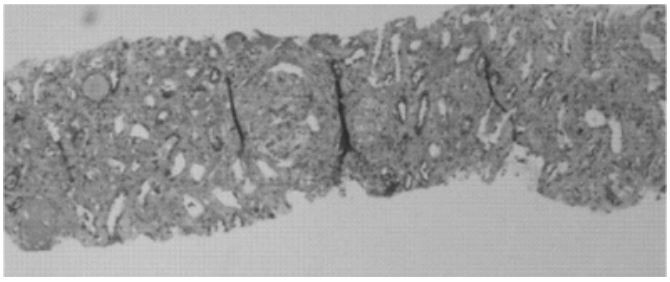
**P-ANCA Crescentic Glomerulonephritis Coincided Hodgkin Lymphoma with Renal Recovery after Lymphoma Treatment** Khaldoun Soudan, Sherry L. Werner, Seema S. Ahuja. *Nephrology and Pathology, The University of Texas Health Science Center and the South Texas Veterans Health Care System, San Antonio, TX.*

**Clinical case**

A 68 year-old male with history of diabetes mellitus and stage 3 chronic kidney disease developed worsening renal function (Creatinine 1.5 to 3.1 mg/dL) with nephrotic range proteinuria of 20g/day. Work-up was notable for a P-ANCA with a pauci-immune crescentic glomerulonephritis on renal biopsy. Due to the severe interstitial fibrosis he did not receive immunosuppressive therapy and was initiated on dialysis.

After one month, patient was diagnosed with Hodgkin Lymphoma [HL] by lymph node biopsy. Chemotherapy (Adriamycin, Bleomycin, Dacarbazine, Vinblastine) was administered with improving lymphadenopathy.

It was noticed after chemotherapy treatment that renal function improved based on an average urea-creatinine split clearance of 36mls/min, increasing urine output and a decrease in proteinuria to 10 g/day. Dialysis was discontinued with stable serum Creatinine of 1.3 mg/dL. P-ANCA titer became undetectable.



**Discussion**

Various solid and lymphproliferative malignancies have been associated with glomerular diseases to include membranous nephropathy, minimal change disease and focal segmental glomerulosclerosis. Effective treatment of the tumor generally leads to reversal of the glomerular injury.

ANCA have been described in HL patients, but is usually not associated with pathological complications such as antibody mediated systemic vasculitis or pauci-immune glomerulonephritis.

This patient had biopsy proven P-ANCA pauci-immune glomerulonephritis with renal failure requiring dialysis that responded to lymphoma treatment.

**Conclusions**

Hodgkins Lymphoma can present initially as P-ANCA pauci-immune crescentic glomerulonephritis.

Significant improvement in renal function is possible after lymphoma treatment even with advanced renal interstitial fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB635**

**Twenty Year Profile of IgA Nephropathy in a Secondary Care Hospital** Sivakumar Sridharan, Vasantha M. Muthuppalaniappan, Michael K. Almond. *Renal Unit, Southend University Hospital, Westcliff-on-Sea, Essex, United Kingdom.*

Aim:

To evaluate the profile of IgA nephropathy cases and the outcomes over 20 years in a secondary care hospital.

Methods:

We identified IgA nephropathy patients from the histopathology reports for the period 1988 to 2008 and their case records were retrieved. Various clinical information pertaining to the study were collected from these records.

Results:

We had a total of 425 renal biopsies during this period, of which there were 40 IgA nephropathy cases. The commonest age group was 40-49 years (27.5% of patients). 75% of patients were males. The commonest presentations were isolated haematuria (35%), combined haematuria and proteinuria (17.5%) and proteinuria (15%). 42.5% of patients had raised creatinine levels at the time of presentation. Urine dipstick testing at the time of first presentation showed blood in 87.5% of patients and protein in 77.5%. Hypertension was present in 32.5% of patients and 25% had smoking history. The commonest histology found was mesangial proliferative type (37.5%) with 5% of patients with crescentic formation. Outcome evaluation showed 22.5% of patients had remission and an equal number developed CKD. 72.5% of patients were managed conservatively, 15% of patients had transplant and 20% of patients had dialysis at sometime during their treatment. Only one patient was treated with short course steroids and none of them had immunosuppressants. 20% of our cohort died over the last 20 years and all of them died of non-renal causes. Outcome was not known in 15% of patients due to non-attendance.

Discussion:

IgA nephropathy is the commonest glomerulonephritis worldwide and it was the commonest diagnosis in our hospital in the past 20 years. Our study showed the commonest age group affected was in the fifth decade of life in comparison to the second and third decades which is common worldwide. Haematuria remains the commonest reason for referral and is present in majority of patients. Crescentic formation predicted poor prognosis as they developed ESRD. Majority of our study patients were treated in a conservative approach and this approach coupled with regular follow-up was found to be successful from a secondary care point of view.

Disclosure of Financial Relationships: nothing to disclose

**PUB636**

**Hypoalbuminemia with Coexisting Protein-Losing Enteropathy and Membranous Nephropathy in a Patient with SLE-Like Mixed Connective Tissue Disease** Donjeta Sulaj, Lin N. Lwin, Hugo J. Villanueva, Jinil Yoo. *Nephrology, Montefiore Medical Center, North Division, Bronx, NY.*

We observed a patient with severe hypoalbuminemia who was found to have SLE - like mixed connective tissue disease (MCTD) with coexisting membranous nephropathy (MN) and protein - losing enteropathy (PLE). Initially she had trace proteinuria (< 0.5 g in 24 - hr urine), but severe hypoalbuminemia (< 1.0 g/dL). Under the presumptive diagnosis (Dx) of PLE, she was placed on methylprednisolone (Medrol) 48 mg p.o. daily. While serum albumin (Salb) level rose to 2.2 g/L in 4 weeks, her proteinuria increased > 3.0 g in 24 - hr urine. With the Dx of MN and persisting muscle weakness, mycophenolate (MMF up to 3.0 g/d) and a low dose of Medrol (18 mg/d) were started.

The case is a 43 - year - old obese lady who was admitted with abdominal pain and diarrhea, and found to have marked muscle weakness (bed-ridden), + pleural effusion, + ascites, serum creatinine (Scr) in ranges of 0.7 to 1.3 mg/dL, Salb 1.6 to 0.9 g/dL, total serum protein 2.2 g/dL, + proteinuria: 418 mg to < 1.0 g in 24 - hr urine, + ANA 1: 1260 speckled, (-) anti - ds DNA, + anti - Ro, + anti - RNP, low serum complement and normal liver enzymes. The kidney biopsy revealed MN Stage I-II and mesangial deposits. She had repeated upper GI endoscopy and colonoscopy which all showed edematous mucosa and nonspecific findings. The presumptive Dx of PLE was made in the view of severe hypoalbuminemia, in which other causes, such as protein malnutrition, heavy proteinuria, or liver diseases have been excluded.

The protein loss in PLE is considered due to increased permeability of intestinal mucosa and/or capillaries induced by inflammatory burden in SLE or MCTD, and responds dramatically to corticosteroids. The pathophysiology of protein loss in PLE is believed to be different from that in glomerular diseases, independent of molecular weight. In our patient, "nephrotic syndrome" due to MN was not evident by masquerading PLE, until her PLE was controlled with Medrol and Salb reached to 2.2 g/dL. Extra renal loss of protein may mask nephrotic syndrome.

Disclosure of Financial Relationships: nothing to disclose

**PUB637**

**Novel Role for alpha-Galactosidase as a CKD Biomarker** Sapna Trivedi,[1] Mehmet M. Altintas,[2] Orlando M. Gutierrez,[2] Jochen Reiser.[2] *[1]Medicine, University of Cambridge, United Kingdom; [2]Medicine, Nephrology, University of Miami, FL.*

**Purpose**: Blood activity levels of alpha galactosidase (a-GAL) are usually measured in the context of diagnosing Fabry's disease. After reliably setting up the testing system, we sought to analyze the relationship of a-GAL activity levels in patients with different stages of Chronic Kidney Disease (CKD) independent of Fabry's disease, to evaluate its use as a potential biomarker in CKD.

**Method:** Patients with renal failure (CKD 2 - 60<GFR<89, CKD 3 - 30<GFR<59, CKD 4 - 15<GFR<29, CKD 5 - GFR<15) were identified. Blood samples were obtained by fingerprick testing and a-GAL measurement and analysis was undertaken using the protocol by Nestor Chamoles[1]. A-GAL activity was correlated with clinical data.

**Results:** eGFR was significantly associated with a-GAL levels in this dataset (P=0.04). The results demonstrate the relationship between eGFR and a-GAL levels (log transformed to achieve a normal distribution) is different by gender with men but not women showing a significant linear association between a-GAL and eGFR. Levels of a-GAL decrease with decreasing quartiles of eGFR in men (P = 0.03) but not women (*P* = 0.6).

Interaction between eGFR and gender

| Source | DF | Type III SS | Mean Square | F Value | PR>F |
|---|---|---|---|---|---|
| Sex | 1 | 2.76 | 2.76 | 4.56 | 0.0353 |
| eGFR | 1 | 1.14 | 1.14 | 1.88 | 0.1739 |
| eGFR*sex | 1 | 3.18 | 3.18 | 5.25 | **0.0242** |

**Conclusion:** In models stratified by gender, eGFR remained linearly associated with a-gal levels in men independently of age, race, body mass index, diabetes, and hypertension (a-gal levels increase 1.5 for every 10 ml/min/1.73 m² increase in eGFR, *P* = 0.004). In contrast, there was no relationship between eGFR and a-GAL levels among women in unadjusted or multivariable adjusted regression analyses (*P* = 0.9). This may in part be due to the known unreliability of the fingerspot testing method in females or due to the fact that two copies of alpha-galactosidase are able to compensate in conditions of CKD in women. In summary, our data suggests that a-GAL activity may have a role as a biomarker for CKD stage in males. We are currently exploring the molecular mechanisms that underlie our findingr]

1) Chamoles N. et al.Clin Chim Acta, 2001. **308**(1-2)

Disclosure of Financial Relationships: nothing to disclose

**PUB638**

**Localization of (Pro)renin Receptor in Glomeruli of IgA Nephropathy, Diabetic Nephropathy, and Minimal Change Nephropathy Using Renal Biopsy Specimen** Satoko Ueda. *1Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Nankoku, Kochi, Japan.*

Activated prorenin plays a key role in the regulation of the tissue RAS, and a direct renin inhibitor has been reported to reduce proteinuria in diabetic nephropathy through inhibiting the tissue RAS. However, little information is available regarding the localization of activated prorenin in human kidney under pathophysiological conditions. We examined the localization of activated prorenin in kidney biopsy specimens of the patients with IgA nephropathy(IgAN), diabetic nephropathy(DN), and minimal change nephropathy(MCN). The antiserum against activated prorenin was raised in a rabbit by injecting the peptide fragment corresponding to the gate region of human prorenin. The antibodies for nephrin and CD34 were used as markers for podocyte and endothelial cells, respectively. Immunohistochemical analyses were performed by confocal microscopy.

We analyzed 19 renal biopsy specimens (n=8 IgAN, n=4 DN, n=7 MCN). Immunohistochemistry with the anti-(P)RR antibody showed positive immunostaining in mesangial area in 13 patients (n=5 IgAN, n=4 DN, n=4 MCN) and podocyte area in 12 patients (n=6 IgAN, n=4 DN, n=2 MCN). These were no significant staining for activated prorenin in endothelial cell in glomeruli. These findings indicate that activated prorenin is observed in mesangial area and podocyte, and the staining pattern may be different.

In conclusion, activated prorenin is expressed in mesangial area and podocyte in human kidney and may contribute to the pathophysiology of IgANand DN.

Disclosure of Financial Relationships: nothing to disclose

**PUB639**

**Unusual Presentation of Lupus Nephritis** Bhavani Adusumilli. *Internal Medicine, William Beaumont Hospital, Royal Oak, MI.*

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, characterized by multisystem involvement. SLE predominantly affects women in childbearing age . Late onset SLE represents a specific subgroup and the studies of elderly patients with SLE are scarce. We report a case of lupus nephritis with an unusual presentation.

A, 79 year-old, female with only history of hypertension was admitted to our hospital with complaints of progressive malaise and rash which started over the bridge of the nose, ears eyebrows and dorsum of hands and symmetric polyarthritis involving the small joints of the hands and wrists. The patient was well until 2 months before admission when she began having weakness and was found to have neutropenia and anemia. The patient was not on any drugs likely to cause drug-induced lupus. On examination, she appeared unwell. There was an erythematous plaque like rash involving the face and ears and crusted lesions on the lip. Neck was supple without adenopathy. Joint exam revealed definite synovitis of MCP and MTP joints as well as PIP joints. Labs, showed a white count of 1.7 BIL/L, hemoglobin of 8.6 mg/dl, with serum creatinine of 1.01mg/dl, anti DsDNA positive greater than 1000,ANA titer greater than 1:1280,low C3 and C4, with negative ANCA, SS-A and SS-B.A thorough infection screen, including a viral panel for Herpes Simplex, Herpes Zoster, Epstein-Barr, Cytomegalovirus, Hepatitis B, Hepatitis C, HIV, Echo and Coxsackie viruses was negative. Urine and blood cultures were negative. Urine analysis showed active sediments with protein /Creatinine ratio of 9.7.A percutaneous needle kidney biopsy was performed and was consistent with diffuse proliferative lupus nephritis (WHO type 4 LUPUS). The patient was initiated on high-dose corticosteroid therapy with plan to start CellCept once her WBC count improved.

Late onset SLE is rare and the course of the disease is considered to be more benign. Skin manifestations, photosensitivity , arthritis and nephritis occur rarely in elderly patients . But here we present a case of late onset lupus presenting with skin manifestations and nephritis.There is less clinical awareness of late-onset lupus, leading to a delay between onset and diagnosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB640**

**Tubulointersticial Fibrosis: Potential Mechanisms of Induction and Repair** Mirian A. Boim, Edgar Maquigussa, Luciana Guilhermino Pereira, Carine Prisco Arnoni. *Medicine - Renal Division, Federal University of São Paulo, São Paulo, São Paulo, Brazil.*

Increased glomerular protein filtration, tubular reabsorption and traffic may have direct toxic effects on epithelial cells, which increase the production of chemokines and profibrotic cytokines, such as the transforming growth factor-beta (TGF-β), the most potent inducer of the epithelial-mesenchymal transition (EMT). On the other hand, the bone morphogenetic protein-7 (BMP-7) plays a critical role in the repairing processes of the damaged tubular cells. The endogenous activity of BMP-7 is controlled by BMP antagonists, such as gremlin. Our objective was to evaluate the role of these proteins in the induction of EMT and the reversion of tubulointerstitial fibrosis using models, in vivo (puromicin-induced proteinuria in rats) and in vitro (immortalized human proximal tubular cells, HK-2). HK-2 cells were stimulated with TGF-β (1ng/ml) for up to 48 hours. Adult male Wistar rats were treated with puromycin (100mg/kg, ip) and after 10 weeks the renal function and proteinuria were evaluated. The expression of EMT marker molecules (α-SMA, E-cadherin and FSP1), fibronectin, collagen, TGF-β, BMP-7 and gremlin were estimated by real time PCR, Western blot and immunohistochemistry. HK-2 cells stimulated with TGF-β expressed α-SMA and fibronectin with decreased expression of E-cadherin indicating that the cells were in EMT process. Gremlin expression also increased in cells incubated with TGF-β, suggesting inhibition of BMPs. Puromycin caused severe proteinuria and tubulointerstitial fibrosis evidenced by increases in expression of fibronectin and collagen associated with increased expression of TGF-β, FSP-1 and α-SMA, indicating the presence of EMT also in the in vivo model. There was a rise in BMP-7 and gremlin expression suggesting that despite the activation of a repair mechanism (BMP-7) is probably inhibited by gremlin, facilitating the fibrogenesis. Future experiments will determine the role of BMP-7 and gremlin in the process of reversion of the EMT at the prospect of developing strategies to control the mechanisms of induction and likely the treatment of tubulointerstitial fibrosis.

Disclosure of Financial Relationships: nothing to disclose

**PUB641**

**Epithelial-to-Mesenchymal Transition Contributes to Aristolochic Acid Nephropathy-Induced Tubulointerstitial Fibrosis** Annelies De Beuf,[1] Joelle L. Nortier,[2] Marc E. De Broe,[1] Patrick C. D'Haese.[1] *[1]Laboratory of Pathophysiology, University of Antwerp, Antwerp, Belgium; [2]Nephrology, Université Libre de Bruxelles, Brussels, Belgium.*

Aristolochic acid nephropathy (AAN), due to the use of traditional Chinese medicine and characterized by progressive renal dysfunction and tubulointerstitial fibrosis, has become a worldwide disease. Using a well validated mouse model of AAN, the present study aimed to (i) identify important mediators leading to renal fibrosis and (ii) check whether epithelial-to-mesenchymal transition (EMT) plays a role herein. Male CH3/He mice were administered AA (2.5 mg/kg/day) up to 10 days whilst a group receiving vehicle served as controls. Animals were sacrificed after 1, 2 and 4 days of treatment, and 1 and 20 days posttreatment. The expression of several genes involved in renal fibrosis and the regulation of EMT was investigated using quantitative real-time RT-PCR. In the acute phase after AA treatment, tubular necrosis was observed, whilst renal fibrosis characterized by

lymphocyte/macrophage infiltration, tubular atrophy and collagen deposition, started to develop later and was maximal 20 days posttreatment. TGF-β/Smad and Wnt/β-catenin are two major intracellular signaling pathways involved in the regulation of EMT. The gradually increase of TGF-β and β-catenin during the development of renal fibrosis reaching maximal increases of 10.6 fold and 2.9 fold respectively 20 days posttreatment was accompanied with increased Smad3 and Smad7 expression (3.5 fold). Interestingly, the mRNA expression of two downstream targets of TGF-β/Smad signaling, namely connective tissue growth factor (CTGF), an EMT related key mediator of fibrosis, and CCN3, a negative regulator of CTGF, was significantly increased (9.9 and 5.3 fold respectively). Finally, the development of AA-induced renal fibrosis went along with a marked upregulation of the EMT markers N-cadherin (5.1 fold) and vimentin (19.8 fold). Results of this study suggest a central role for EMT in the development of AA-induced renal fibrosis and the use of this model allows to identify early mechanisms initiating the fibrotic process and to evaluate the effect of anti-fibrotic compounds.

Disclosure of Financial Relationships: nothing to disclose

**PUB642**

**Dexamethasone Stimulates Fibrogenic Proteins in Rat Mesangial Cells** Noritaka Kawada, Toshiki Moriyama, Harumi Kitamura, Yoshitsugu Obi, Jun-Ya Kaimori, Yoshitsugu Takabatake, Masaru Horio, Hiromi Rakugi, Yoshitaka Isaka. *Division of Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Background. Recent investigations have established the beneficial role of steroid therapy on several glomerulonephritis, including Lupus nephritis, IgA nephropaty and ANCA associated nephritis. The administration of steroid has been shown to reduce tissue inflammation and promote tissue repair. However, steroid, in particular situation, promotes tissue fibrosis, which causes to limit the application of steroid therapy on advanced IgA nephritis. In the present study, we investigated the molecular mechanisms of steroid-induced renal glomerular fibrosis. Results. The administration of Dexamethasone (Dex, 10-6M for 6hrs) in cultured rat mesangial cells reduced the expression of Monocyte chemotactic protein-1 (MCP1) and TGFβ mRNAs (MCP1: -70%, p<0.01, TGFβ: -30%, p<0.05), but increased the expression of fibrogenic protein mRNAs, including Plasminogen activator inhibitor-1 (PAI-1: +130%, p<0.01) and Lysyl oxidase (LOX: +20%, p<0.05). The protein expression of PAI-1 and LOX were not affected by Dex at 6hrs, but significantly induced at 16hrs (PAI-1: +53%, p<0.01, LOX: +79%, p<0.01). The combined administration of Dex and TGFβ (2ng/ml) further induced PAI-1 protein (TGFβ+Dex: +230%)and mRNA (TGFβ: +190%, TGFβ+Dex: +640%) and LOX protein (TGFβ+Dex: +110%) and mRNA (TGFβ: +8%, TGFβ+Dex: +60%). Dex reduced the expression of BMP-1 mRNA (-20%, p<0.05) and the combined administration of TGFβ blunted this response (TGFβ: +3%, TGFβ+Dex: +1%), which may explain a part of the synergistic stimulatory effect of Dex and TGFβ on the LOX protein expression. Conclusions. Dex may enhance fibrosis under the presence of TGFβ partly through the induction of PAI-1 and LOX protein.

Disclosure of Financial Relationships: nothing to disclose

**PUB643**

**The Effect of C3-C3a-C3aR on Renal Epithelial Mesenchymal Transition and Its Possible Mechanism** Fang Liu, Rong Gou, Jun Huang, Ping Fu. *Division of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

**Objective**: The aim of this study is to investigate the effect of C3-C3a-C3aR pathway on renal tubular epithelial mesenchymal transition (TEMT) and its possible mechanism.

**Methods:** Renal proximal tubular cell line HK-2 were cultured in vitro and divided into six groups: control group,TGF-β1 positive control group,10nM anaphylotoxin C3a group,50nM C3a group,100nM C3a and 50 nM C3a+1μM C3a receptor inhibitor(C3aR, SB290257)group. The expressions of E-cadherin, α-SMA, TGF-β1, CTGF, Col-Iand β-catenin were detected by immunohistochemistry, Western blot and real-time PCR. Scanning electron microscope (SEM) was used to observe the cellular morphological changes.

**Results:** Normal HK-2 cells showed the classic cobblestone shape, and SEM showed a loss of apical-basal polarity and microvilli of the cells after stimulated by C3a, and the cells become elongated and invasive. Immunohistochemistry, RT-PCR and Western blot showed that C3a induced the loss of the epithelial marker E-cadherin and increased the expressions of α-SMA, TGF-β1, CTGF, Col-Iand β-catenin in dose-dependent and time-dependent manners. And C3aRA could partly block the decrease of E-cadherin and increase of α-SMA and TGF-β1.

**Conclusions:** C3a could induce tubular epithelial-to-mesenchymal transition, TGF-β1, β-catenin pathway may participate in the mechanism. And C3aRA could partly block the effect of C3a on renal tubular cells. These findings identified a novel pathway that may contribute to renal fibrosis associated with the over-activation of complement in renal injury.

Disclosure of Financial Relationships: nothing to disclose

**PUB644**

**C5a Regulates Tubular Epithelial-Myofibroblast Transdifferentiation *In Vitro*** Fang Liu, Jun Huang, Rong Gou, Ping Fu. *Division of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

**Objective**:

Growing evidences indicated that the activation of complement system was involved in the development of tubulointerstitial injury. But the mechanism is not fully understood. We hypothesize that complement induce the tubular epithelial-myofibroblast transdifferentiation (TEMT) during the tubulointerstitial fibrosis. This study was to investigate the effect of anaphylotoxin C5a on TEMT in vitro.

**Methods**: Renal proximal tubular cell line HK-2 were cultured in vitro and divided into six groups: control group,TGF-β1 positive control group,25nM anaphylotoxin C5a group,50nM C5a group and 25 nM C5a+2.5μM C5a receptor inhibitor group. The expressions of E-cadherin, α-SMA, TGF-β1, CTGF, Col-Iand β-catenin were detected by immunohistochemistry, Western blot and real-time PCR. And scanning electron microscope (SEM) was used to observe the cellular morphological changes.

**Results:** Normal HK-2 cells showed the classic cobblestone shape, and SEM showed a loss of apical-basal polarity and microvilli of the cells stimulated by C5a, and the cells become elongated and invasive. Immunohistochemistry, RT-PCR and Western blot showed that C5a induced the loss of the epithelial marker E-cadherin and increased the expressions of α-SMA, TGF-β1, CTGF, Col-Iand β-catenin in dose-dependent and time-dependent manners. And C5aRA could partly block the decrease of E-cadherin and increase of α-SMA and TGF-β1.

**Conclusion:** C5a could induce TEMT. TGF-β1, β-catenin pathway may participate in the mechanism. And C5aRA could partly block the effect of C5a on renal tubular cells. These findings identified a novel pathway that may contribute to renal fibrosis associated with the over-activation of complement in renal injury.

Disclosure of Financial Relationships: nothing to disclose

**PUB645**

**Endothelial Lineage Impairment and Increased PR3 Expression on Peripheral Cells of Endothelial Phenotype in Wegener´s Granulomatosis** Susann Patschan, Daniel Patschan, Johannes Wessels, Sabine Blaschke, Gerhard A. Mueller. *Department of Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany.*

Wegener´s Granulomatosis (WG) is characterized by microvascular endothelial damage and alterations of the endothelial progenitor cell (EPC) system. Interactions between anti-Proteinase 3 antibodies and their antigens (PR3) on neutrophils are pathogenetically relevant in WG. Aim of this study was (I) to analyze total circulating EPCs and regenerative activity of blood-derived EPCs, and (II) to evaluate PR3 expression on circulating myelomonocytic and endothelial cells in WG.

Blood samples from WG patients were analyzed for total and for Flk-1$^+$ myelomonocytic cells. Healthy donors served as controls. For evaluating the proliferative activity of EPCs, a colony forming unit assay (CFU) was performed. PR3 expression by the cells was quantified by cytometric analysis. Serum Angiopoietin 1 was measured by ELISA technique.

A total of 21 healthy donors (12 female, 9 male [40.3 ±9.2 years]) and 31 WG patients (13 female, 18 male [59.2 ±15.3 years]) were included into the study. The total percentages of EPCs were not different between the two groups. WG patients displayed lower proliferate activity of EPCs (22.3 ±4.1 vs. 45.9 ±6.8, CFU-ECs, p=0.0027). In addition PR3 expression was significantly higher in the total as well as in the Flk-1+ (sub)population of myelomonocytic cells in WG (10.4 ±14.4% vs. 0.3 ±0.4%, p=0.02 bzw. 0.3 ±0.3% vs. 0.1 ±0.1%, p=0.04). Finally, WG patients showed lower serum levels of Angiopoietin 1 as compared to controls (689 ±224 pg/ml vs. 1542 ±315 pg/ml, p=0.034), the levels did not linearily correlate with either clinical activity or the total number of circulating EPCs or the numbers of colonies formed (EPC regeneration).

In addition to reduced EPC regeneration and decreased levels of Angiopoietin 1, both indicating impairement of the endothelial system, patients with WG show significantly increased expression of PR3 in the total and in the Flk-1$^+$ myelomonocytic cell population. Thus PR3 could be involved in the pathogenesis of microvascular endothelial damage in patients with WG.

Disclosure of Financial Relationships: nothing to disclose

**PUB646**

**Association between Renal Tubular Expression of Alpha Five Integrin and Kidney Failure** Kathryn I. Scobie, Daniel A. Muruve, Rick Chin, Kiril Trpkov, Wenjie Wang, Brenda Hemmelgarn. *Nephrology, University of Calgary, Calgary, AB, Canada.*

Chronic kidney disease involves deposition of extracellular matrix and tubulointerstitial fibrosis. Integrins are heterodimeric cell surface proteins that mediate cell-extracellular matrix interactions, cell-cell interaction and adhesion. The α5-integrin subunit is the major fibronectin binding protein and its expression is induced on tubular epithelium during injurious conditions. The purpose of this study was to determine the relationship between tubular epithelial α5-integrin expression in kidney biopsies and risk of kidney failure.

A convenience sample of 158 frozen kidney biopsies from non diabetic patients from our institution were stained for α5-integrin using immunohistochemistry. Of these biopsies 55 did not have adequate tubular epithelial cells and were excluded from the study, for a final study population of 103 subjects. α5-integrin was evaluated by one reviewer and categorized as present (at least 25% of cells within one tubule stained positive) or absent. Patients were followed for a mean of 15.8 months for the primary outcome of development of kidney

failure (a composite of doubling of serum creatinine, end-stage renal disease or death).

Of the 103 biopsies 66 (64.1%) stained positive for α5-integrin. During follow-up 7.8% of patients had a doubling of serum creatinine, 12.6% developed ESRD, and 3.9% died. After adjusting for age, gender, baseline eGFR, diabetes and hypertension, the presence of α5-integrin staining was associated with an almost six-fold increased risk of kidney failure (Odds Ratio 5.9 (CI 1.1 - 30.5; p = 0.036). Our study is limited by its single location and small sample size.

We found a significant association between α5-integrin and development of kidney failure. Further studies are required to confirm these results, and determine the degree of α5-integrin expression which confers an increased risk of adverse clinical outcomes.

Disclosure of Financial Relationships: nothing to disclose

**PUB647**

**CD248 Positive Stromal Cells Are Linked to Determinants of Progressive Chronic Kidney Disease in IgA Nephropathy** Stuart W. Smith,[1] Joel Ogunka Nwosu,[2] Alexander J. Howie,[3] Clare Isacke,[4] Christopher D. Buckley,[1] Caroline O. S. Savage.[1] *[1]Institute of Biomedical Research, University of Birmingham, United Kingdom; [2]University Hospital Birmingham NHS Foundation Trust, Birmingham, United Kingdom; [3]Department of Pathology, University College London, United Kingdom; [4]Breakthrough Breast Cancer Research, The Institute of Cancer Research, United Kingdom.*

CD248 is a stromal marker that is expressed on fibroblasts and pericytes found in the human kidney. Expression is low in normal tissue but becomes upregulated at times of tissue remodelling. We therefore hypothesised that CD248 expression is upregulated in chronic kidney disease(CKD).

Methods: Renal cell populations were isolated from human kidney. CD248 expression was assessed using confocal microscopy, RT-PCR and Western blotting. CD248 expression was characterized in kidney biopsy samples taken from patients with IgA nephropathy using immunohistochemistry(n=93). CD248 staining was quantified using an image analysis system. The extent of chronic renal damage within each biopsy was assessed. CD248 expression was correlated against albuminuria, eGFR and chronic renal damage. Patients were followed up for 1095 days following diagnosis. Confocal microscopy was employed to localise CD248, alpha-smooth muscle actin(αSMA) and Fibroblast specific protein-1(FSP-1).

Results: CD248 was expressed by renal fibroblasts but not by other renal cell populations. CD248 localised to the peritubular and interstitial space in CKD. Expression correlated with albuminuria(0.500; p<0.0000), chronic renal damage (0.539;p<0.0000) and eGFR(-0.679; p<0.0000). This relationship was maintained in a multivariate analysis. In total 19 patients reached a renal endpoint(7 patients doubled their serum creatinine; 12 patients reached end-stage renal failure). Interstitial CD248 staining >9.8% was predictive of poorer renal outcome($\chi 2$=18.28, P=0.0001). Immunofluorescence failed to co-localise CD248 with αSMA or FSP-1.

Conclusion: CD248 defines a subset of renal fibroblasts that are linked to albuminuria and chronic renal damage and may be implicated in the tissue remodelling seen in IgA nephropathy.

Disclosure of Financial Relationships: nothing to disclose

**PUB648**

**Detection and Localization of Advanced Glycation End-Products (AGEs) in Diabetic Kidneys by Raman Microscopy** Vincent Vuiblet,[1] Olivier Piot,[2] Philippe Gillery,[3] Laure-Helene Noel,[5] Michel Manfait,[2] Philippe Birembaut,[4] Philippe Rieu.[1] *[1]Nephrology, University Hospital, Reims, France; [2]UMR 6237, CNRS, Reims, France; [3]Biochemistry Department, University Hospital, Reims, France; [4]Anapathomo-Pathology Department, University Hospital, Reims, France; [5]Unit 507, INSERM, Paris, France.*

While the biochemistry of Advanced Glycation End-Products (AGEs) is rather well defined, the clinical implication of the accumulation of these substances during diabetes and uremia still remains largely unknown. The difficulty to analyse AGEs in tissues is a major limitation to study the role of these pathogenic agents. We therefore investigated the potential of Raman microscopy to identify AGEs in tissues.

Methods: Raman signal of pure carboxymethyllysine (CML) and pentosidine were first obtained for reference. Raman spectral images acquired from renal biopsies of diabetic and non diabetic patients were examined to find out the AGE spectral "fingerprints". Tissue spectral imaging obtained by Raman spectroscopy were then compared with CML- and pentosidine-immunohistochimical staining of the same tissues.

Results: Raman signal of CML and pentosidine showed characteristic spectral "fingerprints" which were also found among the spectral images of diabetic kidneys but not in control kidneys. The tissue localisation of CML and pentosidine obtained by Raman spectroscopy were identical to the results found by immunolocalization.

Conclusion : In this work, we show that the Raman spectroscopy microsopy can detect and localize AGEs in tissues. With further development, this Raman-based approach has the potential for non-invasive examination of AGE adducts in living tissues and ultimately to assess their precise pathogenic role in diabetes-, age- and uremia-related diseases.

Disclosure of Financial Relationships: nothing to disclose

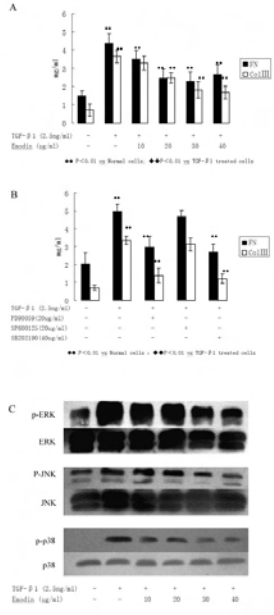
**PUB649**

**Emodin Inhibits ECM Synthesis by Repressing p38 and ERK1/2 in TGF-β1 Stimultated NRK-49F Cells** Bin Zhu, Caifeng Zhu, Yongjun Wang, Ying Lu. *Nephrology, Hangzhou Hospital of Traditional Chinese Medicine (Guangxing Hospital), Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China.*

Objective: To observe the effects of emodin on synthesis of extracellular matrix (ECM) in TGF-β1 stimultated NRK-49F cells.

Methods: NRK-49F were divided into normal control cells; TGF-β1 treated cells and emodin (low, median and high concentration) interfered cells treated with TGF-β1; PD98059, SB202190 and SP600125 were adopted to inhibit ERK1/2, p38 and JNK respectively. Enzyme linked immunosorbent assay (elisa) was performed to detect the secrection of fibronectin (FN) and collagen III in supernatant of cells. Western blot was employed to analyze the phosphorylation of ERK, JNK and P38 respectively .

Results: FN, Collagen III as well as phosphorylation of ERK, P38 were increased in NRK-49F cells stimulated with TGF-β1 as compared with control cells(Fig.1.A); Pretreatment with emodin repressed the expression of FN and Collagen type III in a dose dependent manner in TGF-β1 treated cell(Fig.1.A). PD98059 or SB202190 but not PD98059 significantly attenuated the expression of FN and Collagen type III in TGF-β1 treated cells(Fig.1.B). Emodin also inhibited the phosphorylation of ERK and P38 significantly,but it decreased JNK phosphorylation without significant difference as compared with cells treated with TGF-β1(Fig.1.C).




Conclusions: Emodin inhibits ECM Synthesis by repressing p38 and ERK1/2 but not JNK in TGF-β1 stimultated NRK-49F cells.

Disclosure of Financial Relationships: nothing to disclose

**PUB650**

**Obesity in Patients with Chronic Kidney Disease on Hemodialysis** Bárbara Margareth Menardi Biavo,[2] Clarissa B. B. Uezima,[1] Mary E. C. Costa,[1] Camila Machado de Barros,[2] João Paulo L. B. Martins,[1] Elzo R. Junior,[2] Elvino Barros,[3] Carmen B. Tzanno-Martins.[1] *[1]Integrated Center of Nephrology, Guarulhos, São Paulo, Brazil; [2]Home Dialysis Center, São Paulo, São Paulo, Brazil; [3]Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.*

**Objective:** To determine the number of overweight and obese patients undergoing hemodialysis and verify their relationship to socio-demographic and clinical aspects.

**Methodology:** Cross-sectional study with 464 patients during the month of January 2010. Demographic, clinical and anthropometric data were recorded.

**Results:** Of 464 patients evaluated, 279 (60.1%) were male and 185 were female (39.9%). Patients' mean age was 54.25 ± 15.07 years and the highest prevalence was eutrophic patients (55.6%), followed by overweighed (24.83%), obese (9.9%) and malnourished (9.67%). Given only the obese, we found that the majority are male (62.2%), data that differs from the general population in Brazil. In this population, differences in obesity prevalence between the genres are not observed. For the total sample of patients studied, 153 (32.97%) were diabetics, a higher percentage than that found by the census of the Brazilian Society of Nephrology (SBN - 2008). Of these 32.7% (50/153) were overweighed and 15.03% (20/153) were obese. Assessing the elderly, who account for 23.28% (108/464) of the study population, half have diabetes (54/108). Comparing the elderly patients group with non-diabetics, we observed a higher prevalence of overweight and obesity in the first group - 50% (27/54), while the second group was 25.9% (14/54) of these elderly in these BMI strata.

**Conclusion:** These results demonstrate the relationship of overweight and obesity with DM and aging, both risk factors for the development of CKD. Furthermore, the coincidence of diabetes and CKD with modifiable risk factors (obesity, sedentary lifestyle, poor

dietary habits) increases morbidity and mortality in these patients. Therefore, assessment of nutritional status is important for an adequate clinical and nutritional intervention, in order to help reduce these rates.

Disclosure of Financial Relationships: nothing to disclose

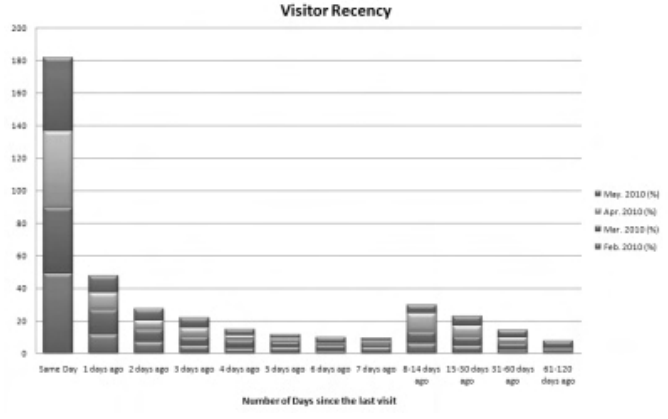
**PUB651**

**Measuring User Interaction with a Nephrology Teaching Resource: Developing a Technologically Advanced Online Teaching Tool** Tejas P. Desai,[1] Cynthia R. Christiano,[1] Paul Bolin, Jr.,[1] Maria E. Ferris.[2] *[1]Medicine, ECU, Greenville, NC; [2]Pediatrics, UNC, Chapel Hill, NC.*

A number of online medical resources do not assess users' level of engagement. Without this information, online teaching tools will not be competitive or successful. We hypothesize that a technologically advanced website will yield, objectively, high user engagement.

Nephrology On-Demand (http://www.nephrologyondemand.org) is a multimedia teaching resource created on the Wordpress platform with direct hyperlink text items, slides available through Slideshare.net, and videos produced through Camtasia Studio 6 and stored on internal servers or YouTube.com. Google Analytics code was inserted into root files and captured data measuring user interaction from February to May 2010. We measured absolute unique visitors and location, duration of visits, visitor loyalty and frequency, and search patterns. Registration is voluntary.

2390 visitors (64 registered) from 84 countries made 3440 visits and viewed 8680 pages. The mean number of page views per visit was 2.59 (SD 0.34) with 61% from new visits. For return users, 47% had visited Nephrology On-Demand 7 times or more; 27% used the site 15 times or more. Many users performed same day returns.




Eighty-seven percent of visitors required 5 pageviews or less to find the desired information. The mean number of minutes required to find the desired information was 2:10 (SD 0:14).

Nephrology On-Demand has an increasing number of visitors who return to the site frequently. Users appear to find desired information with minimal clicks and time. These data suggest users are engaged with the teaching resources available on this website. Future efforts will involve measures to improve the low rate of voluntary registration and evaluations of usability and customer feedback.

Disclosure of Financial Relationships: nothing to disclose

**PUB652**

**Washington University George M O'Brien Center for Kidney Disease Research** Marc R. Hammerman. *Internal Medicine, Nephrology, Washington University School of Medicine, St. Louis, MO.*

The overarching mission of the Washington University (WU) George M. O'Brien Center for Kidney Disease Research (CKDR) (P30 DK079333) is to support investigations designed to gain a better understanding of the way the kidney develops, including the role that particular genes play in the structure and function of the organ and thereby determine how abnormalities in genes and their expression increase an individual's risk of developing kidney disease. The Renal Organogenesis Core focuses on providing researchers access to tissues destined to become kidneys or endocrine pancreas from animal embryos and access to technology designed to transplant such tissues into adult animals as a tool to study kidney development, as a possible technique for replacing damaged kidneys in humans, or developing a viable treatment for diabetes, the major cause of ESRD. The Renal Disease Models Core and imaging center concentrates its efforts on developing transgenic or non-transgenic disease models of kidney disease in mice and rats and evaluating how particular genes and their expression affect kidney development and function. The Kidney Translational Research Core provides access to an established database of biological samples donated with the consent of kidney transplant patients and is expanding the database to include all patients with kidney disease who are treated at WU, St. Louis Children's Hospital and St. Louis University Hospitals. The database contains tissue, blood and DNA samples, which are made available to researchers studying abnormalities in gene expression that occur in kidney disease. Four pilot and Feasibility projects are supported through an Administrative Core. Proceedings of state-of-the-art conferences by invited speakers are published quarterly in the journal *Organogenesis*, as the WU CKDR sponsored Organogenesis Forum. The WUCKDR invites the participation of any qualified investigator. More detailed descriptions of Cores and applications are available on its website: http://renal.wustl.edu/OBRC.html.

Disclosure of Financial Relationships: nothing to disclose

**PUB653**

**Feasibility Studies for Delivering Renvela® (Sevelamer Carbonate) for Oral Suspension Using a Feeding Tube** Chris Y. Ho, Abizer Harianawala, Martin Hanus, Suchitra Venugopal, Sunita Goyal, Hitesh Bhagat. *Drug and Biomaterial R&D, Genzyme Corporation, Waltham, MA.*

Purpose: To demonstrate that Renvela® for oral suspension can be administered to patients using a feeding tube. Sevelamer carbonate is a polymeric amine that binds phosphate and is indicated for the control of serum phosphorus in patients with chronic kidney disease on dialysis. Sevelamer carbonate is hygroscopic but insoluble in water.

Methods: The commercially available feeding tubes ranging from 3.5FR (opening)/58 cm length to 18FR/ 122 cm length composed of silicone, polyurethane or polyvinyl chloride (PVC) were selected for evaluation. To simulate the worst case scenario, for losses in delivery, 0.2 gm dose was used. For clogging studies, a high dose of 2.4 gm was used. The delivered drug was quantitated by using a total titratable amines procedure. The clogging end point was based on the inability of the Renvela® suspension to be delivered from the feeding tube.

Results: Clogging was observed with feeding tubes that had very small openings of 3.5 FR or were very long with length of 125 cm. Acceptable drug delivery (90 to 110% of target dose, with less than 6% RSD) was obtained with all of the feeding tubes that did not clog.

Conclusion: Sevelamer carbonate for oral suspension ranging from 0.2 gm to 2.4 gm dose can be delivered accurately using commercially available feeding tubes.

Disclosure of Financial Relationships: nothing to disclose

**PUB654**

**Investigation of Dose Dividing Techniques for Renvela® (Sevelamer Carbonate) for Oral Suspension 2.4 gm** Chris Y. Ho, Abizer Harianawala, Suchitra Venugopal, Martin Hanus, Sunita Goyal, Hitesh Bhagat. *Drug and Biomaterial R&D, Genzyme Corporation, Waltham, MA.*

Purpose: To develop dose dividing techniques for Renvela 2.4 gm packet using dosing options available over-the-counter.

Background: Each 2.4 gm packet of Renvela® powder contains 2.4 gm of sevelamer carbonate active moiety, which is a non-absorbed, phosphate binding, cross linked polymer. Sevelamer carbonate is indicated for the control of serum phosphorus in patients with chronic kidney disease on dialysis.

Methods: Two different dose dividing techniques were developed using either an oral syringe or a graduated dosing spoon. For the method using an oral syringe, an entire packet of Renvela® powder containing 2.4 gm of sevelamer carbonate was transferred to a cup. Sixty ml of water, measured by a 30 ml or 60 ml oral syringe, was added into the cup to form a suspension. Using the same syringe, 20 ml or 40 ml of the suspension was withdrawn to provide 0.8 gm or 1.6 gm of sevelamer carbonate for dosing, respectively. The remaining sevelamer carbonate suspension was discarded. For the method using a 10 ml over-the-counter graduated dosing spoon, Renvela® powder was poured into the dosing spoon to a specific volume, 2 ml for 0.8 gm and 4 ml for 1.6 gm of sevelamer carbonate. The measured powder was then transferred into a cup, hydrated with water for 5 minutes to form a suspension for dosing. The divided doses obtained by both methods were analyzed *in vitro* for reproducibility and average dose delivered.

Results: Both dose dividing techniques delivered results of 90 to 110% with acceptable % relative standard derivation (RSD) showing that both techniques deliver accurate quantity of sevelamer carbonate from a 2.4 gm Renvela® packet.

Conclusion: Two dose dividing techniques were developed using an oral syringe and graduated dosing spoon available at the pharmacy. *In vitro* testing confirmed that 0.8 gm and 1.6 gm of sevelamer carbonate powder could be dispensed accurately. These practical dose dividing techniques can provide flexibility for clinicians to administer 0.8 gm or 1.6 gm of sevelamer carbonate from a 2.4 gm Renvela® packet.

Disclosure of Financial Relationships: nothing to disclose

**PUB655**

**Nephronpower and the Online Transplant Center; Blogging as an Educational Tool** Kenar D. Jhaveri. *Nephrology, North Shore/LIJ & Hofstra Medical School, Great Neck, NY.*

To help aid the educational material at the disposal to renal fellows in training and transplant physicians in training, I have created two web based teaching tools (blogs).

The first is www.nephronpower.com

The blog focuses on all aspects of nephrology and covers topics from history of nephrology to basic science to quizzes and puzzles. The point is to make short and easy to read blog posts to get the point across. There are several sections:

In the news: which discuss a recent publication of interest with a brief synopsis;

Topic discussion: a specific topic that is discussed in slightly more detail;

Consult rounds: a case based discussion of a specific question; Journal Club: A peer reviewed article that is of interest being discussed.;

Educational material such as quizzes and board type review questions also appear on it to make it a more interactive teaching tool. The entries are entered by faculty and fellows based on interesting topics being discussed in core lectures, consult cases or personal experiences.

The second blog is www.onlinetransplantcenter.blogspot.com ; a specific tool for transplant physicians with a similar educational focus but centered more around the field of transplantation medicine and surgery. There are numerous contributors in this blog from multiple institutions and allows for an educational dialogue of basic science to clinical topics.

These blogs were implemented less than a year ago and there are already 151 blog posts on nephronpower and 51 on the onlinetransplantcenter.

A site-meter reading on nephronpower shows that in the last few months, the average visits have increased from 100/month to 1500/month and the time spent per visit is on average 4 minutes. The world map depicts visitors from all continents visiting this site regularly.

I believe that tools like these allow fellows to actively learn and faculty to actively teach in a more creative manner. Links to other popular blogs that are internal medicine and nephrology related enhance the website and allow for cross transfer of knowledge.

Disclosure of Financial Relationships: nothing to disclose

## PUB656

**CROWNWeb – Expanding CPM Collections** Matthew J. Mcdonough. *Renal Requirements Communications and Training, FMQAI - The Florida ESRD Network, Tampa, FL.*

CROWNWeb software application is a mandatory-use ESRD data collection application developed by CMS for all Medicare-certified dialysis facilities.

The Centers for Medicare & Medicaid Service (CMS) will use the CROWNWeb data-collection system to provide the renal community information to help better gauge their facility's performance and patient outcomes by utilizing the system to collect an expanded list of clinical performance Measures (CPMs) including new CPMS related to patients' infections, medication allergies, and hospitalizations.

CROWNWeb currently collects data for the 26 CPMs adopted by CMS on April 1, 2008 to monitor the quality of care dialysis facilities administer to ESRD patients, and will utilize the new CPMs to help determine if these factors might impact a patient's course of treatment.

**Elements of the New CPMs**

Over 112 new CPM data elements have been created for data entry in CROWNWeb. Of those, 69 are mandatory for use in the future CPMs.

**Infections**

Twenty-Five (25) measures will be collected in CROWNWeb. These measures include, but are not limited to: the patient's dialysis access infection type, the source of vascular infection, and the date antibiotics were prescribed.

**Medication Allergies**

CROWNWeb will use over 31 new elements and measures to help report a patient's medication allergies. These refer to the possible Erythropoiesis Stimulating Agent (ESA), iron, vitamin D, etc. to which a dialysis patient may be allergic (i.e. Epoetin Alfa, Calcijex, Zemplar, etc.).

**Hospitalizations**

Fifteen (15) hospitalization attributes will be collected in CROWNWeb, including: the name of the hospital at which the patient was admitted, the hospital discharge date, and the hospital admission date.

**CROWNWeb Release Schedule**

CROWNWeb's second release is scheduled for January 2011. With the beginning of the expanded bundle and the impending national release of CROWNWeb, it is essential for Medicare-certified dialysis facilities to understand the scope of the project and the various gauges by which CMS will monitor facility performance.

Disclosure of Financial Relationships: nothing to disclose

## PUB657

**Prospective Evaluation for Treatment Compliance with Phosphate Binding Agents in Patients on Haemodialysis (HD): Preliminary Results of COMQUELFOS Study** Rafael Perez-Garcia,[1] Dolores Arenas,[2] Ana Blanco,[3] O. Reatiga,[4] M. C. Prados,[5] F. Rios,[6] Jose L. Lerma.[7] *[1]H Infanta Leonor; [2]H Perpetuo Socorro; [3]Dialcentro FMC Madrid; [4]FMC Reus; [5]H Torrecardenas; [6]FMC San Luciano; [7]H Salamanca.*

Introduction: The efficacy of phosphate binders (PB) is directly related with the treatment compliance (TC). Knobel and al. evaluated the Simplified Medication Adherence Questionnaire (SMAQ)[1]. With the criteria of TC and SMAQ, we evaluate the adherence of the patients on HD to PB treatment.

Methods: 288 patients were included in this multicenter, open-label trial, who met the indication for PB treatment. The patients had a basal phosphate (P) levels ≥5.5 mg/dl. It was considered "adherent treatment patients' those that had TC>75%, positive SMAQ and P≤ 5 mg/dl in the following visits (5 months). The therapeutic strategy to improve the adherence was studied.

Results: 105 patients were analyzed (62♂-43♀) with a means age and P were respectively; 61.3 (23-87) years and 6.3 (5.4-10.2) mg/dl. 56 patients (53.3%) were considered non adherent to treatment and continued at subsequent visits. The most common strategies to improve the adherence were: to insist in the compliance (51.4%), change to other PB (35.5%) and to increase the dose (15.9%). Finally, 85.2% of the total patients (56) were adherent. Table1 show, the PB used in these patients.

| Phosphato Binders (PB) | Non adherent patients' (Basal visit) (n=56) | Adherent patients' (5 months)(n=24)* |
|---|---|---|
| | Nº PB / % | Nº PB / % |
| Mean of P (mg/dl) | 6.3 | 4.9 |
| Calcium-based | | |
| Calcium Carbonate | 18 / 24.6% | 7 / 17.5% |
| Calcium Acetate | 7 / 9.6% | 2 / 5% |
| Non calcium-based | | |
| Lantanum carbonate | 17 / 23.2% | 14 / 35% |
| Sevelamer | 22 / 30.1% | 9 / 22.5% |
| Aluminum Hidroxide | 9 / 12.3% | 8 / 20% |
| Total | 73 / 100% | 40 / 100% |

*At the moment of the analysis only 27 completed patients were available.

Discussion: In these preliminary results, it could not possible to know the most efficacies therapeutic strategy to improve the adherence. At last visit, Lanthanum Carbonate was the most frequently PB used in the patients that achieve to become in adherent patients.

[1]Knobel and al. AIDS 2202; 16(4):605-13.

Disclosure of Financial Relationships: nothing to disclose

## PUB658

**Nutrition Education Program in Hemodialysis: Short and Long Term Impact on Hyperphosphataemia Control** Clarissa B. B. Uezima,[1] Bárbara Margareth Menardi Biavo,[2] Mary E. C. Costa,[1] Elzo R. Junior,[2] João Paulo L. B. Martins,[1] Elvino Barros,[3] Carmen B. Tzanno-Martins.[1] *[1]Integrated Center of Nephrology, Guarulhos, São Paulo, Brazil; [2]Home Dialysis Center, São Paulo, São Paulo, Brazil; [3]Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.*

**OBJECTIVE**

To evaluate the short and long term effect in the control of hyperphosphataemia of a nutrition education program performed with hemodialysis patients.

**METHODS**

In January 2009, a dietary intervention was conducted in hyperphosphataemia patients. Study participants were those who had, in the last three months, an average serum phosphorus ≥ 5.5 mg/dL.

In January 2010, the same activities were conducted with participants of the previous year that had showed increased serum phosphorus levels. Concomitantly, the demographic and laboratory parameters were reevaluated.

**RESULTS**

In 2009, 86 patients with hyperphosphatemia participated in the nutritional intervention. Of these, 10 currently are no longer in the clinic due to transfer to another center, transplant or death.

In 2010, 41 (54%) of 76 patients who participated in the first intervention phase showed increased serum levels of phosphorus and they were subjected to educational activities again. The other 35 (46%) had levels of this mineral within normal limits. Both groups make use of phosphate binder.

After the nutritional intervention, a decrease in serum phosphorus was observed from 6.8 ± 0.90 to 5.9 ± 1.35 mg/dL; P <0.001.

Regarding nutritional status, the average BMI of this population was 23.7 ± 3.9 kg/m².

Urea and albumin levels were stable before and after intervention and Kt/V increased (1.2 ± 0.30 to 1.4 ± 0.50 P 0.004).

After the educational plan, there was a decrease in the number of patients in precontemplation and relapse, and an increase in the action stage.

**CONCLUSION**

There is a known link between the attendance of patient groups who share the same problem and achieving positive results in programs to adopt proper eating habits. However, through this study, it was observed that these group activities should be carried out more frequently to ensure compliance to the dietary guidelines in the long run.

Disclosure of Financial Relationships: nothing to disclose

## PUB659

**Variables Associated with CMV Infections after Kidney Transplantation** Willy Aasebo,[1] Hallvard Holdaas.[2] *[1]Medical Depatment, Akershus Universtity Hospital, Lorenskog, Norway; [2]Medical Department, Oslo University Hospital, Rikshospitalet, Oslo, Norway.*

**Background**. Rejection episodes, recipient age and CMV IgG positive donors to CMV IgG negative recipients have been reported as risk factors for CMV infection after kidney transplantation. In this report associations between CMV infection and a wide variety of variables present at the time of transplantation (n = 32) and variables occurring during the first 10 weeks after transplantation (n = 10), were assessed.

**Methods**. All patients >18 years of age who received a kidney allograft in 2005, 2006 or 2007 were included (n = 593). The standard immunosuppressive protocol consisted of steroids, mycophenolate and CNI. Induction therapy with Basiliximab, in addition to lower doses of cyclosporine, was introduced in 2007. CMV-PCR was taken at least once a week during the first 10 weeks after transplantation. Odds ratio (OR) for CMV infection was explored with univarite and multivariate logistic regression. In the multivaiate regression variables from at transplantation and variables acquired during the first 10 weeks after transplantation were separated in analyses.

**Results**. In all 232 (39 %) had CMV infection. The following variables increased OR in univariate regression analysis: CMV IgG +/- (1.6, p = 0.022), recipient age (1.02, p = 0.0003), prophylactic antibiotics (1.8, p = 0.0006), living donor to deceased donor (1.5, p = 0.020), bacterial infections (1.5, p = 0.016), reoperation (1.8, p = 0.0072), rejection (2.7,

p <0.0001), the use of Cyclosporine (2.5, p <0.0001). The following variables decreased OR: Basiliximab induction therapy (0.6, p = 0.0002), use of immunosuppressants before Tx (0.5, p = 0.0007).

**Conclusion**. New variables associated with CMV infection were identified. Of special interest, and after multiple regression analyses, were: prophylactic antibiotics (OR: 1.4, p = 0.008), use of Cyclosporine (OR: 2.6, p <0.0001), Basiliximab (OR: 0.6, p = 0.002) and immunosuppressive treatment before Tx (OR: 0.5, p = 0.006).

Disclosure of Financial Relationships: nothing to disclose

**PUB660**

**Intra-Operative Ureteral Stents: An Overlooked Cause of Severe Proteinuria Following Kidney Transplantation** C. Afaneh,[1] D. Morrone,[2] David Serur,[2] M. Abreu-Goris,[1] Jun B. Lee,[2] Thangamani Muthukumar,[2] Choli Hartono,[2] D. Leeser,[1] S. Kapur,[1] Manikkam Suthanthiran,[2] Darshana Dadhania.[2] *[1]Transplant Surgery, Cornell Univ.; [2]Transplant Medicine, Cornell Univ.*

Post-transplant (txp) proteinuria is a commonly observed phenomenon. Nephrotic range proteinuria can be indicative of recurrent glomerulonephritis and necessitate early biopsy & intervention. Benign reversible causes of proteinuria are rare. However, encrustation of ureteral stents (US) has been associated with urinary protein excretion. It is of interest that US are commonly placed at the time of renal txp. In this study, we investigated the hypothesis that renal allograft recipients with US have increased levels of proteinuria in the early post-txp period & US removal is associated with prompt improvement in urinary protein excretion.

We retrospectively reviewed 19 renal allograft recipients who had US placed. Inclusion criteria was >2g/day of proteinuria in the early post-txp period. We compared proteinuria levels at 2 weeks (wks) post-txp with the US in place to proteinuria at 1 & 4 wks post-US removal.

The median proteinuria with US in place was 2.98 g/day (range 2.30-9.51) & was significantly reduced to 0.50 g/day 1 wk after US removal (P<0.0001) & remained low at 0.36 g/day 4 wks following removal (Fig.1A) The mean (±SE) serum creatinine (SCr) also significantly decreased from 2.19±0.15 mg/dL with US in place to 1.90±0.12 mg/dL at 1 wk & 1.75±0.11 mg/dL at 4 wks post removal (P<0.0001, Fig.1B). There was no correlation between SCr & level of proteinuria at 2 wks post-txp ($r_s$=0.19, P=0.43).

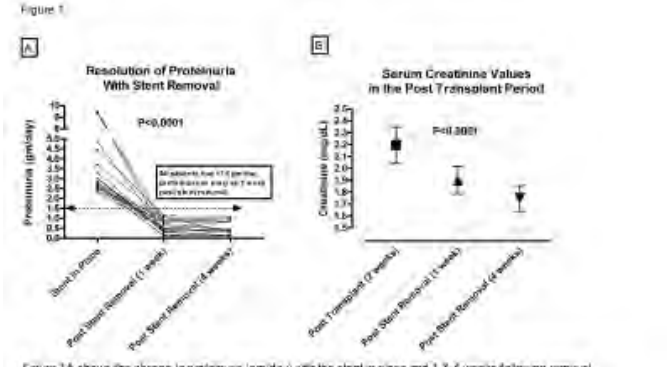



Figure 1A shows the change in proteinuria (gm/day) with the stent in place and 1 & 4 weeks following removal.
Figure 1B shows the mean and standard deviation of the serum creatinine (mg/dL) for the various time points.

Our findings demonstrate that US are associated with significant proteinuria in renal allograft recipients, and their removal is associated with prompt reduction in proteinuria. Thus, US appear to be a new and thus far unrecognized cause of significant proteinuria in renal allograft recipients.

Disclosure of Financial Relationships: nothing to disclose

**PUB661**

**Incidence of BK Viruria in Renal Allograft Recipients with Hepatitis C and Its Correlation with Chronic Allograft Nephropathy** Sana R. Akbar,[1] Sadanand S. Palekar,[1] Sachin H. Sachdev,[1] Adeem Akbar.[2] *[1]Department of Nephrology and Renal And Pancreas Transplant, Newark Beth Israel Medical Center, Newark, NJ; [2]Internal Medicine, St. Luke's Hospital, Bethlehem, PA.*

INTRODUCTION: Chronic allograft nephropathy (CAN) is a major cause of renal allograft failure post renal transplantation. The purpose of our study was to look at patients with hepatitis C who are renal allograft recipients & to see the incidence of BK viruria in this cohort along with its association with CAN.

METHOD: We retrospectively reviewed charts of renal transplant recipients from 1995-2009 at our transplant center who were diagnosed with hepatitis C prior to transplantation. Primary outcome was overall incidence of BK viruria in patients with hepatitis C with renal allografts. Secondary outcome looked at the incidence of CAN in this cohort & presence of other viral infections. CAN was defined as per biopsy findings or creatinine ≥1.5mg/dL with proteinuria in those without a biopsy.

RESULTS: 38 patients with Hepatitis C between ages of 26-78 years were identified.81.6% were males. 9/38 (23.7%)had BK virus urine PCR > 10,000 copies, while 4 patients were not tested. Of 9/38 patients with BK viruria with Hepatitis C, 100% had CMV (IgG).Of these 5/9(55.6%)had CAN. Meanwhile 20/38 patients (52.6%)who had hepatitis C and CMV but no BK viruria, 35% had CAN. There were no cases of CAN in patients who had hepatitis C and BK viruria without CMV. 4/38 (10.5%) patients in this cohort had HIV & 2/4 (50%)had BK viruria & despite presence of hepatitis C & BK viruria did not develop CAN. Overall 15/38(39.5%) patients had CAN. 8/15 had biopsy proven CAN. Overall 3/38(7.9%) allografts were lost to CAN.

CONCLUSION: BK viruria incidence is not very high in renal allograft recipients with Hepatitis C, none the less renal transplant recipients with triple viral infections (hepatitis C, CMV, BK virus) have a much higher incidence with higher risk of CAN than those with dual infection with hepatitis C and CMV only. This also suggests that BK virus may be potentiating CAN in this cohort of patients who are already immunosuppressed & susceptible. The presence of HIV was not related to increased incidence of CAN.

Disclosure of Financial Relationships: nothing to disclose

**PUB662**

**NGAL in Urinary Exosomes as a Source of Kidney Dysfunction Biomarker in Renal Transplantation** Andres Boltansky,[1] Sergio Alvarez,[1] Antonio Vukusich,[1] Margarita Hurtado,[1] Marcela Ursu,[1] Guilio Innocenti,[1] David Carvajal,[1] Cristian Suazo,[2] Sandra Villanueva,[2] Juan Carreno,[2] Rogelio Altuzarra,[2] Constanza Yen,[2] Denisse Tapia,[2] Carlos E. Irarrazabal.[2] *[1]Unidad de trasplante, Clinica Davila, Santiago, Chile; [2]Laboratory of Molecular Physiology, University of Los Andes, Santiago, Chile.*

The End Stage of Renal Disease (ESRD) require for its treatment of permanent dialysis or kidney transplantation (KT). The KT is the best clinical treatment; however, the early function of the allograft varies depending of multiple factors, associated with cold ischemia time (CIT) and the allograft rejection process. It is known that serum creatinine is a late marker for predicting graft recovery after kidney transplantation, mainly in patients with delayed graft function (DGF).

NGAL is produced in the distal nephron and it is one of the most promising novel biomarkers for acute kidney injury (AKI). NGAL has been proposed as a predictor of organ recovery from delayed graft function after kidney transplantation.

The exosomes are vesicles released into the urine from the kidney epithelium and it has been proposed as better source to explore biomarker of renal dysfunction. We propose that determination of NGAL in urinary exosomes is a better predictor of kidney dysfunction after KT than others urinary fractions.

We analyze 15 kidney-allograft recipients (16-60 years): 11 living (LD) and 4 deceased donors (DD). The average of CIT was 14 hours in DD and less than 1hr in LD. Three patient developed DGF. Using Western blot analysis NGAL was detectable from the first post operative day (POD) in cellular and exosomal fraction of the urine, but not in whole urine. The exosomes expressed higher levels of NGAL than cellular fraction. We noticed that NGAL expression in exosomas, but not in the cellular fraction, was elevated in the patients with DGF compared with non-DGF patients (p<0.05) from 1-3 POD and correlates with serum creatinine. This preliminary study suggests the exosomal fraction could be more sensitive substrate to evaluate early biomarkers of DGF after kidney transplantation.

Disclosure of Financial Relationships: nothing to disclose

**PUB663**

**Synchronous Bronchogenic and Gastric Carcinoma in a Renal Transplant Recipient** Amarpali Brar,[1] Fasika M. Tedla,[1] Moro O. Salifu,[1] Dale Distant,[2] Syed I. Shah,[1] Gurinder S. Sidhu.[1] *[1]Medicine, SUNY Downstate Medical center, Brooklyn, NY; [2]Surgery, SUNY Downstate Medical Center, Brooklyn, NY.*

Solid organ transplant recipients (TR) are at increased risk of developing cancer; commonly Kaposi sarcoma and lymphomas. There are limited data to suggest increased risk of epithelial malignancies in TR.

A 60-year-old man with hypertension, diabetes, peripheral vascular disease, coronary artery disease and end stage renal disease underwent renal transplant 2 years prior to presenting with upper abdominal discomfort. He was an active smoker and had smoked for 100 pack years. He denied any family history of cancer. His physical exam at the time was remarkable only for obesity and left leg amputation. His medications included tacrolimus and prednisone. Upper gastrointestinal endoscopy showed a mass in the stomach and pathology revealed the diagnosis of gastric adenocarcinoma. Staging CT scan showed no evidence of metastatic disease in the liver or other parts of the abdomen, but a right upper lobe spiculated mass was found. Trans-thoracic needle biopsy of the lung mass showed a second malignancy- non-small cell lung cancer, TTF-1 positive. The lung cancer was morphologically distinct from the gastric cancer. Patient underwent gastrectomy and pathologic review showed adenocarcinoma of intestinal type with 4/8 nodes involved; T2b N1, TNM stage II. Subsequently he underwent right upper lobectomy with mediastinal and hilar lymph node sampling. The pathologic review showed squamous cell cancer and 0/8 nodes involved; TNM T2N0M0, Stage 1B. Patient did well after both surgeries and was then given adjuvant therapy for gastric cancer with 5-flourouracil and radiotherapy. One year after the initial diagnosis he continues to do well, and is disease free.

Unlike Kaposi sarcoma or lymphoma; epithelial malignancies are not known to be related to immunosuppressed state. This case illustrates a unique scenario with a patient on immunosuppressive therapy having developed two synchronous non-immunosuppression related cancers. The development of the two cancers in this case could be related to smoking. This case is the only known case of two synchronous primary cancers in a TR.

Disclosure of Financial Relationships: nothing to disclose

**PUB664**

**Clinical Implications of Polyomavirus-Associated Nephropathy after Renal Transplantation** Helen P. Cathro,[1] Jason C. Gardenier,[2] Douglas Scott Keith,[3] Robert Sawyer,[2] Kenneth L. Brayman,[2] Hugo Bonatti.[2] *[1]Pathology, University of Virginia, Charlottesville, VA; [2]Surgery, University of Virginia, Charlottesville, VA; [3]Medicine, University of Virginia, Charlottesville, VA.*

BK virus nephropathy (BKVN) develops in ~5% of renal transplants (RT), causing graft loss in 15-80% of cases within 5 years. Most studies suggest that the majority of BKVN occurs during the first post-transplant (PT) year. We noticed an increasing number of cases of late-onset BKVN and conducted a retrospective study.

All renal specimens from patients with biopsy-proven BKVN from 2000-2009 at a single institution were reviewed.

Of 846 RT recipients, 18 had biopsy-proven BKVN (2.1%), 4 of whom had also received pancreatic transplants. The median age of 12 males and 6 females was 52.2 yr (range 27.9-63.8 yr), and the median time PT was 20.1 mn (range 3.2-80.4 mn), with only 4 or 22%, <1 yr out. Fourteen patients were on standard immunosuppression (IS). Screening for BK virus was erratically administered due to geographical/logistical constraints. Five patients had prior biopsy proven episodes of acute rejection and 13 patients (72%) had received intensive prior IS, usually for acute rejection (n=10) or for subsequent pancreas transplantation. Fourteen patients were treated with antiviral therapy, +/- IVIG, +/- IS taper, and single patients were treated with IVIG or IS taper alone. One patient died after returning to dialysis, and 17 were alive at an average of 3.4 yr follow up. Seven of these had returned to dialysis (41%), and 5 had a serum creatinine >2 mg/dL (29%). Only 5 patients had good graft function (29%).

Retrospective analysis of BKVN at a single institution during the current IS era demonstrated 78% of cases occurring after the first PT year, and 39% after the second PT year. Inconsistent screening and over-IS may be playing a role. The poor outcome of 72% of the BKVN cases is in part due to late diagnosis at a severe stage of disease. Late onset BKVN is an underrecognized cause of graft loss and dysfunction, and rigorous screening followed by tapering of IS on positive testing, should be emphasized beyond the first year post-transplantation.

Disclosure of Financial Relationships: nothing to disclose

**PUB665**

**Expanded Criteria Donors and Histological Scoring: Impact on Graft Survival of Kidneys with Prolonged Ischemia and Delayed Graft Function** Carlos R. Chiurchiu,[1,2] Javier De Arteaga,[1,2] Verónica Riva,[3] Maria Virginia Burguesser,[3] Walter Guillermo Douthat,[1,2] Jorge Luis De la Fuente,[1,2] Ana Diller,[3] Pablo U. Massari.[1,2] *[1]Renal Transplant Program, Hospital Privado-Centro Medico de Cordoba, Cordoba, Argentina; [2]Postgraduate School of Nephrology, Catholic University of Cordoba, Cordoba, Argentina; [3]Pathology Service, Hospital Privado-Centro Medico de Cordoba, Cordoba, Argentina.*

The use of expanded donors or kidneys with preexisting chronic damage remains controversial but they offer the opportunity for donor pool expansion. We investigated the impact of these conditions as predictors of graft survival (GS) in a cohort of recipients with prolonged cold ischemia time (CIT) and high incidence of DGF.

Seventy consecutive cadaveric kidney allograft implanted between 2001 and 2005 with an early graft biopsy (mean 7.4 days post-Tx) were included in the study. All kidneys were preserved with Wilconsin and HTK solutions. Donor age was 40.4±15.6 y (37% >50 y), CIT: 22.2±9.6 hours, (median 20 hours, 41% >24 h), 63% with cerebrovascular accident, expanded donors 37%. DGF was present in 84% and oliguria was 13,5±12.5 days. Moderate-severe acute tubular necrosis was present in 71% of early biopsies; moderate histological scoring (MHS: 4-6) 63%, severe (SHS: ≥7) 27%, ideal (1-3) 0% and acute rejection 14,3%.

At 48 months GS was 73,3%, when stratified according MHS vs. SHS: 81% vs. 63% (p: n.s.), donor age (≤ 50 y vs. older): 89% vs 41% (p<0.002), cause of death (cerebrovascular vs. others) 62% vs. 92% (p<0.03), type of donor: (standard vs. expanded) 90% vs 41% (p<0.0003), CIT (>24 h vs rest) 73% vs. 74% (p:n.s.). Multivariate analysis for GS: SHS 1.23 (IC 95% 0.88-1.70, p<0.2), donor age 0.96 (IC 95% 0.87-1.05, p<0.4), death other cerebrovascular accident 0.43 (IC 95% 0.02-6.60, p< 0.5), standard donor 0.12 (IC 95% 0.01-0.87, p< 0.03), days of oliguria 1.02 (IC 95% 0.98-1.05, p< 0.3). Primary non-functioning kidneys were more frequent in expanded donors than standard (20.0 vs 0.0%, p<0.002)

Our results suggest that in allografts with prolonged ischemia and preexisting histological chronicity, only the presence of expanded criteria is a significant independent donor risk factor for graft outcomes.

Disclosure of Financial Relationships: nothing to disclose

**PUB666**

**Deconvolution of the Molecular Mechanisms Underlying the Negative Effects of Calcineurin Inhibitors on the Kidney** Amanda E. Crunk,[1,2] Christopher J. Rivard,[2] Uwe Christians,[1] Jelena Klawitter.[1] *[1]Anesthesiology, University of Colorado Denver, Aurora, CO; [2]Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Modern immunosuppressive drug regimes have significantly increased the one year graft survival rate of kidney grafts, however chronic allograft dysfunction is still a serious problem. Immunosuppressant-mediated nephrotoxicity is one of the major limiting factors in terms of long-term survival. Calcineurin inhibitors (CNIs) like cyclosporine and tacrolimus are known to contribute to chronic allograft dysfunction. Even after 30 years of clinical use, surprisingly, the molecular mechanism of CNI nephrotoxicity is not yet fully understood. Despite the known risks of CNI use, most transplant patients today are still discharged with CNI-based immunosuppressive drug regimes. At an early stage, CNI seem to target proximal tubules, but the effects of CNIs on other regions of the kidney have never systematically been studied. The inner medulla of the kidney, containing the collecting duct cells, is under a significant amount of stress due to its function to concentrate urine and thus being exposed to very high tonicities. We hypothesize that these collecting duct cells are sensitive to CNIs since they require significant amounts of energy to survive in their environment, however, CNIs are known to derail energy metabolism. In order to test this hypothesis we exposed two rat cell lines, the immortalized rat proximal tubules (IRPTC) and the rat inner medullary collecting tubules (RIMCT), to various concentrations of tacrolimus and determined their survival using the Invitrogen Cyquant NF assay. The results showed that the collecting duct cells had an EC50 of 20 µg/ml and the proximal tubules cells had and an EC50 of 60 µg/ml. These data indicate that proximal tubules cells, and not the collecting duct cells, are more resistant to the effects of tacrolimus which is in contrast to histological observations. A systematic proteo-metabonomic approach has focused on the mechanisms of toxicity in RIMCT cells and the effect that CNI-induced endothelial dysfunction may have in sensitizing proximal tubules to CNI nephrotoxicity in vivo.

Disclosure of Financial Relationships: nothing to disclose

**PUB667**

**Hypomagnesaemia after Renal Transplantation (R-TX)** Antonio Di Felice, Emiliana Ferramosca, Marialetizia Soverini, Benedetta Ferri, Antonio Santoro. *Division of Nephrology Dialysis and Hypertension, St. Orsola-Malpighi University Hospital, Bologna, Italy.*

**INTRODUCTION.** Hypomagnesaemia (Hypo-Mg) is an electrolyte disorder frequently present in R-TX, whose pathogenesis is unclear. It has been associated to a cyclosporine-related urine loss, but it can be induced by other drugs, like proton pump inhibitors (PPI), widely employed in gastric protection of R-TX. From the clinical point of view, H-Mg can determine arrhythmias, seizures, hypocalcemia; same experimental studies have suggested that Mg supplementation inhibits cyclosporin-induced chronic nephropathy in experimental animals. Aim of this study is to evaluate incidence of H-Mg after R-TX and its possible relationships with drug therapy.

**PATIENTS AND METHODS.** 96 renal transplanted patients have been studied, age 21-79 yrs; TX age 2-290 months. Creatinine was between 0.8 and 2.1 mg/dl. All patients were on immunosuppressive treatment with calcineurin inhibitors (CNIs), 62% cyclosporin (Cs-A) and 38 % tacrolimus (FK); 61 patients at least (74.4%) were on treatment with PPI.

**RESULTS**. Hypo-Mg was detected on 30 of the 96 examined patients (31.2%), age 56.6±12.6 years (31-76), age of TX 119.3±77.7 months (10-290). 17 of them (56.7%) were on Cs-A therapy (43.4%), and 13 (43.4%) with FK; 27 patients (90%) were on PPI therapy. Only one patient showed a high urinary excretion of Mg. In 10 patients Hypo-Mg persisted despite of oral supplementation. Serum creatinine was 1.34±0.46 mg/dl (0.81-2.36); CNI blood levels were all in therapeutic range.

**CONCLUSION**. 1) A significant incidence of Hypo-Mg after R-TX is confirmed. 2) Hypo-Mg does not appear to be determined exclusively by an elevated urinary loss, but other mechanisms have to be considered (altered abdominal absorption?). 3) Hypo-Mg is present both in Cs-A patients and in FK treated. 4) From the etio-pathogenic point of view, drug hypothesis seems to be relevant, particularly regarding PPI, as well as CNIs. It is therefore necessary to accurately evaluate the indication and the type of gastric protection in R-Tx. 5) More studies are needed to evaluate the possible impact of chronic Hypo-Mg on transplanted kidney long-term function.

Disclosure of Financial Relationships: nothing to disclose

**PUB668**

**Swine Flu (H1N1) in a Liver and Kidney Transplant Patient: A Case Report** Remedios Garofano, Clara Moriana, Manuel Ángel Rodríguez, Mª Dolores Del Pino Pino, Ma del Carmen Prados Soler. *S. Nephrology, C. H. Torrecárdenas, Almería, Andalucía, Spain.*

INTRODUCTION

Swine flu (H1N1) is a new virus resulting from the association of RNA segments of the influenza virus of porcine, aviary and human origin. The incubation period is 1-7 days Contagion between persons occurs via the airway or by contact and is inactivated by desiccation, detergents, alcohol and bleach. Complications include decompensation of the underlying disease or development of viral pneumonia, with respiratory failure and acute respiratory distress syndrome.

CASE REPORT

An 17-year-old woman who received a liver and kidney transplant in June 06 and was being treated with tacrolimus, mycophenolate mofetil and steroids. She was referred to the hospital in July 09 with a diarrhea with mild anemia and leucopoenia and slight worsening of renal function. On admission, the dosage of MMF was reduced and the diarrhea ceased, coproculture and PCR-CMV were negative. The patient's status worsened progressively with onset of high fever, severe worsening of renal function with a plasma creatinine and moderate pancytopenia but with severe leucopoenia, requiring withdrawal of MMF and treatment with filgrastim, plus empiric antibiotic therapy. Blood and urine cultures were positive for E. coli. The infectious symptoms of urinary origin remitted with specific antibiotherapy, the fever ceased and the pancytopenia and renal function improved. Three days later she again had febrile peaks up to 40ºC, accompanied by irritative cough, myalgia and general malaise, compatible with pseudoinfluenza symptoms. PCR for influenza A was reported to be positive on the day the patient was discharged, fully recovered.

CONCLUSION

Since the start of the Swine flu pandemic in Mexico in March 2009, the prevalence of infection has continued to rise. The first confirmed case of Swine flu in Spain was in April 2009. Groups at risk include patients with mild-to-moderate kidney disease and transplant recipients. The most notable characteristics of our case were the early appearance and good evolution of the infection, with no relevant sequelae despite the presence of factors and complications that could give rise to it.

Disclosure of Financial Relationships: nothing to disclose

**PUB669**

**Evaluation of Polyomavirus BK-Associated Nephropathy** Veronica M. Gonzales, Madeleine V. Pahl, Clarence E. Foster III, Renee R. Weng. *University of California, Irvine, Orange, CA.*

*Purpose.* To determine if proposed risk factors for developing polyomavirus BK-associated nephropathy (PVAN) from prior literature exist and what therapies were most effective at preventing graft deterioration in our transplant population. Prior studies have suggested that anti-thymocyte globulin induction, male gender, and deceased donor kidneys may predispose transplant recipients to develop PVAN.

*Methods.* A retrospective chart review was conducted of all renal transplant recipients who had a positive BK virus PCR from Jan. 1st, 2001 to Dec. 31st, 2009. Data collected include demographics, immunosuppression, serum creatinine, PVAN treatment, and renal biopsy results. Based on the identified risk factors and efficacy of treatment options, an evidence based guideline for the screening and management of PVAN will be developed.

*Results.* Twenty three patients were identified from 243 renal transplant recipients. The median time to the first detection of BK virus was 10 months post-transplant. In BK-positive patients, 61% received deceased donor kidneys compared to 72% in the overall transplant population. Basiliximab induction was more common in the positive BK virus group (65% versus 57%). A greater proportion of patients were male in the positive BK virus group (74% versus 62%). There was no association with age, BMI or ethnicity. Treatment of BK virus was reserved for the 16 patients who had viremia and consisted of reduction in immunosuppressive therapy. Eight patients received leflunomide in addition to immunosuppression reduction. Seven patients had BK virus first detected on biopsy and all of those patients progressed to graft dysfunction regardless of treatment.

*Conclusions:* Risk factors from prior studies did not always correlate in our transplant population. The greatest risk was among patients who were less than 12 months post transplant. Routine screening for BK virus allows for early intervention which is critical to treating BK virus and preserving graft function. Decreasing immunosuppressive therapy and the addition of leflunomide were beneficial to patients who were diagnosed early.

Disclosure of Financial Relationships: nothing to disclose

**PUB670**

**Pandemic H1N1 Influenza A in Kidney Transplant Patients: Concerns on Clinical Management** Ana M. Gonzalez Rinne, Alejandra Alvarez, Patricia Delgado Mallen, Domingo Marrero, Lourdes Perez, Jose Gonzalez Posada. *Nephrology, Hospital Universitario Canarias, La Laguna, Tenerife, Spain.*

We studied the incidence, comorbidity and mortality of H1N1 influenza A in our renal transplant population from July to December 2009.

**Patients and methods:** Patients with influenza-like illness who seek medical assistance were tested to confirm diagnosis of pandemic H1N1 influenza A using real-time reverse transcriptase (rRT)-PCR from nasopharyngeal swabs. Prophylaxis with neuraminidase inhibitor (oseltamivir) was started at recommended doses at the begining of symptoms and mantained unless diagnosis was excluded. Only patients with expected complicated clinical course were admitted to Hospital.

**Results:** 12 (3,4%) out of 353 renal transplant patients followed in our center were diagnosed. Mean age was 42.9 years (19 to 69 years). Seven patients had associated comorbid illness and in most patients (75%) immunosuppression was based on combination therapy with prednisone, anticalcineurin inhibitor and mycophenolate. One patient got infected in the early post-transplant period (17 days), the remaining developed the infection in a later post-transplant stage (mean 82,3 months; 8 months to 17 years). Four (33%) cases showed a complicated evolution: one patient, in the early post-transplant period, heavily immunosuppressed for being highly sensitized, developed viral pneumonia with fatal outcome; two had superimposed bacterial infections which led to long hospital stay and in one patient with a severe acute rejection episode, concomitant H1N1 influenza A infection, delayed anti-rejection therapeutic strategy which contributed to graft loss.

**Conclusion:** Despite early treatment with antivirals, renal transplant patients are a population at high risk for serious complications in influenza epidemics. Special preventive strategies must be stressed in the early post-transplant period of highly sensitized (heavily immunossuppressed) patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB671**

**Clinical Relevance of Anti HLA Antibodies in Renal Transplantation in Living Donor Program: A Single Centre Study** Sanjay Gupta, Pranab Mahanta. *Nephrology, AIIMS, New Delhi, India.*

**Background:** Renal transplant recipients may develop de-novo anti HLA antibodies (HLA abs) that may lead to allograft dysfunction. Because of insufficient routine monitoring the exact incidence of humoral alloimmune responses during allograft dysfunction is still uncertain. Aim was to evaluate the clinical relevance of detection of de-novo HLA Abs during the episode of renal allograft dysfunction.

**Methods**: The study group consisted of 41 non HLA-identical living donor first renal transplant recipients (non-sensitized pre-transplant) with an episode of allograft dysfunction needing allograft biopsy. Anti-HLA class I antibodies (HLA-I Abs) and anti-HLA class II antibodies (HLA-II Abs) were tested by ELISA in study group and in the control group with stable graft function.

**Results**: Immunosuppressive regimen was in Tac/MMF/P in 17/41, Tac/Aza/P in 6/41, CsA/MMF/P in 10/41 and CsA/Aza/P in8/41. None received induction. Out of 41 allograft dysfunctions cases, acute cellular rejection (ACR) was seen in 10/41, C4d was positive in 2/10, Chronic rejection (CR) 9/41, Transplant glomerulopathy (TG) 4/41, Focal segmental glomerulosclerosis (FSGS) 7/41, Calcineurin Inhibitor toxicity (CNI) 8/41 and non specific changes 3/41. HLA-I Abs and HLA-II Abs were detected in 14/41 (34%) and 12/41 (29%) respectively. Ten cases had both Abs. Prevalence of HLA-I Abs was 70% in ACR, 44% in CR, 50% in TG, 14% in FSGS and nil in CNI and non specific histology. With HLA-I Abs, odd ratios (OR) for ACR was 8 [95% CI 1.4-157] and HLA-I Abs correlated negatively with CNI. Prevalence of HLA-II Abs was 50% in ACR, 44% in CR, 75% in TG and nil in FSGS, CNI and non specific histology. With HLA-II Abs, the OR for TG was 9 (95% CI 1.04-62.9). HLA-I Abs were present in 13/23 of immune mediated injury (ACR,CR,TG) vs 1/18 of non immune graft dysfunction (FSGS, CNI,non specific) with the OR for immune mediated graft dysfunction as 22 (95% CI 2.5-195).

**Conclusion**: Screening for HLA Abs during the episode of allograft dysfunctions discriminate between immune mediated rejection and non immune allograft dysfunction and help in tailoring immunosuppression. HLA Abs mediated allograft injury exists as a spectrum of renal injury.

Disclosure of Financial Relationships: nothing to disclose

**PUB672**

**Secondary Hyperparathyroidism Due to Ectopic Gland** Clara Moriana, Manuel Ángel Rodríguez, Remedios Garofano, Ma del Carmen Prados Soler, Mª Dolores Del Pino Pino. *S. Nephrology, C. H. Torrecárdenas, Almería, Andalucía, Spain.*

INTRODUCTION:

Chronic kidney disease (CKD) is associated with a cascade of events with negative affects on bone and mineral metabolism. Successful renal transplantation normalizes most uremic disorders seen in patients on dialysis. The incidence of secondary hyperparathyroidism is of 30-50%

CASE REPORT:

The patient was a 64-year-old man with hypertension and CKD. He received a kidney transplant in January 96. In February 07 he was diagnosed with secondary hyperparathyroidism

Ultrasound and scintigraphy of the neck showed no pathological findings. Given the possibility of an ectopic gland the patient underwent a repeat scintigraphy with a thoraco-abdominal scan, again with no findings of note. Treatment of the hypercalcemia was started with cinacalcet up to two full doses of 180 mg/d and bisphosphonates. In December 08, faced with persistent high levels of calcium and intact parathyroid hormone (iPTH), the imaging tests were repeated (ultrasound and scintigraphy). The echogram showed a vascularized, hypoechoic nodule measuring 14x13x12 mm located in the posteroinferior part of the left thyroid lobe, which was reported as an enlarged parathyroid gland. In January 09 the patient underwent parathyroidectomy, no hyperplastic glands or adenomas were found. The hypercalcemia and high iPTH figures persisted, and a CT of the neck and chest showed an intrathoracic, ectopic parathyroid gland in the upper right paratracheal mediastinum, which was removed in July 09

The patient experienced severe worsening of renal function , severe hypocalcemia, anasarca, moderate-severe pericardial effusion, atrial fibrillation and dysphonía. The patient has now completely recovered

CONCLUSIONS:

Management of persistent hyperparathyroidism is an important therapeutic objective after renal transplantation. The cause is usually an occult adenoma, with an incidence of ectopic parathyroid glands of 6-25%, with 55% of these glands situated in the mediastinum. Preoperative scintgraphy with Technetium-99m-Sestamibi and CT can aid the surgeon plan the best initial surgical approach. The complications that can result from parathyroid surgery include hypocalcemia and adynamic bone disease

Disclosure of Financial Relationships: nothing to disclose

**PUB673**

**Treatment of Recurrent FSGS Post Transplant with a Multi-Modal Approach Including High Galactose Diet and Oral Galactose Therapy** Tamim H. Naber,[1] Madhu C. Bhaskaran,[1] Xiaotong Wang,[3] Fouad Boctor,[3] Gregory P. Dilimetin,[5] Ernesto P. Molmenti,[4] Howard Trachtman,[2] Kenar D. Jhaveri.[1] *[1]Nephrology; [2]Pediatrics, NY; [3]Pathology; [4]Surgery, North Shore/LIJ, Hofstra School of Medicine; [5]Nassau Nephrology LLP, NY.*

**Introduction:** FSGS is a leading cause of steroid resistant nephrotic syndrome. 40% of patients with primary FSGS have elevated levels of a circulating factor that increases glomerular permeability to protein. FSGS recurs after transplant in about 30% of cases, with graft loss in up to 50% of these patients. Recurrence typically occurs early, often within hours post transplant, which suggests the causative role of the permeability factor (Palb).

**Case:** We report a case of a 46 year old caucasian woman with biopsy proven primary FSGS diagnosed in 1984 who had recurrence of her disease post living related renal transplant in December 2009. On a basiliximab induction and triple drug regimen, she developed massive proteinuria of 37gm/24 hours within two days after transplantation. This was followed by progressive deterioration in renal function with creatinine of 3.5mg/dL after a nadir of 1.5mg/dL. A kidney biopsy one week post transplant revealed

effacement of podocytes on EM, compared to the zero hour biopsy which was normal. Despite multimodal therapies with 15 treatments of plasmapheresis (TPE), 1 dose of IVIG, and 2 doses of rituximab (1gm); proteinuria remained in the nephrotic range (9 gm/24 hr). Three months after transplantation, she was started on a high galactose diet (Celery, Beets, fresh basil, milk & honey) and supplemental powder galactose 0.2g/kg PO BID. After 2 months, she came off TPE, her urinary spot protein to creatinine ratio stabilized at 0.5, despite a repeat biopsy that showed no change in EM findings. Her creatinine declined to 1.5mg/dL. Palb levels are pending.

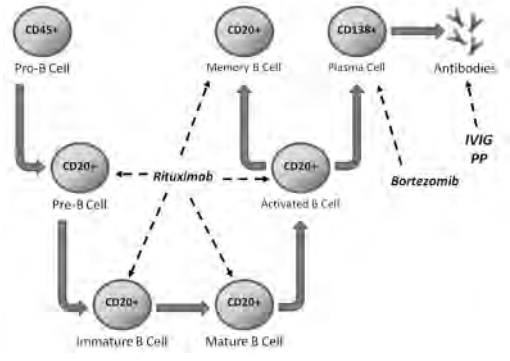
**Conclusion:** This case report suggests that administration of galactose may be a safe and effective means of reducing proteinuria in patients with recurrent FSGS after kidney transplantation. The findings support the performance of randomized clinical trials to assess the efficacy of this novel therapy in patients with this clinical problem.

Disclosure of Financial Relationships: nothing to disclose

**PUB674**

**Bortezomib in Kidney Transplantation: A Review** Rajeev Raghavan,[1] Abdallah Mohamed Jeroudi,[1] Katafan Achkar,[2] A. Osama Gaber,[3] Samir Patel,[3] Abdul A. Abdellatif.[1] *[1]Department s of Medicine and Nephrology, Baylor College of Medicine, Houston, TX; [2]Departments of Nephrology and Surgery, The Kidney Institute and The Methodist Hospital, Houston, TX.*

Introduction: Current therapies for pre-transplant desensitization and antibody mediated rejection (AMR) do not specifically deplete the plasma cells which produce anti-human leukocyte antigen (HLA) antibodies.




Bortezomib is a proteosome inhibitor which induces plasma cell apoptosis. It has been used off label in transplantation since 2005. We review this drug's biological effects, pharmacodynamics, and the current body of literature regarding its use for renal transplantation. Results: Bortezomib interferes with certain cell cycle regulatory proteins causing proteosome inhibition and apoptosis in CD138 positive plasma cells. Neurotoxicity is a dose-related side effect and may occur in up to 30% of treated patients. There are eight published case series using Bortezomib in kidney transplantation. Seven of the eight series show varying success in reducing donor specific antibodies or PRA.

Table 1: Characteristics and outcomes of published cases using Bortezomib as combination or solo therapy for AMR / desensitization (table abdridged for abstract).

| Author | N | Complete Therapy | Results |
|---|---|---|---|
| Wahrman et al (2010) | 2 | 2 cycles bortezomib and steroids | Decreased PRA |
| Walsh et al (2010) | 2 | 1 cycle boretzomib, pharesis, rituximab, and steroids | Reduction donor specific antibodies |
| Sberro-Soussan et al (2010) | 4 | solo therapy Bortzomib | No effect on antibody levels |
| Trivedi et al (2009) | 11 | 1 cycle bortezomib, pharesis, steroids | Reduction in donor specific antibodies |

Conclusions: Limited experience shows Bortezomib may have promise in manipulating the humoral arm of the immune system to manage desensitization and AMR; further clinical trials are needed.

Disclosure of Financial Relationships: nothing to disclose

**PUB675**

**Cases of Swine Flu in Kidney Transplant Patients in Almeria** Manuel Ángel Rodríguez, Remedios Garofano, Clara Moriana, Mª Dolores Del Pino Pino, Ma del Carmen Prados Soler. *S. Nephrology, C. H. Torrecárdenas, Almería, Andalucía, Spain.*

INTRODUCTION

Since the outbreak of the Swine flu pandemic in March 09, the number of cases worldwide has continued to grow. It usually presents as a mild flu-like syndrome, with rapid onset of high fever accompanied by general asthenia, myalgia and respiratory symptoms. Among those at risk are patients with mild-to-moderate renal failure and immunosuppressed persons

CASE 1

17-year-old woman who received a liver and kidney transplant 37 months previously and was on treatment with tacrolimus, mycophenolate mofetil and steroids. She was admitted in July 09 with a febrile syndrome and diarrhea. During her admission she had acute pyelonephritis due to E. coli, severe pancytopenia and acute worsening of renal function. After her total recovery but prior to discharge, she again had high fever and mild respiratory symptoms. A PCR diagnosis of Influenza A was informed on the morning of her discharge

CASE 2

A 38-year-old woman with CRF received a kidney transplant 9 months previously and was on tacrolimus and mycophenolate sodium. She was admitted in October 09 with a history of febrile syndrome and respiratory symptoms, with extensive bilateral pneumonia and acute severe respiratory failure that required admission to the ICU, where she remained for 5 days being treated with intensive oxygen therapy, broad-spectrum antibiotics and oseltamivir. Her course was satisfactory

CASE 3

A 38-year-old woman with CRF received a kidney transplant 2.5 months previously and was on treatment with tacrolimus, mycophenolate mofetil and steroids. She was admitted in November 09 with a two-week history of fever, myalgia, general malaise, cough and expectoration. The patient also had positive blood and urine cultures for E. coli. She was treated with an antibiotic according to the antibiogram and oseltamivir. Her course was satisfactory

CONCLUSION

The three cases of Influenza A in kidney transplant patients recorded in the province of Almeria occurred in young women, shortly after kidney transplantation and with no other risk factors apart from those associated with the transplant itself. From the consideration of the respiratory and renal situation, their course was good

Disclosure of Financial Relationships: nothing to disclose

**PUB676**

**Anemia Control in Kidney Transplant Patients Treated with Mircera® (Methoxy Polyethylene Glycol-Epoetin Beta)** A. Sánchez-Fructuoso,[1] L. Guirado,[2] J. C. Ruiz,[3] Jose-Vicente Torregrosa,[4] M. L. Suarez,[6] R. Gallego.[7] *[1]Hospital Clínico San Carlos, Madrid, Spain; [2]Fundación Puigvert, Barcelona, Spain; [3]Hospital Marqués de Valdecilla, Santader, Spain; [4]Hospital Clinic i Provincial, Barcelona, Spain; [5]Hospital Central de Asturias, Oviedo, Spain; [6]Hospital Dr. Negrín, Gran Canaria, Spain.*

**Background:** Kidney transplant (KT) is considered the gold standard treatment of chronic kidney disease (CKD) but anemia is an increasingly frequent complication after KT. Mircera® provides stable and sustained hemoglobin levels with once-monthly dosing in CKD patients.

**Methods:** Observational, retrospective study to evaluate anemia control in KT patients treated with Mircera® for correction or conversion from other ESAs. Information about demographics, CKD, anemia, blood analyses, treatment and adverse events were collected at baseline and months 1, 3 and 6.

**Results:** Between October 2009-May 2010, 292 patients were evaluated. Mean age: 52.9±13.9 years. Female: 50.7%. Stage 3 CKD: 54.4%. Forty-four(15.1%) patients were in the immediate post-transplant period, 49(16.8%) naïve-treatment and 199(68.2%) converted patients. Eighty-eight(48.9%) of converted patients previously received darbepoetin alfa, 82(45.6%) epoetin beta and 8(4.4%) epoetin alfa. Mean doses of Mircera® at baseline were 75.0±22.2µg/month, 97.0±46.4µg/month and 120.5±58.6µg/month in naïve, converted and immediate post-transplant patients, respectively. Mean Hb varied from baseline to month 6 between 10.2±0.7g/dL and 11.8±0.9g/dL in naïve patients ($p<0.001$), and between 11.4±1.3g/dL and 11.9±1.2g/dL in converted patients ($p<0.001$). In patients in the immediate post-transplant period, mean Hb was maintained between 10.4±1.7g/dL at baseline and 11.5±1.4g/dL in month 3. The only study-drug related adverse event was hypertension. No patient died during the study.

**Conclusions:** These preliminary results suggest that Hb stability can be achieved and maintained after correction or conversion to once-monthly Mircera® in KT recipients. It was well tolerated and the safety profile was as expected and comparable to shorter acting ESAs.

Disclosure of Financial Relationships: nothing to disclose

**PUB677**

**Post Transplant Lymphoproliferative Disorder – A Single Center Experience** Vishwanath Siddini, Sudarshan H. Ballal. *Nephrology, Manipal Hospital, Bangalore, Karnataka, India.*

We report 3 cases of post transplant lymphoproliferative disorders(PTLD) observed at our center.

**MATERIALS AND METHODS:** Retrospective evaluation of 750 renal transplant recipients from 1991 to 2010 was done. All 3 patients diagnosed to have PTLD were on regular follow up. A detailed study of dose and type of immunosuppression, rejection episodes, and clinical, radiological and histological presentation, treatment protocol and outcome was made

**RESULTS:** 3 patients were detected to have PTLD. All 3 were male. Mean age of patients was 40.33 yrs. Mean time of onset from transplantation was 5.6 years. Basiliximab induction was used in one patient. Maintenance immunosuppressions used in two were Cyclosporine, Azathioprine and steroid while Tacrolimus, Azathioprine and steroid was used in one. There were no episodes of rejection necessitating antirejection treatment. Pre-transplant EBV status was unknown in all 3 but post PTLD all had EBV positive status. Manifestation was with weight loss, abdominal pain and pleural effusion.All were extranodal and extrarenal tumors. Patients were treated by reducing the immunosuppression to only steroids along with chemotherapy. 2 patients expired, both within 4 months of diagnosis. One attained remission but relapsed within one month and expired subsequently. One patient is surviving after 3 months of diagnosis and is in remission.

**DISCUSSION:** NHL accounts for 93%. Clinical manifestations are variable. Risk factors are- Immunosuppression, EBV infection & other viral infection. Workup includes routine labs with LDH , histology with molecular genetics, EBV serology, EBV viral load , CMV serology, conventional and PET-CT, bone marrow smear and biopsy , CSF examination. Preventive measures include rapid withdrawal/tapering of agents required for graft acceptance and antiviral prophylaxis (Gancyclovir ). Treatment options include reduction of immunosuppression , chemotherapy (R-CHOP, ACBVP, Pro-MACE-CytaBOM), antiviral drugs (doubtful efficacy), interferon alpha, Rituximab. Prognosis varies with clonality and disease extent.

**CONCLUSION:** High index of suspicion is required. Preventive measures and appropriate treatment helps in improving the prognosis of the disease

Disclosure of Financial Relationships: nothing to disclose

## PUB678

**Corynebacterium Striatum: A Rare but Dangerous Cause of Infective Endocarditis in Renal Transplant Patients** Matthew A. Sparks,[1] Jason J. Eckel,[1] Eric W. Raasch,[1] Ruediger W. Lehrich,[1] Stephen R. Smith,[1] Todd V. Brennan,[2] Matthew Jay Ellis.[1,2] *[1]Division of Nephrology, Department of Medicine, Duke University; [2]Division of Abdominal Transplantation, Department of Surgery, Duke University.*

Typically, coryneform bacteria or diphtheroids are considered skin contaminates when isolated in blood or other cultures. However, in the setting of immunosuppression, this genus of bacteria can lead to serious, life threatening infections. We report two patients who developed C. striatum native mitral valve endocarditis 3-6 months after kidney transplantation, likely from surgical site infections. Ubiquitously found, including the skin and oropharynx, these bacteria are aerobically growing, non-spore forming, non-partially-acid-fast, gram-positive rods of irregular morphology. Specific coryneform species have been identified as important pathogens in both immunocompetent and immunocompromised patients. Previous case reports have identified C. striatum as the cause of endocarditis in immunocompetent hosts. C. jeikeium and C. urealyticum have been reported to cause infections in immunosuppressed patients (solid organ, non kidney transplants), particularly with indwelling devises (i.e. catheters, shunts, valves, urinary catheters etc). Several case reports document coryneform endocarditis in kidney transplant patients; however, no speciation was documented. To our knowledge, these are the first two documented cases of endocarditis caused specifically by C. striatum in kidney transplant recipients (MEDLINE search, 1966-2010, all languages). In conclusion, C. striatum when isolated in blood cultures from immunosuppressed patients should be investigated and treated aggressively, as fatal native or prosthetic valve endocarditis can be seen.

Patient Demographics

| Patient 1 | Patient 2 |
|---|---|
| 41 y/o White Man with DM1 on HD | 43 y/o Black Man with FSGS on PD |
| Living donor transplant | deceased donor transplant |
| Wound dehiscence | Wound dehiscence |
| Acute rejection Before IE | No rejection |
| IE 6 months after transplant | IE 3 months after transplant |
| Native mitral valve | native mitral valve |
| No emboli | Emboli to brain and joints |
| Survived | Died |

Disclosure of Financial Relationships: nothing to disclose

## PUB679

**Predictability of Risk of Infection and Rejection in Renal Transplant Patients Using Adenosine Triphosphate Release Assay** Anurag Tikaria,[Wayne State Uni] Sameh R. Abul-Ezz.[Univ. of Arkans] *[1]Nephrology, Wayne State University, Detroit, MI; [2]Renal Associates, Albaquerque, NM; [3]Nephrology, University of Arkansas for Medical Sciences, Little Rock, AR.*

Assessing the impact of immunosuppressive therapy on the immune system is a major challenge in renal transplant recipients. Currently the gold standard for differentiating rejection from infections associated with nephropathy is allogarft biopsy. Cyclex ImmunKnow assay measures cell mediated immunity by measuring ATP production of CD4+ T cells in response to mitogenic stimulation.

We retrospectively reviewed all kidney transplant recpients who had Cylex Immuknow testing done for allograft dysfunction from 2003- 2006, concurrent with allograft biopsy. Acute rejection of the allograft was diagnosed on histopathology according to the Banff criteria and viral infections with polyoma BK and CMV were tested by plasma PCR.

The number of patients with qualifying events were 136, mean age was 46, 66% were male and 57% were white. Infections were more common when ATP levels were less than 200 ng/ml (RR 2.8, p< 0.001). Acute rejection episodes were more common when ATP levels were more than 200 ng/ml (RR 1.8, p < 0.001).

CD4 + ATP Levels at the time of diagnosis

| ATP levels | Rejection | Infection | None |
|---|---|---|---|
| < 200 | 21(36%) | 14(24%) | 24(30%) |
| >200 | 10(14%) | 34(43%) | 34(43%) |

We conclude that Cylex Immuknow assay can help differentiate patients with acute rejection vs. infection in kideny transplant recipients with acute allograft dysfunction.

Disclosure of Financial Relationships: nothing to disclose

## PUB680

**Recurrence of Complement Factor H-Related Protein 5 Nephropathy in a Renal Transplant** Katherine Anne Vernon,[1] Daniel P. Gale,[2] Elena Goicoechea de Jorge,[1] Adam Mclean,[2] Patrick Maxwell,[3] Matthew C. Pickering,[1] H. Terence Cook.[1] *[1]Centre for Complement and Inflammation Research, Faculty of Medicine, Imperial College, London, United Kingdom; [2]Imperial College Kidney and Transplant Institute, Imperial College, London, United Kingdom; [3]Division of Medicine, University College, London, United Kingdom.*

Complement dysregulation is associated with C3 glomerulonephritis (C3GN). C3GN is characterized by glomerular deposits that contain C3 in the absence of immunoglobulin. A heterozygous internal duplication in the complement factor H-related protein 5 (*CFHR5*) gene has been associated with familial C3GN among Cypriot individuals. This disorder, designated CFHR5 nephropathy, is characterized by microscopic haematuria and renal failure. CFHR5, a plasma protein, has complement regulatory function *in vitro* and co-localises with renal complement deposits *in vivo*, suggesting that it may regulate complement activation within the kidney. The mechanism through which heterozygous internal duplication in CFHR5 predisposes to C3GN remains unknown. Here we report an individual with CFHR5 nephropathy and end-stage renal failure (ESRF) who developed rapid recurrence of CFHR5 nephropathy in an unrelated transplant kidney. A 53-year old Cypriot male with ESRF previously thought to be secondary to type I MPGN following two native renal biopsies, underwent a cadaveric renal transplant. Renal transplantation proceeded with no complications. Although his creatinine initially fell, it subsequently stabilised at 186µmol/l and he underwent a transplant biopsy 46 days after transplantation. The biopsy showed scattered subendothelial and mesangial electron dense deposits with isolated granular C3 staining, features consistent with C3GN. PCR of genomic DNA together with serum CFHR5 western blot analysis revealed heterozygous internal duplication of CFHR5, changes identical to those reported in familial Cypriot CFHR5 nephropathy. CFHR5 nephropathy rapidly recurred in the transplanted kidney, demonstrating that renal synthesis of wild-type CFHR5 cannot prevent the development of CFHR5 nephropathy.

Disclosure of Financial Relationships: nothing to disclose

## PUB681

**Protective Role of TNF-α Converting Enzyme in Rejecting Renal Transplant** Jun Wang, Juan Wang, Zilong Li, Hua Zhou, Lining Wang. *Department of Nephrology, First Affiliated Hospital of China Medical University, Shenyang, Liaoning, China.*

TNF receptors are differentially regulated in rejecting renal transplants. TNF-αconverting enzyme (TACE) regulates the membrane shedding of both receptors and TNF itself. We analyzed the expression and regulation of TACE in rejecting renal transplants. Using immunohistochemistry, epithelial cell culture and TACE plasmid transfection, immunoblotting and ELISA, we showed that TACE was up-regulated mainly in tubular epithelial cells in acute rejecting kidney, where it co-localized with TNFR2. Epithelial cells with increased level of TACE shed more soluble TNFR2 into culture media, and even more after PMA activation of TACE. The shedding could be completely blocked by TACE inhibitor TAPI. Up-regulation of TACE in epithelial cells in acute rejecting kidney could result in TNFR2 shedding and antagonize the pro-inflammatory effect of local TNF.

Disclosure of Financial Relationships: nothing to disclose

## PUB682

**Adherence/Non-Adherence in Renal Transplant Recipients Following Conversion from a Cyclosporine Based Immunosuppressive Regimen to Tacrolimus Monotherapy** Lars Backman. *Uppsala University Hospital, Dept of Transplantation Surgery, Uppsala, Sweden.*

Although difficult to study, non adherence with the immunosuppressive drugs after organ transplantation has been shown to result in significant morbidity and graft loss in organ transplant recipients. One aim of the present study was to evaluate adherence among patients who had converted from Cyclosporine based immunosuppression to Tacrolimus monotherapy.

Patients and methods: 79 renal transplant recipients (58M/21F) with a mean age of 59 (range 25-80) years with Cyclosporine and steroid associated side-effects began the study and 62 (78%) completed it. The patients were converted to a Tacrolimus based regimen with the subsequent withdrawal of steroids and follow-up during a 24 month period. They filled out questionnaires which included questions about their medication.

**Results:** In the first questionnaire in answer to the straightforward question as to how often they missed taking medications during the past 4 weeks, 27 (35%) of the patients who answered admitted to having missed at least once. A total of 51(67%) patients responded that they took their medicine at least 1 hour late. To follow-up questions as to the reasons for missing or taking medicine late, an even larger number, namely 56 patients answered that they just forgot.

Although none of the results were statistically significant would appear that younger patients, male patients, and patients who did not complete the study missed their medicine more often.

An analysis of the sixth questionnaire administered at the end of this 2 year study, showed very similar results with 24 (45%) responding that they missed some medications and 41 (77%) that they sometimes took their medicine more than an hour after the prescribed time.

**Conclusion:** In general our study supports other recent research indicating that despite the patients have undergone a kidney transplant for potentially life threatening condition, there is a large percentage of patients that are non-adherent with there prescribed immunosuppression, putting them at risk for a late rejection and a potential graft loss.

Disclosure of Financial Relationships: nothing to disclose

**PUB683**

**Modification of Cyclosporine-Based Therapy in Long Term Follow Up after Renal Transplantation** Veit Busch,[1] Bernhard Breil,[2] Uta Hillebrand,[1] Stefanie Hanna Reiermann,[1] Barbara M. Suwelack.[1] *[1]University Hospital, Muenster, Germany; [2]Departement of Medical Informatics and Statistics, Muenster, Germany.*

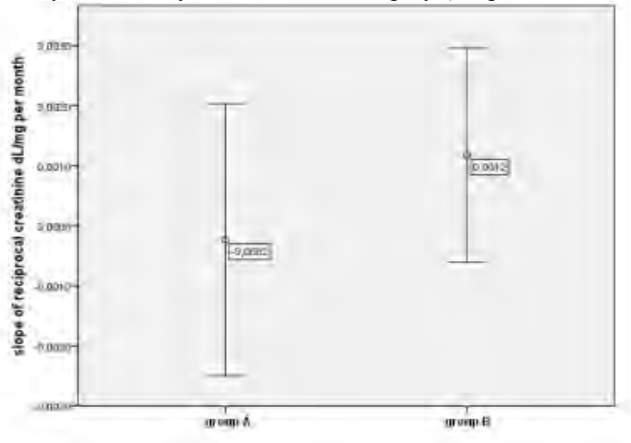
Introduction: In long term care after renal transplantation a modification of a cyclosporine (CSA) based therapy due to side effects, e.g. hyperlipidemia or allograft dysfunction often is necessary.

Methods: Caucasian renal transplant recipients were included into prospective open label trials 8 years after transplantation. In group A (n=54) CSA was replaced by tacrolimus (TAC). In group B (n=51) calcineurin inhibitor (CNI) was tapered and mycophenolate mofetil (MMF) therapy was started or intensified. Group B was subdivided depending on the type of CNI at baseline (Group B1: TAC, n=13; Group B2: CSA, n=38).

Renal function (by reciprocal of serum creatinine: crea-1) bloodpressure and lipid parameters were monitored for 24 months.

Results: At baseline patient and graft characteristics (e.g. crea-1 0,6 versus 0,61 dL/mg) as well as blood pressure, lipid parameters and count of statins and antihypertensive drugs were comparable in both groups.

Slope of crea-1 in 2 years was close to 0 in both groups (no significant difference).




In group A there was a significant drop of s-cholesterol, HDL-cholesterol and triglycerides (baseline vs postinterventional values p≤0,009). Compared to group B LDL-cholesterol-levels were significantly reduced (p=0,02) without an increase in statin therapy.

The subgroup analysis revealed a significantly higher slope of crea-1 in Group B2 as compared to group B1 (+0.0026 versus -0.0028 dL/mg per month, p=0.03) and to group A (p=0.05). Lipid parameters were not different in subgroups.

Conclusions: Even years after kidney transplantation switching to increased dose of MMF from CSA, but not from TAC may improve renal function. A switch from CSA to TAC ameliorates lipid metabolism while preserving renal function.

Disclosure of Financial Relationships: nothing to disclose

**PUB684**

**The Efficacy and Safety of Double Filtration Plasmapheresis (DFPP) as Treatment of Transplantation** Yukiko Hasuike,[1] Naoki Suzuki,[2] Hiroshi Nonoguchi,[1] Takeshi Nakanishi.[1] *[1]Internal Medicine, Division of Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [2]Division of Clinical Engineering, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.*

**Introduction**. DFPP is useful and often performed as a treatment of transplantation to remove pathological IgG. However, excess treatment of DFPP can reduce essential proteins such as albumin and fibrinogen (Fbg) since the reduction rate of Fbg is greater than that of IgG. A patient, who has an operation plan of transplantation, lack of Fbg leads to serious status. It is important to estimate the level of IgG, albumin, and Fbg after DFPP in advance as well as to decide appropriate treatment. To investigate the efficacy and safety of DFPP, a prediction formula for the change of these proteins was established and inspected the agreement.

**Methods**. A patient with acute myeloblastic leukemia who had undergone plasma exchange gave written informed consent to use the effluent of the plasma exchange. The plasma effluent was processed by plasma fractionater with closed circuit *in vitro*, and plasma parameters were determined during the processing. A prediction formula about the parameters was established on the basis of 1 compartment model. The prediction formula was prepared using the parameters before DFPP, circulating blood volume (BV), flow rate of plasma (QF) and drainage (QD), sieving coefficient of plasma separation (SC1) and fractionater (SC2). Using this formula, estimated data from applications the conditions *in vitro* were compared with measured data *in vitro* to inspect the agreement. Likewise *in vitro*, estimated data of clinical DFPP (18 treatments) *in vivo* were compared with measured data *in vivo*.

**Results**. The prediction formula; Concentration at t time = concentration at 0 time*exp [-SC1* {(QF-QD) (1-SC2)+QD}/BV]. The estimated data from the formula were consisted with measured data both *in vitro* and *in vivo* (R2>0.959).

**Discussion**. The formula of DFPP was available to predict the levels of albumin, IgG, and Fbg after DFPP in advance, and to remove IgG without lack of Fbg. Using the prediction formula, suitable DFPP for individual cases could be performed effectively and safely.

Disclosure of Financial Relationships: nothing to disclose

**PUB685**

**Kidney Transplant Patients' Perception, Beliefs & Barriers Related to Regular Nephrology Outpatient Visits** Ajay K. Israni,[1,3] Kathryn R. Goldade,[2] Lisa L. Berndt,[3] Jennifer Vigliaturo,[3] Bertram L. Kasiske,[1,3] Jasjit S. Ahluwalia.[2] *[1]Medicine, Hennepin County Medical Center, University of Minnesota, Minneapolis, MN; [2]Medicine, University of Minnesota, Minneapolis, MN; [3]Minneapolis Medical Research Foundation, Minneapolis, MN.*

**Background:** Our previous work has shown substantial national variation in the frequency of nephrology visits after kidney transplantation. The low frequency of nephrology visits was associated with increased risk of allograft failure.

**Method:** A qualitative study that included interviews and 5 focus groups of transplant recipients.

The study took place at a transplant center in the Upper Midwest. Participants (n=39) were selected if they had at least one of the previously published risk factors for decreased nephrology visit: ethnic minorities, lower median household income, living less than 10 miles from the transplant center.

We assessed the patients' perceptions and beliefs and perceived barriers to regular nephrology outpatient visits.

**Results:** The transplant recipients understood the importance of keeping nephrology appointments and reflected positively on them. Regardless, they perceived barriers to adhering to these visits such as a value on self-reliance which they described increasing over time since transplant; a growing sense that they could interpret their bodies independently without needing to see the doctor as regularly; and, finally, the multitude of physical and mental health challenges post-transplant. There were other factors that motivated patients to keep their regular nephrology visits such as peer support relationships and talking with other patients on dialysis. Patients reported that talking to patients that had received a transplant before them helped them anticipate and cope with the mental and physical challenges inherent to life post-transplant.

**Conclusions:** Even though kidney transplant recipients understood the importance of keeping nephrology appointments, there were significant perceived barriers to these visits. Future interventions should address perceived barriers and motivate patients to keep regular nephrology visits post-transplant as a way to improve allograft outcomes.

Disclosure of Financial Relationships: Research Funding: Research grants >$10,000 from Roche, BMS, Genzyme and Amgen.

**PUB686**

**Perception Is Everything: The Opinions of Potential Renal Transplant Recipients to Non-Standard Donor Options** John F. Johnson, Andrew A. House. *Department of Medicine, Division of Nephrology, London Health Sciences Centre, London, ON, Canada.*

The demand for renal transplantation is much greater than the supply of available donors. Individuals with end stage renal disease often wait years to receive a "standard" deceased-donor transplant. To reduce transplant wait time four non-standard donor options are available; expanded criteria donor (ECD), living donor (LD), pair exchange and xenotransplantation. The last option, which involves the successful transplantation of an animal kidney into a human recipient, remains an experimental donor option. To date, there is limited research about the perception of non-standard donor options by potential recipients. Our study documented the attitudes of potential renal transplant recipients towards these four options. Of 155 individuals surveyed from a transplant waiting list at a single centre, 91 questionnaires were successfully completed (i.e. 59% response rate) and included in our study. The average age of the respondents was 50.41 ($\pm$ 13.53) years. The majority of respondents were (i) male, (ii) on renal replacement therapy for $\geq$ 2 years, and (iii) reported "good" or "very good" health status. We found that only 46% of the respondents would accept an ECD option. In fact, 81% of respondents against an ECD option would not change their opinion in exchange for a decrease in wait time. We also found that, if available, only 53% of respondents would accept an animal donor option. Of the 47% of respondents against xenotransplantation, 89% would not change their opinion in exchange for a decrease in wait time. However, 87% of respondents would accept either an LD or pair exchange option. In conclusion, only about 50% of the potential recipients surveyed would accept an ECD or animal donor option. On the other hand, the majority of potential recipients would accept an LD or pair exchange option. Based on our findings we suggest the following recommendations (i) maintain a separate waiting list for potential recipients who would accept an ECD option, (ii) educate potential recipients about the risks and benefits of an ECD option, and (iii) transplant programs should pursue an LD or pair exchange option wherever possible.

Disclosure of Financial Relationships: nothing to disclose

**PUB687**

**Pilot Study To Evaluate the Efficacy and Safety of Enteric-Coated Mycophenolate Sodium (EC-MPS) in Combination with Sirolimus in *De Novo* Renal Transplant Recipients** V. Ram Peddi, Sofia Recalde, Jennifer Wilson, Lawrence H. Lu, Kimi Ueda Stevenson. *Department of Transplantation, California Pacific Medical Center, San Francisco, CA.*

EC-MPS has different pharmacokinetic and pharmacodynamic profile when compared to Mycopheonalte Mofetil (MMF). Although, there are several studies that have evaluated the safety of MMF and Sirolimus combination, no such data exists for EC-MPS and Sirolimus combination.

**Methods:** In this prospective study (IRB approved), pts received Tacrolimus and EC-MPS immunosuppression initially and were converted to Sirolimus from Tacrolimus between 3 and 6 m post-transplantation. Pts had calculated GFR (Nankivell) pre conversion (baseline) and at months 1, 3, and 6 post conversion. Patients also had measured GFR with radiopharmaceutical I-125 agent (Glofil®) at baseline and at month 6 after conversion.

**Results:** 26 patients consented to the study. Consent was obtained at the time of transplantation. 12 (46%) pts were converted to Sirolimus at a mean of 148 d post-transplantation. 14 pts were not converted and main reason (in 9/14 or 64%) was pts decision not to switch medications given stable allograft function. In this ongoing study all patients completed 1 month of follow-up post conversion. In patients who were switched the mean calculated GFR at baseline was 73.5 mL/m and 78.7 mL/m at 1 month post conversion (7% improvement; p=0.06). 7 pts completed the study and 5 pts are awaiting the month 6 GFR. Measured GFR in the 7 pts at baseline was 69.9 mL/m and 79.6 mL/min at 6 m after conversion (13.9% improvement in GFR; p=0.10). 1 pt had acute rejection 54 days after conversion and was treated successfully with Corticosteroids and remained on Sirolimus. 1 pt had severe edema after conversion and was switched back to Tacrolimus. The mean baseline calculated GFR in the 14 patients who were not converted was 76.2 mL/m at baseline and 79.1 mL/m at 1 month (3.8% improvement; p=0.21).

**Conclusions:** EC-MPS and Sirolimus combination provides safe and effective immunosuppression with low-incidence of complications. Renal function improves after conversion.

Disclosure of Financial Relationships: Research Funding: Novartis, Pfizer, Astellas.

**PUB688**

**Cyclosporine Versus Tacrolimus Maintenance Therapy in Renal Transplantation** Mamdouh N. Albaqumi.[1,2] *[1]Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; [2]Medicine, New York University Medical Center, New York, NY.*

**Background:** Cyclosporine and tacrolimus are important immunosuppressive agents for the prevention of acute rejection. Several studies have shown comparable results in long term graft and patients survival comparing a tacrolimus based therapy to a cyclosporine one while other studies have shown that tacrolimus based regimen had a better renal function, and less episodes of acute rejections. Most of these studies were in Caucasian population. In this study, we describe our experience comparing tacrolimus versus Cyclosporine maintenance therapy in a Saudi population.

**Material and methods:** All patients from 2003 until 2008 in our transplant clinic were identified. A retrospective analysis was carried out comparing patients and graft survival, kidney function, and metabolic profile between the two groups.

**Results:** There was no statistical difference in acute rejection rate between the cyclosporine group and the tacrolimus one (18.7% vs. 20.9% respectively, P=0.756). Mean serum creatinine was not statistically different between the two groups in the first month, 6 months, one year, and two years post transplantation. Patients and graft survival in one and two years were also similar in both groups. Although patient and graft survival were similar in both groups, the cyclosporine group had a higher level of cholesterol compared to the tacrolimus one (4.6±1.03 versus 4.1±0.80 respectively, P= 0.010).

**Conclusion:** In our population, there is no difference in one or two years patients and graft survival between patients on cyclosporine maintenance therapy versus tacrolimus. However, patients on cyclosporine had a higher blood pressure and serum cholesterol level.

Disclosure of Financial Relationships: nothing to disclose

**PUB689**

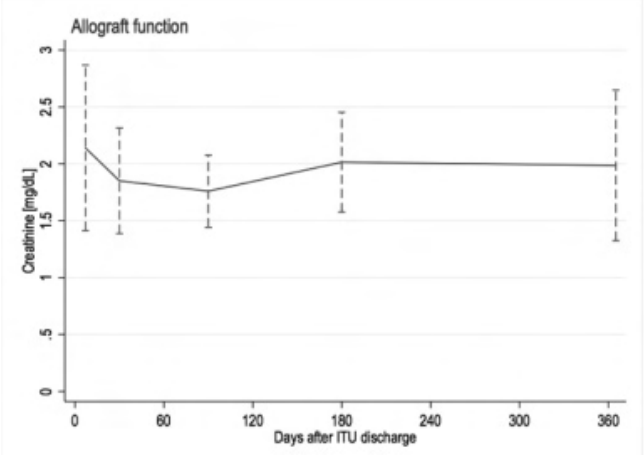
**Long-Term Outcome of Renal Transplant Recipients and Allograft Function after Survival in the ICU** Nishkantha Arulkumaran,[1] Stephen C. West,[1] Maie H. Templeton,[2] Stephen J. Brett,[2] Kakit Chan,[1] David Taube.[1] *[1]Nephrology, Imperial College Kidney and Transplant Institute, London, United Kingdom; [2]Intensive Care Medicine, Imperial College, London, United Kingdom; [3]United Kingdom.*

Purpose: To determine long- term renal transplant recipient survival and graft function after an intensive care unit (ICU) admission

Methods: Retrospective cohort study of renal transplant patients admitted to the ICU over a 4- year period in 2 medical ICUs in a tertiary renal centre

Results: 47 renal transplant patients were admitted to the ICU. The median duration from time of transplantation was 8.8 years. Mean baseline serum creatinine was 1.68mg/dL. Reasons for admission were intracranial pathology (5), cardiac instability (5), pulmonary oedema (4), chest sepsis (12), bacteraemia with septic shock (2), gastrointestinal bleeds and intra- abdominal crises (13), surgical (5), and others (1). Mean APACHE II score was 21, and ICU length of stay (LoS) was 10.5days.

Fifteen patients died on ICU (32%), whilst a further 9 patients (19%) died on the ward. 6 patients (24%) died over a 4 year follow up. Predictors of inpatient mortality were the presence of sepsis (p=0.032) and admission for chest sepsis (p=0.040). On ICU, duration of mechanical ventilation (p=0.019) and ICU LoS (p=0.014) were predictive of mortality. Among patients discharged from hospital, long- term survival was good with a 900- day 50% mortality. Death- censored allograft loss occurred in 5 patients (11%) over a maen of 103 days. Graft function remained stable a year post discharge with no significant change from the baseline creatinine. Mean 1- year creatinine was 1.98mg/dL.




Conclusion: Inpatient mortality of renal transplant recipients admitted to the ICU is significant, though patients who are discharged from hospital do well. Graft loss is not insignificant, though surviving grafts have preserved function.

Disclosure of Financial Relationships: nothing to disclose

**PUB690**

**Retrospective Analysis of Factors Influencing Transplant Function and Survival of Patients with Low Immunological Activity** Cornelia Anneliese Blume, Wilfried Gwinner, Hermann G. Haller. *Nephrology, Medical School Hannover, Hannover, Lower Saxony, Germany.*

**Background:** Standard immunosuppressive regimen after NTX usually contains a calcineurin inhibitor (CNI), an antiproliferative agent and prednisolone. Due to the several side effects, minimization is attractive, but must be safe towards rejection. It remains a challenge to identify patients with low alloimmunity as candidates for minimization. In this study, we retrospectively identified patients with low alloimmunity by stable transplant function during CNI free dual immunosuppression. **Methods and patients:** 99 patients (59 m, 40 f; 54 ± 15 yrs.) transplanted between 1988 and 2006 in Hannover were reduced to mycophenolate and steroids after a mean of 115 ± 77 months. Renal function was analysed. Therapy was reduced in group A (71/99 patients) due to CNI toxicity, proven by biopsy in 43%. Group B patients (28/99) were reduced due to side effects of the CNI. Observation time was 45 ± 27 months. 27/99 patients were treated with mycophenolate directly after transplantation, whereas the rest received the drug at the time of CNI elimination. In 28/99 patients, we performed protocol biopsies within 6 months after NTX. For statistics, the two-sided Wilcoxon analysis was used. **Results:** The mean creatinine of group A was 245 ± 107 µM/l at minimization, whereas group B showed a significantly lower creatinine of 152 ± 73 µM/l ($p < 0.0001$). After an observation time of 45 ± 27 months, transplant function was stable in both groups (group A 289.9 ± 100 µM/l; group B 169.6 +/- 95 µM/l). 5/71 patients of group A lost transplant function in the following. Protocol biopsies in 14/28 patients of group B predicted absence of rejection as well as protocol biopsies in 7/71 group A patients. In 3 patients of group A, protocol biopsies showed signs of humoral rejection (3/5) as predictor of transplant glomerulopathy and chronic damage (c-grade 1-2 according to BANFF 97) or tubulitis as predictor of a chronic allograft failure. **Conclusions:** It is possible to define criteria for an immunologically inert transplant using protocol biopsies. Minimization to mycophenolate and steroids may be safe in a special patient cohort with low immunologic risk.

Disclosure of Financial Relationships: nothing to disclose

**PUB691**

**Listing Pre Dialysis Patients for Transplantation: Is It Worthwhile?** Wendy Brown, Damien Ashby, Peter Hill, Megan Griffith. *Imperial College Kidney & Transplant Institute, London, United Kingdom.*

UK guidelines recommend that people with advanced CKD are activated for transplantation within 6 months of needing to start dialysis. Pre-emptive transplantation may have benefits over transplantation post dialysis. Pre dialysis transplant workup has increased in recent years with evidence that coronary angiography is not detrimental to the rate of decline of GFR. Increased live donor transplantation has also increased rates of pre-emptive transplantation.

Aim: To investigate the outcome of pre dialysis patients listed for transplantation.

Method. All patients deemed fit for transplantation were offered work up/listing for transplantation if anticipated to require dialysis in the next 6 months. The outcomes of those patients listed from Jan 2006 to April 2010 were analysed.

Results: 63/173 patient listed received pre-emptive renal transplants. 34/63 received kidneys from live donors, 29/63 received kidneys from deceased donors, of whom 8 underwent simultaneous kidney pancreas transplantation (SPK). The mean time to live donation was 164 days [14-755], to deceased kidney alone was 314 days [34-958] and to SPK was 198 days [5-754]. 69/173 patients commenced dialysis, the mean time to dialysis was 401 days, but there was great variation between patients [25-1896]. Of these 29 have been transplanted, 13 within 6 months of starting dialysis.

Forty one patients have not yet started dialysis; 10 of these have been suspended from the transplant list for medical reasons or renal function stabilisation; 31 remain active on the transplant waiting list. The mean time these patients have been listed is 392 days [42-1373].

Conclusions: Pre dialysis workup for renal transplantation successfully enables significant numbers of patients to receive pre-emptive renal transplants from both live and cadaveric donors, thus avoiding dialysis and its associated complications; and it also facilitates early transplantation post starting dialysis. However, predicting the start time of dialysis in individual patients is extremely difficult and better ways to assess this are required to optimise the time of listing.

Disclosure of Financial Relationships: nothing to disclose

**PUB692**

**Does Allograft Failure Impact School Attendance in Children on Dialysis? A NAPRTCS Study** Ashton Chen, David B. Kershaw, John C. Magee, Panduranga S. Rao. *University of Michigan, Ann Arbor, MI.*

BACKGROUND: Allograft failure accounts for a significant number of pediatric patients commencing dialysis. There are limited outcomes data in children returning to dialysis after transplant failure. School attendance is an important outcome measure in the pediatric population. METHODS: Using the North American Renal Trials and Collaborative Studies (NAPRTCS) database, we identified school-age patients who initiated dialysis between January 1, 1992 and December 31, 2007 (N=3489). Patients were categorized by transplant history, those with previous allograft failure and those with no history of transplant (transplant naïve). There were 2849 patients of school age with school status data available at 6 months post dialysis initiation; 2262 were transplant naïve and 587 had allograft failure. There were 2052 school age patients with school status available at 12 months after dialysis initiation; 1578 were transplant naïve and 474 had allograft failure. Full-time school attendance at 6 and 12 months after dialysis initiation was compared between the transplant naïve and allograft failure groups using a Chi-square test. Multivariate analysis was performed to determine impact of transplant failure on school attendance. RESULTS: The difference in school attendance between the transplant naïve and transplant failure groups was statistically significant at 6 months (72.3% and 67.2%, p=0.0164), but not at 12 months (72.5% and 68.6%, p=0.103). After covariate adjustment, transplant failure had no impact on school attendance at either 6 or 12 month after dialysis initiation (HR 1.12, CI 0.91-1.39, HR 0.99, CI 0.78-1.27 respectively). CONCLUSION: Children with failed allografts who return to dialysis have comparable full-time school attendance compared to their transplant naïve dialysis counterparts a year after allograft failure. Allograft failure does not appear to be a significant factor impacting school attendance.

Disclosure of Financial Relationships: nothing to disclose

**PUB693**

**Comparison between Direct Renal Measurement as Goal Standard vs Accurancy of Imaging Studies as Well as Renal Weight and Renal Function in Kidneys of Living Renal Donors** Fernardo A. Fajardo, Maria Carmen Avila-Casado. *Nephropatology, Instituto Nacional de Cardiologia Ignacio Chavez, Mexico City, Mexico.*

Regardless immunological factors, number of nephrons can have an impact in the outcome of renal allografts. Using renal imaging: ultrasonogram (USG) and Computed tomography imaging (CTI) in the measurement of donor renal size can be inaccurate. The aim of this study was to compare the renal size by direct measurement of allografts from LRD used as Goal standard, and compare it with the USG and CTI.

**Material and Method**: Twenty-four kidney allografts from LRD were prospectively studied. Donor protocol included: Tc-99m DPTA, direct 24hrs CrCl and calculated MDRD (modification of diet in renal disease); USG and CTI. Number of nephrons was calculated by renal weight (grams). direct measurement of renal size using a rule in cm (metric system) during organ procurement. Renal imaging and renal weight were correlated with short purges of Tcc-99m DPTA. Purges were used for CrCl determination. **Results:** twenty-four kidney allografts from 12male and 12 female donors were included. There was significant correlation between the length by direct measurement with imaging studies (CTI and USG), but CTI was the most accurate imaging technique for the measurement of renal size, and correlated well with the value in length and AP axis (Rho = 0.08, 0.035 respectively). **Conclusions:** Although CTI and USG shown a significant correlation with the goal standard, CTI showed to be most accurate. There was not a significant correlation between kidney weight and 24-Hrs CrCl, MDRD and Tcc-99m DPTA (Rho = 0.189, 0.178, 0.359).

Disclosure of Financial Relationships: nothing to disclose

**PUB694**

**Dermatological Lesions in Renal Transplant Recipients: A Retrospective Study** Marilena Gregorini,[1] Michela Castello,[2] Teresa Rampino,[1] Antonio Dal Canton,[1] Teresa Valsania,[1] Eleonora Pattonieri,[1] Chiara Rocca,[1] Camillo Carrara,[1] Francesca Bosio.[1] *[1]Unit of Nephrology, University and IRCCS Fondazione Policlinico S. Matteo, Pavia, Italy; [2]Unit of Dermatology, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy.*

The chronic use of immunosuppressive drugs in transplantation patients (pts) predispose to opportunistic infections and malignancies. Reports on skin lesions are very few. The aim of our study was to evaluate the relation between immunosuppressive drugs, demographic characteristics and skin lesions.

A retrospective study was performed using medical records of 183 kidney transplant recipients followed for ten years in our Transplant Unit.

Induction therapy consisted of basiliximab and steroids (S); maintenance therapy included cyclosporine, tacrolimus, S, mycophenolate mofetil (MMF), micophenolic acid (MFA), rapamycine, everolimus. Anti-rejection therapy consisted of S and/or tymoglobulines.

The lesion diagnosis has been made by skin, mucous, nails and hair evaluation. Skin biopsies, cultures, serological tests were also performed. Lesions were reported in 95,7% of pts and 54.1% had more than one. 37,7% of pts showed viral lesions, 25,6% showed immunosoppression-related lesions, 20,2% bacterial lesions, 15,8% benign tumors, 14,2% mycosis, 11% precancerosis lesions, 9,2% cutaneous xerosis, 8,7% dermatitis, 8,2%malignant tumors**.** A significative correlation was found between calcineurin inhibitors and gengival hyperplasia ($p<0,001$); mTOR inhibitors and acne/folliculitis ($p<0,05$), MFA and herpes simplex lesions ($p<0,05$); MMF and warts ($p<0,05$). Anti-rejection therapy was related to precancerosis lesions ($p<0,001$), bacterial lesions ($p<0.05$) and gengival hyperplasia ($p<0.001$). Malignant tumors were more frequent in elderly pts ($p<0.001$). Herpes zooster was more frequent in the first two years post-transplant ( $p<0.05$), immunosoppression-related lesions appear in the first year post transplant ( $p<0.001$), precancerosis lesions and malignant tumors in pts after 67,5 months from transplantation. Cutaneous manifestations are frequent in kidney transplanted pts. Early and continuous monitoring is necessary for early diagnosis and an appropriate treatment.

Disclosure of Financial Relationships: nothing to disclose

**PUB695**

**Successful Living Related Renal Transplantation in Factor H Antibody Associated Atypical Hemolytic Uremic Syndrome (aHUS)** Johannes Hofer,[1] Thomas Giner,[1] Therese Jungraithmayr,[1] Alejandra Rosales,[1] Magdalena Riedl,[1] Walter Mark,[2] Lothar Bernd Zimmerhackl.[1] *[1]Medical University, Innsbruck, Austria; [2]Medical University, Kaunas, Lithuania.*

A 12 year old boy presented with sudden onset of nausea, vomiting and deterioration of health. Laboratory examination showed hemolytic anemia (Hb 84 g/l, LDH 1662 U/l), thrombocytopenia (platelets 16 x $10^3$/µl) and acute renal failure (serum creatinine 11.36 mg/dl) indicating aHUS. C3 was decreased to 43 mg/dl. He initially received daily fresh frozen plasma (FFP) infusions over 10 days (5ml/kg), followed by one infusion per week over two weeks and afterwards every other week. The patient did not regain renal function and on the 7th day after disease onset peritoneal dialysis (PD) was started. After one month on PD he developed peritonitis and was switched to hemodialysis (HD). The patient was on HD until renal transplantation, receiving HD 3 times per week (4 hour sessions).

The patient was diagnosed with Factor H antibody (FH-Ab) associated aHUS. The FH-Ab level was initially high (1600 AU/ml). Renal function did not recover. Therefore living related renal transplantation was planned. To reduce FH-Ab titers prior to kidney transplantation one plasma exchange (PE) and a single infusion of IgG (2 g/kg body weight) on the day before transplantation was preformed. In 11/2009 a living related renal transplant from the father was done. Induction therapy with ATG and immunosuppression with Tacrolimus, MMF and Steroids was given. 4 months after transplantation terminal complement complex levels were normal and the antibody titers are in the very low range (<100 AU/ml).

At present many patients with FH-Ab positive aHUS are waiting for renal transplantation. Without evidence of the role of this aHUS subtype before, during and after transplantation they are at high risk for transplant failure because of recurrence of aHUS. IN this case report, the combination of plasma therapy, IVIG and induction therapy was successful. In order to follow these patients consequent evaluation in specific registries are recommended (www.hemolytic-uremic-syndrome.org).

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Disclosure of Financial Relationships: nothing to disclose

**PUB696**

**Successful Renal Transplantation in a 4-Year Girl with Renal Tubular Dysgenesis Due to Mutations of the Angiotensin-Converting Enzyme Gene**

Aya Inaba,[1] Hiroshi Hataya,[1] Yuko Hamasaki,[1] Kenji Ishikura,[1] Hiroyuki Satoh,[1] Kenichi Satomura,[2] Seiichirou Shishido,[3] Masataka Honda.[1] *[1]Nephrology and Transplantation, Tokyo Children's Medical Center, Fuchu, Tokyo, Japan; [2]Pediatric Nephrology, Osaka Medical Center for Maternal and Child Health, Izumi, Osaka, Japan; [3]Pediatric Nephrology, Toho University, Ota, Tokyo, Japan.*

Renal tubular dysgenesis (RTD) is a critical disorder characterized by the Potter sequence, severe hypotension and skull ossification defects. We report a successful renal transplantation in a 4-year-old girl with RTD due to mutations of the angiotensin-converting enzyme (ACE ) gene.

The patient, a Japanese girl, was born at 33 weeks gestation. A hypoplastic lung and diastasis of the cranial sutures were observed. Severe hypotension persisted during the neonatal period, which was treated with plasma expanders and catecholamines. However mild hypotension still persisted. Peritoneal dialysis was required from day 3 after birth. Histopathological findings in the kidney were compatible with RTD and gene analysis revealed compound heterozygotic mutations, two novel deletions in exon 1 and exon 25 of the ACE gene (Eur J Pediatr (2009) 168:207-209) . At 4 years, the patient received a living kidney transplant from her mother. Before transplantation her blood pressure was low (systolic, 60 mmHg) . During the perioperative period, blood pressure was well maintained by adjustment of fluid replacement and catecholamines. A postoperative study showed higher plasma angiotensin II (Ang II) concentrations (194ng/ml) than those measured before transplantation (50ng/ml) . The patient was discharged with a functioning graft on postoperative day 58. At discharge, her blood pressure was higher (systolic, 90mmHg) than that before transplantation.

The major periorperative problem of renal transplantation in our patient was the management of blood pressure and it was maintained successfully without the administration of Ang II. The increase in plasma concentrations of Ang II after transplantation suggests expression of ACE derived from the renal graft and this may have contributed to the perioperative management of blood pressure.

Disclosure of Financial Relationships: nothing to disclose

**PUB697**

**Evaluation of Renal Transplant Recipient in a Single Centre of Bangladesh**

Shahidul Islam, Muhammad Rafiqul Alam, Habibur Rahman, Harun U. R. Rashid. *Department of Nephrology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.*

Renal transplantation (Tx) is the treatment of choice for most patients with end stage renal disease. In our centre renal Tx. started and continue from 1988, but post Tx. Patient evaluation has been not well established.

Total 214 ESRD patients were transplanted from July 1995 to December 2005. All patients were live related donors, mothers were the major donor about 33% followed by brother 29%, sister 16%, father about 14%.

Among the transplanted patient 138 (64.48%) were survival, 19 (8.87%) under went graft failure put on dialysis and 57 (26.73%) were died.

Among the survival patients, 115 recipients were evaluated and reveled 83(72.17%) developed HTN, 32 (27.83%) chest infection (Specific and non-specific), 29 (25.22%) graft dysfunction, 29 (25.22%), 25 (21.70%) UTI, 22 (19.19%) diabetes, 18 (15.65%) having CMV carrier & 9 (7.83%) CVD having post Tx proteinuria.

Over all graft survival in 1st of year 85%, 3rd year 75%, 5th year 65% & 10 year 50%

In conclusion, results of renal Tx. is encouraging as compare to other countries. So, to increase number of transplantation needed to overcome the existing donor scarcity as well as to increase number of transplant centre in our country.

Disclosure of Financial Relationships: nothing to disclose

**PUB698**

**Long-Term Graft Survival over 20 Years after Kidney Transplantation; a Single Center Experience**

Dong Jin Joo, Kyu Ha Huh, Tae-Hyun Yoo, Yu Seun Kim. *Transplantation Center, Yonsei University College of Medicine, Seoul, Korea.*

***Background***: Kidney graft survival has been improving last few decades. We analyzed the clinical differences of the long-term survival graft from others.

***Patients and Methods***: We retrospectively analyzed the 300 kidney recipients who underwent between April, 1979 and November, 1988, which were performed over 20 years ago. Among them, four groups were classified according to the duration of graft survival, which included 6 grafts in short-term survival group (under 1 year, Group 1), 63 grafts in intermediate-term survival group (5-10 years, Group 2), 54 grafts in long-term survival group (10-20 years, Group 3), and 107 grafts in extremely long-term survival group (over 20 years, Group 4). The 70 recipients who survived for 5-10 years and who died with functioning graft within 1 year were excluded.

***Results***: Donor age (30.8±8.8) of Group 4 was younger than other groups (33.7±13.2, 38.8±12.2 and 36.7±10.3; p<0.001). There were no significant differences in HLA mismatching but acute rejection episodes were significantly different (1.00±0.00, 0.59±0.93, 0.19±0.48 and 0.11±0.42; p<0.0001). All recipients in Group 1 were preemptive transplant cases, which was significantly different from others ($p$=0.004). Azathioprin-based immunosuppression was more used in Group 1 (83.3%) than others (20.6%, 29.6%, and 43.0%; $p$<0.0001). Except Group1, Group 2 showed more acute rejection episodes than others ($p$<0.0001; Table 1). Of these different factors, donor age (OR=1.034, $p$<0.0001) and acute rejection episodes (OR=1.461, $p$<0.0001) were significant risk factors that affected on the over-20-years graft survival rate according to the Cox-regression analysis.

***Conclusion***: Long-term graft survivors over 20 years after transplantation showed younger donor age and less acute rejection episodes.

Table 1. Characteristics according to the each survival group

| Variables | Group 1 (N=6) | Group 2 (N=63) | Group 3 (N=54) | Group 4 (N=107) | p-value |
|---|---|---|---|---|---|
| **Recipient factors** | | | | | |
| Sex of recipient (male %) | 100.0 | 74.6 | 79.6 | 75.7 | 0.628 |
| Age of recipient | 37.5±10.1 | 35.3±10.9 | 33.8±9.2 | 32.5±9.1 | 0.243 |
| PreTx Diabetes (%) | 0.0 | 4.8 | 0.0 | 0.0 | 0.147 |
| PreTx HBsAg (%) | 0.0 | 3.2 | 5.6 | 0 | 0.245 |
| Dialysis | | | | | |
| Preemptive (%) | 100.0 | 9.8 | 0 | 5.4 | |
| HD (%) | 0 | 64.7 | 55.9 | 58.1 | 0.041 |
| CAPD (%) | 0 | 8.8 | 32.4 | 16.9 | |
| Acute HD | 0 | 2.9 | 8.3 | 14.9 | |
| Dialysis duration (months) | 0 | 7.7±9.4 | 8.3±12.2 | 12.1±17.4 | 0.261 |
| **Donor factors** | | | | | |
| Sex of donor (male %) | 33.3 | 60.3 | 59.3 | 55.1 | 0.611 |
| Age of donor | 33.7±13.2 | 38.8±12.2 | 36.7±10.3 | 30.8±8.8 | <0.0001 |
| Relation | | | | | |
| LRD:LURD | 4:2 | 40:23 | 33:21 | 62:45 | 0.897 |
| **Immunologic factors** | | | | | |
| HLA mismatching (%) | | | | | |
| 0-1 Ag mismatching | 50.0 | 39.7 | 37.0 | 47.7 | |
| 2-4 Ag mismatching | 50.0 | 60.3 | 61.1 | 52.3 | 0.454 |
| 5-6 Ag mismatching | 0 | 0 | 0 | 0.0 | |
| Number of the HLA Ag mismatching | 1.3±1.2 | 1.9±1.2 | 1.9±1.3 | 1.6±1.3 | 0.175 |
| Main IS (%) | | | | | |
| Azathioprin | 83.3 | 20.6 | 29.6 | 43.0 | 0.001 |
| Cyclosporine A | 16.7 | 79.4 | 70.4 | 57.0 | |
| Number of AR events | 1.0±0.0 | 0.6±0.9 | 0.2±0.5 | 0.1±0.4 | <0.0001 |

Abbreviation: PreTx, pretransplant; HBsAg, hepatitis B-viral surface antigen; HD, hemodialysis; CAPD, continuous ambulatory peritoneal dialysis; LRD, living related donor; LURD, living unrelated donor; IS, immunosuppressants; AR, acute rejection

Disclosure of Financial Relationships: nothing to disclose

**PUB699**

**Predictors of Transplant Recipient Outcome after Renal Allograft Loss**

Hye-Young Kang, Jung Tak Park, Bo Young Nam, Jwa-Kyung Kim, Shin-Wook Kang, Tae-Hyun Yoo. *Brain Korea 21 Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea.*

The number of patients who require dialysis after graft loss is growing with an increase in the number of renal allografts. Patients reinitiating dialysis after graft failure have different characteristics from transplant-naïve patients starting dialysis considering chronic inflammation from allograft rejection and exposure to prolonged immunosuppression. However, data regarding the outcomes of patients on dialysis after graft loss are limited. We therefore explored the characteristics and risk factors for mortality among patients returning to dialysis after graft loss. Study subjects consisted of 294 patients who reinitiated dialysis after graft loss between 1985 and 2006. Glomerular filtration rate (GFR) was calculated using the MDRD equation. New-onset diabetes after transplantation was defined as casual glucose ≥ 200 mg/dl and/or use of hyperglycemic medications. Comorbid conditions were expressed as the Charlson comorbidity index (CCI). Biochemical and clinical data at graft loss were considered as baseline. The mean age of patients at the time of graft loss was 39.7 ± 10.8 years, and 203 (69.5%) patients were male. The mean GFR at dialysis reinitiation was 9.1 ± 4.6 ml/min/1.73 $m^2$ and the most common comorbid conditions were diabetes (20.8%) and malignancy (15.0%). Cardiovascular disease was the most common cause of death during the 67.4 ± 50.6 months of follow-up (28.9%). History of diabetes (HR 6.88, $P$ < 0.001), new-onset diabetes after transplantation (HR 1.84, $P$ = 0.03), high CCI score (HR 1.83, $P$ = 0.02), and low serum albumin level (HR 2.93, $P$ = 0.01) were independent risk factors for mortality. However, GFR at dialysis reinitiation (HR 1.04, $P$ = 0.21), cumulative dose of cyclosporine (HR 1.00, $P$ = 0.33) or prednisolone (HR 1.01, $P$ = 0.16) were not. Comorbid conditions, new-onset diabetes after transplantation, and hypoalbuminemia predicted mortality in patients who returned to dialysis after graft failure. Improving nutritional status and treating comorbid conditions could ameliorate the outcome of these patients.

Disclosure of Financial Relationships: nothing to disclose

**PUB700**

**Steroid Versus Non-Steroid Immunosuppression Protocols in Pediatric Renal Transplant: Comparison of Outcomes**

Juhi Kumar, Heejung Bang, Eduardo M. Perelstein, Valerie L. Johnson. *Pediatric Nephrology/Public Health, Weill Cornell Medical College, New York, NY.*

**Background:** Corticosteroids have been the mainstay of immunosuppression in pediatric renal transplant despite many side effects especially growth suppression in children. Since 2005 our center has been using steroids only as a premedication for thymoglobulin in the initial peri-operative and immediate post transplant period. Prior to that we used a steroid based protocol. Our induction protocol consists of 5 doses of Thymoglobulin and Methylprednisone. Mycophenolate Mofetil is given on day # 0 and Prograf is started on day # 1. Patients are maintained on Prograf and MMF long term.

**Objective:** To compare outcomes between patients on steroid (S) versus non-steroid (NS) based protocols.

**Methods:** Data on demographic, anthropometric, biochemical variables and medications were obtained from patient records. Comparisons between the S and NS groups were made for the following outcomes: graft survival, acute rejection, estimated GFR, height z scores, BMI, number of anti-hypertensives used and use of erythrocyte stimulating agents (ESA). Longitudinal data (at 3 time points up to 2 years) were available on acute rejection,

height z scores and BMI so these data were analyzed by generalized estimating equations accounting for within person correlation, while other cross-sectional data were analyzed by standard linear or logistic regression. In all regression analyses, potential confounders such as age, donor source and time trend were controlled.

**Results:** There were 49 subjects, 20 in the S and 29 in the NS group. Height z scores were significantly higher (p=0.02) and acute rejection rate (p=0.01) and the number of anti-hypertensives used (p=0.02) were significantly lower in the NS group. The differences in graft survival, eGFR, BMI, and use of ESA's were not significant, likely due to insufficient statistical power.

**Limitations:** small sample size, retrospective chart review, historic controls

**Conclusions:** Steroid free immunosuppression provides comparable outcomes in terms of graft survival and graft function but possibly better outcomes for linear growth and incidence of acute rejection in children.

Disclosure of Financial Relationships: nothing to disclose

## PUB701

**Pancreas-Kidney Transplantation: Experience at Single Center in Korea**

Hankyu Lee,[1] Kitae Bang,[1] Jongho Shin,[1] Jongwon Ha,[4] Jaeseok Yang,[3] Curie Ahn.[2] *[1]Nephrology, Internal Medicine, Eulji Hospital, Daejeon, Korea; [2]Department of Internal Medicine, Seoul National University, Seoul, Korea; [3]Transplantation Center, Seoul National University Hospital, Seoul, Korea; [4]Department of Surgery, Seoul National University, Seoul, Korea.*

Introduction. The pancreas transplantation is the curative treatment for diabetes. We report current result of pancrease-kidney transplantation and characteristics in Korea.

Methods. We retrospectively reviewed pancreas-kidney TPL from 2002 to 2010 in Seoul National University Hospital in Korea. 21 patients underwent pancreas transplantation; twenty patients were simultaneous pancreas-kidney and one pancreas after kidney transplantation from deceased donors. 8 patients suffered type 1 diabetes, whereas 13 patients type 2 diabetes.

Results. The mean recipient age was 42.9 years. The mean donor age was 28.5 years. The mean HLA mismatch was 3.9. Immunosuppressive treatment consisted of basiliximab induction followed by tacrolimus, MMF, and Pd. The After a mean follow-up of 45.2 months, survival rate of patients, kidney allograft and pancreas allograft survival rate were 95%, 90%, and 80%, respectively. One patient died of cerebral infarction and small bowel perforation. 2 pancreas allografts were lost due to CMV pancreatitis and small bowel perforation.; Beyond early postoperative period, allograft loss of pancreas and kidney was limited to 1 case of noncompliance to the medications. No more complications occurred during follow including cardiovascular and ophthalmologic ones.

Postoperative complications

| **In-hospital complication** | **36cases / 15pts** |
|---|---|
| Hematoma / Fluid collection | 3 / 3 |
| Small bowel perforation | 2 / 2 |
| Thrombosis in pancreas vein | 2 / 2 |
| Pancreatitis (Unknown, CMV) | 2 / 2 |
| Infection (PCD, Sepsis) | 2 / 2 |
| Cholecystitis | 1 / 1 |
| Urine leakage | 1 / 1 |
| Cerebral infarction | 1 / 1 |
| Bx proven kidney rejection | 11 / 10 |
| Bx proven pancreas rejection | 7 / 6 |
| **Alive at hospital discharge** | **20 pts / 21 pts** |

Conclusion. The pancreas-kidney transplantation is a well-established therapeutic option with tolerable outcomes for patients with diabetes. However, we should be careful about the complications in the immediate postoperative period.

Disclosure of Financial Relationships: nothing to disclose

## PUB702

**Functional Iron Deficiency and Hepcidin in Renal Replacement Therapy**

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Hepcidin has emerged as a key regulator of iron homeostasis and is believed to play an important role in the functional iron deficiency. NGAL (neutrophil gelatinase-associated lipocalin) binds small-iron carrying molecules. We tested the hypothesis that hepcidin and/or NGAL is related to functional iron deficiency (defined as ferritin above 200 ng/ml with TSAT below 20%) in 200 haemodialysis patients and 160 kidney allograft recipients.

Methods: Serum iron, total iron binding capacity, ferritin, transferrin saturation-TSAT, complete blood count, creatinine, albumin were assessed using standard laboratory methods. Soluble transferrin receptor, hsCRP, were measured using commercially available kits. Hepcidin was assayed using kit from Bachem, UK. Serum NGAL was assessed using kits from BIOPORTO, Denmark.

Results: Functional iron deficiency was present in 21% of HD patients and in 23% of kidney allograft recipients. Serum NGAL, hepcidin, ferritin, ESA dose, hsCRP, prevalence of diabetes, use of ACE inhibitors were significantly higher in HD patients with functional iron deficiency, whereas serum albumin, serum iron, TSAT, residual renal function, Kt/V, and serum creatinine pre-and post HD were significantly lower in this population. In addition to a low TSAT and high ferritin, the presence of functional iron deficiency was associated with inflammation and high NGAL in multiple regression analysis. In kidney allograft recipients with functional iron deficiency, serum hepcidin, hsCRP, ferritin were significantly higher, whereas erythrocyte count, serum iron were significantly lower when compared to patients without functional iron deficiency.

Conclusions: Functional iron deficiency is common in patients on renal replacement therapy, and is associated with high hepcidin levels and inflammatory markers. These data support the concept that these patients are inflamed, and this population should be investigated for underlying and potentially reversible causes. Furthermore, hemodialysed patients may benefit from more aggressive dialysis treatment.

Disclosure of Financial Relationships: nothing to disclose

## PUB703

**Experience in the Kidney Transplant Program at the Hospital de Especialidades; Centro Médico de Occidente. IMSS. Guadalajara Jalisco, México**

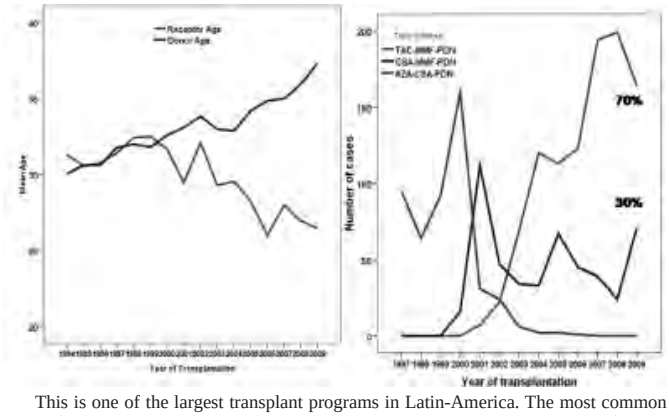
Abel Puentes Camacho,[1] Jorge Andrade-Sierra,[1,2] Enrique Rojas-Campos,[2] Francisco Monteon,[1] Mario Sandoval Sandoval,[1] Carlos Valdespino,[1] Miguel Medina Perez,[1] Luis Alberto Evangelista Carrillo,[1] Javier Castillo Tapia,[1] Alfonso M. Cueto-Manzano,[2] Benjamin Gomez-Navarro.[1] *[1]Nephrology and Organ Transplant, Hospital de Especialidades, Guadalajara, Jalisco, Mexico; [2]Medical Reseach Unit in Renal Diseases, Hospital de Especialidades, Guadalajara, Jalisco, Mexico.*

In the USRDS report, Jalisco (Mexican state) had the highest transplant rate in the world. Therefore this study describes the characteristics of our kidney transplant population.

Clinical charts of 2,319 recipients were retrospectively reviewed (Jan/94-Dec/09).

Main results in Table and Figure.

| Variable | 1994-1999 | 2000-2005 | 2006-2009 |
|---|---|---|---|
| Receptor Age (years) | 32±11 | 30±12 | 27±11 |
| ≤ 19 yrs | 57 (11) | 228 (25) | 261 (30) |
| 20-40 yrs | 346 (67) | 494 (54) | 491 (56) |
| 41-60 yrs | 112 (21) | 183 (20) | 114 (13) |
| ≥ 61 yrs | 3 (1) | 10 (1) | 9 (1) |
| Gender, F/M N(%) | 195(37) / 330(63) | 329(36) / 587(64) | 314 (36) / 564(64) |
| Donor Age(years) | 31±11 | 33±11 | 36±10 |
| Donor F/M N(%) | 261(50) / 260(50) | 475 (52) / 436(48) | 420 (48) / 456(52) |
| Unknown N(%) | 354(69) | 683(74) | 749(85) |
| HLA < 2 Ag | 285 (59%) | 508 (59%) | 203 (24%) |
| HLA ≥ 3 Ag | 125 (26%) | 252 (30%) | 556 (67%) |
| Identical N(%) | 76 (15%) | 95 (11%) | 74 (9%) |
| LRD N(%) | 420 (81) | 668 (73) | 690 (79) |
| Cadaveric D | 37 (7) | 149 (16) | 61 (7) |




This is one of the largest transplant programs in Latin-America. The most common cause of ESRD is unknown. Living donor continuous to be the most common type of donor. In the last decade recipients had shown the most important decrease in age whereas donors have been increasingly older; Immunosuppression have also change, currently the most common scheme is TAC+MMF+PDN .

Disclosure of Financial Relationships: nothing to disclose

## PUB704

**A Six-Year Single Center Experience with Rabbit Anti-Thymocyte Globulin (Thymoglobulin) Containing Induction Regimen in Lowering Acute Rejection Rates in Kidney Transplantation**

Vinayak Ramanath, Preethi Yerram, Diptesh Gupta, Khaled Mohamed. *Division of Nephrology, Univ. of Missouri-Columbia, Columbia, MO.*

**Introduction:** Rates of acute rejection in renal transplantation has been trending down over the last several years owing to more efficacious immunosuppressive regimen. Current acute rejection rate in the US approximates 10-15%.

**Aim:** To determine the acute rejection rate within first 12 months among patients undergoing renal transplantation at the University Hospital, Columbia-Missouri between 2003 and 2009 with Thymoglobulin containing induction regimen, and to compare it with the national average.

**Methods:** After obtaining IRB approval, medical records of all kidney transplant recipients at the University Hospital, Columbia-Missouri between January 2003 and January 2009 were reviewed. Data was collected regarding biopsy proven acute rejection in the first year of transplantation. We defined acute rejection as an episode of renal allograft dysfunction, proven by renal allograft biopsy. Demographic data, donor type, re-transplantation, cold and warm ischemic time, antigen mismatches, CMV status, renal

parameters, type and dose of induction therapy used (thymoglobulin vs other), and details of maintenance immunosuppression.

**Results:** Total of 160 renal transplants were done between January 2003 and January 2009. 26 were from living donors and the remaining 134 from deceased donors. 154 of the 160 patients received thymoglobulin as part of the induction therapy; while four patients did not receive thymoglobulin. No information was available on two patients. All the patients were maintained on a three-drug immunosuppressive regimen; a calcineurin inhibitor, prednisone and an antimetabolite. There were a total of 10 biopsy proven acute graft rejections within the first year, with the rejection rate being 6.2% which is lower than the national average. Four of the ten patients who had rejection were either non-compliant with their immunosuppression or had subtherapeutic levels of the calcineurin inhibitor.

**Conclusion:** Use of thymoglobulin as part of the induction therapy for kidney transplantation is associated with much lower rates of acute rejection.

Disclosure of Financial Relationships: nothing to disclose

**PUB705**

**Higher Education Alleviates Racial Disparities in Access to Kidney Transplantation** Gurprataap Singh Sandhu,[2] Anna Barenbaum,[1] Preeti Rout,[2] Martha Pavlakis,[2,3] Hongying Tang,[3] Joo Heung Yoon,[4] Alexander S. Goldfarb-Rumyantzev.[2,3] *[1]Tel Aviv University, Tel Aviv, Israel; [2]Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [3]Transplant Institute, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [4]Internal Medicine, Massachusetts General Hospital, Boston, MA.*

Racial disparities in access to medical care have been demonstrated before and are difficult to eliminate. We hypothesize that higher education level might result in reduced disparities in access to transplantation.

We used data from the USRDS of incident ESRD patients ≥18 years of age, who started dialysis 1/1/1990-9/1/2007. The outcomes were the likelihood of (1) wait-listed or transplanted without being listed; and (2) of receiving kidney transplant for those patients who were previously listed.

We identified 3,224 ESRD patients for whom education information was available. A mean age of ESRD onset was 57.1 ± 16.2 years, 54.3% were male, 64.2% White, and 50.4% had diabetes. During the study follow-up 35.2% of patients were either listed or transplanted without being listed.

Compared to African Americans greater percentage of White patients graduated from the college (16.7% vs. 10%), smaller percentage of White patients never graduated from the high school (30.8% vs. 38.6%). Compared to Whites African American subjects had a lower likelihood of listed or transplanted without being listed (HR 0.7, p<0.001) and lower likelihood of being transplanted after placed on the waiting list (HR 0.58, p<0.001). When analyzed in groups divided by education level, compared to Whites the likelihood of African American patient being listed or transplanted without listing was lower in three less educated groups: HR 0.67 (p=0.005) for those never completed high school; HR 0.76 (p=0.02) for high school graduates; and HR 0.65 (p=0.003) for those with some college education. However, there was no significant difference in those with highest education level (HR 0.75, p=0.1).

In conclusion, while racial disparities in access to kidney transplantation do exist, the difference lose significance in highly educated individuals.

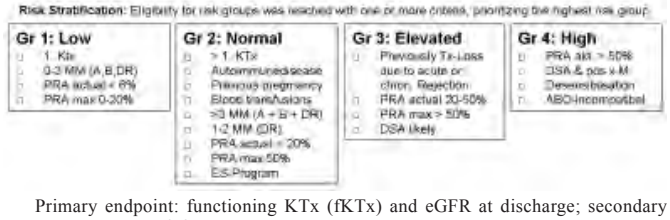
Disclosure of Financial Relationships: nothing to disclose

**PUB706**

**Prospective Immunologic Risk Stratification in Kidney Transplant Recipients – Evaluation of Early Outcome** Lioba V. Schewior,[1] Rolf A. Stahl,[1] Christine Eulenburg,[3] Thomas Eiermann,[4] Lioba Schewior,[2] Björn Nashan,[2] Friedrich Thaiss.[1] *[1]Nephrology, University Medical Center Hamburg-Eppendorf, Germany; [2]Hepatobiliary/Transplantation Surgery, University Medical Center Hamburg; [3]Medical Biometry/Epidemiology, University Medical Center Hamburg; [4]Transplant Immunology/HLA-Laboratory, University Medical Center Hamburg.*

Presensitization of Kidney Transplant Recipients (KTR) has increasing impact on allograft outcome. Still, there is a lack of valid markers to predict the immunologic risk of the allograft/KTR match. The aim was to evaluate an immunologic stratification using established clinical and HLA-laboratory data.

Prior to Kidney Transplant (KTx) stratification was performed




Primary endpoint: functioning KTx (fKTx) and eGFR at discharge; secondary endpoint: delayed graft function (DGF), adverse events and health economic parameters. Immunosuppression (IS) for all KTR were steroids, Basiliximab and Tacrolimus (C0 4-6 ng/dl) or Cyclosporin (C0 80-120 ng/dl) with either MPA or Everolimus. Risk groups 3 and 4 did received additional IS.

N=79 KTR were enrolled. Baseline demographics were comparable (age, cold ischemic time coronary artery disease, diabetes, p=ns). Best KTx function was observed in group 1 and 2, lower in groups 3+4.

KTx function at time of discharge

| Group (n) | fKTx [%] | MDRD4 [mean ml/min] | [+/-sd] | DGF [%] |
|---|---|---|---|---|
| 1 (23) | 95.7 | 58.7 | 27 | 17.4 |
| 2 (44) | 86.4 p=0.008 | 56.5 | 23 ns | 22.7 |
| 3 + 4 (12) | 50.0 p=0.011 | 50.4 | 19 ns | 25.0 |
| Total (79) | 83.5 | 56.6 | 24 | 21.5 |

Two deaths occurred, both due to cardiovascular events (gr 1,2). Other complications were rare with no differences between groups. IS costs were highest in group 4.

In this cohort pretransplant immunologic risk stratification allowed to identify an important proportion of patients at immunologic risk (15%) with an incidence of early allograft failure of 50%.

Disclosure of Financial Relationships: nothing to disclose

**PUB707**

**Comparisons of Enteric-Coated Mycophenolate Sodium (EC-MPS) and Mycophenolate Mofetil (MMF) in Renal Transplant Recipients (RTRs) with Diabetes Mellitus (DM) Prior to Transplant in the Mycophenolic Acid Observational Renal Transplant (MORE) Registry** Fuad S. Shihab,[1] Laurence Chan,[2] Mohanram Narayanan,[3] Michael J. Moritz,[4] Anne Wiland,[5] Kevin M. Mccague,[5] Cataldo Doria.[6] *[1]University of Utah, Salt Lake City, UT; [2]University of Colorado, Denver, CO; [3]Scott and White Clinic, Temple, TX; [4]Lehigh Valley Health Network, Lehigh Valley, PA; [5]Novartis, East Hanover, NJ; [6]Thomas Jefferson University Hospital, Philadelphia, PA.*

**Introduction:** The MORE Registry is a prospective, observational study of de novo RTRs designed to determine effectiveness, tolerability and safety of EC-MPS vs. MMF-based regimens. **Methods:** Based on standard-of-care at 40 US sites, outcomes analyzed included: graft survival (GS), patient survival (PS), first biopsy-proven acute rejection (BPAR), adverse event (AE) rates, serum creatinine (SCr) and percentages of RTRs maintained on at least full recommended mycophenolic acid (MPA) dose (1440/2000 mg/day, EC-MPS/MMF). Preliminary data {229 DM (159 EC-MPS/ 70 MMF), 454 non-DM (304 EC-MPS/ 150 MMF) RTRs; 100% tacrolimus; 58% maintenance steroids} were analyzed. **Results:** Interim results at 1, 3, 6 and 12 mo showed that more of the DM EC-MPS RTRs were maintained on at least full dose of MPA (EC-MPS/ MMF: 79.4/66.7%, p=0.05; 69.2/56.5%, p=0.08; 56.3/47.8%, p=0.38; 50.5/45.7%, p=0.69). The same trend was observed in the non-DM RTRs (EC-MPS/MMF: 81.6/73.0%, p=0.04; 74.5/61.2%, p=0.01; 57.2/44.8%, p=0.04; 47.8/41.9%, p=0.39). Comparable 6-month clinical outcomes were achieved for effectiveness, tolerability and safety in all RTRs. In the DM group of EC-MPS/MMF RTRs, there were no significant differences in GS (99.4/100%, p=0.82), PS (99.3/96.9%, p=0.06), BPAR (6.6/4.3%, p=0.88), mean SCr (1.44/1.54 mg/dL, p=0.33), early AEs by organ system, infections or neoplasia. Similar results were observed in the non-DM group (EC-MPS/MMF: GS 98.6/98.6%; PS 99.6/100%; BPAR 6.8/6.9%: SCr 1.44/1.56 mg/dL). **Conclusion:** A greater percentage of RTRs (± DM) treated with EC-MPS were maintained on full dose MPA, which was previously shown to be a good predictor of long-term graft outcomes.

Disclosure of Financial Relationships: Employer: University of UtahResearch Funding: Novartis; Honoraria: Novartis.

**PUB708**

**Prevalence of and Associations between Low 25-Hydroxyvitamin D Levels and Anemia in Renal Transplant Recipients** Michael Shye, Michal L. Melamed. *Albert Einstein College of Medicine/ Montefiore Medical Center.*

Low 25-Hydroxyvitamin D (25(OH)D) levels have recently been associated with poor outcomes including anemia, infection and all-cause mortality in the general population. Renal transplant recipients (RTR) are a specific population that is more susceptible to these poor outcomes.

We studied all RTR >21 years old who had 25(OH)D values within the first year of transplantation at the Montefiore Medical Center from 2000 to 2008 (n=251) using an electronic medical records database. The baseline 25(OH)D level was defined as the first value obtained after transplantation. We collected information on baseline demographics, co-morbidities, laboratory results and vitamin D use. In addition, infectious hospitalization and mortality was obtained from hospital records and via linkage to the Social Security Administration mortality registry.

Participants had a mean age 48.2 (standard deviation (SD) 13.2), 58% were male, 29% were African-American, 37% were Hispanic and 18% were white, median Charlson score was 3 (IQR 2, 5) and 38% had diabetes mellitus at baseline. Seven % had 25(OH)D levels >=30 ng/mL, 40% had 25(OH)D levels <30 but >=15 ng/mL and 53% had 25(OH)D levels <15 ng/mL. Twenty-eight % had levels <10 ng/mL. 25(OH)D levels were correlated with hemoglobin (hgb) levels (rho=0.18, p=0.003). After multivariable adjustment for demographics, co-morbidities and vitamin D use, for every 1 ng/mL higher 25(OH)D level, hgb was higher by 0.03 g/dL (0.004, 0.05). 119 patients were hospitalized for infectious causes. Patients with baseline 25(OH)D levels <10 ng/mL had a higher incidence rate ratio (IRR) for infectious hospitalization (IRR 1.38 (0.91, 2.12) and those with levels <15 ng/mL had an IRR of 1.28 (0.84, 1.94). Only 25 patients died over follow-up (4 years (IQR 2, 6)). There was no association between baseline 25(OH)D levels and mortality.

We found that in this consecutive sample of RTR with baseline 25(OH)D levels, lower 25(OH)D levels were associated with lower hgb levels. Further research is warranted in this area.

Disclosure of Financial Relationships: nothing to disclose

**PUB709**

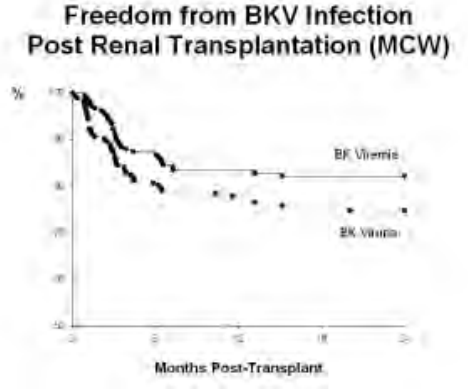
**Prospective Study of BK Virus Infection in Renal Transplant Recipients** Kumar Sujeet, Ankit Sakhuja, Y Ran Zhu, Brahm S. Vasudev, Ehab Saad, Barbara Bresnahan, Sundaram Hariharan. *Division of Nephrology, Medical College of Wisconsin, Milwaukee, WI.*

Introduction: BK virus infection is common in kidney transplant recipients.

Aim: The current prospective single center study evaluated the natural history of BKV infection after renal transplantation.

Methods: A total 189 subjects were enrolled from July 2007 to Sept. 2009. Subjects were followed till May 2010. Subjects were screened at 1, 3,6 and 12 months for viruria and viremia. Detection of BKV DNA in plasma and or urine was considered as BKV infection. Significant BKV infection is defined by BKV DNA copies >10,000/ml in plasma or >100,000 copies/ml in urine. Maintenance Immunosuppressive therapy consisted of MMF, Tacrolimus and Prednisone.

Results: A total of 65 subjects had BKV infection and 124 without. A total 48 had viremia and viruria, and 17 with viruria alone. The donor/recipient demographics, transplant (donor type, CIT, PRA, HLA mismatch) and post-transplant (DGF, induction antibody, acute rejection) variables were not substantially different in subjects with or without infection. Majority of BKV infection occurred six months post transplantation. Significant BKV viremia was seen in 21/189 (11%). These viremic subjects were submitted for transplant renal biopsy. Silent BKV nephritis was detected in 8/21 subjects. All subjects with significant viremia were treated successfully with reduction of immunosuppression without antiviral therapy. Details of appearance and decline in BKV DNA are not shown. Kaplan-Meier estimates of freedom from BKV infection is shown in figure 1.




Conclusion: In conclusion, in renal transplant recipients 1. BKV infection is common, 2. seen within six months post-transplant, 3. silent BKV nephritis can be seen without renal dysfunction, 4. reduction of immunosuppression alone can successfully treat BKV infection.

Disclosure of Financial Relationships: nothing to disclose

**PUB710**

**Cardiovascular Disease Risk Factors in Postoperative Japanese Living Kidney Donors; a Cross-Sectional Study** Masahiko Yazawa,[1] Ryo Kido,[2] Takashi Yasuda,[1] Yugo Shibagaki,[1] Kenjiro Kimura.[1] *[1]Center for Kidney Diseases, St. Marianna University Hospital, Kawasaki, Kanagawa, Japan; [2]Department of Epidemiology and Healthcare Research, Kyoto University, Kyoto, Japan.*

[Background] It is reported that living kidney donors (LKDs) develop to have cardiovascular disease (CVD) risk factors post-donation, however, the prevalence of these risks have not been well studied.

[Purpose] We examined kidney function, albuminiuria and CVD risk factors in 36 prevalent Japanese LKDs to describe those prevalence in current practice.

[Methods] The study design was cross-sectional. All the thirty-six LKDs who underwent donor uninephrectomy in our hospital and agreed to the informed consent were selected. Their kidney functions measured by inulin crearance (Cin) and CVD risk factors (albuminuria, glucose / lipid metabolism, blood pressure by ambulatiory monitoring, uric acid, and BMI) were cross-sectionally evaluated.

[Results] The mean age at donation and at the study were 58.1±9.8 and 61.7±9.2, respectively. The median time from donation to the study was 944 days. The mean kidney function measured by Cin was 55.2±10.3 ml/min/1.73m$^2$, indicating 63.9% of donors were at CKD stage 3. Microalbminuria appeared in as much as 16.7% of LKDs. Prevalence of other CVD risk factors such as hypertension, non-dipper type blood pressure, dyslipidemia, glucose intolerance, and hyperuricemia were 22.2%, 40.6%, 41.7%, 19.4% and 27.8%, respectively, which were almost equal or lower than Japanese general population but still was significant considering that LKDs were cleared from pretransplant evaluation that they were "healthy".

[Conclusion] After kidney donation, although equally or less prevalent than general population, kidney donors have still relatively high prevalence of cardiovascular risk factors, indicating the importance of long-term follow up of donors with special attention to CVD risk factors.

Disclosure of Financial Relationships: nothing to disclose

**PUB711**

**Relationship between Arginine Vasopressin and Aquaporin-2 in Early Postoperative Kidney Transplantation Patients** Yang Wook Kim,[1] Kyu-Bok Jin,[1] Hyun Seung Lee,[2] Tae Hee Kim.[2] *[1]Nephrology, Internal Medicine, Haeundae Paik Hospital, Pusan, Korea; [2]Nephrology, Internal Medicine, Busan Paik Hospital, Pusan, Korea.*

**Background:** The aquaporin-2 (AQP2) water channel is mainly located in the apical plasma membrane of epithelial cells in the collecting ducts in responses to arginine vasopressin (AVP). The level of AVP increases in chronic kidney disease, but mechanism remains unclear. There are few studies about AVP expression in kidney transplantation(KT). This present study was aimed at relationship with AVP and urine AQP2 expression in KT patients prospectively.

**Method:** Fourteen KT patients and eight healthy persons(control) were enrolled. To evaluate urine concentration, plasma AVP, urine AQP2 expression, serum osmolarity, urine osmolarity, urine specific gravity, serum sodium and urine sodium were measured before and after KT.

**Result:** KT did not produce a significant change in serum sodium, urine sodium, serum osmolarity, and urine specific gravity. The urine osmolarity increased significantly after KT but were low compared to control(p=0.002). Pre-KT p-AVP level was elevated inappropriately, but AVP declined to normal range after KT but was high compared to control(P=0.018). The urine AQP2 expression unrelated to the high p-AVP level was suppressed before KT, and increased after KT, however, low compared to control(p=0.005).

**Conclusion:** The relationship of urine AQP2 and p-AVP were almost normalized after KT, but urine AQP2 expression compared to P-AVP level was inappropriately decreased in early period after KT. These results suggest that the correlation between p-AVP and AQP2 expression are not yet regulated by normal feedback mechanisms in early KT period.

Disclosure of Financial Relationships: nothing to disclose

**PUB712**

**Effect of Desensitization Therapy with High Dose IVIG on Anti-HLA Antibodies** Vinay Nair, Deirdre L. Sawinski, Peter S. Heeger, Bernd Schroppel. *Nephrology, Mount Sinai Medical Center, NY, NY.*

Purpose: The aim of this study was to determine the effect of high dose IVIG on anti-HLA antibodies in highly sensitized kidney wait-listed patients.

Methods: 15 patients with a cPRA>40% and high on the wait list were analyzed (12 adult, 3 pediatric). Patients received 2gm/kg IVIG per month for 4 months or until transplanted. HLA antibodies were determined before and after desensitization using Luminex single antigen beads. Antibodies > 10,000 MFI resulted in entering unacceptable antigens into UNOS. Eight patients had IgG subtyping performed. Statistical analysis was performed using Graphpad Prism software.

Results: 66% of IVIG treated patients had a decrease in antibody strength (median decrease 1908 MFI or 26.7% of baseline) while 26% had an increase (median increase 1203 MFI or 40.8% of baseline). Pooled analysis revealed a decrease of 837 MFI translating into an 11.2% decrease from baseline (p<0.0001). There was no difference when analyzing class 1 and class 2 separately. The number of class II antibodies with an MFI>10,000 decreased (7 vs. 3 antibodies; p=0.03) whereas there were more antibodies with an MFI from 1000 to 5000 after IVIG (26 vs. 31 antibodies; p=0.05). 5 patients had a change in calculated PRA greater than 10% (4 decreased (range 12-27%) 1 increased by 17%). If cPRA was less than 90% there was a greater likelihood that IVIG would result in a change. There were no differences in the response of IgG class 1 vs. class 2 after desensitization. The 5 patients subsequently transplanted had lower cPRA pre and post desensitization.

Conclusion: Desensitization with high dose IVIG did not result in a clinically meaningful reduction in antibody number, strength, or cPRA in the majority of patients. The effect of IVIG may be greater in patients with a lesser degree of sensitization. However IVIG may have other beneficial effects in highly sensitized patients and change in antibody strength should not be the only measure of successful desensitization.

Disclosure of Financial Relationships: nothing to disclose

**PUB713**

**Tumorectomised ABO-Incompatible Renal Transplant Amidst Donor Specific Antibodies** Vinod Venkataraman. *Renal Medicine, Royal Melbourne Hospital, Melbourne, VIC, Australia.*

INTRODUCTION

The gulf between survival on dialysis and transplantation led to overcoming of ABO incompatibility and donor specific anti-HLA antibodies (DSAb) in live donor renal transplants. Eculizumab, an inhibitor of the C5 complement molecule has been used to prevent antibody mediated rejection (AbMR) associated with DSAb. Additional strategy was implantation of tumorectomised donor kidney. We describe a transplant of tumorectomised ABO incompatible (ABOi) kidney amidst positive flow crossmatch to DSAb.

METHODS

A 57 years old patient with a donor against whom she was both ABOi and had DSAb was planned for a paired kidney donation (PKD). An incidental small tumour of donor kidney prevented this. Alternatively she had pre transplant plasma exchanges and received tumorectomised kidney with Eculizumab infusions.

The anti-B ortho titre fell from 16 to 4 and the DSAb dropped from MFIs of 16848 to HLA- B50and 2327 to HLA- B38 to 5648 and 249 respectively post-plasmapharesis. The patient received Eculizumab and Basiliximab induction prior to theatre and maintained on Tacrolimus, Mycophenolate and Prednisolone.

After removal from the donor, the kidney was sighted to have the original lesion along with a second small tumour abutting the antero-medial border. Papillary renal cell cancer was found on complete excision.

RESULTS

There was immediate graft function and creatinine reached nadir of 115 umol/ l. ABO titres and DSAb remained low. Renal function was stable with no proteinuria but marginal microalbuminuria.

Protocol biopsies at 3 months showed no cellular rejection, though there were occasional areas of focal glomerulitis. There were no other features AbMR and C4d was negative. There were no evidence of transplant glomerulopathy**.** Eculizumab infusions finished 3 months post transplant after re-assurance from renal biopsy and low DSAb levels.

Surveillance imaging at 3 months showed no recurrence in transplant, but a lesion was seen in the native kidney. Nephrectomy showed clear cell renal cancer with no invasion of renal vein.

CONCLUSION

Innovative surgical techniques and new immunosuppressive therapies enables renal transplantation in previously unimaginable circumstances.

Disclosure of Financial Relationships: nothing to disclose

**PUB714**

**Excessive Activation of the Complement System Underlies the Progression of Autosomal Dominant Polycystic Kidney Disease** Zhen Su,[1] Changlin Mei.[2] *[1]Nephrology, Changzheng Hospital, Second Military Medical University, Shanghai, China; [2]Nephrology, Changzheng Hospital, Second Military Medical University, Shanghai, China.*

**Objective** To explore the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD), and seek new therapeutic targets. **Methods** Urine glycoproteins were identified using mass spectrometry(MS). Urine glycoproteins was reconfirmed by western blot, in-gel digestion and MS. Complement factor B (CFB) and C9 were detected by immunohistochemistry and western blot. C3 and C4 were detected by immunoturbidimetry. Han:SPRD rats were interfered with rosmarinic acid. **Results** 113 glycoproteins and 261 peptide segments were identified, of which 39 glycoproteins expressed differentially in the urine of ADPKD patients. Compared with the normal control group, the expression of CFB, SERPING1 and C9 increased remarkably with the progression of ADPKD, while the expression of C1RL, CD55 and CD59 decreased gradually. Western blot, in-gel digestion and MS reconfirmed that the change of the 6 proteins was consistent with that of MS. Immunohistochemistry showed that CFB and C9 expressed highly in cyst-lining epithelial cells and renal tubular epithelial cells of ADPKD patients and rats. Western blot showed that the expression of C9 and CFB in the kidney of ADPKD patients and rats were increased. Serum C3 and C4 were normal in ADPKD patients. Rosmarinic acid was able to delay deterioration of renal dysfunction of rats. **Conclusion** The alternative complement pathway expressed highly in ADPKD kidney tissue, and complement inhibitor rosmarinic acid was able to delay the progression of renal dysfunction in Han: SPRD rats, suggesting that it participated in the progression of ADPKD, and implying that inhibiting complement activation may be a potential effective strategy for the treatment of ADPKD.

Disclosure of Financial Relationships: nothing to disclose

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